A prospective study of the effects of erythropoietin and early sustained hypothermia on renal function and renal biomarkers in patients with traumatic brain injury

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General declaration

In accordance with Monash University Doctorate Regulation 17/ Doctor of Philosophy and Master of Philosophy (MPhil) regulations the following declarations are made:

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes five original papers published in peer reviewed journals. The core theme of the thesis is exploring treatments and early biomarkers for acute kidney injury in patients with traumatic brain injury. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the Department of Epidemiology and Preventive Medicine under the supervision of Rinaldo Bellomo, Alistair Nichol, Michael Bailey and additional supervisor, Robert Atkins. The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of chapters 1 and 2 my contribution to the work involved the following:

Thesis chapter	Publication title	Publication status*	Nature and extent of candidate's contribution
1	The meaning of acute kidney injury and its relevance to intensive care and anaesthesia.	Published	Principal author, responsible for literature review and interpretation, and development and writing up of the manuscript. Responsible author who accepts overall responsibility for the publication.
1	The incidence of acute kidney injury in patients with traumatic brain injury.	Published	Principal author, involved in study conception. Responsible for study design, literature review, data collection, analysis, interpretation, and development and writing up of the manuscript. Responsible author who accepts overall responsibility for the publication.
2	Erythropoietin (EPO) as a novel brain and kidney protective agent.	Published	Principal author, responsible for literature review and interpretation, and development and writing up of the manuscript.
2	Therapeutic hypothermia: Benefits, mechanisms and potential clinical applications in	Published	Principal author, responsible for literature review and interpretation, and development and writing up

	neurological, cardiac and kidney injury.		of the manuscript. Responsible author who accepts overall responsibility for the publication.
2	Biomarkers of acute kidney injury in anaesthesia, intensive care and major surgery: from the bench to clinical research to clinical practice.	Published	Principal author, responsible for literature review and interpretation, and development and writing up of the manuscript.

I have / **have not** (circle that which applies) renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Signed: ……

Date: **20 / 11 / 2013** ……

Statement regarding confidentiality of thesis

The findings in this thesis must remain confidential until publication of the results of the parent EPO-TBI and POLAR trials. Blinded supervisors, Rinaldo Bellomo and Alistair Nichol, have had no access to the Renal substudy data and no involvement in the Results section of the thesis nor any part of the thesis that refers to these results. An additional Monash supervisor who had no direct access to any trial data was enlisted to critique drafts of the Results chapter and sections referring to the results.

EPO-TBI and POLAR trial management committee members and the Australian and New Zealand Intensive Care Society (ANZICS) Clinical Trials Group Executive Committee will have the opportunity to review and approve the non-confidential sections of the thesis.

Thesis examiners will sign a confidentiality agreement not to disclose the information to other parties. For Monash staff and students no confidentiality undertaking is required because both are bound by obligations under their employment contract or obligations signed up to when becoming a student at Monash (per Maria Stevenberg, Contracts Associate, Monash Solicitors).

The thesis will be embargoed with restricted access on the Monash repository and results will not be published until the EPO-TBI and POLAR trials are published.

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Summary

Acute Kidney Injury (AKI) is the consensus term developed and validated within the last 10 years that encompasses all classes or stages of kidney injury; it refers to a continuum of injury. AKI is common in critically ill patients and is associated with adverse outcomes such as longer length of stay, progression to Chronic Kidney Disease, and mortality. Research into therapies to treat AKI has been largely unsuccessful, which is partly attributable to the inadequacy of conventional methods (creatinine, urea and urine output) to detect AKI early enough for therapies to be effectively implemented. As a result, the investigation of new therapies for AKI and of novel biomarkers for the early detection of AKI, have become a research priority.

Two treatments were to be given to patients with traumatic brain injury in clinical trials commencing in 2010, to investigate their benefit for neurological function. The treatments: erythropoietin (a tissue protective drug) and early, sustained hypothermia, had also been shown to protect the kidney from injury and to hasten renal recovery in animal studies. However, few studies of their clinical effectiveness had been undertaken in humans. This was a perfect opportunity to investigate potential therapies for AKI while also incorporating investigation of early novel biomarkers for AKI; thereby addressing two of the priority areas in renal research. In this thesis we have taken advantage of the unique opportunity that the EPO-TBI and POLAR trials present, to investigate whether erythropoietin and hypothermia (respectively) reduce the occurrence, severity, and duration of AKI. Furthermore, in a nested cohort of patients from each trial several promising new biomarkers for AKI have been evaluated. We have assessed whether these novel biomarkers predicted the occurrence, severity and duration of AKI and whether erythropoietin and hypothermia reduced the release of these renal biomarkers.

Our findings indicate that in terms of AKI classification, a) AKI is considerably more common than previously thought in patients with traumatic brain injury making further investigation into causes, its impact on outcomes, and prevention relevant, and b) serum creatinine is an unreliable marker of AKI in this patient group so potential alternatives identified need to be used and further investigated.

Of the treatments under investigation, to our surprise, we found that hypothermia is associated with an increased frequency of AKI compared to normothermia, and that there is a suggestion in the evidence (including biomarker measurements) that EPO may be associated with a negative impact in patients with traumatic brain injury.

The earliest, statistically significant predictors for AKI were plasma cystatin C and urine Neutrophil Gelatinase-Associated Lipocalin (NGAL) at 48 hours post dose/injury.

In response to erythropoietin and hypothermia, urine Kidney Injury Molecule-1 (KIM-1) and plasma NGAL showed the greatest difference in response between treated and control groups for both therapies.

Overall, there were a number of unexpected findings, several of which can be checked when the EPO-TBI and POLAR trial data is complete, and others that warrant further dedicated investigation. However, it is clear that given the higher incidence of AKI in patients receiving hypothermia versus normothermia, the risks and benefits of hypothermia in these patients need to be reassessed. It is also clear from the extent of AKI in this patient group that a serious review of patient monitoring and management is required. AKI is independently associated with mortality and other adverse outcomes and attention to its prevention could help to improve outcomes for patients with traumatic brain injury.

List of abbreviations and definitions

Abbreviations/acronyms are listed in alphabetical order

ACEI	Angiotensin-converting-enzyme inhibitor
ACTRN	Australian-New Zealand clinical trials registry number
AIF	Apoptosis inducing factor
AKI	Acute kidney injury
AKIN	Acute kidney injury network
Akt	Protein kinase B
ALP	Alkaline phosphatise
ANZ	Australia and New Zealand
ANZICS CTG	Australian and New Zealand intensive care clinical trials group
APACHE III	Acute physiology and chronic health evaluation score (refined and published in 1991)
ASA	American society of anaesthesiologists
ATN	Acute tubular necrosis
AUC	Area under the curve (of the receiver operating characteristic [ROC] curve)
Bad	A pro-apoptotic member of the Bcl-2 protein family
BART	Blood conservation using antifibrinolytics in a randomized trial
Bcl-2	B-cell lymphoma 2
Bcl-x_L	Antiapoptotic protein: B-cell lymphoma-extra large
BFU-Es	Burst-forming unit-erythroid cells
BMI	Body mass index
CD-131	Common beta subunit to certain cytokine receptors
CEPO	Carbamylated EPO
CHEST	Crystalloid versus hydroxyethyl starch trial
CI-AKI	Contrast-induced acute kidney injury
CKD	Chronic kidney disease
CLP	Caecal ligation and puncture
CPB	Cardiopulmonary bypass
CPP	Cerebral perfusion pressure
Cr	Creatinine
CRASH 2	Clinical randomisation of an antifibrinolytic in significant haemorrhage trial (evaluated the effect of tranexamic acid in TBI patients)
CRP	C-reactive protein
CRRT	Continuous renal replacement therapy
CT	Computerised tomography
CyC	Cystatin C
DAI	Diffuse axonal injury
DECRA	Decompressive craniectomy trial (in diffuse traumatic brain injury)

DeLiT	Dexamethasone, light anaesthesia and tight glucose control trial
DNA	Deoxyribonucleic acid
DTI	Diffusion tensor imaging
DVT	Deep vein thrombosis
EARLY ARF	Early intervention in acute renal failure trial (to evaluate the effectiveness of EPO in preventing renal failure)
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme linked immunosorbent assay
EPO	Erythropoietin
EPOR	Erythropoietin receptor
EPO-TBI	Erythropoietin in traumatic brain injury (EPO-TBI)
FA	Fatty acid
FACCT	Fluids and catheters treatment trial
FFAs	Free fatty acid
FOXO3a	Forkhead transcription factor
GCS	Glasgow coma scale
GFR	Glomerular filtration rate
GGT	γ-glutamyl transpeptidase
GOSE	Extended glasgow outcome scale
GSK3β	Glycogen synthase kinase 3β
GST	Glutathione S-transferase
HSP70	Heat shock protein 70
ICP	Intracranial pressure
ICU	Intensive care unit
IL-18 / 6 etc	Interleukin-18 / 6 etc
IMPACT	International mission for prognosis and clinical trial
ISRCTN	International Standard Randomised Controlled Trials Number
JAK2	Member of the janus-type protein tyrosine kinase family which is bound to the common beta subunit of the EPOR
JAK2-STAT5	A signalling pathway that transmits information from chemical signals outside the cell, through the cell membrane, and into gene promoters on the DNA in the cell nucleus, which causes DNA transcription and activity in the cell
KDIGO	Kidney disease: improving global outcomes
KIM-1	Kidney injury molecule-1
L-FABP	Liver-type fatty acid binding protein
MAPK	Mitogen-activated protein kinase
MDRD'175' equation	A modification of diet in renal disease equation to estimate GFR (use recommended in Australia in 2007)
MOD	Multiple organ dysfunction
mPTP	Mitochondrial permeability transition pore
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MRS	Magnetic resonance spectroscopy
NAC	N-acetylcysteine

NCT	National clinical trial number, another term for the ClinicalTrials.gov registry number
NF-kB	Nuclear factor-kappaB
NGAL	Neutrophil gelatinase-associated lipocalin
NHMRC	National health and medical research council
NO	Nitric oxide
NSAID	Non-steroidal anti-inflammatory drug
OR	Odds ratio
PE	Pulmonary embolism
PEG-SOD	Polyethylene glycol–superoxide dismutase
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
POLAR	The prophylactic hypothermia trial to lessen traumatic brain injury
pRIFLEmax	Term that may be used in paediatric RIFLE classification of AKI
ProTECT III	Progesterone for the treatment of traumatic brain injury trial
RAAS	Renin-angiotensin-aldosterone system
RBF	Renal blood flow
RCRI	Revised cardiac risk index
RELIEF	Restrictive versus liberal fluid therapy in major abdominal surgery trial
RENAL	Randomized evaluation of normal versus augmented level replacement therapy trial
RHuEPO	Recombinant human erythropoietin
RIFLE	Classification system for acute kidney injury: risk, injury, failure, loss, endstage
RINSE	Rapid infusion of cold saline during CPR for patients with cardiac arrest
RRT	Renal replacement therapy
SCr	Serum creatinine
SGK1	Serum and glucocorticoid-regulated kinase-1
SOFA	Sequential organ failure assessment
STATs	Signal transducer and activators of transcription e.g. STAT5
SyNAPSe	Study of the neuroprotective activity of progesterone in severe traumatic brain injuries
TBI	Traumatic brain injury
TH	Therapeutic hypothermia
TNF-alpha	Tumour necrosis factor alpha: cytokine involved in the acute phase reaction of systemic inflammation
TRANSFUSE	Standard issue transfusion versus fresher red blood cell use in intensive care trial
USA	United States of America
VEGF	Vascular endothelial growth factor
XIAP	X-chromosome-linked inhibitor of apoptotic protein

List of publications, presentations, awards and grants

Listed below are the candidate's publications, presentations, and awards relevant to the period of candidature.

PUBLICATIONS

Journal articles relevant to thesis

1. Moore E, Bellomo R, Nichol A. Biomarkers of acute kidney injury in anesthesia, intensive care and major surgery: from the bench to clinical research to clinical practice. Minerva Anestesiol 2010 Jun;76(6):425-40.
2. Moore EM, Bellomo R, Nichol AD, Harley N, MacIsaac C, Cooper DJ. The incidence of acute kidney injury in patients with traumatic brain injury. Ren Fail 2010 Oct; 32(9).
3. Moore EM, Bellomo R, Nichol AD. Erythropoietin (EPO) as a novel brain and kidney protective agent. Anaesthesia and Intensive Care. 2011 May;39(3):356-72
4. Moore EM, Nichol AD, Bernard SA, Bellomo R. Therapeutic hypothermia: Benefits, mechanisms and potential clinical applications in neurological, cardiac and kidney injury. Injury. 2011 Sep;42(9):843-54. Epub 2011 Apr 9.
5. E. Moore R. Bellomo. Novel Biomarkers of acute kidney injury: ready for clinical application? Current Opinion in Critical Care 2010, 16:523–525
6. Moore EM, Bellomo R, Nichol AD. The Meaning of Acute Kidney Injury and its Relevance to Intensive Care and Anaesthesia. Anaesthesia and Intensive Care. 2012 Nov;40(6):929-48.

Editorial

1. Moore E, Bellomo R. Erythropoietin (EPO) in acute kidney injury, Annals of Intensive Care 2011,1:3. Available at http://www.annalsofintensivecare.com/content/1/1/3

Journal articles not specific to thesis

1. Moore EM, Tobin AE. The association between preoperative eGFR and outcomes in cardiac surgical patients. Crit Care Resusc 2009 Dec;11(4):310.
2. Moore EM, Simpson JA, Tobin A, Santamaria J. Preoperative estimated glomerular filtration rate and RIFLE-classified postoperative acute kidney injury predict length of stay post coronary bypass surgery in an Australian setting. Anaesth Intensive Care 2010 Jan;38(1):113-21.
3. Schneider AG, Bellomo R, Reade M, Peck L, Young H, Eastwood G, Garcia M, Moore E, Harley N. Safety evaluation of a trial of lipocalin-directed sodium bicarbonate infusion for renal protection in at-risk critically ill patients. Crit Care Resusc 2013 Jun;15(2):126-33.

Book Chapters

1. Moore E, Bernard S, Cooper DJ, Nichol A. Therapeutic Hypothermia: Clinical History, Mechanisms of Action, Common Complications, Current Indications and Future Research Directions in the Critically Ill. In: Valdez Delgado JI, Fernandez Garza VG, editors. Hypothermia: Prevention, Recognition and Treatment. Hauppauge, N.Y.: Nova Science Publishers, 2012. pp.43-68. Available at:

https://www.novapublishers.com/catalog/product_info.php?products_id=33761

2. Moore E, Bernard S, Cooper DJ, Nichol A. Prophylactic Hypothermia in Traumatic Brain Injury. In: Valdez Delgado JI, Fernandez Garza VG, editors. Hypothermia: Prevention, Recognition and Treatment. Hauppauge, N.Y.: Nova Science Publishers, 2012. pp.69-90. Available at: https://www.novapublishers.com/catalog/product_info.php?products_id=33761

PRESENTATIONS

Oral presentations: National / International

Oct 2010:
Australia and New Zealand Intensive Care Society (ANZICS) Clinical Trials Group meeting, Melbourne
Presentation of the Renal Assessment of the EPO-TBI (Erythropoietin for Traumatic Brain Injury) trial for endorsement
E. Moore, R. Bellomo, A. Nichol, M. Bailey

Mar 2011:
Australia and New Zealand Intensive Care Society (ANZICS) Clinical Trials Group meeting, Noosa
Presentation of the Renal Substudy of the POLAR (Prophylactic hypothermia in Traumatic Brain Injury) trial for endorsement
E. Moore, R. Bellomo, A. Nichol, M. Bailey

Mar 2011:
Australasian Cardiovascular Nursing College - Annual Scientific meeting, Melbourne
Preoperative eGFR and Postoperative RIFLE AKI predict length of stay after CABG surgery
E. Moore, J. Simpson, A. Tobin, J. Santamaria.

Mar 2011:
Australian College of Critical Care Nurses, Victorian Symposium, Melbourne
The incidence of Acute Kidney Injury in Traumatic Brain Injury
E. Moore, R. Bellomo, A. Nichol, N. Harley, C. MacIsaac, D. J. Cooper.

Apr 2011:
International Neurotrauma Symposium, Shanghai, China
The incidence and implications of Acute Kidney Injury in Traumatic Brain Injury
E. Moore, R. Bellomo, A. Nichol, N. Harley, C. MacIsaac, D. J. Cooper.

Nov 2012:
AMREP Early Career Researcher, Second Biannual Symposium
Acute kidney injury in traumatic brain injury: frequency and implications
E. Moore, R. Bellomo, A. Nichol, N. Harley, C. MacIsaac, D. J. Cooper.

Mar 2013:
Australian and New Zealand Intensive Care Society (ANZICS) Clinical Trials Group meeting, Noosa
The Renal substudies of the EPO-TBI and POLAR trial: an update.
E.Moore, R. Bellomo, A. Nichol, M. Bailey

Mar 2013:
ANZICS ICU Research Coordinator Interest Group, Annual meeting, Noosa
The THIRST study: an overview.
E.Moore

Oct 2013: ANZICS and Australian College of Critical Care Nurses (ACCCN) ASM, Hobart
Fluid accumulation post cardiac surgery and risk for renal replacement therapy.
E. Moore, A. Tobin, D. Reid, J. Santamaria

Oral presentations: Local

Mar 2011:
Alfred Hospital, ICU Renal Special Interest Group study day, Melbourne
The Renal Substudies of EPO-TBI and POLAR / The RIFLE classification system
E. Moore

May 2011:
Royal Melbourne Hospital, Research Week
The incidence and implications of Acute Kidney Injury in patients with Traumatic Brain Injury
E. Moore, R. Bellomo, A. Nichol, N. Harley, C. MacIsaac, D. J. Cooper.

Aug 2011:
Alfred Hospital, ICU Renal Special Interest Group study day, Melbourne
The Renal Substudies of EPO-TBI and POLAR / Renal Research in Alfred ICU
E. Moore

Nov 2012:
AMREP Early Career Researcher, Second Biannual Symposium
Acute kidney injury in traumatic brain injury: frequency and implications
E. Moore, R. Bellomo, A. Nichol, N. Harley, C. MacIsaac, D. J. Cooper.

Poster presentations

Sep 2010:
Australian Epidemiological Association Annual Conference
The incidence of acute kidney injury in patients with traumatic brain injury
E. Moore, R. Bellomo, A. Nichol, N. Harley, C. MacIsaac, D. J. Cooper (*Poster)*

Oct 2010:
ACCCN/ANZICS ASM, Melbourne
The incidence of acute kidney injury in patients with traumatic brain injury
E. Moore, R. Bellomo, A. Nichol, N. Harley, C. MacIsaac, D. J. Cooper (*Poster)*

Nov 2010:
Alfred Research Week Poster Display, Melbourne
The incidence of acute kidney injury in patients with traumatic brain injury
E. Moore, R. Bellomo, A. Nichol, N. Harley, C. MacIsaac, D. J. Cooper *(Poster)*

Nov 2011:
National Trauma Research Institute: 'From Roadside to Recovery - Successful Systems of Trauma Care'
Acute Kidney Injury in TBI: Incidence and implications
E. Moore, R. Bellomo, A. Nichol, N. Harley, C. MacIsaac, D. J. Cooper. (*Poster)*

AWARDS

Mar 2009:
StV Intensive Care Staff Award for outstanding contribution to St Vincent's Health Service.
Intensive Care Unit, St Vincent's Hospital, Melbourne

Mar 2009-Sep 2012:
Australian Postgraduate Award Scholarship
Awarded by the Australian government and Monash University to students of exceptional research potential who undertake a higher degree by research $78,476

Nov 2009 and Mar 2012/13:
Members Equity/ANF Education award
For assistance to attend the ANZICS/ACCCN Annual Scientific Meeting 2009 and the ANZICS Clinical Trials Group annual meetings in 2012 and 2013 $600

Sep 2010:
Department of Epidemiology and Preventive Medicine, Monash University: Conference support
For attendance at the Australian Epidemiological Association Annual Conference to present:
The incidence of acute kidney injury in patients with traumatic brain injury (*Poster)*
$400

Mar 2011:
Monash Postgraduate Travel Grant Award
For travel to Noosa, Queensland to present The Renal Substudy of the POLAR trial for endorsement at the Australian and New Zealand Intensive Care Society, Clinical Trials Group Meeting $450

Apr 2011:
Monash Postgraduate Travel Grant Award
For travel to Shanghai, China to present at the International Neurotrauma Symposium - The Incidence and implications of Acute Kidney Injury in Traumatic Brain Injury
$1,035

GRANTS

2010-2011 Intensive Care Foundation - Project grant $13,600
Project: Acute kidney Injury: Investigating potential therapies (erythropoietin and cooling) and early biomarkers in patients with traumatic brain injury.
Rinaldo Bellomo, Elizabeth Moore, Alistair Nichol, Michael Bailey

1 Introduction

1.1 Overview of Introduction

Acute Kidney Injury (AKI) is common in critically ill patients and is associated with longer length of stay, increased cost of hospital stay, mortality, and increased risk for progression to Chronic Kidney Disease. Conventional diagnostic markers of AKI become abnormal late in the course of injury (creatinine and urea) or lack specificity (urine output). As a result, the investigation of treatments for AKI has been severely hindered with interventions being implemented too late to be effective. Subsequently, it has become a research priority to find new early markers for AKI and to investigate potential therapies for AKI.

The EPO-TBI and POLAR trials are investigating therapies (erythropoietin and hypothermia respectively) for patients with traumatic brain injury (TBI). Evidence suggests that these therapies may also be protective of the kidneys; therefore, the trials presented a unique opportunity to investigate these two potential therapies for AKI in renal substudies. We have taken this opportunity to address two priority areas of renal research; potential new therapies for AKI and potential early biomarkers of AKI, in the Renal substudies of the EPO-TBI and POLAR trials.

Patients with TBI compose the patient group under investigation in the Renal substudies of the EPO-TBI and POLAR trials. In the Introduction to this thesis we review the central elements of the investigation: AKI, the condition for which we are seeking effective therapies and new early biomarkers; and TBI, the patient population in which we are assessing treatments and biomarkers.

The incidence of AKI in patients with TBI was unknown before this project commenced. Thus, it was necessary to ascertain whether the frequency of AKI in the TBI population was sufficient to warrant continuing with the planned investigation. The published preliminary study to address this question is included later in the Introduction followed by an outline of the thesis.

1.2 Acute Kidney Injury

Acute Kidney Injury (AKI) is now the consensus term that encompasses all classes or stages of kidney injury from 'renal impairment' to 'acute renal failure' (to use terms that have now become superseded). Therefore, AKI refers to a continuum of injury.

AKI is the focal topic in this thesis, which involves the exploration of treatments for and biomarkers of AKI in patients with TBI. The following review conducted by the candidate and colleagues sets the scene by providing a comprehensive introduction to AKI; synthesising the latest information on the epidemiology of AKI, diagnostic methods and treatments, changing views on its pathophysiology, and the current direction of AKI research. The review was published in a journal with an anaesthetic and critical care focus and was pitched at this readership.

Moore EM, Bellomo R, Nichol AD. The Meaning of Acute Kidney Injury and its Relevance to Intensive Care and Anaesthesia. Anaesthesia and Intensive Care. 2012 Nov;40(6):929-48.

Declaration for Thesis Chapter

Moore EM, Bellomo R, Nichol AD. The meaning of acute kidney injury and its relevance to intensive care and anaesthesia. Anaesth Intensive Care. 2012 Nov;40(6):929-48.

Declaration by candidate

In the case of section, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Searched and reviewed the literature, collated and interpreted the information, developed and wrote up drafts.	60 %

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
R Bellomo	Involved in conception of the article, reviewed and drafted manuscript.	N/A
A Nichol	Reviewed and drafted manuscript.	N/A

Candidate's Signature	Elizabeth Moore	Date 20/1/2013

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below.

Location(s)	Australian and New Zealand Intensive Care Research Centre, Department of Epidemiology and Preventive Medicine, Monash University, Alfred campus

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

	Date
	20/1/2013
PDF to Word	20/1/2013

Anaesth Intensive Care 2012; **40:** 929-948

Review

The meaning of acute kidney injury and its relevance to intensive care and anaesthesia

E. M. MOORE*, R. BELLOMO†, A. D. NICHOL‡

Australian and New Zealand Intensive Care Research Centre, School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia

SUMMARY

Acute kidney injury (AKI) is the new consensus term for acute renal failure. The term describes a continuum of kidney injury, a common condition in the critically ill and after major surgery, which is associated with increased mortality. The incidence of AKI in intensive care unit patients in Australia is >30% and sepsis is a major contributory factor. However, there is limited knowledge about its incidence after major surgery, except for cardiac surgery. The creation of staged AKI classification systems (RIFLE [Risk, Injury, Failure, Loss, End-stage], Acute Kidney Injury Network and the new Kidney Disease: Improving Global Outcomes criteria) has accelerated progress in critical care nephrology research by showing that even small changes in serum creatinine are associated with increased risk of death and that this risk increases progressively with severity of AKI. Recent thought and research has cast doubt over previously accepted pathophysiological views of AKI. Moreover, terms such as 'prerenal azotaemia' and 'acute tubular necrosis' are now being challenged as lacking validity, having little supportive evidence and carrying limited clinical utility. In this review, we explore the limitations of animal and human models of AKI and the implications of recent research on our current understanding of the pathophysiology of AKI. In addition, we describe conventional and novel diagnostic methods and therapies, and explore the clinical implications of the effect of fluid administration and perioperative management. Finally, we identify priorities for clinical investigations and future directions in AKI research.

Key Words: acute kidney injury, intensive care, anaesthesia

Acute kidney injury (AKI) is a consensus term now used to describe the continuum of injury previously called acute renal failure[1]. It is a common complication in the critically ill and is one of the most serious complications of critical illness and after cardiac surgery, being independently associated with an increased risk of death[2] and with prolonged intensive care unit (ICU) and hospital length-of-stay[3,4], which implies an increase in resource use and cost (Figure 1). As the severity of AKI increases, so does the risk of adverse outcomes[2,4,5]. AKI refers to a 'syndrome' involving a rapid reduction in renal excretory capacity with accumulation, among others, of nitrogen waste products such as creatinine and urea (called azotaemia). The concentration of potassium, phosphate and metabolic acids also typically increases. The term AKI implies a continuum of injury; studies confirm that even small increases in serum creatinine are associated with increased mortality and risk rises progressively as serum creatinine increases[2].

The International Acute Dialysis Quality Initiative consensus conferences led to the development of the RIFLE (Risk, Injury, Failure, Loss, End-stage) classification system to define AKI[1] (Figure 2). This classification system incorporates change in renal function from baseline, is easy to use and is clinically applicable across centres. The RIFLE system provides a framework to aid stratification and randomisation of AKI patients into clinical trials. It has facilitated comparison of studies on a large scale and has enhanced progress in scientific investigation of critical care nephrology. The recently developed Acute Kidney Injury Network (AKIN) criteria for AKI are based on the RIFLE system with minor modifications: more 'permissive' criteria for AKIN stage 1 (equivalent to RIFLE 'risk' category) and the

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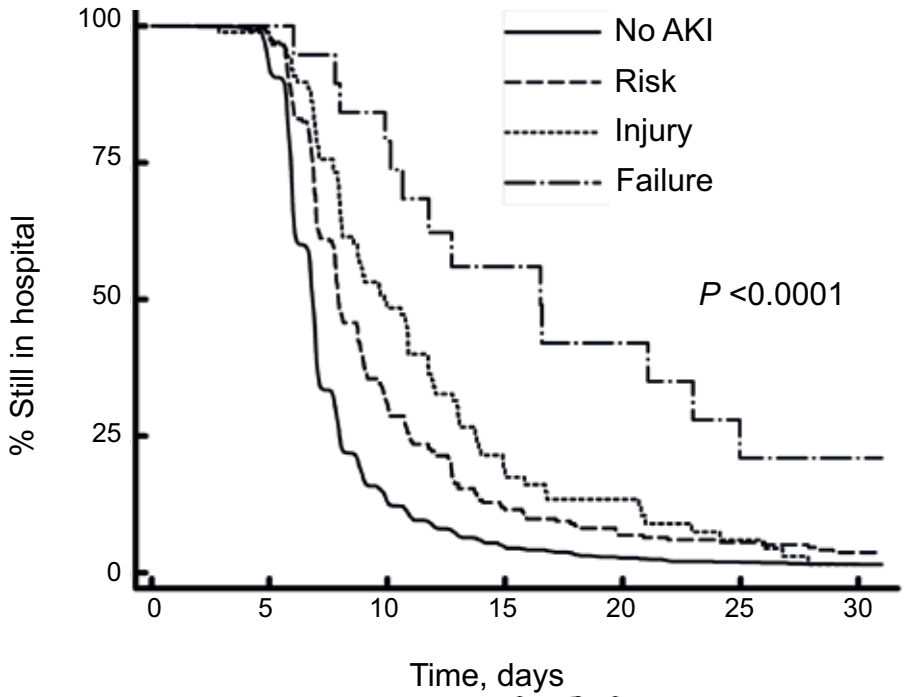

Figure 1: Time to hospital discharge after coronary bypass graft surgery for postoperative acute kidney injury categories (n=2998). AKI=acute kidney injury. Figure modified with permission from Moore et al[4].

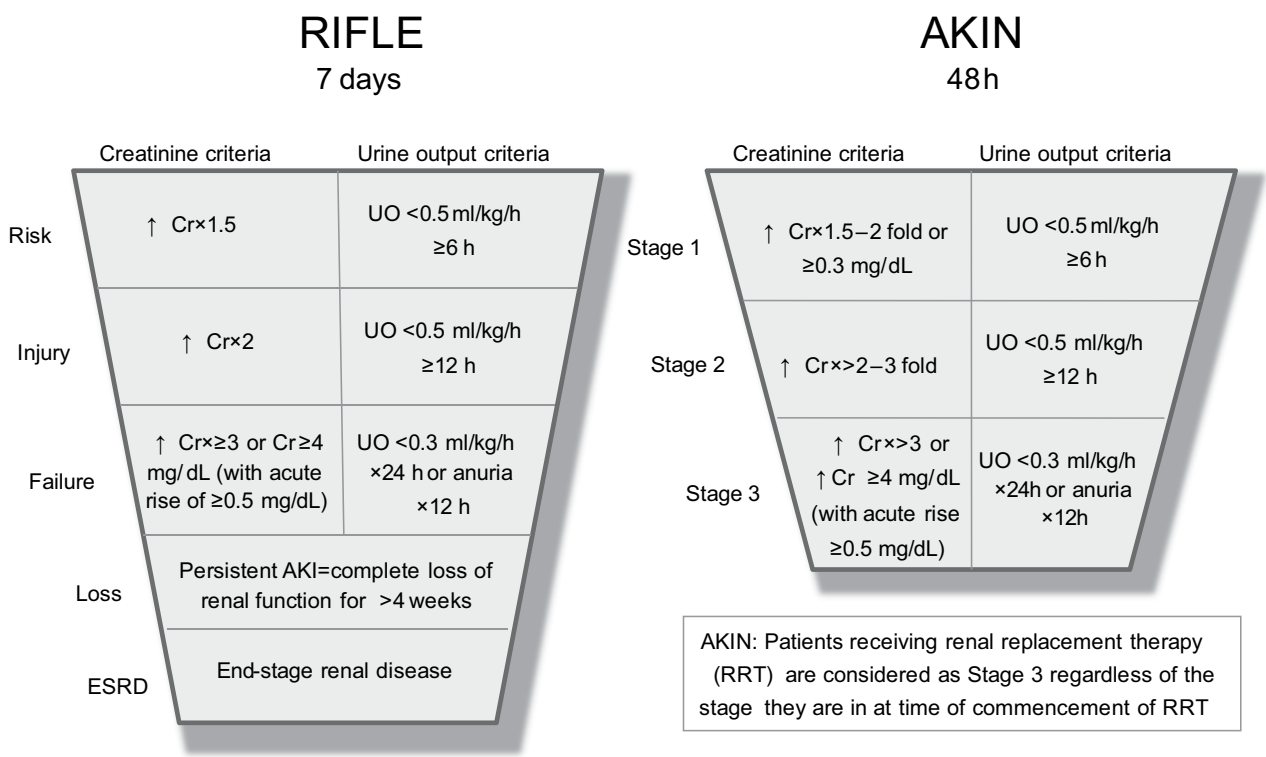

Figure 2: RIFLE and AKIN classifications for AKI. RIFLE= Risk, Injury, Failure, Loss, End stage, AKIN=Acute Kidney Injury Network, ↑=increased, Cr=creatinine, UO=urine output, AKI=acute kidney injury, ESRD=end-stage kidney disease, RRT=renal replacment therapy. Figure from Bellomo et al[1] and Mehta et al[6]. Reproduced with permission from BioMed Central.

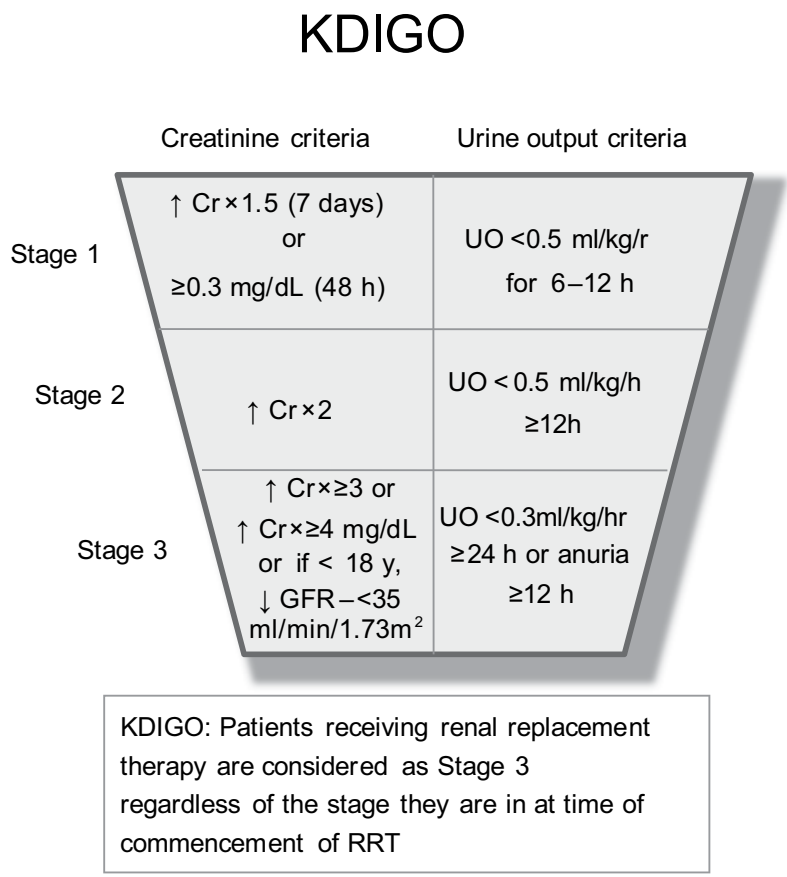

Figure 3: The KDIGO classification for acute kidney injury (AKI). KDIGO=Kidney Disease: improving global outcomes, ↑=increased, Cr=creatinine, UO=urine output, ↓=decreased, GFR=glomerular filtration rate, RRT=renal replacement therapy.

use of a 48-hour time constraint for the diagnosis of AKI[6] (Figure 2). The more recently developed Kidney Disease: Improving Global Outcomes (KDIGO) classification system[7] combines the RIFLE and the AKIN criteria with its own minor modifications (Figure 3).

Although the AKIN criteria were designed to improve sensitivity in diagnosing AKI, this has not been demonstrated and comparative studies show that AKIN does not improve the ability to predict outcomes compared to RIFLE[8–11]. The RIFLE criteria have been more extensively used and validated to classify renal function in clinical studies to date, involving over 550,000 subjects worldwide[12]. Furthermore, a recent multicentre study of thousands of intensive care unit (ICU) patients found RIFLE to be a more robust measure, with a higher detection rate of AKI in the first 48 hours after ICU admission[9]. In this study, the AKIN criteria were more likely to misclassify patients as non-AKI compared with the RIFLE criteria. The novel KDIGO criteria have been formulated very recently and require validation.

INCIDENCE

The incidence of AKI (RIFLE R, I or F) in critically ill patients in large Australian and European studies is about 36%[13,14], and sepsis is one of the major contributors to its development[15]. Data from 57 Australian and New Zealand ICUs (n=120, 123 patients) found that 27.8% of all patients had a sepsis-related diagnosis, 42% of patients with sepsis had AKI and of all patients with AKI, 32.4% had sepsis[16]. In more severe sepsis the occurrence of AKI is even more common[17].

In developed countries, after sepsis, cardiac surgery is the second most common trigger of AKI[4]. Trauma is also an important cause of AKI. In Australia and New Zealand, the crude incidence of early AKI (within 24 hours of admission) in critically ill trauma patients (n=9449) was 18.1% and around 10% in patients with traumatic brain injury[18,19].

The incidence of AKI in intensive care and cardiac surgical settings has increased[20,21] and, despite progress in many aspects of medicine, mortality rates in patients with AKI have improved only marginally[20]. This

suggests an increase in the burden of illness associated with AKI. Moreover, the marginal improvement in mortality rates, despite an increased incidence of AKI, suggests greater cost and resource use in the treatment of these patients[20].

AETIOLOGY

The causes or triggers of AKI have been traditionally divided into prerenal, postrenal and intrinsic causes to simplify this continuum of injury, which results from multiple mechanisms. Certain syndromes also lead to the development of AKI, as do certain drugs.

Prerenal AKI

This term refers to AKI occurring in response to extrarenal diseases such as septic shock, cardiogenic shock, hypovolaemia and bleeding. Most severe AKIs stem from a prerenal cause[22].

Renal AKI

Renal, 'intrinsic' or 'parenchymal' AKI occurs as a result of pathological processes often of an inflammatory nature that primarily affect the renal tissue itself, (e.g. glomerulonephritis, acute interstitial nephritis, pyelonephritis and vasculitis).

The term 'Renal AKI' also traditionally encompasses ischaemia-induced structural injury to the kidneys (often referred to as acute tubular necrosis [ATN]) typically resulting from severe 'prerenal AKI'. Histological changes in ATN are believed to involve localised necrosis of the renal tubule epithelium, with focal rupture or loss of basement membrane. Necrotic cells (casts) are shed into the tubule lumen, obliterating it and causing AKI. If the tubular basement membrane is intact, regeneration is possible. In this setting, the boundaries of functional prerenal injury and structural changes become blurred, as prolonged renal ischaemia leads to increased renal damage and 'prerenal AKI' becomes 'renal AKI'[23].

Postrenal AKI

Post-renal AKI occurs as a result of obstruction of urinary outflow (e.g. renal calculi, papillary necrosis and surgical ureteric injury). Elevated intra-abdominal pressure may cause AKI.

Problems with traditional aetiological concepts

ATN and prerenal azotaemia are two key concepts related to AKI. Both are considered to stem from prerenal causes. It is thought that prerenal azotaemia is not associated with histopathological changes and patients with prerenal azotaemia are considered likely to respond to fluid therapy, whereas ATN involves structural injury and changes. If it is prolonged, prerenal azotaemia (no histological changes) is taken to lead to ATN, a generally unconfirmed histological diagnosis[24]. There are inconsistencies and conceptual flaws here[24,25]. In the absence of confirmation of histological changes (renal biopsies are rarely performed) these terms that assume the presence or absence of structural injury cannot be reliably assigned. Moreover, the use of separate diagnoses to identify conditions that may refer to different stages of a progressive disease does not reflect the complicated continuum of injury that AKI represents. Indeed, the terms ATN and prerenal azotaemia may have limited clinical utility.

SPECIFIC AKI SYNDROMES

Hepatorenal syndrome

The hepatorenal syndrome describes a type of AKI occurring with severe liver disease. It involves progressive oliguria with very low urine sodium levels (<10 mmol/l). However, other causes of AKI are in fact more common in patients with severe liver disease (e.g. sepsis, cardiomyopathy or hypovolaemia due to diuresis/paracentesis). AKI in this setting mirrors sepsis in that it seems to occur without renal histopathological changes. However, unlike animal models of sepsis, renal vasoconstriction is intense along with significant renin–angiotensin–aldosterone system (RAAS) activation, suggesting neurohormonal involvement. Hypotension due to splanchnic vasodilation seems to be involved in activating vasoconstriction and RAAS[26], which bolsters the systemic circulation, but the renal circulation might suffer. In postoperative systemic inflammatory response syndrome a similar situation may occur, with elevated noradrenaline, renin and angiotensin II levels leading to AKI. However, this has not been investigated. Recently, in patients with hepatorenal syndrome, terlipressin, a long-acting vasopressin derivative is being used to improve glomerular filtration rate (GFR) with effect[27,28]. Its mechanism of action is thought to be through splanchnic vasoconstriction and redistribution of blood flow away from a dilated splanchnic bed which may increase central circulatory volume and renal blood flow.

Cardiorenal syndrome

The co-existence of heart failure and kidney disease in many patients has emerged as a focus of epidemiological and therapeutic interest. Cardiorenal syndrome involves deleterious interaction between these two organs. Whether the heart or kidney fails primarily, this precipitates the failure of the other organ. Neurohormonal activity, anaemia, oxidative

stress and renal sympathetic activity are potential contributors to this complex syndrome. The occurrence of AKI with chronic kidney disease in such patients may be triggered by an acute decompensation of their heart failure. Indeed, initial work in this area hints that a congestive state, more than decreased forward output or pressure, may contribute more to the development of AKI[29].

Rhabdomyolysis-induced AKI

Rhabdomyolysis is typically seen following major trauma, drug overdose, vascular embolism and in response to agents that induce muscular injury. It can also develop as a complication of cardiac surgery. All causes have a common pathway involving prerenal, renal and postrenal factors. Calcium release from the sarcolemma and cytosolic calcium overload lead to muscle cell damage and the release of myocyte constituents into the circulation. One of the key compounds released is myoglobin, a haem-containing protein that plays a major role in the development of AKI[30]. Indeed, creatine kinase and free myoglobin levels are used to diagnose rhabdomyolysis[31]. Randomised, controlled trials have not been performed in this area, so management is based on data from small studies and includes early, aggressive fluid resuscitation, elimination of causative agents, compartment syndrome correction, urine alkalinisation to pH >6.5 and diuresis (>300 ml/hour).

Drug-induced AKI

Drugs appear to contribute to AKI, particularly in ICU patients, in approximately 20% of cases[32]. Drugs that can rapidly induce prerenal AKI include non-steroidal anti-inflammatory drugs, angiotensin-converting-enzyme inhibitors and cyclosporin. Non-steroidal anti-inflammatory drugs, which inhibit prostaglandin synthesis, reduce GFR by blocking the effect of renal vasodilatory prostaglandins in hypoperfused states[33]. Angiotensin-converting-enzyme inhibitors may induce prerenal AKI by

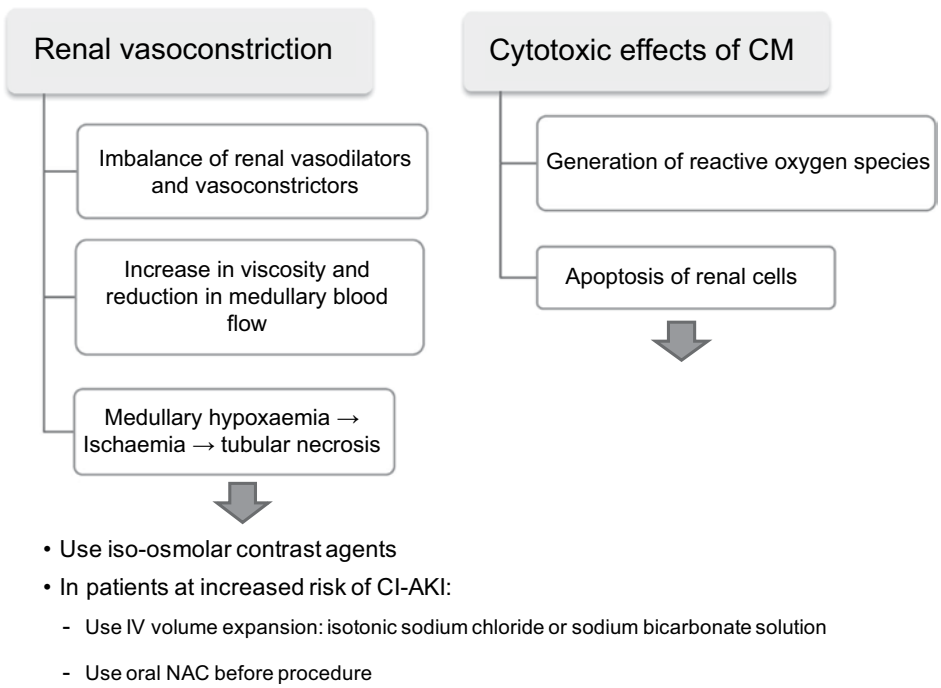

Figure 4. Proposed pathogenesis of contrast-induced nephropathy. Pathogenesis of contrast-induced AKI (CI-AKI) is linked to renal vasoconstriction and cytotoxicity of contrast. Renal vasoconstriction is mediated by contrast-induced release of endothelin and adenosine and by the high osmolality of the contrast. Vasodilators such as nitric oxide (NO) are decreased by depletion of NO synthesis cofactors (e.g. tetrahydrobiopterin), the modification of NO substrates such as L-arginine, and the interference with NO synthesis through nuclear factor Kappa B[37]. The imbalance between vasoconstrictors and vasodilators leads to medullary ischaemia, hypoxia and eventual endothelial dysfunction[36]. N-acetylcysteine (NAC) targets this vasoconstriction. NAC increases NO production, thus reversing renal vasoconstriction. Contrast alters the mitochondrial function of renal cells, resulting in the generation of reactive oxygen species and apoptosis. NAC acts as an antioxidant to protect against free radical damage[7]. The mechanisms by which volume expansion protects against CI-AKI are unknown but neurohumoral effects of volume expansion, suppression of vasopressin and inhibition of the renin-angiotensin axis, may attenuate medullary hypoxia. Volume expansion may also reduce cellular damage by diluting the contrast and decreasing tubular fluid viscosity. CM=contrast media. Reprinted from the Journal of Vascular Surgery, 54/2, Rundback et al, Contrast-induced nephropathy, 575-579, 2011, with permission from Elsevier[37].

reducing the production of angiotensin II, which maintains glomerular capillary hydrostatic pressure by constricting the glomerular efferent arterioles[34]. Cyclosporin A and other immunosuppressive drugs, such as tacrolimus and sirolimus, also cause vasoconstriction of the afferent arterioles which reduces renal perfusion, thus decreasing GFR and inducing AKI[35].

Other nephrotoxic drugs that can cause or contribute to the development of AKI include aminoglycosides, amphotericin B, sulphonamides, acyclovir, methotrexate, angiotensin receptor blockers and radiocontrast agents. Importantly, in drug-induced AKI, the causative agent can often be identified and removed or replaced by a less nephrotoxic agent.

Contrast-induced AKI

Contrast-induced AKI (CI-AKI) is a common iatrogenic cause of AKI after angiographic procedures and angioplasty and is associated with adverse outcomes. Proposed mechanisms of the pathogenesis of CI-AKI include two main theories: renal vasoconstriction resulting in medullary hypoxaemia (mediated by changes in nitric oxide, endothelin or adenosine) and the direct cytotoxic effects of the contrast[36,37] (Figure 4). Evidence shows that CI-AKI can be reduced by the use of iso-osmolar agents[38], and the incidence of postoperative AKI in patients undergoing elective cardiac surgery may be reduced by delaying surgery beyond 24 hours after exposure to contrast agents and by minimising their use[39]. The utility of fenoldopam to reduce renal injury in CI-AKI has been refuted in two randomised controlled trials[40,41]. Volume expansion is recommended to count-eract the effect of radiocontrast in the KDIGO clinical practice guidelines (Figure 4). While there is a potent-ial (but inconsistent) benefit of bicarbonate solutions, the KDIGO guidelines recommend the use of either isotonic sodium chloride or sodium bicarbonate intravenous solutions[7]. There is no clear evidence regarding optimal rate and duration of infusion, however, most studies suggest starting fluids ≥1 hour before and continuing for three to six hours after contrast administration[7]. The evidence for an overall benefit of N-acetylcysteine (NAC) is not consistent[42], but oral NAC has low-risk for adverse events and a low cost, and therefore its use prior to radiocontrast procedures is acceptable[7]. A large factorial design study called *Prevention of serious adverse events following angiography* (NCT01467466, n=8680, see Further Reading) is soon to start in the USA, Australia and New Zealand. It will compare N-acetylcysteine versus placebo and sodium bicarbonate versus saline, looking at differences in mortality, dialysis or persistent decline in kidney function at 90 days. This trial could provide definitive answers regarding the use of NAC and bicarbonate for CI-AKI prevention.

RISK FACTORS FOR AKI

Risk factors for AKI vary depending on the patient type. Few studies have focused on the risk factors for developing AKI in the general ICU population. One study of 40 ICUs in 16 countries evaluated risk factors using the Sequential Organ Failure Assessment score (n=1411)[43]. The most important risk factors for AKI were age >65 years, infection, acute circulatory or respiratory failure, past history of chronic heart failure, lymphoma/leukaemia or cirrhosis (odds ratio >1.4, P <0.05)[43]. A recent study of risk factors for AKI after major non-cardiac surgery in patients with previously normal renal function (n=1166) found that age, American Society of Anesthesiologists physical status, emergency surgery, high-risk surgery, ischaemic heart disease, congestive heart disease and Revised Cardiac Risk Index score were independent preoperative determinants for AKI[44].

In the many studies of risk factors for AKI after cardiac surgery, a wide variety of causes have been implicated. Factors most consistently associated with development of postoperative AKI are duration of cardiopulmonary bypass and advanced age[45–47], followed by preoperative impaired renal function, diabetes, the need for perioperative intra-aortic balloon pump or evidence of ventricular dysfunction. Interestingly, one study that focused on potentially modifiable risk factors for AKI and compared results of three definitions of AKI (>25, >50 and >75% decrease in estimated GFR, n=3460)[47] found that preoperative anaemia, perioperative red blood cell transfusions and need for surgical re-exploration were also strongly associated with AKI using all three definitions.

The use and age of blood products in critically ill patients and their potential association with poor outcomes, including the development of AKI, is an area of increasing investigation and of relevance to anaesthetists and intensive care clinicians. A landmark study by Koch and colleagues[48] (n=2872) comparing outcomes in patients receiving 'newer' versus 'older' stored red cells (blood stored for ≤14 days or for >14 days respectively) found that patients given 'older' blood were more likely to have kidney injury (2.7 vs 1.6%, P=0.003). An Australian study called *Standard issue transfusion versus fresher red blood cell use in intensive care—a randomised controlled trial* (NCT01638416, n=5000) will investigate the effect of using the freshest available red blood cells compared

to standard practice (oldest available red blood cell unit) on 90-day mortality and need for renal replacement therapy (RRT) in critically ill patients.

Another area of concern is the potential association of hydroxyethyl starch use and the development of AKI. In a recent Cochrane review[49], patients treated with hydroxyethyl starch compared with other fluid therapies had a relative risk for kidney failure of 1.5 (95% confidence interval 1.2 to 1.87, n=1199) and 1.38 for requiring RRT (0.89 to 2.16, n=1236). Interestingly, the risk of developing kidney failure in studies including septic patients was higher than in surgical and trauma patients.

In a recent trial of hydroxyethyl starch versus a balanced crystalloid solution in patients with severe sepsis (n=798), those receiving starch had an increased risk of death at day 90 and were more likely to require renal-replacement therapy[50]. Starch based solutions may also have an adverse effect on coagulation and inflammatory markers[51,52]. Histological renal tubular damage is observed with experimental hydroxyethyl starch administration[53]. Moreover, hypertonic hydroxyethyl starch may induce a pathological state known as 'osmotic nephrosis'[54], whereby hydroxyethyl starch molecules enter renal proximal tubular cells by pinocytosis. The resulting vacuoles fuse with each other and with lysosomes to form larger vacuoles containing hydroxyethyl starch molecules and hydrolytic enzymes. This may cause swelling of tubular cells and vacuolar retention if the agent is only slowly digestible by the lysosomal enzymes. This lysosomal alteration is an early sign of cell damage and is reversible but may be a first step in the development of irreversible tubular cell lesions.

The results of a multicentre Australian and New Zealand randomised controlled trial (n=7000), which completed recruitment early in 2012 (NCT00935168), further support the lack of benefit of starch solutions for critically ill patients and further highlight their negative impact on renal function.

Finally, other general interventions may be of relevance to the kidney. For example, the protective effect of erythropoietin and hypothermia for the kidneys is also under investigation in sub-studies of two Australian-based multicentre clinical trials (ACTRN12609000827235 and ACTRN12609000764235, respectively).

PATHOGENESIS

The development of AKI in critically ill and postoperative patients is complex and multifactorial, and in developed countries is largely the result of

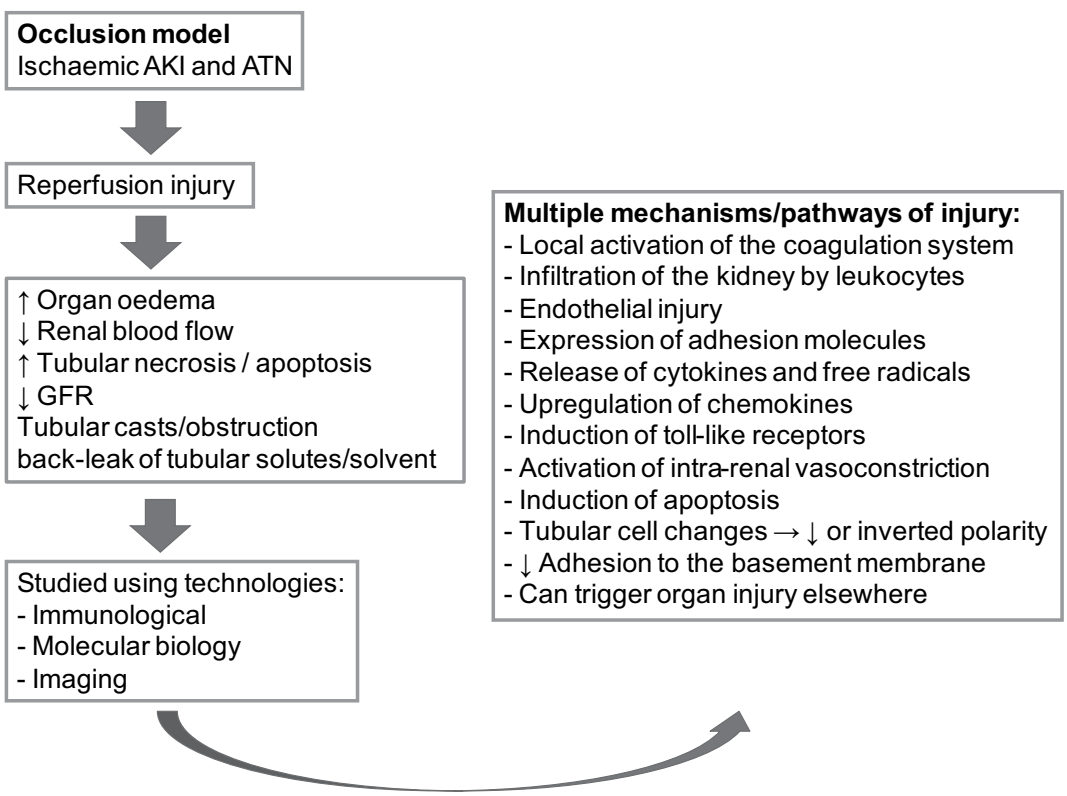

FIGURE 5: Effects and mechanisms suggested by the animal 'Occlusion model' of AKI[56–59]. AKI=acute kidney injury, ATN=acute tubular necrosis, ↑=increased, ↓=decreased, GFR=glomerular filtration rate, →=lead to.

prerenal causes[2,22]. Consequently, we will focus on this area, rather than AKI resulting from less common inflammatory or obstructive disease or vascular events.

AKI: animal models

Our understanding of the pathogenesis of prerenal AKI comes largely from animal models because the invasive investigations necessary to our understanding of the pathogenesis of AKI cannot be performed in man. These models predominantly involve the occlusion of the arterial blood supply to the kidney to induce ischaemia, although models of ureteric obstruction and transplant are also in use[55]. AKI and acute tubular necrosis are induced by the occlusion model of ischaemia (Figure 5 summarises the reported biological effects of subsequent reperfusion injury in these models). Studies using several laboratory technologies have shown that a multitude of pathways and mechanisms are most likely involved (Figure 5)[56–59].

Unfortunately such models lack clinical relevance for the conditions that most commonly trigger AKI—sepsis, major surgery (most importantly cardiac surgery) and acute heart failure—none of which involve occlusion of the renal artery[60,61]. More relevant models are required.

Models of septic AKI that adequately reflect human AKI are challenging to develop. Endotoxin bolus induces hypodynamic shock with low cardiac output in small animals[60,62], which confounds the model by combining septic and cardiogenic shock, a state very different from human sepsis where a hyperdynamic state is most common. Interestingly, when hyperdynamic sepsis is induced in large animals (mostly sheep), AKI develops as expected, but marked global renal vasodilation occurs, renal blood flow (RBF) increases above normal levels and there is no histopathological injury[62,63] despite severely decreased urine output and increased serum creatinine. Thus, experimental septic AKI can occur while RBF is abnormally high. This observation challenges the notion that all prerenal AKI must be due to hypoperfusion. It also raises questions about how GFR can be lost in the setting of markedly increased RBF. Possible explanations include efferent arteriolar vasodilatation with decreased intraglomerular filtration pressure and/or intrarenal blood flow redistribution (shunting).

Irrespective of the complexity of intrarenal events, different septic models lead to variable systemic and renal haemodynamic states, and it is not possible to know which models are most relevant to human sepsis. It seems clear, however, that current animal models of ischaemic AKI have limited explanatory value in the setting of sepsis, which is the most common trigger of AKI in patients in developed countries.

RBF could also increase in humans with septic AKI and there is currently no way to disprove this possibility[64]. Moreover, recent studies suggest that variations to the renal microcirculation may have more impact on renal function than changes in global RBF[65,66]. Even if success in increasing urine output was achieved by a better understanding of mechanisms responsible for its decrease, restoring urine output when the kidneys are under physiological stress may be harmful due to increased oxygen demand[67]. Furthermore, we cannot be certain that acute tubular necrosis occurs in human sepsis if there is evidence that septic animals and humans lose their renal function without any histopathological changes[68,69]. These observations highlight the many uncertainties that surround septic AKI and its pathogenesis.

Anaesthesia-related models of AKI

Anaesthesia-related rodent models of ischaemia-reperfusion AKI often use excision of the right kidney followed by occlusion of the left renal artery using clamps[70]. More recently, occlusion via a hanging-weight system has been introduced[71], which reportedly creates highly reproducible injury, thus minimising the variability associated with clamping. Bilateral nephrectomy[70] and transplant[72] models have also been used. While factors contributing to the development of surgical AKI may include a reduction in RBF, renal artery occlusion and bilateral nephrectomy do not adequately represent the conditions they aim to replicate. Caecal ligation and puncture-induced sepsis models have been used to evaluate the effect of anaesthesia on sepsis-induced AKI. Caecal ligation and puncture mice become hypotensive without a hyperdynamic phase[73], although vigorous fluid resuscitation provides an early hyperdynamic phase[74]. Caecal ligation and puncture-induced sepsis mimics more clinical features and drug responses of human sepsis than endotoxin bolus models but does not consistently lead to the development of AKI[60].

Models of postoperative AKI

These models generally seek to evaluate the effect of certain human surgical procedures on the development of AKI and are fairly representative replications in rodents of such surgery, but have the inherent limitations of many non-human models. Such models include orthotopic liver transplant[75], kidney transplant and juxtarenal abdominal aortic aneurysm repair[76]. Conventional surgical ischaemic AKI models involve a laparotomy with renal pedicle occlusion[77] or left renal artery clamp occlusion[78]. Once again, such injury is not a realistic replication of what occurs in

human abdominal surgery outside of specific vascular interventions.

Models of cardiac surgery and AKI

Cardiopulmonary bypass models attempt to replicate in animals the effects of a bypass procedure[79,80], with the limitations of non-human models. Most conventional ischaemia-reperfusion mouse models use renal pedicle clamping (both artery and vein). However, most patients who develop cardiac surgery-associated AKI have only renal artery hypoperfusion[77]. A recent study found that renal artery occlusion and pedicle occlusion produce less tubular injury than renal vein occlusion, due to less kidney congestion[77]. Understanding such differences is important for the design of future experimental studies in mice and highlights the poorly appreciated role of congestion compared with ischaemia.

Human research

Although more human investigation would seem logical considering the problems with animal models of AKI, the fact that there is no known effective intervention for histologically confirmed AKI makes this difficult and renal biopsies cannot be justified. In addition, mechanistic investigations in humans are extraordinarily difficult or even sometimes impossible. Thus, investigations are limited to post-mortem studies with their inherent problems: selection bias, pre-mortal ischaemia and hypoxia. Similarly, there is no accurate, continuous, non-invasive technique to measure RBF in humans[64]. New non-invasive methods to measure RBF such as magnetic resonance techniques and contrast enhanced ultrasonography are under development[81,82], but they are difficult to apply outside the research environment. Data for RBF from invasive techniques[83] should be viewed with caution as they are taken in patients with AKI who may have organ oedema, tubular damage, back-leak and increased tubular luminal pressure, which could produce changes in RBF. Reduced RBF may therefore be the result of AKI and not the causative mechanism, or it could be both. Human models of AKI in cardiac surgery have, unfortunately, not provided specific insights into its pathophysiology. Renal transplant models, on the other hand[84], allow tissue examination but are affected by nephrotoxic drugs, and results from such models where kidneys are kept under artificial conditions cannot be extrapolated to common triggers such as sepsis or major surgery.

Neurohormonal response

In the presence of AKI, kidney-specific neurohormonal responses seem to be activated[85]. Experimental and human studies of AKI indicate that RAAS, the tubuloglomerular feedback system and the renal sympathetic system are activated[85,86]. In the setting of sepsis, it is thought that infection results in nitric oxide synthase induction with subsequent vasodilation. This leads to arterial volume depletion and baroreceptor activation, triggering sympathetic system activation, which induces heightened RAAS activity and renal vasoconstriction. Vasopressin is also released enhancing water retention[85]. However, the relative importance of each mechanism and the timing of onset and duration of their occurrence remain uncertain. This hinders the development of new interventions.

Whether neurohormonal changes cause intrarenal shunting, and whether this shunting contributes to reduced GFR and ischaemia of the renal medulla, is unknown. Shunting may be linked to microcirculatory changes, and assessment of the microcirculation should be performed with any global RBF measurements performed in animals (limited to animals as techniques are invasive) to enhance our understanding of AKI.

Renal biomarker studies in the critically ill suggest the involvement of different underlying pathophysiological processes in septic and non-septic AKI[87].

Effects of anaesthesia and surgery on renal function

Volatile anaesthetics may reduce GFR as a result of a reduction in renal perfusion pressure either by decreasing systemic vascular resistance (e.g. isoflurane or sevoflurane) or cardiac output (e.g. halothane). This reduction in GFR is exacerbated by hypovolaemia and catecholamine, and antidiuretic hormone release in response to painful stimuli during surgery[88]. Recent studies have found that volatile anaesthetics that reduce GFR, such as isoflurane, also ameliorate renal injury with a subsequent reduction in hepatic and intestinal multiorgan failure after renal ischaemia-reperfusion injury[70,89]. This may be related to a reduction in necrosis, inflammation and the severity of kidney injury when isoflurane activates certain cytoprotective signalling pathways in human proximal tubule cells[89]. Sevoflurane, previously thought to be a potential cause of kidney injury due to fluoride toxicity[90], is now considered safe, even at low flows[91]. Similarly, propofol may inhibit pro-inflammatory cytokines in septic animal models[92,93]. While the mechanisms are unclear, recent animal studies indicate that propofol could decrease inflammatory cytokines and oxidative stress by inhibiting free radical generation, increasing bone morphogenetic protein-7 expression during septic AKI and regulating other inflammatory factors[92]. The use of methoxyflurane as a general anaesthetic was

abandoned in the 1970s due to its dose-dependent nephrotoxicity. It is now used in Australia as an emergency short-term analgesic, largely in the pre-hospital setting via the self-administered Penthrox® inhaler (Medical Developments International Limited, Springwood, Victoria)[94]. Used in this way at a low and limited dose with a contraindication for its use in people with any degree of kidney injury, there is no indication of a harmful effect on the kidneys[95].

Perioperative procedures can contribute to the development of AKI. Positive pressure ventilation during general anaesthesia can decrease cardiac output leading to catecholamine, renin and angiotensin II release with sympathoadrenal activation and reduction in renal blood flow, increasing the risk for AKI. Abdominal insufflation during laparoscopic surgery, with the associated increased intra-abdominal pressure, has a similar effect on RBF and GFR[96]. During liver transplantation, aortic cross-clamping or inferior vena cava occlusion can cause AKI, thus increasing mortality and morbidity[97].

In the setting of pre-existing kidney injury, the dependence of many anaesthetic drugs on renal excretion should always be considered. In these patients, increased sensitivity to barbiturates and benzodiazapines occurs due to decreased protein-binding, resulting in an increase in active drug-causing pharmacological effects. Judicious use of opioids with a preference for fentanyl and hydromorphone over morphine and pethidine is advised[91]. Fentanyl has a rapid onset, a shorter half-life and greater potency than morphine. It is highly lipid-soluble facilitating its passage across the blood–brain barrier[98]. The severe side-effects of pethidine (serotonin syndrome, seizures, delirium, dysphoria and tremor) are due to the action of its metabolite norpethidine which has convulsant and hallucinogenic effects[99]. Compared to pethidine, hydromorphone has a shorter half-life, less severe side-effects and is thought to have a lower risk of dependency. Suxamethonium should

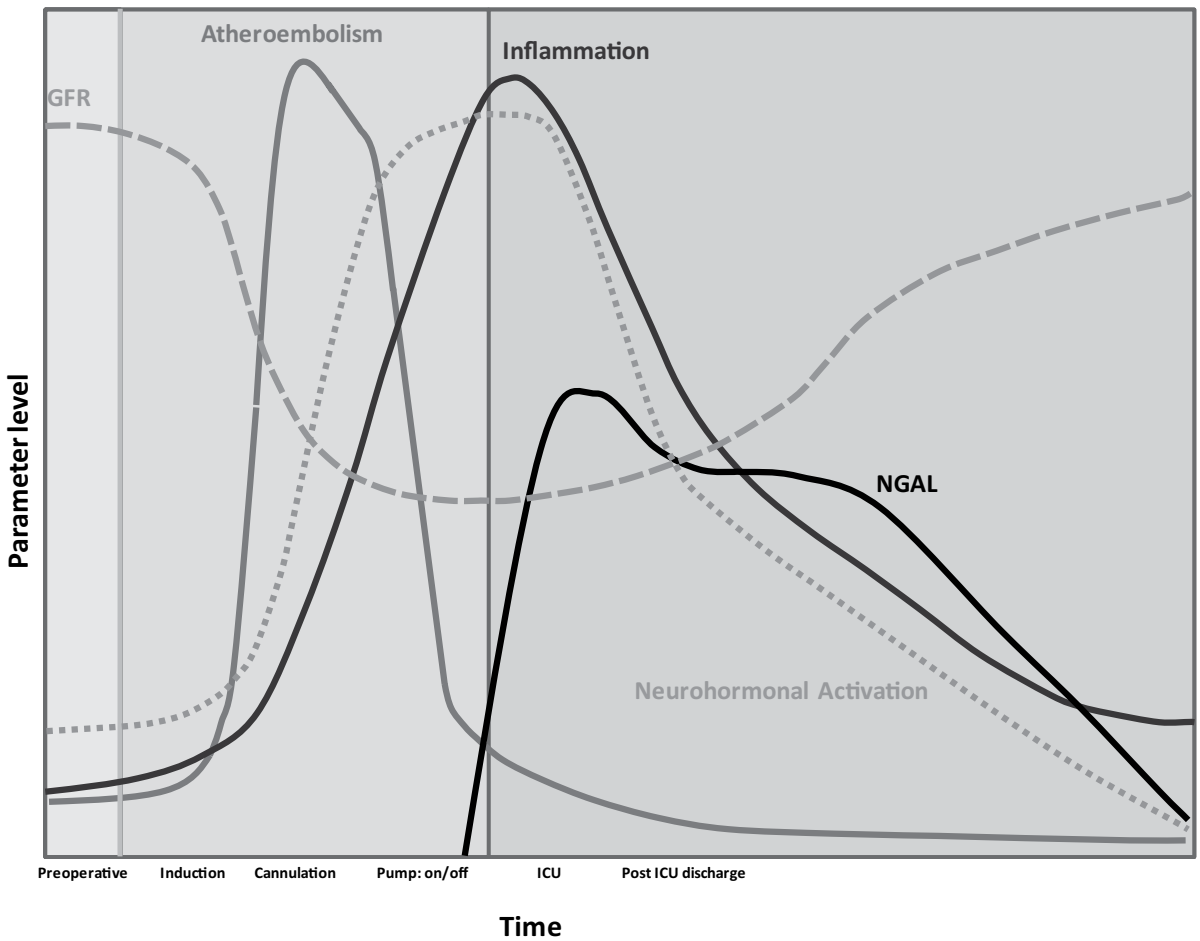

FIGURE 6: Illustration of the estimated time-course of major mechanisms of cardiac surgery-associated kidney injury. Likely changes in GFR and NGAL are shown. GFR=glomerular filtration rate, NGAL=neutrophil gelatinase-associated lipocalin, ICU=intensive care unit. Figure adapted from R. Bellomo, S. Auriemma, A. Fabbri, A D'Onofrio, N. Katz, P. A. McCullough et al[103]. The pathopysiology of cardiac surgery-associated acute kidney injury (CSA-AKI). Int J Artif Organs 2008; 31:166-178 with permission from the Publisher.

be avoided if the patient's potassium level is high or unknown; it consists of two acetylcholine molecules and if cellular acetylcholine receptors remain open, potassium can move out to the extracellular fluid causing hyperkalaemia. Non-depolarising muscle relaxants cisatracurium and atracurium do not rely on renal excretion for their elimination and are preferred to other neuromuscular blockers whose effects may be prolonged[100]. Antimicrobials should be dosed according to renal function, and non-steroidal anti-inflammatory drugs may exacerbate renal injury and should be avoided.

Patients with kidney disease or a high-risk for postoperative AKI may benefit from a sympathetic block for T4–T10 to attenuate catecholamine-induced renal vasoconstriction and suppress cortisol and adrenaline release[101]. Similarly, the use of postoperative epidural anaesthesia, where appropriate, may reduce the incidence of AKI, but this has not yet been definitely established[102]. For elective surgical patients with chronic kidney disease, dialysis is recommended the day before surgery, and patients with pre-existing AKI undergoing major surgery require invasive monitoring and frequent postoperative biochemical monitoring.

AKI after cardiac surgery

Several pathophysiological mechanisms come into play with cardiac surgery and collectively contribute to the development of AKI at different stages (Figure 6)[103]. Furthermore, worsening of preoperative renal dysfunction is associated with longer length-of-stay, increasing operative mortality, prolonged mechanical ventilation and increasing need for RRT[4,104].

As described earlier, preoperative use of certain antibiotics, non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors or contrast agents can cause or predispose patients to AKI. However, apart from contrast-induced AKI, their impact is minimal.

Pro-thrombotic drugs such as aprotinin, aminocaproic acid and tranexamic acid are given after cardiopulmonary bypass and may cause renal injury. In adult cardiac surgery, aprotinin use is associated with an increased risk for AKI[105,106], and its supply worldwide was suspended after the Blood conservation using Antifibrinolytics in a Randomised Trial[107] found the risk of death was higher with aprotinin use compared to the use of lysine analogues. However, recent paediatric cardiac surgery trials revealed mixed results[108], and a recent US 'post-aprotinin era' study[109] showed that the relative risk of early postoperative kidney injury and mortality have not improved since the suspension of aprotinin, while intraoperative blood product use has increased. Recent news that the suspension of aprotinin in Europe may be lifted[110] indicates that controversy remains.

Metabolic factors

Diabetes is an independent predictor of postoperative AKI in patients with normal preoperative kidney function[111] and hyperglycaemia is associated with a higher incidence of postoperative AKI. However, intensive intraoperative insulin therapy has not been shown to reduce the incidence of AKI compared to conventional glucose control[112]. A factorial design trial (NCT00995501) to investigate the effects of dexamethasone, tight glucose control and depth-of-anaesthesia on peri-operative inflammation and morbidity in major non-cardiac surgery may help to clarify this. Obesity (body mass index 30 to 40) and, conversely, a body mass index <20 and albumin <25 g/l (measures of malnutrition) are associated with a higher incidence of postoperative AKI[113,114].

Neurohormonal activation

Preoperatively, this commonly occurs in patients with chronic heart failure. Low cardiac output syndromes lead to elevated sympathetic nervous system activity, activation of the renin-angiotensin-aldosterone system and higher levels of circulating vasopressin[103]. This then leads to general vasoconstriction, reduced renal filtration and sodium, and water retention, which increases the renal risk in heart surgery. Plasma levels of adrenaline, noradrenaline, vasopressin, dopamine, a trial natriuretic factor and prolactin rise during cardiopulmonary bypass, as does renin activity. Their levels fall at different times with a net effect of reduced renal blood flow and GFR, thus increasing the risk for kidney injury[103]. After cardiac surgery, patients are overloaded with fluid. Natriuretic peptides may be of benefit in 'at risk' patients with syndromes of excessive neurohormonal activation by helping to increase GFR and promote diuresis and natriuresis[115,116], however this requires further investigation.

Inflammation

There is a strong association between postoperative complications and preoperative C-reactive protein (area under the curve=0.86) and IL-6 (area under the curve=0.79)[117]. The level of baseline systemic inflammation appears to be related to levels immediately after cardiac surgery[118]. After cardiopulmonary bypass in children, surges in novel markers of injury were followed by inflammatory markers (C-reactive protein and interleukins), peaking at 24 hours after bypass[119]. However, a recent Cochrane Review showed no benefit of corticosteroid use in cardiopulmonary bypass[120]. Inflammation and levels

of inflammatory mediators appear to be associated with the development of AKI[121,122].

Oxidative stress

This is thought to be an important mechanism of renal injury. As free radicals are difficult to measure, levels of antioxidant defences or the products of oxidative damage are assayed as indirect measures of oxidative stress. Contrast-induced nephropathy may be mediated by oxidative stress[123], and gentamicin, iron and radiocontrast may promote the generation of oxygen metabolites[124]. Animal studies have shown that antioxidant therapy with NAC prevented lipid peroxidation and caused increased antioxidant activity in renal tissue after cardiopulmonary bypass[80]. However, a recent study of cardiac bypass surgery patients found that markers of oxidative stress did not correlate with the progression of AKI, but the levels of plasma neutrophil gelatinase-associated lipocalin (NGAL) in the first few hours after bypass were predictive of AKI[125]. This suggests a greater involvement of inflammatory processes in the development of AKI at this stage, which is consistent with findings of no benefit of NAC on renal function in clinical trials[126,127].

In summary, the pathogenesis of post cardiopulmonary bypass AKI is extraordinarily complex and no intervention has been consistently shown to attenuate this injury.

DIAGNOSIS

AKI generally occurs in the setting of other acute illness and carries no reliable clinical signs or symptoms. Oliguria may develop early but is non-specific. Other signs and symptoms do not present until advanced loss of function has occurred. In most cases, AKI is diagnosed in a high-risk setting, such as sepsis, haemorrhagic shock, cardiac surgery, trauma, major emergency surgery, advanced liver disease or heart failure. Its diagnosis is based on the clinical context, changes in urine output and the measurement of waste products of nitrogen metabolism (creatinine or urea) and renally excreted electrolytes (especially potassium and phosphate). To rule out the possibility of chronic kidney disease in patients with an elevated serum creatinine, the clinical context must be taken into account. Factors suggestive of chronic kidney disease include prior elevated serum creatinine, known risk factors for chronic kidney disease, a very high serum creatinine and/or phosphate, anaemia, a slow clinical course or small kidneys on ultrasound.

Serum creatinine is the most commonly used marker of renal function. However, its use as a marker of AKI has several limitations. First, its release varies with age, gender, muscle mass, diet, exercise and drugs. Second, its secretion in the tubules accounts for 10–40% of creatinine clearance and can mask a decrease in GFR. Finally, a decrease of at least 50% in GFR is needed before serum creatinine becomes abnormal, and more than 24 hours may pass before a sufficient increase in blood concentration is detectable. Of relevance to anaesthetists and often not fully appreciated, fluid resuscitation and a positive fluid balance may artificially lower serum creatinine, confounding diagnosis. In a post hoc analysis of the Fluid and Catheter Therapy Trial cohort (n=1000), Liu and colleagues found that the incidence of AKI (creatinine-classified) before adjustment for fluid balance was greater in patients managed with a conservative fluid protocol (57 vs 51%, $P=0.04$). However, after adjustment for fluid balance, the incidence of AKI was greater in patients managed with the liberal fluid protocol (66 vs 58%)[128]. Moreover, those with AKI identified after adjustment for a positive fluid balance had a higher mortality rate than patients who did not meet AKI criteria both before and after adjustment for fluid balance (31 vs 12%, $P < 0.001$). The impact of fluid balance on classification of AKI should therefore be considered in future studies of AKI.

The RIFLE and RIFLE/AKIN classification systems for AKI, which use serum creatinine, have been discussed earlier. If estimated GFR is used to classify renal function, the Modification of Diet in Renal Disease abbreviated equation, which incorporates creatinine, age and gender, is widely used and validated for its calculation[129]. Estimated GFR has been successfully used to classify acute kidney injury in critically ill patients using the RIFLE GFR criteria[4,18,104].

Like creatinine, plasma urea also has limited reliability in estimating GFR. Its production and renal clearance are not constant and levels can be altered by changes in circulatory volume, protein intake and gastrointestinal bleeding. Urea also requires time to accumulate and delays diagnosis, making it a poor measure of GFR.

Urine output is monitored in surgery and ICUs by indwelling catheters. Trends in urine output help gauge kidney function and may better reflect a change in renal haemodynamics than solute clearance[1]. Urine output lacks specificity and sensitivity and is affected by several drugs (i.e. diuretics and vasopressors) reducing its utility as a marker of renal function.

Other methods for diagnosing AKI include inulin clearance which has been regarded as the most accurate method for determining GFR for research in the past. However, an intravenous infusion is

required, and inulin measurement is expensive and labour-intensive, making it an impractical option. Radionuclide-based tests have performed well, although such methods are too complex for clinical use.

Novel biomarkers for AKI

The lack of adequate methods for the diagnosis of AKI has opened up a new field of research in AKI[130–134]. Innovative technologies such as proteomics have facilitated the detection of several new biomarkers of AKI (Table 1)[135]. Amongst the most promising of these are NGAL and cystatin C, which have been evaluated, predominantly after cardiac surgery, in general ICU patients and on emergency department presentation[133,136]. They assess complementary aspects of renal injury (NGAL: tubular stress; cystatin C: GFR)[137]. The utility of different biomarkers for each RIFLE classification stage is summarised in Table 1.

Certain key points can be made. First, the concentration of these biomarkers seems to change earlier than creatinine in patients who develop AKI. If AKI was detected early, clinicians might be able to treat it sooner and have a better chance of preventing or reducing injury. Second, the biomarkers respond to treatment or recovery making them potentially useful to evaluate the effect of interventions such as perioperative haemodynamic optimisation of high-risk surgical patients on renal function and as safety markers to monitor toxicity[138,139]. Third, they appear to be more sensitive than creatinine and respond to 'subclinical' degrees of kidney injury that pass undetected by creatinine, yet are associated with worse outcomes. Indeed, recent studies suggest that NGAL may detect subclinical states of AKI that are associated with increased risk of RRT, hospital mortality or length-of-stay[140,141]. Furthermore, biomarker combinations may offer improved prediction of AKI beyond clinical models, and provide severity and prognostic information at an early stage in the development of injury. This would allow the application of potential therapies at appropriate time points. Finally, biomarkers may help elucidate the potential mechanisms of injury of AKI; this is manifested in a recent investigation surrounding the pathophysiological processes of septic AKI[87].

The arrival of new biomarkers of AKI has allowed a re-evaluation of mechanisms, timing and pathogenesis in this area, and new biomarkers continue to emerge[130,132,133,142]. This field could lead to therapeutic benefit and eventual modification of the definition of AKI. However, no biomarker-guided interventional study has yet been conducted and their ability to guide therapy remains unknown.

TABLE 1

Biomarkers indicated for use in different RIFLE classification stages of AKI

RIFLE classification	Biomarker use	Biomarkers
Risk of kidney injury increased	Early detection	Serum: NGAL, CyC Urine: NGAL, IL-18, KIM-1, GST, L-FABP, Hepcidin
Injury to the kidney	Diagnosis	Tubular stress/injury Serum: NGAL, CyC Urine: NGAL, KIM-1, Hepcidin Reduction in GFR Serum: creatinine, urea, CyC
Failure of the kidney	Prognosis	Serum: NGAL, CyC, creatinine, urea, IL-6 Urine: NGAL, KIM-1, Hepcidin

RIFLE=Risk, Injury, Failure, Loss, End-stage classification system of kidney function, AKI=acute kidney injury, NGAL=neutrophil gelatinase-associated lipocalin, CyC=cystatin C, IL-18=interleukin 18, KIM-1=kidney injury molecule 1, GST=glutathione S-transferase, L-FABP=liver-type fatty acid binding protein, IL-6=interleukin 6, GFR=glomerular filtration rate.

PREVENTION AND TREATMENT

Investigation of pharmacotherapies to prevent or ameliorate AKI has been disappointing. Loop diuretics do not reduce the incidence of AKI[143]. The proposed nephroprotective effect of 'low-dose' dopamine was refuted in postoperative and general critically ill patients[144]. Fenoldopam, a vasodilator, may reduce the need for RRT after cardiac surgery[42] but has shown inconsistent results in different settings. Natriuretic peptides have only shown unconfirmed promise in major surgery[42]. Calcium channel antagonists show no benefit on postoperative renal function after cardiac surgery and unconvincing evidence for a benefit in non-cardiac surgery[42]. Despite promising results in experimental studies, N-acetylcysteine after radiocontrast and before cardiac surgery to prevent AKI has shown unconvincing results[145,146], and it is doubtful whether a perioperative intravenous sodium bicarbonate infusion for cardiac surgical patients is nephroprotective[147,148], despite promising results from an early pilot study[149].

The lack of success in finding a consistently effective treatment for AKI has several explanations. First, multiple causes and disease processes contribute to the development of AKI and this heterogeneity of causes and likely pathogenesis means finding a common treatment will be unlikely. Second, limited knowledge of the disease processes involved in the development of AKI makes planning of investigations into the prevention or treatment of AKI difficult.

Third, if interventions are only guided by conventional markers (creatinine, urea, urine output) which only become deranged very late in the course of injury, they may be initiated too late for treatment to be effective. This problem has profoundly hindered therapeutic progress in this area.

Timely fluid resuscitation (avoiding overload) and appropriate titration of inotropes/vasopressors to maintain adequate perfusion pressure and cardiac output remain the only widely accepted therapy to prevent or treat AKI. Indeed, protocolised perioperative optimisation of haemodynamic and oxygenation parameters using oxygen, fluids and inotropes/vasopressors in high-risk surgical patients with septic shock may reduce mortality and the risk of AKI[150]. However, the optimal perioperative fluid regimen is uncertain and increasing evidence suggests that better outcomes are associated with more restrictive fluid replacement practice[151,152]. A recent systematic review of randomised controlled trials of goal-directed therapy-based, haemodynamically-targeted fluid resuscitation found that this practice was associated with decreased incidence of AKI after major surgery[153]. However, the benefit could not be attributed to the provision of greater volumes of fluid to hypovolaemic patients, but rather, the use of inotropes with goal-directed therapy and an equivalent amount of fluid resuscitation between groups seemed to be associated with a lower incidence of AKI. Perhaps providing fluid earlier and faster with inotropic support minimises the harmful effects of fluid overload. In Australia this has led to the development and design of the first large multicentre, randomised controlled trial to determine whether a restrictive versus liberal (current practice) perioperative fluid regimen decreases mortality (NCT01424150). This trial will be the first phase III trial of fluid therapy in the perioperative period ever conducted.

Further measures to prevent/treat AKI include avoidance of nephrotoxic drugs, maintenance of adequate oxygenation and haemoglobin concentration, monitoring of volume status with preference for euvolaemia and maintaining plasma glucose between 8–10 mmol/l in critically ill patients receiving insulin therapy[7]. The administration of fluids containing starch should be avoided due to a demonstrated lack of benefit for critically ill patients and for their association with increased need for RRT[49,50]. Whether the pleiotropic effects of erythropoietin are protective of the kidneys requires more investigation in humans[154,155].

Patients who develop progressive azotaemia, hyperkalaemia, metabolic acidosis or fluid overload will generally require RRT as supportive therapy.

Renal Replacement Treatment

There is insufficient evidence to indicate the best time to commence RRT, although observational studies[156] suggest benefit from early initiation[157]. The recognition of the contribution of even low levels of fluid overload (10–20%) to increased risk of adverse clinical outcomes in AKI patients[158], lends more weight to the recommendation of early RRT for severely oliguric patients.

Studies do not show a survival advantage between available RRTs commonly used in developed countries such as intermittent haemodialysis, slow low-efficiency dialysis or continuous renal replacement therapy (CRRT)[159]. Of note, in RRT patients, RIFLE seems less effective in predicting risk of death, which may be due to the inability to discriminate between the R, I and F classes in such a sick population[2]. Nonetheless, in the RENAL trial (almost exclusively CRRT), renal recovery was far greater than in the ATN trial (considerable use of intermittent haemodialysis), supporting the potential benefit of CRRT for renal recovery[160]. In addition, there is a consensus that in patients who are vasopressor dependent, CRRT should be the treatment of choice[159,160]. In Australia and New Zealand, CRRT is the dominant approach to RRT in the ICU.

Regarding the intensity of RRT, both the ATN and RENAL trials showed no benefit from the use of a higher intensity of RRT[161,162]. A dose of 25–30 ml/kg/hour of effluent generation is recommended[163]. How to decide when to stop CRRT is uncertain, however, a spontaneous urine output of over 500 ml/day may help in determining when to consider a trial off CRRT[164].

THE FUTURE

The mortality associated with severe AKI, particularly in critically ill patients, remains very high. The ATN and RENAL trials revealed mortality rates of 53 and 44.7%, respectively. Moreover, postoperative AKI is one of the most serious complications in surgical patients and accounts for 18–47% of all cases of hospital-acquired AKI[165]. Of equal concern, recent studies indicate a link between AKI and subsequent progression to chronic kidney disease, end-stage kidney disease and mortality[166,167]. This implies that a short episode of AKI may contribute to kidney/patient morbidity and mortality in the long-term, which would increase the human and financial costs of AKI far beyond current projections. Whether this increased risk of chronic kidney disease is a direct effect of AKI or whether AKI is simply yet another marker of patients predisposed to chronic kidney disease, or both, warrants further investigation.

There is growing interest in the potential renal protection that remote ischaemic preconditioning could provide for patients undergoing major surgery[168] and larger trials are currently underway (NCT01067703, n=2070). The apparent negative effect of saline and other chloride-rich fluids on renal function is also of increasing interest[169]. A recent observational study in abdominal surgery patients found that the use of 0.9% saline compared to a balanced fluid resulted in an increased risk for dialysis requirement[170].

The need for earlier detection of AKI and investigation into effective treatments is evident, with the evaluation of novel early biomarkers for AKI and development of targeted therapies becoming a priority. Certain biomarkers appear to rise and respond to specific subgroups of AKI in different time frames and at different levels. We may, therefore, see the development of biomarker 'panel' tests, which might detect the presence of AKI early and perhaps give an indication of the type/cause of AKI. This, in turn, would facilitate the investigation and development of targeted treatments for AKI with biological agents (e.g. erythropoietin) or broad biological modifiers (e.g. cooling).

Erythropoietin and therapeutic hypothermia are two treatments that have shown potential as kidney protective therapies. They are being investigated in renal sub-studies of two large multicentre randomised controlled trials: *Erythropoietin in traumatic brain injury* (ACTRN12609000827235) and *Prophylactic hypothermia to lessen traumatic brain injury* (ACTRN12609000764235). These sub-studies will include the use of multiple biomarkers to evaluate the effects of the interventions and the diagnostic, prognostic and treatment monitoring value of the biomarkers when used in combination.

CONCLUSION

AKI is a major contributing factor to adverse outcomes after major surgery and in critically ill patients. Its consensus classification has facilitated AKI research, and the intense investigation of early biomarkers of AKI may lead to expanded definitions that incorporate the new concept of 'subclinical' AKI and open the door to earlier and better targeted interventions. As the pathophysiology of AKI becomes better understood, some previously held beliefs are being questioned and our ability to develop interventions and implement them at the optimum time will improve. The results of the *Crystalloid versus hydroxyethyl starch trials* (NCT00935168), the future *Restrictive versus liberal fluid therapy in major abdominal surgery: RELIEF study* (NCT01424150) and several new lines of AKI research will provide greater insight into the prevention and treatment of AKI and assist anaesthetists and intensive care clinicians in providing better care to their increasingly high-risk patients.

CONFLICT OF INTEREST

R. Bellomo and A. Nichol have received funding from the National Health and Medical Research Council and the Victorian Neurotrauma Initiative (now a research program of the Transport Accident Commission) for the *Erythropoietin in traumatic brain injury* and *Prophylactic hypothermia to lessen traumatic brain injury* trials; investigating the benefit of erythropoietin and prophylactic hypothermia respectively on six-month neurological outcomes in patients with traumatic brain injury. In addition, R. Bellomo, E. Moore and A. Nichol have received funding from the Intensive Care Foundation to conduct the renal substudies of the *Erythropoietin in traumatic brain injury* and *Prophylactic hypothermia to lessen traumatic brain injury* trials.

FURTHER READING

Further information regarding the current and future trials mentioned in this article can be found by entering the relevant trial registration number at apps.who.int/trialsearch.

REFERENCES

1. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure—definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004; 8:R204-212.
2. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: a systematic review. Kidney Int 2008; 73:538-546.
3. Chertow GM, Burdick E, Honour M, Bonventre J, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol 2005; 16:3365-3370.
4. Moore EM, Simpson JA, Tobin A, Santamaria J. Preoperative estimated glomerular filtration rate and RIFLE-classified postoperative acute kidney injury predict length of stay post-coronary bypass surgery in an Australian setting. Anaesth Intensive Care 2010; 38:113-121.
5. Uchino S, Bellomo R, Goldsmith D, Bates S, Ronco C. An assessment of the RIFLE criteria for acute renal failure in hospitalized patients. Crit Care Med 2006; 34:1913-1917.
6. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG et al. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care 2007; 11:R31.
7. Khwaja A. KDIGO Clinical Practice Guidelines for Acute Kidney Injury. Nephron Clin Pract 2012; 120:179-184.

8. Bagshaw SM, George C, Bellomo R. A comparison of the RIFLE and AKIN criteria for acute kidney injury in critically ill patients. Nephrol Dial Transplant 2008; 23:1569-1574.
9. Joannidis M, Metnitz B Bauer P, Schusterschitz N, Moreno R, Druml W et al. Acute kidney injury in critically ill patients classified by AKIN versus RIFLE using the SAPS 3 database. Intensive Care Med 2009; 35:1692-1702.
10. Lopes JA, Fernandes P, Jorge S, Goncalves S, Alvarez A, Costa e Silva Z et al. Acute kidney injury in intensive care unit patients: a comparison between the RIFLE and the Acute Kidney Injury Network classifications. Crit Care 2008; 12:R110.
11. Robert AM, Kramer RS, Dacey LJ, Charlesworth DC, Leavitt BJ, Helm RE et al. Cardiac surgery-associated acute kidney injury: a comparison of two consensus criteria. Ann Thorac Surg 2010; 90:1939-1943.
12. Srisawat N, Murugan R, Wen Xi, Singbartl K, Clermont G, Eiam-Ong S et al. Recovery from acute kidney injury: determinants and predictors. Contrib Nephrol 2010; 165:284-291.
13. Bagshaw SM, George C, Dinu I, Bellomo R. A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. Nephrol Dial Transplant 2008; 23:1203-1210.
14. Ostermann M, Chang RWS. Acute kidney injury in the intensive care unit according to RIFLE. Crit Care Med 2007; 35:1837-1843; quiz 1852.
15. Parmar A, Langenberg C, Wan L, May CN, Bellomo R, Bagshaw SM. Epidemiology of septic acute kidney injury. Curr Drug Targets 2009; 10:1169-7811.
16. Bagshaw SM, George C, Bellomo R. Early acute kidney injury and sepsis: a multicentre evaluation. Crit Care 2008; 12:R47.
17. Bagshaw SM, Lapinsky S, Dial S, Arabi Y, Dodek P, Wood G et al. Acute kidney injury in septic shock: clinical outcomes and impact of duration of hypotension prior to initiation of antimicrobial therapy. Intensive Care Med 2009; 35:871-881.
18. Bagshaw SM, George C, Gibney RTN, Bellomo R. A multicenter evaluation of early acute kidney injury in critically ill trauma patients. Ren Fail 2008; 30:581-589.
19. Moore EM, Bellomo R, Nichol A, Harley N, Macisaac C, Cooper DJ. The incidence of acute kidney injury in patients with traumatic brain injury. Ren Fail 2010; 32:1060-1065.
20. Bellomo R. The epidemiology of acute renal failure: 1975 versus 2005. Curr Opin Crit Care 2006; 12:557-560.
21. Thakar CV, Worley S, Arrigain S, Yared JP, Paganini EP. Improved survival in acute kidney injury after cardiac surgery. Am J Kidney Dis 2007; 50:703-711.
22. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S et al. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA 2005; 294:813-818.
23. Reineck HJ, O'Connor GJ, Lifschitz MD, Stein JH. Sequential studies on the pathophysiology of glycerol-induced acute renal failure. J Lab Clin Med 1980; 96:356-362.
24. Langenberg C, Bagshaw SM, May CN, Bellomo R. The histopathology of septic acute kidney injury: a systematic review. Crit Care 2008; 12:R38.
25. Bellomo R, Bagshaw S, Langenberg C, Ronco C. Pre-renal azotemia: a flawed paradigm in critically ill septic patients? Contrib Nephrol 2007; 156:1-9.
26. Moreau R, Lebrec D. Acute kidney injury: new concepts. Hepatorenal syndrome: the role of vasopressors. Nephron Physiol 2008; 109:73-79.
27. Gluud LL, Christensen K, Christensen E, Krag A. Systematic review of randomized trials on vasoconstrictor drugs for hepatorenal syndrome. Hepatology 2010; 51:576-584.
28. Sagi SV, Mittal S, Kasturi KS, Sood GK. Terlipressin therapy for reversal of type 1 hepatorenal syndrome: a meta-analysis of randomized controlled trials. J Gastroenterol Hepatol 2010; 25:880-885.
29. Damman K, Navis G, Smilde TDJ, Voors AA, van der Bij W, van Veldhuisen DJ et al. Decreased cardiac output, venous congestion and the association with renal impairment in patients with cardiac dysfunction. Eur J Heart Fail 2007; 9:872-878.
30. Slater MS, Mullins RJ. Rhabdomyolysis and myoglobinuric renal failure in trauma and surgical patients: a review. J Am Coll Surg 1998; 186:693-716.
31. Bosch X, Poch E, Grau JM. Rhabdomyolysis and acute kidney injury. N Engl J Med 2009; 361:62-72.
32. Bentley ML, Corwin HL, Dasta J. Drug-induced acute kidney injury in the critically ill adult: recognition and prevention strategies. Crit Care Med 2010; 38:S169-174.
33. Markowitz GS, Perazella MA. Drug-induced renal failure: a focus on tubulointerstitial disease. Clin Chim Acta 2005; 351:31-47.
34. Verme-Gibboney C. Oral angiotensin-converting-enzyme inhibitors. Am J Health Syst Pharm 1997; 54:2689-2703.
35. Olyaei AJ, de Mattos AM, Bennett WM. Immunosuppressant-induced nephropathy: pathophysiology, incidence and management. Drug Saf 1999; 21:471-488.
36. Heyman SN, Rosenberger C, Rosen S. Regional alterations in renal haemodynamics and oxygenation: a role in contrast medium-induced nephropathy. Nephrol Dial Transplant 2005; 20 Suppl 1:i6-11.
37. Rundback JH, Nahl D, Yoo V. Contrast-induced nephropathy. J Vasc Surg 2011; 54:575-579.
38. McCullough PA, Bertrand ME, Brinker JA, Stacul F. A meta-analysis of the renal safety of isosmolar iodixanol compared with low-osmolar contrast media. J Am Coll Cardiol 2006; 48:692-699.
39. Ranucci M, Ballotta A, Kunkl A, De Benedetti D, Kandil H, Conti D et al. Influence of the timing of cardiac catheterization and the amount of contrast media on acute renal failure after cardiac surgery. Am J Cardiol 2008; 101:1112-1118.
40. Allaqaband S, Tumuluri R, Malik AM, Gupta A, Volkert P, Shalev Y et al. Prospective randomized study of N-acetylcysteine, fenoldopam, and saline for prevention of radiocontrast-induced nephropathy. Catheter Cardiovasc Interv 2002; 57:279-283.
41. Stone GW, McCullough PA, Tumlin JA, Lepor NE, Madyoon H, Murray P et al. Fenoldopam mesylate for the prevention of contrast-induced nephropathy: a randomized controlled trial. JAMA 2003; 290:2284-2291.
42. Patel NN, Rogers CA, Angelini GD, Murphy GJ. Pharmacological therapies for the prevention of acute kidney injury following cardiac surgery: a systematic review. Heart Fail Rev 2011; 16:553-567.
43. de Mendonca A, Vincent JL, Suter PM, Moreno R, Dearden NM, Antonelli M et al. Acute renal failure in the ICU: risk factors and outcome evaluated by the SOFA score. Intensive Care Med 2000; 26:915-921.
44. Abelha FJ, Botelho M, Fernandes V, Barros H. Determinants of postoperative acute kidney injury. Crit Care 2009; 13:R79.
45. Bahar I, Akgul A, Ozatik MA, Vural KM, Demirbag AE, Boran M et al. Acute renal failure following open heart surgery: risk factors and prognosis. Perfusion 2005; 20:317-322.
46. Bove T, Calabro MG, Landoni G, Aletti G, Marino G, Crescenzi G et al. The incidence and risk of acute renal failure after cardiac surgery. J Cardiothorac Vasc Anesth 2004; 18:442-445.

47. Karkouti K, Wijeysundera DN, Yau TM, Callum JL, Cheng DC, Crowther M et al. Acute kidney injury after cardiac surgery: focus on modifiable risk factors. Circulation 2009; 119:495-502.
48. Koch CG, Li L, Sessler DI, Figueroa P, Hoeltge GA, Mihaljevic T et al. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008; 358:1229-1239.
49. Dart AB, Mutter TC, Ruth CA, Taback SP. Hydroxyethyl starch (HES) versus other fluid therapies: effects on kidney function. Cochrane Database Syst Rev 2010; 1:CD007594.
50. Perner A, Haase N, Guttormsen AB, Tenhunen J, Klemenzson G, Aneman Anders M et al. Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. N Engl J Med 2012; 367:124-134.
51. Nielsen VG. Effects of Hextend hemodilution on plasma coagulation kinetics in the rabbit: role of factor XIII-mediated fibrin polymer crosslinking. J Surg Res 2006; 132:17-22.
52. Schramko A, Suojaranta-Ylinen R, Kuitunen A, Raivio P, Kukkonen S, Niemi T. Hydroxyethylstarch and gelatin solutions impair blood coagulation after cardiac surgery: a prospective randomized trial. Br J Anaesth 2010; 104:691-697.
53. Lipfert B, Standl T, Dullmann J, Helmchen U, Schulte am Esch J, Lorke DE. Histology and ultrastructure of liver and kidney following blood exchange with ultrapurified, polymerised bovine hemoglobin in comparison with hydroxyethyl starch. Lab Invest 1999; 79:573-582.
54. Dickenmann M, Oettl T, Mihatsch MJ. Osmotic nephrosis: acute kidney injury with accumulation of proximal tubular lysosomes due to administration of exogenous solutes. Am J Kidney Dis 2008; 51:491-503.
55. Heyman SN, Lieberthal W, Rogiers P, Bonventre JV. Animal models of acute tubular necrosis. Curr Opin Crit Care 2002; 8:526-534.
56. Kato N, Yuzawa Y, Kosugi T, Hobo A, Sato W, Miwa Y et al. The E-selectin ligand basigin/CD147 is responsible for neutrophil recruitment in renal ischemia/reperfusion. J Am Soc Nephrol 2009; 20:1565-1576.
57. Kwon O, Hong SM, Ramesh G. Diminished NO generation by injured endothelium and loss of macula densa nNOS may contribute to sustained acute kidney injury after ischemia-reperfusion. Am J Physiol Renal Physiol 2009; 296:F25-33.
58. Thuillier R, Favreau F, Celhay O, Macchi L, Milin S, Hauet T. Thrombin inhibition during kidney ischemia-reperfusion reduces chronic graft inflammation and tubular atrophy. Transplantation 2010; 90:612-621.
59. Versteilen AMG, Blaauw N, Di Maggio F, Groeneveld ABJ, Sipkema P, Musters RJP et al. rho-Kinase inhibition reduces early microvascular leukocyte accumulation in the rat kidney following ischemia-reperfusion injury: roles of nitric oxide and blood flow. Nephron Exp Nephrol 2011; 118:e79-86.
60. Doi K, Leelahavanichkul A, Yuen PST, Star RA. Animal models of sepsis and sepsis-induced kidney injury. J Clin Invest 2009; 119:2868-2878.
61. Ishikawa K, May CN, Gobe G, Langenberg C, Bellomo R. Pathophysiology of septic acute kidney injury: a different view of tubular injury. Contrib Nephrol 2010; 165:18-27.
62. Langenberg C, Wan L, Egi M, May CN, Bellomo R. Renal blood flow in experimental septic acute renal failure. Kidney Int 2006; 69:1996-2002.
63. Langenberg C, Wan L, Egi M, May CN, Bellomo R. Renal blood flow and function during recovery from experimental septic acute kidney injury. Intensive Care Med 2007; 33:1614-1618.
64. Wan L, Yang N, Hiew CY, Schelleman A, Johnson L, May C et al. An assessment of the accuracy of renal blood flow estimation by Doppler ultrasound. Intensive Care Med 2008; 34:1503-1510.
65. Le Dorze M, Legrand M, Payen D, Ince C. The role of the microcirculation in acute kidney injury. Curr Opin Crit Care 2009; 15:503-508.
66. Molitoris BA, Sandoval RM. Multiphoton imaging techniques in acute kidney injury. Contrib Nephrol 2010; 165:46-53.
67. Bragadottir G, Redfors B, Nygren A, Sellgren J, Ricksten SE. Low-dose vasopressin increases glomerular filtration rate, but impairs renal oxygenation in post-cardiac surgery patients. Acta Anaesthesiol Scand 2009; 53:1052-1059.
68. Bagshaw SM, Uchino S, Bellomo R, Morimatsu H, Morgera S, Schetz M et al. Septic acute kidney injury in critically ill patients: clinical characteristics and outcomes. Clin J Am Soc Nephrol 2007; 2:431-439.
69. Lerolle N, Nochy D, Guerot E, Bruneval P, Fagon JY, Diehl JL et al. Histopathology of septic shock induced acute kidney injury: apoptosis and leukocytic infiltration. Intensive Care Med 2010; 36:471-478.
70. Kim M, Park SW, Kim M, D'Agati VD, Lee HT. Isoflurane activates intestinal sphingosine kinase to protect against renal ischemia-reperfusion-induced liver and intestine injury. Anesthesiology 2011; 114:363-373.
71. Grenz A, Hong JH, Badulak A, Ridyard D, Luebbert T, Kim JH et al. Use of a hanging-weight system for isolated renal artery occlusion. J Vis Exp 2011.
72. Kong HY, Zhu SM, Wang LQ, He Y, Xie HY, Zheng SS. Sevoflurane protects against acute kidney injury in a small-size liver transplantation model. Am J Nephrol 2010; 32:347-355.
73. Ganopolsky JG, Castellino FJ. A protein C deficiency exacerbates inflammatory and hypotensive responses in mice during polymicrobial sepsis in a cecal ligation and puncture model. Am J Pathol 2004; 165:1433-1446.
74. Hollenberg SM, Dumasius A, Easington C, Colilla SA, Neumann A, Parrillo JE. Characterization of a hyperdynamic murine model of resuscitated sepsis using echocardiography. Am J Respir Crit Care Med 2001; 164:891-895.
75. Wang J, Du Z, Zhang W, Han B, Peng C, Chen N. Post liver transplantation acute kidney injury in a rat model of syngeneic orthotopic liver transplantation. Lab Invest 2011; 91:1158-1169.
76. Yeung KK, Richir M, Hanrath P, Teerlink T, Kompanowska-Jezierska E, Musters RJP et al. Infrarenal aortic-clamping after renal ischaemia aggravates acute renal failure. Eur J Clin Invest 2011; 41:605-615.
77. Li X, Liu M, Bedja D, Thoburn C, Gabrielson K, Racusen L et al. Acute renal venous obstruction is more detrimental to the kidney than arterial occlusion: implication for murine models of acute kidney injury. Am J Physiol Renal Physiol 2012; 302:F519-525.
78. Aravindan N, Natarajan M, Shaw AD. Fenoldopam inhibits nuclear translocation of nuclear factor kappa B in a rat model of surgical ischemic acute renal failure. J Cardiothorac Vasc Anesth 2006; 20:179-186.
79. Murphy GJ, Lin H, Coward RJ, Toth T, Holmes R, Hall D et al. An initial evaluation of post-cardiopulmonary bypass acute kidney injury in swine. Eur J Cardiothorac Surg 2009; 36:849-855.
80. Zhu J, Yin R, Shao H, Dong G, Luo L, Jing H. N-acetylcysteine to ameliorate acute renal injury in a rat cardiopulmonary bypass model. J Thorac Cardiovasc Surg 2007; 133:696-703.
81. Prowle JR, Molan MP, Hornsey E, Bellomo R. Cine phase-contrast magnetic resonance imaging for the measurement of renal blood flow. Contrib Nephrol 2010; 165:329-336.

82. Schneider A, Johnson L, Goodwin M, Schelleman A, Bellomo R. Bench-to-bedside review: contrast enhanced ultrasonography—a promising technique to assess renal perfusion in the ICU. Crit Care 2011; 15:157.
83. Prowle JR, Ishikawa K, May CN, Bellomo R. Renal blood flow during acute renal failure in man. Blood Purif 2009; 28:216-225.
84. Alejandro V, Scandling JD Jr, Sibley RK, Dafoe D, Alfrey E, Deen W et al. Mechanisms of filtration failure during postischemic injury of the human kidney. A study of the reperfused renal allograft. J Clin Invest 1995; 95:820-831.
85. Schrier RW, Wang W. Acute renal failure and sepsis. N Engl J Med 2004; 351:159-169.
86. Loutzenhiser R, Griffin K, Williamson G, Bidani A. Renal autoregulation: new perspectives regarding the protective and regulatory roles of the underlying mechanisms. Am J Physiol Regul Integr Comp Physiol 2006; 290:R1153-1167.
87. Abstracts of Sepsis 2011. Beijing, China. October 26-28, 2011. Crit Care 2011; 15 (Suppl 3):P1-46.
88. Kusudo K, Ishii K, Rahman M, Aki Y, Miyatake A, Kosaka H et al. Blood flow-dependent changes in intrarenal nitric oxide levels during anesthesia with halothane or sevoflurane. Eur J Pharmacol 2004; 498:267-273.
89. Kim M, Kim M, Park SW, Pitson SM, Lee HT. Isoflurane protects human kidney proximal tubule cells against necrosis via sphingosine kinase and sphingosine-1-phosphate generation. Am J Nephrol 2010; 31:353-362.
90. Eger EI 2nd, Koblin DD, Bowland T, Ionescu P, Laster MJ, Fang Z et al. Nephrotoxicity of sevoflurane versus desflurane anesthesia in volunteers. Anesth Analg 1997; 84:160-168.
91. Wagener G, Brentjens TE. Anesthetic concerns in patients presenting with renal failure. Anesthesiol Clin 2010; 28:39-54.
92. Hsing CH, Chou W, Wang JJ, Chen HW, Yeh CH. Propofol increases bone morphogenetic protein-7 and decreases oxidative stress in sepsis-induced acute kidney injury. Nephrol Dial Transplant 2011; 26:1162-1172.
93. Song XM, Wang YL, Li JG, Wang CY, Zhou Q, Zhang ZZ et al. Effects of propofol on pro-inflammatory cytokines and nuclear factor kappaB during polymicrobial sepsis in rats. Mol Biol Rep 2009; 36:2345-2351.
94. Mazze RI. Methoxyflurane revisited: tale of an anesthetic from cradle to grave. Anesthesiology 2006; 105:843-846.
95. Grindlay J, Babl FE. Review article: efficacy and safety of methoxyflurane analgesia in the emergency department and prehospital setting. Emerg Med Australas 2009; 21:4-11.
96. Annat G, Viale JP, Bui XB, Hadj AO, Benzoni D, Vincent M et al. Effect of PEEP ventilation on renal function, plasma renin, aldosterone, neurophysins and urinary ADH, and prostaglandins. Anesthesiology 1983; 58:136-141.
97. Rymarz A, Serwacki M, Rutkowski M, Pakosinski K, Grodzicki M, Patkowski W et al. Prevalence and predictors of acute renal injury in liver transplant recipients. Transplant Proc 2009; 41:3123-3125.
98. Mayes S, Ferrone M. Fentanyl HCl patient-controlled iontophoretic transdermal system for the management of acute postoperative pain. Ann Pharmacother 2006; 40:2178-2186.
99. Latta KS, Ginsberg B, Barkin RL. Meperidine: a critical review. Am J Ther 2002; 9:53-68.
100. Craig RG, Hunter JM. Neuromuscular blocking drugs and their antagonists in patients with organ disease. Anaesthesia 2009; 64 (Suppl 1):55-65.
101. Li Y, Zhu S, Yan M. Combined general/epidural anesthesia (ropivacaine 0.375%) versus general anesthesia for upper abdominal surgery. Anesth Analg 2008; 106:1562-1565.
102. Guay J. The benefits of adding epidural analgesia to general anesthesia: a metaanalysis. J Anesth 2006; 20:335-340.
103. Bellomo R, Auriemma S, Fabbri A, D'Onofrio A, Katz N, McCullough PA et al. The pathophysiology of cardiac surgery-associated acute kidney injury (CSA-AKI). Int J Artif Organs 2008; 31:166-178.
104. Cooper WA, O'Brien SM, Thourani VH, Guyton RA, Bridges CR, Szczech LA et al. Impact of renal dysfunction on outcomes of coronary artery bypass surgery: results from the Society of Thoracic Surgeons National Adult Cardiac Database. Circulation 2006; 113:1063-1070.
105. Grant MC, Kon Z, Joshi A, Christenson E, Kallam S, Burris N et al. Is aprotinin safe to use in a cohort at increased risk for thrombotic events: results from a randomized, prospective trial in off-pump coronary artery bypass. Ann Thorac Surg 2008; 86:815-822.
106. Henry D, Carless P, Fergusson D, Laupacis A. The safety of aprotinin and lysine-derived antifibrinolytic drugs in cardiac surgery: a meta-analysis. CMAJ 2009; 180:183-193.
107. Fergusson DA, Hebert PC, Mazer CD, Fremes S, MacAdams C, Murkin JM et al. A comparison of aprotinin and lysine analogues in high-risk cardiac surgery. N Engl J Med 2008; 358:2319-2331.
108. Leyvi G, Nelson O, Yedlin A, Pasamba M, Belamarich PF, Nair S et al. A comparison of the effect of aprotinin and epsilon-aminocaproic acid on renal function in children undergoing cardiac surgery. J Cardiothorac Vasc Anesth 2011; 25:402-406.
109. DeSantis SM, Toole JM, Kratz JM, Uber WE, Wheat MJ, Stroud MR et al. Early postoperative outcomes and blood product utilization in adult cardiac surgery: the post-aprotinin era. Circulation 2011; 124:S62-69.
110. European Medicines Agency. Press release: European Medicines Agency recommends lifting suspension of aprotinin. From: http://www.ema.europa.eu/ema/index.jsp?curl=pages/home/Home_Page.jsp&mid=. Accessed April 2012.
111. Doddakula K, Al-Sarraf N, Gately K, Hughes A, Tolan M, Young V et al. Predictors of acute renal failure requiring renal replacement therapy post cardiac surgery in patients with preoperatively normal renal function. Interact Cardiovasc Thorac Surg 2007; 6:314-318.
112. Gandhi GY, Nuttall GA, Abel MD, Mullany CJ, Schaff HV, Williams BA et al. Intraoperative hyperglycemia and perioperative outcomes in cardiac surgery patients. Mayo Clin Proc 2005; 80:862-866.
113. Wigfield CH, Lindsey JD, Munoz A, Chopra PS, Edwards NM, Love RB. Is extreme obesity a risk factor for cardiac surgery? An analysis of patients with a BMI > or = 40. Eur J Cardiothorac Surg 2006; 29:434-440.
114. Engelman DT, Adams DH, Byrne JG, Aranki SF, Collins JJ Jr, Couper GS et al. Impact of body mass index and albumin on morbidity and mortality after cardiac surgery. J Thorac Cardiovasc Surg 1999; 118:866-873.
115. Mentzer RM Jr, Oz MC, Sladen RN, Graeve AH, Hebeler RF Jr, Luber JM Jr et al. Effects of perioperative nesiritide in patients with left ventricular dysfunction undergoing cardiac surgery: the NAPA Trial. J Am Coll Cardiol 2007; 49:716-726.
116. Sezai A, Hata M, Niino T, Yoshitake I, Unosawa S, Wakui S et al. Results of low-dose human atrial natriuretic peptide infusion in nondialysis patients with chronic kidney disease undergoing coronary artery bypass grafting: the NU-HIT (Nihon University working group study of low-dose HANP Infusion Therapy during cardiac surgery) trial for CKD. J Am Coll Cardiol 2011; 58:897-903.
117. Amar D, Zhang H, Park B, Heerdt PM, Fleisher M, Thaler HT. Inflammation and outcome after general thoracic surgery. Eur J Cardiothorac Surg 2007; 32:431-434.

118. Magovern JA, Singh D, Teekell-Taylor L, Scalise D, McGregor W. Preoperative clinical factors are important determinants of the inflammatory state before and after heart surgery. ASAIO J 2007; 53:316-319.
119. Agirbasli M, Nguyen ML, Win K, Kunselman AR, Clark JB, Myers JL et al. Inflammatory and hemostatic response to cardiopulmonary bypass in pediatric population: feasibility of seriological testing of multiple biomarkers. Artif Organs 2010; 34:987-995.
120. Dieleman JM, van Paassen J, van Dijk D, Arbous MS, Kalkman CJ, Vandenbroucke JP et al. Prophylactic corticosteroids for cardiopulmonary bypass in adults. Cochrane Database Syst Rev 2011; 5:CD005566.
121. Chawla LS, Seneff MG, Nelson DR, Williams M, Levy H, Kimmel PL et al. Elevated plasma concentrations of IL-6 and elevated APACHE II score predict acute kidney injury in patients with severe sepsis. Clin J Am Soc Nephrol 2007; 2:22-30.
122. Simmons EM, Himmelfarb J, Sezer MT, Chertow GM, Mehta RL, Paganini EP, et al. Plasma cytokine levels predict mortality in patients with acute renal failure. Kidney Int 2004; 65:1357-1365.
123. Bakris GL, Lass N, Gaber AO, Jones JD, Burnett JC Jr. Radiocontrast medium-induced declines in renal function: a role for oxygen free radicals. Am J Physiol 1990; 258:F115-120.
124. Baliga R, Ueda N, Walker PD, Shah SV. Oxidant mechanisms in toxic acute renal failure. Am J Kidney Dis 1997; 29:465-477.
125. Prabhu A, Sujatha DI, Ninan B, Vijayalakshmi MA. Neutrophil gelatinase associated lipocalin as a biomarker for acute kidney injury in patients undergoing coronary artery bypass grafting with cardiopulmonary bypass. Ann Vasc Surg 2010; 24:525-531.
126. Burns KE, Chu MW, Novick RJ, Fox SA, Gallo K, Martin CM et al. Perioperative N-acetylcysteine to prevent renal dysfunction in high-risk patients undergoing cabg surgery: a randomized controlled trial. JAMA 2005; 294:342-350.
127. Haase M, Haase-Fielitz A, Bagshaw SM, Reade MC, Morgera S, Seevenayagam S et al. Phase II, randomized, controlled trial of high-dose N-acetylcysteine in high-risk cardiac surgery patients. Crit Care Med 2007; 35:1324-1331.
128. Liu KD, Thompson BT, Ancukiewicz M, Steingrub JS, Douglas IS, Matthay MA et al. Acute kidney injury in patients with acute lung injury: impact of fluid accumulation on classification of acute kidney injury and associated outcomes. Crit Care Med 2011; 39:2665-2671.
129. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med 2003; 139:137-147.
130. Bonventre JV. Pathophysiology of acute kidney injury: roles of potential inhibitors of inflammation. Contrib Nephrol 2007; 156:39-46.
131. Devarajan P. Proteomics for the investigation of acute kidney injury. Contrib Nephrol 2008; 160:1-16.
132. Heller F, Frischmann S, Grunbaum M, Zidek W, Westhoff TH. Urinary calprotectin and the distinction between prerenal and intrinsic acute kidney injury. Clin J Am Soc Nephrol 2011; 6:2347-2355.
133. Moore E, Bellomo R, Nichol A. Biomarkers of acute kidney injury in anesthesia, intensive care and major surgery: from the bench to clinical research to clinical practice. Minerva Anestesiol 2010; 76:425-440.
134. Taes Youri EC, Wuyts B, Boelaert JR, De Vriese AS, Delanghe JR. Prohepcidin accumulates in renal insufficiency. Clin Chem Lab Med 2004; 42:387-389.
135. Devarajan P, Krawczeski CD, Nguyen MT, Kathman T, Wang Z, Parikh CR. Proteomic identification of early biomarkers of acute kidney injury after cardiac surgery in children. Am J Kidney Dis 2010; 56:632-642.
136. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis 2009; 54:1012-1024.
137. Moore E, Bellomo R. Novel biomarkers of acute kidney injury: ready for clinical application? Curr Opin Crit Care 2010; Epub ahead of print.
138. Capuano F, Goracci M, Luciani R, Gentile G, Roscitano A, Benedetto U et al. Neutrophil gelatinase-associated lipocalin levels after use of mini-cardiopulmonary bypass system. Interact Cardiovasc Thorac Surg 2009; 9:797-801.
139. Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT. Increased incidence of acute kidney injury with aprotinin use during cardiac surgery detected with urinary NGAL. Am J Nephrol 2008; 28:576-582.
140. Haase M, Devarajan P, Haase-Fielitz A, Bellomo R, Cruz D, Wagener G et al. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. J Am Coll Cardiol 2011; 57:1752-1761.
141. Nickolas TL, Schmidt-Ott KM, Canetta P, Forster C, Singer E, Sise M et al. Diagnostic and prognostic stratification in the emergency department using urinary biomarkers of nephron damage: a multicenter prospective cohort study. J Am Coll Cardiol 2012; 59:246-255.
142. Prowle JR, Ostland V, Calzavacca P, Licari E, Ligabo EV, Echeverri JE et al. Greater increase in urinary hepcidin predicts protection from acute kidney injury after cardiopulmonary bypass. Nephrol Dial Transplant 2012; 27:595-602.
143. Bagshaw SM, Delaney A, Haase M, Ghali WA, Bellomo R. Loop diuretics in the management of acute renal failure: a systematic review and meta-analysis. Crit Care Resusc 2007; 9:60-68.
144. Bellomo R, Chapman M, Finfer S, Hickling K, Myburgh J. Low-dose dopamine in patients with early renal dysfunction: a placebo-controlled randomised trial. Australian and New Zealand Intensive Care Society (ANZICS) Clinical Trials Group. Lancet 2000; 356:2139-2143.
145. Gonzales DA, Norsworthy KJ, Kern SJ, Banks S, Sieving PC, Star RA et al. A meta-analysis of N-acetylcysteine in contrast-induced nephrotoxicity: unsupervised clustering to resolve heterogeneity. BMC Med 2007; 5:32.
146. Nigwekar SU, Kandula P. N-acetylcysteine in cardiovascular-surgery-associated renal failure: a meta-analysis. Ann Thorac Surg 2009; 87:139-147.
147. Heringlake M, Heinze H, Schubert M, Novak Y, Guder J, Kleinebrahm M et al. A perioperative infusion of sodium bicarbonate does not improve renal function in cardiac surgery patients: a prospective observational cohort study. Crit Care 2012; 16:R156.
148. McGuinness S, Parke RL, Bellomo R, Van Haren F. Sodium bicarbonate infusion to reduce cardiac surgery associated acute kidney injury; a phase II multi-centre, double-blind, randomized controlled trial. Am J Respir Crit Care Med 2012; 185:A6857.
149. Haase M, Haase-Fielitz A, Bellomo R, Devarajan P, Story D, Matalanis G et al. Sodium bicarbonate to prevent increases in serum creatinine after cardiac surgery: a pilot double-blind, randomized controlled trial. Crit Care Med 2009; 37:39-47.

150. Brienza N, Giglio MT, Marucci M, Fiore T. Does perioperative hemodynamic optimization protect renal function in surgical patients? A meta-analytic study. Crit Care Med 2009; 37:2079-2090.
151. Gonzalez-Fajardo JA, Mengibar L, Brizuela JA, Castrdeza J, Vaquero-Puerta C. Effect of postoperative restrictive fluid therapy in the recovery of patients with abdominal vascular surgery. Eur J Vasc Endovasc Surg 2009; 37:538-543.
152. Rahbari NN, Zimmermann JB, Schmidt T, Koch M, Weigand MA, Weitz J. Meta-analysis of standard, restrictive and supplemental fluid administration in colorectal surgery. Br J Surg 2009; 96:331-341.
153. Prowle JR, Chua HR, Bagshaw SM, Bellomo R. Clinical review: volume of fluid resuscitation and the incidence of acute kidney injury—a systematic review. Crit Care 2012; 16:230.
154. Endre ZH, Westhuyzen J. Early detection of acute kidney injury: emerging new biomarkers. Nephrology (Carlton) 2008; 13:91-98.
155. Song YR, Lee T, You SJ, Chin HJ, Chae DW, Lim C et al. Prevention of acute kidney injury by erythropoietin in patients undergoing coronary artery bypass grafting: a pilot study. Am J Nephrol 2009; 30:253-260.
156. Bagshaw SM, Uchino S, Bellomo R, Morimatsu H, Morgera S, Schetz et al. Timing of renal replacement therapy and clinical outcomes in critically ill patients with severe acute kidney injury. J Crit Care 2009; 24:129-140.
157. Garcia-Fernandez N, Perez-Valdivieso JR, Bes-Rastrollo M, Vives M, Lavilla J, Herreros J et al. Timing of renal replacement therapy after cardiac surgery: a retrospective multicenter Spanish cohort study. Blood Purif 2011; 32:104-111.
158. Prowle JR, Echeverri JE, Ligabo EV, Ronco C, Bellomo R. Fluid balance and acute kidney injury. Nat Rev Nephrol 2010; 6:107-115.
159. Bagshaw SM, Berthiaume LR, Delaney A, Bellomo R. Continuous versus intermittent renal replacement therapy for critically ill patients with acute kidney injury: a meta-analysis. Crit Care Med 2008; 36:610-617.
160. Uchino S, Bellomo R, Kellum JA, Morimatsu H, Morgera S, Schetz MR et al. Patient and kidney survival by dialysis modality in critically ill patients with acute kidney injury. Int J Artif Organs 2007; 30:281-292.
161. Bellomo R, Cass A, Cole L, Finfer S, Gallagher M, Lo S et al. Intensity of continuous renal-replacement therapy in critically ill patients. N Engl J Med 2009; 361:1627-1638.
162. Palevsky PM, Zhang JHo, O'Connor TZ, Chertow GM, Crowley ST, Choudhury D et al. Intensity of renal support in critically ill patients with acute kidney injury. N Engl J Med 2008; 359:7-20.
163. Prowle JR, Schneider A, Bellomo R. Clinical review: optimal dose of continuous renal replacement therapy in acute kidney injury. Crit Care 2011; 15:207.
164. Uchino S, Bellomo R, Morimatsu H, Morgera S, Schetz M, Tan I et al. Discontinuation of continuous renal replacement therapy: a post hoc analysis of a prospective multicenter observational study. Crit Care Med 2009; 37:2576-2582.
165. Carmichael P, Carmichael AR. Acute renal failure in the surgical setting. ANZ J Surg 2003; 73:144-153.
166. Coca SG, Yusuf B, Shlipak MG, Garg AX, Parikh CR. Long-term risk of mortality and other adverse outcomes after acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis 2009; 53:961-973.
167. Waikar SS, Winkelmayer WC. Chronic on acute renal failure: long-term implications of severe acute kidney injury. JAMA 2009; 302:1227-1229.
168. Venugopal V, Laing CiM, Ludman A, Yellon DM, Hausenloy D. Effect of remote ischemic preconditioning on acute kidney injury in nondiabetic patients undergoing coronary artery bypass graft surgery: a secondary analysis of 2 small randomized trials. Am J Kidney Dis 2010; 56:1043-1049.
169. Reid F, Lobo DN, Williams RN, Rowlands BJ, Allison SP. (Ab)normal saline and physiological Hartmann's solution: a randomized double-blind crossover study. Clin Sci 2003; 104:17-24.
170. Shaw AD, Bagshaw SM, Goldstein SL, Scherer LA, Duan M, Schermer CR et al. Major complications, mortality, and resource utilization after open abdominal surgery: 0.9% saline compared to Plasma-Lyte. Ann Surg 2012; 255:821-829.

1.3 Traumatic Brain Injury

Traumatic Brain Injury (TBI) refers to brain injury acquired through a traumatic event such as a traffic accident or a blow to the head. The patients receiving the treatments (erythropoietin and prophylactic hypothermia) that we are investigating for AKI in the parent trials (EPO-TBI and POLAR, respectively) have sustained a TBI. This section provides an overview of the TBI patient population and a general review of TBI including classification, pathogenesis, diagnosis, treatment and future directions in TBI research. We will focus on patients with TBI due to blunt, non-penetrating trauma as participants in the EPO-TBI and POLAR trials are recruited from this population.

1.3.1 Epidemiology / Incidence

TBI is the most common cause of death and disability in young people in developed countries[1] and in Australia is largely caused by vehicular trauma (61%) involving young males, and falls in elderly patients (25%) as our population ages[2]. In Europe, TBI is responsible for more years of disability than any other cause[3] and the financial burden of TBI in the USA has been estimated at over US$60 billion per year[4]. Death shortly after injury and morbidity are common in patients with TBI and the proportion of all severe TBI patients with favorable neurological outcomes (extended Glasgow Outcomes Score [GOSE]) at 6 months was less than 50% in a recent study[2]. This is even more tragic when the youth and unreached potential of the TBI population is taken into account.

1.3.2 Classification

TBI had traditionally been classified by mechanism into closed versus penetrating injury. Clinical severity of TBI is classified using the Glasgow Coma Scale (GCS) which has evolved into a universal classification system for the severity of TBI (Table 1)[5].

Table 1 Glasgow Coma Scale

Score	1	2	3	4	5	6
Eyes	No eye opening	Opens eyes in response to pain	Opens eyes in response to voice	Opens eyes spontaneously	NA	NA
Verbal	No verbal response	Incomprehensible sounds	Utters inappropriate words	Confused, disoriented	Oriented, converses normally	NA
Motor	No motor response	Extension to pain (decerebrate posture)	Abnormal flexion to pain (decorticate posture)	Flexion/withdrawal to pain	Localises to pain	Obeys commands

Pain= a peripheral pain stimulus, such as squeezing the lunula area of the patient's fingernail, NA=not applicable (maximum scores: eyes=4, verbal=5, motor=6)

Patients with TBI are classified as having a mild, moderate, or severe injury according to the following GCS groupings[6]:

Mild TBI GCS $\geq$ 13
Moderate TBI GCS 9 to 12
Severe TBI GCS $\leq$ 8.

The severity of TBI may be more accurately predicted by also considering CT scan findings and factors such as context (i.e. drug or alcohol intoxication). It is preferable to assess the GCS after the patient has undergone cardiopulmonary or fluid resuscitation and before sedation because the examination is sensitive to hypotension, hypoxia and sedative medication.

Assessment of structural damage using neurological imaging is not prone to such confounding. The Marshall classification system for TBI (Table 2) focuses on the presence or not of a mass lesion and differentiates diffuse injury using signs of raised intracranial pressure on CT imaging. It is used in many clinical trials involving TBI. However, this system could mask patients with diffuse axonal injury *and* a mass lesion, and does not make full use of prognostic CT information[7].

Table 2 The Marshall CT classification of TBI[8]

Category	Definition
Diffuse injury I	No visible intra-cranial pathology seen on CT scan
Diffuse injury II	Cisterns are present with midline shift < 5 mm and/or lesion densities present No high- or mixed-density lesion > 25 ml, may include bone fragments and foreign bodies
Diffuse injury III	Cisterns compressed or absent with mid-line shift 0–5 mm No high- or mixed-density lesion > 25 ml
Diffuse injury IV	Mid-line shift > 5 mm No high- or mixed-density lesion > 25 ml
Evacuated mass lesion	Any lesion surgically evacuated
Non-evacuated mass lesion	High or mixed-density lesion >25 mL, not surgically evacuated

TBI is frequently associated with extracranial injuries which increase the risk of secondary brain injury due to hypoxia, hypotension, pyrexia and coagulopathy. Therefore, to assist prognosis, the severity of extracranial injuries should be recorded using the Injury Severity Score which is derived from the Abbreviated Injury Scale for 6 body regions. This data is collected by Trauma Registries in Australia.

1.3.3 Pathogenesis

We will focus on the pathogenesis of TBI resulting from blunt trauma.

Primary Injury: TBI from blunt trauma occurs as a result of direct impact, or rapid acceleration or deceleration by external forces. The extent and type of damage caused depends on the nature, intensity and direction of these forces. Primary injuries can be focal; including intracranial haematomas and contusions; or diffuse (diffuse axonal injury [DAI]). Damage includes shearing of white matter tracts that connect distant regions of the brain and diffuse swelling. DAI is distinguished by many small lesions in white matter tracts. With high shear rates, blood vessels can also tear causing haemorrhages[3].

Cellular processes in early neurotrauma (minutes to hours post injury) that cause damage include increased permeability of membranes, ion-pump failure, and structural changes to proteins[3]. Contributing factors to ischaemic damage, which is often involved

in the primary brain injury are microvascular occlusion, excitotoxic injury, and impaired cerebral perfusion and oxygenation[3].

Secondary injury: The processes involved in secondary brain injury develop over hours to days and include excessive neurotransmitter release, calcium-mediated damage, free-radical overload, mitochondrial dysfunction, gene activation, apoptosis and inflammation; which ultimately lead to axonal failure, neuronal energy metabolic failure and neuronal death[9].

Cerebral ischaemia resulting from TBI initiates a complex cascade of events (Hypothermia review, section 2.3) leading to excessive calcium influx into brain cells and the release of large amounts of the excitatory transmitter, glutamate[10-12]. This process worsens astrocytic swelling and contributes to brain swelling and elevated intracranial pressure. Along with free radical damage this is a major cause of cell death. Further neuronal death is induced by early gene activation and the release of caspases and other proapoptotic molecules[9]. Autophagy, which is activated in TBI may also play a role in cell death[13].

Proinflammatory mediators are released after traumatic brain injury from 1 hour to 5 days, with a maximum response within a few days. The inflammatory response is necessary to clean up cell debris after injury, and can trigger regeneration. However, associated cytokine release leads to opening of the blood-brain barrier, activation of cell death and triggering of apoptosis. If the inflammatory response is controlled it can be neuroprotective but in excess, it can be deleterious[14].

Mitochondrial dysfunction plays an important role in acute post-traumatic neurodegeneration. Neuronal death or damage occurring after acute traumatic brain injury is mainly due to energy failure from inhibition of the electron transport chain and collapse of the mitochondrial membrane potential[15]. Mitochondria play a pivotal role in regulating intracellular calcium that is massively increased in TBI[16-18]. This calcium buffering induces mitochondrial swelling, which appears to be reversible over time if cell repair mechanisms are intact, and the mitochondrial permeability transition pore (mPTP) does not open. Several studies identify the mPTP as a critical trigger that induces an apoptotic cascade when opened, determining cell survival after TBI[15-17]. The loss of membrane potential, further mitochondrial swelling, and rupture of the outer mitochondrial membrane ensues[15]. Opening of the mPTP is modulated by several

factors after severe TBI during early reperfusion, of which, cytosolic and mitochondrial calcium overload during ischemia, the release of reactive oxygen species, and cellular alkalosis during reperfusion may be particularly important[19,20]. Other factors that may alter mitochondrial function after TBI, such as decreased cerebral blood flow, cerebral ischemia, hypoglycaemia, seizures, pyrexia (which may induce oxidative stress), may favour the opening of the mPTP, which may therefore occur several days after injury[15].

Brain trauma is accompanied by regional alterations of brain metabolism, reduction in metabolic rates and possible energy crisis. A study using Positron emission tomography and microdialysis in patients with TBI found a 25% incidence rate of metabolic crisis (elevated lactate/pyruvate ratio) but only 1% incidence of ischaemia[21], thus indicating that TBI may lead to a state of enduring metabolic / energy crisis that is largely related to mechanisms other than ischemia.

1.3.4 Diagnosis

CT imaging is the preferred method of assessment of patients admitted to emergency departments with head injury. It is quick, accurate and widely available. Current guidelines recommend CT examination in TBI patients with a GCS of 14 or below, or with a GCS of 15 and risk factors such as loss of consciousness, prolonged duration of amnesia, abnormal/altered pupil reaction, vomiting, old age and anticoagulant therapy[22-24]. CT imaging allows detection of structural damage, intracranial haematomas and fractures. In the sub-acute and chronic stages of TBI, MRI allows better detection of lesions in white matter in patients with DAI[25]. Follow-up CT is suggested if lesions are present on the initial scan, if the patient deteriorates or in the presence of high ICPs[26].

1.3.5 Treatment

The complex pathophysiology of TBI and the heterogeneity of this disease present challenges to the investigation of therapies for TBI, which have been largely unsuccessful. For a therapy to be effective in TBI it must act on several mechanisms or pathways involved rather than target one particular factor.

Several drugs have been investigated for the treatment of TBI; however most have proven to be ineffective. Pegorgotein (PEG-SOD)[27], magnesium[28], bradykinin inhibitors (e.g. deltibant)[29,30] and N-methyl-d-aspartate receptor antagonists (e.g. selfotel[31],

dexanabinol[32]) were ineffective in clinical trials. Other neuroprotective agents that were effective in animal models of TBI but disappointed in clinical trials were, calcium channel blockers[33], free radical scavengers and corticosteroids[34]. Similarly, there is no reliable evidence that beta-2 receptor antagonists reduce mortality or disability after TBI[35].

The CRASH 2 nested intracranial bleeding study that evaluated the effect of tranexamic acid in TBI patients hinted that tranexamic acid administration might improve outcome in TBI patients and may be worth evaluating further[36]. In phase II studies progesterone has shown promise; short-and long-term progesterone treatment was associated with an improvement in neurological outcome in patients with traumatic brain injury[37-39]. However results of larger phase III, randomised, multicentre, clinical trials are awaited (ProTECT III, NCT00822900; SyNAPSe, NCT01143064). Similarly, cyclosporin A, which inhibits the mitochondrial permeability transition pore, has shown encouraging results for safety and efficacy in phase II studies[40-42]. A continuous infusion commenced within 8 hours of injury as opposed to 12 and lasting for 72 hours versus 24 may be preferable; the optimal therapeutic window and dose for cyclosporin A administration remain to be determined[43]. Success in larger, later phase trials for the treatment of moderate and severe TBI is pending, including the EPO-TBI and POLAR trials

Decompressive craniectomy: The recent DECRA trial (bifrontotemporoparietal decompressive craniectomy for severe diffuse TBI) found that in adults with refractory intracranial hypertension, craniectomy decreased ICP, time on mechanical ventilation, and ICU length of stay compared to standard care. However, contrary to the study hypothesis it was associated with worse long term functional outcomes (Extended Glasgow Outcome Scale score)[44]. Results may have several explanations; the intervention may lead to, a) excessive expansion of the swollen brain and axonal stretch / neuronal injury; or b) unwanted changes to blood flow and metabolism. Alternatively, differences associated with this procedure in comparison with a unilateral craniectomy or another bilateral surgical method may influence the result[44].

1.3.6 Future

Regulated epidemiological monitoring of TBI patients should be performed to establish a sound information base of clinical variables to inform prognostic algorithms such as

those based on data from the International Mission for Prognosis And Clinical Trial (IMPACT) database. The establishment of centralised trauma systems, with care of the most severe TBI patients concentrated in specialised centres from emergency care to rehabilitation is recommended[3].

Despite advances being made in developed countries in TBI prevention through public health measures, there has been little success in the investigation of treatments for TBI. Cooling has historically shown promise in the treatment of TBI, however, has several associated complications. We continue in attempts to overcome these by modifying implementation, and a current trial (POLAR NCT00987688) will shed more light on future directions and the feasibility of continued trials in this area. There is substantial experimental evidence, and supportive clinical trial evidence to suggest a neuroprotective effect of erythropoietin in acute brain injury and a trial in TBI patients (EPO-TBI, NCT00987454) is investigating this question.

The opening of the mitochondrial permeability transition pore (mPTP) is a critical trigger in acute neurodegeneration following TBI. Given this pivotal role, the multiple factors that modulate its opening, and the ensuing chain of events that have been elucidated in the experimental setting, it seems reasonable to focus on mPTP in future neuroprotective research in humans[15].

1.3.7 Conclusion

The financial burden of TBI in Australia is enormous. Potential treatments need to influence multiple pathways to have any chance of success given the complexity of the pathophysiology of TBI and the heterogeneity of this disease. Promising new diagnostic/prognostic techniques may help identify patients who are more likely to experience a favourable outcome, and may help to tailor therapy to patients' needs. Stronger evidence and clarification is required in specific areas such as the management of raised ICP. The investigation of treatments for TBI has been largely unsuccessful; results of several current trials are awaited and more focus on a critical trigger involved in the neurological deterioration following TBI may be advisable.

1.4 The incidence of Acute Kidney Injury in patients with Traumatic Brain Injury

The principal aim in this thesis was to investigate the effect of erythropoietin and hypothermia on AKI. These interventions were delivered to ICU patients with moderate or severe TBI (GCS < 13) in the EPO-TBI and POLAR parent trials. No previous dedicated investigation of AKI had been performed in the TBI population; therefore it was necessary to gauge the incidence of AKI in these patients in order to confirm our belief that continuing with the investigation was warranted. The following study conducted by the candidate and colleagues was the first dedicated investigation of the occurrence of AKI in patients with TBI and based on the results the intended investigation continued.

Moore EM, Bellomo R, Nichol AD, Harley N, MacIsaac C, Cooper DJ. The incidence of acute kidney injury in patients with traumatic brain injury. Ren Fail 2010 Oct; 32(9).

Declaration for Thesis Chapter

Moore EM, Bellomo R, Nichol AD, Harley N, MacIsaac C, Cooper DJ. The incidence of acute kidney injury in patients with traumatic brain injury. Ren. Fail. 2010;32(9): 1060-65.

Declaration by candidate

In the case of Chapter , the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Reviewed the literature; designed the study; obtained ethical approval; collected, analysed and interpreted the data; developed and wrote up drafts, submitted the manuscript, and was responsible for the response to reviewers.	90 %

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
R Bellomo	Involved in conception of the study, analysis and interpretation of data, review and drafting of manuscript.	N/A
A Nichol	Contributed to review and drafting of manuscript.	N/A
N Harley	Contributed to review and drafting of manuscript.	N/A
C MacIsaac	Contributed to review and drafting of manuscript.	N/A
DJ Cooper	Contributed to review and drafting of manuscript.	N/A

Candidate's Signature	Elizabeth Moore	**Date** 13 Mar 2012

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s)	**Australian and New Zealand Intensive Care Research Centre, Department of Epidemiology and Preventive Medicine, Monash University, Alfred campus** **Intensive Care Unit, Alfred Hospital** **Intensive Care Unit, Royal Melbourne Hospital**

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

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CLINICAL STUDY

The incidence of acute kidney injury in patients with traumatic brain injury

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Abstract

There is limited information on the incidence of acute kidney injury (AKI) in patients with traumatic brain injury (TBI) although AKI may contribute to morbidity and mortality. We investigated the incidence of AKI in patients with moderate and severe TBI and the association of AKI with risk factors and outcomes in these patients.

We studied all TBI patients over 16 years of age admitted to the two designated trauma hospitals in the state of Victoria, Australia from 1 January to 31 December 2008. Patients were included if they had head trauma and presented with a Glasgow coma scale (GCS) <13. Prospectively collected data from the hospital trauma registries, ICUs, and pathology databases were analyzed retrospectively. Risk injury failure loss end (RIFLE) criteria were used to categorize renal function.

The incidence of AKI was 9.2% (19/207). Patients who developed AKI were older, had higher severity of illness scores, and a lower GCS. Overall 42.1% of these patients died in hospital compared with 18.1% in patients without AKI. In univariable linear regression analysis, age, severity of illness, and admitting hospital were associated with AKI. After multivariable logistic regression, the occurrence of AKI was associated with age ($p < 0.001$) and higher APACHE III scores ($p = 0.016$).

AKI is relatively common even in patients with TBI. Its association with age and APACHE III scores helps identify patients at higher risk of AKI.

Keywords: kidney failure, acute; renal insufficiency, acute, brain injuries, craniocerebral trauma; acute kidney injury; traumatic brain injury

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INTRODUCTION

There is limited information on the incidence of acute kidney injury (AKI) in patients with traumatic brain injury (TBI). A few studies have broadly investigated non-neurological outcomes in TBI and reported a low incidence of 'renal failure' in TBI patients.[1–3] However, these studies used Sequential Organ Failure Assessment (SOFA) or Multiple Organ Dysfunction (MOD) scores to identify the presence of AKI and classify its severity. These scores have not been validated for AKI and use single serum creatinine (SCr) cutoffs to classify renal function, which likely only identify patients with severe AKI. Accordingly, they are insensitive to less severe changes in kidney function, which, in many studies, have been shown to be independent predictors of AKI.[4–6] Moreover, none of these studies focused on AKI over time, or used change in renal function from baseline to measure renal injury. This approach is now considered more appropriate than single SCr cutoff measurements.[7,8]

Recently, AKI has been defined and classified according to a consensus system called Risk injury failure loss end (RIFLE).[9] This system has been validated in more than 250,000 patients in multiple studies from different countries and centers, and in different patient

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populations.[7,10,11] The RIFLE system uses estimates of glomerular filtration rate (GFR) to assess baseline function if this is unknown and calculates changes in GFR to assess whether a patient has developed AKI. This approach has been confirmed accurate in studies involving thousands of patients[7,10,12] and is particularly useful in populations, such as TBI patients, where a baseline SCr is typically missing.

A recent analysis of data from the Australian and New Zealand Intensive Care Society (ANZICS) adult patient database using the RIFLE criteria suggested that 11% of patients with TBI are likely to develop AKI within the first 24 hours of ICU admission.[10] These data, however, did not extend beyond the first day after admission. Given that the burden of AKI in patients with TBI may be greater than previously expected,[10] the paucity of data on the incidence of AKI in TBI,[1–3] the limitations of the ANZICS database study (e.g., renal function data for 24 hours only),[10] and the potential health resource implications, further investigation appears desirable.

In this study, the primary aim was to provide a more reliable estimate of the incidence of AKI as classified by the RIFLE criteria in patients with TBI in the state of Victoria, Australia. In addition we described their clinical characteristics stratified by AKI and investigated risk factors for AKI and associations between AKI and outcomes.

METHODS

The Alfred and Royal Melbourne Hospital Ethics Committees approved the study and waived the need for informed consent. We studied all patients, 16 years and over, with moderate or severe TBI (head injury resulting from trauma; GCS < 13), and admitted to the intensive care units of the two designated trauma centers of the state of Victoria, Australia, from 1 January 2008 to 31 December 2008. Those patients with no SCr data beyond the day of admission or the following day were excluded. Routine, prospectively collected data from the two hospital trauma registries and their intensive care unit and pathology databases were collected retrospectively.

We applied the RIFLE classification system to the assessment of renal function, but did not include urine output. Because no baseline creatinine was available in these patients, their baseline GFR was estimated using normative age and gender-stratified eGFR values.[13] Those with no SCr measurements beyond the first 2 days were excluded to eliminate early deaths and to differentiate those who may be responsive to interventions. As the first available SCr was potentially confounded by creatinine release from muscle injury (Figure 1), we used the peak SCr from the day after admission to the first 10 days in the modification of diet in renal disease (MDRD)-175 equation[14] to calculate the worst eGFR. Then maximal decline from baseline was calculated to establish RIFLE level of dysfunction.

STATISTICAL ANALYSIS

Associations between RIFLE categories and potential confounders were investigated using the chi-square test for categorical confounders, one way analysis of variance for normally distributed continuous confounders, and the Kruskall–Wallis test for non-normally distributed continuous confounders. Univariable relationships with the percent decline in eGFR were determined using linear regression. Multivariable models were constructed using multivariable regression analysis with both stepwise and backward elimination techniques. Comparison of the sample with and without those with no SCr measurements beyond the first 2 days was performed for descriptive, univariable, and multivariable analyses. Intercooled Stata, version 9 and above (Statacorp, College Station, Texas, USA) was used for analyses.

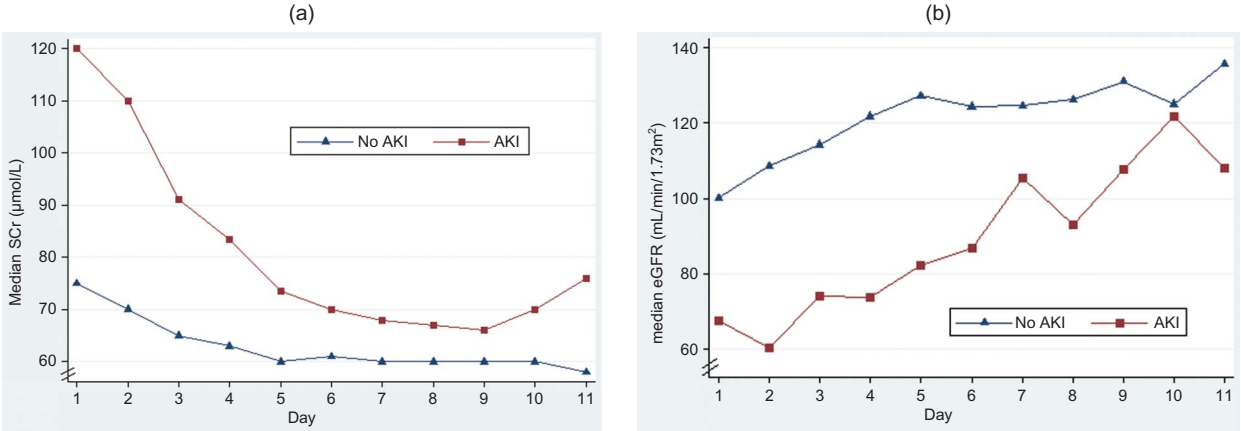

Figure 1. (a) Median serum creatinine: day of admission to day 10. (b) Median eGFR: day of admission to day 10.

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RESULTS

In the year 2008, we identified 216 patients fitting the inclusion criteria. Nine patients with no SCr data beyond the day of admission (making calculation of their peak level impossible) were excluded leaving a total of 207 patients. A further 20 patients with no SCr beyond the day after admission or the following day were excluded for part of the analysis. These 20 patients are referred to as 'those with no SCr beyond day 2' in Tables 1 and 2. Exclusion of these patients left 187 moderate or severe TBI patients with SCr values beyond the day after admission or the following day. TBI patients were relatively young and mostly male (median age 33; 76.8% males) (Table 1). Of the 20 patients with no SCr beyond day 2, 80% died, 80% were male, only 25% underwent operations, and the hospital length of stay for all but 2 of them was ≤2 days. This was reflected in the comparisons with and without exclusions (Tables 1 and 2).

The incidence of AKI (RIFLE Risk/Injury/Failure) was 9.2% (19/207, Table 1). Patients with AKI tended to be older and have higher severity of illness scores and a lower Glasgow coma scale (GCS). AKI patients had a higher mortality (Table 1).

In univariable linear regression analysis, age, severity of illness, and admitting hospital were associated with decrease in eGFR; in contrast, GCS and low blood pressure were not associated with lower eGFR (Table 2). Logistic regression analysis revealed no association of AKI with mortality. In the final multivariable linear regression analysis, the percent decline in eGFR was associated with older age, higher severity of illness, and hospital of admission (Table 2).

DISCUSSION

Statement of principal findings

We conducted a retrospective epidemiological study to establish the incidence and risk factors for and outcome of AKI in patients admitted to ICU because of TBI. We found that the incidence of AKI approximates 10%. In addition we found that age, severity of illness, low GCS, and hospital of admission were risk factors for the development of AKI, and that patients who have both TBI and AKI have a mortality rate of greater than 40%.

Comparison to literature

The incidence of AKI in TBI patients was explored by Zygun et al.[2] who found that only 1 of 209 patients with severe TBI developed 'renal failure' and 7% developed 'renal dysfunction' (MOD score). However, these investigators used an unvalidated tool to assess for AKI, which has limited sensitivity. More recently Wahlstrom et al.[1] found that in a cohort of 93 TBI patients none developed severe renal failure. However,

Table 1. Patient characteristics grouped by 'percent decline in eGFR', including and excluding those with no SCr beyond day 2.

Variable	Including those with no SCr beyond day 2			Excluding those with no SCr beyond day 2		
	Normal ≤25% ↓ (n = 188)	Risk and Injury >25% ↓ (n = 19)[a]	p-value	Normal ≤25% ↓ (n = 173)	Risk and Injury >25% ↓ (n = 14)[a]	p-Value
Median age (years)	31	50	0.007	32	45	0.147
Gender (male)	78.2%	63.2%	0.139	77.5%	64.3%	0.264
MV hours (median)	100.9	73.2	0.712	119	106	0.400
Median APACHE III[b]	50	80	<0.001	48	69	0.002
Median APACHE II[b]	16	24	<0.001	16	21.5	0.016
GCS (median)	7	3	0.038	7	3	0.085
Mean MAP high	98.2	95.4	0.850	97.4	92.4	0.734
Mean MAP low	68.8	65.3	0.032	69.0	68.6	0.508
ISS (mean)[c]	32.5	32.3	0.366	32.3	31.5	0.392
Surgical case	29.8%	31.6%	0.871	29.5%	42.9%	0.296
Hosp LOS (median)	13.4	13.2	0.418	14.5	17.6	0.469
Home discharge[d]	7.8%	15.8%	0.238	7.3%	21.4%	0.068
Hospital death	18.1%	42.1%	0.013	13.3%	21.4%	0.398
Pk Cr d 1–10 (mean)	74.6	114.8		74.8	114.6	
Worst eGFR (mean)	104.0	55.0		103.5	56.5	
↓ in eGFR (mean)	−19.5	29.3		−19.0	27.8	
% ↓ in eGFR (mean)	−23.0	34.8		−22.4	32.9	

Notes: MV hours, hours on mechanical ventilation; APACHE II or III, severity of disease scores within the first 24 hours of ICU admission; GCS, Glascow coma scale; MAP high/low, highest/lowest mean arterial blood pressure within 24 hours of ICU admission; ISS, injury severity score; Hosp LOS, hospital length of stay; Home discharge, discharge to home versus elsewhere (includes death); Hosp death, died in this hospital admission; Pk Cr d 1–10, peak serum creatinine from day 1 to 10; Worst eGFR, eGFR calculated with the peak serum creatinine from day 1 to 10; ↓ in eGFR, decrease in eGFR from baseline to worst.

[a]Categories combined due to low numbers in injury category: Risk n = 17 (8.2%), Injury n = 2 (1%).

[b]n = 199 for APACHE II and III variables.

[c]n = 204 for ISS variable.

[d]n = 198 for home discharge variable.

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Table 2. Comparison of univariable and multivariable estimates for linear regression models with 'percent decline in eGFR': including and excluding those with no SCr beyond day 2.

Predictors of percentage decline in eGFR	Including those with no SCr beyond day 2				Excluding those with no SCr beyond day 2			
	Univariable		Multivariable		Univariable		Multivariable	
	Param Est ± SE	*p*-value	Param Est ± SE	*p*-value	Param Est ± SE	*p*-value	Param Est ± SE	*p*-value
Age (year)	0.57 ± 0.12	<0.001	0.63 ± 0.11	<0.001	0.45 ± 0.13	0.001	0.53 ± 0.12	<0.001
Hospital	−28.13 ± 4.21	<0.001	−33.79 ± 3.80	<0.001	−30.28 ± 4.21	<0.001	−34.04 ± 3.97	<0.001
APACHE III	0.32 ± 0.09	0.001	0.19 ± 0.08	0.016	0.31 ± 0.11	0.004	0.18 ± 0.09	0.044
APACHE II	0.94 ± 0.40	0.020			0.80 ± 0.45	0.080		
Mech Vent (hours)	0.01 ± 0.02	0.349			0.02 ± 0.02	0.215		
GCS	−0.79 ± 0.74	0.285			−0.81 ± 0.77	0.293		
MAP high (24 hours)	−0.09 ± 0.15	0.552			−0.12 ± 0.17	0.461		
MAP low (24 hours)	−0.26 ± 0.24	0.271			−0.06 ± 0.25	0.818		
ISS	0.04 ± 0.18	0.831			0.01 ± 0.19	0.938		
Gender (male)	−7.34 ± 5.40	0.176			−6.34 ± 5.53	0.253		
Surgical case	7.59 ± 4.97	0.129			9.64 ± 5.07	0.059		

Notes: Param Est, parameter estimate; SE, standard error; Hospital, difference attributable to admitting hospital (difference in serum creatinine assays between hospitals likely accounts for most of this difference); APACHE II or III, severity of disease scores within the first 24 hours of ICU admission; GCS, Glascow coma scale; MAP high/low, highest/lowest mean arterial blood pressure within 24 hours of ICU admission; ISS, injury severity score.

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these investigators used the SOFA score criteria for AKI (creatinine ≥300 μmol/L, urine output ≤500 mL/day), which, unlike the consensus RIFLE criteria, are highly insensitive and only detect patients with the most advanced form of AKI. Bagshaw et al.[10] in their study on AKI in 9449 trauma patients calculated baseline SCr from the MDRD equation assuming a lower limit of normal baseline GFR (75 mL/min) which has been used in numerous studies[7,12,15–17] and found that, in the first 24 hours, the incidence of AKI was 11%, a finding similar to that reported in this study. The RIFLE criteria measure change in renal function from a baseline value,[9] have been extensively used and validated to classify renal function in studies cumulatively involving >250,000 subjects,[6,7,10] and seem appropriate for this kind of assessment. Our study using a similar methodology found similarly that the incidence of AKI in TBI patients is approximately 10%. No other studies have defined the risk factors for AKI in this population. Indeed, recent experimental evidence[18] confirming prevention of renal injury as a result of the neuroprotective effect of two interventions (erythropoietin and carbamylated erythropoietin) given in the presence of brain death makes quantification of the extent of AKI in patients with brain injury even more relevant.

Implications of study findings

AKI can worsen cerebral edema,[19] and is associated with a greater risk for death.[6] Severe cases require costly treatments and can result in long-term damaging effects on the kidneys.[20] Accordingly, it is desirable to prevent development of AKI. The observation that older patients are at a greater risk of its development (Table 1) suggests the need to be particularly focused on the resuscitation efforts of older patients with TBI. The high mortality seen in these patients allows appreciation of the fact that the presence of AKI in these patients is also an additional predictor of poor outcome.

The association of AKI with a lower GCS (Table 1) may reflect the severity of trauma and its impact on the kidneys. However, AKI can also directly contribute to worsen cerebral edema. The fact that the injury severity score (an indication of the severity of trauma) is not associated with AKI (Tables 1 and 2) suggests that the association of AKI with GCS may be direct and/or due to its predictive ability of poor outcome.

Notably, patients without AKI tended to have a significant improvement in eGFR of approximately 20% (Table 1). This effect may likely follow fluid resuscitation and possible related changes in GFR induced by it. However, it may also represent a dilutional effect on SCr induced by fluid administration (pseudo-increase in GFR) or both dilution and related GFR changes. These dynamic effects highlight the difficulties associated with evaluating renal function in trauma patients.

Strengths and limitations

The strength of this study is that it uses a sufficiently large cohort with prospectively collected data and electronically collected and stored laboratory data and applies such data to the estimation of the incidence of AKI using modern and validated criteria (RIFLE). As the sample is young (median age 33) which characterizes TBI patients[21] and given that TBI patients tend to have reasonable pre-morbid health, in the absence of baseline information, we sought a normative baseline eGFR (males 85, females 83) in accordance with a healthy young population.[13] Furthermore, we used percent decline in eGFR from 'normative' baseline to worst eGFR in the first 10 days, in preference to estimating baseline SCr given recent doubts surrounding this method.[22] This methodology has to be taken into

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account when interpreting the findings. However, despite concerns that the use of eGFR is not accurate when patients are not in steady state, it has been used in this context and still predicts association with known risk factors and outcomes.[10,23–25]

The cohort is from two hospitals in the state of Victoria, Australia, and may not apply to other jurisdictions. However, the Victorian trauma system has many similarities with other trauma systems elsewhere in the developed world.

Between hospitals there was a 16 μmol/L difference in mean/median SCr. While there were some differences in characteristics of hospital samples, this is probably largely because of differences in SCr measurement methodology. This likely laboratory effect was adjusted for in multivariable linear regression models.

The pattern of SCr levels was typically elevated in the first available SCr level followed by a dip (Figure 1). The early peak may have been due to muscular injury caused by trauma or decreased perfusion pressure causing an increase in muscle breakdown and higher SCr.

Although the data were collected prospectively as part of routine data collection during 2008, this is a retrospective analysis and therefore control over quality of the data is limited. Where duplicate data were available from two or more sources, checks revealed high reliability of the data.

Future research

The presence of AKI in 10% of patients and its association with increased mortality allow the identification of whether pharmacologic interventions which appear to be beneficial in neurotrauma can simultaneously protect brain and kidney and decrease the incidence of AKI. Erythropoietin and carbamylated erythropoietin are two such treatments which have been shown to protect the brain and kidney in brain dead rats because of inhibition of the inflammatory processes caused by brain injury.[18] Their potential for a similar dual effect in patients with TBI is worthy of investigation. Such studies may provide useful insights into AKI in this setting and be of relevance to other settings.

CONCLUSIONS

AKI is relatively common even in patients with TBI and identifies patients with a high risk of death. Its association with age, hypotension, and APACHE III scores helps identify patients at higher risk of AKI which may be targets of future trials of drugs that are simultaneously neuroprotective and nephroprotective.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Wahlstrom MR, Olivecrona M, Nystrom F, Koskinen LO, Naredi S. Fluid therapy and the use of albumin in the treatment of severe traumatic brain injury. *Acta Anaesthesiol Scand*. 2009;53:18–25.

[2] Zygun D, Kortbeek J, Fick G, Laupland KB, Doig CJ. Non-neurologic organ dysfunction in severe traumatic brain injury. *Crit Care Med*. 2005;33:654–660.

[3] Zygun DA, Doig CJ, Gupta AK, et al. Non-neurological organ dysfunction in neurocritical care. *J Crit Care*. 2003;18:238–244.

[4] Cooperative Antimicrobial Therapy of Septic Shock Database Research Group, Bagshaw SM, Lapinsky S, et al. Acute kidney injury in septic shock: Clinical outcomes and impact of duration of hypotension prior to initiation of antimicrobial therapy. *Intensive Care Med*. 2009;35:871–881.

[5] Dasta JF, Kane-Gill SL, Durtschi AJ, Pathak DS, Kellum JA. Costs and outcomes of acute kidney injury (AKI) following cardiac surgery. *Nephrol Dial Transplant*. 2008;23:1970–1974.

[6] Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. *Kidney Int*. 2008;73:538–546.

[7] Bagshaw SM, George C, Dinu I, Bellomo R. A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. *Nephrol Dial Transplant*. 2008;23:1203–1210.

[8] Ricci Z, Ronco C. Today's approach to the critically ill patient with acute kidney injury. *Blood Purif*. 2009;27:127–134.

[9] Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P, Acute Dialysis Quality Initiative Workgroup. Acute renal failure – definition, outcome measures, animal models, fluid therapy and information technology needs: The second international consensus conference of the acute dialysis quality initiative (ADQI) group. *Crit Care*. 2004;8:R204–R212.

[10] Bagshaw SM, George C, Gibney RT, Bellomo R. A multi-center evaluation of early acute kidney injury in critically ill trauma patients. *Ren Fail*. 2008;30:581–589.

[11] Kuitunen A, Vento A, Suojaranta-Ylinen R, Pettilä V. Acute renal failure after cardiac surgery: Evaluation of the RIFLE classification. *Ann Thorac Surg*. 2006;81:542–546.

[12] Ostermann M, Chang RWS. Acute kidney injury in the intensive care unit according to RIFLE. *Crit Care Med*. 2007;35:1837–1843.

[13] Wetzels JF, Kiemeney LA, Swinkels DW, Willems HL, den Heijer M. Age- and gender-specific reference values of estimated GFR in Caucasians: The Nijmegen Biomedical Study. *Kidney Int*. 2007;72:632–637.

[14] Mathew TH, Johnson DW, Jones GRD. On behalf of the Australasian Creatinine Consensus Working Group. Chronic kidney disease and automatic reporting of estimated glomerular filtration rate: Revised recommendations. *Med J Aust*. 2007;187:459–463.

[15] Guitard J, Cointault O, Kamar N, et al. Acute renal failure following liver transplantation with induction therapy. *Clin Nephrol*. 2006;65:103–112.

[16] Heringlake M, Knappe M, Vargas Hein O, et al. Renal dysfunction according to the ADQI-RIFLE system and clinical practice patterns after cardiac surgery in Germany. *Minerva Anestesiol*. 2006;72:645–654.

[17] Uchino S, Bellomo R, Goldsmith D, Bates S, Ronco C. An assessment of the RIFLE criteria for acute renal failure in hospitalized patients. *Crit Care Med*. 2006;34:1913–1917.

[18] Nijboer WN, Ottens PJ, van Dijk A, van Goor H, Ploeg RJ, Leuvenink HG. Donor pretreatment with carbamylated

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erythropoietin in a brain death model reduces inflammation more effectively than erythropoietin while preserving renal function. *Crit Care Med*. 2010;38:1155–1161.

[19] Davenport A. Renal replacement therapy in the patient with acute brain injury. *Am J Kidney Dis*. 2001;37:457–466.

[20] Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol*. 2005;16: 3365–3370.

[21] Berry C, Ley EJ, Tillou A, Cryer G, Margulies DR, Salim A. The effect of gender on patients with moderate to severe head injuries. *J Trauma*. 2009;67:950–953.

[22] Bagshaw SM, Uchino S, Cruz D, et al. A comparison of observed versus estimated baseline creatinine for determination of RIFLE class in patients with acute kidney injury. *Nephrol Dial Transplant*. 2009;24:2739–2744.

[23] Cooper WA, O'Brien SM, Thourani VH, et al. Impact of renal dysfunction on outcomes of coronary artery bypass surgery – results from the society of thoracic surgeons national adult cardiac database. *Circulation*. 2006;113:1063–1070.

[24] Hillis GS, Croal BL, Buchan KG, et al. Renal function and outcome from coronary artery bypass grafting – impact on mortality after a 2.3-year follow-up. *Circulation*. 2006;113: 1056–1062.

[25] Bagshaw SM, Uchino S, Bellomo R, et al. Timing of renal replacement therapy and clinical outcomes in critically ill patients with severe acute kidney injury. *J Crit Care*. 2009;24:129–140.

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1.5 Outline of Thesis

The Introduction to this thesis (Chapter 1) has provided a comprehensive review of AKI (the focal point of the project) and an overview of the traumatic brain injury patient group that is under investigation. The preliminary study assessing the incidence of AKI in patients with TBI which was performed to confirm our belief that the conduction of the Renal substudies of the EPO-TBI and POLAR trials was warranted, is also included in this section. In the following Literature Review (Chapter 2) the therapies under investigation; erythropoietin and hypothermia, are reviewed as are the novel biomarkers we have investigated in the Renal substudies of the EPO-TBI and POLAR trials.

The Renal substudies and associated methods are described in Chapter 3, and in Chapter 4 the study results are provided. Finally, thesis findings and their significance are discussed, and conclusions and future research directions are outlined in Chapter 5.

2 Literature Review

2.1 Overview of Literature Review

Erythropoietin and early and sustained hypothermia are the two potential treatments for AKI investigated in the Renal substudies of the EPO-TBI and POLAR trials, on which this thesis is based. Several novel renal biomarkers for AKI are also assessed in the Renal substudies. As key elements in the investigation, separate sections of the literature review are therefore dedicated to each of the potential treatments for AKI, and to the novel biomarkers as a group.

2.2 Erythropoietin: properties and potential as a kidney protective agent

Erythropoietin (EPO) is well-known for its erythropoietic effects; however the more recently discovered pleiotropic effects of EPO have highlighted its tissue-protective potential for the kidneys. The candidate and colleagues synthesise the literature on this relatively new area of research in the following comprehensive published review; examining the properties and mechanisms of action of EPO, and the plausibility of, and evidence surrounding its prophylactic use for brain and kidney injury.

Moore EM, Bellomo R, Nichol AD. Erythropoietin (EPO) as a novel brain and kidney protective agent. Anaesthesia and Intensive Care. 2011 May;39(3):356-72

Declaration for Thesis Chapter

Moore EM, Bellomo R, Nichol AD. Erythropoietin as a novel brain and kidney protective agent. Anaesth. Intensive Care;39(3):356-72.

Declaration by candidate

In the case of Chapter , the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Searched and reviewed the literature, collated and interpreted the information, wrote up drafts and submitted the review manuscript, was responsible for the response to reviewers.	85 %

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
R Bellomo	Involved in interpretation of the literature, reviewed and drafted manuscripts	N/A
A Nichol	Involved in interpretation of the literature, reviewed and drafted manuscripts	N/A

Candidate's Signature		**Date** 30/4/2012

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s)	**Australian and New Zealand Intensive Care Research Centre, School of Public Health and Preventive Medicine, Monash University, Alfred campus**

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

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Anaesth Intensive Care 2011; **39:** 356-372

Erythropoietin as a novel brain and kidney protective agent

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SUMMARY

Erythropoietin is a 30.4 kDa glycoprotein produced by the kidney, which is mostly known for its physiological function in regulating red blood cell production in the bone marrow. Accumulating evidence, however, suggests that erythropoietin has additional organ protective effects, which may specifically be useful in protecting the brain and kidneys from injury. Experimental evidence suggests that these protective mechanisms are multi-factorial in nature and may include inhibition of apoptotic cell death, stimulation of cellular regeneration, inhibition of deleterious pathways and promotion of recovery.

In this article we review the physiology of erythropoietin, assess previous work that supports the role of erythropoietin as a general tissue protective agent and explain the mechanisms by which it may achieve this tissue protective effect. We then focus on specific laboratory and clinical data that suggest that erythropoietin has a strong brain protective and kidney protective effect. In addition, we comment on the implications of these studies for clinicians at the bedside and for researchers designing controlled trials to further elucidate the true clinical utility of erythropoietin as a neuroprotective and nephroprotective agent.

Finally, we describe EPO-TBI, a double-blinded multi-centre randomised controlled trial involving the authors that is being conducted to investigate the organ protective effects of erythropoietin on the brain, and also assesses its effect on the kidneys.

Key Words: erythropoietin, tissue protective, neuroprotective, nephroprotective

Erythropoietin (EPO) is a complex molecule, first isolated in 1977[1] and now well-known for its physiological role in the regulation of red blood cell production in the bone marrow. Recombinant human EPO, first cloned in 1985[2], is now commercially available and widely used in the treatment of anaemia. In recent years, additional tissue/organ protective properties of EPO have become apparent, particularly for the brain and kidneys. There has been considerable study of the mechanisms and pathways involved in the tissue protective effects of EPO given the substantial therapeutic potential it presents. However, further investigation of these effects is required. This is in part due to the current incomplete understanding of its mechanisms of action, potential side-effects, and uncertainty regarding its non-erythropoietic clinical indications. These concerns have been highlighted by a number of adverse events reported in recent clinical trials (i.e. increased rates of thromboembolic events in some studies).

In this review, we assess the evidence supporting EPO as a general tissue protective drug and discuss the potential mechanisms by which it may achieve this general effect. We then focus on EPO's neurological and renal protective effects and the potential mechanisms through which it may confer this specific protection. Finally, we review the experimental studies and clinical trials of EPO in neurological and acute kidney injury (AKI), discuss risks, lessons learned and the need for further multi-centre randomised studies in humans before any change in clinical practice is considered.

PHYSIOLOGICAL PROPERTIES OF ERYTHROPOIETIN

Structure of the erythropoietin molecule

EPO is a 30.4 kD glycoprotein and class I cytokine consisting of 165 amino acids[3]. EPO has four acidic oligosaccharide side chains (3 N-linked and 1 O-linked) and contains up to 14 sialic acid residues (Table 1[3-8], see recombinant human EPO); its carbohydrate portion contributes 40% of its molecular weight[3]. The N-linked polysaccharide side

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chains appear to be important for the biosynthesis and secretion of EPO. They are thought to enhance stability in the blood and to limit hepatic clearance, thus facilitating the systemic transit of EPO from the kidney (main production site of EPO) to target cells in the bone marrow[9].

The polypeptide skeleton of the human EPO molecule has a constant amino acid sequence; however, the carbohydrate side-chains are heterogenic in sugar content and structure. The variable nature of the sialic acid content gives rise to EPO isoforms with differences in charge. As the number of sialic acid groups on the carbohydrate portion of EPO increase, so does its serum half-life (Table 1), while receptor-binding capacity decreases[10-13]. It is important, however, to note that clearance appears to have a stronger influence on in vivo activity than receptor-binding affinity.

Each EPO molecule has two EPO receptor (EPOR) binding sites, located on opposite faces of the molecule. There are two affinities of the EPOR for EPO in solution; one of high (~1 nM) and one of low affinity (~1 microM), perhaps reflecting two non-equivalent receptor binding sites on each EPO molecule[14].

Physiological stimuli for erythropoietin production/release

Approximately 90% of systemic EPO in adults is produced by peritubular interstitial fibroblasts in the renal cortex and outer medulla of the kidney. Most of the remaining production comes from hepatocytes in the liver, with some expression in the brain, spleen, lungs, testes and bone marrow[15]. A feedback mechanism involving oxygen delivery to the tissues appears to regulate EPO production[16]. Hypoxia-inducible factor regulates transcription of the EPO gene in the kidney and liver which determines EPO synthesis. This process is dependent on local oxygen tension. Hypoxia-inducible factor is continually transcribed at the messenger riboneucleic acid level but, at the protein level, it is only stable in hypoxic cells. Hypoxia-inducible factor is quickly destroyed by well-oxygenated cells through ubiquitylation (tagging for degradation in the proteasome) by the von Hippel-Landau tumour suppressor protein. However when oxygen delivery decreases, the von Hippel-Landau tumour suppressor protein ceases its proteolysis of hypoxia-inducible factor, increasing the levels of hypoxia-inducible factor which subsequently increases EPO production[15,17].

Structure of erythropoietin receptors

The EPOR is a 66kD membrane glycoprotein typically consisting of 484-amino acids and two peptide chains. It belongs to a large cytokine and growth factor receptor family, which includes IL-3, -4, -6 receptors, granulocyte macrophage colony stimulating factor receptor and the growth hormone receptor, and has some common signalling mechanisms[3]. The EPOR has a single transmembrane domain, an extracellular domain composed of two parts and an intracellular domain[3]. Binding studies have demonstrated that the EPOR has different affinities for EPO with perhaps an accessory component of the EPOR that increases the binding

TABLE 1
Erythropoeitin analogues[3-8]

	rHuEPO	Darbepoietin	AsialoEPO	Carbamylated erythropoietin
Molecular weight, kDa	30.4	38	30-34	40
N-linked oligosaccharide chains	3	5	0	Carbamylation of lysines in EPO → homocitrulline residues
Sialic acid residues	10-14	≤22	<0.02 sialic acids/molecule	Carbamylation of lysines in EPO → homocitrulline residues
IV half life, h	5	25.3	0.023	3.3
SC half life, h	25	48.8	2.5	3-6 (rats – same as EPO)
Clearance, ml/h/kg	4-15 (↓ with dose)	1.6±0.3	rapid	–
Erythropoietic activity	Yes	Yes	No	No
Use	Anaemia Rx-CKD	Anaemia Rx-CKD	Potential: neuro injury	Potential: neuro-, cardio-, renoprotective

AsialoEPO[7,8]: Desialylation →high clearance. Half life is too short for erythropoiesis as continuous EPOR stimulation is required. Has same or increased affinity for EPOR. Neuroprotection appears to be retained.

Carbamylated erythropoietin[4-6]: No interaction with classical EPOR. Less affinity for EPOR but retains cytoprotection. May interact with alternative receptor e.g. heterodimer with only 1 EPOR. Colony-stimulating factor levels comparable to EPO.

rHuEPO=recombinant human erythropoietin, asialoEPO=asialo erythropoietin, EPO=erythropoietin, →=leads to, IV=intravenous, SC=subcutaneous, ↓=reduced, Rx=treatment, CKD=chronic kidney disease, EPOR=receptor.

affinity of EPO. Alternatively this may be due to differences in the two distinct EPO molecule receptor binding sites; however, this is yet to be ascertained[14]. It is thought that EPOR isoforms with higher affinity for EPO binding may be responsible for the erythropoietic effects of EPO, while isoforms with a lower affinity for EPO binding may have non-erythropoietic effects such as tissue protection[18].

The cytoplasmic domains of the EPOR contain a number of phosphotyrosines that are phosphorylated by the activation of a member of the Janus-type protein tyrosine kinase family (JAK2), which is bound to the common beta subunit of the EPOR[19]. In addition to activating the mitogen-activated protein kinase, phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt) pathway (Figure 114), phosphotyrosines also serve as docking sites for signal transducer and activators of transcription (STAT) such as STAT5[14]. These pathways are further described in "Post-receptor (intracellular) effects of erythropoietin" below. Dephosphorylation of JAK can be induced by phosphatase with the consequent internalisation and degradation of the EPO/EPOR complex which marks the end of EPO activity. This prevents over-activation that may lead to excessive erythrocytosis[20].

The erythropoiesis stimulating effects of erythropoietin

The principal physiological function of EPO is red blood cell production, which results from a tightly controlled proliferation and differentiation pathway[21]. Early haematopoietic progenitor cells differentiate into burst-forming unit-erythroid cells on which EPORs first appear. However, EPO is not required at this stage. Burst-forming unit-erythroid cells differentiate into colony-forming unit-erythroid cells, dependent on EPO for survival, with a corresponding increase in EPOR expression[21]. Continuous stimulation with EPO triggers the differentiation of colony-forming unit-erythroid cells into erythroblasts, which lose their nuclei to form reticulocytes. After a few days reticulocytes lose reticulin and become erythrocytes (red blood cells). Reticulocytes and erythrocytes stop expressing EPOR and cease being responsive to EPO[21]. The steady-state lag time between effect-site EPO stimulation and reticulocyte appearance in the human systemic circulation was estimated at 10.8 hours[22] and the steady-state reticulocyte residence time reported in humans is one day, which increases two- to three-fold during stress erythropoiesis[23].

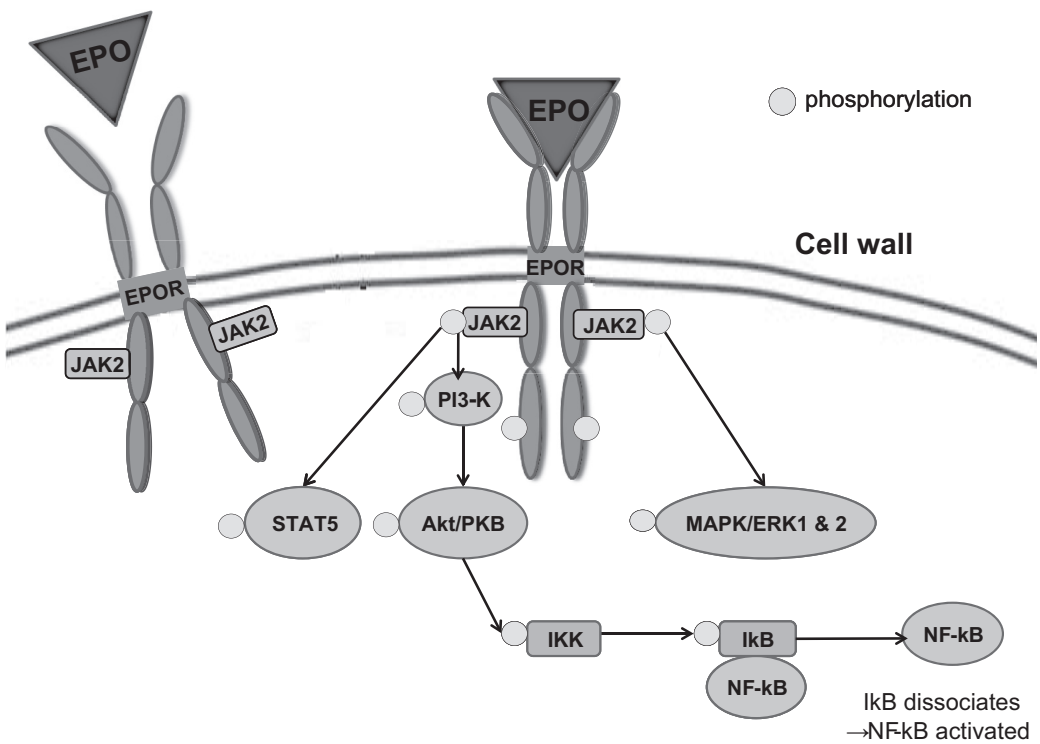

Figure 1: The main pathways of erythropoietin (EPO). The intracellular domain of the erythropoietin receptor contains phosphotyrosines that are phosphorylated by activation of a member of the Janus-type protein tyrosine kinase family (JAK2), which is bound to the beta subunit of the EPO receptor. These phosphotyrosines then serve as docking sites for Signal transducer and activator of transcription 5 (STAT5) and activate the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt) pathway[14]. Akt stimulates IkappaB kinase (IKK) to phosphorylate which phosphorylates the inhibitory IkB protein that then dissociates from nuclear factor-kappaB (NF-kB) resulting in its activation.

EPO-binding to EPORs on erythroid progenitor cells leads to activation of the JAK2-STAT5 signalling pathway and phosphorylation of PI3K and Akt[24] (Figure 1). Akt-mediated phosphorylation of Bad (a pro-apoptotic member of the B-cell lymphoma 2 protein family) in the Bad-B-cell lymphoma-extra large (Bcl-x_L) complex releases the antiapoptotic protein Bcl-x_L (B-cell lymphoma-extra large), which suppresses erythroid progenitor cell apoptosis[25]. Akt is also involved in several pathways that promote cell survival and anti-apoptotic effects through inhibition of forkhead transcription factor (FOXO3a), inactivation of glycogen synthase kinase 3β (GSK3β), induction of x-chromosome-linked inhibitor of apoptotic protein (XIAP), inactivation of caspases, and prevention of cytochrome C release (Figure 2[19,26,27]). These effects not only enhance the erythropoietic properties of EPO but appear to be important in the protection of other cell types as well and may contribute to the reported neuronal and renal protective effects[24].

PLEIOTROPIC EFFECTS OF ERYTHROPOIETIN

The tissue protective effects of EPO may be elicited through the EPOR homodimer via JAK2-STAT5 activation and mediation of apoptosis. However, the interaction and contribution of the various downstream cellular signalling pathways may differ for each different cell type. Alternatively or additionally, tissue protection may be mediated by a second EPOR isoform; a heterodimer composed

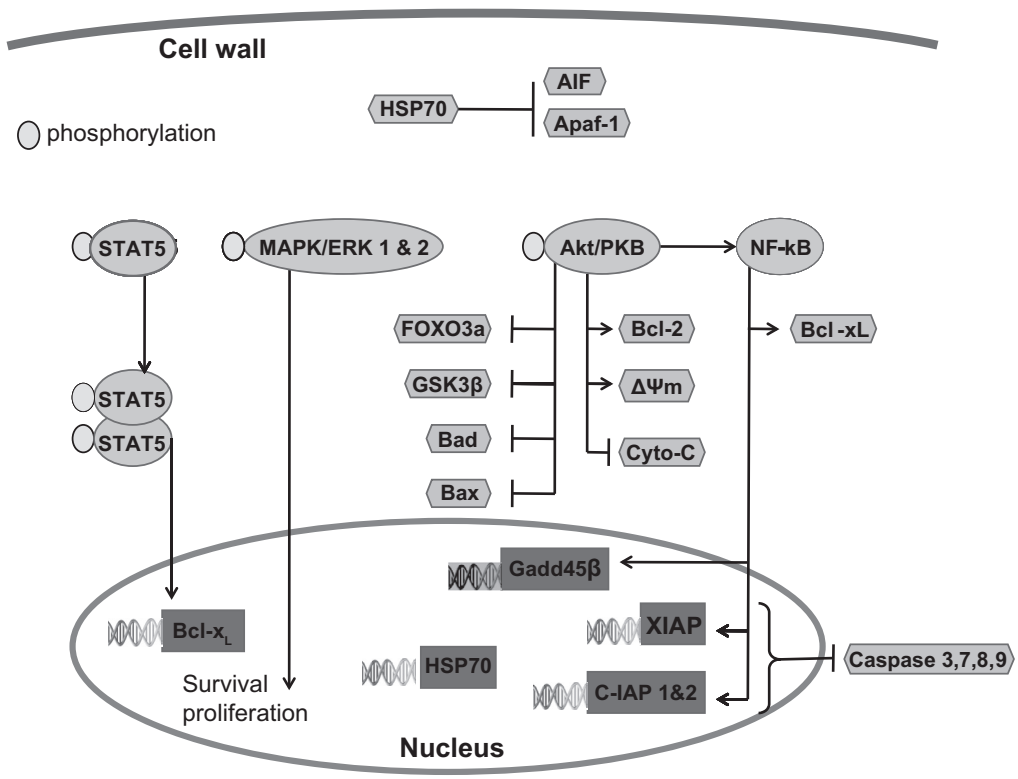

FIGURE 2: Apoptotic pathways influenced by erythropoietin that may confer tissue protection. Activated and dimerised signal transducer and activators of transcription 5 (STAT5) moves to the nucleus and promotes transcription of pro-mitogenic and anti-apoptotic genes, including antiapoptotic protein B-cell lymphoma-extra large (Bcl-xL)[19,27]. Erythropoietin can phosphorylate extracellular signal-related kinases (ERK), one of three groups of mitogen-activated protein kinase (MAPK). The ERK pathway (ERK 1 and 2) is associated with apoptotic regulation and can be cytoprotective[26].
Akt promotes cell survival and anti-apoptotic effects by 1) inhibiting forkhead transcription factor (FOXO3a), a trigger for apoptosis; 2) inactivating glycogen synthase kinase 3β (GSK3β), thus preventing cell injury; 3) reducing the activity of pro-apoptotic proteins Bad and B-cell lymphoma 2-associated X protein (Bax); 4) increasing the activity of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2); 5) maintaining mitochondrial membrane potential, thus preventing cytochrome C (Cyto-C) release.
Nuclear factor-kappaB (NF-kB) prevents apoptosis by 1) inducing expression of the inhibitors of apoptotic protein (IAP) C-IAP 1 and 2, and the x-chromosome-linked IAP (XIAP), which can inhibit active caspase 3, 7 and 9; and inhibit caspase 8 activation (C-IAP 1 and 2); 2) promoting induction of Growth arrest and DNA damage protein (Gadd45β) by TNF-α, which is associated with cell cycle and DNA repair, and suppression of apoptosis; 3) by activating Bcl-xL.
EPO induces heat shock protein 70 (HSP70) which inhibits apoptosis inducing factor (AIF) moving into the nucleus, thus avoiding DNA fragmentation and apoptosis. HSP70 also prevents apoptotic protease-activating factor-1 (Apaf-1)/cytochrome C (Cyto-C) binding; a complex involved in pro-apoptotic caspase activation. PKB=protein kinase B.

of an EPOR monomer and the cytokine receptor, common beta subunit. This signal-transducing subunit is also common to the granulocyte-macrophage colony stimulating factor and the IL-3 and IL-5 receptors[28,29]. Carbamylated EPO (CEPO) does not bind to the classical EPOR isoform, and is devoid of classic haematopoietic activity[5,30] (Table 1), yet has been shown to provide tissue protection in the brain, heart[31] and kidney[30], supporting the existence of a heteroreceptor EPO isoform, which mediates tissue protection.

Carbamylated erythropoietin and tissue protection

The administration of CEPO, provides renal tissue-protective effects. In an ischaemia-reperfusion rat model, CEPO markedly reduced apoptosis and increased tubular epithelial cell proliferation. Moreover, CEPO was more protective against ischaemia-reperfusion injury to tubular epithelial cells than EPO in this study[4]. In an in vitro model developed by the same team, CEPO promoted more capillary formation than EPO and also appeared to protect the kidneys from ischaemia-reperfusion injury by promotion of angiogenesis[32]. This protective effect requires mitogenesis, and endothelial progenitor cell differentiation, proliferation and migration. The anti-apoptotic, cell proliferative, angiogenesis-promoting properties of CEPO may indicate involvement of the following signalling pathways: JAK2-STAT5, PI3K/Akt and downstream anti-apoptotic pathways, and mitogen-activated protein kinase. Whether different conformations of the EPOR activate different intracellular pathways or whether the single EPOR chain (in the case of CEPO) dimerises with other membrane proteins to provide the protective effect is currently not known[18]. Different classes of EPOR with respect to affinity for EPO-binding have also been described: high affinity EPOR appear to mediate the haematopoietic effects and low affinity receptors may be principally involved in tissue protection by EPO[18].

It is clear that the relationship of EPO with its receptor is extremely complex. Therefore further investigation is required to fully understand the EPOR heterodimer isoform, and the mechanisms and pathways involved in its tissue protective activity. These findings could inform the future development of tissue-protective cytokines.

Post-receptor (intracellular) effects of erythropoietin

There are a number of common pathways through which EPO exerts its erythropoietic effects that also appear to confer tissue protection. As mentioned, it is uncertain whether the pathways for tissue-protection are activated by EPOR homodimers or heterodimers, both, or another receptor conformation[18]. EPO 'classically' binds to two EPORs which become joined as a homodimer and change. This activates JAK2, which is bound to the common beta subunit of the EPOR[19], and leads to phosphorylation of tyrosine residues of the EPOR, which activates a number of signalling pathways discussed below (Figures 1 and 2).

Signal transducer and activators of transcription 5

EPO classically signals through the STAT5 pathway. The STAT proteins are direct substrates of Janus kinases (JAK); activation of JAK results in tyrosine phosphorylation of the STATs (Figures 1 and 2). Activation of JAK2 also leads to phosphorylation of the PI3K and subsequent phosphorylation of Akt (also known as protein kinase B) (Figure 1).

Akt

Akt (also called protein kinase B) is important because it is the principal component in a variety of pathways that promote cell survival and anti-apoptotic effects (Figure 2). It is involved in inactivating caspases which mediate apoptosis, mitochondrial dysfunction and subsequent release of cytochrome C[27], leading to cell injury and genomic DNA degradation. Cytochrome C binds to apoptotic protease-activating factor-1 which activates pro-apoptotic caspase pathways and results in cell injury and death[27]. Akt reduces caspase activation by increasing the activity of B-cell lymphoma 2 and Bcl-x_L which, in turn, prevent B-cell lymphoma 2-associated X protein translocation to the mitochondria, maintain mitochondrial membrane potential and prevent cytochrome C release from the mitochondria[19]. EPO's ability to maintain cellular integrity and prevent inflammatory apoptosis is closely linked to maintenance of mitochondrial membrane potential; modulation of apoptotic protease-activating factor-1; inhibition of cytochrome C release and inhibition of caspase 1, 3, 8 and 9 activation.

Recent data also indicate that PI3K-regulated serum and glucocorticoid-regulated kinase-1 may contribute to the mediation of EPO's anti-apoptotic effects and possibly renal protective effects[33].

Mitogen-activated protein kinase

The phosphorylation of mitogen-activated protein kinases appears to also contribute to the cell protection which EPO confers (Figure 2).

Protein kinase C

Protein kinase C is also involved in the signal transduction pathways of EPO and inhibition of apoptosis and cell survival. It regulates the EPO-

induced erythroid proliferation and differentiation[34]. Indeed, inhibition of protein kinase C activity interferes with phosphorylation of the EPOR which suggests that protein kinase C may be an upstream modulator of the EPOR[27].

Calcium

EPO may be involved in modulation of cellular calcium homeostasis given that classical protein kinase C isoenzymes depend on calcium for activity[35]. EPO can elicit a prompt rise in intracellular free calcium in bone marrow, stimulate calcium channel activity and enhance intracellular calcium concentration of neuronal cells[35-37]. This suggests that EPO influences calcium homeostasis by increasing calcium influx[38]. A quick, transient increase in calcium by EPO may be required for cytoprotection during excitotoxicity, since a reduction of intracellular calcium can cancel the protective effect of EPO during glutamate administration[39]. EPO may enhance the function and viability of neurons through a calcium mechanism[37,40].

Nuclear factor-kappaB

Nuclear factor-kappaB (NF-kB), a mediator of inflammatory and cytokine response is also implicated in EPO signalling. The expression and cytoprotection of EPO partly depend on Akt and subsequent NF-kB activation (Figures 1 and 2). NF-kB plays a role in the release of EPO during hypoxia-inducible factor-1 induction; Akt can increase NF-kB and hypoxia-inducible factor-1 activation with resultant increase in EPO expression[41].

Heat shock protein 70

Induction of heat shock protein 70 by EPO is related to renal protection in ischaemic kidneys[42]. Heat shock protein 70 prevents apoptosis a) by inhibiting movement of apoptosis inducing factor to the nucleus[43] and b) by preventing apoptotic protease-activating factor-1/cytochrome C binding in the cytosol[44] (Figure 2).

Potential new tissue protective mechanisms

There are other mechanisms by which EPO may confer cytoprotection. It has recently been demonstrated that local nitric oxide bioavailability increases when EPO activates endothelial nitric oxide synthase and this effect on the endothelium may be critical for the renal tissue protective effects of EPO. EPO is an extremely potent stimulator of endothelial progenitor cells, whose function is partly dependent on nitric oxide bioavailability. Endothelial progenitor cells appear to be involved in endothelial recovery after injury and the formation of new blood vessels in ischaemic areas[18]. In the kidney, AKI triggers apoptosis, inducing an inflammatory response. EPO limits these negative effects in part by stimulating vascular repair and by mobilising endothelial progenitor cells and increasing tubular cell proliferation[28]. These findings suggest that EPO may exert protective effect via an interaction with the microvasculature.

Angiogenesis and EPO's renal protective effects may also be influenced by vascular endothelial growth factor (VEGF). In a number of studies, the presence of VEGF appears to be associated with tissue protection[45-47]. EPO was the first described hypoxia-inducible factor target gene but many more have been discovered including VEGF which has additional potential to provide renal protection[28]. Nakano and colleagues found that the vascular EPO/EPOR system promoted post-ischaemic angiogenesis by upregulating the VEGF/VEGF receptor system, both directly by promoting neo-vascularisation and indirectly by mobilising endothelial progenitor cells and bone marrow-derived proangiogenic cells[48]. It appears that angiogenesis is impaired and blood vessels are less responsive to VEGF in the absence of EPOR.

In animal studies, preconditioning of the hypoxia-inducible factor system using short episodes of ischaemia has been found to protect the kidney from ischaemic and toxic injury. Hypoxia-inducible factor is capable of inducing a range of protective gene products including glucose transporters and heme oxygenase-1 with renal protective potential. Comparison of the protective capacities of EPO and hypoxia-inducible factor activation in the kidneys may be worthwhile[28]. Moreover, it is possible that some effects of EPO such as the promotion of angiogenesis may be responsible for some of the benefit provided by ischaemic preconditioning. The tissue protective or 'pleiotropic' effects of EPO beyond erythropoiesis have been shown in the brain, heart and kidney in many animal and some clinical studies.

Neurological protection

Traumatic brain injury and the potential protective effect of erythropoietin

Brain injury, particularly as a result of trauma, is a leading cause of mortality and long-term disability, particularly affecting young people. It is associated with significant human and financial costs. Complementary to measures to prevent injury, treatment can be directed at decreasing morbidity after primary injury[49]. Extensive research shows that, after brain trauma (primary injury), several

biochemical pathways are activated, leading to secondary brain injury[50]. These pathways include inflammation, oxidative stress, increased vascular permeability and excitotoxic mechanisms (release of chemicals toxic to brain cells)[50]. Secondary injury greatly increases brain cell death, stroke size and overall brain injury[51]. Despite intense investigation, no specific treatment has conclusively been shown to attenuate secondary brain injury, and current management of traumatic brain injury (TBI) is supportive, seeking to prevent or rapidly treat complications which worsen secondary injury[52,53]. It is therefore a priority to investigate promising therapies for traumatic or acute brain injury and EPO is one such intervention.

Mechanisms

Human neurons, astrocytes and microglial cells produce EPO and express EPOR[54]. The increase in production and secretion of EPO and enhanced expression of the EPOR in the human central nervous system in response to oxygen deficiency suggests a possible physiological role for EPO to attenuate secondary brain injury[27]. The receptor complex mediating the neuroprotective effects of EPO may be associated with the common beta receptor subunit, CD131. Increasing evidence suggests that EPOR activation after EPO binding inhibits neuronal apoptosis[55,56]. EPO-EPOR binding may induce JAK2 activation with phosphorylation of the inhibitor of NF-kB, leading to transcription of neuro-protective genes[57] (Figure 2). In addition, EPO seems to prevent neurological apoptosis through an Akt-dependent mechanism[58]. Amongst a number of cell-survival promotion pathways, Akt blocks cell apoptosis by inhibiting glycogen synthase kinase 3β[59] (Figure 2). Furthermore, an in vitro study indicated that EPO attenuated glutamate excitotoxicity when given after its induction, and promoted nerve terminal sprouting in motor neurons by direct effect involving the JAK2 pathway[60].

EPO's neuroprotective mechanisms could also include activation of calcium channels to protect from glutamate toxicity, antioxidant enzyme production and neo-angiogenesis that improves blood flow and oxygenation in border zones of ischaemic areas[61]. EPO also blocks free radicals, normalises cerebral blood flow, affects neuro-transmitter release (preventing excitotoxicity) and has anti-inflammatory effects, thus playing a crucial role in neuroprotection of the central and peripheral nervous system[62]. It is therefore clear that EPO modulates a number of key pathways which could reduce neural injury and provide protective effects.

Animal studies

In animal models, EPO has been shown to decrease cerebral infarct volume, oedema and neuronal apoptosis, while neuronal survival and cerebral function increased[63-65]. Following experimental subarachnoid haemorrhage, EPO was shown to reduce neuronal death and vasoconstriction and improve functional recovery and cerebral blood flow autoregulation[63-65]. EPO's potential to prevent cortical injury has been investigated in spinal cord models, the ocular system and the peripheral nervous system in animals with promising results[66-70]. Importantly, administration of EPO up to 24 hours after a traumatic brain injury has been shown to be protective and to improve functional neurological outcomes in rats[71]. These results suggest that EPO not only attenuates gross anatomical injury but that it may also improve functional outcomes when administered many hours after the primary insult in animal models of brain injury.

Clinical trials

In humans, EPO dramatically improved functional neurological outcomes in a randomised controlled trial when administered to patients with subarachnoid haemorrhage[72]. Furthermore, in a double-blind proof-of-concept study, high dose EPO improved clinical outcome of patients at one month after acute ischaemic stroke and displayed a trend for reduction in infarct size. Moreover, EPO appeared to reduce neurocognitive dysfunction at two months after coronary artery bypass graft surgery and was considered safe to use in a double-blind Canadian randomised control trial[73]. In chronic neurological disease EPO may also provide benefit. High-dose EPO appeared to improve cognitive performance in a small study of schizophrenic men with cognitive deficit, compared to placebo-treated patients[74]. A small open label exploratory study of high-dose EPO for patients with chronic progressive multiple sclerosis also suggested potential benefit[75]. EPO has the potential to provide benefit for neurological dysfunction in numerous settings.

A large multicentre intensive care unit (ICU) randomised controlled trial[53] found that EPO significantly decreased 29-day mortality compared to placebo (3.5% vs 6.6%) in a pre-planned subgroup analysis of trauma patients (many with traumatic brain injury). This occurred despite a lack of effect on transfusion requirement. However, an increase in thrombotic events was noted. In a recent German double-blind randomised controlled trial of EPO in acute ischaemic stroke, systemic thrombolysis (rtPA) was concurrently administered with EPO in

TABLE 2

Animal studies of erythropoietin in acute kidney injury relevant to non-haematopoietic functions: ischaemia-reperfusion models

Year, reference	Type	AKI model/follow-up	EPO protocol	Outcome
2009[98]	Dogs	IR: nephrectomy, 2 weeks recovery, then renal artery occlusion, ischaemia 1 h; reperfusion 28 days	EPO 500 U/kg IV pre-ischaemia ±90 min abdominal insufflation	↓microalbuminuria, ↑renal function recovery at 4 weeks; IV EPO better than mannitol for renal IR injury protection
2009[86]	Rats	IR: transplanted with male bone marrow cells; reperfusion 2 or 4 weeks	EPO 5000 U 30 min pre-ischaemia	↑GFR (4 weeks), →proteinuria/Hb (2 and 4 weeks), ↓tubulointerstitial changes. EPO did not enhance bone marrow cell recruitment
2008[93]	Rats	IR: occlusion of infrarenal abdominal aorta, ischaemia 30 min, reperfusion 60 min vs sham	EPO 1000 U/kg s.c. 5 min pre-ischaemia	↓MDA levels, ↓SOD activity, ↓catalase (↓oxidative stress so not required), ↓histopathological changes: ↓focal glomerular necrosis, ↓dilation of Bowman's capsule, ↓degeneration/necrosis in tubular epithelium, ↓interstitial inflammatory infiltration and ↓blood vessel congestion
2007[4]	Rats	IR: bilateral renal pedicle occlusion 1 day after last EPO injection, ischaemia 45 min; reperfusion 24, 72 h and 1 week	EPO 100U/kg or 100U/kg CEPO s.c. every 2 days for 2 weeks (6 injections) vs saline	CEPO: (no erythropoiesis) ↓apoptosis, ↓α-SMA expression, ↑tubular epithelial cell proliferation, ↓SCr. EPO: ↑Hb, ↓in apoptosis and α-SMA (not as marked as CEPO). CEPO more therapeutic than EPO
2007[46]	Rats	IR: bilateral renal pedicle occlusion 1 day after last EPO injection, ischaemia 60 min; reperfusion 24, 72 h	EPO 100 U/kg s.c. every 2 days for 2 weeks (6 injections) vs saline	↑HIF-1alpha-positive cells, ↑VEGF mRNA expression, ↓tubular hypoxia, ↓apoptotic and α-SMA-positive interstitial cells
2008[32]	Rats	In vitro: endothelial tube formation assay; IR: bilateral renal pedicle occlusion 1 day after last EPO dose, ischaemia 45 min; reperfusion 24, 72 h and 1 week	EPO 100 U/kg or 100 U/kg CEPO s.c. every 2 days for 2 weeks (6 injections) vs saline	EPO: tendency of increased tube formation; CEPO: more capillary-like formation than EPO; ↑peritubular capillary endothelial cells. CEPO may protect kidneys from IR injury by promoting angiogenesis
2007[88]	Pigs	IR: unilateral nephrectomy; occlusion renal artery for 1 h 1 week later, reperfusion 5 days	EPO 5000 U/kg IV at ischaemia, then 1000 U/kg s.c. daily, reperfusion 5 days vs no treatment	↓renal dysfunction, ↓cell death (histology at 5 days)
2006[100]	Rats	IR: bilateral renal pedicle occlusion, ischaemia 45 min; reperfusion 48 h vs sham/saline	EPO 500 U/kg i.p. 20 min pre-ischaemia	↓SCr, ↓urea, ↓histological injury, ↓tubular apoptosis
2006[92]	Rats and in vitro	IR: bilateral renal pedicle occlusion, ischaemia 45 min; reperfusion 1-7 days vs sham/vehicle	EPO 5000 U/kg or DPO 25 μg/kg i.p. at time of ischaemia or 6 h post reperfusion	EPO and DPO at T0 and T6 -↓tubular apoptosis, ↓plasma Cr/urea, ↑tubular regeneration (cell proliferation and mitosis)
2005[83]	Rats	IR: right nephrectomy, clamp left pedicle 45 min and reperfusion 45 min and 24 h	EPO 1000U/kg and genistein (tyrosine kinase inhibitor) 10 mg/kg 2 h before ischaemia	↓SCr, ↓urea, ↓TNF-α and IL-2 expression (pro-inflammatory mediators of IR injury), ↓LDH (indicates lipid peroxidation), ↓histological injury; genistein reversed benefits of EPO
2004[102]	Rats and in vitro	IR: uni/bilateral renal artery occlusion, 30 min ischaemia, 24 or 48 h reperfusion vs sham/vehicle. In vitro: exposure of human PTCs to 1 or 21% O_2 for up to 24 h; or 1% O_2 16 h then 21% O_2 24 h	EPO 5000 U/kg i.p. 30 min pre-ischaemia. In vitro: 6.25-400 U/ml rHuEPO vs vehicle, incubated for 5 or 24 h	↓apoptosis, ↑regeneration, ↓casts, ↓plasma Cr. In vitro: ↓apoptosis, ↑mitosis/DNA synthesis
2004[97]	Mice	IR: bilateral renal artery occlusion, 30 min ischaemia, 24 h reperfusion vs sham/vehicle	EPO 1000 U/kg/day s.c. 3 days pre IR, or EPO 1000 U/kg sc on reperfusion	↓plasma Cr, ↓plasma AST, ↓histological injury, ↓kidney MPO and MDA levels
2004[99]	Rats	IR: bilateral renal pedicle occlusion, 45 min ischaemia, 6 h reperfusion vs sham/saline	EPO 300 U/kg IV 30 min pre-ischaemia, 5 min pre-reperfusion, or 30 min post reperfusion	↓tubular apoptosis, ↓tubular (NAG) and reperfusion (AST) injury, ↓histological injury, ↓SCr, better urine flow, ↑creatinine clearance

TABLE 2

Animal studies of erythropoietin in acute kidney injury relevant to non-haematopoietic functions: ischaemia-reperfusion models (continued)

Year, reference	Type	AKI model/follow-up	EPO protocol	Outcome
2004[90]	Rats	IR: bilateral renal artery occlusion, 40 min ischaemia, 48 (1) or 96 (2) h reperfusion vs sham/vehicle	1. EPO 200 U/kg i.p. at start ischaemia and 6, 24 h post reperfusion; or 2. 200 U/kg IV and 4, 10, 24, 48 h post reperfusion	↓plasma Cr, ↓polyuria, ↓FENa, ↑AQP/NHE/TSC expression (prevented down-regulation of AQPs and Na+ transporters – may imrove ischaemia-induced urine concentrating defects and impairment of tubular Na+ reabsorption)
2003[42]	Rats and in vitro	IR: bilateral renal artery occlusion, 45 min ischaemia, ≤72 h reperfusion vs sham/ saline	EPO 3000 U/kg 24 h pre IR injury	↓SCr, ↓tubular necrosis, ↓tubular apoptosis, ↓tubular cell proliferation (renoprotection may be independent of growth promotion), ↑bcl-2 protein, ↓caspase 3 activity, ↓JNK expression, dose-dependent ↑HSP70 expression, inhibition of HSP70 expression lead to ↓renoprotection
2001[95]	Rats	IR: right kidney occlusion, 30 or 45 min ischaemia, simultaneous left nephrectomy, ≤96 h reperfusion vs sham/ vehicle	EPO 500 or 3000 U/kg IV at ischaemia then s.c. 24, 48 h post	↑HCt, ↓mortality (severe ischaemia group), →SCr/ weight

AKI=acute kidney injury, EPO=erythropoeitin, IV=intravenous, IR=ischaemia-reperfusion, ↓=decreased, ↑=increased, IV=intravenous, GFR=glomerular filtration rate, →=the same level, MDA=malondialdehyde (indicates free radical generation), SOD=superoxide dismutase (an antioxidant), CEPO=carbamylated EPO, α-SMA=alpha-smooth muscle actin (associated with renal injury), SCr=serum creatinine, HIF-1 alpha=hypoxia inducible factor-alpha, VEGF=vascular endothelial growth factor, mRNA=messenger RNA, i.p.=intraperitoneal, DPO=darbepoietin, Cr=creatinine, TNF-α=tumour necrosis factor-alpha, IL-2=interleukin-2, LDH=lactate dehydrogenase, rHuEPO=recombinant human erythropoietin, PTC=proximal tubule cell, MPO=myeloperoxidase, NAG=N-acetylglutamate, AST=aspartate aminotransferase (indicates reperfusion injury), FENa-fractional excretion of Na+, AQP=aquaporin, NHE=Na+/H+ exchanger, TSC=thiazide-sensitive sodium chloride cotransporter, bcl-2=oncogene activated by chromosome translocation in human B-cell lymphomas, JNK=c-Jun N-terminal kinase, HSP70=heat shock protein 70.

most patients (63%)[76]. Contrary to existing clinical evidence[77] from a small single-centre study, this trial showed a negative effect, which may be related to the concurrent use of thrombolysis and/or factors related to the multi-centre nature of this trial. Notably, intracerebral haemorrhage was the main cause of death, and stroke severity was higher in those who died in the EPO group. Furthermore, pre-clinical combination therapy safety studies had not been performed[78]. Of note, it is stated that one of the authors of this study holds a patent on the use of EPO for the treatment of cerebral ischaemia[76].

There is substantial experimental evidence, a plausible biological rationale and supportive clinical evidence from clinical trials to suggest a possible beneficial effect of EPO in acute and chronic brain injury. In addition, a recent small case controlled study suggests that EPO used in conjunction with hypothermia may increase survival and improve neurological outcomes[79]. However, as these clinical studies suggest, EPO treatment may also result in clinically important side-effects.

Renal protection

Animal and in vitro studies

EPORs have been found in both vascular and non-vascular renal tissue[80]. Many animal studies have shown that EPO administration protects kidney tissue from damage and improves renal function in ischaemia-reperfusion and contrast-induced injury models of AKI (Tables 2 and 3[4,8,24,32,42,46,47,81-102]). These investigations invariably found that EPO reduced kidney dysfunction by decreasing apoptosis. It has also been shown that different intracellular 'survival' pathways in the kidney such as the PI3K/Akt pathway are activated by EPO[103,104]. Furthermore, in a recent in vitro study[24], EPO was shown to reduce cisplatin-induced apoptosis in human proximal tubule epithelial cells, while phosphorylation of STAT5 and Akt increased. Involvement of the JAK2-STAT5 pathway has also been implicated in cell protection[8,24]. STAT5 activation can modulate proliferation and protect against cell apoptosis. In addition, EPO has been shown to reduce the expression of pro-inflammatory mediators, TNF-alpha and IL-2, in ischaemia-reperfusion renal injury and reverse the effect of endotoxin on the anti-oxidant, renal superoxide dismutase[94]. These anti-inflammatory properties of EPO also suggest involvement of the NF-kB pathway in its kidney protection.

Erythropoietin in acute kidney injury

AKI as classified by the RIFLE criteria (an extensively used and validated classification system for renal function) is common in the ICU and occurs in approximately 36% of critically ill patients[105,106]. AKI

TABLE 3

Animal studies of erythropoietin in acute kidney injury relevant to non-haematopoietic functions: other models

Year, reference	Type	AKI model/follow-up	EPO protocol	Outcome
2010[96]	Rats	Brain death + perfused kidney model	10 μg/kg EPO or CEPO IV, 4 h brain death, then kidney reperfusion in perfused kidney model	EPO and CEPO: ↓expression of proinflammatory genes, ↓infiltration of polymorphonuclear cells in kidney, preserved vascular integrity. CEPO more effective than EPO. Kidney function fully restored with EPO and CEPO
2010[91]	Mice	Aristolochic acid nephropathy	DPO 0.1 μg/kg weekly from day of aristolochic acid administration or on day 28	↑survival of tubular cells lead to ↓acute tubular injury, interstitial inflammation and interstitial fibrosis
2009[82]	In vitro	Oxidative stress: human proximal tubule cells 2 h in H_2O_2	EPO-alpha 100 and 400 U/ml	EPO 100 U/ml: little effect; 400 U/ml: ↑damage due partly to ↓activation of cell survival/ prolifer'n signalling pathways; ↓phosphorylation of Akt, GSK-3B, mTOR, ERK1/ERK2, and forkhead transcription factor of H_2O_2-treated cells
2008[8]	Rats and in vitro	CIN: Ioversol 2.9 g/kg iodine + inhibition of prostaglandin and NO synthesis. In vitro: Ioversol 100 mg/ml iodine to induce proximal tubular cell injury	In vitro and EPO 10,000 U/kg or asialoEPO 80 ng/g IV 1 h before Ioversol	EPO and asialoEPO: ↓renal dysfunction and histological injury, ↓apoptosis, ↓caspase3-activated apoptosis in renal porcine epithelial cells in vitro with ↑JAK2/STAT5 phosphorylation and HSP70 expression; ↑JAK2/ STAT5 phosphorylation and HSP70 expression in rat kidneys in vivo
2008[24]	Rats and in vitro	In vitro/CIN: cisplatin 5.5 mg/kg IV	EPO 5000 U/kg IV OR equivalent peptide mass of inactive EPO OR DPO 25 μg/kg pre-cisplatin OR saline	In vitro: in human RPTE cells EPO ↓ apoptosis at ≥100 U/ml, ↑ STAT5 and Akt/PKB phosphorylation. JAK2 inhibitor, tyrphostin AG-490 reduced EPO protection. Rats: EPO ↑HCt, ↓SCr; DPO also ↑HCt, ↓SCr. Clearance studies: GFR and renal blood flow confirmed DPO renal protection. ↓tubular apoptosis and necrosis with DPO. DPO 48 h post cisplatin was renoprotective
2008[85]	Mice and in vitro	CIN: i.p. cisplatin injection (10 mg/kg/day) for 2 days vs placebo. Follow-up 6 days	EPO 1000 U/kg i.p. daily ≤3 days before cisplatin vs vehicle (or bone marrow isolation/ blood cultures)	↓urea, ↓casts, ↑MSC numbers in vitro and in vivo (in bone marrow and spleen and mobilised into the peripheral circulation). Is protective effect due to ↑ in MSC numbers after EPO or their mobiliaation into circulation?
2007[94]	Mice	Endotoxaemia: 2.5 mg/kg endotoxin i.p. (lipopolysaccharide); follow-up 16 h later	EPO 4000 U/kg 30 min before endotoxin vs vehicle	↑GFR (inulin clearance), →MAP, →renal bood flow, ↑CRP, →serum NO, EPO reversed the endotoxin effect on renal SOD activity (SOD ↓ in control group)
2006[89]	Rats	CIN: (iothalamate), following inhibition of NO and prostaglandin synthesis with indomethacin and Nω nitro-L-arginine methyl ester	EPO 3000 U/kg and 600 U/ kg IV 24 and 2 h pre CIN induction vs saline	Cr clearance preserved, results inconclusive but may indicate protective trend
2005[47]	Rats and in vitro	Chronic kidney disease	DPO s.c. 0.4 μg/kg/week into 5/6 remnant kidney rats after renal mass reduction, sacrificed at 1, 2 and 12 weeks	↑microvascular density, ↑endothelial proliferation, preserved renal function (↓SCr), ↓scarring, ↑VEGF expression; In vitro: ↑cell proliferation, ↑VEGF, ↓hypoxia-induced apoptosis
2004[87]	Pig/mouse: in vitro	In vitro: exposure of LLC-PK1 (pig kidney epithelial cell line) and mouse mesangial cells to protaglandin D_2 synthase, campothecin, hydrogen peroxide or hypoxia	DPO 6.25-100 ng/ml incubation for 16 h	↓tubular apoptosis (for toxic and hypoxic stimuli)
2004[81]	Rats	Haemorrhagic shock and endotoxic shock	EPO 300 U/kg IV pre resuscitation	↓renal dysfunction in haemorrhagic but not endotoxic shock

TABLE 3

Animal studies of erythropoietin in acute kidney injury relevant to non-haematopoietic functions: other models (continued)

Year, reference	Type	AKI model/follow-up	EPO protocol	Outcome
2001[84]	Rats	CIN: cisplatin toxicity – i.p. cisplatin injection 6 mg/kg vs placebo	EPO 100 U/kg i.p. pre cisplatin then daily for 9 days vs placebo	↑renal bld flow/GFR at 9 days, ↑tubular regeneration, ↑tubular cell proliferation, ↑functional recovery
1994[101]	Rats	CIN: cisplatin toxicity – i.p. cisplatin injection 7 mg/kg vs placebo	EPO 100 U/kg i.p. post cisplatin then daily for 9 days vs placebo	↑functional recovery, ↑tubular regeneration, ↑DNA synthesis

AKI=acute kidney injury, EPO=erythropoietin, CEPO=carbamylated EPO, ↓=decreased, IV=intravenous, DPO=darbepoietin, ↑=increased, H_2O_2=hydrogen peroxide, Akt/JAK2/STAT5/=signalling pathways of EPO, GSK-3β=glycogen synthase kinase 3 beta, mTOR=mammalian target of rapamycin, ERK=extracellular signal-regulated kinase, CIN=contrast induced nephropathy, i.p.=intraperitoneal, NO=nitric oxide, asialoEPO=asialoEPO, HSP70=heat shock protein 70, RPTE=renal proximal tubular epithelial, PKB=protein kinase B, SCr=serum creatinine, MSC=marrow stem cell, GFR=glomerular filtration rate, →=the same level, MAP=mean arterial pressure, CRP=C-reactive protein, SOD=superoxide dismutase (an anti-oxidant), Cr=creatinine, VEGF=vascular endothelial growth factor.

is independently associated with increased mortality and with prolonged length of stay. It escalates both the human and financial costs of care. Therefore, it seems desirable to investigate treatments with potential to ameliorate or prevent AKI.

Some injury pathways for AKI in the critically ill include exposure to endogenous and exogenous toxins, metabolic factors, ischaemia and reperfusion insults, neurohormonal activation, inflammation and oxidative stress. Of these, ischaemia-reperfusion may be the most common. EPO can prevent or reduce injury and assist renal repair and recovery through limitation of apoptosis, promotion of neovascularisation, anti-inflammatory action and tissue regeneration.

Investigation of potential treatments for AKI has had limited success to date. However, from the results of animal and some limited preliminary human studies, therapeutic use of EPO seems promising for those 'at risk' for AKI[8,86,96,107].

Clinical trials of erythropoietin in acute kidney injury

One randomised clinical pilot trial of preoperative EPO/placebo in 71 patients undergoing elective coronary artery bypass graft surgery showed renal protective results[107] (Table 4[107,108]). EPO 300 μ/kg intravenous given immediately preoperatively seemed to reduce the incidence of AKI, (8% EPO vs 29% placebo, $P=0.035$); and improved postoperative renal function as indicated by a smaller increase in SCr (%SCr↑ at 24 hours: EPO 1±3, placebo 15±7, $P=0.04$) and a smaller decline in estimated glomerular filtration rate (% estimated glomerular filtration rate ↓ at 24 hours: EPO 3±3, placebo -5±4, $P=0.04$) postoperatively (Table 4). A more recent and slightly larger (n=162) study assessed EPO's effect in ICU patients at risk for AKI (defined by a cut-off value of two proximal tubular enzymes in urine: γ-glutamyl transpeptidase and alkaline phosphatase) (Table 4). EPO 500 μ/kg intravenous was given after a high γ-glutamyl transpeptidase ×alkaline phosphatase product was detected and again 24 hours later. The primary outcome was the average percent SCr increase from baseline over four to seven days. This trial found no renal protective effect of EPO as determined by the average percent SCr increase from baseline over four to seven days. Table 4 compares the 'coronary artery bypass graft' and 'EARLY ARF' EPO trials. The reasons for these contradictory findings may be related to the differences in design and methods used.

Potential risks of erythropoietin

Pure red cell aplasia

Despite the numerous benefits of EPO there are also some risks. Pure red cell aplasia is a rare adverse event characterised by anaemia, low reticulocyte count, absence of erythroblasts, resistance to EPO and neutralising antibodies against EPO[109,110]. Only three cases were reported in EPO patients from 1988 to 1998, after which incidence increased to a peak in 2001. This was attributed to a number of factors that were subsequently addressed. Since the introduction of Teflon-coated plungers (2003), and changes to EPO formulation and the regulation of EPO administration (2002 to 2004), there have been only six reported cases of pure red cell aplasia, converting it once again into an extremely rare occurrence.

Cancer patients

EPO administration in patients with cancer, given to reduce chemotherapy or radiotherapy induced anemia, has been associated with increased mortality and enhanced tumour growth[17,111]. The underlying mechanisms remain uncertain, but EPO may serve as a growth factor to cancer cells and may promote

TABLE 4
Comparison of articles: EARLYARF vs EPO in CABG surgery

	EPO in CABG[107]	EARLYARF[108]
Sample size	71 (EPO=36, placebo=35)	162 (EPO=84, placebo=78)
Patient population	Elective CABG	Aim: ICU patients at high risk of AKI; Obtained: critically ill patients
Country, centre(s)	Seoul, Korea, single centre	Christchurch and Dunedin, New Zealand, 2 centres
Study design	Prospective randomised double-blind, placebo-controlled trial of EPO	2 parts: a) Prospective randomised double-blind placebo-controlled, parallel group trial of EPO; b) Observational study to assess GGT×ALP to early identify patients at high risk of AKI
EPO type and dose	1 dose preop: 300 μ/kg EPO (Recormon, Roche) or normal saline IV	2 doses: EPO-beta 500 U/kg to max 50,000 U or normal saline IV; 1st within 6 h of ↑GGT×ALP; 2nd 24 h later
Inclusion criteria	>18, elective CABG	↑ in GGT and ALP urine concentration product by >46.3
Exclusion criteria	Emergent CABG, pre-existing AKI, on RRT, uncontrolled HT, nephrotoxic drugs within 3 days of operation, previous use of EPO	<16 y, no IDC, haematuria, rhabdomyolysis, myoglobinuria, polycythemia, cytotoxic chemotherapy, RRT or needs in 48 h, stay ≤24 h, survival ≤72 h, prior RIFLE 'failure'
Measurements	Baseline SCr preop and 24, 72, 120 h post	Baseline Cr: various versions of preop/pre-ICU Cr including lowest on ICU admit/last ICU Cr/minimum at 12 months. Triage: GGTxALP within 1 h of ICU admission, at 12 and 24 h, daily to 7 days; Intervention: Blood for Cr and Cyst C and start for 4/24 Cr clearance were at the same timepoints
Age (mean)	66.7±9.6	61.6±15.4
Study groups: EPO vs placebo	Baseline and intraop: no significant differences, most OPCABG (77%) (+3 valves in EPO group)	EPO group older (P=0.011), ↓likelihood for neuro surgery, injury, seizure or ICH (P <0.05) and ↑ likelihood for sepsis (P <0.05). More placebo pts had AKI (not significant)
Primary outcome	Incidence of AKI after CABG	A priori: average % plasmaCr↑ from baseline over 4-7 days
Secondary outcomes	Changes in SCr and eGFR (first 5 days postop), ICU and hospital LOS, in-hospital mortality	Intervention trial: AKIN and RIFLE AKI definitions, plasma cystatin C, need for dialysis, death within 7/30/90 days; triage trial: mean time to randomisation after collecting urine sample with ↑GGT×ALP, identification of patients at ↑ risk of AKI/dialysis/death
AKI: definition	≥50%↑ in SCr from preop baseline (first 5 days postop); eGFR: Cockroft-Gault equation	AKIN (Cr and UO) and RIFLE (Cr) definitions
AKI: proportion (after CABG/ GGT×ALP >46.3)	EPO 8%, placebo 29%; P=0.035	AKIN Cr: EPO 45.2%, placebo 47.4%; RIFLE Cr: EPO 23.8%, placebo 19.2%; AKIN UO: EPO 70.2%, placebo 51.3% (P=0.016)
Results	%SCr↑ at 24 h: EPO 1±3, placebo 15±7 (P=0.04). %SCr↑ at 120 h: EPO 7±4, placebo 27±8 (P=0.01). %eGFR↓ at 24 h: EPO 3±3, placebo -5±4 (P=0.04). %eGFR↓ at 120 h: EPO -4±3, placebo -13±5 (P=0.01). ICU LOS h: EPO 65±77, placebo 84±113 (P=0.262). Hosp LOS days 10.1±7, placebo 11.1±5.5 (P=0.442)	No significant difference in 1° outcome or 2° outcomes except AKI (AKIN UO). Of randomised patients without AKI initially (n=104) EPO patients had higher %plasmaCr↑: EPO 8.5±27 (n=61), placebo -4.6±18 (n=47) (P=0.004). No difference in ICU or hosp stay. GGT×ALP: poor predictive value for AKI, low for RRT/ death
Onset of injury	Initiation of CABG operation	Heterogenous. Time of injury estimated for subdivision analysis. Samples from 6-12 h after putative insult more predictive for AKI (AUC=0.69), dialysis and death
Possible mechanism of injury	CVS compromise, CPB exposure, ↑catecholemines, atheroembolism, IR injury, nephrotoxic agents, inflammation	Heterogenous
Safety	No symptomatic thrombosis or other adverse events in EPO patients	No evidence for ↑intravascular thrombosis. EPO not associated with ↑ in adverse events

EPO=erythropoietin, CABG=coronary artery bypass graft, ICU=intensive care unit, AKI=acute kidney injury, preop=preoperatively, GGT=γ-glutamyl transpeptidase, ALP=alkaline phosphatase, IV=intravenous, RRT=renal replacement therapy, HT=hypertension, IDC=indwelling catheter, RIFLE and AKIN=AKI classification systems, post=postoperatively, (S)Cr=(serum) creatinine, Cyst C=cystatin C, intraop=intraoperatively, OPCABG=off pump CABG, ICH=intra-craneal haemorrhage, ↑=increased, eGFR=estimated glomerular filtration rate, LOS=length of stay, UO=urine output criteria, ↓=decreased, AUC=area under curve, CVS=cardiovascular system, CPB=cardiopulmonary bypass, IR=ischaemia reperfusion.

tumour angiogenesis[17]. Furthermore, patients with certain malignancies may be in a hypercoagulable state, making EPO administration unadvisable. Consequently patients with a known malignancy should be excluded from future EPO trials.

Thrombosis

Recent studies and clinical trials have found an increased rate of thrombosis with EPO[53,112,113] which has mainly been observed in patient groups with higher than conventional levels of haemoglobin (>120 g/l)[113]. Putative mechanisms are increased blood viscosity, increased platelet count and reactivity, decreased protein C and S plasma levels, enhanced thrombin generation and factor VIII antigen plasma levels. Exclusion of patients with haemoglobin >120 g/l from clinical trials of EPO minimises the risk for thrombosis[113]. Nonetheless, systematic assessment for thrombosis should be performed in any EPO trials of critically ill patients as they have an increased risk for thrombosis.

Hypertension

Hypertension occurs in about 30% of patients receiving long-term EPO treatment[3]. This was partly attributed in the past to the rise in haematocrit, but now appears to involve increased endothelin release, upregulation of tissue renin and angiotensin production, changes in the balance of vasoactive substances (prostaglandin/prostacyclin/thromboxane), and an elevation of calcium by EPO (at least in chronic kidney disease) that impairs the vasodilating action of nitric oxide[27]. It is advisable that patients with uncontrolled hypertension do not participate in trials of EPO in AKI.

Carbamylated EPO, a cytoprotective, non-erythropoietic derivative of EPO (Table 1), may not exhibit the same risks as EPO and holds great interest as a future tissue-protective therapy. However, it requires further experimental testing before it can be safely evaluated in clinical trials.

The EPO-TBI trial and EPO-AKI substudy

EPO-TBI, a randomised, double-blind, controlled trial of EPO in ICU patients with TBI in Australia, New Zealand and Saudi Arabia, has recently commenced recruitment (ACTRN12609000827235) at the Alfred and Royal Melbourne hospitals in Victoria. The trial will soon roll out to a total of 19 sites. EPO-TBI is endorsed by the Australian and New Zealand Clinical Trials Group and has National Health and Medical Research Council and Victorian Neurotrauma Initiative funding. With a cohort of 606 patients, it will be the largest randomised controlled trial of EPO in patients with TBI ever performed. Furthermore, it is one of the largest TBI trials currently being conducted. Participants are moderate and severe TBI patients admitted to ICU with a GCS ≤12, aged between 15 and 65 years, with a haemoglobin <120 g/l, within 24 hours of injury and expected to stay ≥48 hours. Patients will be randomised to receive either EPO 40,000 IU subcutaneously or placebo in a 1:1 ratio, weekly for up to three weeks while in ICU. Pharmaceutical and mechanical venous thrombo-embolism prophylaxis will be prescribed if not contraindicated, and compression Doppler ultrasound examinations to monitor patients for the development of proximal deep vein thrombosis will be performed prior to or within 48 hours of the first EPO/placebo dose, then twice in each week following each dose. The primary outcome is the proportion of unfavourable neurological outcomes at six months: defined as severe disability (Glasgow outcome scale, extended=2 to 4) or death (Glasgow outcome scale, extended=1).

'EPO-AKI' and 'EPO-Biomarkers' comprise the Intensive Care Foundation-funded renal sub-study of the EPO-TBI trial. This sub-study assesses the effect of EPO on the development of acute kidney injury and the response to treatment using multiple renal biomarkers with different time profiles. All of these biomarkers have been extensively investigated and patterns in response to injury are known. Having recently sustained a timed physical injury and with a moderate incidence of AKI[114,115], this homogeneous cohort lends itself to such a study. AKI will be classified using methods based on the RIFLE criteria; a classification system used extensively and validated to classify renal function in several populations with studies cumulatively involving over 250,000 subjects. Baseline renal function will be taken from a consistent source for all patients. With a cohort of 606 patients, this will also be the largest study of EPO to protect against AKI ever performed, increasing the probability of detecting a treatment effect. Furthermore, it will be the first EPO trial to incorporate active risk assessment for thrombotic episodes. Weekly intervals separate the doses of EPO (up to three) in this trial to allow time for clearance and avoid excessively high levels of EPO. In addition, patients with a known malignancy and/or uncontrolled hypertension will be excluded, thus minimising risk to patients. The EPO-TBI trial provides a unique opportunity to clarify the potential benefit of EPO as a brain protective and kidney protective agent. This trial may also provide valuable insight into the mechanisms of EPO in AKI and pave the way for further dedicated large scale trials of EPO in AKI.

DECLARATION OF INTEREST

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REFERENCES

1. Miyake T, Kung CK, Goldwasser E. Purification of human erythropoietin. J Biol Chem 1977; 252:5558-5564.
2. Lin K, Suggs S, Lin CH, Browne JK, Smalling R, Egrie JC et al. Cloning and expression of the human erythropoietin gene. Proc Natl Acad Sci USA 1985; 82:7580-7584.
3. Mocini D, Leone T, Tubaro M, Santini M, Penco M. Structure, production and function of erythropoietin: implications for therapeutical use in cardiovascular disease. Curr Med Chem 2007; 14:2278-2287.
4. Imamura R, Isaka Y, Ichimaru N, Takahara S, Okuyama A. Carbamylated erythropoietin protects the kidneys from ischemia-reperfusion injury without stimulating erythropoiesis. Biochem Biophys Res Commun 2007; 353:786-792.
5. Leist Ml, Ghezzi P, Grasso G, Bianchi R, Villa P, Fratelli M et al. Derivatives of erythropoietin that are tissue protective but not erythropoietic. Science 2004; 305:239-242.
6. Torup L, Leist M. Development of Non-Erythropoietic Erythropoietin Variants for Neuroprotection. In: Hoke A, ed. Erythropoietin and the Nervous System: Novel Therapeutic Options for Neuroprotection. New York: Springer US 2006. p. 211-219.
7. Wang X, Zhu C, Wang X, Gerwien JG, Schrattenholz A, Sandberg M et al. The nonerythropoietic asialoerythropoietin protects against neonatal hypoxia-ischemia as potently as erythropoietin. J Neurochem 2004; 91:900-910.
8. Yokomaku Y, Sugimoto T, Kume S, Araki S, Isshiki K, Chin-Kanasaki M et al. Asialoerythropoietin prevents contrast-induced nephropathy. J Am Soc Nephrol 2008; 19:321-328.
9. Boissel JP, Lee WR, Presnell SR, Cohen FE, Bunn HF. Erythropoietin structure-function relationships. Mutant proteins that test a model of tertiary structure. J Biol Chem 1993; 268:15983-15993.
10. Catlin DH, Breidbach A, Elliott S, Glaspy J. Comparison of the isoelectric focusing patterns of darbepoetin alfa, recombinant human erythropoietin, and endogenous erythropoietin from human urine. Clin Chem 2002; 48:2057-2059.
11. Elliott S, Egrie J, Browne J, Lorenzini T, Busse L, Rogers N. Control of rHuEPO biological activity: the role of carbohydrate. Exp Hematol 2004; 32:1146-1155.
12. Rush RS, Derby PL, Smith DM, Merry C, Rogers G, Rohde MF et al. Microheterogeneity of erythropoietin carbohydrate structure. Anal Chem 1995; 67:1442-1452.
13. Rush RS, Derby PL, Strickland TW, Rohde MF. Peptide mapping and evaluation of glycopeptide microheterogeneity derived from endoproteinase digestion of erythropoietin by affinity high-performance capillary electrophoresis. Anal Chem 1993; 65:1834-1842.
14. Middleton SA, Barbone FP, Johnson DL, Thurmond RL, You Y, McMahon FJ et al. Shared and unique determinants of the erythropoietin (EPO) receptor are important for binding EPO and EPO mimetic peptide. J Biol Chem 1999; 274:14163-14169.
15. Weidemann A, Johnson RS. Nonrenal regulation of EPO synthesis. Kidney Int 2009; 75:682-688.
16. Jelkmann W. Erythropoietin after a century of research: younger than ever. Eur J Haematol 2007; 78:183-205.
17. Diskin CJ, Stokes TJ, Dansby LM, Radcliff L, Carter TB. Beyond anemia: the clinical impact of the physiologic effects of erythropoietin. Semin Dial 2008; 21:447-454.
18. Bahlmann FH, Fliser D. Erythropoietin and renoprotection. Curr Opin Nephrol Hypertens 2009; 18:15-20.
19. Johnson DW, Forman C, Vesey DA. Novel renoprotective actions of erythropoietin: new uses for an old hormone. Nephrology (Carlton) 2006; 11:306-312.
20. Percy MJ, Lee FS. Familial erythrocytosis: molecular links to red blood cell control. Haematologica 2008; 93:963-967.
21. Elliott S, Pham E, Macdougall IC. Erythropoietins: a common mechanism of action. Exp Hematol 2008; 36:1573-1584.
22. Ramakrishnan R, Cheung WK, Wacholtz MC, Minton N, Jusko WJ. Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after single and multiple doses in healthy volunteers. J Clin Pharmacol 2004; 44:991-1002.
23. Freise KJ, Widness JA, Schmidt RL, Veng-Pedersen P. Pharmacodynamic analysis of time-variant cellular disposition: reticulocyte disposition changes in phlebotomized sheep. J Pharmacokinet Pharmacodyn 2007; 34:519-547.
24. Salahudeen AK, Haider N, Jenkins J, Joshi M, Patel H, Huang H et al. Antiapoptotic properties of erythropoiesis-stimulating proteins in models of cisplatin-induced acute kidney injury. Am J Physiol Renal Physiol 2008; 294:F1354-1365.
25. Silva M, Benito A, Sanz C, Prosper F, Ekhterae D, Nunez G et al. Erythropoietin can induce the expression of bcl-x(L) through STAT5 in erythropoietin-dependent progenitor cell lines. J Biol Chem 1999; 274:22165-22169.
26. Guillard C, Chretien S, Pelus A-S, Porteu F, Muller O, Mayeux P et al. Activation of the mitogen-activated protein kinases Erk1/2 by erythropoietin receptor via a G(i) protein beta gamma-subunit-initiated pathway. J Biol Chem 2003; 278:11050-11056.
27. Li F, Chong ZZ, Maiese K. Erythropoietin on a tightrope: balancing neuronal and vascular protection between intrinsic and extrinsic pathways. Neurosignals 2004; 13:265-289.
28. Bernhardt WM, Eckardt K-U. Physiological basis for the use of erythropoietin in critically ill patients at risk for acute kidney injury. Curr Opin Crit Care 2008; 14:621-626.
29. Brines M, Grasso G, Fiordaliso F, Sfacteria A, Ghezzi P, Fratelli M et al. Erythropoietin mediates tissue protection through an erythropoietin and common beta-subunit heteroreceptor. Proc Natl Acad Sci USA 2004; 101:14907-14912.
30. Chatterjee PK. Novel pharmacological approaches to the treatment of renal ischemia-reperfusion injury: a comprehensive review. Naunyn Schmiedebergs Arch Pharmacol 2007; 376:1-43.
31. Joyeux-Faure M, Godin-Ribuot D, Ribuot C. Erythropoietin and myocardial protection: what's new? Fundam Clin Pharmacol 2005; 19:439-446.
32. Imamura R, Okumi M, Isaka Y, Ichimaru N, Moriyama T, Imai E et al. Carbamylated erythropoietin improves angiogenesis and protects the kidneys from ischemia-reperfusion injury. Cell Transplant 2008; 17:135-141.
33. Rusai K, Prokai A, Szebeni B, Fekete A, Treszl A, Vannay A et al. Role of serum and glucocorticoid-regulated kinase-1 in the protective effects of erythropoietin during renal ischemia/reperfusion injury. Biochem Pharmacol 2010; 79:1173-1181.

34. Myklebust JH, Smeland EB, Josefsen D, Sioud M. Protein kinase C-alpha isoform is involved in erythropoietin-induced erythroid differentiation of CD34(+) progenitor cells from human bone marrow. Blood 2000; 95:510-518.
35. Mladenovic J, Kay NE. Erythropoietin induces rapid increases in intracellular free calcium in human bone marrow cells. J Lab Clin Med 1988; 112:23-27.
36. Assandri R, Egger M, Gassmann M, Niggli E, Bauer C, Forster I et al. Erythropoietin modulates intracellular calcium in a human neuroblastoma cell line. J Physiol 1999; 516 (Pt 2):343-352.
37. Masuda S, Nagao M, Takahata K, Konishi Y, Gallyas F Jr, Tabira T et al. Functional erythropoietin receptor of the cells with neural characteristics. Comparison with receptor properties of erythroid cells. J Biol Chem 1993; 268:11208-11216.
38. Miller BA, Barber DL, Bell LL, Beattie BK, Zhang MY, Neel BG et al. Identification of the erythropoietin receptor domain required for calcium channel activation. J Biol Chem 1999; 274:20465-20472.
39. Morishita E, Masuda S, Nagao M, Yasuda Y, Sasaki R. Erythropoietin receptor is expressed in rat hippocampal and cerebral cortical neurons, and erythropoietin prevents in vitro glutamate-induced neuronal death. Neuroscience 1997; 76:105-116.
40. Koshimura K, Murakami Y, Sohmiya M, Tanaka J, Kato Y. Effects of erythropoietin on neuronal activity. J Neurochem 1999; 72:2565-2572.
41. Figueroa YG, Chan AK, Ibrahim R, Tang Y, Burow ME, Alam J et al. NF-kappaB plays a key role in hypoxia-inducible factor-1-regulated erythropoietin gene expression. Exp Hematol 2002; 30:1419-1427.
42. Yang CW, Li C, Jung JY, Shin SJ, Choi BS, Lim SW et al. Preconditioning with erythropoietin protects against subsequent ischemia-reperfusion injury in rat kidney. FASEB J 2003; 17:1754-1755.
43. Lui JC, Kong SK. Heat shock protein 70 inhibits the nuclear import of apoptosis-inducing factor to avoid DNA fragmentation in TF-1 cells during erythropoiesis. FEBS Lett 2007; 581:109-117.
44. Beere HM, Wolf BB, Cain K, Mosser DD, Mahboubi A, Kuwana T et al. Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. Nat Cell Biol 2000; 2:469-475.
45. Bahlmann FH, Song R, Boehm SM, Mengel M, von Wasielewski R, Lindschau C et al. Low-dose therapy with the long-acting erythropoietin analogue darbepoetin alpha persistently activates endothelial Akt and attenuates progressive organ failure. Circulation 2004; 110:1006-1012.
46. Imamura R, Moriyama T, Isaka Y, Namba Y, Ichimaru N, Takahara S et al. Erythropoietin protects the kidneys against ischemia reperfusion injury by activating hypoxia inducible factor-1alpha. Transplantation 2007; 83:1371-1379.
47. Kang DH, Park EY, Yu ES, Lee YS, Yoon KI. Renoprotective effect of erythropoietin (EPO): possibly via an amelioration of renal hypoxia with stimulation of angiogenesis in the kidney. Kidney Int 2005; 67:1683.
48. Nakano M, Satoh K, Fukumoto Y, Ito Y, Kagaya Y, Ishii N et al. Important role of erythropoietin receptor to promote VEGF expression and angiogenesis in peripheral ischemia in mice. Circ Res 2007; 100:662-669.
49. Murray GD, Teasdale GM, Braakman R, Cohadon F, Dearden M, Iannotti F et al. The European Brain Injury Consortium survey of head injuries. Acta Neurochir (Wien) 1999; 141:223-236.
50. Bramlett HM, Dietrich WD. Pathophysiology of cerebral ischemia and brain trauma: similarities and differences. J Cereb Blood Flow Metab 2004; 24:133-150.
51. Yatsiv I, Grigoriadis N, Simeonidou C, Stahel PF, Schmidt OI, Alexandrovitch AG et al. Erythropoietin is neuroprotective, improves functional recovery, and reduces neuronal apoptosis and inflammation in a rodent model of experimental closed head injury. FASEB J 2005; 19:1701-1703.
52. Brain Trauma Foundation, American Association of Neurological Surgeons, Congress of Neurological Surgeons. Guidelines for the management of severe traumatic brain injury. J Neurotrauma 2007; 24:S1-106.
53. Corwin HL, Gettinger A, Fabian TC, May A, Pearl RG, Heard S et al. Efficacy and safety of epoetin alfa in critically ill patients. N Engl J Med 2007; 357:965-976.
54. Gluhovschi G, Gluhovschi C, Bob F, Velciov S, Trandafirescu V, Petrica L et al. Multiorgan-protective actions of blockers of the renin-angiotensin system, statins and erythropoietin: common pleiotropic effects in reno-, cardio- and neuroprotection. Acta Clin Belg 2008; 63:152-169.
55. Celik M, Gokmen N, Erbayraktar S, Akhisaroglu M, Konakc S, Ulukus C et al. Erythropoietin prevents motor neuron apoptosis and neurologic disability in experimental spinal cord ischemic injury. Proc Natl Acad Sci USA 2002; 99:2258-2263.
56. Digicaylioglu M, Lipton SA. Erythropoietin-mediated neuroprotection involves cross-talk between JAK2 and NF-kappaB signalling cascades. Nature 2001; 412:641-647.
57. Egrie JC, Dwyer E, Browne JK, Hitz A, Lykos MA. Darbepoetin alfa has a longer circulating half-life and greater in vivo potency than recombinant human erythropoietin. Exp Hematol 2003; 31:290-299.
58. Belayev L, Khoutorova L, Zhao W, Vigdorchik A, Belayev A, Busto R et al. Neuroprotective effect of darbepoetin alfa, a novel recombinant erythropoietic protein, in focal cerebral ischemia in rats. Stroke 2005; 36:1071-1076.
59. Brines ML, Ghezzi P, Keenan S, Agnello D, de Lanerolle NC, Cerami C et al. Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. Proc Natl Acad Sci USA 2000; 97:10526-10531.
60. Yoo JY, Won YJ, Lee JH, Kim JU, Sung IY, Hwang SJ et al. Neuroprotective effects of erythropoietin posttreatment against kainate-induced excitotoxicity in mixed spinal cultures. J Neurosci Res 2009; 87:150-163.
61. Grasso G, Sfacteria A. The many faces of erythropoietin: from erythropoiesis to a rational neuroprotective strategy. Expert Opin Investig Drugs 2008; 17:1407-1409.
62. Lykissas MG, Korompilias AV, Vekris MD, Mitsionis GI, Sakellariou E, Beris AE. The role of erythropoietin in central and peripheral nerve injury. Clin Neurol Neurosurg 2007; 109:639-644.
63. Bernaudin M, Marti HH, Roussel S, Divoux D, Nouvelot A, MacKenzie ET et al. A potential role for erythropoietin in focal permanent cerebral ischemia in mice. J Cereb Blood Flow Metab 1999; 19:643-651.
64. Calapai G, Marciano MC, Corica F, Allegra A, Parisi A, Frisina N et al. Erythropoietin protects against brain ischemic injury by inhibition of nitric oxide formation. Eur J Pharmacol 2000; 401:349-356.
65. Sakanaka M, Wen TC, Matsuda S, Masuda S, Morishita E, Nagao M et al. In vivo evidence that erythropoietin protects neurons from ischemic damage. Proc Natl Acad Sci USA 1998; 95:4635-4640.

66. Bocker-Meffert S, Rosenstiel P, Rohl C, Warneke N, Held-Feindt J, Sievers J et al. Erythropoietin and VEGF promote neural outgrowth from retinal explants in postnatal rats. Invest Ophthalmol Vis Sci 2002; 43:2021-2026.
67. Campana WM, Myers RR. Exogenous erythropoietin protects against dorsal root ganglion apoptosis and pain following peripheral nerve injury. Eur J Neurosci 2003; 18:1497-1506.
68. Chong ZZ, Kang J-Q, Maiese K. Hematopoietic factor erythropoietin fosters neuroprotection through novel signal transduction cascades. J Cereb Blood Flow Metab 2002; 22:503-514.
69. Kaptanoglu E, Solaroglu I, Okutan O, Surucu HS, Akbiyik F et al. Erythropoietin exerts neuroprotection after acute spinal cord injury in rats: effect on lipid peroxidation and early ultrastructural findings. Neurosurg Rev 2004; 27:113-120.
70. Sugawa M, Sakurai Y, Ishikawa-Ieda Y, Suzuki H, Asou H. Effects of erythropoietin on glial cell development; oligodendrocyte maturation and astrocyte proliferation. Neurosci Res 2002; 44:391-403.
71. Lu D, Mahmood A, Qu C, Goussev A, Schallert T, Chopp M. Erythropoietin enhances neurogenesis and restores spatial memory in rats after traumatic brain injury. J Neurotrauma 2005; 22:1011-1017.
72. Tseng M-Y, Hutchinson PJ, Richards HK, Czosnyka M, Pickard JD, Erber WN et al. Acute systemic erythropoietin therapy to reduce delayed ischemic deficits following aneurysmal subarachnoid hemorrhage: a Phase II randomized, double-blind, placebo-controlled trial. Clinical article. J Neurosurg 2009; 111:171-180.
73. Haljan G, Maitland A, Buchan A, Arora RC, King M, Haigh J et al. The erythropoietin neuroprotective effect: assessment in CABG surgery (TENPEAKS): a randomized, double-blind, placebo controlled, proof-of-concept clinical trial. Stroke 2009; 40:2769-2775.
74. Ehrenreich H, Hinze-Selch D, Stawicki S, Aust C, Knolle-Veentjer S, Wilms S et al. Improvement of cognitive functions in chronic schizophrenic patients by recombinant human erythropoietin. Mol Psychiatry 2007; 12:206-220.
75. Ehrenreich H, Fischer B, Norra C, Schellenberger F, Stender N, Stiefel M et al. Exploring recombinant human erythropoietin in chronic progressive multiple sclerosis. Brain 2007; 130:2577-2588.
76. Ehrenreich H, Weissenborn K, Prange H, Schneider D, Weimar C, Wartenberg K et al. Recombinant human erythropoietin in the treatment of acute ischemic stroke. Stroke 2009; 40:e647-656.
77. Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M et al. Erythropoietin therapy for acute stroke is both safe and beneficial. Mol Med 2002; 8:495-505.
78. Tufekci KU, Genc K. Neurovascular protection by erythropoietin: from the bedside back to the bench. Stroke 2010; 41:e462.
79. Cariou A, Claessens Y-E, Pene F, Marx J-S, Spaulding C, Hababou C et al. Early high-dose erythropoietin therapy and hypothermia after out-of-hospital cardiac arrest: a matched control study. Resuscitation 2008; 76:397-404.
80. Westenfelder C, Biddle DL, Baranowski RL. Human, rat, and mouse kidney cells express functional erythropoietin receptors. Kidney Int 1999; 55:808-820.
81. Abdelrahman M, Sharples EJ, McDonald MC, Collin M, Patel NSA, Yaqoob MM et al. Erythropoietin attenuates the tissue injury associated with hemorrhagic shock and myocardial ischemia. Shock 2004; 22:63-69.
82. Andreucci M, Fuiano G, Presta P, Lucisano G, Leone F, Fuiano L et al. Downregulation of cell survival signalling pathways and increased cell damage in hydrogen peroxide-treated human renal proximal tubular cells by alpha-erythropoietin. Cell Prolif 2009; 42:554-561.
83. Ates E, Yalcin AU, Yilmaz S, Koken T, Tokyol C. Protective effect of erythropoietin on renal ischemia and reperfusion injury. ANZ J Surg 2005; 75:1100-1105.
84. Bagnis C, Beaufils H, Jacquiaud C, Adabra Y, Jouanneau C, Le Nahour G et al. Erythropoietin enhances recovery after cisplatin-induced acute renal failure in the rat. Nephrol Dial Transplant 2001; 16:932-938.
85. Bi B, Guo J, Marlier A, Lin SR, Cantley LG. Erythropoietin expands a stromal cell population that can mediate renoprotection. Am J Physiol Renal Physiol 2008; 295:F1017-1022.
86. Esposito C, Pertile E, Grosjean F, Castoldi F, Diliberto R, Serpieri N et al. The improvement of ischemia/reperfusion injury by erythropoetin is not mediated through bone marrow cell recruitment in rats. Transplant Proc 2009; 41:1113-1115.
87. Fishbane S, Ragolia L, Palaia T, Johnson B, Elzein H, Maesaka JK. Cytoprotection by darbepoetin/epoetin alfa in pig tubular and mouse mesangial cells. Kidney Int 2004; 65:452-458.
88. Forman CJ, Johnson DW, Nicol DL. Erythropoietin administration protects against functional impairment and cell death after ischaemic renal injury in pigs. BJU Int 2007; 99:162-165.
89. Goldfarb M, Rosenberger C, Ahuva S, Rosen S, Heyman SN. A role for erythropoietin in the attenuation of radiocontrast-induced acute renal failure in rats. Ren Fail 2006; 28:345-350.
90. Gong H, Wang W, Kwon T-H, Jonassen T, Li C, Ring T et al. EPO and alpha-MSH prevent ischemia/reperfusion-induced down-regulation of AQPs and sodium transporters in rat kidney. Kidney Int 2004; 66:683-695.
91. Hamano Y, Aoki T, Shirai R, Hatano M, Kimura R, Ogawa M et al. Low-dose darbepoetin alpha attenuates progression of a mouse model of aristolochic acid nephropathy through early tubular protection. Nephron Exp Nephrol 2010; 114:e69-81.
92. Johnson DW, Pat B, Vesey DA, Guan Z, Endre Z, Gobe GC. Delayed administration of darbepoetin or erythropoietin protects against ischemic acute renal injury and failure. Kidney Int 2006; 69:1806-1813.
93. Kiris I, Kapan S, Kilbas A, Yilmaz N, Altuntas I, Karahan N et al. The protective effect of erythropoietin on renal injury induced by abdominal aortic-ischemia-reperfusion in rats. J Surg Res 2008; 149:206-213.
94. Mitra A, Bansal S, Wang W, Falk S, Zolty E, Schrier RW. Erythropoietin ameliorates renal dysfunction during endotoxaemia. Nephrol Dial Transplant 2007; 22:2349-2353.
95. Nemoto T, Yokota N, Keane WF, Rabb H. Recombinant erythropoietin rapidly treats anemia in ischemic acute renal failure. Kidney Int 2001; 59:246-251.
96. Nijboer WN, Ottens PJ, van Dijk A, van Goor H, Ploeg RJ, Leuvenink HGD. Donor pretreatment with carbamylated erythropoietin in a brain death model reduces inflammation more effectively than erythropoietin while preserving renal function. Crit Care Med 2010; 38:1155-1161.
97. Patel NSA, Sharples EJ, Cuzzocrea S, Chatterjee PK, Britti D, Yaqoob MM et al. Pretreatment with EPO reduces the injury and dysfunction caused by ischemia/reperfusion in the mouse kidney in vivo. Kidney Int 2004; 66:983-989.
98. Phillips CK, Hruby GW, Mirabile G, Motamedinia P, Lehman DS, Okhunov Z et al. Erythropoietin-induced optimization of renal function after warm ischemia. J Endourol 2009; 23:359-365.

99. Sharples EJ, Patel N, Brown P, Stewart K, Mota-Philipe H, Sheaff M et al. Erythropoietin protects the kidney against the injury and dysfunction caused by ischemia-reperfusion. J Am Soc Nephrol 2004; 15:2115-2124.
100. Spandou E, Tsouchnikas I, Karkavelas G, Dounousi E, Simeonidou C, Guiba-Tziampiri O et al. Erythropoietin attenuates renal injury in experimental acute renal failure ischaemic/reperfusion model. Nephrol Dial Transplant 2006; 21:330-336.
101. Vaziri ND, Zhou XJ, Liao SY. Erythropoietin enhances recovery from cisplatin-induced acute renal failure. Am J Physiol 1994; 266:F360-366.
102. Vesey DA, Cheung C, Pat B, Endre Z, Gobe G, Johnson DW. Erythropoietin protects against ischaemic acute renal injury. Nephrol Dial Transplant 2004; 19:348-355.
103. Ribeil J-A, Zermati Y, Vandekerckhove J, Cathelin S, Kersual J, Dussiot M et al. Hsp70 regulates erythropoiesis by preventing caspase-3-mediated cleavage of GATA-1. Nature 2007; 445:102-105.
104. Xu B, Dong G, Liu H, Wang Y, Wu H, Jing H. Recombinant human erythropoietin pretreatment attenuates myocardial infarct size: a possible mechanism involves heat shock Protein 70 and attenuation of nuclear factor-kappaB. Ann Clin Lab Sci 2005; 35:161-168.
105. Bagshaw SM, George C, Dinu I, Bellomo R. A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. Nephrol Dial Transplant 2008; 23:1203-1210.
106. Ostermann M, Chang Rene WS. Acute kidney injury in the intensive care unit according to RIFLE. Crit Care Med 2007; 35:1837-1843.
107. Song YR, Lee T, You SJ, Chin HJ, Chae D-W, Lim C et al. Prevention of acute kidney injury by erythropoietin in patients undergoing coronary artery bypass grafting: a pilot study. Am J Nephrol 2009; 30:253-260.
108. Endre ZH, Walker RJ, Pickering JW, Shaw GM, Frampton CM, Henderson SJ et al. Early intervention with erythropoietin does not affect the outcome of acute kidney injury (the EARLYARF trial). Kidney Int 2010; 77:1020-1030.
109. Bennett CL, Luminari S, Nissenson AR, Tallman MS, Klinge SA, McWilliams N et al. Pure red-cell aplasia and epoetin therapy. N Engl J Med 2004; 351:1403-1408.
110. McKoy JM, Stonecash RE, Cournoyer D, Rossert J, Nissenson AR, Raisch DW et al. Epoetin-associated pure red cell aplasia: past, present, and future considerations. Transfusion 2008; 48:1754-1762.
111. Bohlius J, Schmidlin K, Brillant C, Schwarzer G, Trelle S, Seidenfeld J et al. Recombinant human erythropoiesis-stimulating agents and mortality in patients with cancer: a meta-analysis of randomised trials. Lancet 2009; 373:1532-1542.
112. Bohlius J, Schmidlin K, Brillant C, Schwarzer G, Trelle S, Seidenfeld J et al. Erythropoietin or Darbepoetin for patients with cancer – meta-analysis based on individual patient data. Cochrane Database Syst Rev 2009:CD007303.
113. Singh AK, Szczech L, Tang KL, Barnhart H, Sapp S, Wolfson M et al. Correction of anemia with epoetin alfa in chronic kidney disease. N Engl J Med 2006; 355:2085-2098.
114. Bagshaw SM, George C, Gibney RTN, Bellomo R. A multi-center evaluation of early acute kidney injury in critically ill trauma patients. Ren Fail 2008; 30:581-589.
115. Moore EM, Bellomo R, Nichol AD, Harley N, MacIsaac C, Cooper DJ. The incidence of acute kidney injury in patients with traumatic brain injury. Ren Fail 2010; 32:1060-1065.

2.3 Hypothermia

In the last 20 years there has been a renewed interest in the therapeutic use of hypothermia for brain injury and investigation of its mechanisms of action has led to a greater understanding of the complications involved in its use and safer application of hypothermia. The mechanisms of action of hypothermia could also potentially provide protection for AKI given that ischaemia-reperfusion injury and possibly inflammation are often involved in the development of AKI.

In the following published review we explore the evidence surrounding the use of therapeutic hypothermia for brain injury and recent evidence supporting its potential protective effect on the kidneys. The optimal method of employment of hypothermia to achieve maximal benefit is examined and the future of therapeutic hypothermia as a kidney protective therapy is discussed.
In the POLAR trial, attempts have been made to incorporate current evidence in the method of delivering prophylactic hypothermia to optimise its protective effect. The Renal substudy of the POLAR trial, on which part of this thesis is based, examines the effect of prophylactic hypothermia on renal function and the incidence of AKI.

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Declaration for Thesis Chapter

Moore EM, Nichol AD, Bernard SA, Bellomo R. Therapeutic hypothermia: Benefits, mechanisms and potential clinical applications in neurological, cardiac and kidney injury. Injury;42(9):843-54.

Declaration by candidate

In the case of Chapter , the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Searched and reviewed the literature, collated and interpreted the information, developed and wrote up drafts, submitted the manuscript, was responsible for the response to reviewers.	85 %

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
R Bellomo	Reviewed and drafted the manuscript.	N/A
A Nichol	Assisted in interpretation of the literature, reviewed and drafted the manuscript .	N/A
S Bernard	Reviewed and drafted the manuscript.	N/A

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Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
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Review

Therapeutic hypothermia: Benefits, mechanisms and potential clinical applications in neurological, cardiac and kidney injury

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ABSTRACT

Therapeutic hypothermia involves the controlled reduction of core temperature to attenuate the secondary organ damage which occurs following a primary injury. Clinicians have been increasingly using therapeutic hypothermia to prevent or ameliorate various types of neurological injury and more recently for some forms of cardiac injury. In addition, some recent evidence suggests that therapeutic hypothermia may also provide benefit following acute kidney injury.

In this review we will examine the potential mechanisms of action and current clinical evidence surrounding the use of therapeutic hypothermia. We will discuss the ideal methodological attributes of future studies using hypothermia to optimise outcomes following organ injury, in particular neurological injury. We will assess the importance of target hypothermic temperature, time to achieve target temperature, duration of cooling, and re-warming rate on outcomes following neurological injury to gain insights into important factors which may also influence the success of hypothermia in other organ injuries, such as the heart and the kidney. Finally, we will examine the potential of therapeutic hypothermia as a future kidney protective therapy.

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Introduction

Therapeutic hypothermia (TH) involves the controlled reduction of a patient's core temperature in an attempt to protect an organ at risk of injury. To date, TH has principally been used as a protective therapy following various brain insults; however there

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is emerging evidence that it may also be useful in the protection of other organs when at risk of injury.

The current main clinical indications for TH for cerebral protection in adults are out-of-hospital cardiac arrest[1,2] and, in neonates, hypoxic ischaemic encephalopathy[3] with randomised clinical trials showing the neurological benefit of TH.[1] Furthermore, recent meta-analyses have suggested potential outcome benefit with TH following traumatic brain injury (TBI).[4,5] The safety and efficacy of TH have resulted in TH being increasingly applied by clinicians to comatose patients of various etiologies (i.e. stroke, hepatic encephalopathy, etc.) in an attempt to decrease brain injury.[6,7] These new but as yet unproven indications follow from the established use of TH to improve outcomes in operations involving significant risk of cerebral ischaemia during circulatory arrest in cardiac and neurosurgery.[8–10] In addition to its protective neurological effects, hypothermia may decrease infarct size in patients with acute myocardial infarction after emergency percutaneous coronary intervention[11] and reduce the risk of renal failure after renal ischaemia–reperfusion injury in animals.[12,13]

Although evidence is limited, studies suggest five key factors that could explain the failure of these previous studies and need to be addressed in future studies of TH: time to induction of hypothermia after injury, target temperature attained, duration of cooling, rate of re-warming and prevention of side effects/complications from hypothermia. In this article we will review the evidence for potential clinical applications of TH, describe its mechanisms of action and side effects, and, within this setting, discuss optimal methods for its implementation in future clinical trials. The focus is on TBI and on how the lessons learned with TBI may help effectively apply TH to the treatment of acute kidney injury.

Cooling: physiological aspects of induction, maintenance and re-warming

In order to successfully and safely implement cooling, awareness of the physiological effects and appropriate management of the side effects of hypothermia are required. There are 3 commonly recognised phases of hypothermic management: induction, maintenance and re-warming.

In the induction phase the aim is to reduce the temperature to target as quickly as possible. In TBI, clinical studies indicate that the temperature range associated with better outcomes appears to be 32–35 °C[4] (Table 1). As this phase involves the highest risk for immediate side effects such as electrolyte disorders, hyperglycaemia and shivering (Table 1), it is preferable to reach the stable maintenance phase quickly.[14] Continual monitoring of ventilation, blood pressure, sedation, blood sugar, and electrolytes is required in this phase.

Several methods are used to induce hypothermia[14]:

Surface cooling by air: Traditional methods such as exposing the skin to air, which may be combined with sponge baths, are effective, and air-circulating cooling blankets are also available.
Surface cooling by fluid: This includes methods from ice packs to water-circulating cooling blankets, pads, and wrapping garments, as well as hydrogel-coated water-circulating pads.
Core-cooling: The infusion of ice-cold fluids is effective in inducing hypothermia. Invasive devices such as intravascular catheters with saline-filled cold balloons or cooled metal components are also used for core-cooling as well as antipyretic agents.

By combining different cooling methods the target temperature is more rapidly achieved.[14]

In the maintenance phase, core temperature should be tightly controlled to ensure patient stability. During this period, prevention of side effects such as nosocomial infections and pressure ulcers is important particularly if the duration of hypothermia is prolonged.

The re-warming phase involves the very slow increase of the temperature of the patient to normal levels. This is done slowly for several reasons: to minimise electrolyte disturbances caused by shifts between intra- and extracellular compartments, and to reduce insulin sensitivity and the risk for hypoglycaemia if the patient is receiving insulin.[15] Very slow rewarming is also necessary to prevent the exacerbation of damaging mechanisms in the injured brain which are associated with rapid re-warming; and to minimise the degree of vasodilation with warming in an attempt to maintain systemic blood pressure and cerebral perfusion pressure. Following rewarming, hyperthermia is commonly seen. However, normothermia should be maintained since fever is independently associated with adverse outcomes in many forms of brain injury.[14]

Potential mechanisms of the neuroprotective effects of therapeutic hypothermia and common side effects

In out-of-hospital cardiac arrest, hypoxic ischaemic encephalopathy in neonates, traumatic brain injury (TBI), stroke and hepatic encephalopathy, TH is used to reduce the potential neurological complications of evolving secondary brain tissue injury. The mechanisms of action of hypothermia are complex (Table 1) but principally they act to attenuate the cascade of destructive processes (secondary injury), which occurs in the minutes to hours following initial tissue injury (primary injury). We will discuss these processes with particular regard to cerebral insults, where the majority of the research to date has focused, however, these protective processes are expected to be replicated in other organs during TH. Side effects which consequently affect patient management are also addressed (Table 1).

Metabolism/electrolytes: Until the 1990s, it was assumed that the neurological protective effects of hypothermia were solely due to a reduction in cerebral metabolism.[16] Whilst there is indeed a decrease in metabolism, it is now understood that this is only one of many mechanisms behind the protective effects of hypothermia. As the core temperature drops and the metabolic rate decreases, oxygen and glucose consumption, and carbon dioxide production also decrease, thus helping to prevent or ameliorate injury when oxygen supply is interrupted or limited.[17]

The reduction in metabolic rate induced by hypothermia may require adjustments in ventilator settings to maintain normocapnia. Decreases in insulin sensitivity and secretion may also require changes to insulin infusion rates. The rate of these changes depends on the rapidity of induction and rewarming.[14]

Electrolyte levels are also affected by hypothermia due to tubular dysfunction and intracellular shift.[18] Considering that magnesium may ameliorate cerebral injury; that low phosphate levels are linked to higher risk of infection[19]; and that low magnesium and potassium levels can increase the risk of arrhythmias,[19,20] these electrolytes should be kept in the high-normal range during and after hypothermia.[19] However, caution is required with potassium supplementation during cooling as there is a risk of rebound hyperkalaemia during rewarming.

Apoptosis and mitochondrial dysfunction: Following ischaemia with subsequent reperfusion, cells may recover to varying degrees, become necrotic, or enter a pathway to programmed cell death (apoptosis). Whilst hypothermia appears to block the apoptotic pathway in its early stages[21–23] there is a finite window for interventions such as hypothermia to affect this process.

Ion pumps and neuroexcitotoxicity: Hypothermia is thought to inhibit damaging neuroexcitatory processes that occur with ischaemia–reperfusion injury (Fig. 1 and Table 1). When cerebral oxygen supply is interrupted, levels of adenosine triphosphate

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Table 1
Potential mechanisms of action, risks and changes with hypothermia.

Mechanism/change	Explanation	When/treatment
Metabolic changes	↓ Cerebral metabolic rate by 6–8% per 1 °C ↓ in core T → ↓ in O_2 consumption and CO_2 production. Excessive ↑ in CO_2 can ↑ cerebral oedema, and excessive ↓ in CO_2 can ↑ ischaemia.	Acute in induction/frequent BGs and ventilator setting adjustments to maintain normocapnia, slow rewarming
Electrolytes	Cooling → ↑ renal tubular dysfunction → ↑ electrolyte excretion Cooling → intracellular electrolyte shift → ↓ K^+, Mg^{2+}, PO_4^- → ↑ risk for arrhythmias Rewarming → intracellular K^+ released → hyperkalaemia	Keep electrolytes in high-normal range, slow rewarming (0.25 °C/h post cardiac arrest, slower for severe TBI)
Apoptosis and mitochondrial dysfunction	Post IR injury mitochondrial dysfunction (mitochondria = cells' energy source), disturbed energy metabolism in cell, and caspase enzymes can → apoptosis Hypothermia blocks apoptotic pathway early by: ↓ caspase enzyme activation, ↓ mitochondrial dysfunction, ↓excitatory neurotransmitters, and modifying intracellular ion concentrations	Starts late in post-reperfusion phase, can continue for 72 h or more → In theory wide window for treatment
Ion pumps and neuroexcitotoxicity	IR injury → ↓ brain O_2 supply → quick ↓ in ATP and phosphocreatine levels. This initiates a complex cascade of events involving excessive calcium influx into brain cells, excessive glutamate receptor activation and neuronal hyperexcitability (excitotoxic cascade) which can lead to further injury and cell death even after reperfusion and normalisation of glutamate levels.[25–32] Hypothermia can ↓ damage from neuroexcitatory cascade	Disturbed Ca^{2+} homeostasis begins minutes after injury and may continue for many hours → may be treatable. Animal studies suggest to initiate treatment early in the neuro-excitatory cascade
Inflammation	Brain injury → Proinflammatory mediators released ++ → leukocytes drawn across BBB → ↑ inflammatory cells in brain → passage of neutrophils, phagocytic monocytes and macrophages into brain → phagocytic action and toxin production → further injury by stimulating further immune reactions Some of this is neuroprotective, but if continual and excessive → ↑ injury. Hypothermia → ↓ ischaemia-induced inflammatory and immune reactions, ↓ NO production (key agent in developing brain injury post-ischaemia), ↓ neutrophil/macrophage function and ↓ WCC	Begins ~ 1 h after ischaemia and persists for up to 5 days, suggesting a therapeutic window for these mechanisms
Free radicals	IR injury → ↑free radicals that oxidise and damage cell components → brain's defence mechanisms likely overwhelmed. Hypothermia → ↓ release of free radicals → endogenous antioxidants more able to meet demand	
Blood–brain barrier/vascular permeability	Traumatic/IR injury can disrupt BBB → brain oedema. Mild hypothermia ↓ BBB disruptions and vascular permeability after IR injury → ↓brain oedema. Brain oedema and ICH play key role in neurological injury in severe TBI and ischaemic stroke, and ICH is a marker for neurological injury → plausible that therapies to ↓ ICP may also improve neurological outcome Hypothermia has been used to ↓ ICP in neurological injury including TBI, ischaemic stroke, meningitis and SAH	Brain oedema peaks after 24–72 h → this mechanism could offer a wide therapeutic window
Acidosis and cellular metabolism	Ion-pump failure, mitochondrial dysfunction, cellular hyperactivity and ↓ in cell membrane integrity →intracellular acidosis → ↑harmful processes. Hypothermia can alleviate this, may improve brain glucose metabolism and when induced early enhances speed of metabolic recovery → ↓ toxic metabolite accumulation → ↓ acidosis	
Brain temperature	Brain temperature slightly higher than core temperature and can ↑ 0.1–2.0 °C post-injury (more with fever). Injured areas are hotter than uninjured areas due to cellular hyperactivity. Dissipation of heat by lymph/venous drainage is hampered by local brain oedema (cerebral thermo-pooling) → ↑ hyperthermia-related injury Hypothermia in brain-injured patients may ↓ potential hyperthermia-related adverse effects	
Coagulation	Activation of coagulation seems to be involved in developing IR injury Its reversal, whilst targeting other mechanisms, could improve outcomes Hypothermia induces anticoagulatory effects: mild platelet dysfunction at 33–35 °C; can affect clotting factors at ≤33 °C, and a potential reduction in platelet count, may influence synthesis and kinetics of clotting enzymes and plasminogen activator inhibitors. This anticoagulation effect could provide protection, but not investigated. Cooling to 35 °C – no effect on coagulation	Assess risk versus benefit

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Table 1 (*Continued*)

Mechanism/change	Explanation	When/treatment
Vasoactive mediators	Secretion of vasoactive substances endothelin and TxA_2 (vasoconstrictors) and prostaglandin I_2 (vasodilator) is affected by hypothermia. TxA_2 and prostaglandin I_2 regulate cerebral blood flow. Their balanced production is required to maintain homeostasis. If disrupted by ischaemia/trauma TxA_2 production increases which can → vasoconstriction and hypoperfusion in injured brain Hypothermia → ↓imbalance, but regulation of cerebral perfusion is complex and influenced by cerebral autoregulation and patient management. Influence of hypothermia on secretion of vasoactive mediator in brain-injured patients requires further investigation	
Improved tolerance of ischaemia (pre-conditioning)	In animal models 'preconditioning' with hypothermia improves tolerance for ischaemia. As brain injury is frequently complicated by ischaemic events after the initial insult, this could be a valuable neuroprotective mechanism	
Reduction of epileptic activity	Epileptic activity without signs and symptoms (non-convulsive) occurs frequently in brain-injured patients and if it occurs in the acute phase of brain injury the combined effect is destructive. Evidence indicates that hypothermia ↓ epileptic activity; another mechanism through which it could provide neuroprotection	
Early gene activation	Hypothermia → ↑early gene activation which is part of the protective cellular stress response to injury and → ↑production of cold shock proteins that can be cytoprotective in the presence of ischaemic and traumatic injury	
Shivering	↑ metabolic rate, O_2 consumption, work of breathing, heart rate and myocardial O_2 consumption	34–35 °C/opiates, sedation, paralysis if required, other agents
Insulin sensitivity and secretion	↓ with cooling → hyperglycaemia or ↑ insulin required ↑ with rewarming → hypoglycaemia or ↓ insulin required	Induction and rewarming/frequent BGL checks and insulin adjustments, slow rewarming
Cardiovascular/ haemodynamic effects	Mild hypothermia: In euvolaemic, adequately sedated pts ↓ HR, ↑ myocardial contractility, → or slightly ↑ BP, ↓ CO. ↓ metabolic rate matches or exceeds ↓ CO → balance maintained Initial transient ↑ HR due to ↑ venous return (↑ if sedation inadequate, shivering untreated) Stabilises cell membranes → ↓risk of arrhythmias, ↑ successful defibrillation. Deep hypothermia: (≤30 °C) ↓ contractility, ↑ risk for arrhythmias, ↓ successful defibrillation, ↓ response to antiarrhythmics Cold diuresis: the result of ↑ venous return (due to peripheral vessel constriction), atrial natriuretic peptide activation, ↓ ADH and renal ADH receptor levels, and tubular dysfunction → hypovolaemia. Risk ↑ if diuretic agents used e.g. mannitol	Sedate adequately Allow ↓ HR 45–55 at 33 °C (artificial ↑ HR → ↓ contractility) Avoid and correct hypovolaemia. Avoid stimulating HR
Coronary perfusion	↓ metabolic rate and HR protects ischaemic myocardium, ↑ coronary vasodilation and perfusion. But in severely atherosclerosed coronaries, vasoconstriction can occur → may affect result of hypothermia Shivering can ↑ myocardial O_2 consumption	Sedate adequately-prevent shivering
Drug clearance	Most enzyme-based reactions slowed → ↓ drug clearance by liver. Tubular dysfunction may also affect clearance, and response to some drugs alters e.g. ↓ effect of adrenaline and noradrenaline. BUT most drug levels ↑ → ↑strength and duration of effect	Modify doses of certain drugs
Infection	↓ leukocyte migration and phagocytosis, ↓ proinflammatory cytokine synthesis → ↓ proinflammatory response → may protect against damaging neuroinflammation, but ↑ risk for infection (↑ risk with ↑ duration) ↑ risk for wound infection due to cutaneous vasoconstriction Signs of infection: e.g. fever and possibly CRP and WCC ↓.	Low threshold for antibiotic treatment may be advisable ↑ in 'cooling power' required may indicate fever and infection
Gut	↓ gut function and gastric emptying, ↓ metabolic rate	Reduce feeding target in maintenance phase

↓: decrease (d), →: leads to, ↑: increase (d), *T*: temperature, O_2: oxygen, CO_2: carbon dioxide, BGs: blood gases, IR: ischaemia reperfusion, ATP: adenosine triphosphate, Ca^{2+}: calcium, BBB: blood–brain barrier, NO: nitric-oxide, WCC: white cell count, ICH: intracranial hypertension, ICP: intracranial pressure, TxA_2: thromboxane A_2, °C: degrees celcius, BGL: blood glucose level, K^+: potassium, Mg^{2+}: magnesium, PO_4^-: phosphate, TBI: traumatic brain injury, HR: heart rate, BP: blood pressure, CO: cardiac output, ADH: antidiuretic hormone, CRP: C-reactive protein.

(ATP) and phosphocreatine decrease rapidly.[24] This initiates a complex cascade of events involving excessive calcium influx into brain cells, excessive glutamate receptor activation and neuronal hyperexcitability (excitotoxic cascade) which can lead to further injury and cell death even after reperfusion and normalisation of glutamate levels.[25–32] Hypothermia seems to prevent or ameliorate damage from this neuroexcitatory cascade. Some animal studies suggest interventions need to be initiated early in the neuroexcitatory cascade for treatment to be effective,[24,33,34] again implying a narrow therapeutic time window for effective TH.

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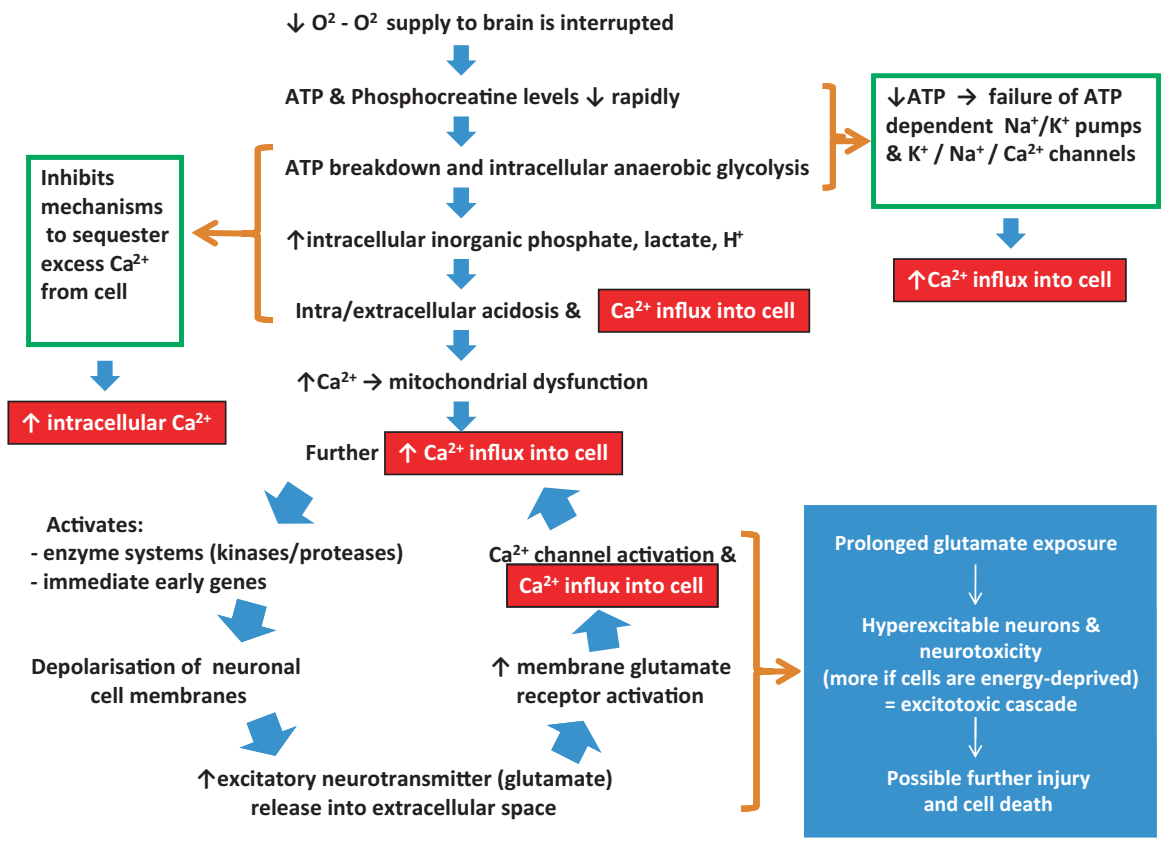

Fig. 1. The mechanism of neuroexcitotoxicity. When oxygen supply to the brain is interrupted, levels of adenosine triphosphate (ATP) and phosphocreatine decrease within seconds.[24] This initiates a complex cascade of events leading to excessive calcium influx into brain cells leading to the extracellular release of large amounts of the excitatory neurotransmitter, glutamate. This is followed by excessive glutamate receptor activation and neuronal hyperexcitability (excitotoxic cascade) which can lead to further injury and cell death even after reperfusion and normalisation of glutamate levels.[25–32] Hypothermia seems to prevent or ameliorate damage from this neuroexcitatory cascade.

Inflammation: In most brain injury, an inflammatory response begins about 1 h after ischaemia–reperfusion and persists for up to 5 days.[24,35] Large amounts of proinflammatory mediators are released inducing inflammatory and immune responses which can cause additional injury.[35] This response if controlled is adaptive and can be neuroprotective, but if continued and excessive, injury increases.[35–38] Hypothermia has been shown to suppress these reactions.[39–41] Moreover, there again appears to be a defined time window during which hypothermia could provide a therapeutic effect on these mechanisms.

Free radicals: Following ischaemia–reperfusion injury, the production of free radicals that can oxidise and damage cell components is increased, and the cell defence mechanisms to prevent such injury are likely to be overwhelmed.[29,42] With hypothermia, the release of free radicals is considerably decreased allowing endogenous anti-oxidative mechanisms to prevent or attenuate oxidative damage to cells.[29,42]

Blood brain barrier/vascular permeability: Traumatic or ischaemia–reperfusion injury may disrupt the blood–brain barrier which can result in brain oedema.[43,44] Mild hypothermia reduces such disruptions and also reduces vascular permeability after ischaemia–reperfusion injury and this may decrease brain oedema.[43–45] These findings have prompted studies investigating the use of hypothermia to attenuate secondary brain injury and therefore the development of cerebral oedema (prophylactic hypothermia) and also of 'rescue' hypothermia as a specific treatment of raised intracranial pressure (Eurotherm3235 Trial ISRCTN34555414). It is possible that therapies that reduce intra-cranial pressure such as hypothermia also improve neurological outcome,[17] however the association between controlled intra-cranial pressure and improvement in neurological outcome is uncertain.[16,46,47]

Intra-/extra-cellular acidosis and cellular metabolism: The reduction in cell membrane integrity, ion-pump failure, mitochondrial dysfunction, and cellular hyperactivity all contribute to the development of intracellular acidosis which stimulates many of these harmful processes.[48,49] These potentially harmful processes can be attenuated by hypothermia.[48,49]

Elevated brain temperature: The temperature of the brain is normally slightly higher than the core temperature[50,51] and this difference can increase between 0.1 and 2.0 °C following brain injury and further still with fever.[52–54] Animal studies indicate that hyperthermia increases the risk and degree of brain injury[55–57] and clinical studies confirm fever as an independent predictor of adverse outcomes after stroke and traumatic brain injury.[58–60] Therefore, the use of hypothermia in brain-injured patients may prevent or ameliorate potential hyperthermia-related adverse effects.

Coagulation: Activation of coagulation appears to play a role in the development of ischaemia–reperfusion injury.[17,61] Reversal of this mechanism, combined with targeting of other mechanisms, could improve outcomes.[17] Hypothermia induces some anticoagulatory effects which could in theory provide neurological protection.[17]

The effect of hypothermia on coagulation is summarised in Table 1. Whilst there have been no significant bleeding problems in many studies of patients with severe TBI, stroke or cardiac arrest, patients with active bleeding were generally excluded from these studies.[14,16] However, as the effect on clotting factors only

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becomes significant below 33 °C, it may be that cooling to 35 °C can be safely undertaken in patients with active bleeding.[17] Once bleeding is controlled in such patients, further cooling to 33 °C may be reasonable.[17]

Vasoactive mediators: A number of studies indicate that the secretion of vasoactive substances (endothelin and thromboxane A_2 [vasoconstrictors], prostaglandin I_2 [vasodilator]) at sites including the brain is affected by hypothermia. Thromboxane A_2 and prostaglandin I_2 are key regulators of cerebral blood and balanced production is required to maintain homeostasis.[62,63] Disruption to such production by ischaemia/trauma can cause damaging results to perfusion.[64,65] Evidence suggests that hypothermia attenuates such imbalances[27,66]; however, the regulation of cerebral perfusion after neurological injury is complex and influenced by cerebral autoregulation and patient management factors.[17] Therefore, the influence of hypothermia on the secretion of vasoactive mediators in brain-injured patients requires further investigation taking this contextual complexity into account.

Improved tolerance of ischaemia: In several animal models, 'preconditioning' with hypothermia improves the subsequent tolerance for ischaemia.[67,68] Considering that brain injury is frequently complicated by ischaemic events occurring after the initial insult, this may also be a valuable neuroprotective mechanism.

Reduction of epileptic activity: Epileptic activity without clinical signs and symptoms (non-convulsive) occurs frequently in brain-injured patients. When non-convulsive epileptic activity occurs in the setting of acute brain injury the combined effect is injurious.[69,70] Evidence indicates that hypothermia suppresses epileptic activity[71,72] and this is an additional mechanism through which hypothermia could provide neuroprotection.

Early gene activation: Hypothermia stimulates early gene activation which is part of the protective cellular stress response to injury and promotes production of cold shock proteins that can be cytoprotective in the presence of ischaemic and traumatic injury.[17]

Shivering: The unfavourable effects of shivering can generally be suppressed by administering adequate sedation and paralytic agents during induction of cooling. In addition, sedation may lead to vasodilation which promotes heat loss by surface cooling. The neuroprotective effect of hypothermia may be lost if adequate sedation is not administered.[73]

Cardiovascular/haemodynamic effects: The effects of hypothermia on the myocardium and its contractility partly depend on the patient's volume status and adequacy of sedation.[17] Table 1 summarises the changes to heart function related to hypothermia. The decrease in metabolic rate usually matches or exceeds the reduction in cardiac output thus maintaining or improving the equilibrium between supply and demand.[14] The heart rate decreases with a lower temperature (40–50 at 33 °C) and it is generally advisable to allow this decrease.[17] Myocardial contractility improves when heart rate decreases with hypothermia, and artificially increasing heart rate during hypothermia markedly decreases myocardial contractility.[74,75] Hypothermia can also produce 'cold diuresis' causing hypovolemia.[17] Prompt correction of hypovolaemia may prevent hypotension.

Coronary perfusion: Hypothermia could provide protection for ischaemic myocardium, however this may be affected by coronary artery disease.[76,77] There is preliminary evidence that suggests that early induction of hypothermia after myocardial infarction may ameliorate subsequent myocardial injury.[16]

Drug clearance: Clearance of drugs by the liver and the kidneys is decreased during hypothermia.[14,16,78] Therefore, the levels of most drugs increase with hypothermia and it may be reasonable to modify doses of certain drugs.[17]

Infection: Hypothermia suppresses the pro-inflammatory response. Whilst this may protect against damaging neurological injury, the risk for infection is potentially increased.[14]

Other: Hypothermia is linked with reduced gut function.[14] In addition, fat metabolism increases with hypothermia leading to higher levels of free fatty acids, ketones and lactate, which may reduce pH. Liver enzymes may also become elevated.[17]

In summary, hypothermia may attenuate secondary injury and provide protection in the presence of many injurious processes after ischaemic or traumatic injury. The large range of mechanisms of action through which hypothermia may confer its protective effects is a key to its efficacy compared with treatments that target only one or two mechanisms.[17] A better understanding of the destructive mechanisms and side effects involved in different types and phases of injury will potentially enable the application of hypothermia more effectively in the future.

What is the clinical evidence?

Hypothermia is the first treatment shown to be potentially efficacious in clinical trials for postischaemic injury. We will examine the evidence for the use of TH for neurological injury, ischaemic cardiac injury and the early evidence of benefit in acute kidney injury.

Neurological injury

Out-of-hospital cardiac arrest: After positive results from animal studies[33,79,80] small clinical trials in patients who remained comatose following cardiac arrest showed improved outcomes compared to historical controls.[81–85] Subsequently, randomised controlled trials showed positive neurological results for cooling after cardiac arrest.[1,2] The initial cardiac rhythm in these studies was ventricular fibrillation or pulseless ventricular tachycardia. Bernard et al.[1] recruited 77 patients and cooling was initiated during CPR at the scene or early during transport to hospital with a target temperature of 33 °C for 12 h. The rate of favourable neurological outcomes was 49% (21/43) in the hypothermia group, and 26% (9/34) in the control group ($p = 0.046$). The adjusted odds ratio for good outcome in the hypothermia versus normothermia group in multivariate analysis was 5.25 (1.47–18.76, $p = 0.011$). Similarly, in the European study[2] ($n = 273$) favourable neurological outcome rate was 55% (75/136) in the hypothermia group and 39% in controls. Cooling was started after a median time of 105 min and maintained for 24 h at 32–34 °C (target temperature was reached at an average of 8 h after return of circulation). American Heart Association[86] and European Resuscitation Council guidelines[87] now recommend using TH after cardiac arrest with an initial rhythm of ventricular tachycardia or pulseless ventricular fibrillation (VF) and consideration of its use for other rhythms.

Recently a further trial by Bernard et al. of TH for out-of-hospital VF cardiac arrest patients found that paramedic cooling with a rapid infusion of cold fluid decreased core temperature at hospital arrival.[88] However, it was not shown to improve outcome at hospital discharge compared with cooling commenced in the hospital. The difference in patient temperature between the groups was modest (0.8 °C) and of relatively short duration, possibly due to the short transport times in a large metropolitan network of hospitals. Such a brief difference in core temperature may have been insufficient to significantly affect neurological outcomes at hospital discharge. The RINSE trial (NCT01172678) which is currently recruiting in Australia, may help to clarify the benefit that immediate induction of hypothermia provides for any patient with an out-of-hospital cardiac arrest receiving cardiopulmonary resuscitation. This RCT aims to determine whether immediate cooling during cardiopulmonary resuscitation by paramedics

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using a rapid infusion of cold saline, improves outcome at hospital discharge, compared with standard care.

Peri-natal hypoxic ischaemic encephalopathy: Early studies indicated that prolonged mild hypothermia after perinatal hypoxic-ischaemic encephalopathy reduced brain injury and improved neurological outcome.[89] However, the results of several clinical trials of hypothermia in such patients were inconclusive.[90–93] When neurological outcome at ≥18 months was assessed, a composite outcome of death or disability was used making interpretation difficult, and estimates for therapeutic benefit rarely reached statistical significance.[94] A recent meta-analysis including more recent studies has helped to clarify this issue, finding that moderate hypothermia is associated with a reduction in mortality and neurological dysfunction at 18 months in infants with hypoxic-ischaemic encephalopathy.[94] Cumulatively, these findings are gradually leading to a practice change and increasing adoption of hypothermia in treatment of this group.[95,96]

Traumatic brain injury: Experimental models of TBI indicate that hypothermia induced pre-injury reduces neurological damage and mortality, and improves neurological outcomes.[97–99] Animal studies also suggest that if hypothermia is induced within a few hours of the primary injury, it may also improve neurological outcomes.[100–104]

Over the last 20 years, numerous clinical studies have investigated hypothermia in TBI patients and many studies support a likely benefit, however they vary in study design in a number of key factors such as rate of induction, duration of hypothermia, and rate of re-warming.[105–108] There is still considerable uncertainty surrounding the use of hypothermia to treat TBI. Six adult patient meta-analyses have been performed with different inclusion criteria.[4,5,109–112] The most recent meta-analyses found an increase in favourable long-term neurological outcomes and/or mortality in patients receiving hypothermia versus normothermia.[4,5] This resulted in the first grade III recommendation for the use of hypothermia in adult patients with severe TBI.[4]

Although most single centre studies found hypothermia to be effective, the Clifton study found no improvement in outcomes for adults[46] and the Hutchison study found no improvement in children,[113] instead there was a trend towards higher mortality in children receiving hypothermia ($p = 0.06$). However, these two trials[46,113] had significant methodological limitations which may have prevented detection of benefit and in the hypothermia groups may have caused harm.

In these two studies, induction of hypothermia only commenced on hospital arrival and was further delayed by the lack of rapid cooling methods. In the Clifton study, the time to target temperature was 8.4 h[46] and in the Hutchison study it was 10.2 h.[113] Animal models suggest that the 'therapeutic window' may have been missed due to these delays, decreasing the likelihood that these trials would show a beneficial effect of hypothermia. In the Clifton trial a subgroup of patients from the hypothermia group who were hypothermic on arrival to hospital had significantly better long-term neurological outcome compared to normothermic patients,[46] which may support the proposed beneficial effect of early hypothermia. In order to test whether earlier hypothermia is beneficial, paramedic initiated hypothermia at the location of injury would be ideally tested in future trials.

The duration of hypothermia in the 2 multi-centre trials was 24 h in the Hutchison trial[113] and 48 h in the Clifton trial[46] and this may not be sufficient given that cerebral oedema is often most marked at this time. Brain tissue swelling in severe TBI often lasts 3–5 days[114] and in recent adult meta-analyses hypothermia applied for >48 h was associated with reductions in mortality and more favourable neurological outcomes.[4,5] Future trials should therefore maintain therapeutic hypothermia for >48 h.

In the Hutchison[113] and Clifton[46] multicentre studies, re-warming was performed at a pre-set time, regardless of the patients' intracranial pressure (ICP). Initiation of re-warming in TBI patients with high ICP may account for the increased use of vasopressors and hyperventilation in the hypothermic group of children.[113] On the other hand, recent meta-analyses found that re-warming rate was not an independent risk factor for survival,[4,5] nevertheless in future trials, re-warming may be better guided by a clinically relevant physiological trigger such as ICP and re-warming occur over a prolonged period (≤0.25 °C/h) to prevent rebound and uncontrolled intracranial hypertension.[114]

Another consideration in the Hutchison trial was that neurological outcomes were better than expected in normothermic patients.[113] This may have been due to prevention of hyperpyrexia and it may be that the benefit of hypothermia in previous studies is attributable to the prevention of hyperpyrexia rather than a therapeutic effect of hypothermia.[114] Future trials should therefore include strict temperature control and prevention of hyperpyrexia in the control group. Another criticism of the Clifton trial was the variance in effect of hypothermia between centres which may have decreased the ability to detect an overall beneficial effect of hypothermia.[114]

Clifton and colleagues have recently completed a further trial of prophylactic hypothermia in patients with severe TBI which was prematurely terminated, likely as a result of methodological issues. TBI patients were enrolled within 2.5 h of injury addressing the question of whether prophylactic hypothermia had been commenced too late in the past; however a 48 h duration of hypothermia may not be sufficient, and rewarming at 48 h without allowance for individualized management of ICP in relation to cooling is problematic. In addition, the protocol allowed for large volumes of IV fluid administration which could lead to intracranial hypertension. These and other concerns make this trial's findings inconclusive and the question still remains as to whether prophylactic hypothermia for severe TBI improves neurological outcomes. Further investigation is required.

Peri-operative hypothermia: Intraoperative hypothermia is widely used during cardiac and neurological surgery but without strong evidence from randomised controlled trials. As the ischaemic injury is predictable, hypothermia can commence pre-injury and theoretically prevent damaging processes.

In cardiac surgery, results of clinical studies to assess the benefit of hypothermia are inconclusive. A meta-analysis by Rees et al. examining the effect of hypothermia during cardiopulmonary bypass on neurological outcomes found a trend towards a reduced incidence of non fatal strokes in the hypothermic group (OR 0.68, 95%CI 0.43–1.05).[115] On the other hand, there was a trend for a higher number of non-stroke related perioperative deaths in the hypothermic group (OR1.46, 0.9–2.37).[115] Nathan et al. found that patients taken off cardiopulmonary bypass at a lower temperature (34 °C versus 37 °C) showed better neurocognitive performance 1 week and 3 months after cardiac bypass surgery,[10] however this benefit was not sustained at 5 year follow-up.[116] Inconsistencies in study designs may explain some of this variation in results: patient population; site and method for temperature determination; cardiopulmonary bypass techniques; temperature regime; myocardial protection; surgical techniques; rewarming rates and postoperative management of hyperthermia may all differ between patients and hospitals which can affect outcome. Cognitive deficits post cardiopulmonary bypass surgery could potentially be linked to rapid re-warming which decreases venous oxygen saturation.[117] Stronger evidence supports the avoidance of fast rewarming[118] and hyperthermia[119] during or after cardiopulmonary bypass surgery.

In aortic surgery, animal studies[120–123] and early clinical trials using cooling to protect the spinal cord[17–119] showed promising

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results. On the other hand, hypothermia showed no difference in outcome in a large clinical study of patients undergoing cerebral aneurysm clipping.[124] However, these patients received rapid rewarming and there is evidence that re-warming rapidly can decrease the benefits of hypothermia or even worsen outcomes.

Importantly, postoperative hyperthermia may be associated with more cognitive deficits.[125,126] Conflicting results in some studies of peri-operative therapeutic hypothermia may be due to the harmful effects of rapid re-warming. Therefore, slower re-warming should be adopted in future studies using intraoperative hypothermia.

Ischaemic stroke: Animal models[127–130] suggest hypothermia could limit neurological injury in stroke. However, the clinical studies have been relatively small with late initiation of hypothermia when much of the injury may have become irreversible.[131–137] Some recent studies have combined hypothermia with thrombolysis to simultaneously restore blood flow and prevent reperfusion injury.[138,139] Whilst this approach appears to be safe, larger prospective studies are needed to assess benefit. However, the requirement for close monitoring and management of temperature in non-sedated patients may require ICU admission and this presents a challenge for future studies.

Cardiac injury

Animal studies and preliminary human studies suggest a protective effect of hypothermia on the ischaemic heart,[140] and several studies have shown that mild hypothermia is feasible and safe to apply in the setting of acute myocardial infarction.[11,141] However, mild hypothermia has not been shown in prospective randomised trials to significantly reduce infarct size or mortality rate.[142] Based on current evidence, the use of hypothermia to reduce infarct size or improve heart function cannot be recommended[140,142] but if patients are rapidly cooled to target temperature quickly after cardiac arrest, and this is maintained at 32–34 °C for the recommended period, then their myocardial function and neurological outcome may both be improved.[140] However, further studies are required before recommendations regarding the induction of hypothermia following cardiac injury could be made.

Kidney injury

Whilst some of the mechanisms of action of hypothermia previously mentioned are more applicable to brain injury, many of these protective mechanisms could potentially provide benefit if hypothermia was used prophylactically for the kidneys after injury or in patients at high risk for kidney injury undergoing procedures recognised as injurious for the kidneys.

In a renal ischemia-reperfusion injury model of hypothermia, the temperature of the rats during the ischemia phase significantly affected the severity of injury. Rats kept hyperthermic at around 40 °C developed more severe and unrecoverable renal injury (measured by creatinine), rats kept at ~37 °C developed 'recoverable' renal injury, whereas those at ~33 °C had reduced renal injury.[12] Furthermore, Zager et al. found that hypothermia was very effective during ischemia in rats, and also effective but to a lesser extent when applied during early reflow post-ischemia, and that these benefits were additive.[13] However, there has been little clinical investigation of the renal effects of TH. A pilot study of 30 patients undergoing angiographic procedures to assess whether mild hypothermia reduces the incidence of contrast-induced nephropathy in patients with pre-existing renal injury (serum creatinine rise from baseline ≥25%) found the incidence to be 10% in those who received hypothermia compared to 40% in historical controls.[16]

In the European multi-centre trial[85] which evaluated the neurological effects of 24 h of mild hypothermia (32–34 °C) after cardiac arrest, the effect on renal function was also evaluated.[143] There was no difference between groups in the incidence of acute kidney injury or the need for renal replacement therapy. There was a delayed improvement in renal function in the cooled group indicated by creatinine clearance (but not in serum creatinine) that occurred within 4 weeks.[143]

Two randomised trials have been conducted to evaluate mild hypothermia for 24 h and rewarming on kidney function after coronary artery bypass graft surgery.[144] In one trial, 223 patients were cooled to 32 °C during cardiopulmonary bypass and randomised to rewarm to 37 °C or 34 °C.[144] In this trial, patients rewarmed to 37 °C had a higher incidence of renal injury (25% increase in serum creatinine or a 25% decrease in creatinine clearance) than those rewarmed to 34 °C (17% versus 9%, $p = 0.07$). In a second trial, 267 patients were randomised to mild hypothermia at 34 °C or normothermia (37 °C).[144] In this trial, mild hypothermia provided no benefit in serum creatinine levels or reduction in incidence of renal injury compared to normothermia (20%: 34 °C versus 15%: 37 °C, $p = 0.28$). Rewarming on cardiopulmonary bypass in this study was an independent risk factor for renal injury. Given the association of cardiopulmonary bypass technique with increased risk for kidney injury,[145,146] the use of this method of re-warming to assess the effect of hypothermia on renal function may not be optimal. Instead, continuation of cooling postoperatively using advanced techniques followed by slow rewarming might be considered in this setting.

Overall, there is some experimental evidence and a plausible rationale to suggest a potential benefit of prophylactic hypothermia for kidney injury, however clinical evidence is inconclusive. Further investigation in this area is warranted, particularly considering the largely unsuccessful attempts to find effective therapies for acute kidney injury (AKI).[147]

Hypothermia and AKI

AKI is common in the ICU and occurs in approximately 36% of critically ill patients.[148,149] AKI is independently associated with increased mortality,[150] and with prolonged length of stay.[146,151] It increases both the human and financial costs of care. Therefore, it is important to investigate treatments with potential to ameliorate or prevent AKI.

The most extensively used and validated consensus definition and method of classifying AKI is the RIFLE classification system.[152] The serum creatinine cutoffs of the RIFLE criteria are most commonly used to classify the severity of AKI: no AKI: <50% increase in SCr, risk of AKI: 50–200% increase, kidney injury: >200–300% increase, failure of kidney function: >300% increase or SCr ≥4 mg/dl with a rise ≥0.5 mg/dl.

Some injury pathways for AKI in the critically ill include exposure to endogenous and exogenous toxins, metabolic factors, ischaemia and reperfusion insults, neurohormonal activation, inflammation, and oxidative stress. Of these, ischaemia–reperfusion may be the most common. Hypothermia may prevent or reduce injury and assist renal recovery through a reduction in metabolic demand, decreased production of free radicals, promotion of cellular integrity, limitation of apoptosis and through anti-inflammatory effects.[17]

Investigations of potential treatments for AKI have had limited success to date,[147] however, from the results of animal and human studies, further investigation of hypothermia as a prophylactic therapy for kidney injury appears warranted for patients at high risk for AKI.[12,13,16,143] The POLAR trial which has commenced recruitment in Australia and New Zealand with the primary aim to assess the effect of prophylactic hypothermia on neurological

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outcome, is doing just this, by also investigating the effect of hypothermia on renal function in its renal substudy.

Prophylactic hypothermia: POLAR and POLAR-acute kidney injury

POLAR (*P*rophylactic Hyp*O*thermia to *L*essen tr*A*umatic brain inju*R*y), is a randomised, blinded, controlled trial of hypothermia in ICU patients with severe traumatic brain injury (TBI). The trial is being conducted at 6 sites in Australia and New Zealand and recruitment has commenced (ACTRN12609000764235). POLAR is endorsed by the Australian and New Zealand Clinical Trials Group (ANZICS CTG) and has NHMRC and Victorian Neurotrauma Initiative funding. The trial has a planned cohort of 500 patients and is one of the largest TBI trials currently being conducted. Participants will be intubated, severe TBI patients (GCS $\leq$ 8), aged between 18 and 60 years who are able to be randomised within 3 h of injury. Patients will be randomised to receive either early and sustained hypothermia (33 °C) for $\geq$72 h (maximum 7 days) using state of the art cooling systems, followed by slow controlled rewarming (0.17 °C/h = 4 °C/24 h); or normothermia (36.5–37.5 °C). Hypothermia will be induced as quickly and early as possible and rewarming will proceed after 72 h of cooling if the ICP is controlled and the patient has achieved a degree of stability. Pre-hospital randomisation at the scene of injury and cooling with cold fluid (0.9% saline at 4 °C) will be performed by paramedics. In addition, early emergency department recruitment will occur. Previous Australian experience with pre-hospital cooling of cardiac arrest patients will be applied.[88] Guidelines for standard care applicable to both groups have been provided and a run-in phase is underway. Patient recruitment is being undertaken at sites that receive larger volumes of major trauma and have experience in the care of TBI patients to reduce the effect of variance in patient care. The primary outcome measure is the proportion of favourable neurological outcomes at 6 months post-injury (Glasgow outcome scale, extended (GOSE) 5–8). This is considered the most appropriate primary outcome given the large human and financial benefit that may result from an increase in favourable neurological outcomes in TBI.[114]

'POLAR-AKI' and 'POLAR-Biomarkers' comprise the Intensive Care Foundation-funded renal substudy of the POLAR trial to assess the effect of prophylactic TH on the development of AKI and the response to this treatment using multiple renal biomarkers with different time profiles. This cohort of patients are ideal for a study of hypothermia for renal injury since there is a moderate incidence of AKI in this group,[148,153] and all patients have recently sustained a timed physical injury. AKI will be classified using methods based on the RIFLE criteria; a classification system used extensively and validated to classify renal function in several populations with studies cumulatively involving over 250,000 subjects.[152] With a cohort of 500 patients (all POLAR trial participants), this will be the largest study of hypothermia to protect against AKI yet performed, increasing the probability of detecting a treatment effect of TH. The POLAR trial provides a unique opportunity to clarify the potential benefit of hypothermia as a brain protective and kidney protective therapy. This trial when completed may also provide insight into the mechanisms of action of therapeutic hypothermia in AKI.

Conflict of interest statement

Alistair Nichol and Stephen Bernard are investigators of the POLAR trial, a National Health and Medical Research Council/Victorian Neurotrauma Initiative funded clinical trial of Hypothermia in Traumatic Brain Injury (NCT00987688). Elizabeth Moore, Alistair Nichol and Rinaldo Bellomo are investigators of the Renal Substudy of the POLAR trial.

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References

1. Bernard SA, Gray TW, Buist MD, *et al.* Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med* 2002; **346**:557–63.
2. Hypothermia after Cardiac Arrest Study Group. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. *N Engl J Med* 2002;**346**:549–56.
3. Jacobs S, Hunt R, Tarnow-Mordi W, Inder T, Davis P. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev* 2007. CD003311.
4. Brain Trauma Foundation. American Association of Neurological Surgeons. Congress of Neurological Surgeons. Guidelines for the management of severe traumatic brain injury. *J Neurotrauma* 2007;**24**(Suppl 1):S1–106.
5. Peterson K, Carson S, Carney N. Hypothermia treatment for traumatic brain injury: a systematic review and meta-analysis. *J Neurotrauma* 2008;**25**:62–71.
6. Bernard S. New indications for the use of therapeutic hypothermia. *Critical Care (London England)* 2004;**8**:E1.
7. Bernard SA, Buist M. Induced hypothermia in critical care medicine: a review. *Crit Care Med* 2003;**31**:2041–51.
8. Apostolakis E, Shuhaiber JH. Antegrade or retrograde cerebral perfusion as an adjunct during hypothermic circulatory arrest for aortic arch surgery. *Expert Rev Cardiovasc Ther* 2007;**5**:1147–61.
9. Mack WJ, Ducruet AF, Angevine PD, *et al.* Deep hypothermic circulatory arrest for complex cerebral aneurysms: lessons learned. *Neurosurgery* 2007;**60**:815–27.
10. Nathan HJ, Wells GA, Munson JL, Wozny D. Neuroprotective effect of mild hypothermia in patients undergoing coronary artery surgery with cardiopulmonary bypass: a randomized trial. *Circulation* 2001;**104**:I85–91.
11. Dixon SR, Whitbourn RJ, Dae MW, *et al.* Induction of mild systemic hypothermia with endovascular cooling during primary percutaneous coronary intervention for acute myocardial infarction. *J Am Coll Cardiol* 2002;**40**:1928–34.
12. Delbridge MS, Shrestha BM, Raftery AT, El Nahas AM, Haylor JL. The effect of body temperature in a rat model of renal ischemia-reperfusion injury. *Transplant Proc* 2007;**39**:2983–5.
13. Zager RA, Gmur DJ, Bredl CR, Eng MJ. Degree and time sequence of hypothermic protection against experimental ischemic acute renal failure. *Circ Res* 1989;**65**:1263–9.
14. Polderman KH, Herold I. Therapeutic hypothermia and controlled normothermia in the intensive care unit: practical considerations, side effects, and cooling methods. *Crit Care Med* 2009;**37**:1101–20.
15. van den Broek MP, Groenendaal F, Egberts AC, *et al.* Effects of hypothermia on pharmacokinetics and pharmacodynamics: a systematic review of preclinical and clinical studies. *Clin Pharmacokinet* 2010;**49**:277–94.
16. Polderman KH. Induced hypothermia and fever control for prevention and treatment of neurological injuries. *Lancet* 2008;**371**:1955–69.
17. Polderman KH. Mechanisms of action, physiological effects, and complications of hypothermia. *Crit Care Med* 2009;**37**:S186–202.
18. Polderman KH, Peerdeman SM, Girbes AR. Hypophosphatemia and hypomagnesemia induced by cooling in patients with severe head injury. *J Neurosurg* 2001;**94**:697–705.
19. Weisinger JR, Bellorin-Font E. Magnesium and phosphorus. *Lancet* 1998;**352**: 391–6.
20. Polderman KH, van Zanten AR, Girbes AR. The importance of magnesium in critically ill patients: a role in mitigating neurological injury and in the prevention of vasospasms. *Intensive Care Med* 2003;**29**:1202–3.
21. Liou AK, Clark RS, Henshall DC, Yin XM, Chen J. To die or not to die for neurons in ischemia, traumatic brain injury and epilepsy: a review on the stress-activated signaling pathways and apoptotic pathways. *Prog Neurobiol* 2003;**69**:103–42.
22. Ning XH, Chen SH, Xu CS, *et al.* Hypothermic protection of the ischemic heart via alterations in apoptotic pathways as assessed by gene array analysis. *J Appl Physiol* 2002;**92**:2200–7.
23. Xu L, Yenari MA, Steinberg GK, *et al.* Mild hypothermia reduces apoptosis of mouse neurons in vitro early in the cascade. *J Cereb Blood Flow Metab* 2002;**22**:21–8.
24. Small DL, Morley P, Buchan AM. Biology of ischemic cerebral cell death. *Prog Cardiovasc Dis* 1999;**42**:185–207.
25. Auer RN. Non-pharmacologic (physiologic) neuroprotection in the treatment of brain ischemia. *Ann N Y Acad Sci* 2001;**939**:271–82.
26. Baker AJ, Zornow MH, Grafe MR, *et al.* Hypothermia prevents ischemia-induced increases in hippocampal glycine concentrations in rabbits. *Stroke* 1991; **22**:666–73.
27. Busto R, Dietrich WD, Globus MY, *et al.* Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. *J Cereb Blood Flow Metab* 1987;**7**:729–38.

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28. Busto R, Globus MY, Dietrich WD, *et al.* Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. *Stroke* 1989;**20**:904–10.
29. Globus MY, Alonso O, Dietrich WD, *et al.* Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. *J Neurochem* 1995;**65**:1704–11.
30. Hall ED, Braughler JM. Free radicals in CNS injury. *Res Publ Assoc Res Nerv Ment Dis* 1993;**71**:81–105.
31. Leker RR, Shohami E, Leker RR, Shohami E. Cerebral ischemia and trauma-different etiologies yet similar mechanisms: neuroprotective opportunities. *Brain Res Brain Res Rev* 2002;**39**:55–73.
32. Siesjo B, Bengtsson F, Grampp W, Theander S. Calcium, excitotoxins, and neuronal death in the brain. *Ann N Y Acad Sci* 1989;**568**:234–51.
33. Kuboyama K, Safar P, Radovsky A, *et al.* Delay in cooling negates the beneficial effect of mild resuscitative cerebral hypothermia after cardiac arrest in dogs: a prospective, randomized study. *Crit Care Med* 1993;**21**:1348–58.
34. Takata K, Takeda Y, Sato T, *et al.* Effects of hypothermia for a short period on histologic outcome and extracellular glutamate concentration during and after cardiac arrest in rats. *Crit Care Med* 2005;**33**:1340–5.
35. Schmidt OI, Heyde CE, Ertel W, Stahel PF. Closed head injury—an inflammatory disease? *Brain Res Brain Res Rev* 2005;**48**:388–99.
36. Merrill JE, Benveniste EN. Cytokines in inflammatory brain lesions: helpful and harmful. *Trends Neurosci* 1996;**19**:331–8.
37. Morganti-Kossmann MC, Rancan M, Stahel PF, *et al.* Inflammatory response in acute traumatic brain injury: a double-edged sword. *Curr Opin Crit Care* 2002;**8**:101–5.
38. Patel HC, Boutin H, Allan SM. Interleukin-1 in the brain: mechanisms of action in acute neurodegeneration. *Ann N Y Acad Sci* 2003;**992**:39–47.
39. Aibiki M, Maekawa S, Ogura S, *et al.* Effect of moderate hypothermia on systemic and internal jugular plasma IL-6 levels after traumatic brain injury in humans. *J Neurotrauma* 1999;**16**:225–32.
40. Kimura A, Sakurada S, Ohkuni H, *et al.* Moderate hypothermia delays proinflammatory cytokine production of human peripheral blood mononuclear cells. *Crit Care Med* 2002;**30**:1499–502.
41. Suehiro E, Fujisawa H, Akimura T, *et al.* Increased matrix metalloproteinase-9 in blood in association with activation of interleukin-6 after traumatic brain injury: influence of hypothermic therapy. *J Neurotrauma* 2004;**21**:1706–11.
42. Novack TA, Dillon MC, Jackson WT. Neurochemical mechanisms in brain injury and treatment: a review. *J Clin Exp Neuropsychol Off J Int Neuropsychol Soc* 1996;**18**:685–706.
43. Chi OZ, Liu X, Weiss HR. Effects of mild hypothermia on blood–brain barrier disruption during isoflurane or pentobarbital anesthesia. *Anesthesiology* 2001;**95**:933–8.
44. Huang ZG, Xue D, Preston E, *et al.* Biphasic opening of the blood–brain barrier following transient focal ischemia: effects of hypothermia. *Can J Neurol Sci* 1999;**26**:298–304.
45. Jurkovich GJ, Pitt RM, Curreri PW, Granger DN. Hypothermia prevents increased capillary permeability following ischemia-reperfusion injury. *J Surg Res* 1988;**44**:514–21.
46. Clifton GL, Miller ER, Choi SC, *et al.* Lack of effect of induction of hypothermia after acute brain injury. *N Engl J Med* 2001;**344**:556–63.
47. Polderman KH, Ely EW, Badr AE, Girbes ARJ. Induced hypothermia in traumatic brain injury: considering the conflicting results of meta-analyses and moving forward. *Intensive Care Med* 2004;**30**:1860–4.
48. Ding D, Moskowitz SI, Li R, *et al.* Acidosis induces necrosis and apoptosis of cultured hippocampal neurons. *Exp Neurol* 2000;**162**:1–12.
49. Natale JA, D'Alecy LG. Protection from cerebral ischemia by brain cooling without reduced lactate accumulation in dogs. *Stroke* 1989;**20**:770–7.
50. Crowder CM, Tempelhoff R, Theard MA, *et al.* Jugular bulb temperature: comparison with brain surface and core temperatures in neurosurgical patients during mild hypothermia. *J Neurosurg* 1996;**85**:98–103.
51. Nybo L, Secher NH, Nielsen B, *et al.* Inadequate heat release from the human brain during prolonged exercise with hyperthermia. *J Physiol (Lond)* 2002;**545**:697–704.
52. Childs C, Vail A, Protheroe R, *et al.* Differences between brain and rectal temperatures during routine critical care of patients with severe traumatic brain injury. *Anaesthesia* 2005;**60**:759–65.
53. McIlvoy L, McIlvoy L. Comparison of brain temperature to core temperature: a review of the literature. *J Neurosci Nurs* 2004;**36**:23–31.
54. Verlooy J, Heytens L, Veeckmans G, Selosse P. Intracerebral temperature monitoring in severely head injured patients. *Acta Neurochir (Wien)* 1995; **134**:76–8.
55. Baena RC, Busto R, Dietrich WD, *et al.* Hyperthermia delayed by 24 hours aggravates neuronal damage in rat hippocampus following global ischemia. *Neurology* 1997;**48**:768–73.
56. Hickey RW, Kochanek PM, Ferimer H, *et al.* Induced hyperthermia exacerbates neurologic neuronal histologic damage after asphyxial cardiac arrest in rats. *Crit Care Med* 2003;**31**:531–5.
57. Wass CT, Lanier WL, Hofer RE, *et al.* Temperature changes of > or =1 degree C alter functional neurologic outcome and histopathology in a canine model of complete cerebral ischemia. *Anesthesiology* 1995;**83**:325–35.
58. Hajat C, Hajat S, Sharma P. Effects of poststroke pyrexia on stroke outcome: a meta-analysis of studies in patients. *Stroke* 2000;**31**:410–4.
59. Reith J, Jorgensen HS, Pedersen PM, *et al.* Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. *Lancet* 1996;**347**:422–5.
60. Soukup J, Zauner A, Doppenberg EM, *et al.* The importance of brain temperature in patients after severe head injury: relationship to intracranial pressure, cerebral perfusion pressure, cerebral blood flow, and outcome. *J Neurotrauma* 2002;**19**:559–71.
61. Gando S, Kameue T, Nanzaki S, Nakanishi Y. Massive fibrin formation with consecutive impairment of fibrinolysis in patients with out-of-hospital cardiac arrest. *Thromb Haemost* 1997;**77**:278–82.
62. de Leval X, Hanson J, David JL, *et al.* New developments on thromboxane and prostacyclin modulators part II: prostacyclin modulators. *Curr Med Chem* 2004;**11**:1243–52.
63. Dogne JM, de Leval X, Hanson J, *et al.* New developments on thromboxane and prostacyclin modulators part I: thromboxane modulators. *Curr Med Chem* 2004;**11**:1223–41.
64. Chen ST, Hsu CY, Hogan EL, *et al.* Thromboxane, prostacyclin, and leukotrienes in cerebral ischemia. *Neurology* 1986;**36**:466–70.
65. Hsu CY, Halushka PV, Hogan EL, *et al.* Alteration of thromboxane and prostacyclin levels in experimental spinal cord injury. *Neurology* 1985;**35**:1003–9.
66. Aibiki M, Maekawa S, Yokono S. Moderate hypothermia improves imbalances of thromboxane A2 and prostaglandin I2 production after traumatic brain injury in humans. *Crit Care Med* 2000;**28**:3902–6.
67. Yuan HB, Huang Y, Zheng S, *et al.* Hypothermic preconditioning increases survival of purkinje neurons in rat cerebellar slices after an in vitro simulated ischemia. *Anesthesiology* 2004;**100**:331–7.
68. Yunoki M, Nishio S, Ukita N, *et al.* Hypothermic preconditioning induces rapid tolerance to focal ischemic injury in the rat. *Exp Neurol* 2003;**181**:291–300.
69. Jordan KG. Nonconvulsive status epilepticus in acute brain injury. *J Clin Neurophysiol* 1999;**16**:332–40.
70. Krumholz A. Epidemiology and evidence for morbidity of nonconvulsive status epilepticus. *J Clin Neurophysiol* 1999;**16**:314–22.
71. Lundgren J, Smith ML, Blennow G, *et al.* Hyperthermia aggravates and hypothermia ameliorates epileptic brain damage. *Exp Brain Res* 1994;**99**:43–55.
72. Yager JY, Armstrong EA, Jaharus C, *et al.* Preventing hyperthermia decreases brain damage following neonatal hypoxic-ischemic seizures. *Brain Res* 2004;**1011**:48–57.
73. Sessler DI. Thermoregulatory defense mechanisms. *Crit Care Med* 2009;**37**: S203–210.
74. Lewis ME, Al-Khalidi AH, Townend JN, *et al.* The effects of hypothermia on human left ventricular contractile function during cardiac surgery. *J Am Coll Cardiol* 2002;**39**:102–8.
75. Mattheussen M, Mubagwa K, Van Aken H, *et al.* Interaction of heart rate and hypothermia on global myocardial contraction of the isolated rabbit heart. *Anesth Analg* 1996;**82**:975–81.
76. Frank SM, Satitpunwaycha P, Bruce SR, *et al.* Increased myocardial perfusion and sympathoadrenal activation during mild core hypothermia in awake humans. *Clin Sci* 2003;**104**:503–8.
77. Nabel EG, Ganz P, Gordon JB, *et al.* Dilation of normal and constriction of atherosclerotic coronary arteries caused by the cold pressor test. *Circulation* 1988;**77**:43–52.
78. Tortorici MA, Kochanek PM, Poloyac SM. Effects of hypothermia on drug disposition, metabolism, and response: a focus of hypothermia-mediated alterations on the cytochrome P450 enzyme system. *Crit Care Med* 2007;**35**:2196–204.
79. Safar P, Xiao F, Radovsky A, *et al.* Improved cerebral resuscitation from cardiac arrest in dogs with mild hypothermia plus blood flow promotion. *Stroke* 1996;**27**:105–13.
80. Xiao F, Safar P, Radovsky A. Mild protective and resuscitative hypothermia for asphyxial cardiac arrest in rats. *Am J Emerg Med* 1998;**16**:17–25.
81. Bernard SA, Jones BM, Horne MK. Clinical trial of induced hypothermia in comatose survivors of out-of-hospital cardiac arrest. *Ann Emerg Med* 1997;**30**:146–53.
82. Felberg RA, Krieger DW, Chuang R, *et al.* Hypothermia after cardiac arrest: feasibility and safety of an external cooling protocol. *Circulation* 2001;**104**: 1799–804.
83. Nagao K, Hayashi N, Kanmatsuse K, *et al.* Cardiopulmonary cerebral resuscitation using emergency cardiopulmonary bypass, coronary reperfusion therapy and mild hypothermia in patients with cardiac arrest outside the hospital. *J Am Coll Cardiol* 2000;**36**:776–83.
84. Yanagawa Y, Ishihara S, Norio H, *et al.* Preliminary clinical outcome study of mild resuscitative hypothermia after out-of-hospital cardiopulmonary arrest. *Resuscitation* 1998;**39**:61–6.
85. Zeiner A, Holzer M, Sterz F, *et al.* Mild resuscitative hypothermia to improve neurological outcome after cardiac arrest. A clinical feasibility trial. Hypothermia After Cardiac Arrest (HACA) Study Group. *Stroke* 2000;**31**:86–94.
86. Bernard S. Hypothermia after cardiac arrest: expanding the therapeutic scope. *Crit Care Med* 2009;**37**:S227–233.
87. Nolan JP, Deakin CD, Soar J, *et al.* European Resuscitation Council. European Resuscitation Council guidelines for resuscitation 2005. Section 4. Adult advanced life support. *Resuscitation* 2005;**67**(Suppl. 1):S39–86.
88. Bernard SA, Smith K, Cameron P, *et al.* Induction of therapeutic hypothermia by paramedics after resuscitation from out-of-hospital ventricular fibrillation cardiac arrest: a randomized controlled trial. *Circulation* 2010;**122**:737–42.
89. Gunn AJ, Gunn TR. The 'pharmacology' of neuronal rescue with cerebral hypothermia. *Early Hum Dev* 1998;**53**:19–35.
90. Azzopardi DV, Strohm B, Edwards AD, *et al.* Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009;**361**:1349–58.

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91. Eicher DJ, Wagner CL, Katikaneni LP, *et al.* Moderate hypothermia in neonatal encephalopathy: efficacy outcomes. *Pediatr Neurol* 2005;**32**:11–7.
92. Gluckman PD, Wyatt JS, Azzopardi D, *et al.* Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 2005;**365**:663–70.
93. Shankaran S, Laptook AR, Ehrenkranz RA, *et al.* Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* 2005;**353**: 1574–84.
94. Edwards AD, Brocklehurst P, Gunn AJ, *et al.* Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. *BMJ* 2010; **340**:c363.
95. Long M, Brandon DH. Induced hypothermia for neonates with hypoxic-ischemic encephalopathy. *J Obstet Gynecol Neonatal Nurs* 2007;**36**:293–8.
96. Selway LD. State of the science: hypoxic ischemic encephalopathy and hypothermic intervention for neonates. *Adv Neonatal Care* 2010;**10**:60–6.
97. Clifton GL, Jiang JY, Lyeth BG, *et al.* Marked protection by moderate hypothermia after experimental traumatic brain injury. *J Cereb Blood Flow Metab* 1991;**11**:114–21.
98. Jiang JY, Lyeth BG, Kapasi MZ, *et al.* Moderate hypothermia reduces blood-brain barrier disruption following traumatic brain injury in the rat. *Acta Neuropathol (Berl)* 1992;**84**:495–500.
99. Lyeth BG, Jiang JY, Robinson SE, *et al.* Hypothermia blunts acetylcholine increase in CSF of traumatically brain injured rats. *Mol Chem Neuropathol* 1993;**18**:247–56.
100. Clark RS, Kochanek PM, Marion DW, *et al.* Mild posttraumatic hypothermia reduces mortality after severe controlled cortical impact in rats. *J Cereb Blood Flow Metab* 1996;**16**:253–61.
101. Colbourne F, Corbett D. Delayed postischemic hypothermia: a six month survival study using behavioral and histological assessments of neuroprotection. *J Neurosci* 1995;**15**:7250–60.
102. Lawrence EJ, Dentcheva E, Curtis KM, *et al.* Neuroprotection with delayed initiation of prolonged hypothermia after in vitro transient global brain ischemia. *Resuscitation* 2005;**64**:383–8.
103. Markgraf CG, Clifton GL, Moody MR. Treatment window for hypothermia in brain injury. *J Neurosurg* 2001;**95**:979–83.
104. Roelfsema V, Bennet L, George S, *et al.* Window of opportunity of cerebral hypothermia for postischemic white matter injury in the near-term fetal sheep. *J Cereb Blood Flow Metab* 2004;**24**:877–86.
105. Liu WG, Qiu WS, Zhang Y, *et al.* Effects of selective brain cooling in patients with severe traumatic brain injury: a preliminary study. *J Int Med Res* 2006;**34**:58–64.
106. Marion DW, Penrod LE, Kelsey SF, *et al.* Treatment of traumatic brain injury with moderate hypothermia. *N Engl J Med* 1997;**336**:540–6.
107. Qiu WS, Liu WG, Shen H, *et al.* Therapeutic effect of mild hypothermia on severe traumatic head injury. *Chin J Traumatol* 2005;**8**:27–32.
108. Zhi D, Zhang S, Lin X. Study on therapeutic mechanism and clinical effect of mild hypothermia in patients with severe head injury. *Surg Neurol* 2003;**59**: 381–5.
109. Alderson P, Gadkary C, Signorini DF. Therapeutic hypothermia for head injury. *Cochrane Database Syst Rev* 2004. CD001048.
110. Harris OA, Colford Jr JM, Good MC, *et al.* The role of hypothermia in the management of severe brain injury: a meta-analysis. *Arch Neurol* 2002;**59**: 1077–83.
111. Henderson WR, Dhingra VK, Chittock DR, *et al.* Hypothermia in the management of traumatic brain injury. A systematic review and meta-analysis. *Intensive Care Med* 2003;**29**:1637–44.
112. McIntyre LA, Fergusson DA, Hebert PC, *et al.* Prolonged therapeutic hypothermia after traumatic brain injury in adults: a systematic review. *JAMA* 2003;**289**:2992–9.
113. Hutchison JS, Ward RE, Lacroix J, *et al.* Hypothermia therapy after traumatic brain injury in children. *N Engl J Med* 2008;**358**:2447–56.
114. Nichol AD, Cooper DJ, Polar Study Investigators on behalf of the ANZICS-Clinical Trials Group, E.P. O. Study Investigators on behalf of the ANZICS-Clinical Trials Group. Can we improve neurological outcomes in severe traumatic brain injury? Something old (early prophylactic hypothermia) and something new (erythropoietin). Injury 2009; 40:471–478.
115. Rees K, Beranek-Stanley M, Burke M, Ebrahim S. Hypothermia to reduce neurological damage following coronary artery bypass surgery. *Cochrane Database Syst Rev* 2001. CD002138.
116. Nathan HJ, Rodriguez R, Wozny D, *et al.* Neuroprotective effect of mild hypothermia in patients undergoing coronary artery surgery with cardiopulmonary bypass: five-year follow-up of a randomized trial. *J Thorac Cardiovasc Surg* 2007;**133**:1206–11.
117. Kawahara F, Kadoi Y, Saito S, *et al.* Slow rewarming improves jugular venous oxygen saturation during rewarming. *Acta Anaesthesiol Scand* 2003;**47**:419–24.
118. Grigore AM, Grocott HP, Mathew JP, *et al.* The rewarming rate and increased peak temperature alter neurocognitive outcome after cardiac surgery. *Anesth Analg* 2002;**94**:4–10.
119. Hogue Jr CW, Palin CA, Arrowsmith JE, *et al.* Cardiopulmonary bypass management and neurologic outcomes: an evidence-based appraisal of current practices. *Anesth Analg* 2006;**103**:21–37.
120. Allen BT, Davis CG, Osborne D, Karl I. Spinal cord ischemia and reperfusion metabolism: the effect of hypothermia. *J Vasc Surg* 1994;**19**:332–9. discussion 339–340.
121. Isaka M, Kumagai H, Sugawara Y, *et al.* Cold spinoplegia and transvertebral cooling pad reduce spinal cord injury during thoracoabdominal aortic surgery. *J Vasc Surg* 2006;**43**:1257–62.
122. Matsumoto M, Iida Y, Sakabe T, *et al.* Mild and moderate hypothermia provide better protection than a burst-suppression dose of thiopental against ischemic spinal cord injury in rabbits. *Anesthesiology* 1997;**86**:1120–7.
123. Naslund TC, Hollier LH, Money SR, *et al.* Protecting the ischemic spinal cord during aortic clamping. The influence of anesthetics and hypothermia. *Ann Surg* 1992;**215**:409–15.
124. Todd MM, Hindman BJ, Clarke WR, Torner JC. Intraoperative Hypothermia for Aneurysm Surgery Trial I. Mild intraoperative hypothermia during surgery for intracranial aneurysm. *N Engl J Med* 2005;**352**:135–45.
125. Grigore AM, Mathew J, Grocott HP, *et al.* Prospective randomized trial of normothermic versus hypothermic cardiopulmonary bypass on cognitive function after coronary artery bypass graft surgery. *Anesthesiology* 2001;**95**: 1110–9.
126. Grocott HP, Mackensen GB, Grigore AM, *et al.* Postoperative hyperthermia is associated with cognitive dysfunction after coronary artery bypass graft surgery. *Stroke* 2002;**33**:537–41.
127. Baker CJ, Onesti ST, Solomon RA. Reduction by delayed hypothermia of cerebral infarction following middle cerebral artery occlusion in the rat: a time-course study. *J Neurosurg* 1992;**77**:438–44.
128. Liu Y, Barks JD, Xu G, *et al.* Topiramate extends the therapeutic window for hypothermia-mediated neuroprotection after stroke in neonatal rats. *Stroke* 2004;**35**:1460–5.
129. Ohta H, Terao Y, Shintani Y, *et al.* Therapeutic time window of post-ischemic mild hypothermia and the gene expression associated with the neuroprotection in rat focal cerebral ischemia. *Neurosci Res* 2007;**57**:424–33.
130. Zausinger S, Scholler K, Plesnila N, Schmid-Elsaesser R. Combination drug therapy and mild hypothermia after transient focal cerebral ischemia in rats. *Stroke* 2003;**34**:2246–51.
131. Guluma KZ, Hemmen TM, Olsen SE, *et al.* A trial of therapeutic hypothermia via endovascular approach in awake patients with acute ischemic stroke: methodology. *Acad Emerg Med* 2006;**13**:820–7.
132. Kammersgaard LP, Rasmussen BH, Jorgensen HS, *et al.* Feasibility and safety of inducing modest hypothermia in awake patients with acute stroke through surface cooling: a case–control study: the Copenhagen Stroke Study. *Stroke* 2000;**31**:2251–6.
133. Krieger DW, De Georgia MA, Abou-Chebl A, *et al.* Cooling for acute ischemic brain damage (cool aid): an open pilot study of induced hypothermia in acute ischemic stroke. *Stroke* 2001;**32**:1847–54.
134. Schwab S, Georgiadis D, Berrouschot J, *et al.* Feasibility and safety of moderate hypothermia after massive hemispheric infarction. *Stroke* 2001;**32**: 2033–5.
135. Schwab S, Schwarz S, Aschoff A, *et al.* Moderate hypothermia and brain temperature in patients with severe middle cerebral artery infarction. *Acta Neurochir Suppl* 1998;**71**:131–4.
136. Schwab S, Schwarz S, Spranger M, *et al.* Moderate hypothermia in the treatment of patients with severe middle cerebral artery infarction. *Stroke* 1998; **29**:2461–6.
137. Steiner T, Friede T, Aschoff A, *et al.* Effect and feasibility of controlled rewarming after moderate hypothermia in stroke patients with malignant infarction of the middle cerebral artery. *Stroke* 2001;**32**:2833–5.
138. Hemmen TM, Raman R, Guluma KZ, *et al.* Intravenous thrombolysis plus hypothermia for acute treatment of ischemic stroke (ICTuS-L): final results. *Stroke* 2010;**41**:2265–70.
139. Martin-Schild S, Hallevi H, Shaltoni H, *et al.* Combined neuroprotective modalities coupled with thrombolysis in acute ischemic stroke: a pilot study of caffeinol and mild hypothermia. *J Stroke Cerebrovasc Dis* 2009; **18**:86–96.
140. Kelly FE, Nolan JP. The effects of mild induced hypothermia on the myocardium: a systematic review. *Anaesthesia* 2010;**65**:505–15.
141. Ly HQ, Denault A, Dupuis J, *et al.* A pilot study: the Noninvasive Surface Cooling Thermoregulatory System for Mild Hypothermia Induction in Acute Myocardial Infarction (the NICAMI Study). *Am Heart J* 2005;**150**:933.
142. Parham W, Edelstein K, Unger B, *et al.* Therapeutic hypothermia for acute myocardial infarction: past, present, and future. *Crit Care Med* 2009;**37**:S234–237.
143. Zeiner A, Sunder-Plassmann G, Sterz F, *et al.* The effect of mild therapeutic hypothermia on renal function after cardiopulmonary resuscitation in men. *Resuscitation* 2004;**60**:253–61.
144. Boodhwani M, Rubens FD, Wozny D, Nathan HJ. Effects of mild hypothermia and rewarming on renal function after coronary artery bypass grafting. *Ann Thorac Surg* 2009;**87**:489–95.
145. Massoudy P, Wagner S, Thielmann M, *et al.* Coronary artery bypass surgery and acute kidney injury—impact of the off-pump technique. *Nephrol Dial Transplant* 2008;**23**:2853–60.
146. Moore EM, Simpson JA, Tobin AE, Santamaria JD. Preoperative eGFR and RIFLE-classified postoperative AKI predict length of stay post coronary bypass surgery in an Australian setting. *Anaesth Intensive Care* 2010;**38**:113–21.
147. Moore E, Bellomo R, Nichol A. Biomarkers of acute kidney injury in anesthesia, intensive care and major surgery: from the bench to clinical research to clinical practice. *Minerva Anestesiol* 2010;**76**:425–40.
148. Bagshaw SM, George C, Gibney RTN, Bellomo R. A multi-center evaluation of early acute kidney injury in critically ill trauma patients. *Ren Fail* 2008;**30**: 581–9.

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149. Ostermann M, Chang RWS. Acute kidney injury in the intensive care unit according to RIFLE. *Crit Care Med* 2007;**35**:1837–43.
150. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: a systematic review. *Kidney Int* 2008;**73**:538–46.
151. Chertow GM, Burdick E, Honour M, *et al.* Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 2005;**16**:3365–70.
152. Bellomo R, Ronco C, Kellum JA, *et al.* Acute renal failure—definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Critical Care* 2004;**8**:R204–12.
153. Moore EM, Bellomo R, Nichol AD, *et al.* The incidence of acute kidney injury in patients with traumatic brain injury. *Ren Fail* 2010;**32**:1060–5.

2.4 Novel Renal Biomarkers

Reliance on conventional biomarkers to diagnose AKI has led to delays in the implementation of therapies for AKI; creatinine and urea become deranged late in the course of injury, and urine output lacks specificity. This has hampered AKI research, particularly in interventional studies where commencement of therapy is guided by conventional markers.

Consequently, there has been intense investigation of new early biomarkers for AKI over the last 10 years. The following published article (section 2.4.1) reviews in detail several of the more promising biomarkers of AKI that have emerged; it has been given an anaesthetics slant to cater to the journal readership. Section 2.4.2 provides a short summary of biomarkers measured in the Renal substudies of the EPO-TBI and POLAR trials that are not addressed in the published review, or are only briefly covered.

The initial intention in the Renal substudies was to include measurement of Liver-type fatty acid binding protein (L-FABP) which is reviewed in this publication, however, for reasons of finance and logistics, L-FABP was not eventually measured in these studies.

2.4.1 Biomarkers of AKI

Moore E, Bellomo R, Nichol A. Biomarkers of acute kidney injury in anesthesia, intensive care and major surgery: from the bench to clinical research to clinical practice. Minerva Anestesiol 2010 Jun;76(6):425-40.

Declaration for Thesis Chapter

Moore E, Bellomo R, Nichol A. Biomarkers of acute kidney injury in anesthesia, intensive care and major surgery: from the bench to clinical research to clinical practice. Minerva Anestesiol. 2010;76(6):425-40.

Declaration by candidate

In the case of Chapter , the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Searched and reviewed the literature, collated and interpreted the information, wrote up drafts and submitted the manuscript, was responsible for the response to reviewers.	80 %

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
R Bellomo	Assisted with interpretation of literature, and designing and drafting the manuscript	N/A
A Nichol	Reviewed and drafted manuscript	N/A

Candidate's Signature	Elizabeth Moore	Date 30/4/2012

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s)	**Australian and New Zealand Intensive Care Research Centre, Department of Epidemiology and Preventive Medicine, Monash University, Alfred campus Department of Intensive Care, Austin Hospital**

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

	Date 30/4/12
	13/3/12

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REVIEW

Biomarkers of acute kidney injury in anesthesia, intensive care and major surgery: from the bench to clinical research to clinical practice

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ABSTRACT

Acute kidney injury (AKI) is common after major surgery and reportedly occurs in approximately 36% of ICU patients (RIFLE Risk/Injury/ Failure categories). It is associated with increased mortality, greater cost, and prolonged Intensive Care Unit (ICU) and hospital stay, despite attempts to develop therapies to prevent or attenuate AKI, which have had limited success. One major reason for this lack of success may be the result of delayed implementation due to the inability to detect AKI early. Traditional biomarkers of AKI (creatinine and urea) do not detect injury early enough. Thus, it is a priority to find reliable, early biomarkers that predict subsequent AKI. Innovative technologies such as functional genomics and proteomics have facilitated detection of several promising early biomarkers of AKI, such as neutrophil gelatinase-associated lipocalin (NGAL), cystatin C (CyC), liver-type fatty acid binding protein (L-FABP), interleukin-18 (IL-18), and kidney injury molecule-1 (KIM-1). These biomarkers have many potential applications during anesthesia and in the ICU. They can be used to evaluate the effect of new techniques and therapies on kidney function, as safety markers to monitor toxicity and as measures of treatment effect. For example, NGAL and cystatin C have been used in a safety monitoring trial of hydroxyethylstarch therapy and to detect AKI early, during or immediately after cardiac surgery. Clinical use beyond research settings is rapidly expanding.
(Minerva Anestesiol 2010;76:425-40).

Key words: Kidney failure - Creatinine - Urea - Biological markers - Lipocalins - Cystatin C - Fatty acid-binding proteins - Interleukin-18.

AKI is common with major surgery and critical illness

Acute kidney injury (AKI) is the consensus term now used to describe the continuum of the condition previously called acute renal failure. AKI as classified by the RIFLE criteria [1] (acronym for Risk, Injury, Failure, Loss and Endstage) has been reported to occur in approximately 36% of critically ill patients and is common after major surgery such as open heart surgery.[2, 3] Although the Acute Kidney Injury Network (AKIN) criteria, based on the RIFLE classification system are being used increasingly and are presumed to improve sensitivity in diagnosing AKI, they have not been shown to improve the ability to predict outcomes.[4, 5] The RIFLE criteria have been extensively used and validated to classify renal function in several populations with studies cumulatively involving >250 000 subjects.[6] Furthermore, a

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recent study found RIFLE to be more robust, with a higher detection rate of AKI than AKIN in the first 48 hours post-ICU admission.[7] Therefore, we favor the RIFLE classification for AKI.

AKI is independently associated with an increased risk of death[6] and with prolonged length of stay.[8, 9] Severe cases require costly treatment, can result in prolonged kidney dysfunction, and escalate the human and financial costs of care. Therefore, it seems desirable to detect AKI as early as possible in order to develop or implement potentially protective therapy.

Why therapies have been unsuccessful

To date, attempts to develop therapies to prevent or attenuate AKI have failed to show consistently protective effects. The use of diuretics has not proven to be of benefit. Fenoldopam, a vasodilator, has shown promise in certain populations [10] but not in others; [11, 12] natriuretic peptides may be of use in major surgery but have also not been effective in other situations.[13, 14] The capacity of N-acetyl cysteine to prevent AKI after radiocontrast has been tested with unconvincing results, [15-17] and the benefits of perioperative IV sodium bicarbonate infusion in cardiac surgical patients [18] are yet to be confirmed. The most widely accepted treatment to prevent or treat AKI (although untested in controlled trials) remains prompt fluid resuscitation of circulatory volume and appropriate use of inotropes/vasopressors to maintain adequate cardiac output and perfusion pressure.[19-21] Beyond these measures, cases unresponsive to fluid resuscitation in the presence of hyperkalemia, metabolic acidosis or fluid overload commonly receive renal replacement therapy/dialysis to support the kidneys.

There are several reasons why no reproducibly and consistently effective treatment for AKI has been found. First, AKI can occur as a result of multiple causes and disease processes. Whereas certain treatments may benefit subgroups, heterogeneity of disease makes finding one treatment for all types of AKI unlikely. Second, understanding of the pathogenesis of AKI is limited. An incomplete knowledge of the mechanisms involved has resulted in difficulties in developing logical approaches to prevention or treatment. Third, interventions are implemented too late. This delay is due to our reliance on conventional biomarkers (creatinine, urea, urine output) to diagnose AKI. Such biomarkers either do not detect injury in real-time and become abnormal many hours later in the course of injury (creatinine or urea) or lack specificity (urine output). To draw a parallel with the treatment for acute myocardial infarction, one can easily imagine how difficult it would be to show the benefits of thrombolysis or stenting in the absence of troponin, electrocardiogram changes or the presence of chest pain to allow early and specific diagnosis within minutes instead of hours or a day later. By analogy, if we could detect AKI early, we would treat it more rapidly and should have a better chance of preventing or reducing injury, as is the case for several other acute syndromes in medicine.

The limitations of traditional biomarkers

The traditional clinical biomarkers for the detection of AKI are creatinine, urea, and urine output. All have serious limitations as early detectors of AKI. Creatinine is the product of the breakdown of creatine to phosphocreatine in skeletal muscle and of the subsequent liver metabolism of creatine to form creatinine.[22] It is produced and released into plasma at a fairly constant rate and is filtered by the glomerulus. A small amount is also secreted into the urine. Creatinine is not reabsorbed in the tubules or metabolized by the kidney. If filtering of creatinine is deficient, blood levels rise with an inverse relationship with GFR. Unfortunately, there are several limitations to serum creatinine (SCr) as a biomarker of AKI.[22] First, its release varies with age, gender, diet, muscle mass, drugs, and vigorous exercise. Second, secretion accounts for 10-40% of creatinine clearance, [22] which could mask a decrease in GFR. Third, the accuracy of SCr assays can be reduced by artifact. Fourth, creatinine becomes abnormal only when more than 50% of GFR is lost and may require up to 24 hours before sufficient increases in blood concentration are detectable.

Urea is a water-soluble, low molecular weight by-product of protein metabolism. Its level is also inversely related to GFR, but several factors affect its production and clearance, limiting its reliability in estimating GFR. Urea production is incon-

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TABLE I.—*Characteristics, function and significance of NGAL, cystatin C and L-FABP.*

Biomarker	Molecular weight	Origin		Normal concentration		Physiological function	Significance of rise in level
				Urine	Plasma/Serum		
NGAL	21 kDa	Urine NGAL - mostly local synthesis in kidney (distal nephron) in response to injury - secreted into urine *Circulating NGAL* - synthesised sytemically in response to renal injury → filtered by glomerulus → uptake by proximal tubule epithelia → pooled and little secreted into urine *Neutrophils/macrophages* may be a third source of renal NGAL - under investigation	Adults Children	1.0-20.0 ng/mL 1.0-20.0 ng/mL	70-105 ng/mL 30-80 ng/mL	*Bacteriostatic effect* - binds to iron-carrying molecules (siderophores) which are synthesised by specific bacteria to gather iron. By doing this, NGAL reduces bacterial growth *Antioxidant effect* - binds to "human siderophores" to transport iron into target cells thus stopping free and reactive iron from producing oxygen radicals which can cause oxidative stress and cell injury *Scavenges intracellular iron* for extracellular export (hypothesis) *Acts as a growth factor* - regulates cell proliferation, apoptosis and differentiation (supported by increasing evidence)	Tubular stress/injury. There is an earlier rise in urine than serum
Cystatin C	13.3 kDa	Produced at a constant rate by nucleated cells → filtered by the glomerulus → almost completely reabsorbed and catabolised (but not secreted) in the proximal tubules		<0.1 mg/g Cr [24]	<1-1.5 mg/L	Potent inhibitor of lysosomal proteases (inhibits breakdown of lysosomal protein) and extracellular inhibitor of cysteine proteases (prevents breakdown of extracellular protein)	Change in GFR is reflected in changes in serum and urine levels of cystatin C - acts as a marker of GFR
L-FABP	14.3 kDa	Expressed in the liver, intestine, pancreas, lung stomach, and kidneys NoiriDoi09. Production in the liver seems to determine blood levels. Renal L-FABP is found in cytoplasm of the proximal tubules	Children (CPB) Adults (CIN)	<10 ng/mg Cr/L Portilla *et al.*[27] 5-20 µg/g Cr [25] Nakamura *et al.*[25]	60-110 ng/mL[27]	Renal L-FABP helps maintain low levels of free FAs in the cytoplasm by a) binding to them and transporting them to cell components to accelerate FA metabolism and by b) binding to free FAs and being excreted from the proximal tubule cells into urine for elimination [26]	Tubular stress/injury, which results in an accumulation of free FAs in the proximal tubules [23] There is an earlier rise in urine than serum

stant, and values can be altered by changes in circulatory volume, protein intake, gastrointestinal bleeding, among other parameters. Rate of renal clearance of urea is not constant; 40-50% of filtered urea may be reabsorbed in the tubules.[22] Consequently, urea is a poor measure of GFR, requires time to accumulate, does not reflect real-time changes in GFR and delays diagnosis.

Urine output is measured routinely in operating rooms and ICUs with indwelling catheters. A trend in urine output is a crude gauge of kidney function and may be a more sensitive indicator of changes in renal hemodynamics than a measure of solute clearance.[1] Nonetheless, many patients with AKI do not have oliguria, and many patients with oliguria in the operating room or ICU do

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TABLE II.—*Performance of NGAL in diagnosis of AKI in adults: studies with AUC-ROC analysis.*

Year	Authors	Setting	Age	SexF %	Existing renal disease excluded	% AKI	AKI Definition (↑ in SCr)	Timing (AKI)	Sample size
NGAL									
2009	Liangos *et al.*[38]	CSA	68	28	* †	13	≥50%	72 h	103
2009	Tuladhar *et al.*[39]	CSA	67	30	Y	18	>44.2 µmol/L	48 h	50
2009	Han *et al.*[37]	CSA	NA	NA	NA	40	≥26.5 µmol/L	72 h	90
2009	Haase-Fielitz *et al.*[36]	CSA	70	39	Y	23	>50%	5 days	100
2009	Haase-Fielitz *et al.*[35]	CSA	70	39	* ‡	39	>25%	5 days	100
2009	Haase *et al.*[34]	CSA	70	39	* ‡	46	>50% or >26.5 µmol/L or ¥	48 h	100
2008	Xin *et al.*[40]	CSA	38	56	N	27	>50% or ≥26.5 µmol/L or ¥	48 h	33
2008	Wagener G. *et al.*[45]	CSA	63	34	§	20	≥50% or >0 26.5 µmol/L	48 h	426
2008	Koyner *et al.*[24]	CSA	65	29	Y	47	≥25% or RRT	72 h	72
2006	Wagener G. *et al.*[41]	CSA	68	35	*	20	≥50%	10 days	81
2009	Makris *et al.*[43]	Trauma	NA	19	Y	NA	NA	5 days	31
2008	Ling W. *et al.*[42]	CIN	67	40	N	NA	≥25% or > 44.2 µmol/L	72 h	40
2008	Nickolas *et al.*[44]	ED	60	49	*	5	≥50% or 25%↓ in eGFR	Hosp stay	635

CSA: cardiac surgery associated AKI; CIN: contrast induced nephropathy; ED: emergency department; Y: yes; N: no; NA: not available or not applicable; RRT: renal replacement therapy; Hosp stay: hospital stay; Quantikine® - R&D systems. *: dialysis, †: transplant, ‡: SCr >300 µmol/L; §: endstage renal disease; ¥: urine output <0.5 mL/kg/h for >6 h.

not develop AKI. Finally, many drugs used in the operating room or ICU (*i.e.*, diuretics and vasopressors) act as additional confounders.

In response to these problems, innovative technologies such as functional genomics and proteomics have facilitated the detection of several potential earlier biomarkers of AKI. Some of the most promising biomarkers include neutrophil gelatinase-associated lipocalin (NGAL), cystatin C (CyC), and liver-type fatty acid binding protein (L-FABP), which have now been evaluated in several populations.

NGAL

The expression of NGAL in early, acute tubular injury was identified using functional genomics. NGAL is a measure of tubular stress (Table I); its concentration increases dramatically in response to tubular injury and precedes rises in SCr by >24 hours.[28] NGAL normally exerts protective bacteriostatic and antioxidant effects involving iron transport and is thought to act as an iron scavenger and growth factor [29, 30] (Table I). The proposed role of NGAL and its twin molecule, hepcidin (a "master regulator" of iron [29]) are summarized in Figure 1.

NGAL has been intensely investigated in recent years, predominantly in adult cardiac surgery. ELISA techniques have been used to measure NGAL; however, accurate and sensitive point of care tests that reduce costs and the potential for measurement error are now available [32, 33] (Tables II-IV).

Cardiac surgery – children

In a landmark study, diagnosis of AKI by SCr occurred 1-3 days after surgery, whereas plasma and urine NGAL levels were powerful independent predictors of AKI within 2 hours of surgery (Table IV). Subsequent studies confirmed these findings [27, 32, 33] (Table IV). Moreover, plasma NGAL predicted duration of AKI, length of stay and mortality.

Cardiac surgery – adults

The findings in children were broadly confirmed

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Instrument	Time from procedure/ admission (h)	Cutoff		Urine		Plasma/Serum		AUC-ROC	
		Urine	Plasma	Sensitivity %	Specificity %	Sensitivity %	Specificity %	Urine %	Plasma %
Quantikine®	2	166 ng/mg		67	11			50	
ELISA	2	393 ng/mL	426 ng/mL	93	78	80	67	96	85
NA	0	NA		NA	NA			59	
	3	NA		NA	NA			65	
Triage®Biosite	ICU/6hrs		150 ng/mL			79	78		80
	24		90 ng/mL			91	76		87
Triage®Biosite	ICU/6hrs		145 ng/mL			68	64		67
Triage®Biosite	ICU	>150 ng/mL		73	74			77	
ELISA kit	2	250 µgL		71	73			88	
	2	250 µg/mmol		81	78			93	
ELISA kit	0	23.5 ng/mL		31	81			57	
	3	18.1 ng/mL		38	78			60	
	18	15.6 ng/mL		39	78			61	
ELISA	ICU	300 ng/mg		67	62	NA	NA	71	53
	ICU+6hrs	300 ng/mg		34	86	NA	NA	70	46
Immunoblot	3	213 ng/mL		69	65			74	
	18	213 ng/mL		73	78			80	
ELISA	0	25 ng/mL		91	95			98	
ELISA kit	24	9.85 ng/mL		77	70			73	
Immunoblot	0	130 µg/gCr		90	100			95	

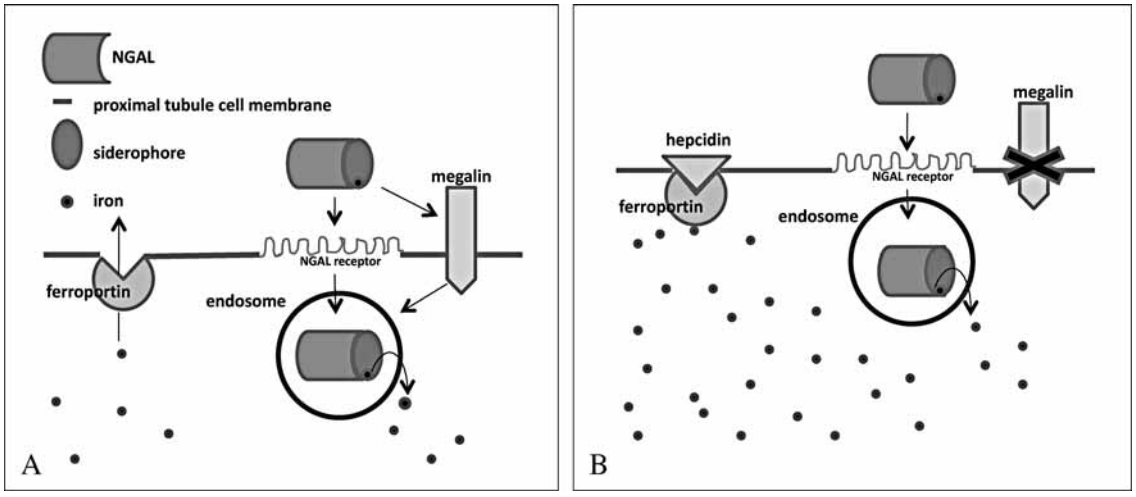

Figure 1.—A) Proposed model of NGAL and hepcidin-mediated iron trafficking in the proximal tubule. NGAL bound to siderophore and iron, delivers iron to cells via megalin and NGAL receptors. In the endosome, NGAL releases iron, which promotes expression of iron-dependent genes (*e.g.*, ferritin), stimulates epithelial proliferation and reduces renal damage. Iron moves out of cells *via* the ferroportin pathway.[28] Diagram modified from Schmidt-Ott *et al.* 2007.[30] B) Model representing potential mechanisms and pathways present in renal injury. Local and systemic NGAL expression increases. Tubular injury results in disruption to megalin receptor function, reduced NGAL-mediated intracellular iron uptake, and subsequent increase in urine and serum NGAL concentration. With inflammation, hepcidin is up-regulated and binds to ferroportin, which is internalized, endocytosed and degraded. Absence of the ferroportin pathway for iron removal promotes the accumulation of intracellular iron, which reduces extracellular free reactive iron, thereby, reducing oxidative stress. The association of higher hepcidin levels with those that do not develop AKI vs. those that develop AKI after cardiopulmonary bypass surgery is consistent with this proposed mechanism.[31]

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TABLE III.—*Performance of Cystatin C in diagnosis of AKI in adults: studies with AUC-ROC analysis.*

Year	Authors	Setting	Age	SexF %	Existing renal disease excluded	% AKI	AKI Definition (↑ in SCr)	Timing (AKI)	Sample size
Cystatin C									
2009	Liangos *et al.*[38]	CSA	68	28	* †	13	≥50%	72 h	103
2009	Haase-Fielitz *et al.*[36]	CSA	70	39	N	23	≥50%	5 days	100
			70	40	Y	23	≥50%	5 days	73
2009	Haase *et al.*[34]	CSA	70	39	* ‡	46	≥50% or >26.5 µmol/L or §	48 h	100
2008	Koyner *et al.*[24]	CSA	65	29	Y	47	≥25% or RRT	72 h	72
2007	Ling *et al.*[48]	Tplant-liver	47	10	NA	NA	GFR<80 mL/min/1.73 m^2	1 wk	30
2004	Daniel *et al.*[47]	Tplant-ren'	40	35	NA	27	Inulin Cl <90 mL/min	NA	60
2005	Villa *et al.*[49]	Crit ill	54	32	Y	50	Cr Cl <80 mL/min/1.73 m^2	NA	50
2004	Ahlstrom *et al.*[46]	Crit ill	55	32	N	27	≥50%	ICU stay	202

CSA: cardiac surgery associated AKI; Tplant-liver/ren': post-liver or renal transplant; Crit ill: critically ill; NA: not available or not applicable; Inulin Cl: inulin clearance; Cr Cl: creatinine clearance; Y: yes; N:no. *: dialysis; †: transplant; ‡: SCr >300 µmol/L; §: urine output <0.5 mL/kg/h for >6 h. BNII automat and PENIA - Dade Behring. ELISA kit-CyC - Biovendor LLC.

TABLE IV.—*Performance of biomarkers in diagnosis of AKI in children: studies with AUC-ROC analysis.*

Year	Authors	Setting	Age	SexF %	Existing renal disease excluded	% AKI	AKI Definition (↑ in SCr)	Timing (AKI)	Sample size
NGAL									
2008	Portilla *et al.*[27]	CSA	3.5	48	Y	52	≥50%	5 days	40
2008	Bennett *et al.*[32]	CSA	4	48	Y	51	≥50%	3 days	196
2007	Dent *et al.*[33]	CSA	4	47	Y	37	≥50%	3 days	120
2005	Mishra *et al.*[50]	CSA	4	37	Y	28	≥50%	3 days	71
2008	Wheeler *et al.*[53]	SIRS/SS	3	35	Y	15	>176.82 µmol/L or *	7 days	143
2007	Hirsch *et al.*[52]	CIN	7	43	Y	12	≥50%	24 h	91
Cystatin C									
2007	Herrero-Morin *et al.*[51]	Crit ill	2.9	44	Y	56	Cr Cl <80 ml/min/1.73m^2	ICU stay	25
L-FABP									
2008	Portilla *et al.*[27]	CSA	3.5	48	Y	50	≥50%	5 days	16

CSA: cardiac surgery associated AKI; CIN: contrast induced nephropathy; Crit ill: critically ill; SIRS/SS: systemic infammatory response syndrome/Septic shock; NA: not available or not applicable; Cr Cl: creatinine clearance; Y: yes; N: no; Wblot: Western blot. *: urea >100 mg/dL. Triage® - Biosite, ARCHITECT® - Abbott.

in adults, albeit with somewhat diminished accuracy [34, 36, 45] (Table II), with an AUC of 0.77-0.96 [34, 36, 39, 40] (Table II). Moreover, plasma NGAL was an independent predictor of AKI duration and severity, [34] of length of ICU stay, [34] and of the need for renal replacement therapy, and of hospital death [35, 36] (Table V). The predictive value of NGAL post cardiac surgery was stronger with a strict definition of AKI (>50% *versus* >25% increase in SCr) and increased with progressive severity of AKI (Table II).[35]

Contrast-induced nephropathy (CIN)

In children with congenital heart disease, Hirsch *et al.*[52] found that both serum and urine NGAL were excellent predictors for AKI at 2 hours after contrast (Table IV). In adults with normal SCr, [58] NGAL levels were significantly higher in urine at

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Instrument	Time from procedure/ admission (h)	Cutoff		Urine		Plasma/Serum		AUC-ROC	
		Urine	Plasma	Sensitivity %	Specificity %	Sensitivity %	Specificity %	Urine %	Plasma %
PENIA	2	192 ng/mg		42	86			50	
Nephelometry	ICU/6 h		>1.1 mg/L			77	86		83
	24		>1.2 mg/L			91	64		84
Nephelometry	ICU/6 h		>1.1 mg/L			75	89		78
	24		>1.2 mg/L			86	80		84
Nephelometry	ICU		>1.1 mg/L			74	67		76
ELISA kit-CyC	ICU	0.35 mg/g		58	72	NA	NA	69	62
	ICU+6 h	0.11 mg/g		45	84	NA	NA	72	63
NA	day 1,4,7		1.57 mg/L			85	85		94
BNII automat	~6.7 mths post		1.18 mg/L			72	80		NA
	~6.7 mths post		1.52 mg/L			60	87		NA
Nephelometry	NA		NA			NA	NA		93
BNII automat	24		NA			NA	NA		89

Instrument	Time from procedure/ admission (h)	Cutoff		Urine		Plasma/Serum		AUC-ROC	
		Urine	Plasma	Sensitivity %	Specificity %	Sensitivity %	Specificity %	Urine %	Plasma %
ELISA	4	100 ng/mgCr		100	100			100	
ARCHITECT®	2	100 ng/mL		82	90			95	
Triage®	2		150 ng/mL			84	94		96
ELISA & Wblot	2	25 µg/L	25 µg/L	100	98	70	94	100	91
	2	50 µg/L		100	98			100	
ELISA	day 1		139 ng/mL			86	39		68
ELISA	2	100 ng/mL	100 ng/ml	73	100	73	98	92	91
	6	100 ng/mL	100 ng/ml	90	99	73	100	97	95
Nephelometry	1-6days		0.6 mg/L			85	63		85
ELISA & Wblot	4	486 ng/mgCr		71	68			81	

2 hours and in serum 4 hours post contrast in patients who developed CIN, whereas SCr was significantly higher at 48 hours. Consistent with this, Ling *et al.*[42] found urine NGAL performed well in the early diagnosis of CIN (Table II).

Critically ill

Wheeler [53] found a significant difference in serum NGAL between children who were healthy, critically ill with SIRS, and critically ill with septic shock (medians 80, 108 and 303 ng/mL, respectively). Furthermore, NGAL was significantly elevated in those with AKI compared to those without. In addition, Zappitelli *et al.*[55] found that there was a progressive increase in urine NGAL concentration with worsening pRIFLEmax and that urine NGAL was a good diagnostic marker for persistent AKI (Table VI). In critically ill adults,[54]

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TABLE V.—*Performance of biomarkers in prognosis of outcomes: studies with AUC-ROC analysis.*

Year	Authors	Setting	Age	SexF %	Existing renal disease excluded	% AKI	AKI Definition (↑ in SCr)	Timing (AKI)	Sample size
NGAL									
2009	Haase-Fielitz *et al.*[36]	CSA	70	39	*	23	>50%	5 days	100
2009	Siew *et al.*[54]	Crit ill	53	58	† ‡ §	14	≥50% or ¥	24 h	451
						22	≥50% or ¥	48 h	451
2007	Zappitelli *et al.*[55]	Crit ill	~6	46	§	74	≥50%	14 days	140
Cystatin C									
2004	Herget-Rosenthal *et al.*[56]	Risk for AKI	67	36	N	52	≥50%	NA	85
2004	Herget-Rosenthal *et al.*[57]	ATN	69	36	N	All	ATN	NA	73
2004	Ahlstrom *et al.*[46]	Crit ill	55	32	N	27	≥50%	24 h	202

CSA: cardiac surgery associated AKI; Crit ill: critically ill; ATN: acute tubular necrosis; Y: yes; N: no; BNII Automat - Dade Behring; NA: not applicable; R minus 1/2: 1 or 2 days before SCr R-criteria (RIFLE) was fulfilled; RRT: renal replacement therapy; AKI 24/48 h: sustained AKI within 24/48 h; AKI next 48 h: persistent AKI in the next 48 h; H death: in-hospital mortality. *: SCr >300 µmol/L; †: dialysis; ‡: transplant; §: endstage renal disease; ¥: >26.5 µmol/L.

the median urine NGAL at enrolment was significantly higher in those who developed AKI within 48 hours.

Kidney transplant

In a multicenter study, urine NGAL measured on the day of transplant predicted delayed graft function and dialysis (AUC 0.9).[59] In addition, Kusaka *et al.*[60] found that a decrease in NGAL predicted organ recovery before a decrease in SCr or recovery of urine output.

NGAL and chronic kidney disease (CKD)

NGAL in serum and urine appears to reflect the presence, severity [61, 62] and progression [63] of CKD. NGAL seems to be a better predictor of GFR than SCr (and than CyC when GFR <30 mL/min [62, 64]) in patients with CKD.[61]

Urine NGAL was also strongly predictive of AKI in children with diarrhea-associated hemolytic uremic syndrome, [65] in multitrauma [43] and in emergency department [44] patients (Table II).

A recent systematic review and meta-analysis of NGAL studies using standardized data sheets sent to authors, confirmed the value of NGAL as an early predictor of AKI across settings. Urine and plasma/serum NGAL performed similarly well, and the performance of NGAL improved with standardized laboratory platforms *versus* research-based assays (cutoff >150 ng/mL). NGAL level had prognostic value for renal replacement therapy and mortality.[66]

NGAL is now the most promising novel renal biomarker [39, 50, 52] in urine and plasma. The cutoff values for NGAL range widely, with higher values used for adults *versus* children (effect of age and co-morbidities) and for cardiac surgical studies *versus* CIN (lower magnitude of injury in CIN). Therefore, it seems that each clinical setting would require the establishment of a "normal" range and cutoff value. As a general rule, however, a concentration >150 ng/mL can identify patients at high risk for AKI, and a level >350 ng/mL, those at high risk for renal replacement therapy.

If we compare the performance of NGAL in diagnosing AKI to widely used markers like troponin I, which has carried an AUC in the range of 0.7-0.8 for diagnosis of acute myocardial infarction,[39, 67] its performance would seem sufficient for clinical adoption and inclusion into an early diagnostic panel for AKI. Clinical use of NGAL beyond confirmation of its utility is rapidly expanding.[18, 68-70]

Cystatin C

CyC is a low molecular weight cysteine protease inhibitor (Table I, Figure 2). Its serum level is determined by glomerular filtration, in contrast to NGAL, which responds to stimuli and is a measure of tubular stress (Table I, Figure 2). Therefore,

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Instrument	Time from procedure/ admission (h)	Cutoff		Urine		Plasma/Serum		AUC-ROC		Outcome
		Urine	Plasma	Sensitivity %	Specificity %	Sensitivity %	Specificity %	Urine %	Plasma %	
Triage®Biosite	NA		340 ng/mL			75	100		83	RRT
ELISA kit	day 1	NA		NA	NA			70		AKI 24 hrs
ELISA kit	day 1	NA		NA	NA			66		AKI 48 hrs
ELISA	NA	0.2 ng/mg		78	67			79		AKI next 48hrs
Nephelometry	R minus 2		NA			53	82		69	RRT
	R minus 1		NA			76	93		75	RRT
Nephelometry	NA	1 g/molCr		92	83			92		RRT
BNII automat	NA		NA			75	50		62	H death

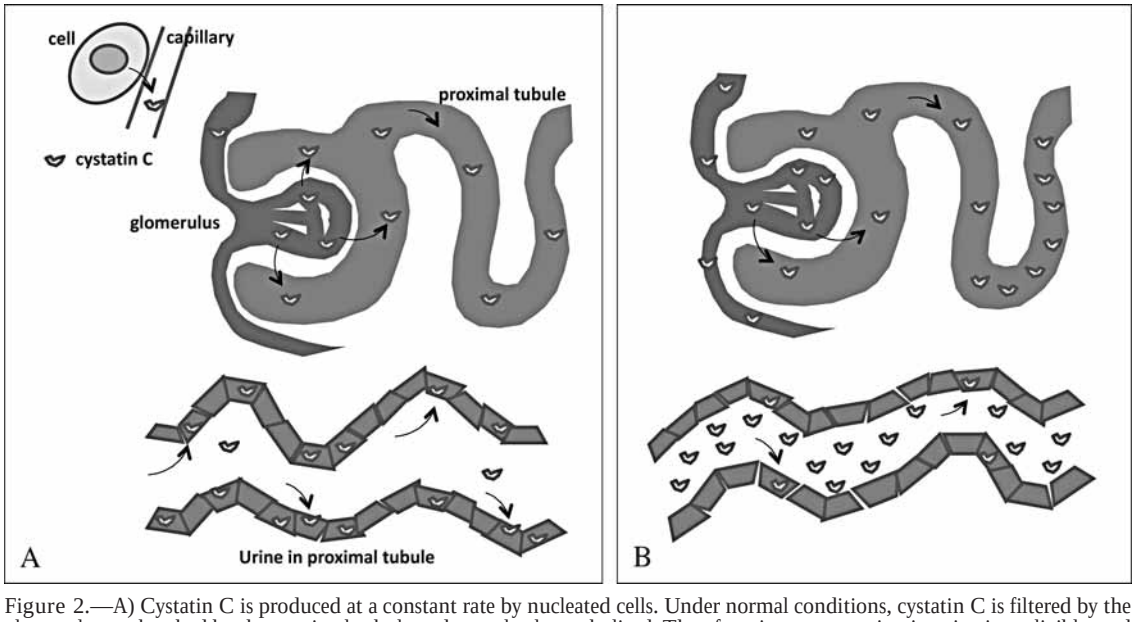

Figure 2.—A) Cystatin C is produced at a constant rate by nucleated cells. Under normal conditions, cystatin C is filtered by the glomerulus, reabsorbed by the proximal tubule and completely catabolized. Therefore, its concentration in urine is negligible, and in blood is low. B) With tubular injury, there is less filtration of cystatin C at the glomerulus due to proteinuria/ blockage/cell damage, therefore, the concentration of cystatin C in the blood increases. Less reabsorption of cystatin C by the proximal tubule due to injury also leads to increased concentration of cystatin C in the urine.

changes in serum and urine levels of CyC reflect changes in GFR. Given that its levels are not significantly affected by age, gender, race, muscle mass, infection, liver disease or inflammatory disease and that it is not secreted by the tubules, CyC is probably a better measure of glomerular function than SCr.[49, 71]

Comparison with SCr

In a systematic review of 24 studies, Laterza *et al*.[72] found CyC superior to SCr in detecting "impaired GFR" (0.95 *vs*. 0.91, P=0.003). Apart from two unfavorable results,[46, 73] the superiority of CyC over SCr as a diagnostic marker for AKI is supported by several studies of ICU patients.[49, 51,

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[56, 57, 74] Using the combined results of 11 datasets, Royakkers *et al*.[75] found the diagnostic accuracy for GFR determination of CyC superior to that of SCr: AUC 0.93 *vs*. SCr 0.84, and CyC is now often used in this role.[68, 76] The use of CyC as a biomarker for AKI has been investigated in several settings.

Cardiac surgery

In adults, despite an inconclusive result from Heise *et al*.,[77] other studies found that CyC predicted AKI early before SCr but that it was not superior to NGAL (Table II-III).[24, 36] Notably, after excluding those with preoperative renal impairment, Haase-Fielitz *et al*. found that the predictive performance of CyC for AKI was reduced from 0.83 to AUC 0.78, whereas that of NGAL remained the same (Table II-III).[36] In this setting, the predictive value of CyC appears to be partly as a marker of chronic renal injury rather than acute injury, [34] and, as such, it may be useful as a complementary marker to NGAL. CyC displayed considerable prognostic value in Haase-Fielitz' study; in this study, serum CyC and plasma NGAL were independent predictors of AKI and excellent predictors of the need for renal replacement therapy and of hospital death.[36] In addition, Haase *et al*.[34] found that CyC was an independent predictor of severity and duration of AKI after adult cardiac surgery.

Post transplant

After kidney transplant, CyC predicted delayed graft function, but the prediction was relatively late [78, 79] (at three days). In contrast, after liver transplants, Hei *et al*.[80] found that postoperative CyC predicted AKI earlier and more accurately than SCr (within 24 h) and that preoperative serum CyC also predicted postoperative AKI. Ling *et al*.[48] confirmed these findings (Table III).

Post contrast

Rickli *et al*.[81] found that, after contrast application, serum CyC levels increased before SCr. Furthermore, Kimmel *et al*.[82] found that serum CyC reflected contrast-induced changes better than SCr. Another study[83] found that urine CyC rose significantly at 8 hours ($P<0.05$) and at 24 h ($P<0.01$) after contrast, with no change in creatinine.

Thus, CyC is an earlier and more accurate marker of AKI than SCr, but it is generally preceded by NGAL in detecting AKI; elevated preoperative CyC before liver transplant predicts postoperative AKI; CyC is more sensitive to contrast-induced changes than SCr; and CyC is a better marker of chronic renal impairment and its effect on outcomes, [34, 80] which accounts for part of its diagnostic value. As both rise sequentially, CyC could complement NGAL, which can lose diagnostic accuracy in the presence of co-morbidities. Given that automated, standardized immunonephelometric assays are commercially available and provide results in minutes, CyC represents a feasible and promising biomarker for AKI. Its inclusion in a sequential AKI diagnostic panel with NGAL appears logical.

L-FABP

L-FABP is expressed in various organs including liver and kidney.[26] Its function in the kidney is presumed to be the same as that in the liver: cellular uptake of fatty acids (FAs) from plasma and promotion of intracellular FA metabolism. Free FAs are easily oxidized, leading to oxidative stress that can induce cellular injury. Through its involvement in regulation of FA metabolism, L-FABP may inhibit the accumulation of intracellular FAs (Table I, Figure 3), thereby preventing oxidation of free FAs.[84] L-FABP may be an important cellular antioxidant during oxidative stress.

L-FABP can be filtered *via* glomeruli and reabsorbed in the proximal tubule cells due to its small size, which could partly explain the increase of L-FABP in proximal tubular cell injury. However, an experimental study [23] revealed that renal L-FABP expression was up-regulated and that urinary L-FABP excretion was accelerated by accumulation of free FAs. Renal L-FABP may help maintain low levels of free FAs in the cytoplasm by facilitating their intracellular metabolism and their excretion in urine (Table I, Figure 3).[26] This is consistent with the early and exponential rise in urine L-FABP compared with a later and more modest rise in serum L-FABP in contrast-induced nephropathy in mice [85] and post-cardiac surgery in

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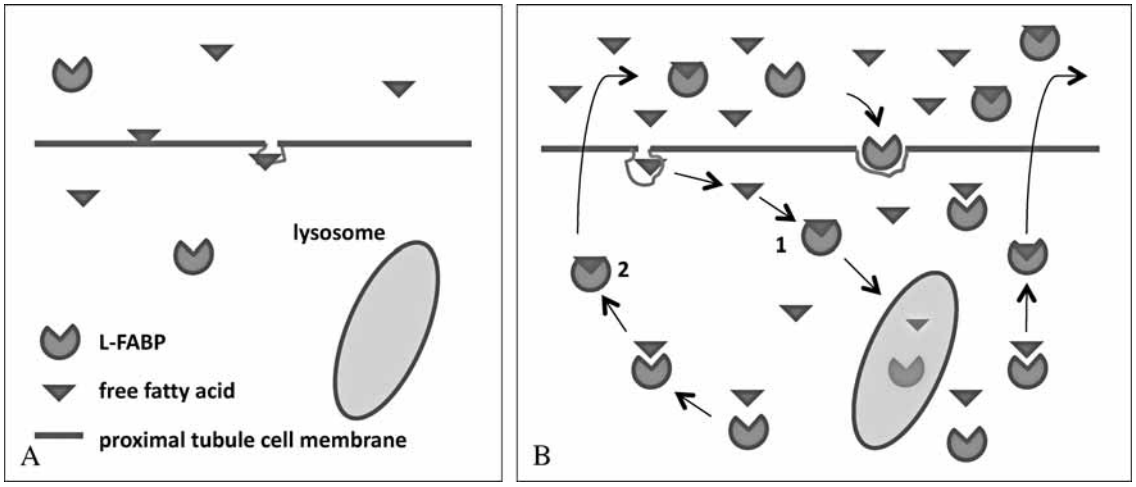

Figure 3.—A) Under normal conditions, the urinary L-FABP concentration is low. B) Proposed model of renal L-FABP up-regulation in the presence of fatty acid accumulation in proximal tubule cells after injury. Renal L-FABP and reabsorbed circulating L-FABP, filtered by the glomerulus, maintain low levels of Free Fatty Acids (FFAs) in the cytoplasm by 1) binding FFAs and transporting FAs to lysosomes to accelerate FA metabolism and by 2) binding FFAs and removing FAs from the cell for elimination *via* urine.

children.[27] ELISA techniques have been used to measure L-FABP levels; however, a urine dipstick kit has been developed and requires evaluation.[26]

In an experimental study, urine L-FABP showed great potential for early and accurate detection of histological and functional decline in both nephrotoxin-induced and ischemia-reperfusion injury in mice.[86] Dose-response to injury was well-reflected in L-FABP levels in this study; severity of histological injury increased with ischemia time and cisplatin dose and correlated well with L-FABP levels. Urine L-FABP increased after 1 h, even in mice subjected to only 5 min of ischemia.[86]

In a clinical study,[25] 13 of 66 patients had significantly elevated urine L-FABP before non-emergency angiogram; later, all 13 showed contrast-induced nephropathy, whereas no patient with low urine L-FABP showed signs of nephropathy. Furthermore, pre-contrast SCr showed no difference between the AKI/no AKI groups. Urine L-FABP appears to be a more sensitive predictor of AKI than SCr and could serve as a clinical predictor of contrast-induced nephropathy. In a further study, higher urine L-FABP levels differentiated patients with septic shock from those with severe sepsis, from those with AKI, and from healthy controls.[87] Of the septic shock patients; urine L-FABP levels in survivors were reduced by treatment. Non-survivors had higher urine levels with a smaller reduction after treatment compared with survivors. Thus, L-FABP may be useful in treatment evaluation. Urine L-FABP can predict AKI in pediatric cardiopulmonary bypass surgery [27] with an AUC at 4 h post-surgery of 0.81 (Table IV).

Urine L-FABP shows promise as an early, accurate biomarker of AKI; however, it appears to rise later than NGAL (pediatric bypass surgery, 4 h L-FABP *vs.* 2 h NGAL). The predictive ability of L-FABP for AKI requires further clinical confirmation in different patient populations.

Interleukin-18 (IL-18)

IL-18 is a proinflammatory cytokine and a powerful mediator of ischemia-induced AKI in animal models. It is induced and cleaved in the proximal tubule and is detected in urine following experimental AKI.[88, 89] In a cross-sectional study, IL-18 levels were significantly greater in patients with established AKI but not in those with urinary tract infections, pre-renal azotemia or CKD.[90] The AUC for the diagnosis of established AKI (acute tubular necrosis) was 0.95. Consistent with this finding, the AUC of IL-18 as an early predictor of AKI in patients after kidney transplant, in patients after pediatric cardiac surgery, and in those with Acute Respiratory Distress Syndrome [59, 91, 92] showed good performance (0.70-0.9), with the

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strongest predictive value post transplant. However, in critically ill children, IL-18 performed weakly.[93] Haase *et al.* concluded that IL-18 may be a non-specific marker of inflammation but that it did not predict AKI post cardiac surgery.[94] In general, IL-18 has displayed low sensitivity and high specificity. There have been weak positive results for the prognostic ability of urine IL-18: at 4 hours after cardiac surgery, IL-18 weakly correlated with number of days with AKI, [92] and in non-septic critically ill children, IL-18 predicted severity of AKI and mortality.[93] Furthermore, IL-18 predicts mortality in critically ill adults.[91] IL-18 is specific to ischemic AKI but may also be a non-specific marker of inflammation and has shown inconsistent results. Its inclusion in urinary panels requires further evaluation.

Kidney injury molecule-1 (KIM-1)

KIM-1 is a transmembrane glycoprotein that is not expressed in normal kidneys but that is upregulated in proximal tubular cells after ischemic or nephrotoxic injury. The ectodomain segment of KIM-1 is shed and is detected in urine.[95, 96] In a cross-sectional study,[97] KIM-1 was markedly induced in proximal tubules in biopsies from patients with established AKI (largely ischemic), and it differentiated ischemic AKI from pre-renal azotemia and CKD. In another cross-sectional study [98] of hospitalized patients, the AUC for KIM-1 for differentiating those with AKI from controls was 0.9. A further case-control study [98] found an AUC of 0.83 for KIM-1 for the diagnosis of AKI at 12 hours post-cardiopulmonary bypass. In a recent prospective study of 90 adults undergoing cardiac surgery, urinary KIM-1, N-acetyl-β-D-glucosaminidase (NAG - a lysosomal glucosidase abundant in tubular cells which is excreted in the urine when proximal tubule cells are damaged), and NGAL were measured.[37] The AUC for KIM-1 to predict AKI immediately post-surgery (0.68), although low, was higher than those for NAG and NGAL. Combining the three biomarkers enhanced the sensitivity of early detection of postoperative AKI, and AUCs became 0.75 and 0.78. Furthermore, in a study that examined the relationship between KIM-1 and a composite end-point (dialysis or death) in hospitalized patients, there was a suggestion that elevated urinary KIM-1 levels are associated with adverse outcomes in hospitalized patients who develop AKI.[99] The strength of KIM-1 appears to be detection of existing AKI. Its inclusion in a urinary AKI panel requires further investigation.

Conclusions

Given the heterogeneity of AKI and the settings in which it occurs, it is likely that diagnosis and classification of AKI will not be possible using one biomarker alone, and a panel of biomarkers comparable to the panel of cardiac enzymes used to diagnose and assess severity of acute myocardial infarction will be required. Current key renal biomarkers are NGAL and CyC, which 1) show great promise and utility, 2) have commercially available assays to provide immediate results, and 3) assess complementary aspects of renal injury (NGAL - tubular stress; CyC - GFR). In terms of sequence post-cardiac surgery, NGAL and L-FABP have higher predictive accuracy for AKI in urine and/or plasma early, at 2-4 h, which later wanes; in contrast, the predictive accuracy of CyC, IL-18 and KIM-1 increases at 12-24 h.[100] Such information can be used to include appropriate biomarkers in sequential predictive panels, which would open the door to a whole new area of research and, perhaps, interventions.

Novel renal biomarkers can be used to evaluate the effect of new techniques and therapies on kidney function and to provide safety markers for monitoring toxicity and AKI associated with established treatments.[18, 69, 101] To this end, NGAL, L-FABP and CyC have superior sensitivity and detect AKI earlier than SCr, enhancing the ability to demonstrate benefits and to justify the implementation of therapies or kidney protective techniques in evaluation studies.

Optimum perioperative hemodynamic management and measures such as preoperative hydration for high risk patients could be more effectively explored. In patients undergoing liver transplantation or major surgery, in which AKI is common,[48, 102] early detection of AKI with novel biomarkers has great potential. Novel protective therapies or those that have previously been difficult to evaluate or administer in time to prevent or

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ameliorate AKI (*e.g.*, N-acetyl cysteine, bicarbonate or fenoldopam) could be more appropriately assessed or administered in a targeted manner or, in patients receiving nephrotoxins (such as calcineurin inhibitors or aminoglycosides), renal injury could be detected much earlier and drug therapy adjusted. The potential benefit and mechanisms of volatile anesthetics for kidney protection [103-106] could also be investigated using novel biomarkers. The above information is highly relevant to the anesthesiologist; if early point-of-care biomarker measurements (*e.g.*, NGAL) were performed during or immediately after major surgery, this would allow timely implementation and evaluation of potential protective therapies, either intra-operatively or on ICU admission, targeted to those at high risk of AKI. Indeed, the consideration of pre- to postoperative changes in NGAL concentration could also aid medical decision-making, which might lead to improved outcomes. Furthermore, if NGAL reflected the likelihood for timely discharge, it may be useful in surgical case and ICU bed planning.

References

1. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P, and the ADQI workgroup. Acute renal failure definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004;8:R204-R12.
2. Bagshaw SM, George C, Dinu I, Bellomo R. A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. Nephrol Dial Transplant 2008;23:1203-10.
3. Ostermann M, Chang RWS. Acute kidney injury in the intensive care unit according to RIFLE. Crit Care Med 2007;35:1837-43.
4. Bagshaw SM, George C, Bellomo R, Committe ADM, Bagshaw SM, George C *et al*. A comparison of the RIFLE and AKIN criteria for acute kidney injury in critically ill patients. Nephrol Dial Transplant 2008;23:1569-74.
5. Lopes JA, Fernandes P, Jorge S, Goncalves S, Alvarez A, Costa e Silva Z *et al*. Acute kidney injury in intensive care unit patients: a comparison between the RIFLE and the Acute Kidney Injury Network classifications. Crit Care 2008;12:R110.
6. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. Kidney Int 2008;73:538-46.
7. Joannidis M, Metnitz B, Bauer P, Schusterschitz N, Moreno R, Druml W *et al*. Acute kidney injury in critically ill patients classified by AKIN *versus* RIFLE using the SAPS 3 database. Intensive Care Med 2009;35:1692-702.
8. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol 2005;16:3365-70.
9. Moore EM, Simpson JA, Tobin AE, Santamaria JD. Preoperative eGFR and RIFLE-classified postoperative AKI predict length of stay post coronary bypass surgery in an Australian setting. Anaesth Intensive Care 2010 [In press].
10. Landoni G, Biondi-Zoccai GG, Tumlin JA, Bove T, De Luca M, Calabro MG *et al*. Beneficial impact of fenoldopam in critically ill patients with or at risk for acute renal failure: a meta-analysis of randomized clinical trials. Am J Kidney Dis 2007;49:56-68.
11. Allaqaband S, Tumuluri R, Malik AM, Gupta A, Volkert P, Shalev Y *et al*. Prospective randomized study of N-acetylcysteine, fenoldopam, and saline for prevention of radiocontrast-induced nephropathy. Catheter Cardiovasc Interv 2002;57:279-83.
12. Stone GW, McCullough PA, Tumlin JA, Lepor NE, Madyoon H, Murray P *et al*. Fenoldopam mesylate for the prevention of contrast-induced nephropathy: a randomized controlled trial. JAMA 2003;290:2284-91.
13. Nigwekar SU, Hix JK, Nigwekar SU, Hix JK. The role of natriuretic peptide administration in cardiovascular surgery-associated renal dysfunction: a systematic review and meta-analysis of randomized controlled trials. J Cardiothorac Vasc Anesth 2009;23:151-60.
14. Nigwekar SU, Navaneethan SD, Parikh CR, Hix JK, Nigwekar SU, Navaneethan SD *et al*. Atrial natriuretic peptide for management of acute kidney injury: a systematic review and meta-analysis. Clin J Am Soc Nephrol 2009;4:261-72.
15. Gonzales DA, Norsworthy KJ, Kern SJ, Banks S, Sieving PC, Star RA *et al*. A meta-analysis of N-acetylcysteine in contrast-induced nephrotoxicity: unsupervised clustering to resolve heterogeneity. BMC Med 2007;5:32.
16. Nigwekar SU, Kandula P, Nigwekar SU, Kandula P. N-acetylcysteine in cardiovascular-surgery-associated renal failure: a meta-analysis. Ann Thorac Surg 2009;87:139-47.
17. Tepel M, van der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W. Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. N Engl J Med 2000;343:180-4.
18. Haase M, Haase-Fielitz A, Bellomo R, Devarajan P, Story D, Matalanis G *et al*. Sodium bicarbonate to prevent increases in serum creatinine after cardiac surgery: a pilot double-blind, randomized controlled trial. Crit Care Med 2009;37:39-47.
19. Brienza N, Giglio MT, Marucci M, Fiore T, Brienza N, Giglio MT *et al*. Does perioperative hemodynamic optimization protect renal function in surgical patients? A meta-analytic study. Crit Care Med 2009;37:2079-90.
20. Joannidis M. Medical therapy of acute kidney injury. Acta Clinica Belgica - Supplementum 2007;(2):353-6.
21. Mahon P, Shorten G. Perioperative acute renal failure. Curr Opin Anaesthesiol 2006;19:332-8.
22. Bagshaw SM, Gibney RTN. Conventional markers of kidney function. Crit Care Med 2008;36(4 Suppl):S152-8.
23. Kamijo A, Sugaya T, Hikawa A, Okada M, Okumura F, Yamanouchi M *et al*. Urinary excretion of fatty acid-binding protein reflects stress overload on the proximal tubules. Am J Pathol 2004;165:1243-55.
24. Koyner JL, Bennett MR, Worcester EM, Ma Q, Raman J, Jeevanandam V *et al*. Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery. Kidney Int 2008;74:1059-69.
25. Nakamura T, Sugaya T, Node K, Ueda Y, Koide H. Urinary excretion of liver-type fatty acid-binding protein in contrast medium-induced nephropathy. Am J Kidney Dis 2006;47:439-44.
26. Noiri E, Doi K, Negishi K, Tanaka T, Hamasaki Y, Fujita T *et al*. Urinary fatty acid-binding protein 1: an early predictive biomarker of kidney injury. Am J Physiol Renal Physiol 2009;296:F669-79.
27. Portilla D, Dent C, Sugaya T, Nagothu KK, Kundi I, Moore P *et al*. Liver fatty acid-binding protein as a biomarker of acute kidney injury after cardiac surgery. Kidney Int 2008;73:465-72.

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28. Schmidt-Ott KM, Mori K, Kalandadze A, Li J-Y, Paragas N, Nicholas T *et al*. Neutrophil gelatinase-associated lipocalin-mediated iron traffic in kidney epithelia. Curr Opin Nephrol Hypertens 2006;15:442-9.
29. Kell DB. Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. BMC Medical Genomics 2009;2(2).
30. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P *et al*. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 2007;18:407-13.
31. Ho J, Lucy M, Krokhin O, Hayglass K, Pascoe E, Darroch G *et al*. Mass spectrometry-based proteomic analysis of urine in acute kidney injury following cardiopulmonary bypass: a nested case-control study. Am J Kidney Dis 2009;53:584-95.
32. Bennett M, Dent CL, Ma Q, Dastrala S, Grenier F, Workman R *et al*. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol 2008;3:665-73.
33. Dent CL, Ma Q, Dastrala S, Bennett M, Mitsnefes MM, Barasch J *et al*. Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. Crit Care 2007;11:R127.
34. Haase M, Bellomo R, Devarajan P, Ma Q, Bennett MR, Mockel M *et al*. Novel biomarkers early predict the severity of acute kidney injury after cardiac surgery in adults. Ann Thorac Surg 2009;88:124-30.
35. Haase-Fielitz A, Bellomo R, Devarajan P, Bennett M, Story D, Matalanis G *et al*. The predictive performance of plasma neutrophil gelatinase-associated lipocalin (NGAL) increases with grade of acute kidney injury. Nephrol Dial Transplant 2009;24:3349-54.
36. Haase-Fielitz A, Bellomo R, Devarajan P, Story D, Matalanis G, Dragun D *et al*. Novel and conventional serum biomarkers predicting acute kidney injury in adult cardiac surgery—a prospective cohort study. Crit Care Med 2009;37:553-60.
37. Han WK, Wagener G, Zhu Y, Wang S, Lee HT. Urinary biomarkers in the early detection of acute kidney injury after cardiac surgery. Clin J Am Soc Nephrol 2009;4:873-82.
38. Liangos O, Tighiouart H, Perianayagam MC, Kolyada A, Han WK, Wald R *et al*. Comparative analysis of urinary biomarkers for early detection of acute kidney injury following cardiopulmonary bypass. Biomarkers 2009;14:423-31.
39. Tuladhar SM, Puntmann VO, Soni M, Punjabi PP, Bogle RG. Rapid detection of acute kidney injury by plasma and urinary neutrophil gelatinase-associated lipocalin after cardiopulmonary bypass. J Cardiovasc Pharmacol 2009;53:261-6.
40. Xin C, Yulong X, Yu C, Changchun C, Feng Z, Xinwei M. Urine neutrophil gelatinase-associated lipocalin and interleukin-18 predict acute kidney injury after cardiac surgery. Ren Fail 2008;30:904-13.
41. Wagener G, Jan M, Kim M, Mori K, Barasch JM, Sladen RN *et al*. Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. Anesthesiology 2006;105:485-91.
42. Ling W, Zhaohui N, Ben H, Leyi G, Jianping L, Huili D *et al*. Urinary IL-18 and NGAL as early predictive biomarkers in contrast-induced nephropathy after coronary angiography. Nephron 2008;108:c176-81.
43. Makris K, Markou N, Evodia E, Dimopoulou E, Drakopoulos I, Ntetsika K *et al*. Urinary neutrophil gelatinase-associated lipocalin (NGAL) as an early marker of acute kidney injury in critically ill multiple trauma patients. Clin Chem Lab Med 2009;47:79-82.
44. Nickolas TL, O'Rourke MJ, Yang J, Sise ME, Canetta PA, Barasch N *et al*. Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. Ann Intern Med 2008;148:810-9.
45. Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT. Urinary neutrophil gelatinase-associated lipocalin and acute kidney injury after cardiac surgery. Am J Kidney Dis 2008;52:425-33.
46. Ahlstrom A, Tallgren M, Peltonen S, Pettila V. Evolution and predictive power of serum cystatin C in acute renal failure. Clin Nephrol 2004;62:344-50.
47. Daniel J-P, Chantrel F, Offner M, Moulin B, Hannedouche T. Comparison of cystatin C, creatinine and creatinine clearance vs. GFR for detection of renal failure in renal transplant patients. Ren Fail 2004;26:253-7.
48. Ling Q, Xu X, Li JJ, Chen J, Shen JW, Zheng SS. Alternative definition of acute kidney injury following liver transplantation: based on serum creatinine and cystatin C levels. Transplant Proc 2007;39:3257-60.
49. Villa P, Jimenez M, Soriano M-C, Manzanares J, Casasnovas P. Serum cystatin C concentration as a marker of acute renal dysfunction in critically ill patients. Crit Care 2005;9:R139-43.
50. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C *et al*. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2005;365:1231-8.
51. Herrero-Morin JD, Malaga S, Fernandez N, Rey C, Dieguez MA, Solis G *et al*. Cystatin C and beta2-microglobulin: markers of glomerular filtration in critically ill children. Crit Care 2007;11:R59.
52. Hirsch R, Dent C, Pfriem H, Allen J, Beekman RH 3rd, Ma Q *et al*. NGAL is an early predictive biomarker of contrast-induced nephropathy in children. Pediatr Nephrol 2007;22:2089-95.
53. Wheeler DS, Devarajan P, Ma Q, Harmon K, Monaco M, Cvijanovich N *et al*. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. Crit Care Med 2008;36:1297-303.
54. Siew ED, Ware LB, Gebretsadik T, Shintani A, Moons KGM, Wickersham N *et al*. Urine neutrophil gelatinase-associated lipocalin moderately predicts acute kidney injury in critically ill adults. J Am Soc Nephrol 2009;20:1823-32.
55. Zappitelli M, Washburn KK, Arikan AA, Loftis L, Ma Q, Devarajan P *et al*. Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: a prospective cohort study. Critical Care 2007;11:R84.
56. Herget-Rosenthal S, Marggraf G, Husing J, Goring F, Pietruck F, Janssen O *et al*. Early detection of acute renal failure by serum cystatin C. Kidney Int 2004;66:1115-22.
57. Herget-Rosenthal S, Poppen D, Husing J, Marggraf G, Pietruck F, Jakob H-G *et al*. Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. Clin Chem 2004;50:552-8.
58. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Dobrzycki S. Neutrophil gelatinase-associated lipocalin (NGAL) correlations with cystatin C, serum creatinine and eGFR in patients with normal serum creatinine undergoing coronary angiography. Nephrol Dial Transplant 2007;22:295-6.
59. Parikh CR, Jani A, Mishra J, Ma Q, Kelly C, Barasch J *et al*. Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation. Am J Transplant 2006;6:1639-45.
60. Kusaka M, Kuroyanagi Y, Mori T, Nagaoka K, Sasaki H, Maruyama T *et al*. Serum neutrophil gelatinase-associated lipocalin as a predictor of organ recovery from delayed graft function after kidney transplantation from donors after cardiac death. Cell Transplant 2008;17:129-34.
61. Bolignano D, Lacquaniti A, Coppolino G, Campo S, Arena A, Buemi M. Neutrophil gelatinase-associated lipocalin reflects the severity of renal impairment in subjects affected by chronic kidney disease. Kidney Blood Press Res 2008;31:255-8.

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62. Mitsnefes MM, Kathman TS, Mishra J, Kartal J, Khoury PR, Nickolas TL *et al*. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in children with chronic kidney disease. Pediatr Nephrol 2007;22:101-8.
63. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR *et al*. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. Clin J Am Soc Nephrol 2009;4:337-44.
64. Goldstein SL, Devarajan P. Progression from acute kidney injury to chronic kidney disease: a pediatric perspective. Adv Chronic Kidney Dis 2008;15:278-83.
65. Trachtman H, Christen E, Cnaan A, Patrick J, Mai V, Mishra J *et al*. Urinary neutrophil gelatinase-associated lipocalcin in D+HUS: a novel marker of renal injury. Pediatr Nephrol 2006;21:989-94.
66. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A, Ngal Meta-analysis Investigator Group. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis 2009;54:1012-24.
67. Ilva T, Eriksson S, Lund J, Porela P, Mustonen H, Pettersson K *et al*. Improved early risk stratification and diagnosis of myocardial infarction, using a novel troponin I assay concept. Eur J Clin Invest 2005;35:112-6.
68. Boldt J, Brosch C, Ducke M, Papsdorf M, Lehmann A. Influence of volume therapy with a modern hydroxyethylstarch preparation on kidney function in cardiac surgery patients with compromised renal function: a comparison with human albumin. Crit Care Med 2007;35:2740-6.
69. Capuano F, Goracci M, Luciani R, Gentile G, Roscitano A, Benedetto U *et al*. Neutrophil gelatinase-associated lipocalin levels after use of mini-cardiopulmonary bypass system. Interact Cardiovasc Thorac Surg 2009;9:797-801.
70. Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT. A comparison of urinary neutrophil gelatinase-associated lipocalin in patients undergoing on- *versus* off-pump coronary artery bypass graft surgery. J Cardiothorac Vasc Anesth 2009;23:195-9.
71. Dharnidharka VR, Kwon C, Stevens G, Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis 2002;40:221-6.
72. Laterza OF, Price CP, Scott MG, Laterza OF, Price CP, Scott MG. Cystatin C: an improved estimator of glomerular filtration rate? Clin Chem 2002;48:699-707.
73. Mazul-Sunko B, Zarkovic N, Vrkic N, Antoljak N, Bekavac Beslin M, Nikolic Heitzler V *et al*. Proatrial natriuretic peptide (1-98), but not cystatin C, is predictive for occurrence of acute renal insufficiency in critically ill septic patients. Nephron 2004;97:c103-7.
74. Delanaye P, Lambermont B, Chapelle JP, Gielen J, Gerard P, Rorive G *et al*. Plasmatic cystatin C for the estimation of glomerular filtration rate in intensive care units. Intensive Care Med 2004;30:980-3.
75. Royakkers AANM, van Suijlen JDE, Hofstra LS, Kuiper MA, Bouman CSC, Spronk PE *et al*. Serum cystatin C-A useful endogenous marker of renal function in intensive care unit patients at risk for or with acute renal failure? Curr Med Chem 2007;14:2314-7.
76. Kaartinen K, Syrjanen J, Porsti I, Hurme M, Harmoinen A, Pasternack A *et al*. Inflammatory markers and the progression of IgA glomerulonephritis. Nephrol Dial Transplant 2008;23:1285-90.
77. Heise D, Waeschle RM, Schlobohm J, Wessels J, Quintel M. Utility of cystatin C for assessment of renal function after cardiac surgery. Nephron 2009;112:c107-14.
78. Lebkowska U, Malyszko J, Lebkowska A, Koc-Zorawska E, Lebkowski W, Malyszko JS *et al*. Neutrophil gelatinase-associated lipocalin and cystatin C could predict renal outcome in patients undergoing kidney allograft transplantation: a prospective study. Transplant Proc 2009;41:154-7.
79. Poge U, Gerhardt T, Bokenkamp A, Stoffel-Wagner B, Klehr HU, Sauerbruch T *et al*. Time course of low molecular weight proteins in the early kidney transplantation period—influence of corticosteroids. Nephrol Dial Transplant 2004;19: 2858-63.
80. Hei Z-q, Li X-y, Shen N, Pang H-y, Zhou S-l, Guan J-q. Prognostic values of serum cystatin C and beta2 microglobulin, urinary beta2 microglobulin and N-acetyl-beta-D-glucosaminidase in early acute renal failure after liver transplantation. Chin Med J (Engl) 2008;121:1251-6.
81. Rickli H, Benou K, Ammann P, Fehr T, Brunner-La Rocca HP, Petridis H *et al*. Time course of serial cystatin C levels in comparison with serum creatinine after application of radiocontrast media. Clin Nephrol 2004;61:98-102.
82. Kimmel M, Butscheid M, Brenner S, Kuhlmann U, Klotz U, Alscher DM *et al*. Improved estimation of glomerular filtration rate by serum cystatin C in preventing contrast induced nephropathy by N-acetylcysteine or zinc — preliminary results. Nephrol Dials Transplant 2008;23:1241-5.
83. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Pawlak K, Mysliwiec M *et al*. Could neutrophil-gelatinase-associated lipocalin and cystatin C predict the development of contrast-induced nephropathy after percutaneous coronary interventions in patients with stable angina and normal serum creatinine values? Kidney Blood Press Res 2007;30:408-15.
84. Kamijo-Ikemori A, Sugaya T, Kimura K. Urinary fatty acid binding protein in renal disease. Clin Chim Acta 2006;374:1-7.
85. Negishi K, Noiri E, Maeda R, Portilla D, Sugaya T, Fujita T. Renal L-type fatty acid-binding protein mediates the bezafibrate reduction of cisplatin-induced acute kidney injury. Kidney Int 2008;73:1374-84.
86. Negishi K, Noiri E, Doi K, Maeda-Mamiya R, Sugaya T, Portilla D *et al*. Monitoring of urinary L-type fatty acid-binding protein predicts histological severity of acute kidney injury. Am J Pathol 2009;174:1154-9.
87. Nakamura T, Sugaya T, Koide H. Urinary liver-type fatty acid-binding protein in septic shock: effect of polymyxin B-immobilized fiber hemoperfusion. Shock 2009;31:454-9.
88. Melnikov VY, Ecder T, Fantuzzi G, Siegmund B, Lucia MS, Dinarello CA *et al*. Impaired IL-18 processing protects caspase-1-deficient mice from ischemic acute renal failure. J Clin Invest 2001;107:1145-52.
89. Melnikov VY, Faubel S, Siegmund B, Lucia MS, Ljubanovic D, Edelstein CL *et al*. Neutrophil-independent mechanisms of caspase-1- and IL-18-mediated ischemic acute tubular necrosis in mice. J Clin Invest 2002;110:1083-91.
90. Parikh CR, Jani A, Melnikov VY, Faubel S, Edelstein CL, Parikh CR *et al*. Urinary interleukin-18 is a marker of human acute tubular necrosis. Am J Kidney Dis 2004;43: 405-14.
91. Parikh CR, Abraham E, Ancukiewicz M, Edelstein CL, Parikh CR, Abraham E *et al*. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. J Am Soc Nephrol 2005;16:3046-52.
92. Parikh CR, Mishra J, Thiessen-Philbrook H, Dursun B, Ma Q, Kelly C *et al*. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. Kidney Int 2006;70:199-203.
93. Washburn KK, Zappitelli M, Arikan AA, Loftis L, Yalavarthy R, Parikh CR *et al*. Urinary interleukin-18 is an acute kidney injury biomarker in critically ill children. Nephrol Dial Transplant 2008;23:566-72.
94. Haase M, Bellomo R, Story D, Davenport P, Haase-Fielitz A. Urinary interleukin-18 does not predict acute kidney injury after adult cardiac surgery: a prospective observational cohort study. Crit Care 2008;12:R96.
95. Bagshaw SM, Langenberg C, Haase M, Wan L, May CN, Bellomo R *et al*. Urinary biomarkers in septic acute kidney injury. Intensive Care Med 2007;33:1285-96.

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96. Zhang Z, Humphreys BD, Bonventre JV, Zhang Z, Humphreys BD, Bonventre JV. Shedding of the urinary biomarker kidney injury molecule-1 (KIM-1) is regulated by MAP kinases and juxtamembrane region. J Am Soc Nephrol 2007;18:2704-14.
97. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV, Han WK *et al*. Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. Kidney Int 2002;62:237-44.
98. Han WK, Waikar SS, Johnson A, Betensky RA, Dent CL, Devarajan P *et al*. Urinary biomarkers in the early diagnosis of acute kidney injury. Kidney Int 2008;73:863-9.
99. Liangos O, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H *et al*. Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. J Am Soc Nephrol 2007;18:904-12.
100. Thurman JM, Parikh CR. Peeking into the black box: new biomarkers for acute kidney injury. Kidney Int 2008;73:379-81.
101. Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT. Increased incidence of acute kidney injury with aprotinin use during cardiac surgery detected with urinary NGAL. Am J Nephrol 2008;28:576-82.
102. Biancofiore G, Davis CL, Biancofiore G, Davis CL. Renal dysfunction in the perioperative liver transplant period. Curr Opin Organ Transplant 2008;13:291-7.
103. De Hert SG, Turani F, Mathur S, Stowe DF, De Hert SG, Turani F *et al*. Cardioprotection with volatile anesthetics: mechanisms and clinical implications. Anesth Analg 2005;100:1584-93.
104. Julier K, da Silva R, Garcia C, Bestmann L, Frascarolo P, Zollinger A *et al*. Preconditioning by sevoflurane decreases biochemical markers for myocardial and renal dysfunction in coronary artery bypass graft surgery: a double-blinded, placebo-controlled, multicenter study. Anesthesiology 2003;98:1315-27.
105. Landoni G, Turi S, Bignami E, Zangrillo A. Organ protection by volatile anesthetics in non-coronary artery bypass grafting surgery. Future Cardiol 2009;5:589-603.
106. Pagel PS, Pagel PS. Cardioprotection by volatile anesthetics: established scientific principle or lingering clinical uncertainty? J Cardiothorac Vasc Anesth 2009;23:589-93.

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2.4.2 Renal substudies: Novel Biomarkers of AKI requiring further description

2.4.2.1 IL-18

IL-18 is a pro-inflammatory cytokine that binds to the IL-18 receptor and along with IL-12 induces cell-mediated immunity after microbial infection. IL-18 is also able to induce severe inflammatory reactions and it is a powerful mediator of ischemia-induced AKI in animal models. It is induced and cleaved in the proximal tubule and is detected in urine following experimental AKI[45,46]. IL-18 has shown promise in detecting cases of established AKI[47] (AUC=0.95). Consistent with this, the AUC of IL-18 as an early predictor of AKI in patients after kidney transplant, paediatric cardiac surgery, and in patients with Acute Respiratory Distress Syndrome[48-50], showed good performance (0.70-0.9), with strongest predictive value post transplant. In non-septic critically ill children[51] and in critically ill adults[48] IL-18 has predicted mortality; however, results have been inconsistent[50,52]. In a cross-sectional study, IL-18 levels were significantly greater in patients with established AKI but not in those with urinary tract infections, pre-renal azotemia or chronic kidney disease[47]. It is important to note that IL-18 measurements may be influenced by coexisting inflammatory conditions such as arthritis, inflammatory bowel disease and systemic lupus erythematosus. In general, IL-18 has displayed low sensitivity and high specificity; it is specific to ischemic AKI but may also be a non-specific marker of inflammation and has shown inconsistent results. The use of IL-18 in studies as an indicator of kidney injury has become more prevalent[53].

2.4.2.2 KIM-1

KIM-1 is a transmembrane glycoprotein not expressed in normal kidneys but is up-regulated in proximal tubular cells after ischemic or nephrotoxic injury that results in dedifferentiation of the epithelium (early manifestation of response to injury[54]). KIM-1 is also expressed in lymphocytes, but at significantly lower levels[55].

KIM-1 enables epithelial cells to detect and phagocytose dead cells that obstruct the tubule lumen in the post-ischaemic kidney in AKI[55]. KIM-1 is a phosphatidylserine receptor that detects apoptotic cells and directs them to lysosomes[56]. KIM-1 may also play an important role in limiting the autoimmune response to injury, given that phagocytosis of apoptotic bodies is one mechanism for limiting the proinflammatory

response in many systems. It appears that KIM-1 protein is also expressed in the proximal tubules in chronic renal failure[57].

KIM-1 has shown to be a more sensitive marker of kidney injury than creatinine in experimental studies with many types of injury, including toxin-induced injury[58-61]. Its performance in humans reflects this; the concentration of KIM-1 was found to be higher in patients with AKI (including so-called 'pre-renal AKI'[62]) and it increased before the presence of casts in urine in patients with so-called 'ATN'[63]. Furthermore, in less severe cases of AKI such as contrast-induced injury, the concentration of KIM-1 appears to be reduced suggesting that levels reflect the severity of disease[63]. In paediatric cardiac surgery, it appears to be a sensitive diagnostic tool for AKI[63], and in renal transplant recipients levels have predicted AKI and graft loss[59], and are associated with histological changes indicating tubular damage[64]. In a cross-sectional study[20] of hospitalised patients, the AUC for KIM-1 for differentiating those with AKI from controls was 0.9[65]. A further case-control study found an AUC of 0.83 for KIM-1 for the diagnosis of AKI at 12 hours post cardiopulmonary bypass. KIM-1 can now be used in the evaluation of kidney injury in FDA drug review processes. Urinary KIM-1 also appears to be associated with future risk of kidney disease and may identify people at risk for CKD[66].

KIM-1 is a true marker of injury (as opposed to a marker of GFR, such as creatinine). It is not expressed normally in the kidneys and its upregulation and insertion into the proximal tubule membrane with injury, and presence until the cells have completely recovered, are properties that make it a very strong candidate as a biomarker of AKI[66]. Increasingly, KIM-1 along with other biomarkers are being used to detect 'sub-clinical' degrees of AKI in studies[62], as is now recommended[67]. The recent availability of a fast urine dipstick for KIM-1[68] will facilitate its evaluation in future studies. In future, the role of KIM-1 as a therapeutic target in addition to that of a marker of AKI may be explored and if an AKI urinary biomarker panel were developed, its inclusion would be almost guaranteed.

2.4.2.3 Alpha- and Pi-GST

The cytosolic dimeric isoenzymes alpha- and pi-Glutathione S-transferase (GST) metabolise chemotherapeutic drugs, carcinogens, environmental pollutants, and a range

of other harmful foreign compounds (xenobiotics). GSTs play a key role in enzymic detoxification, involving the conversion of xenobiotics (often electrophilic and lipophilic) into more water-soluble, less toxic metabolites that can be eliminated more easily from the cell[69]. GSTs also play an important role as intracellular binding proteins[70].

Low levels of GST are released into the urine in healthy people and in the presence of renal tubular injury, intracellular GST is released into urine by damaged tubular cells in larger amounts (alpha-GST is present in the proximal tubule, and pi-GST is largely in the distal tubule)[69,70]. Assays to measure their concentration in urine are now commercially available, and more widespread testing could increase the understanding of their clinical utility. Concurrent measurement of urinary alpha- and pi-GST may allow discrimination between proximal and distal tubular injury.

Levels of urinary alpha-GST have been shown to rise in nephrotoxicity[71], surgery[65], acute kidney failure and renal transplants[72,73]. Alpha-GST plays an important role in cellular antioxidant defence mechanisms[74], and has performed quite well as a biomarker for AKI. Alpha-GST is also a biomarker of liver damage and is involved in the biosynthesis of sex steroids[75]. Pi-GST plays a role in inhibition of the Jun N-terminal kinase (JNK), protecting cells from hydrogen peroxide-induced cell death[70]. Alpha- and pi-GSTs have been increasingly studied as early biomarkers of acute kidney injury in patients after cardiac surgery[76] and in ICU patients with sepsis[65].

2.4.2.4 Cathepsin S

Plasma cathepsin S is a lysosomal cysteine proteinase regulated by its main endogenous inhibitor cystatin C. Apart from its role in protein breakdown, experimental data suggest that there is a close connection between cathepsin S and the regulation of inflammatory activity[77]; higher levels are associated with increased inflammation. Cathepsin S is also associated with heart disease[78] and cancer[79]. Circulating levels are higher in people with obesity, diabetes and cardiovascular disease[80-82] and weight loss is reported to reduce levels.

Experimental studies suggest that Cathepsin S promotes formation of atherosclerotic plaques and destabilises advanced plaques[78,83-85]. It degrades low-density lipoprotein cholesterol and reduces cholesterol efflux from macrophage foam cells (lipid-loaded macrophages), thus suggesting that Cathepsin S aggravates the formation and maintenance of foam cells in atherosclerotic lesions.

Its role in the development of cancer may be through stimulating cancer cell migration and tumour angiogenesis and proliferation[79,86-88]. Cathepsin S is highly expressed in malignant tissue and may be involved early in the process of the development of clinically diagnosed cancer[79,88].

In a recent community-based study of elderly men and women, higher serum cathepsin S was associated with an increased risk for mortality[89]. Given its potential role in many diseases, cathepsin S has been proposed as a promising target for pharmaceutical development of an inhibitor[90]. Further research of the utility of cathepsin S measurement in clinical settings is required. As we were measuring its inhibitor, cystatin C, it was considered of interest to measure cathepsin S levels as well.

3 The Renal Substudies: Methods

The Renal substudies of the EPO-TBI and POLAR trials provide the structure of the investigation in this thesis. They were designed by the PhD candidate and are incorporated in the protocols of the respective trials; the substudies have many similarities in their design. The Renal substudies and associated methods are described in this section treated as a single project. The protocols of the EPO-TBI and POLAR trials, hard copies of the respective electronic case report forms and example consent forms are included as appendices to this thesis to provide the context of the substudies.

3.1 Aims

We plan to investigate treatments and new biomarkers for the early detection of acute kidney injury (AKI) by analysing changes in renal function and biomarkers in the Renal Substudies of the EPO-TBI and POLAR multi-centre randomised controlled trials. The specific aims of this project are to determine whether in patients with TBI:

a) EPO therapy and early and sustained hypothermia reduce the risk for AKI compared to placebo and normothermia respectively.
b) the release of biomarkers NGAL, cystatin-C, IL-18, KIM-1, Cathepsin S and alph- and pi-GST, is reduced in those treated with EPO and in those receiving early and sustained hypothermia, compared to those in the respective control groups.
c) the release of these biomarkers predicts the occurrence, severity, duration of and recovery from AKI.

3.2 Background

Acute kidney injury (AKI) is a rapid deterioration of kidney function and is the consensus term now used to describe acute renal failure[91]; it is reported to occur in one third of ICU patients[92,93]. Information on the incidence of AKI in patients with TBI is very limited. We performed what was to the best of our knowledge the first dedicated study to determine the incidence of AKI in patients with TBI (see section 1.4). We found the incidence of AKI (Risk/Injury/Failure) using the RIFLE criteria was 9.2% in

patients with moderate and severe TBI, in two adult major trauma centres in Victoria, Australia.[94] A further retrospective single-centre study set in Shanghai, China[95] (n=136) found the incidence of AKI using the AKIN classification system, which has more permissive Stage 1 criteria (RIFLE Risk equivalent), was 23%. The occurrence of AKI in patients with TBI was considered sufficient to continue the conduction of the Renal substudies of the EPO-TBI and POLAR trials.

AKI increases the risk of aggravating cerebral oedema[96], and is also associated with increased mortality[97] and prolonged ICU and hospital length of stay[98,99]. Severe cases require costly treatments and can result in extended injury and delayed recovery, escalating the human and financial costs associated with trauma cases. The reversibility of AKI is more likely with prompt treatment[100], however current markers of AKI (creatinine and urine output) are unreliable or detect AKI too late to prevent or ameliorate kidney injury with treatment. This has hindered investigation of potential treatments for AKI. It is therefore a priority to investigate markers which may facilitate earlier detection of AKI and treatments which may reduce the incidence of AKI. Cerebral oedema is relatively common amongst severe to moderate TBI patients. As the risk of aggravating cerebral oedema increases with AKI, patients with TBI could directly benefit from the investigation of new treatments for AKI.

Two treatments: erythropoietin (a tissue-protective drug currently in use for other purposes) and early and sustained hypothermia are being given to patients with TBI in the EPO-TBI and POLAR clinical trials respectively, to investigate their benefit for neurological outcome. Both of these treatments also show promise for treating AKI, and we propose to take advantage of this unique opportunity and study their effect on kidney function in these trials. Although evidence for the renoprotective effects of EPO and hypothermia can be found in section 2.2 and 2.3, a summary is provided below for the reader's convenience.

3.3 EPO: Renal effects

In addition to neuroprotective effects, extensive animal studies support the nephroprotective effect of EPO following ischemia-reperfusion injury, contrast-induced nephropathy, and in endotoxin or septic models[101-107]. Recent experimental evidence also confirmed the prevention of renal damage as well as a neuroprotective effect when

erythropoietin was given in the presence of brain death[108]. The potential for a similar dual effect in patients with traumatic brain injury is worthy of investigation. The neurological and renal cytoprotective effects of EPO appear related to promotion of neovascularisation and tissue regeneration, limitation of apoptosis, and anti-inflammatory action[109]. Several trials are currently investigating the effect of EPO on incidence of AKI in cardiac surgical patients; on kidney graft function after kidney transplant; and in ICU patients with AKI; in small cohorts[110,111]. Results of a small clinical pilot trial on cardiac surgical patients (n=71), indicated a reduction in the incidence of AKI, and improved postoperative renal function in patients receiving EPO[112]. However, results are inconsistent; two small trials of EPO in cardiac surgery (n=80)[113], and in ICU patients at risk for AKI (n=162)[114] found no renoprotective effect of EPO. Methodical issues could have contributed to the latter result, and whether more sensitive definitions of AKI using alternative biomarkers would provide different results is unknown. Certainly, further investigation is required. There has been no such investigation in patients with TBI. The renal substudy of EPO-TBI presents a unique opportunity to fill this gap at minimal cost.

3.4 Hypothermia: Renal effects

Evidence suggests that inducing mild hypothermia (32-35°C) in the first hours after an ischaemic event can prevent or ameliorate permanent injury not only in the brain as for TBI but also in the kidneys[115,116]. In experimental studies, hypothermia (~33°C) at the time of and immediately after induced renal ischaemia/ reperfusion injury dramatically reduced the risk of renal failure[115,116]. In animals involved in kidney ischemia-reperfusion models it is well-acknowledged that temperature control is important to avoid confounding because hypothermia exerts a marked protective effect[117]. Some mechanisms through which hypothermia may protect the kidneys include: the blockade of excitotoxic mechanisms, modulation of the inflammatory response and modulation of apoptosis[118]. There has been little clinical investigation of the renal effects of hypothermia[119-122] and with inconclusive results, and no such trial in patients with TBI. A plausible rationale suggests the potential benefit of prophylactic hypothermia for kidney injury and the POLAR trial presents a unique opportunity to clinically investigate this question.

3.5 Biomarkers

Creatinine and urine output are acknowledged as insensitive and non-specific markers of AKI. Changes in serum creatinine occur late in the injury process, when initiation of kidney protective measures is likely too late[123], which has hindered investigation into treatments for AKI. As a result in recent years many alternative early biomarkers for AKI have been explored. In the Renal substudies of EPO-TBI and POLAR we measured the following novel biomarkers to investigate their potential as early predictors of AKI and their response to therapy in patients with TBI: NGAL, cystatin-C and Cathepsin S in plasma; and NGAL, cystatin-C, IL-18, KIM-1, and alpha- and pi-GST in urine. Cathepsin S is the only 'odd man out' here; it appears to play a role in many disease processes, and as we were measuring its inhibitor, cystatin C, it was considered of interest, and was feasible to measure. Descriptions of the potential mechanisms of action of these biomarkers and evidence of their utility as early markers for AKI can be found in section 2.4.

Several of the biomarkers measured are potential alternatives to creatinine for the definition and early diagnosis of AKI, either alone or in synergy with other biomarkers. If their value as early renal biomarkers is confirmed in further clinical studies in a variety of critically ill populations they may change the definition, diagnosis, prognosis and timing of therapy for AKI. No study has analysed the biomarkers we have measured for their response to therapy or as markers of renal function in patients with TBI. The POLAR and EPO-TBI trials provide an unprecedented opportunity to analyse the biomarker response to EPO and hypothermia which could elucidate mechanisms involved in the body's response to these therapies. This project is an example of resourceful and cost-efficient research which would otherwise have a prohibitive price tag.

3.6 Methods

This project comprises the Renal Substudies of the EPO-TBI and POLAR multicentre randomised controlled trials. The aim is to assess the effect of EPO and early and sustained hypothermia, on renal function, and renal biomarker response in patients with TBI. Renal function will be assessed for all patients in the POLAR (n=500) and EPO-TBI (n=606) trials using urine output and creatinine-based criteria of the extensively

used and validated RIFLE classification system[91]. In small nested cohorts (n=30 EPO-TBI; n=53 POLAR) within each trial, the level of the novel renal biomarkers in urine and plasma as appropriate was measured at 4 timepoints before and during therapy.

3.6.1 Inclusion / Exclusion criteria

Inclusion criteria: The same as for the EPO-TBI and POLAR trials; all patients recruited to these trials will participate in the substudies.

Inclusion criteria- EPO-TBI: ICU patients with non-penetrating moderate or severe TBI (pre-intubation GCS <13) who are a) ≥ 15 and ≤ 65 years, b) ≤ 24 hours since primary traumatic injury, c) expected to stay ≥ 48 hours, d) have a haemoglobin not exceeding the upper limit of the applicable normal reference range in use at the treating institution, and e) have written informed consent from the legal surrogate.

Inclusion criteria- POLAR: a) Non–penetrating severe TBI (GCS < 9), b) estimated age ≥ 18 and < 60 years of age and c) patient is intubated or intubation is imminent.

Exclusion criteria: The same as for the EPO-TBI and POLAR trials with the following addition: those undergoing renal replacement therapy while conventional renal markers are being measured will be excluded from analyses to compare risk for AKI.

Exclusion criteria EPO-TBI: a) GCS = 3 + fixed dilated pupils, b) history of DVT/ PE /other thromboembolic event, c) a chronic hypercoagulable disorder including any known malignancy, d) treatment with EPO in the last 30 days, e) first dose unable to be given within 24 hours of injury, f) pregnancy or lactation, g) uncontrolled hypertension, h) history of epilepsy/seizures in past 3 months, i) acute myocardial infarct in last 12 months, j) imminent death and k) inability to perform lower limb ultrasounds, l) sensitivity to mammalian cell derived products, m) hypersensitivity to the active substance or additives, n) pure red cell aplasia, o) end stage renal failure (receives chronic dialysis), p) severe pre-existing physical or mental disability or severe co morbidity that may interfere with the assessment of outcome q) spinal cord injury, r) treatment with investigational drug within 30 days of enrolment, s) treating physician believes it is not in the best interests of the patient to be randomized to this trial.

Exclusion criteria POLAR – pre-hospital: a) drug or alcohol intoxication diagnosed as predominant cause of coma, b) randomisation not possible within 3 hours of injury, c) estimated transport time to hospital > 2.5hrs at the scene d) intubation possible without drugs e) systolic BP <90mmHg, f) heart rate > 120bpm pre-hospital, g) GCS=3 + un-reactive pupils, h) obvious/known pregnancy, i) receiving hospital is not a study

site, j) evidence of anti-coagulant treatment, k) cardiac arrest at the scene or in transit, l) penetrating neck/torso injury, m) known to be carer-dependent due to pre-existing neurological condition.

***Additional POLAR exclusion criteria once in the Emergency Department*:** clinically significant bleeding likely to require haemostatic intervention, positive urine or blood pregnancy test, the treating clinician believes that cooling is not in the patient's best interest.

3.6.2 Power calculations and sample size

The power calculations for EPO/POLAR-AKI for the complete sample when recruitment is finished (n=606 EPO-TBI; n=500 POLAR), and for the anticipated complete sample size for EPO/POLAR-Biomarkers (n=50) can be found in the Renal substudy sections of the EPO-TBI and POLAR trial protocols in the appendices (EPO-TBI: Section 11, page 33; POLAR: section 30, page54 of protocols). Due to timeframes for PhD completion, data was extracted before most of these target samples were reached. Sample sizes for extracted thesis data are: EPO-AKI n= 123, POLAR-AKI n=70, EPO-Biomarkers n=30, POLAR-Biomarkers n=53.

3.6.3 Consent

The Renal substudies of the EPO-TBI and POLAR trials are integral components of these trials; separate consent was not obtained for participation in the substudies or for specimen collection at the Alfred and Royal Melbourne hospital sites where biomarker data was obtained. Consent forms at the 2 'Biomarker sites' were slightly different to those at the other sites (see Appendices), with additional information on biomarker specimen collection and rationale. Once biomarker specimen collection ceased, the consent forms at the 'Biomarker sites' were changed accordingly. Ethical committee approval was granted at all sites before commencing the trials.

Unconscious patients with TBI cannot provide informed consent so consent was obtained from the legal surrogate in the EPO-TBI trial, in accordance with section 4.4 of the NHMRC National Statement with variations according to different state and territory legislations. The POLAR trial used delayed consent, in accordance with section

4.4 of the NHMRC/ARC National Statement. This had been used successfully in previous NHMRC funded randomised trials by the trial investigators.

3.6.4 Randomisation

In EPO-TBI, a well-tested web-based system including block randomisation at each site is used by the ICU research coordinators. Treatment allocation is stratified by centre and severity of head injury. In the POLAR trial, randomisation is performed by the paramedics and emergency department staff using a tried and effective closed envelope system including block randomization. Treatment allocation will be stratified by ambulance and by centre.

3.6.5 Treatments

EPO-TBI: Patients received either subcutaneous EPO (epoietin alfa 40,000 units) or placebo on day 1, 8 and 15 while in ICU. Measures to prevent and monitor risk of thrombosis were implemented.

POLAR: Patients received prophylactic hypothermia or normothermia. Hypothermia was induced by exposure and ice-cold infusion (up to 2 litres) to 35°C by paramedics. Cooling continued in hospital with surface pads at 35°C until a clinically significant high risk for bleeding was ruled out, then they were cooled to 33°C, which was maintained for 72 hours. The maximum period of hypothermia was 7 days (rewarming and recooling may occur in the event of bleeding, or prior to surgery for stabilised bleeding). Patients were then re-warmed slowly to 37°C and maintained normothermic up to a maximum of 7 days. Normothermia patients were maintained at 36.5-37.5 °C with surface pads if required for a maximum of 7 days. All patients received protocolised evidence-based care.

3.6.6 Recruitment

All patients recruited to the EPO-TBI and POLAR trials were included in the renal substudies, and underwent RIFLE renal function assessment. All patients recruited to the EPO-TBI and POLAR trials at the Alfred and Royal Melbourne hospitals up until late 2011, in addition, had blood and urine specimens taken for biomarker

measurement, with the aim for at least 50 patients for the EPO-TBI trial and 50 patients for the POLAR trial recruited between the 2 hospitals. Due to PhD time constraints, specimens were obtained from 30 patients in the EPO-TBI trial and 53 patients in the POLAR trial. Study sites were not co-enrolling pre-hospital patients with severe TBI into other trials during the recruitment period.

3.6.7 Data Collection

Data collection instruments and procedures were developed using experience from previous TBI trials with involvement of the Australian and New Zealand Intensive Care Research Centre (ANZIC-RC). Data available for the Renal substudies at January 31, 2012 (EPO-TBI) and February 27, 2012 (POLAR) was extracted. Estimated time for completion of recruitment is late 2014 for the EPO-TBI trial and 2016 for the POLAR trial. Data collection included baseline demographics, relevant medical history, primary diagnoses, estimated weight, physiological parameters, severity of TBI, diagnostic interventions, and therapeutic interventions.

The data collected specifically for the Renal substudies were: first available post trauma creatinine and time taken, highest daily creatinine (for 21 days: EPO-TBI trial; 10 days: POLAR trial), need for renal replacement therapy, and RIFLE urine output criteria for the first 7 days while in the Intensive Care Unit i.e. was the urine output a) < 0.5mL/kg/h for ≥ 6 hours, b) < 0.5mL/kg/h for ≥ 12 hours, or c) < 3mL/kg/h for 24 hours or was the patient anuric for 12 hours (total urine output ≤ 50mL for this period).

Creatinine: All patients had routine bloods taken on arrival in the emergency department from which baseline creatinine was taken because a 'normal' pre-illness serum creatinine within the last 12 months was invariably not available in the hospital records in this young (mean age 33) and generally, previously healthy population. Daily serum creatinine was collected from the routine daily bloods while in ICU and from any bloods taken once discharged to the ward for 21 days EPO-TBI/10days POLAR.

Urine output: Urine output was measured hourly in ICU. RIFLE urine output criteria were calculated daily for 7 days post injury. Patient weight was generally estimated.

Urine and blood specimens were collected for all but one of the 30 patients randomised into the EPO-TBI trial from May 2010 to November 2011 (one patient refused specimen collection); and from all but 2 of the 53 patients randomised into the POLAR trial from December 2010 to November 2011 (2 patients unexpectedly deteriorated

rapidly and died before specimens could be taken) between the Royal Melbourne and Alfred hospitals. Specimen collection was performed by the Renal substudies project manager or the Research Coordinators at the sites.

In the EPO-TBI trial, urine and blood specimens for biomarker measurements were collected shortly before study dose administration (given up to 24 hours after injury) and at 24, 48, and 72 hours after the first dose of study drug. In the POLAR trial, specimens were collected as soon as possible after admission to hospital (preferably before cooling), then at 24, 48 and 72 hours after injury. Biomarker specimens could be taken within 4 hours of the due time. The aim of measuring biomarkers was to assess their effectiveness as predictors of AKI and their response to injury / treatments over time.

Data collected for each trial can be seen in the hard copy version of the case report form for each trial (see Appendices); however data were captured through online data entry by sites to electronic case report forms. Full data was collected for all patients including those excluded at any stage in accordance with the POLAR / EPO-TBI schedules. Patient data is de-identified and confidentiality was maintained in the production of the thesis observing good clinical practice. The Renal substudies required no alteration to treatments for participants in the POLAR and EPO-TBI trials.

3.6.8 Defining AKI

Data available at January 31, 2012 for the Renal Assessment of the EPO-TBI trial, and at February 27, 2012 for the Renal Substudy of the POLAR trial were extracted and analysed. To define AKI we used the glomerular filtration rate (GFR) and urine output criteria of the RIFLE (Risk, Injury, Failure, Loss, Endstage) classification system[91] (AKI review, section 1.2):

No AKI:	GFR decrease ≤ 25% and UO does not fit any of the criteria below
Risk:	GFR decrease > 25% or UO < 0.5 ml/kg/hr over 6 hours
Injury:	GFR decrease > 50% or UO < 0.5 ml/kg/hr over 12 hours
Failure:	GFR decrease > 75% or UO < 0.3 ml/kg/hr over 24 hours.

Classification of AKI is challenging in patients with TBI due to the early rise in creatinine, which may be due to muscular injury caused by trauma or a reduced

perfusion pressure leading to increased muscle breakdown and higher serum creatinine soon after injury. The absence of a true baseline or peak SCr for trauma patients[94] makes the determination of the incidence of AKI in this population very challenging. To calculate the decrease in estimated GFR (eGFR) from baseline we used the difference between peak creatinine from day 1 to 7 (converted to eGFR using the MDRD 175 formula) for each patient, and a normative baseline eGFR (age and sex-stratified for each patient) taken from Dutch eGFR tables[124] (Appendix 7, Tables 1a and 1b), expressed as a percent decrease in eGFR. The Dutch Nijmegen eGFR tables provide us with a baseline eGFR that is age and sex stratified for a healthy population, which reflects the generally healthy pre-injury status of TBI patients. The tables correspond to a majority caucasion population and while they may not completely reflect an Australian population, they share many similarities with local characteristics. This method has been used to calculate change in eGFR in a previous study for a similar population[94]. Patients were considered to have AKI if either their decrease in eGFR or urine output met the AKI criteria, whichever gave the most severe classification.

This method was compared to others (Tables 4, 7 and 11): the widely used and validated Acute Kidney Injury Network (AKIN) criteria[125], the recently established Kidney Disease: Improving Global Outcomes (KDIGO) classification system[126], and another version of the RIFLE criteria using the first post-trauma serum creatinine as baseline and the peak creatinine from the first 7 days for change in creatinine from baseline. Diagrams summarising the RIFLE, AKIN and KDIGO AKI classification criteria can be found in the article in section 1.2.

The new EPI-CKD equation has been recently recommended by the Australasian Creatinine Consensus Working Group to calculate eGFR in replacement of the MDRD 175 equation[127]. After all analyses had been performed with the MDRD 175 equation we tested the new EPI-CKD equation and found that there was no change in the direction of the results.

Creatinine collection continued for 21 days for the EPO-TBI patients and 10 days for the POLAR patients to assess the duration of AKI. Duration of data collection is different for the two trials due to the timeframes of therapy (EPO-TBI: EPO is given day 1, 8, 15, POLAR cooling is received for 72 hours). To calculate AKI-free days we employed the same method used to define AKI above with the highest creatinine for each of the first 7 days, to determine whether the patient had AKI on each of these days. Seven days was used as urine output data was available for this period and the majority of patients were classified using urine output criteria. A percent decrease in eGFR of > 50% and or urine output < 0.5 ml/kg /hr over 12 hours was used to define an 'AKI day'. Any new day with AKI was considered an 'AKI day' and the patient was considered to have AKI until a result of a decrease in eGFR of ≤ 50% was obtained. Any

day out of hospital before day 7 was an AKI-free day and if a patient died they had no further AKI-free days.

Outcomes examined were solely renal outcomes as preferred by the trial management committees to maintain confidentiality of trial data and results. Renal replacement therapy was not examined as an outcome as it was only required for 1 patient (placebo) in the EPO-TBI trial and 2 patients (one hypothermia, one control) in the POLAR trial.

3.6.9 Laboratory measurements

Blood specimens were centrifuged at 3000rpm for 15mins at 4°C, then the plasma was fractioned and frozen at -80°C. Urine was fractioned and frozen at -80°. Plasma NGAL was measured using the Biosite Triage® NGAL MeterPro, a point of care measurement device. The rest of the samples were transported frozen on dry ice by World Courier to the respective laboratories for measurement. Urine NGAL was measured in clinical laboratories at the Cincinnati Children's Hospital Medical Center using the ARCHITECT platform (Abbott laboratories, Abbott Park, Illinois). IL-18 levels were measured by a sensitive commercially available ELISA (Medical and Biological laboratories, Nagoya, Japan), and KIM-1 was measured using an ELISA assay. Alpha- and pi-GST were measured at Argutus Medical laboratories, Trinity Technology and Enterprise Campus, Dublin, using the Argutus Medical Alpha and Pi GST EIA platforms BIO-91 and BIO-85 respectively. At the Academiska Sjukhuset in Uppsala, Sweden, in the Department of Clinical Chemistry and Pharmacology, urine and plasma cystatin C measurements were performed on a Mindray BS-380 analyzer (Shenzhen Mindray Bio-medical Electronics CO., LTD, Shenzhen, China). Urine creatinine measurements were performed with a Jaffe method (Synermed, Burgess Hill, UK), and plasma levels of cathepsin S were measured by ELISA (human cathepsin S [total], DY1183; R&D Systems, Minneapolis, MN, USA). The intra-assay coefficient of variation was 7%.

3.6.10 Outcome measures

EPO/POLAR-AKI

Primary outcomes: Proportion with Acute Kidney Injury (Risk/Injury/Failure RIFLE categories) in those receiving EPO v. placebo; and those receiving hypothermia v. normothermia.

Secondary outcomes:

Severity of AKI (measured as percent increase in serum creatinine from baseline to peak value while in ICU)

Duration of AKI measured as AKI free days at 21 days (EPO-TBI) or 10 days (POLAR) after randomization

EPO/POLAR-Biomarkers

Primary outcomes: Levels of biomarkers NGAL, cystatin-C and Cathepsin S in plasma; and NGAL, cystatin-C, IL-18, KIM-1, and GST alpha and pi in urine, in patients receiving EPO v. placebo; and those receiving hypothermia v. normothermia, and comparison of urine and plasma samples of NGAL and cystatin C.

Secondary outcomes: Relationships between biomarkers and change in serum creatinine, and combined RIFLE class Risk/Injury/Failure.

3.6.11 Statistical Analysis

For the original statistical analysis plan, see the EPO-TBI and POLAR trial protocols in the Appendices (Section 11 and section 30 respectively in each protocol).

AKI analysis

Pooled EPO-TBI/POLAR data: To investigate the incidence of AKI in patients with TBI we pooled the EPO-TBI and POLAR datasets to provide a larger more representative sample. Treated and control/placebo patients from both trials were all included in this pooled analysis. Patient characteristics that were available for both trials were compared between AKI/No AKI groups using the student t-test for normally distributed continuous variables, the Wilcoxon Rank Sum test for non-parametric and ordinal variables, and the chi-square test for binomial variables (or Fishers exact where numbers where small).

EPO-TBI and POLAR data (separate analysis of each trial): Patient characteristics and AKI categories were compared between treatment /control groups using the appropriate tests per variable described above. Serum creatinine was log-transformed and univariable associations with the log of creatinine were determined using mixed linear modeling adjusting for repeated measures. The levels of creatinine and GFR for treated versus control patients were plotted for 21 days in the EPO-TBI study and 10 days in the POLAR study. For the POLAR study, serum creatinine measurements of cooled patients taken before, during and after rewarming were collected to confirm on an individual level the increase in creatinine associated with rewarming.

Biomarker analysis

Pearson correlation coefficients for linear associations between the biomarkers (using log transformed biomarker data) were calculated and the respective scatter plots of these relationships were plotted using pooled EPO-TBI / POLAR biomarker data. Results for the biomarker data are presented as summary statistics for each biomarker and as separate analyses: pooled EPO-TBI/POLAR biomarker data (for AKI), EPO-TBI biomarkers and POLAR biomarkers.

Changes over time for biomarker concentrations at 0, 24, 48 and 72 hours were analysed using repeated measures analysis of variance fitting main effects for group, time and the interaction between group and time. These analyses were calculated for a) AKI groupings in combined EPO-TBI/POLAR data, b) EPO versus placebo in EPO-TBI data, c) hypothermia versus normothermic controls in POLAR data.

Patient characteristics and median biomarker concentration were compared between AKI / No AKI groups (pooled EPO-TBI/POLAR data), EPO versus placebo, and hypothermic versus normothermic control patients using the appropriate tests per variable described above. In addition to this the change in biomarker concentration from baseline to 24 hours was compared between treated and control groups in the EPO-TBI and POLAR analyses. In the pooled EPO-TBI/POLAR biomarker analysis, the area under the ROC curve was also calculated for each biomarker concentration at 24, 48 and 72 hours to evaluate diagnostic capacity, and a graph of the mean serum creatinine concentration for the first 4 study days was plotted.

Statistical analysis was performed using SAS Version 9.2 (SAS Institute, Cary, NC) and Stata version 9 and above (StataCorp, College Station, Texas, USA).

3.6.12 Minimising bias

Consent for both trials incorporates the Renal substudies thus minimising possibility for recruitment bias. The incorporation of Renal substudy requirements in training sessions, trial manuals, data quality control checks, and random audits for both trials promotes data quality and protocol standardisation and thus helps to minimise bias. In addition, bias is minimised in both trials by concealed random treatment allocation and in the POLAR trial by protocolised treatment in both groups.

4 Results

4.1 Overview of Results

This PhD project entails the Renal substudies of two clinical trials each of which is composed of two parts, EPO and POLAR-AKI and EPO and POLAR-Biomarkers (see trial protocols in Appendices). EPO and POLAR-AKI assess the effects of erythropoietin and early and sustained hypothermia respectively on renal function in patients from the EPO-TBI and POLAR trials. EPO and POLAR-Biomarkers assess the effects of erythropoietin and early and sustained hypothermia respectively on renal biomarkers in nested cohorts of patients from these trials.

Data from patients in the EPO-TBI and POLAR trials were pooled (including treated and control patients) to provide more power to assess the occurrence, severity and duration of AKI in the combined TBI population. These results are presented first (section 4.2) followed by the results of the impact of erythropoietin and hypothermia on the development of AKI in patients with TBI (EPO and POLAR-AKI, section 4.3).

Data for patients in the nested biomarker cohorts from the EPO-TBI and POLAR trials were also combined to increase the power to evaluate the biomarkers as predictors of AKI in patients with TBI. Results on the predictive ability of the biomarkers for AKI and the effect of erythropoietin and hypothermia on the release of these biomarkers are presented in section 4.4. To preserve confidentiality of the data and results of the EPO-TBI and POLAR trials, non-renal outcomes are not investigated in this thesis at the request of the management committees of both trials.

The main findings for sections 4.2 to 4.4 will be summarised at the beginning of each section.

4.2 AKI in patients with TBI: combined EPO-TBI and POLAR data

The main findings in the pooled EPO-TBI/POLAR-AKI analysis were that the occurrence of AKI was much greater than previously found in retrospective studies, which highlights the need for further investigation into causes, the impact of AKI on outcomes, and potential measures to reduce the development of AKI in this patient group. Also of interest was the surprising number of patients classified with AKI by the urine output criteria, consistent with previous findings of a greater sensitivity of urine output for AKI and underlining the unreliability of serum creatinine as a marker of AKI in this patient group and the need to seek alternatives.

The EPO-TBI and POLAR datasets were pooled (including treated and control patients) to assess the overall incidence of AKI (defined by conventional methods) in patients with TBI. Variables in this dataset are those common to both trials. Non-renal outcomes were not investigated in this thesis as preferred by the management committees of the parent trials to preserve confidentiality of the trial data and results. Renal replacement therapy was not investigated as an outcome because it was used for only 3 patients who developed renal failure in the combined studies (1 patient in the EPO-TBI trial [placebo] and 2 patients in the POLAR trial [1 cooled, 1 control]). Complete creatinine or urine output data were available for 193 patients between the two trials. These patients had a mean age of 33, and 161 patients (83.4%) were male which is fairly consistent with existing data[2,94] on patients with any degree of TBI. Patients who developed AKI were heavier in weight, with a lower pre-intubation respiratory rate and higher systolic blood pressure on hospital admission than those who did not develop AKI (Table 3). Those with AKI had fewer AKI free days than patients with no AKI (5 versus 7, $p<0.001$), and mean and peak serum creatinine was higher in the AKI versus no AKI group as expected (Mean: 67 versus 59, Peak: 82 versus 69, $p\leq0.01$).

Table 3 Comparison of characteristics between AKI groupings in the combined EPO-TBI / POLAR dataset

Variable	No AKI (n=96)	AKI* (n=97)	P value
Weight, kg	75.7 ± 1.9	84.4 ± 1.6	0.004
Respiratory Rate	23.2 ± 0.95	20.3 ± 0.86	0.008
Systolic BP	134 ± 2.8	143 ± 3.5	0.01
Pre-intubation GCS	6.5 ± 0.26	5.9 ± 0.26	0.10
Mass lesion evacuation performed %	32% (30)	36% (35)	0.11
Pupils equal & reactive %	76% (73)	81.4% (79)	0.13
Motor Score from pre-intubation GCS*	4 (2-5)	3. 5 (1-4.5)	0.13
INR closest to randomisation	1.21 ± 0.02	1.17 ± 0.02	0.16
Platelets closest to randomisation	202 ± 7	214 ± 7	0.20
Median ICH Index, day 1-7*	0 (0-10.4)	4.2 (0-11.6)	0.29
Female %	15.6% (15)	17.5% (17)	0.32
Age	33 ± 1.3	33 ± 1.4	0.35
Craniectomy performed %	22.3% (21)	25.7% (25)	0.36
Both pupils non reactive %	6.2% (6)	7.2% (7)	0.54
Median CPP index, day 1-7*	0 (0-8.3)	4.5 (0-10.3)	0.56
Bifrontal decompressive craniectomy %	6.3% (6)	8.2% (8)	0.70
APTT closest to randomisation	30 ± 0.46	30 ± 0.5	0.95
Earliest post trauma creatinine	76 ± 2.2	77 ± 1.9	0.25
Mean Serum Creatinine, day 1-7	59 ± 1.2	67 ± 1.4	0.01
Peak serum creatinine, day 1-7	69 ± 1.5	82 ± 2.2	0.001
AKI-free days (no AKI v. AKI)*	7 (7-7)	5 (5-6)	<0.001
AKI-free days (GFR↓> 50% v. ≤ 50%)*	7 (7-7)	6 (6-7)	0.004

Values are reported as mean ± standard error, median (IQR)*, or % (n).
AKI=AKI (R/I/F): RIFLE urine output or GFR criteria using normative baseline GFR and worst GFR for day 1-7 to calculate percent decrease in GFR (section 3.6.8), Respiratory Rate=last unassisted respiratory rate, Systolic BP=first systolic blood pressure on hospital admission, GCS=Glasgow Coma Score; ICH index=Intracranial Hypertension Index: the number of end-hourly measures of intracranial pressure of more than 20mmHg divided by the total number of measurements, multiplied by 100, CPP index=cerebral perfusion pressure index: the number of observations of cerebral perfusion pressure of less than 60mmHg divided by the total number of measurements, multiplied by 100.

In the combined EPO-TBI / POLAR dataset 50.3% of patients developed some degree of AKI. Similar results were found using alternative classification methods (Table 4). Of 193 patients in the combined dataset, 48 developed AKI class R (Risk, 24.9%), 46 developed AKI class I (Injury, 23.8%) and 3 developed AKI class F (Failure, 1.6%).

Table 4 Patients with AKI in the combined EPO-TBI and POLAR dataset using different classification systems and definitions for AKI

	Total=193 n (%)
1. RIFLE: using normative baseline GFR and worst GFR (day 1-7) or UO	
No AKI: GFR decrease ≤ 25%	96 (49.7%)
Risk: GFR decrease > 25%	48 (24.9%)
Injury: GFR decrease > 50%	46 (23.8%)
Failure: GFR decrease > 75%	3 (1.6%)
2. RIFLE: using first post-trauma creatinine baseline and peak creatinine (day 1-7) or UO	
No AKI	96 (49.7%)
Risk	46 (23.8%)
Injury	49 (25.4%)
Failure	2 (1%)
3. AKIN category	
No AKI	91 (47.2%)
AKIN Stage 1	50 (25.9%)
AKIN Stage 2	49 (25.4%)
AKIN Stage 3	3 (1.6%)
4. KDIGO category	
No AKI	88 (45.6%)
KDIGO Stage 1	51 (26.4%)
KDIGO Stage 2	51 (26.4%)
KDIGO Stage 3	3 (1.6%)

RIFLE=classification system for Acute kidney Injury (AKI): Risk, Injury, Failure, Loss, Endstage; GFR=glomerular filtration rate; UO=urine output; AKIN= Acute Kidney Injury Network classification system for AKI; KDIGO=Kidney Disease: Improving Global Outcomes classification system

AKI occurred in 97 patients; 88 (91%) of those with AKI were classified by urine output alone, and 2% by both urine output and GFR. Only 7% of patients with AKI were classified solely by RIFLE GFR criteria (Table 5). Considering that the urine output criteria are the same for all classification systems it is not surprising that similar rates of AKI were found for each system (Table 4).

Table 5 AKI determined by GFR, urine output or both

Combined EPO-TBI / POLAR data		
RIFLE: using normative baseline GFR and worst GFR for day 1-7 or UO	**Criteria for AKI**	**(n=193) n (%)**
No AKI: GFR decrease ≤ 25%		
	Both	96 (49.7%)
Risk: GFR decrease > 25%	GFR	7 (3.6%)
	UO	40 (20.8%)
	Both	1 (0.5%)
Injury: GFR decrease > 50%	GFR	0
	UO	46 (23.9%)
	Both	0
Failure: GFR decrease > 75%	GFR	0
	UO	2 (1.0%)
	Both	1 (0.5%)

GFR=glomerular filtration rate, UO=urine output

4.3 Treatments for AKI

The EPO-TBI and POLAR trials which assess the effect of erythropoietin and early and sustained hypothermia respectively on long-term neurological outcome for patients with TBI, presented unique opportunities for the additional investigation of the effect of these therapies on renal function and renal biomarkers. In this section we will present the results for the effect of erythropoietin (4.3.1) and hypothermia (4.3.2) on the development of AKI in patients with TBI.

One of the main findings in this section was that EPO does not appear to protect the kidneys as we might have expected. In fact, trends hint that EPO may have a slightly negative impact on renal function. Hypothermia, the other therapy under investigation was associated with a significantly greater occurrence of AKI than normothermia, which may be related to the negative side effects of rewarming.

4.3.1 EPO-AKI: Does EPO reduce the risk for, severity of, and duration of AKI versus placebo in patients with TBI?

Of the 130 patients recruited to the EPO-TBI trial when data was extracted, seven patients were excluded as no creatinine results or urine output data were available for them, leaving 123 patients in the analysis. Baseline characteristics of the two study groups were similar (Table 6). Mean age was 33, most patients were male with a mean APACHE II score of 20 and Injury Severity Score of 19, and 73% had sustained a severe traumatic brain injury. Patients receiving EPO or placebo were given a median of 2 doses. Physiological, neurological and operative characteristics post-trauma were similar between groups. Road transport accidents were the major cause of trauma. Mean serum creatinine was slightly higher in patients who received EPO, however this difference was not clinically significant and there was no difference between groups in the number of AKI-free days in ICU from day 1 to 7 (Table 6).

Table 6 Baseline, operative and renal patient characteristics by treatment allocation

Characteristics	EPO (n=63)	Placebo (n=60)	p-value
Age	32.6 ±13.6	33.6 ± 14.2	0.7
Male %	84% (53)	77% (46)	0.3
APACHE II Score	19.7 ± 7.8	20 ± 6.4	0.78
Haemoglobin at randomisation	116 ± 19.5	115 ± 21.3	0.82
INR closest to randomisation	1.2 ± 0.2	1.2 ± 0.2	0.18
APTT closest to randomisation	30 ± 4.4	29.7 ± 4.1	0.64
Platelets closest to randomisation	203 ± 74.7	212 ± 68.3	0.49
Systolic BP	134. ± 38.8	137 ± 24.8	0.61
Respiratory Rate	24.2 ± 7.4	23.1 ± 8.3	0.46
Lowest Systolic BP pre-randomisation	90.1 ± 20	93.2 ± 16.2	0.35
Lowest Sao2	91.8 ± 14.7	94.3 ± 6.4	0.21
Weight	79.9 ± 22	76.9 ± 15.3	0.39
Pupils equal & reactive %	83% (52)	85% (51)	0.71
Pre-intubation GCS	6.7 ± 2.7	6.7 ± 2.8	0.97
Motor Score from pre-intubation GCS	4 (2-5)	4 (1-5)	0.82
Sustained Severe Traumatic Brain Injury	73% (46)	73% (44)	0.97
Total vol. hypertonic saline, day 1-7 (mL)	691 ± 1380	579 ± 1342	0.65
Total volume of Mannitol, day 1-7 (mL)	59.7 ± 201	40.7 ± 153	0.56
Total volume of RBC, day 1-7	299 ± 475	374 ± 582	0.44
Median CPP index, day 1-7	6.1 ± 8.7	5 ± 11	0.61
Median ICH index, day 1-7	8 ± 17.5	7.3 ± 12.2	0.84
Mass lesion evacuation performed %	34% (21)	33% (20)	0.9
Craniectomy performed %	28% (17)	22% (13)	0.43
Bifrontal decompressive craniectomy %	10% (6)	8% (5)	0.77
Injury severity score	19.4 ± 14.7	19.4 ± 15	0.98
Number of study doses	2 (1-3)	2 (1-2)	0.57
Earliest post trauma creatinine	79.5 ± 23.9	75.9 ± 19.3	0.37
Mean Serum Creatinine: days 1 to 7	61.9 (55.2-69)	54.8 (50.3-68.1)	0.015
Peak serum creatinine: days 1 to 7	75 ± 20.1	70.6 ± 16.5	0.19
AKI-free days (pts with GFR↓ > 25%)	6.16 ± 1.2	6.33 ± 1	0.43
AKI-free days (pts with GFR↓ > 50%)	6.65 ± 0.8	6.68 ± 0.7	0.99

All values are reported as mean ± standard deviation, median (IQR) or % (n).
Systolic BP=first systolic blood pressure on hospital admission, Respiratory Rate=last unassisted respiratory rate, Lowest SaO2=lowest oxygen saturation of arterial blood pre-randomisation, GCS=Glasgow Coma Score; vol.=volume, CPP index=cerebral perfusion pressure index: the number of observations of cerebral perfusion pressure of less than 60mmHg divided by the total number of measurements, multiplied by 100; ICH index=Intracranial Hypertension Index: the number of end-hourly measures of intracranial pressure of more than 20mmHg divided by the total number of measurements, multiplied by 100.

Of the 123 patients included in the analysis, 51 (41.5%) had some degree of AKI according to the GFR/urine output RIFLE criteria, and of those with AKI, 27 (53%) fell into the Injury / Failure categories (Table 7). There was no significant difference between the EPO and placebo groups in the proportion of patients with AKI, and the proportions in each AKI category or stage were similar between groups (Table 7). Similar results were found when alternative classification methods were used (Table 7).

Table 7 Patients with Acute Kidney Injury: comparing erythropoietin versus placebo groups using different classification systems and definitions

	EPO (n=63) n (%)	Placebo (n=60) n (%)	p-value
1. RIFLE: using normative baseline GFR and worst GFR (day 1-7) or UO			
No AKI: GFR decrease ≤ 25%	35 (56%)	37 (61%)	0.49
Risk: GFR decrease > 25%	14 (22%)	10 (17%)	0.44
Injury: GFR decrease > 50%	12 (19%)	12 (20%)	0.89
Failure: GFR decrease > 75%	2 (3%)	1 (2%)	1.00
2. RIFLE: using first post-trauma creatinine baseline and peak creatinine (day 1-7) or UO			
Rifle0	36 (57%)	36 (60%)	0.75
Rifle1	12 (19%)	10 (17%)	0.73
Rifle2	14 (22%)	13 (21%)	0.94
Rifle3	1 (2%)	1 (2%)	1.00
3. AKIN category			
No AKI	32 (51%)	33 (55%)	0.64
AKIN Stage 1	16 (25%)	14 (23%)	0.79
AKIN Stage 2	13 (21%)	12 (20%)	0.93
AKIN Stage 3	2 (3%)	1 (2%)	1.00
4. KDIGO category			
No AKI	32 (51%)	32 (53%)	0.78
KDIGO Stage 1	15 (24%)	14 (23%)	0.95
KDIGO Stage 2	14 (22%)	13 (22%)	0.94
KDIGO Stage 3	2 (3%)	1 (2%)	1.00

See sections 1.2 and 3.6.8 for details of each definition / classification of AKI; EPO=erythropoietin; RIFLE=classification system for Acute kidney Injury (AKI): Risk, Injury, Failure, Loss, Endstage; GFR=glomerular filtration rate; UO=urine output; AKIN= Acute kidney Injury Network classification system for AKI; KDIGO=Kidney Disease: Improving Global Outcomes

Of 51 patients classified as having some degree of AKI, 47 (92%) were classified using urine output alone, and only 3 (6%) were classified using RIFLE GFR criteria alone (Table 8). One patient was classified with AKI according to both RIFLE urine output and GFR criteria.

Table 8 Acute Kidney Injury (AKI) determined by GFR, urine output or both

RIFLE: using normative baseline GFR and worst GFR (day 1-7) or UO	Criteria for AKI	Total (n=123) n (%)
No AKI: GFR decrease ≤ 25%	Both	72 (58.6%)
Risk: GFR decrease > 25%	GFR	3 (2.4%)
	UO	21 (17.1%)
	Both	0
Injury: GFR decrease > 50%	GFR	0
	UO	24 (19.5%)
	Both	0
Failure: GFR decrease > 75%	GFR	0
	UO	2 (1.6%)
	Both	1 (0.8%)

GFR=glomerular filtration rate; RIFLE=Risk, Injury, Failure, Loss, Endstage: classification system for AKI; UO=urine output

Creatinine was found to be well approximated by a log-normal distribution, so was log-transformed prior to analysis. Using mixed linear modeling adjusting for repeat measures, the percent decrease in GFR from day 1 to 7 was associated with log creatinine (from which the definition of AKI is partly derived) as expected. Table 4 shows the relationship of other variables with log creatinine. A higher creatinine was associated with male gender, shorter clotting time (INR), heavier weight, and unreactive pupils. Multivariable analysis was not performed because the idea was not to create a predictive model and it was beyond the scope of the thesis.

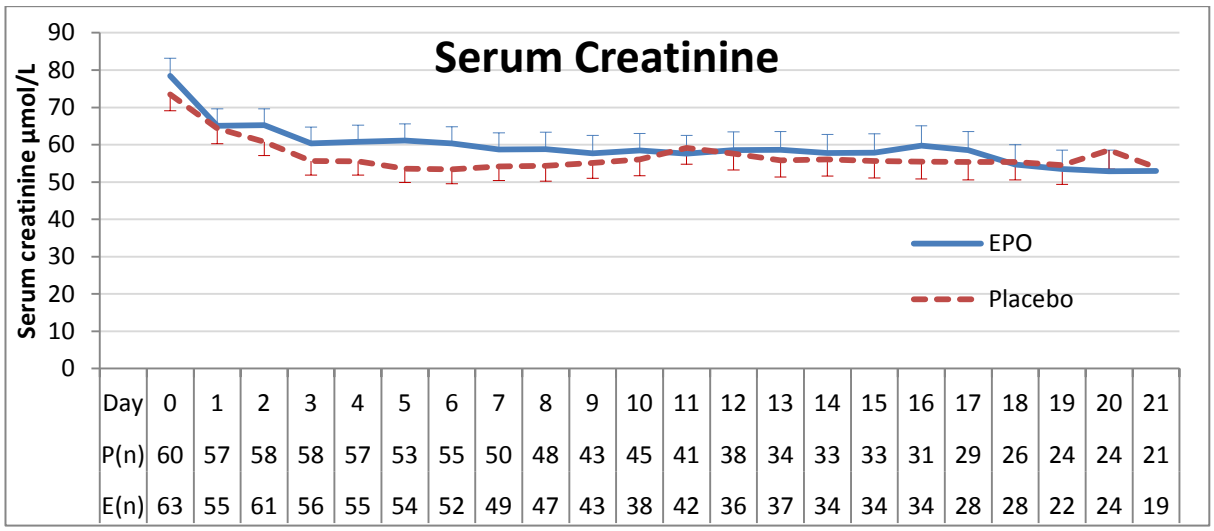
Table 9 Univariable estimates for linear regression models with log creatinine adjusted for repeat measures

Variable	Param Est ± SE	p-value
% Decrease in GFR (day 1-7)	-0.27 ± 0.05	<0.001
Peak serum creatinine (day 1-7)	0.006 ± 0.001	<0.001
Female	-0.19 ± 0.04	<0.001
INR closest to randomisation	-0.25 ± 0.10	0.008
Weight, kg	0.002 ± 0.001	0.01
Both pupils non-reactive	0.18 ± 0.09	0.03
Platelets administered	-0.14 ± 0.07	0.04
Admitting hospital	<0.001	0.05
Red Blood Cells administered	-0.07 ± 0.04	0.07
Pelvic Fracture sustained	-0.09 ± 0.05	0.08
Lowest Sao2	-0.003 ± 0.002	0.09
APACHE II Score	-0.004 ± 0.003	0.11
FFP administered	-0.07 ± 0.05	0.14
One pupil non-reactive	-0.09 ± 0.06	0.16
Last unassisted Respiratory Rate	0.003 ± 0.002	0.17

Variables have p-value ≤ 0.2 for parameter estimate. Param Est=Parameter estimate, SE=standard error, GFR=glomerular filtration rate, INR=International Normalized Ratio: a measure of coagulation, APACHE II=a severity of illness score, FFP=fresh frozen plasma

The levels of creatinine and GFR for EPO versus placebo patients were plotted for 21 days (Figure 1 and 2). The graphs of creatinine and GFR 'mirrored' each other as expected (GFR is derived from an equation using creatinine, age and gender) despite the use of a normative baseline for GFR. There is some separation between groups in the first week.

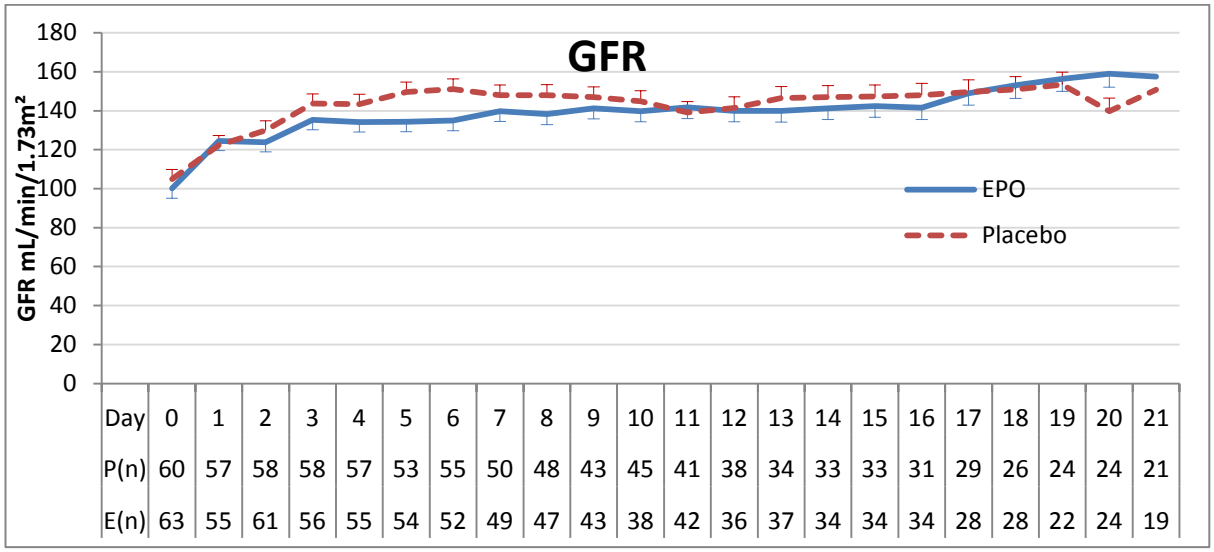
Figure 1 **Serum creatinine concentration for erythropoietin versus placebo patients plotted for 21 days**



Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
P(n)	60	57	58	58	57	53	55	50	48	43	45	41	38	34	33	33	31	29	26	24	24	21
E(n)	63	55	61	56	55	54	52	49	47	43	38	42	36	37	34	34	34	28	28	22	24	19

Geometric mean serum creatinine with 95% confidence interval is displayed. The number of patients per group for whom creatinine is available per day is displayed. Error bars are unidirectional to avoid graph crowding.

Figure 2 **Level of GFR for erythropoietin versus placebo patients plotted for 21 days**



Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
P(n)	60	57	58	58	57	53	55	50	48	43	45	41	38	34	33	33	31	29	26	24	24	21
E(n)	63	55	61	56	55	54	52	49	47	43	38	42	36	37	34	34	34	28	28	22	24	19

Mean GFR with 95% confidence interval is displayed and the number of patients per group for whom calculation of GFR was possible per day is displayed.

4.3.2 POLAR-AKI: Does early and sustained hypothermia reduce the risk for, severity of, and duration of AKI versus placebo in patients with TBI?

Of the 83 patients recruited to the POLAR trial when data was extracted, 2 were pilot study patients so were excluded and 11 patients were excluded as no creatinine results or urine output data were available for them, leaving 70 patients in the analysis. Baseline characteristics of the hypothermia and control arms were similar with some differences (Table 10). Mean age was 33, patients were predominantly male (89%) and mean GCS and Injury Severity Score were 5 and 27 respectively. Pre-hospital randomisation occurred in 46% of cases. Differences in the early post-trauma period were found in motor score from the pre-intubation GCS (Control patients displayed better motor response than cooled patients), number of patients with bilateral unreactive pupils (more in the hypothermia group), platelet count and mean heart rate in the first 12 hours. The mean temperature in the first 72 hours was significantly different as desired, and in the first 7 days there was a difference in the mean highest potassium.
AKI-free days were calculated for the first 7 days of patients' ICU stay and compared between groups. Cooled patients experienced fewer AKI-free days than control patients (Table 10). There was no significant difference in mean or peak serum creatinine between groups.

Table 10 POLAR trial patient characteristics: baseline and first 7 days by treatment allocation

Characteristics	Hypothermia (n=35)	Controls (n=35)	p-value
Age	31.2 ± 11.9	35.3 ± 11.6	0.15
Male %	86% (30)	91% (32)	0.45
Randomised pre-hospital %	43% (15)	49% (17)	0.63
Weight	82.4 ± 15.1	83.8 ± 15.6	0.71
Pre-intubation GCS	4.9 ± 2.3	5.8 ± 1.9	0.11
Motor Score from pre-intubation GCS	1 (1-4)	4 (1-5)	0.05
Hypotensive pre-hospital %	14% (5)	6% (2)	0.26
Hypoxic pre-hospital %	12% (4)	6% (2)	0.67
Fluid volume given pre-hospital	1919 ± 1280	1657 ± 1382	0.42
First Systolic BP in ED	141 ± 31	145 ± 24	0.56
Pupils equal & reactive in ED %	71% (25)	69% (24)	0.79
Both pupils Non-reactive in ED %	17% (6)	3% (1)	0.05
WCC closest to randomisation	16.7 ± 8.8	13.8 ± 5.7	0.12
INR closest to randomisation	1.1 ± 0.4	1.1 ± 0.4	0.79
APTT closest to randomisation	26.3 ± 11.2	28.5 ± 9.0	0.37
Platelets closest to randomisation	228 ± 58	189 ± 51	0.005

Haemoglobin closest to randomisation	133 ± 23	128 ± 27	0.42
Glucose closest to randomization	7.8 ± 4.1	8.4 ± 4.7	0.62
Mean MAP for 12h post-randomisation	89.1 ± 9.7	85.1 ± 9.5	0.10
Mean HR for 12h post-randomisation	78.2 ± 18.7	86.9 ± 15	0.04
Mass lesion evacuation performed %	34% (12)	34% (12)	1.00
Craniectomy performed %	26% (9)	20% (7)	0.57
Bifrontal decompressive craniectomy %	0% (0)	9% (3)	0.24
ICP Monitor inserted %	86% (30)	83% (29)	0.74
Mean temperature in first 24h	34.5 ± 1.4	36.8 ± 0.6	<0.001
Mean temperature in first 72h	34.4 ± 1.6	36.8 ± 0.7	<0.001
Injury severity score	24.3 ± 9.9	29.0 ± 11.2	0.10
Median ICH index (day 1-7)	6.4 (2.2-12.7)	2.9 (0-10.7)	0.18
Median CPP index (day 1-7)	7.4 (2.4-11.2)	8 (1.4-12.8)	0.98
No. days Noradrenaline > 15mcg/min	1 (0-3)	2 (0-3)	0.57
No. days Noradrenaline given	5 (2-6)	4 (1-6)	0.34
Volume hypertonic saline, day 1-7	100 (0-700)	75 (0-332)	0.45
Total Thiopentone given (grams)	2.3 ± 6.1	1.2 ± 4.9	0.39
Volume of Albumin, day 1-7 (mL)	163 ± 436	29 ± 118	0.08
Volume of Colloids, day 1-7 (mL)	131 ± 415	85 ± 274	0.59
Volume of Crystalloids, day 1-7 (mL)	16000 ± 5666	14183 ± 5938	0.08
Volume of FFP, day 1-7 (mL)	226 ± 569	310 ± 486	0.51
Fluid Input, day 1-7 (mL)	23374 ± 9272	22041 ± 9609	0.56
Fluid Output, day 1-7 (mL)	18113 ± 8245	17910 ± 8214	0.92
Fluid balance, day 1-7 (mL)	5262 ± 44962	4131± 5158	0.35
Highest APTT	38.2 ± 8.3	36.3 ± 5.8	0.27
Highest INR	1.4 ± 0.3	1.4 ± 0.2	0.52
Highest Potassium	4.8 ± 0.4	4.5 ± 0.4	0.01
Lowest Potassium	3.1 ± 0.7	3.3 ± 0.3	0.15
Highest White Cell Count	19.5 ± 8.3	17.1 ± 6.3	0.18
Lowest White Cell Count	9.5 ± 6.1	9.2 ± 5.5	0.85
Lowest Platelets	155 ± 59	134 ± 44	0.09
Earliest post trauma creatinine	77.1 ± 16.2	72.3 ± 18.5	0.26
Average Serum Creatinine: days 1 to 7	67.7 ± 14.4	64.8 ± 14.2	0.41
Peak serum creatinine: days 1 to 7	85.1 ± 22.3	76.8 ± 18.5	0.09
AKI-free days (pts with GFR↓ > 25%)	5 (4-6)	6 (6-7)	0.002
AKI-free days (pts with GFR↓ > 50%)	6 (5-7)	7 (6-7)	0.04

All values are reported as mean ± standard deviation, % (n) or median (IQR). GCS=Glasgow Coma Score, BP= blood pressure, ED=Emergency department, WCC=White cell count, INR=International normalized ratio: measure of the extrinsic pathway of coagulation, APTT= activated partial thromboplastin time, MAP=mean arterial blood pressure, HR=heart rate, ICP=intracranial pressure; the Injury severity score is derived from scores from the Abbreviated Injury Scale; ICH index=Intracranial Hypertension Index: the number of end-hourly measures of intracranial pressure of more than 20mmHg divided by the total number of measurements, multiplied by 100; CPP index=cerebral perfusion pressure index: the number of observations of cerebral perfusion pressure of less than 60mmHg divided by the total number of measurements, multiplied by 100; No.=number, GFR↓=decrease in GFR.

Of the 70 patients included in the analysis, 46 (66%) had some degree of AKI according to the GFR/urine output RIFLE criteria, with 22 patients (48% of those with AKI) in the Injury category (Table 11). While the difference for each AKI category between cooled versus normothermic control patients was not significant, when AKI categories were combined, 80% of all cooled patients versus 51% of all control patients had developed some degree of AKI (p=0.012) (Table 11). Similar results were found using alternative classification methods although the difference in AKI between study groups was more pronounced using the RIFLE GFR classification.

Table 11 Patients with Acute Kidney Injury: comparing Cooled versus Control groups using different classification systems and definitions

	Cooled (n=35) n (%)	Controls (n=35) n (%)	p-value
1. RIFLE: using normative baseline GFR and worst GFR (day 1-7) or UO			
No AKI: GFR decrease ≤ 25%	7 (20%)	17 (49%)	0.012
Risk: GFR decrease > 25%	14 (40%)	10 (28%)	0.31
Injury: GFR decrease > 50%	14 (40%)	8 (23%)	0.12
2. RIFLE: using first post-trauma creatinine baseline and peak creatinine (day 1-7) or UO			
No AKI: SCr increase < 50%	8 (23%)	16 (46%)	0.044
Risk: SCr increase ≥ 50%	13 (37%)	11 (31%)	0.61
Injury: SCr increase ≥ 200%	14 (40%)	8 (23%)	0.12
3. AKIN category			
No AKI	9 (26%)	17 (49%)	0.048
AKIN Stage 1	11 (31%)	9 (26%)	0.6
AKIN Stage 2	15 (43%)	9 (26%)	0.13
4. KDIGO category			
No AKI	8 (23%)	16 (46%)	0.044
KDIGO Stage 1	12 (34%)	10 (29%)	0.61
KDIGO Stage 2	15 (43%)	9 (26%)	0.13

see sections 1.2 and 3.6.8 for details of each definition / classification of AKI; RIFLE=classification system for Acute kidney Injury (AKI); Risk, Injury, Failure, Loss, Endstage; GFR=glomerular filtration rate; UO=urine output; SCr=serum creatinine; AKIN= Acute kidney Injury Network classification system for AKI; KDIGO=Kidney Disease: Improving Global Outcomes. The 'Failure' or 'Stage 3' categories have been left out as no patients fit the criteria for these categories.

Of the 46 participants with AKI, 89% (41/46) were classified as developing AKI (any category) as a result of urine output classification alone, 8.7% (4/46) as a result of GFR alone and 1 patient as a result of both (Table 12).

Table 12 AKI determined by GFR, urine output or both

RIFLE: using normative baseline GFR and worst GFR for the first 7 days or UO	Criteria for AKI	Total (n=70) n (%)
No AKI: GFR decrease ≤ 25%	Both	24 (34.3%)
Risk: GFR decrease > 25%	GFR	4 (5.7%)
	UO	19 (27.1%)
	Both	1 (1.5%)
Injury: GFR decrease > 50%	GFR	0
	UO	22 (31.4%)
	Both	0
Failure: GFR decrease > 75%	No patients	0

GFR=glomerular filtration rate, UO=urine output

Using mixed linear modeling adjusting for repeated measures, the percent decrease in GFR from day 1 to 7 was negatively associated with log creatinine (from which the definition of AKI is partly derived) as expected. The relationship of other variables with log creatinine can be seen in Table 13. A higher creatinine was associated with male gender; higher potassium level; volume of fluid input including albumin, FFP and colloids; noradrenaline administration; longer clotting times; higher glucose; more severe head injury and hypoxia pre-hospital. Multivariable analysis was not performed because the idea was not to create a predictive model and it was beyond the scope of the thesis.

Table 13 Univariable estimates for linear regression models with log creatinine adjusted for repeat measures

Variable	Param Est ± SE	p-value
≥25% ↓ in GFR, day 1-7	-0.21 ± 0.05	<0.001
Peak serum creatinine, day 1-7	0.008 ± 0.001	<0.001
Highest Potassium	0.21 ± 0.06	<0.001
Female	-0.28 ± 0.07	<0.001
Weight, kg	0.006 ± 0.002	0.001
Volume of Albumin, day 1-7 (mL)	$2.39*10^{-4} \pm 7.76*10^{-5}$	0.002
Highest APTT	0.009 ± 0.03	0.01

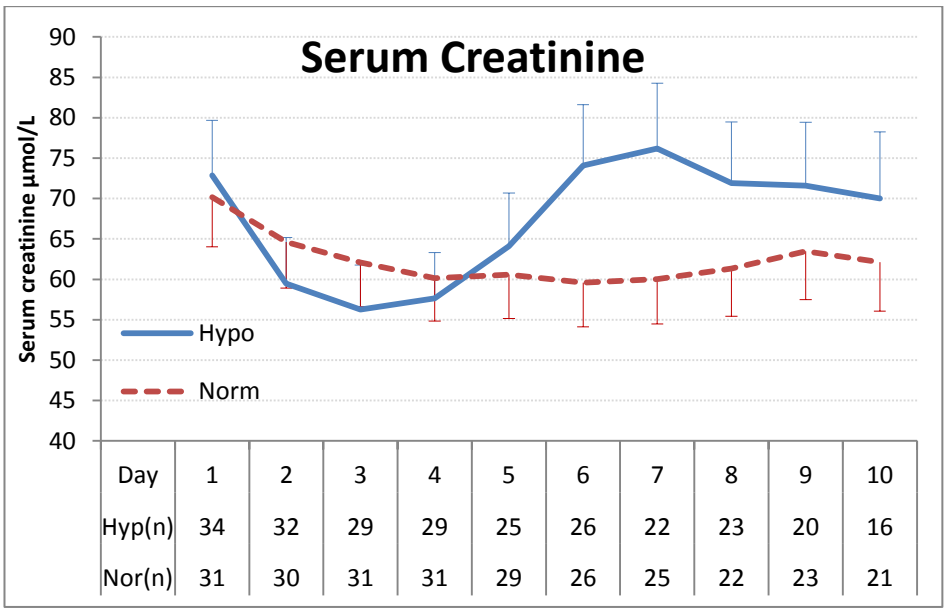
Volume of FFP, day 1-7 (mL)	$1.12*10^{-4} \pm 4.75*10^{-5}$	0.02
No. days Noradrenaline given	0.02 ± 0.01	0.02
ICH index	0.003 ± 0.001	0.02
Glucose closest to randomization	0.01 ± 0.006	0.03
Bifrontal decompressive craniectomy	0.27 ± 0.13	0.04
Volume of Colloids, day 1-7 (mL)	$1.53*10^{-4} \pm 7.31*10^{-5}$	0.04
Highest INR	0.23 ± 0.11	0.04
Fluid Input, day 1-7 (mL)	$5.70*10^{-6} \pm 2.82*10^{-6}$	0.04
Hypoxic pre-hospital %	0.19 ± 0.10	0.05
Abbreviated Injury Scale: Head/Neck	0.04 ±0.02	0.07
Highest White cell count	0.007 ± 004	0.07
Platelets closest to randomisation	0.001 ± 0.0005	0.10
Lowest White cell count	0.008 ± 0.001	0.10
Fluid output, day 1-7 (mL)	$5.09*10^{-6} \pm 3.29*10^{-6}$	0.12
Total amount Thiopentone, g	0.007 ± 0.005	0.16
CPP index	0.002 ± 0.001	0.18

Variables have p ≤ 0.2 for parameter estimate. Values are calculated from data for day 1-7 unless otherwise indicated. Param Est=Parameter estimate, SE=standard error, GFR=glomerular filtration rate, APTT= activated partial thromboplastin time, FFP=fresh frozen plasma, ICH index=Intracranial Hypertension Index, INR=International normalized ratio: measure of the extrinsic pathway of coagulation.

The levels of creatinine and GFR for cooled versus control patients were plotted for 10 days (Figure 3 and 4). The graphs of creatinine and GFR 'mirrored' each other as expected (GFR is derived from an equation using creatinine, age and gender) despite the use of a normative baseline for GFR. The graph for the normothermia (control) group is similar in pattern to that of the placebo group in the erythropoietin versus placebo comparison in Figure 1. In contrast the graph plotted for the hypothermia group displays a distinct dip in creatinine from day 1 to 5, the period in which patients are cooled and commence rewarming. After cooling the 'hypothermia' graph does not then follow the normothermia group graph, instead there appears to be a peak in creatinine after rewarming and creatinine in the cooled group then remains somewhat higher than the control group.

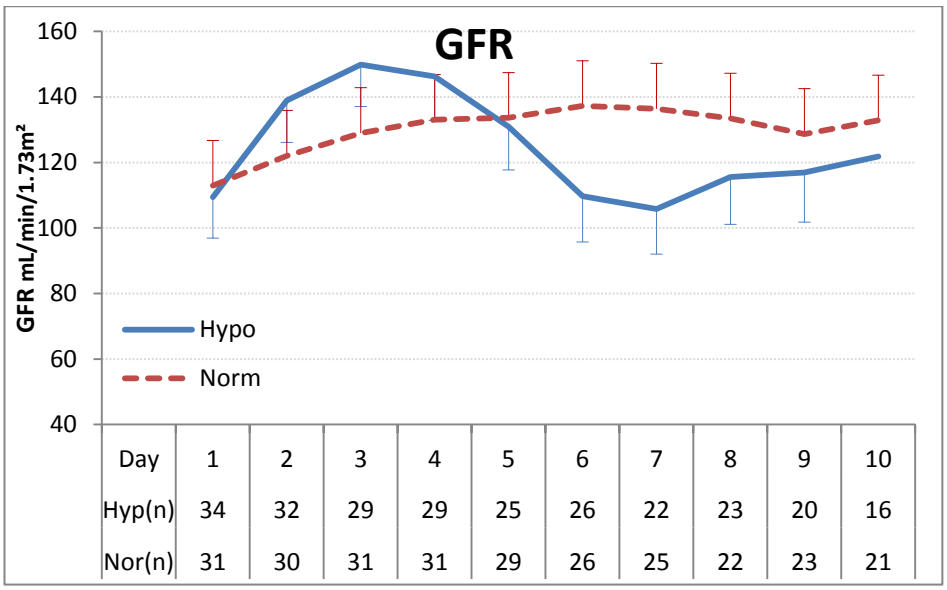
In a post-hoc analysis we collected serum creatinine measurements of cooled patients taken before, during and after rewarming to confirm on an individual level the increase in creatinine associated with rewarming. Creatinine levels increased progressively through these stages (Figure 5).

Figure 3 **Serum creatinine concentration for Hypothermia versus Normothermia patients plotted for 10 days**



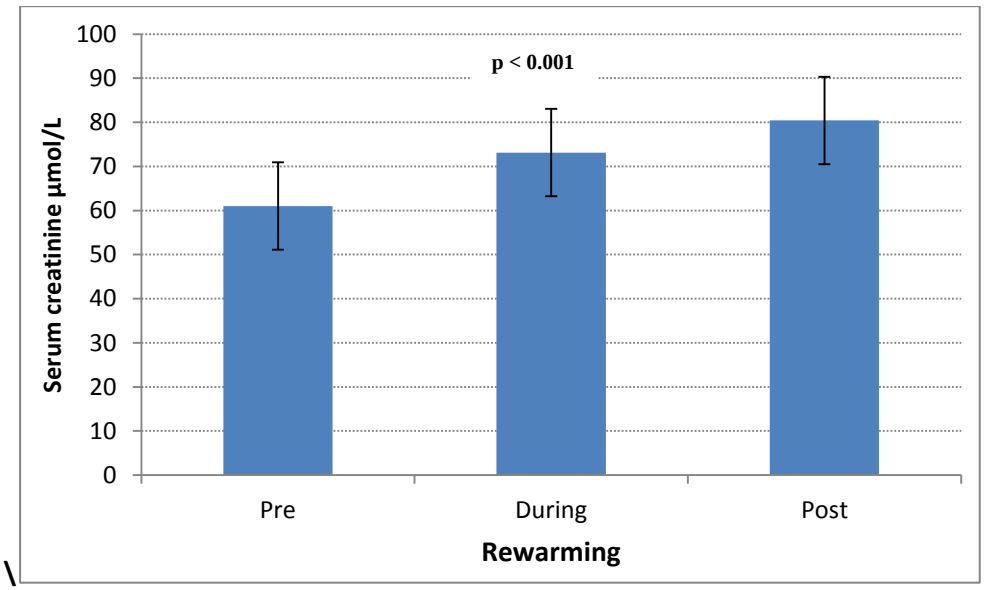
Geometric mean with 95% confidence interval is displayed. The number of patients per group for whom creatinine is available per day is displayed. Error bars are unidirectional to avoid graph crowding.

Figure 4 **Level of GFR for Hypothermia versus Normothermia control patients plotted for 10 days**



Mean with 95% confidence interval is displayed. The number of patients per group for whom calculation of GFR was possible per day is displayed. Error bars are unidirectional to avoid graph crowding.

Figure 5 **Serum creatinine concentration of hypothermia group patients taken before, during and after rewarming**



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Bar graphs are shown with 95% confidence intervals. P-value is for overall difference between periods.

4.4 New biomarkers for AKI

4.4.1 Overview, rationale for choice of biomarkers, and main findings

Recent intense investigation of new early biomarkers for AKI has revealed several promising contenders[63,128-134]. In the following section (4.4.2) correlations between all novel biomarkers under investigation are first examined, then expected responses and summary statistics for each biomarker are described. Results of the combined EPO/POLAR Biomarker data analysis follow (section 4.4.3) where data for all patients (treated and controls) in the nested biomarker cohorts from the EPO-TBI and POLAR trials were combined to increase the power to assess the biomarkers as predictors of AKI in patients with TBI. Finally, the results of the EPO-Biomarkers and POLAR-Biomarkers sections of the trials follow in sections 4.4.4 and 4.4.5 to assess the effect of erythropoietin and hypothermia on biomarker release.

Rationale for biomarker choice

We have measured the biomarkers under investigation for several reasons. Urine NGAL, KIM-1, and IL-18 profile as a group of biomarkers that are relatively easy to measure in urine and that respond to different triggers thereby complementing each other. NGAL is a marker of tubular stress and injury, KIM-1 is upregulated in ischaemic and nephrotoxic AKI, and IL-18 identifies inflammatory states. Urine cystatin C (CyC) would further complement these biomarkers as a marker of GFR. Cystatin C rises more gradually than the aforementioned markers of AKI.

In blood, plasma cystatin C is an alternative marker of GFR while plasma NGAL concentration also rises in response to AKI as a result of systemic NGAL synthesis and has shown to be an effective marker for AKI in certain groups including children. In general, the concentration of plasma biomarkers increases more gradually compared to urine markers. Plasma cathepsin S measurement was offered to us as a laboratory 'add-on'. As we were measuring its inhibitor, CyC, it was considered of interest to measure Cathepsin S levels as well. It is not a marker of renal function but data suggest it is involved in the regulation of inflammation and it is also associated with heart disease and cancer[79-83].

Urine Alpha- and Pi-GST are relatively new and less-tested kidney biomarkers and the laboratory that undertook their measurement was interested in participating in the study. They are markers of renal tubular injury and Alpha-GST has been shown to rise with nephrotoxicity.

Main findings

The concentration of all urine biomarkers increased in patients given EPO and decreased in the placebo group from baseline to 24 hours suggesting that EPO may be associated with a negative impact on patients with TBI which may involve the kidneys. Alternatively, EPO's stimulation of new growth and angiogenesis could be responsible for an increase in the concentration of some of the biomarkers.

Plasma CyC and urine NGAL were the biomarkers that showed more promise as alternative biomarkers for AKI. Their concentration was significantly higher in patients with AKI at 48 hours after dose/injury.

In response to erythropoietin and hypothermia, urine KIM-1 and plasma NGAL showed the greatest differentiation in response between treated and control groups for both therapies. The change in urine NGAL concentration between EPO and placebo groups was also notable.

4.4.2 Correlations between biomarkers and biomarker summary statistics

4.4.2.1 Correlations between biomarkers

The concentration of each biomarker was measured at baseline, then 24, 48 and 72 hours after study dose/injury in the first 30 patients (except one) recruited to the EPO-TBI trial and the first 53 patients (except two) recruited to the POLAR trial between the Royal Melbourne and Alfred hospitals in Victoria, Australia.

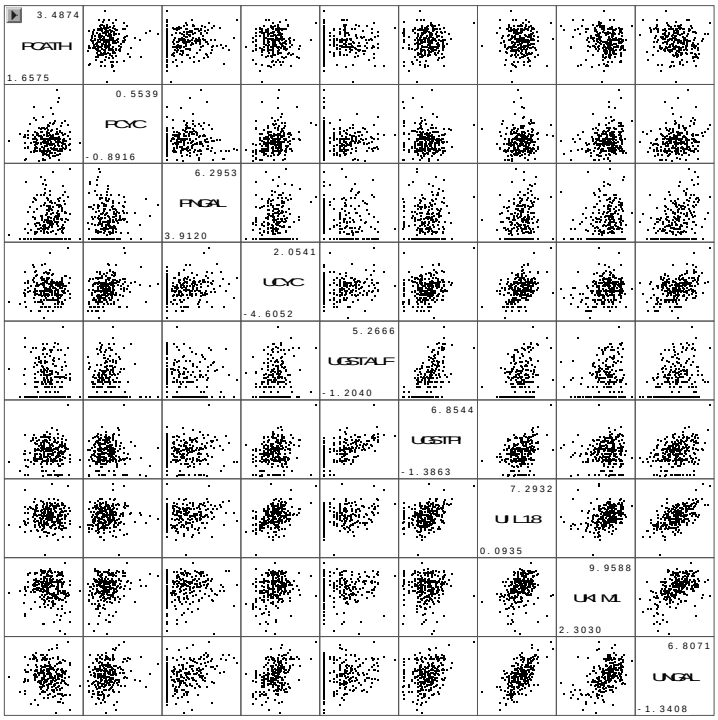
The following table and figure display a) Pearson correlation coefficients for linear associations between the biomarkers for the combined data at all timepoints (Table 14) and b) the respective scatter plots providing a visual representation of these linear correlations (Figure 6). Statistically significant relationships between biomarkers are marked in Table 14 below.

Table 14 Pearson correlation coefficients for colinearity between log-transformed biomarker data

Variable	pCath	pCyC	pNGAL	uCyC	uGSTalf	uGSTpi	uIL-18	uKIM-1	uNGAL
pCath S	1.00	0.16†	0.19*	0.07	-0.04	-0.07	0.05	-0.09	-0.11
pCyC	0.16†	1.00	0.12	0.26*	-0.08	-0.03	0.17†	0.23*	0.19*
pNGAL	0.19*	0.12	1.00	0.19*	-0.06	-0.05	0.16†	0.16†	0.31*
uCyC	0.07	0.26*	0.19*	1.00	0.18*	0.26*	0.46*	0.26*	0.43*
uGSTalf	-0.04	-0.08	-0.06	0.18*	1.00	0.62*	0.31*	0.02	0.18*
uGSTpi	0.07	-0.03	0.05	0.26*	0.62*	1.00	0.33*	-0.04	0.23*
uIL-18	0.05	0.17†	0.16†	0.46*	0.31*	0.33*	1.00	0.29*	0.66*
uKIM-1	-0.09	0.23*	0.16†	0.26*	0.02	-0.04	0.29*	1.00	0.49*
uNGAL	-0.11	0.19*	0.31*	0.43*	0.18*	0.23*	0.66*	0.49*	1.00

*** p≤0.01, † p≤0.05 (cells with significant results are highlighted in grey). 'p' precedes plasma biomarkers, 'u' precedes urine biomarkers, Cath=cathepsin S, CyC=cystatin C, NGAL=neutrophil gelatinase –associated lipocalin, Cr=creatinine, GSTalf=alpha-glutathione S-transferase (GST), GSTpi=pi-GST, IL-18=interleukin-18, KIM-1=kidney injury molecule-1.**

Figure 6 Scatter plots showing linear correlation between biomarkers



'P' precedes plasma biomarkers, 'U' precedes urine biomarkers, Cath=cathepsin S, CyC=cystatin C, NGAL=neutrophil gelatinase–associated lipocalin, CrSW=creatinine, GSTal=alpha-glutathione S-transferase (GST), GSTpi=pi-GST, IL18=interleukin-18, KIM1=kidney injury molecule-1

In the following sections (4.4.2.2 to 4.4.2.10) a brief summary of results for each biomarker is provided followed by the results for the biomarker analyses for AKI (pooled EPO-TBI/POLAR data: section 4.4.3), and the biomarker analyses for the EPO-TBI and POLAR trials (sections 4.4.4 and 4.4.5 respectively).

4.4.2.2 Plasma NGAL

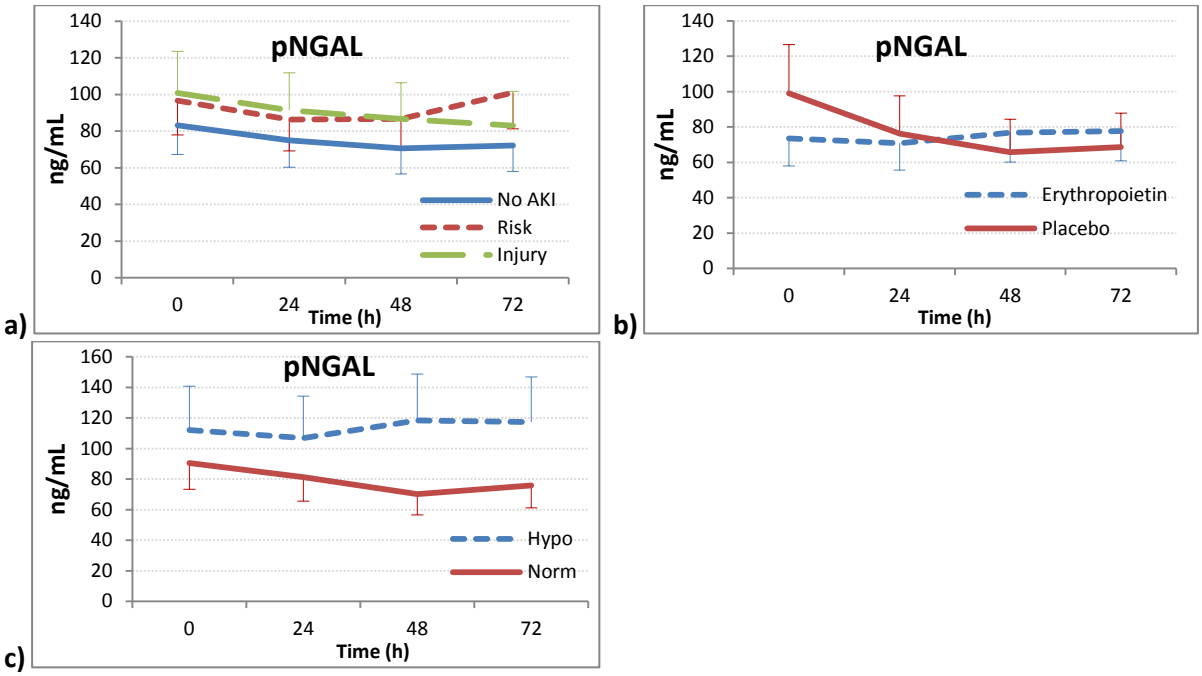
Figure 7 compares the concentration of plasma NGAL at the 4 timepoints (baseline, then 24, 48 and 72 hours after dose/injury) in 3 analyses: in patients with varying degrees of AKI (in pooled data from both trials), in patients receiving EPO versus placebo, and in patients undergoing cooling versus normothermic controls. The biomarker concentration refers to the geometric mean concentration. The expected responses to traumatic injury, AKI and the treatments are summarised in Table 15.

Table 15 Plasma NGAL: expected responses to injury / AKI / treatment

Item	Expected response in biomarker
Traumatic injury	Concentration may ↑ very soon after physical injury then ↓
AKI (Risk/Injury)	Possible ↑ from baseline: systemic synthesis in response to renal injury[134]
EPO	Concentration may be lower in EPO group due to EPO's tissue protective effects[135]
Hypothermia	Concentration may ↑: there is some tubular dysfunction in response to cooling[136]

In the pooled data for AKI, the concentration of plasma NGAL trended down in all groups for 48 hours (Figure 7a). Plasma NGAL concentration in patients with AKI (Risk or Injury groups) was higher than in patients with no-AKI but the difference between groups was not significant. In EPO versus placebo patients, plasma NGAL concentration behaved differently over time; it decreased in patients receiving placebo and remained constant in patients receiving EPO (Figure 7b, p=0.01). Plasma NGAL concentration was significantly higher in cooled patients compared to normothermic controls for all timepoints (Figure 7c, p=0.01).

Figure 7 **Graphs of plasma NGAL concentrations at 0, 24, 48 and 72 hours from baseline for a) AKI groupings in combined EPO-TBI/POLAR data, b) EPO versus placebo in EPO-TBI data, c) hypothermia versus normothermic controls in POLAR data**



pNGAL=plasma NGAL, Hypo=hypothermia, Norm=Normothermic controls

Combined EPO-TBI/POLAR data and AKI: In the pooled data analysis, there was no significant difference in the median concentration of plasma NGAL between patients with AKI (RIFLE Risk and Injury groups combined) and those with no AKI at any timepoint (Table 16).

Table 16 **Combined EPO-TBI/POLAR biomarker data: plasma NGAL concentration per timepoint with AKI**

Variable	No AKI Pts with GFR↓ ≤ 25% (n=26)	AKI Pts with GFR↓ > 25% (n=54)	p-value	AUC
pNGAL baseline	88 (71-94)	85 (50-154)	0.43	0.56
pNGAL 24h	56 (50-104)	82 (50-118)	0.10	0.62
pNGAL 48h	50 (50-90)	91 (50-123)	0.07	0.63
pNGAL 72h	58 (50-96)	87 (50-135)	0.11	0.62

Values are reported as median (interquartile range [IQR]), units are ng/mL, AKI=AKI (R/I/F): RIFLE urine output or glomerular filtration rate (GFR) criteria using normative baseline GFR and worst GFR for day 1-7 to calculate percent decrease in GFR (section 3.6.8), AUC=area under the ROC curve, pNGAL=plasma NGAL

EPO-TBI trial: There was no significant difference in concentration of plasma NGAL between patients who received EPO or placebo at any timepoint (Table 17).

Table 17 EPO-TBI biomarker data: plasma NGAL concentration compared between groups

Variable	EPO (n=15)	Placebo (n=14)	p-value
pNGAL baseline	62 (50-82)	102 (50-154)	0.16
pNGAL 24h	65 (50-94)	66 (50-110)	0.94
pNGAL 48h	72 (62-103)	50 (50-71)	0.11
pNGAL 72h	92 (50-113)	50 (50-100)	0.29
Change in pNGAL (0 to 24h)	-4 (-32-14)	-27 (-47-0)	0.23

Values are reported as median (IQR), units are ng/mL, pNGAL=plasma NGAL

POLAR trial: Plasma NGAL concentration was significantly different between the hypothermia and control groups at 48 (p=0.001) and 72 (p=0.02) hours after injury (Table 18).

Table 18 POLAR biomarker data: plasma NGAL concentration compared between groups

Variable	Hypothermia (n=24)	Normothermia (n=27)	p-value
pNGAL baseline	89 (72-184)	92 (67-121)	0.45
pNGAL 24h	87 (71-174)	86 (50-119)	0.11
pNGAL 48h	130 (63-190)	67 (50-95)	<0.01
pNGAL 72h	127(50-179)	65 (50-82)	0.02
Change in pNGAL (0 to 24h)	0.5 (-24-19)	-11 (-37-12)	0.91

Values are reported as median (IQR), units are ng/mL. pNGAL=plasma NGAL

4.4.2.3 Urine NGAL

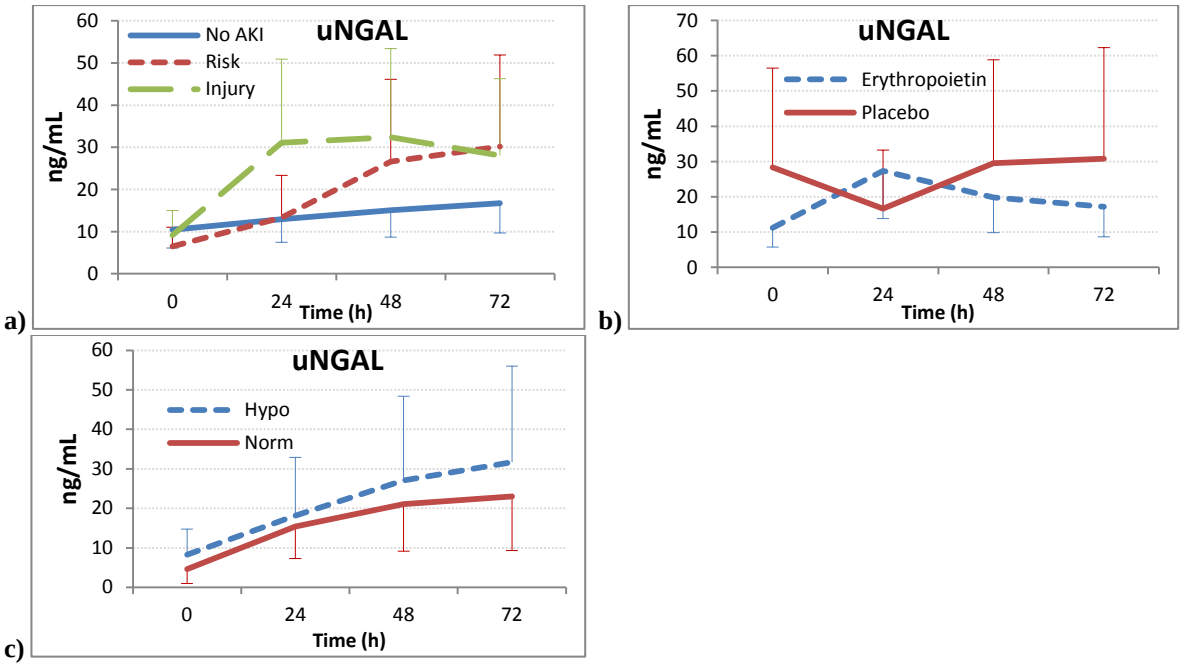
Figure 8 compares the concentration of urine NGAL at the 4 timepoints (baseline, then 24, 48 and 72 hours after dose/injury) in 3 analyses: in patients with varying degrees of AKI (in combined data from both trials), in patients receiving EPO versus placebo, and in patients undergoing cooling versus normothermic controls. The biomarker concentration refers to the geometric mean concentration. The expected responses to traumatic injury, AKI and the treatments are summarised in Table 19.

Table 19 Urine NGAL: expected responses to injury / AKI / treatment

Item	Expected response in biomarker
Traumatic injury	Concentration will remain low after injury as for 'No AKI' group in Makris et al.[137]
AKI (Risk/Injury)	↑ from baseline[133]
EPO	Concentration may be lower in EPO group due to EPO's tissue protective effects[135]
Hypothermia	Concentration may ↑: there is some tubular dysfunction in response to cooling[136]

In the pooled data for AKI, there was a significant difference between groups over time ($p=0.05$); the concentration of urine NGAL rose acutely in the 'Injury' group from baseline to 24 hours and rose with progressively lower acuity in the 'Risk' and 'No AKI' groups (Figure 8a). In the EPO versus placebo groups there was also a significant difference between groups over time ($p=0.03$); urine NGAL concentration in patients who received EPO rose from baseline to 24 hours while it decreased in patients who received placebo (Figure 8b). There was no significant difference in urine NGAL concentration between cooled and normothermic control groups (Figure 8c).

Figure 8 **Graphs of urine NGAL concentrations at 0, 24, 48 and 72 hours from baseline for a) AKI groupings in combined EPO-TBI/POLAR data, b) EPO versus placebo in EPO-TBI data, c) hypothermia versus normothermic controls in POLAR data.**



uNGAL=urine NGAL, Hypo=hypothermia, Norm=Normothermic controls

Combined EPO-TBI/POLAR data and AKI: In the pooled data, there was a significant difference in the median concentration of urine NGAL between patients with AKI (RIFLE Risk and Injury groups combined) and those with no AKI at 48 hours after study dose/injury (Table 20).

Table 20 **Combined EPO-TBI/POLAR biomarker data: urine NGAL concentration per timepoint with AKI**

Variable	AKI Pts with GFR↓ ≤ 25% (n=26)	No AKI Pts with GFR↓ > 25% (n=54)	p-value	AUC
uNGAL baseline	13 (3-39)	7 (2-25)	0.30	0.59
uNGAL 24h	15 (4-39)	23 (9-52)	0.16	0.59
uNGAL 48h	19 (8-28)	25 (17-62)	0.04	0.65
uNGAL 72h	18 (9-29)	24 (11-81)	0.20	0.60

Values are reported as median (IQR), units are ng/mL, AKI=AKI (R/I/F): RIFLE urine output or GFR criteria using normative baseline GFR and worst GFR for day 1-7 to calculate percent decrease in GFR (see Methods), AUC=area under the ROC curve, uNGAL=urine NGAL

EPO-TBI trial: There was no significant difference in the median concentration of urine NGAL between patients who received EPO or placebo at any timepoint (Table 21). The difference in the change in urine NGAL concentration from baseline to 24 hours between groups approached significance (p=0.07).

Table 21 EPO-TBI biomarker data: urine NGAL concentration compared between groups

Variable	EPO (n=15)	Placebo (n=14)	p-value
uNGAL baseline	11 (3-48)	102 (50-154)	0.10
uNGAL 24h	23 (10-63)	14 (5-93)	0.35
uNGAL 48h	32 (7-40)	20 (15-121)	0.98
uNGAL 72h	18 (9-55)	24 (13-43)	0.45
Change in uNGAL (0 to 24h)	12 (2-35)	-8 (-24-19)	0.07

Values are reported as median (IQR), units are ng/mL, uNGAL=urine NGAL

POLAR trial: Median urine NGAL concentration was not significantly different between the hypothermia and control groups at any timepoint (Table 22).

Table 22 POLAR biomarker data: urine NGAL concentration compared between groups

Variable	Hypothermia (n=24)	Normothermia (n=27)	p-value
uNGAL baseline	6 (2-25)	5 (2-8)	0.36
uNGAL 24h	15 (6-41)	16 (7-32)	0.77
uNGAL 48h	25 (12-78)	22 (12-42)	0.40
uNGAL 72h	31 (14-111)	18 (10-63)	0.36
Change in uNGAL (0 to 24h)	5 (-7-25)	7 (1-23)	0.90

Values are reported as median (IQR), units are ng/mL, uNGAL=urine NGAL

4.4.2.4 Plasma Cystatin C

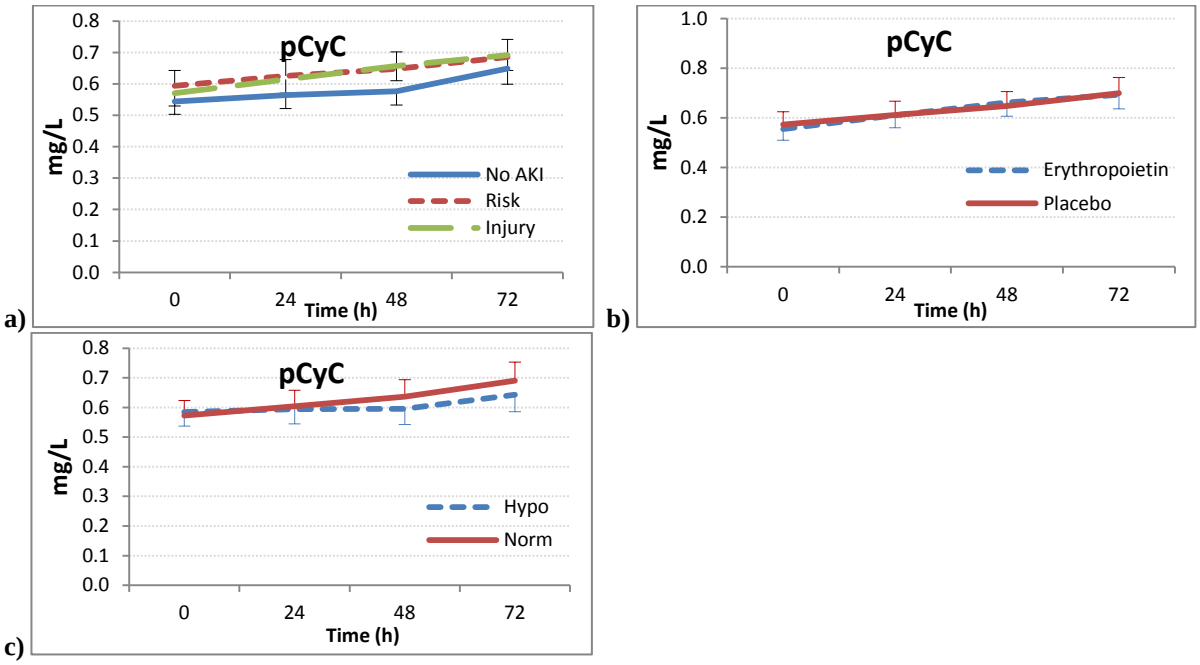
Figure 9 compares the concentration of plasma cystatin C (CyC) at the 4 timepoints (baseline, then 24, 48 and 72 hours after dose/injury) in 3 analyses: in patients with varying degrees of AKI (in combined data from both trials), in patients receiving EPO versus placebo, and in patients undergoing cooling versus normothermic controls. The expected responses to traumatic injury, AKI and the treatments are summarised in Table 23.

Table 23. Plasma CyC: expected responses to injury / AKI / treatment

Item	Expected response in biomarker
Traumatic injury	Concentration may ↑ slowly after injury due to post trauma kidney injury
AKI (Risk/Injury)	Higher with AKI and ↑ from baseline due to ↓ filtration at glomerulus[134]
EPO	Concentration may be lower in EPO group due to EPO's tissue protective effects[135]
Hypothermia	Concentration may be lower in cooled group due to ↓ metabolic rate[9]

In the pooled data for AKI, although plasma CyC concentration was slightly lower in patients with no AKI, there was no significant difference between groups (Figure 9a). In the EPO versus placebo patients there was no difference in plasma CyC between groups (Figure 9b), and there was no significant difference in plasma CyC concentration between cooled and normothermic control groups (Figure 9c). Overall there was a slow rise in plasma CyC over time in all groups in the 3 graphs (Figure 9).

Figure 9 Graphs of plasma cystatin C concentrations at 0, 24, 48 and 72 hours from baseline for a) AKI groupings in combined EPO-TBI/POLAR data, b) EPO versus placebo in EPO-TBI data, c) hypothermia versus normothermic controls in POLAR data.



pCyC=plasma cystatin C, Hypo=hypothermia, Norm=Normothermic controls

Combined EPO-TBI/POLAR data and AKI: In the pooled data, there was a significant difference in the median concentration of plasma CyC between patients with AKI

(RIFLE Risk and Injury groups combined) and those with no AKI at 48 hours after study dose/injury (Table 24).

Table 24 Combined EPO-TBI/POLAR biomarker data: plasma cystatin C concentration per timepoint with AKI

Variable	No-AKI Pts with GFR↓ ≤ 25% (n=26)	AKI Pts with GFR↓ > 25% (n=54)	p-value	AUC
pCyC baseline	0.53 (0.48-0.63)	0.58 (0.51-0.63)	0.33	0.59
pCyC 24h	0.57 (0.51-0.62)	0.60 (0.54-0.67)	0.17	0.62
pCyC 48h	0.59 (0.51-0.66)	0.66 (0.56-0.74)	0.01	0.70
pCyC 72h	0.63 (0.59-0.69)	0.65 (0.60-0.75)	0.34	0.60

Values are reported as median (IQR), units are mg/L, AKI=AKI (R/I/F): RIFLE urine output or GFR criteria using normative baseline GFR and worst GFR for day 1-7 to calculate percent decrease in GFR (section 3.6.8), AUC=area under the ROC curve, pCyC=plasma cystatin C

EPO-TBI trial: There was no significant difference in the median concentration of plasma CyC between patients who received EPO or placebo at any timepoint (Table 25).

Table 25 EPO-TBI biomarker data: plasma cystatin C concentration compared between groups

Variable	EPO (n=15)	Placebo (n=14)	p-value
pCyC baseline	0.56 (0.51-0.62)	0.58 (0.5-0.63)	0.49
pCyC 24h	0.63 (0.54-0.67)	0.60 (0.57-0.65)	0.90
pCyC 48h	0.68 (0.60-0.72)	0.60 (0.57-0.76)	0.77
pCyC 72h	0.69 (0.64-0.74)	0.67 (0.59-0.79)	0.67
Change in pCyC (0 to 24h)	0.05 (0.01-0.11)	0.04 (-0.01-0.07)	0.52

Values are reported as median (IQR), units are mg/L, pCyC=plasma cystatin C

POLAR trial: Median plasma CyC concentration was not significantly different between the hypothermia and control groups at any timepoint (Table 26).

Table 26 POLAR biomarker data: plasma cystatin C concentration compared between groups

Variable	Hypothermia (n=24)	Normothermia (n=27)	p-value
pCyC baseline	0.60 (0.51-0.65)	0.56 (0.51-0.65)	0.84
pCyC 24h	0.60 (0.53-0.64)	0.56 (0.53-0.69)	0.50
pCyC 48h	0.59 (0.52-0.70)	0.62 (0.54-0.71)	0.26
pCyC 72h	0.62 (0.56-0.71)	0.64 (0.60-0.73)	0.24
Change in pCyC (0 to 24h)	0.02 (-0.05-0.07)	0.03 (-0.03-0.08)	0.58

Values are reported as median (IQR), units are mg/L, pCyC=plasma cystatin C

4.4.2.5 Urine Cystatin C

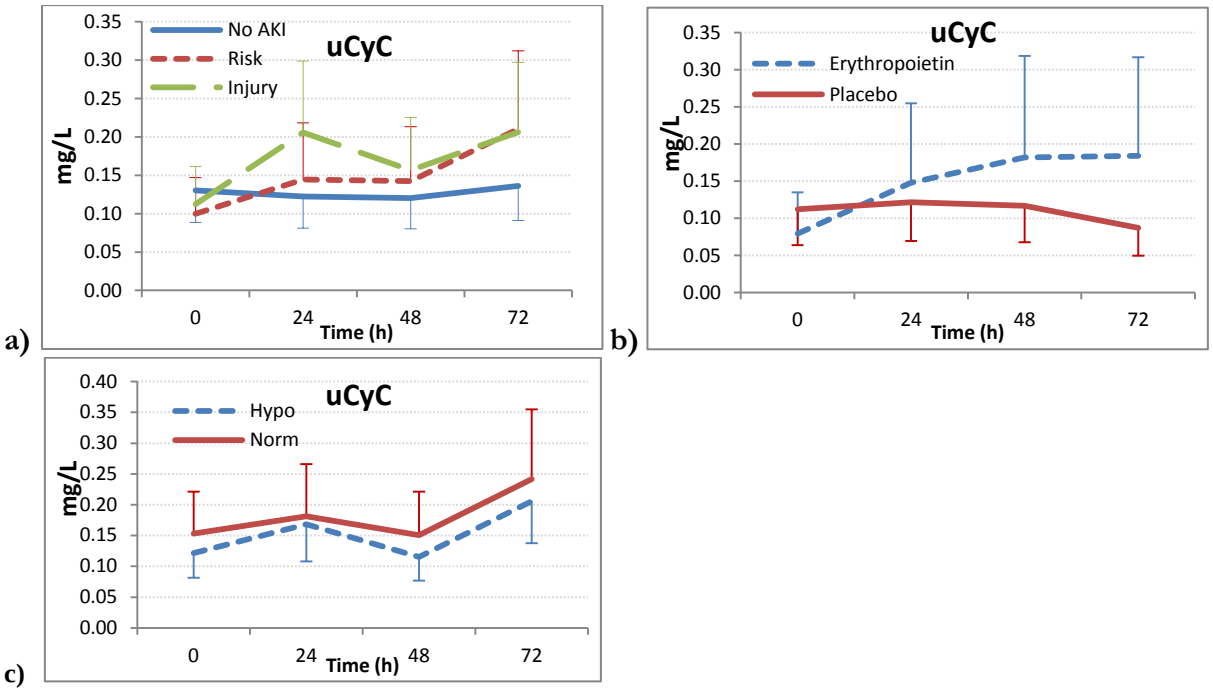
Figure 10 compares the concentration of urine cystatin C (CyC) at the 4 timepoints (baseline, then 24, 48 and 72 hours after dose/injury) in 3 analyses: in patients with varying degrees of AKI (in combined data from both trials), in patients receiving EPO versus placebo, and in patients undergoing cooling versus normothermic controls. The expected responses to traumatic injury, AKI and the treatments are summarised in Table 27.

Table 27 Urine CyC: expected responses to injury / AKI / treatment

Item	Expected response in biomarker
Traumatic injury	Concentration may ↑ slowly after injury
AKI (Risk/Injury)	Higher with AKI and ↑ from baseline due to ↓ reabsorption by tubules[134]
EPO	Concentration may be lower in EPO group due to tissue protective effects, or may be higher due to EPO's cell proliferative, angiogenesis-promoting properties[135]
Hypothermia	Concentration may be lower in cooled group due to ↓ metabolic rate[9]

Urine CyC concentration rose from baseline to 24 hours post study dose/injury in the patients who developed some degree of AKI (Risk / Injury categories), compared to the 'No AKI' group whose levels remained constant (Figure 10a) however there were no significant differences between groups. The concentration of this biomarker also rose continually (more acutely in the first 24 hours) in patients who received EPO, while it trended down over time in the placebo group, but again this difference was not statistically significant (Figure 10b). There was no significant difference in urine CyC levels between patient groups in the POLAR trial (Figure 10c).

Figure 10 **Graphs of urine cystatin C concentrations at 0, 24, 48 and 72 hours from baseline for a) AKI groupings in combined EPO-TBI/POLAR data, b) EPO versus placebo in EPO-TBI data, c) hypothermia versus normothermic controls in POLAR data.**



uCyC=urine Cystatin C, Hypo=hypothermia, Norm=Normothermic controls

Combined EPO-TBI/POLAR data and AKI: There was no significant difference in the concentration of urine CyC for any of the timepoints between patients with some degree of AKI (RIFLE Risk and Injury groups combined) and those with no AKI (Table 28).

Table 28 **Combined EPO-TBI/POLAR biomarker data: urine cystatin C concentration per timepoint with AKI**

Variable	No AKI Pts with GFR↓ ≤ 25% (n=26)	AKI Pts with GFR↓ > 25% (n=54)	p-value	AUC
uCyC baseline	0.13 (0.06-0.20)	0.11 (0.06-0.16)	0.36	0.57
uCyC 24h	0.17 (0.08-0.23)	0.15 (0.10-0.30)	0.57	0.54
uCyC 48h	0.14 (0.06-0.25)	0.17 (0.09-0.31)	0.57	0.54
uCyC 72h	0.16 (0.06-0.23)	0.22 (0.10-0.38)	0.11	0.62

Values are reported as median (IQR), units are mg/L, AKI=AKI (R/I/F): RIFLE urine output or GFR criteria using normative baseline GFR and worst GFR for day 1-7 to calculate percent decrease in GFR (see Methods), AUC=area under the ROC curve, uCyC=urine cystatin C

EPO-TBI trial: The continual rise in urine CyC concentration in the EPO group over the first 72 hours from study dose, and its trend downward in the placebo group, results in a near significant difference in urine CyC concentration between study groups at 72 hours (Table 29, p=0.06). For all other timepoints and for the comparison of change in urine CyC concentration from baseline to 24 hours, there is no significant difference between groups.

Table 29 EPO-TBI biomarker data: urine cystatin C concentration compared between groups

Variable	EPO (n=15)	Placebo (n=14)	p-value
uCyC baseline	0.08 (0.04-0.14)	0.11 (0.05-0.15)	0.58
uCyC 24h	0.14 (0.07-0.29)	0.12 (0.08-0.23)	0.66
uCyC 48h	0.20 (0.10-0.31)	0.13 (0.09-0.21)	0.30
uCyC 72h	0.16 (0.10-0.29)	0.06 (0.03-0.28)	0.06
Change in uCyC (0 to 24h)	0.04 (-0.02-0.15)	-0.01 (-0.06-0.13)	0.43

Values are reported as median (IQR), units are mg/L, uCyC=urine cystatin C

POLAR trial: There was no notable difference in concentration of urine CyC between cooled patients and normothermic controls for any of the timepoints (Table 30).

Table 30 POLAR biomarker data: urine cystatin C concentration compared between groups

Variable	Hypothermia (n=24)	Normothermia (n=27)	p-value
uCyC baseline	0.11 (0.07-0.21)	0.16 (0.09-0.26)	0.22
uCyC 24h	0.16 (0.08-0.33)	0.18 (0.14-0.30)	0.63
uCyC 48h	0.14 (0.03-0.24)	0.21 (0.09-0.27)	0.33
uCyC 72h	0.17 (0.13-0.35)	0.23 (0.15-0.36)	0.46
Change in uCyC (0 to 24h)	0.06 (0.01-0.11)	0.01 (-0.03-0.10)	0.22

Values are reported as median (IQR), units are mg/L, uCyC=urine cystatin C

4.4.2.6 Urine KIM-1

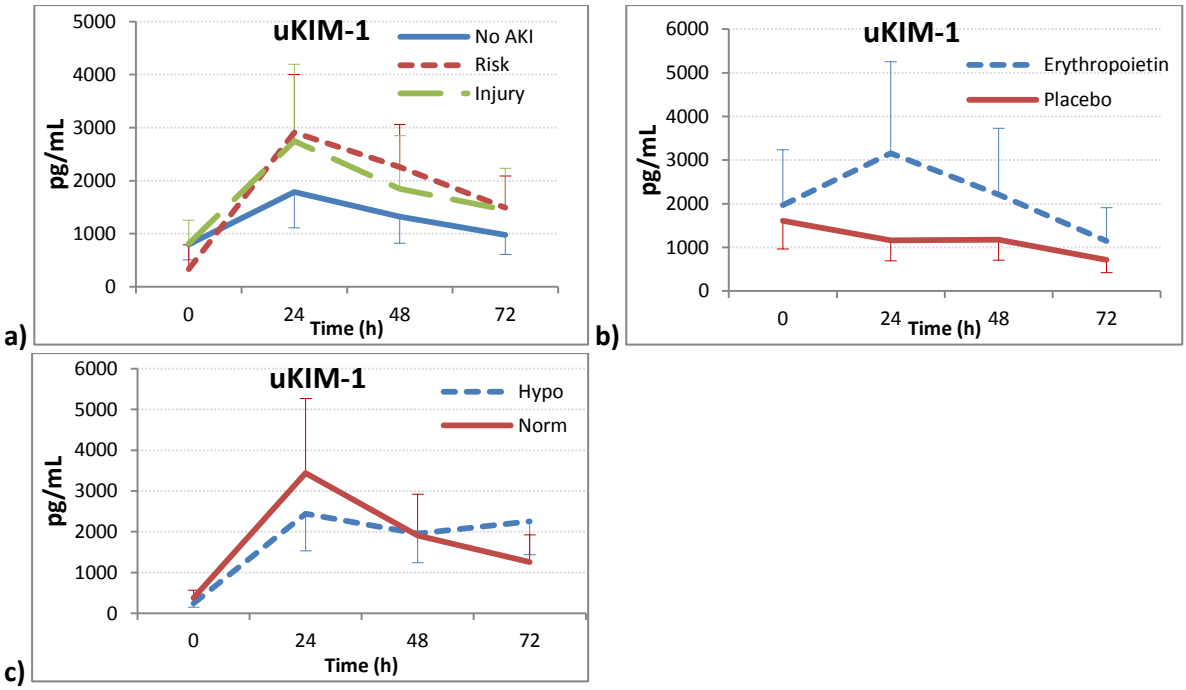
Figure 11 compares the concentration of urine Kidney Injury Molecule-1 (KIM-1) at the 4 timepoints (baseline, then 24, 48 and 72 hours after dose/injury) in 3 analyses: in patients with varying degrees of AKI (in combined data from both trials), in patients receiving EPO versus placebo, and in patients undergoing cooling versus normothermic controls. The expected responses to traumatic injury, AKI and the treatments are summarised in Table 31.

Table 31 Urine KIM-1: expected responses to injury / AKI / treatment

Item	Expected response in biomarker
Traumatic injury	Concentration may be unchanged or ↑ (due to renal injury) after physical injury
AKI (Risk/Injury)	Higher with AKI and ↑ from baseline due to upregulation in tubules[134]
EPO	Concentration may be lower in EPO group due to tissue protective effects[135]
Hypothermia	Concentration may be lower in cooled group due to ↓ metabolic rate[9]

Urine KIM-1 concentration rose more acutely from baseline to 24 hours post study dose/injury in the patients who developed some degree of AKI (Risk / Injury categories) compared to the No AKI group (Figure 11a). There was a significant difference between the graphs for these groups over time (p=0.01). The concentration of urine KIM-1 also rose from baseline to 24 hours after study dose in patients who received EPO compared to its decrease in the placebo group, and there was a significant difference between the graphs for the 2 groups (p=0.04) (Figure 11b). For the POLAR trial the graphs for the two groups behaved differently over time (p=0.03), however, there was no significant difference overall between patient groups (Figure 11c).

Figure 11 **Graphs of urine KIM-1 concentrations at 0, 24, 48 and 72 hours from baseline for a) AKI groupings in combined EPO-TBI/POLAR data, b) EPO versus placebo in EPO-TBI data, c) hypothermia versus normothermic controls in POLAR data.**



uKIM-1=urine KIM-1, Hypo=hypothermia, Norm=Normothermic controls

Combined EPO-TBI/POLAR data and AKI: There was no significant difference in the concentration of urine KIM-1 for any of the timepoints between patients with some degree of AKI (RIFLE Risk and Injury groups combined) and those with no AKI. (Table 32).

Table 32 **Combined EPO-TBI/POLAR biomarker data: urine KIM-1 concentration per timepoint with AKI**

Variable	No AKI Pts with GFR↓ ≤ 25% (n=26)	AKI Pts with GFR↓ > 25% (n=54)	p-value	AUC
uKIM-1 baseline	677 (418-2041)	707 (200-1605)	0.34	0.56
uKIM-1 24h	2464 (1255-3126)	2770 (1561-5404)	0.24	0.59
uKIM-1 48h	1823 (1001-3084)	2553 (1406-4351)	0.10	0.62
uKIM-1 72h	1140 (458-1934)	1579 (671-2902)	0.19	0.60

Values are reported as median (IQR), units are pg/mL, AKI=AKI (R/I/F): RIFLE urine output or GFR criteria using normative baseline GFR and worst GFR for day 1-7 to calculate percent decrease in GFR (section 3.6.8), AUC=area under the ROC curve, uKIM-1=urine Kidney Injury Molecule-1

EPO-TBI trial: Median urine KIM-1 concentration was significantly different between groups at 24 hours after dose (Table 33) which is reflected in the graph in Figure 11b.

Table 33 EPO-TBI biomarker data: urine KIM-1 concentration compared between groups

Variable	EPO (n=15)	Placebo (n=14)	p-value
uKIM-1 baseline	1814 (917-5375)	1950 (726-2626)	0.59
uKIM-1 24h	2974 (1679-6045)	1491 (730-2566)	0.01
uKIM-1 48h	2482 (1513-3131)	1318 (791-2519)	0.13
uKIM-1 72h	1228 (656-1627)	1133 (414-1845)	0.53
Change in uKIM-1 (0 to 24h)	922 (-288-3251)	-198 (-1843-621)	0.12

Values are reported as median (IQR), units are pg/mL, uKIM-1=urine Kidney Injury Molecule-1

POLAR trial: The continued elevation of urine KIM-1 at 72 hours after injury in the cooled group and its decrease in the normothermic group resulted in a significant difference between the groups at 72 hours.

Table 34 POLAR biomarker data: urine KIM-1 concentration compared between groups

Variable	Hypothermia (n=24)	Normothermia (n=27)	p-value
uKIM-1 baseline	228 (144-611)	483 (194-726)	0.25
uKIM-1 24h	2537 (1326-4454)	2974 (2359-5735)	0.13
uKIM-1 48h	3084 (1608-3941)	1917 (1331-3327)	0.17
uKIM-1 72h	2183 (1174-5223)	1206 (772-2237)	0.03
Change in uKIM-1 (0 to 24h)	2491 (1191-3613)	2389 (1317-4854)	0.5

Values are median (IQR), units are pg/mL. uKIM-1 =urine Kidney Injury Molecule-1

4.4.2.7 Urine IL-18

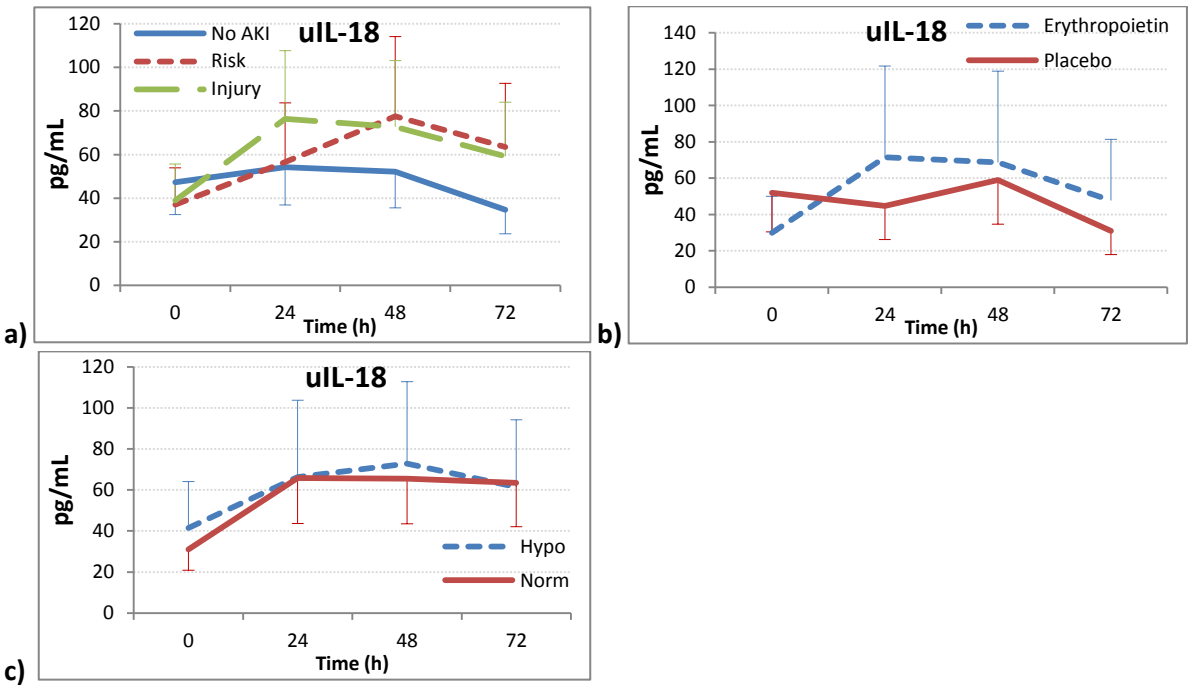
Figure 12 compares the concentration of urine Interleukin-18 (IL-18) at the 4 timepoints (baseline, then 24, 48 and 72 hours after dose/injury) in 3 analyses: in patients with varying degrees of AKI (in combined data from both trials), in patients receiving EPO versus placebo, and in patients undergoing cooling versus normothermic controls. The expected responses to traumatic injury, AKI and the treatments are summarised in Table 35.

Table 35 Urine IL-18: expected responses to injury / AKI / treatment

Item	Expected response in biomarker
Traumatic injury	Concentration may ↑ after physical injury (proinflammatory cytokine)[134]
AKI (Risk/Injury)	Higher with AKI and ↑ from baseline due to upregulation in tubules[134]
EPO	Concentration may be lower in EPO group due to tissue protective effects[135]
Hypothermia	Concentration may be lower in cooled group due to ↓ metabolic rate[9]

Urine IL-18 concentration appears to rise more acutely in patients who developed AKI (peak at 24 hours) compared to those who were at risk for developing AKI (peak at 48 hours), while the graph for 'no-AKI' remains relatively flat (Figure 12a). However there was no significant difference between graphs. There was no significant difference between the graphs for the EPO-TBI groups, although urine IL-18 concentration rose from baseline to 24 hours in patients receiving EPO and decreased in the placebo group (Figure 12b). In the POLAR trial there was no difference in urine IL-18 concentration between groups (Figure 12c).

Figure 12 Graphs of urine IL-18 concentrations at 0, 24, 48 and 72 hours from baseline for a) AKI groupings in combined EPO-TBI/POLAR data, b) EPO versus placebo in EPO-TBI data, c) hypothermia versus normothermic controls in POLAR data.



uIL-18=urine IL-18, Hypo=hypothermia, Norm=Normothermic controls

Combined EPO-TBI/POLAR data and AKI: The concentration of urine IL-18 at 72 hours post dose/injury was significantly higher in patients with a decrease in GFR > 25% (61 [33-121]) compared to those with a decrease in GFR of ≤ 25% (34 [24-69]), p=0.05 (Table 36).

Table 36 Combined EPO-TBI/POLAR biomarker data: urine IL-18 concentration per timepoint with AKI

Variable	No AKI Pts with GFR↓ ≤ 25% (n=26)	AKI Pts with GFR↓ > 25% (n=54)	p-value	AUC
uIL-18 baseline	44 (26-96)	39 (20-77)	0.29	0.58
uIL-18 24h	54 (39-89)	84(37-120)	0.11	0.60
uIL-18 48h	60 (29-89)	76 (38 -113)	0.23	0.59
uIL-18 72h	34 (24-69)	61(33-121)	0.04	0.65

Values are reported as median (IQR), units are pg/mL, AKI=AKI (R/I/F): RIFLE urine output or GFR criteria using normative baseline GFR and worst GFR for day 1-7 to calculate percent decrease in GFR (section 3.6.8), AUC=area under the ROC curve, uIL-18=urine Interleukin-18

EPO-TBI trial: Median urine IL-18 concentration was not significantly different between EPO and placebo groups at any timepoint (Table 37).

Table 37 EPO-TBI biomarker data: urine IL-18 concentration compared between groups

Variable	EPO (n=15)	Placebo (n=14)	p-value
uIL-18 baseline	42 (21-84)	45 (32-98)	0.37
uIL-18 24h	89 (43-112)	61 (14-91)	0.38
uIL-18 48h	63 (56-78)	78 (30-92)	0.99
uIL-18 72h	47.24 (30-94)	32 (14-79)	0.36
Change in uIL-18 (0 to 24h)	36 (-5-70)	12 (-28-30)	0.10

Values are reported as median (IQR), units are pg/mL, uIL-18= urine Interleukin-18

POLAR trial: Median urine IL-18 concentration was not significantly different between groups at any timepoint (Table 38).

Table 38 POLAR biomarker data: urine IL-18 concentration compared between groups

Variable	Hypothermia (n=24)	Normothermia (n=27)	p-value
uIL-18 baseline	47 (20-116)	38 (17-67)	0.32
uIL-18 24h	76 (30-137)	65 (36-117)	0.69
uIL-18 48h	82(41-121)	64 (33-96)	0.55
uIL-18 72h	54 (37-117)	76 (29-135)	1.00
Change in uIL-18 (0 to 24h)	17 (1-101)	19 (-2-76)	0.95

Values are reported as median (IQR), units are pg/mL, uIL-18= urine Interleukin-18

4.4.2.8 Urine Alpha-Glutathione S-transferase

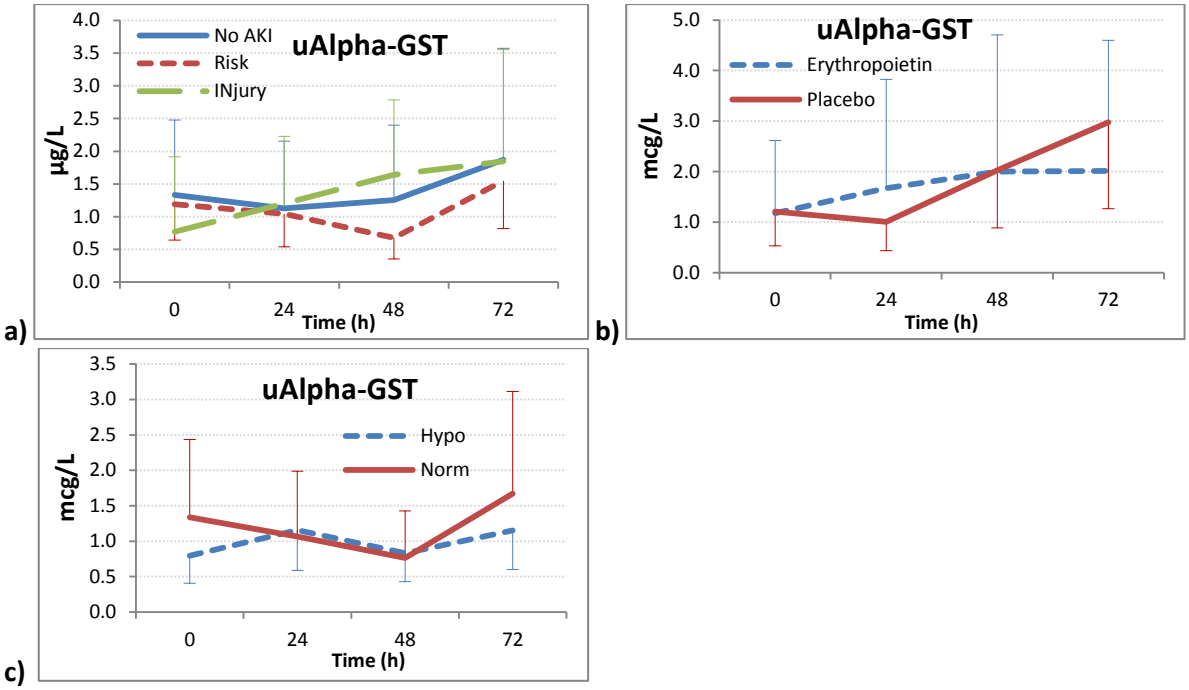
Figure 13 compares the concentration of urine alpha-glutathione S-transferase (alpha-GST) at the 4 timepoints (baseline, then 24, 48 and 72 hours after dose/injury) in 3 analyses: in patients with varying degrees of AKI (in combined data from both trials), in patients receiving EPO versus placebo, and in patients undergoing cooling versus normothermic controls. The expected responses to traumatic injury, AKI and the treatments are summarised in Table 39.

Table 39 Urine Alpha-GST: expected responses to injury / AKI / treatment

Item	Expected response in biomarker
Traumatic injury	Concentration may remain the same after physical injury
AKI (Risk/Injury)	↑ from baseline (section 2.4.2)
EPO	Concentration may be lower in EPO group due to tissue protective effects[135]
Hypothermia	Concentration may be lower in cooled group due to ↓ metabolic rate[9]

There is no significant difference between groups in any of the graphs for the above analyses (Figure 13).

Figure 13 **Graphs of urine alpha -GST concentrations at 0, 24, 48 and 72 hours from baseline for a) AKI groupings in combined EPO-TBI/POLAR data, b) EPO versus placebo in EPO-TBI data, c) hypothermia versus normothermic controls in POLAR data.**



uAlpha-GST=urine alpha-GST, Hypo=hypothermia, Norm-Normothermic controls

Combined EPO-TBI/POLAR data and AKI: In the pooled data analysis, there was no significant difference in the concentration of urine alpha-GST between patients with some degree of AKI (RIFLE Risk and Injury groups combined) and those with no AKI at any timepoint (Table 40).

Table 40 **Combined EPO-TBI/POLAR biomarker data: urine Alpha-GST concentration per timepoint with AKI**

Variable	No AKI Pts with GFR↓ ≤ 25% (n=26)	AKI Pts with GFR↓ > 25% (n=54)	p-value	AUC
uAlpha-GST baseline	1.3 (0.3-2.9)	0.3 (0.3-2.5)	0.22	0.59
uAlpha-GST 24h	1.0 (0.3-2.8)	0.8 (0.3-3.3)	0.77	0.49
uAlpha-GST 48h	1.0 (0.3-3.3)	0.8 (0.3-2.5)	0.62	0.55
uAlpha-GST 72h	2.8 (0.3-5.3)	1.6 (0.3-5.3)	0.68	0.47

Values are median (IQR), units are mcg/L, 0.3 mcg/L was the lowest detectable concentration, AKI=AKI (R/I/F): RIFLE urine output or GFR criteria using normative baseline GFR and worst GFR for day 1-7 to calculate percent decrease in GFR (section 3.6.8), AUC=area under the ROC curve, uAlpha-GST=urine Alpha-glutathione S-transferase

EPO-TBI trial: Median urine alpha-GST concentration was not significantly different between EPO and placebo groups at any timepoint (Table 41).

Table 41 EPO-TBI biomarker data: urine Alpha-GST concentration compared between groups

Variable	EPO (n=15)	Placebo (n=14)	p-value
uAlpha-GST baseline	1.0 (0.3-3.5)	0.6 (0.3-3.5)	0.96
uAlpha-GST 24h	1.3 (0.8-3.8)	1.3 (0.3-2.8)	0.41
uAlpha-GST 48h	2 (0.3-4.3)	1.8 (0.3-6.0)	1.00
uAlpha-GST 72h	2.6 (0.3-5.3)	3.3 (0.8-8.8)	0.53
Change in uAlpha-GST (0 to 24h)	0 (-2.3-1.0)	0 (-0.8-0.5)	0.31

Values are reported as median (IQR), units are mcg/L, 0.3 mcg/L was the lowest detectable concentration, uAlpha-GST=urine Alpha-glutathione S-transferase

POLAR trial: Median urine alpha-GST concentration was not significantly different between the hypothermia and control groups at any timepoint (Table 42).

Table 42 POLAR biomarker data: urine Alpha-GST concentration compared between groups

Variable	Hypothermia (n=24)	Normothermia (n=27)	p-value
uAlpha-GST baseline	0.3 (0.3-1.75)	1.75 (0.3-3.5)	0.14
uAlpha-GST 24h	0.3 (0.3-3.38)	0.55 (0.3-2.75)	0.98
uAlpha-GST 48h	0.3 (0.3-2)	0.63 (0.3-1.63)	0.64
uAlpha-GST 72h	1 (0.3-2.25)	2.13 (0.3-4.88)	0.33
Change in uAlpha-GST (0 to 24h)	0 (-2.2-0.25)	0 (-2.25-1.5)	0.39

Values are reported as median (IQR), units are mcg/L, 0.3 mcg/L was the lowest detectable concentration, Alpha-GST=urine Alpha-glutathione S-transferase

4.4.2.9 Urine Pi-Glutathione S-transferase

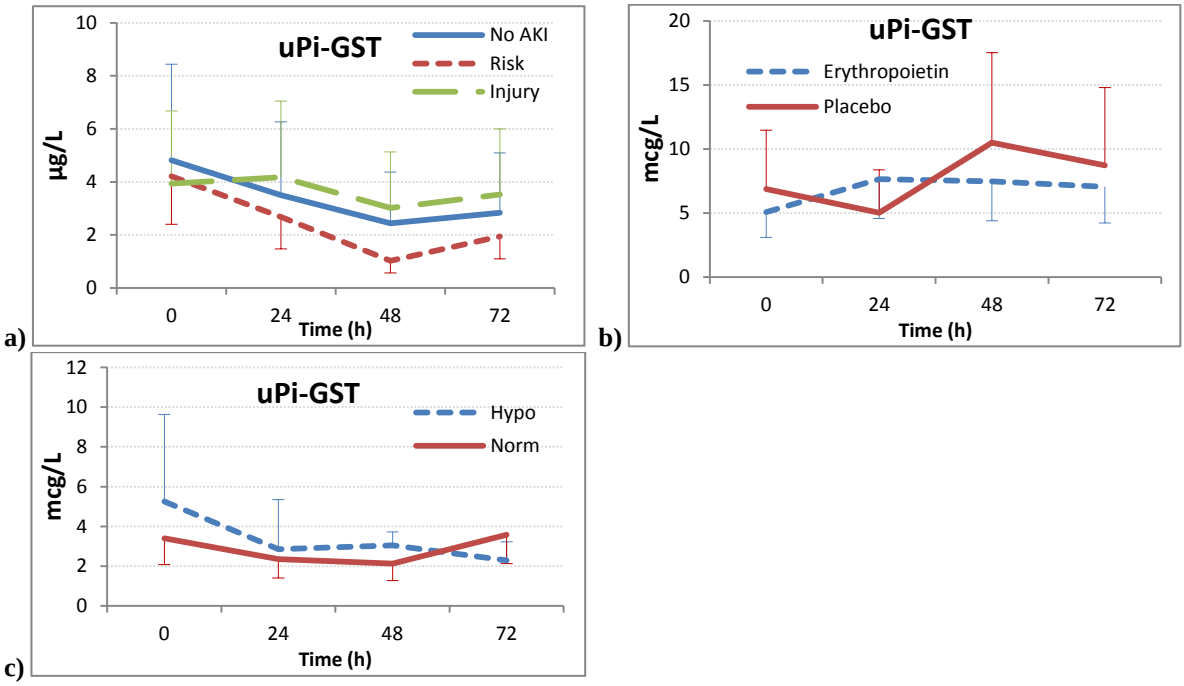
Figure 14 compares the concentration of urine pi-glutathione S-transferase (pi-GST) at the 4 timepoints (baseline, then 24, 48 and 72 hours after dose/injury) in 3 analyses: in patients with varying degrees of AKI (in combined data from both trials), in patients receiving EPO versus placebo, and in patients undergoing cooling versus normothermic controls. The expected responses to traumatic injury, AKI and the treatments are summarised in Table 43.

Table 43 Urine Pi-GST: expected responses to injury / AKI / treatment

Item	Expected response in biomarker
Traumatic injury	Concentration may remain the same after physical injury
AKI (Risk/Injury)	↑ from baseline (section 2.4.2)
EPO	Concentration may be lower in EPO group due to tissue protective effects[135]
Hypothermia	Concentration may be lower in cooled group due to ↓ metabolic rate[9]

There is no significant difference between groups in any of the graphs for the analyses and no interpretable findings (Figure 14).

Figure 14 Graphs of urine pi-GST concentrations at 0, 24, 48 and 72 hours from baseline for a) AKI groupings in combined EPO-TBI/POLAR data, b) EPO versus placebo in EPO-TBI data, c) hypothermia versus normothermic controls in POLAR data.



uPi-GST=urine pi-GST, Hypo=hypothermia, Norm=Normothermic controls

Combined EPO-TBI/POLAR data and AKI: In the pooled data analysis, there was no significant difference in the concentration of urine pi-GST between patients with some degree of AKI (RIFLE Risk and Injury groups combined) and those with no AKI at any timepoint (Table 44).

Table 44 Combined EPO-TBI/POLAR biomarker data: urine Pi-GST concentration per timepoint with AKI

Variable	No AKI Pts with GFR↓ ≤ 25% (n=26)	AKI Pts with GFR↓ > 25% (n=54)	p-value	AUC
uPi-GST baseline	4.4 (2.4-12.1)	4.3 (2.3-8.8)	0.51	0.54
uPi-GST 24h	3.5 (1.8-6.8)	4 (1.8-6.5)	0.83	0.47
uPi-GST 48h	4.0 (1.8-9.0)	3.6 (1.8-10.5)	0.96	0.51
uPi-GST 72h	5.4 (1.8-12.0)	5.3 (2.0-13.0)	0.68	0.53

Values are reported as median (IQR), units are mcg/L, AKI=AKI (R/I/F): RIFLE urine output or GFR criteria using normative baseline GFR and worst GFR for day 1-7 to calculate percent decrease in GFR (see Methods), AUC=area under the ROC curve, uPi-GST=urine Pi-glutathione S-transferase

EPO-TBI trial: Median urine pi-GST concentration was not significantly different between EPO and placebo groups at any timepoint (Table 45).

Table 45 EPO-TBI biomarker data: urine Pi-GST concentration compared between groups

Variable	EPO (n=15)	Placebo (n=14)	p-value
uPi-GST baseline	4.5 (3.2-10.5)	7.8 (3.3-14.5)	0.46
uPi-GST 24h	5.1 (4.8-11.0)	3.6 (3.0-14.5)	0.19
uPi-GST 48h	9.3 (3-13.3)	8.8 (3.8-32)	0.48
uPi-GST 72h	6.8 (4.5-13.5)	9.8 (6.8-12.8)	0.38
Change in uPi-GST (0 to 24h)	0.4 (-1.8-3.8)	-2.3 (-7.8-4.8)	0.28

Values are reported as median (IQR), units are mcg/L, uPi-GST=urine Pi-glutathione S-transferase

POLAR trial: Median urine pi-GST concentration was not significantly different between the hypothermia and control groups at any timepoint (Table 46).

Table 46 POLAR biomarker data: urine Pi-GST concentration compared between groups

Variable	Hypothermia (n=24)	Normothermia (n=27)	p-value
uPi-GST baseline	3.8 (2.5-9.0)	3.1 (1.8-7.3)	0.43
uPi-GST 24h	2.5 (1.6-5.4)	2.5 (1.3-5.9)	0.69
uPi-GST 48h	2.8 (1.8-5.8)	1.8 (1.4-3.9	0.11
uPi-GST 72h	2.55 (0.5-6)	5.15 (1.65-7.3)	0.26
Change in uPi-GST (0 to 24h)	-1.3 (-7.0-1.0)	-1 (-5.3-3.3)	0.60

Values are reported as median (IQR), units are mcg/L, uPi-GST=urine Pi-glutathione S-transferase

4.4.2.10 Plasma Cathepsin S

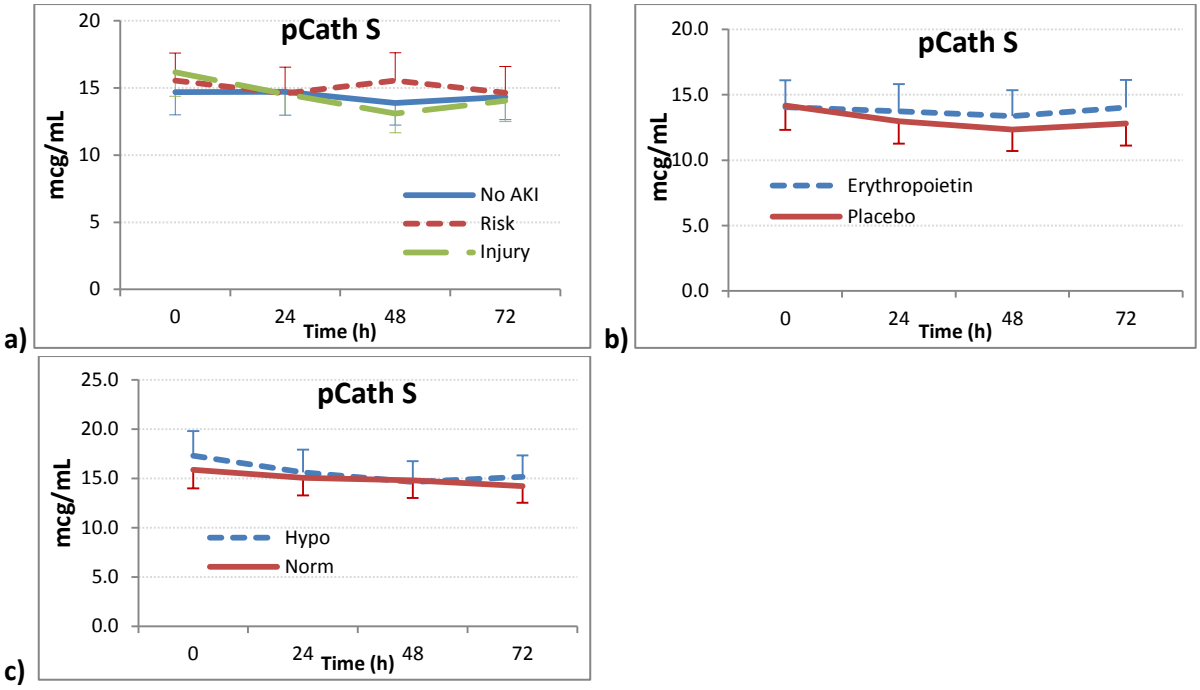
Figure 15 compares the concentration of plasma cathepsin S (Cath S) at the 4 timepoints (baseline, then 24, 48 and 72 hours after dose/injury) in 3 analyses: in patients with varying degrees of AKI (in combined data from both trials), in patients receiving EPO versus placebo, and in patients undergoing cooling versus normothermic controls. The expected responses to traumatic injury, AKI and the treatments are summarised in Table 47.

Table 47 Plasma Cath S: expected responses to injury / AKI / treatment

Item	Expected response in biomarker
Traumatic injury	Concentration may ↓ in response to an ↑ in cystatin C, its inhibitor (section 2.4.2)
AKI (Risk/Injury)	Concentration may remain constant – it is not a marker of AKI (section 2.4.2)
EPO	Concentration may ↓ with EPO due to anti-inflammatory effect (pCath S is associated with inflammatory activity, section 2.4.2)
Hypothermia	Concentration may be lower in cooled group due to ↓ metabolic rate and anti-inflammatory effect of cooling (pCath S is associated with inflammatory activity, section 2.4.2)

Visually there is little difference between groups in any of the graphs and there is no significant group effect for any of the graphs (Figure 15). The concentration of plasma cathepsin S remains fairly constant over time in all graphs, however the very slow gradual reduction in plasma cathepsin S concentration in the EPO-TBI and POLAR graphs is consistent with Cystatin C (which gradually rises, Figure 9) being an inhibitor of Cathepsin S.

Figure 15 **Graphs of plasma cathepsin S concentrations at 0, 24, 48 and 72 hours from baseline for a) AKI groupings in combined EPO-TBI/POLAR data, b) EPO versus placebo in EPO-TBI data, c) hypothermia versus normothermic controls in POLAR data.**



pCath S=plasma cathepsin S, Hypo=hypothermia, Norm-Normothermic control

Combined EPO-TBI/POLAR data and AKI: In the pooled data analysis, there was no significant difference in the concentration of plasma cathepsin S between patients with some degree of AKI (RIFLE Risk and Injury groups combined) and those with no AKI at any timepoint (Table 48).

Table 48 Combined EPO-TBI/POLAR biomarker data: plasma Cathepsin S values per timepoint with AKI

Variable	No AKI Pts with GFR↓ ≤ 25% (n=26)	AKI Pts with GFR↓ > 25% (n=54)	p-value	AUC
pCath S baseline	15 (13-18)	15 (13-21)	0.28	0.56
pCath S 24h	15 (13-17)	14 (12-18)	0.73	0.51
pCath S 48h	14 (11-16)	14 (12-17)	0.41	0.54
pCath S 72h	13 (11-19)	14 (11-18)	0.61	0.53

Values are reported as median (IQR), units are mcg/mL, AKI=AKI (R/I/F): RIFLE urine output or GFR criteria using normative baseline GFR and worst GFR for day 1-7 to calculate percent decrease in GFR (section 3.6.8), AUC=area under the ROC curve, pCath S=plasma Cathepsin S

EPO-TBI trial: Median plasma cathepsin S concentration was not significantly different between EPO and placebo groups at any timepoint (Table 49).

Table 49 EPO-TBI biomarker data: plasma Cathepsin S concentration compared between groups

Variable	EPO (n=15)	Placebo (n=14)	p-value
pCath S baseline	14.5 (11.9-15.1)	15.0 (12.3-18.3)	0.75
pCath S 24h	14.1 (11.2-15.3)	13.6 (11.1-14.8)	0.77
pCath S 48h	12.7 (11.5-13.9)	13.3 (10.0-15.4)	0.61
pCath S 72h	13.4 (11.2-16.0)	13.0 (10.6-15.7)	0.52
Change in pCath S (0 to 24h)	-0.3 (-1.0-0.3)	-0.4 (-4.0-0.4)	0.64

Values reported as median (IQR), units are mcg/mL, pCath S=plasma Cathepsin S

POLAR trial: Median plasma cathepsin S concentration was not significantly different between the hypothermia and control groups at any timepoint (Table 50).

Table 50 POLAR biomarker data: plasma Cathepsin S values compared between hypothermia / normothermia groups

Variable	Hypothermia (n=24)	Normothermia (n=27)	p-value
pCath S baseline	18.7 (13.3-21.4)	16.6 (13.0-20.6)	0.30
pCath S 24h	15.7 (12.9-19.8)	15.8 (11.8-18.8)	0.45
pCath S 48h	14.8 (11.5-17.0)	15.6 (13.1-17.4)	0.90
pCath S 72h	14.9 (12.1-19.0)	14.3 (11.9-18.5)	0.58
Change in pCath S (0 to 24h)	-1.6 (-3.6-0.1)	-1.6 (-2.8-0.6)	0.37

Values reported as median (IQR), units are mcg/mL, pCath S=plasma Cathepsin S

4.4.3 Combined EPO-TBI/POLAR biomarker data: Does the release of biomarkers predict the occurrence severity, and duration of AKI?

Biomarker specimens were collected for the first 51eligible patients recruited to the POLAR trial and for 29 of the first 30 patients recruited to the EPO-TBI trial between the Royal Melbourne and Alfred Hospitals in Victoria, Australia. This left 80 patients with biomarker specimens collected between the two hospitals. Fifty four of these 80 patients (67.5%) were classified with AKI (any category) using GFR criteria of the RIFLE classification system. Normative baseline GFR (Appendix 7, Figure 1a and 1b) and worst GFR from day 1 to 7 were used to define percent decrease in GFR (section 3.6.8). Patient characteristics and renal data for this combined dataset are compared in Table 51 for patients with no AKI versus some degree of AKI. There was no significant difference in characteristics between groups, however a greater proportion of patients from the AKI group had craniectomies performed. As expected, mean and peak serum creatinine were higher, and AKI-free days were fewer in the AKI group. When viewing the combined results we should keep in mind the effect of the two treatments in the two trials that could have influenced biomarker concentration creating background 'noise'.

Table 51 Combined EPO/POLAR-Biomarkers: Patient characteristics and renal data compared between Acute Kidney Injury groupings

Variable	GFR↓≤ 25% (n=26)	GFR↓ > 25% (n=54)	p-value
Age	34 ± 13	33 ± 13	0.73
Male %	80.8% (21)	81.5% (44)	0.94
INR closest to randomisation	1.2 ± 0.1	1.2 ± 0.2	0.56
APTT closest to randomisation	31 ± 4.7	31 ± 4.9	0.82
Platelets closest to randomisation	181 ± 36.7	202 ± 58.8	0.10
Systolic BP	138 ± 25.7	147 ± 34.2	0.22
Respiratory Rate	19.7 ± 9.3	19.2 ± 7.4	0.82
Weight	76 (70-82)	80 (78-85)	0.07
Pupils equal & reactive %	73.1% (19)	83.3% (45)	0.28
Both pupils non reactive %	3.8% (1)	5.6% (3)	1.00
Pre-intubation GCS	6.6 ± 2.7	6 ± 2.7	0.36
Motor Score from pre-intubation GCS	5 (1-5)	4 (1-5)	0.06
Mass lesion evacuation performed %	26.9% (7)	29.6% (16)	0.80
Craniectomy performed	7.7% (2)	20.4% (11)	0.15
Bifrontal decompressive craniectomy %	3.8% (1)	9.3% (5)	0.39
Injury severity score	29.8 ± 9.8	30.9 ± 8.6	0.63
Earliest post trauma creatinine	65.4 ± 13.4	73.5 ± 17.4	0.04
Mean serum creatinine: days 1 to 7	57.9 (51.7-66.7)	62.8 (57.6-72.3)	0.04
Peak serum creatinine: days 1 to 7	66.6 ± 12.4	80.5 ± 20.9	0.002
AKI-free days (pts with GFR↓ > 25%)	7 (7-7)	5 (4-6)	<0.001
AKI-free days (pts with GFR↓ > 50%)	7 (7-7)	6 (6-7)	<0.001

GFR↓=decrease in glomerular filtration rate, AKI=GFR↓ > 25%, values are reported as mean ± standard deviation, % (n) or median (interquartile range), INR=International normalized ratio: measure of the extrinsic pathway of coagulation, APTT=activated partial thromboplastin time, Systolic BP=first systolic blood pressure on hospital admission, Respiratory Rate=last unassisted respiratory rate, GCS=Glasgow Coma Score.

The proportion of patients in each AKI category is displayed in Table 52. Classification of AKI by urine output occurred in 91% (49/54) of patients. The number of patients classified by RIFLE urine output or GFR criteria for the EPO-TBI and POLAR trials can be found in sections 4.3.1 and 4.3.2.

Table 52 Patients in RIFLE GFR groupings

RIFLE category		n (%)
No AKI:	**GFR↓ ≤ 25%**	26 (32.5%)
Risk:	**GFR↓ > 25% to 50%**	26 (32.5%)
Injury:	**GFR↓ > 50% to 75%**	28 (35%)
Failure:	**GFR↓ > 75%**	0

GFR↓=decrease in GFR

The biomarker concentrations were compared between AKI groupings for the 24, 48 and 72 hour timepoints in Table 53 and are presented in graphs in Figure 16. The biomarker concentration refers to the geometric mean concentration. The strongest predictors for AKI at 48 hours were plasma CyC (p= 0.008), and urine NGAL (p=0.04) (Table 53).
The strongest predictor for AKI at 72 hours was IL-18 (p= 0.04, Table 53).

Table 53 **Combined EPO/POLAR-Biomarkers: concentration of biomarkers at 24, 48 and 72 hours after dose/injury compared between AKI groupings in descending order of AUC value**

24 HOURS				
Biomarker	**No AKI (n=26)**	**AKI (n=54)**	**p-value**	**AUC**
pNGAL	56 (50-104)	82 (50-118)	0.10	0.62
pCyC	0.57 (0.51-0.62)	0.61 (0.54-0.67)	0.17	0.62
uIL-18	54 (39-89)	83 (37-120)	0.11	0.60
uKIM-1	2464 (1255-3126)	2770 (1561-5404)	0.24	0.59
uNGAL	15 (4-39)	23 (9-52)	0.16	0.59
uCyC	0.17 (0.08-0.23)	0.15 (0.10-0.30)	0.57	0.54
pCath	15 (13-17)	14 (12-18)	0.73	0.51
uAlpha-GST	1 (0.3-2.8)	0.75 (0.3-3.3)	0.77	0.49
uPi-GST	3.5 (1.8-6.8)	4 (1.8-6.5)	0.83	0.47
48 HOURS				
Biomarker	**No AKI (n=26)**	**AKI (n=54)**	**p-value**	**AUC**
pCyC	0.59 (0.51-0.66)	0.66 (0.56-0.74)	0.008	0.70
uNGAL	19 (8-28)	25 (17-62)	0.04	0.65
pNGAL	50 (50-90)	91 (50-123)	0.07	0.63
uKIM-1	1823(1001-3084)	2553 (1406-4351)	0.10	0.62
uIL-18	60 (29-89)	76 (38-113)	0.23	0.59
uPi-GST	4.0 (1.8-9.0)	3.6 (1.8-10.5)	0.96	0.51
uAlpha-GST	1.0 (0.3-3.3)	0.8 (0.3-2.5)	0.62	0.55
uCyC	0.14 (0.06-0.25)	0.17 (0.09-0.31)	0.57	0.54
pCath	14 (11-16)	14 (12-17)	0.41	0.54
72 HOURS				
Biomarker	**No AKI (n=26)**	**AKI (n=54)**	**p-value**	**AUC**
uIL-18	34 (24-69)	61 (33-121)	0.04	0.65
uCyC	0.16 (0.06-0.23)	0.22 (0.10-0.38)	0.11	0.62
pNGAL	58 (50-96)	87 (50-135)	0.11	0.62
pCyC	0.63 (0.59-0.69)	0.65 (0.60-0.75)	0.34	0.60
uKIM-1	1140 (458-1934)	1579 (671-2902)	0.19	0.60
uNGAL	18 (9-29)	24 (11-81)	0.20	0.60
pCath	13 (11-19)	14 (11-18)	0.61	0.53
uPi-GST	5.4 (1.8-12.0)	5.3 (2.0-13.0)	0.68	0.53
uAlpha-GST	2.8 (0.3-5.3)	1.6 (0.3-5.3)	0.68	0.47

AKI=Patients with a decrease in GFR > 25% (section 3.6.8), values are reported as median (IQR). p=plasma, u=urine, NGAL=neutrophil gelatinase-associated lipocalin (ng/mL), CyC=cystatin C (mg/L), IL-18=interleukin 18 (pg/mL), KIM-1=Kidney Injury Molecule-1 (pg/mL), Cath S=Cathepsin S (mcg/mL), Alpha-GST=Alpha-glutathione S-transferase (mcg/L)

In Figure 16 the graphs of biomarker concentration over the first 4 days after injury are plotted for the AKI versus No AKI groups (Risk and Injury RIFLE GFR categories for AKI are separated in the graphs). For urine KIM-1 and NGAL the categories for AKI display significant difference between groups over time (p=0.01 and 0.05 respectively).

Baseline to 24 hours: For patients classified with kidney injury all urine biomarkers (except urine Pi-GST) show a rise in concentration from baseline to 24 hours while the graph for those with no AKI remains relatively flat.

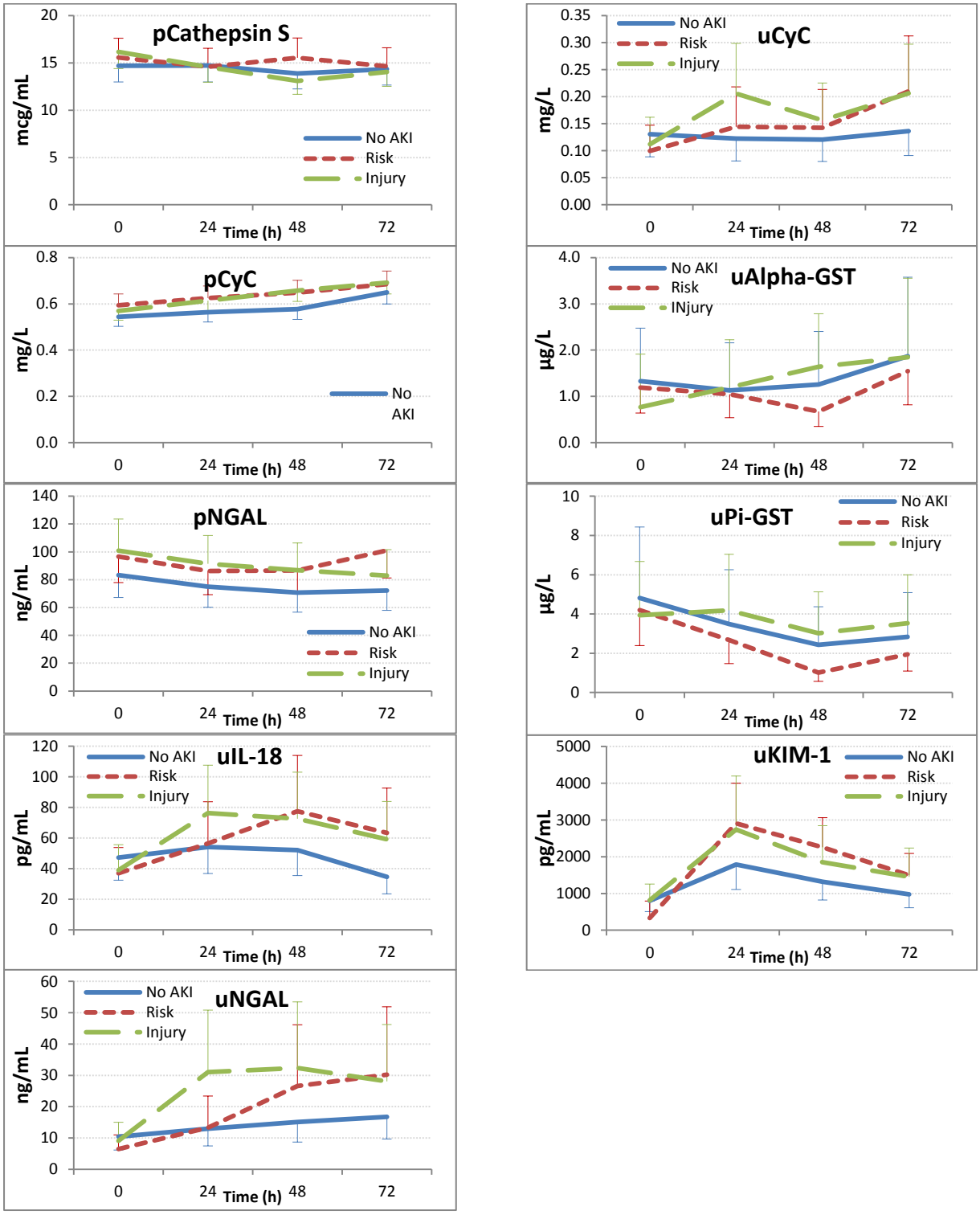
Plasma biomarkers: Plasma NGAL concentration decreases over the 72 hours, with lower concentrations in those with no AKI. Plasma CyC rises slowly and consistently throughout the 72 hour period for all groups with lower concentrations for those with no AKI.

Peaks: Graphs for those classified with kidney injury for all urine biomarkers (apart from the GST markers) peak at 24 hours. Urine IL-18 and NGAL concentrations remain raised for these patients at 48 hours on the graphs. For patients in the Risk category, urine IL-18 and NGAL concentrations rise more slowly to peak at 48 hours.

Plasma versus urine: The graphs for plasma and urine biomarkers display different patterns over time. Graphs for plasma biomarkers are 'flatter'.

AKI versus no-AKI: In graphs for patients with no AKI the concentration of each biomarker is lower than the concentration for those with AKI at 24, 48 and 72 hours (with the exception of Cathepsin S and the GST biomarkers).

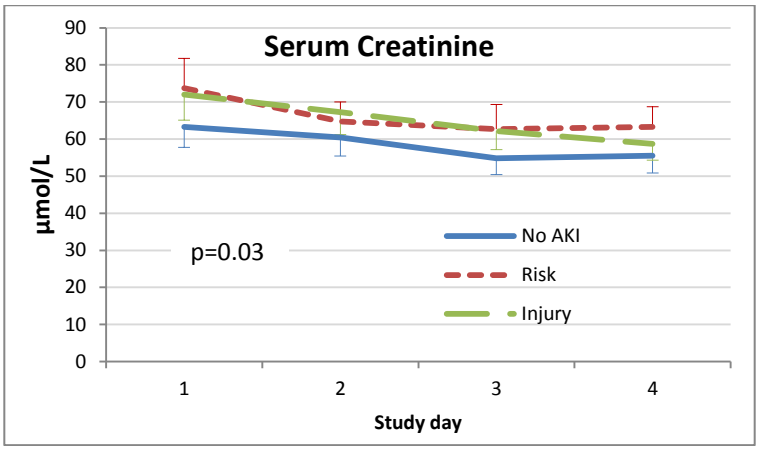
Figure 16 **Combined EPO/POLAR-Biomarkers: Graphs of biomarker concentrations at 0, 24, 48 and 72 hours from baseline for AKI groupings**



p=plasma, u=urine, CyC=cystatin C, NGAL=neutrophil gelatinase-associated lipocalin, Cr=creatinine, GST=Glutathione S-transferase, IL-18=interleukin 18, KIM-1=Kidney Injury Molecule-1

The mean serum creatinine concentration for the first 4 study days for the combined EPO-TBI and POLAR trial patients is displayed in Figure 17. Although the difference in mean value between those with and without AKI would not be considered clinically significant, the serum creatinine in those with no AKI is statistically significantly lower than those with any degree of AKI (p=0.03) despite the fact that 91% of patients were classified by urine output not serum creatinine.

Figure 17 Combined EPO/POLAR data: Graph of mean serum creatinine concentration for days 1 to 4 for AKI groupings



The p-value is for the difference between patients with any degree of AKI versus No AKI

4.4.4 EPO-Biomarkers: Is the release of renal biomarkers reduced / changed by EPO versus placebo in TBI patients?

Biomarker specimens were collected on all (but one) of the first 30 patients recruited to the EPO-TBI trial between the Royal Melbourne and Alfred Hospitals. Permission to take specimens was refused for one patient in the trial. Baseline characteristics of the two biomarker study groups were similar considering the small sample size (Table 54). The mean cerebral perfusion pressure (CPP) index was higher in the group receiving EPO. The number of AKI-free days in ICU in patients classified with any degree of AKI (RIFLE GFR criteria) was greater in the group receiving placebo, however, there was no significant difference in mean or peak serum creatinine (day 1-7) between groups.

Table 54 Baseline, operative and renal patient characteristics compared between treatment groups

Characteristics	EPO (n=15)	Placebo (n=14)	p-value
Age	25.6 (22.6-45.9)	26.5 (22.4-53.1)	0.50
Male %	67% (10)	67% (10)	1.00
APACHE II Score	22.1 ± 5.7	21.6 ± 6.7	0.82
Haemoglobin at randomisation	110 ± 19	105 ± 14	0.41
INR closest to randomisation	1.2 ± 0.1	1.2 ± 0.1	0.17
APTT closest to randomisation	31 ± 4	30 ± 4	0.63
Platelets closest to randomisation	182 ± 55	187 ± 51	0.82
Systolic BP	150 ± 37	137 ± 35	0.33
Respiratory Rate	21.8 ± 6.1	19.8 ± 3.8	0.37
Lowest Systolic BP pre-randomisation	93 ± 16	93 ± 17	0.94
Lowest Sao2	93 ± 16	93 ± 17	0.94
Weight	75 ± 18	81 ± 9	0.28
Pupils equal & reactive %	100% (15)	93% (14)	0.31
Pre-intubation GCS	8.3 ± 2.6	7.1 ± 3.7	0.31
Motor Score from pre-intubation GCS*	4 (3-5)	4 (1-5)	0.51
Sustained Severe Traumatic Brain Injury	53% (8)	60% (9)	0.71
Total vol. hypertonic saline, day 1-7 (mL)	757 ± 1456	231 ± 377	0.23
Total volume of Mannitol, day 1-7 (mL)	3.3 ± 12.9	13.3 ± 51.6	0.47

Characteristics	EPO (n=15)	Placebo (n=14)	p-value
Total volume of RBC, day 1-7	233 ± 318	541 ± 676	0.12
Median CPP index, day 1-7	6.7 (4.2-16.7)	0 (0-5.9)	0.04
Median ICH index, day 1-7	4.2 (0-8.3)	0 (0-12.5)	0.62
Mass lesion evacuation performed %	40% (6)	27% (4)	0.44
Craniectomy performed %	27% (4)	13% (2)	0.65
Bifrontal decompressive craniectomy %	7% (1)	13% (2)	1.00
Injury severity score	32 ± 10	33 ± 14	0.72
Number of study doses*	2 (2-3)	1 (1-2)	0.02
Earliest post trauma creatinine	70 ± 11	63 ± 14	0.14
Mean Serum Creatinine: days 1 to 7*	61 ± 10	58 ± 10	0.41
Peak serum creatinine: days 1 to 7	71 ± 14	66 ± 13	0.26
AKI-free days (pts with GFR↓ > 25%)	5 (4-6)	6 (6-7)	0.04
AKI-free days (pts with GFR↓ > 50%)	7 (6-7)	7 (6-7)	0.91

All values are reported as mean ± standard deviation, median (interquartile range) or % (n). Systolic BP=first systolic blood pressure on hospital admission, Respiratory Rate=last unassisted respiratory rate, Lowest SaO2=lowest oxygen saturation of arterial blood pre-randomisation, GCS=Glasgow Coma Score; vol.=volume, CPP index=cerebral perfusion pressure index: the number of observations of cerebral perfusion pressure of less than 60mmHg divided by the total number of measurements, multiplied by 100; ICH index=Intracranial Hypertension Index: the number of end-hourly measures of intracranial pressure of more than 20mmHg divided by the total number of measurements, multiplied by 100.

The concentration of each biomarker at baseline, then 24, 48 and 72 hours after first dose was calculated and reported as geometric means. In Figure 18 the graphs of biomarker concentration over time are plotted for the EPO versus placebo groups.

Baseline to 24 hours: For all urine biomarkers from pre-dose (0 hours) to 24 hours the concentration rises in patients receiving EPO and decreases or remains 'flat' in patients receiving placebo and the concentration of all urine biomarkers at 24 hours is greater in EPO versus placebo patients.

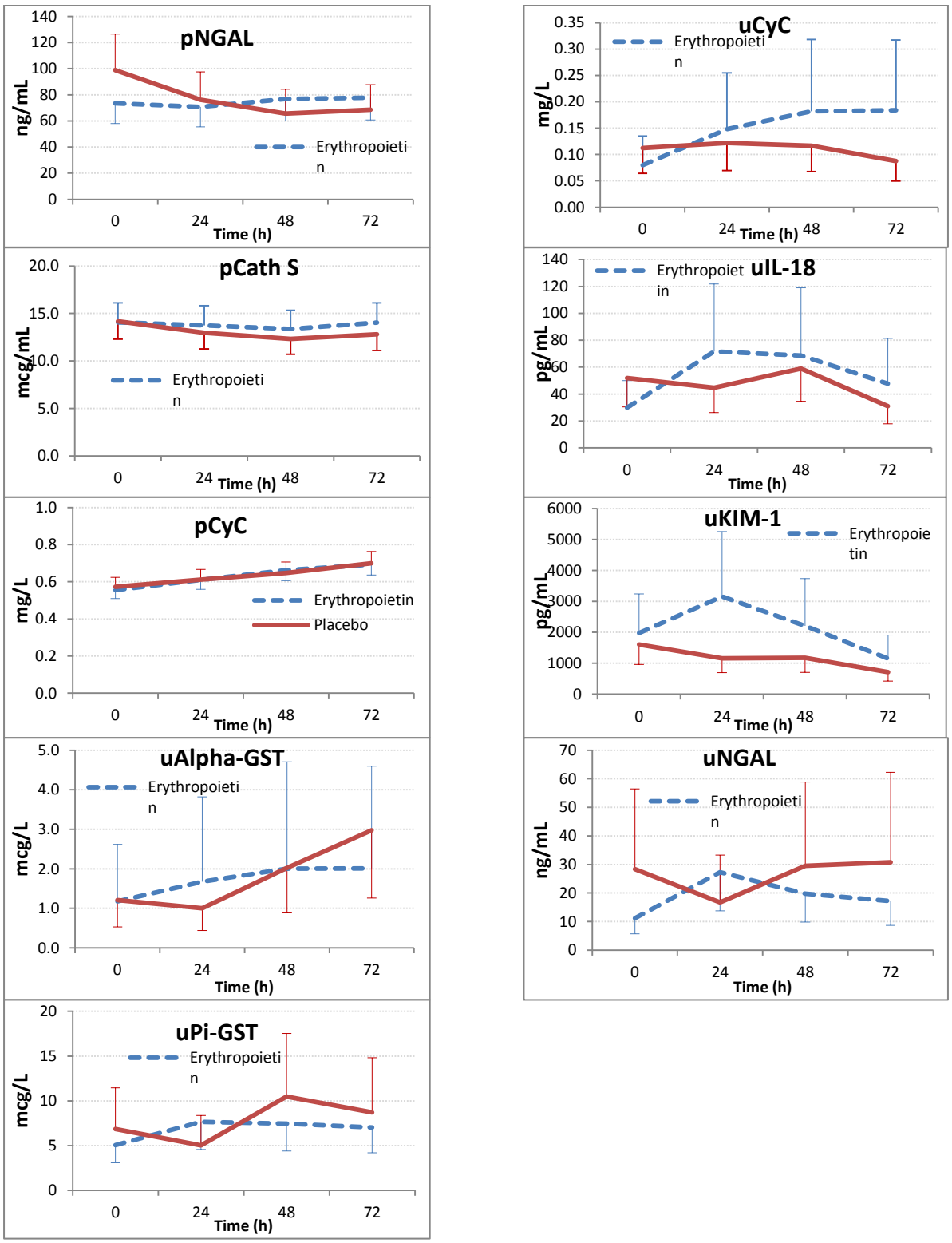
Plasma biomarkers: For plasma NGAL, the concentration in EPO patients remains virtually unchanged over time while the concentration in placebo patients decreases over time (p=0.01). Plasma CyC behaves differently to the other biomarkers in that the EPO and placebo graphs are inseparable and rise gradually over the 72 hour period.

From 24 to 48 hours: All urine biomarker graphs for patients receiving placebo rise or plateau in this period.

Urine biomarkers: For the urine biomarkers; IL-18, KIM-1 and NGAL, EPO graphs peak at 24 hours (while the placebo graphs dip). The peak in the EPO graph for urine IL-18 remains raised up to 48 hours. For urine NGAL there is a significant difference in concentration between groups over time ($p=0.03$) and there is a significant difference in urine KIM-1 concentration between groups ($p=0.04$).

Plasma versus urine: The graphs for plasma and urine NGAL and CyC display different patterns over time.

Figure 18 **Graphs of biomarker concentrations at 0, 24, 48 and 72 hours from study dose for the EPO versus Placebo groups**



pCyC=plasma cystatin C, pNGAL=plasma neutrophil gelatinase-associated lipocalin, uAlpha-GST=urine Alpha-Glutathione S-transferase, uPi-GST=urine Pi-GST, uIL-18=urine interleukin 18, uKIM-1=urine Kidney Injury Molecule-1, uNGAL=urine NGAL

The concentration of each biomarker at each timepoint was calculated and is compared in Table 55 for the 24, 48 and 72 hour timepoints respectively. The change in concentration of each biomarker from baseline to 24 hours was also compared between EPO and placebo groups (Table 56). At 24 hours the concentration of KIM-1 was significantly higher in the EPO versus placebo group (0.01, Table 55). At 72 hours the concentration of urine CyC was higher in the EPO versus placebo group (p= 0.06, Table 55).

Table 55 Comparison of biomarker concentration between groups at 24, 48 and 72 hours after injury in ascending order of p-value

24 HOURS			
Biomarker	**EPO (n=15)**	**Placebo (n=14)**	**p-value**
uKIM-1	2974 (1679-6045)	1491 (730-2566)	0.01
uPi-GST	5.1 (4.8-11.0)	3.6 (3.0-14.5)	0.19
uNGAL	23 (10-63)	14 (5-93)	0.35
uIL-18	89 (43-112)	61 (14-91)	0.38
uAlpha-GST	1.3 (0.8-3.8)	1.3 (0.3-2.8)	0.41
uCyC	0.14 (0.07-0.29)	0.12 (0.08-0.23)	0.66
pCath	14.5 (11.9-15.1)	13.6 (11.1-14.8)	0.77
pCyC	0.63 (0.54-0.67)	0.60 (0.57-0.65)	0.90
pNGAL	65 (50-94)	66 (50-110)	0.94
48 HOURS			
Biomarker	**EPO (n=15)**	**Placebo (n=14)**	**p-value**
pNGAL	72 (62-103)	50 (50-71)	0.11
uKIM-1	2482 (1513-3131)	1318 (791-2519)	0.13
uCyC	0.20 (0.10-0.31)	0.13 (0.09-0.21)	0.30
uPi-GST	9 (3-13)	9 (4-32)	0.48
pCath	12.7 (11.5-13.9)	13.3 (10.0-15.4)	0.61
pCyC	0.68 (0.60-0.72)	0.60 (0.57-0.76)	0.77
uNGAL	32 (7-40)	20 (15-121)	0.98
uIL-18	63 (56-78)	78 (30-92)	0.99
uAlpha-GST	2.0 (0.3-4.3)	1.8 (0.3-6.0)	1.00
72 HOURS			
Biomarker	**EPO (n=15)**	**Placebo (n=14)**	**p-value**
uCyC	0.16 (0.10-0.29)	0.06 (0.03-0.28)	0.06
pNGAL	92 (50-113)	50 (50-100)	0.29
uIL-18	47 (30-94)	32 (14-79)	0.36
uPi-GST	6.8 (4.5-13.5)	9.8 (6.8-12.8)	0.38
uNGAL	18 (9-55)	25 (13-43)	0.45
pCath	13 (11-16)	13 (11-16)	0.52
uKIM-1	1228 (656-1627)	1133 (414-1845)	0.53
uAlpha-GST	2.6 (0.3-5.3)	3.3 (0.8-8.8)	0.53
pCyC	0.69 (0.64-0.74)	0.67 (0.59-0.79)	0.67

Values are reported as median (IQR). p=plasma, u=urine, NGAL=neutrophil gelatinase-associated lipocalin (ng/mL), CyC=cystatin C (mg/L), IL-18=interleukin 18 (pg/mL), KIM-1=Kidney Injury Molecule-1 (pg/mL), Cath S=Cathepsin S (mcg/mL), Alpha-GST=Alpha-glutathione S-transferase (mcg/L)

The change in concentration of urine NGAL from baseline to 24 hours approached significance between EPO and placebo groups (p= 0.07, Table 56). Its concentration

increased in the EPO group and decreased in the placebo group from baseline to 24 hours.

Table 56 Comparison of the change in biomarker concentration between groups from baseline to 24 hours after first study dose

Biomarker	EPO (n=15)	Placebo (n=14)	p-value
uNGAL	12 (2-35)	-8 (-24-19)	0.07
uIL-18	36 (-5-70)	12 (-28-30)	0.10
uKIM-1	922 (-288-3251)	-198 (-18438-621)	0.12
pNGAL	-4 (-32-14)	-27 (-47-0)	0.23
uPi-GST	0.4 (-1.8-3.8)	-2.3 (-7.8-4.8)	0.28
uAlpha-GST	0 (-2.3-1.0)	0 (-0.8-0.5)	0.31
uCyC	0.04 (-0.02-0.15)	-0.01 (-0.06-0.13)	0.43
pCyC	0.05 (0.01-0.11)	0.04 (-0.01-0.07)	0.52
pCath	-0.3 (-1.0-0.3)	-0.36 (-4.03-0.42)	0.64

Values are reported as median (interquartile range). p=plasma, u=urine, NGAL=neutrophil gelatinase-associated lipocalin (ng/mL), CyC=cystatin C (mg/L), IL-18=interleukin 18 (pg/mL), KIM-1=Kidney Injury Molecule-1 (pg/mL), Cath S=Cathepsin S (mcg/mL), Alpha-GST=Alpha-glutathione S-transferase (mcg/L)

4.4.5 POLAR-Biomarkers: Is the release of renal biomarkers reduced/changed by early, sustained hypothermia versus normothermia in TBI patients?

Biomarker specimens were collected on the first 51 eligible patients recruited to the POLAR trial between the Royal Melbourne and Alfred Hospitals. Baseline characteristics of the two biomarker study groups were similar (Table 57) with a few exceptions. Patients treated with hypothermia had a higher platelet count close to randomisation, a lower pre-intubation GCS motor score, and more of them were hypotensive compared to the normothermic control group.

Table 57 Baseline, operative and renal characteristics of POLAR-Biomarkers patients

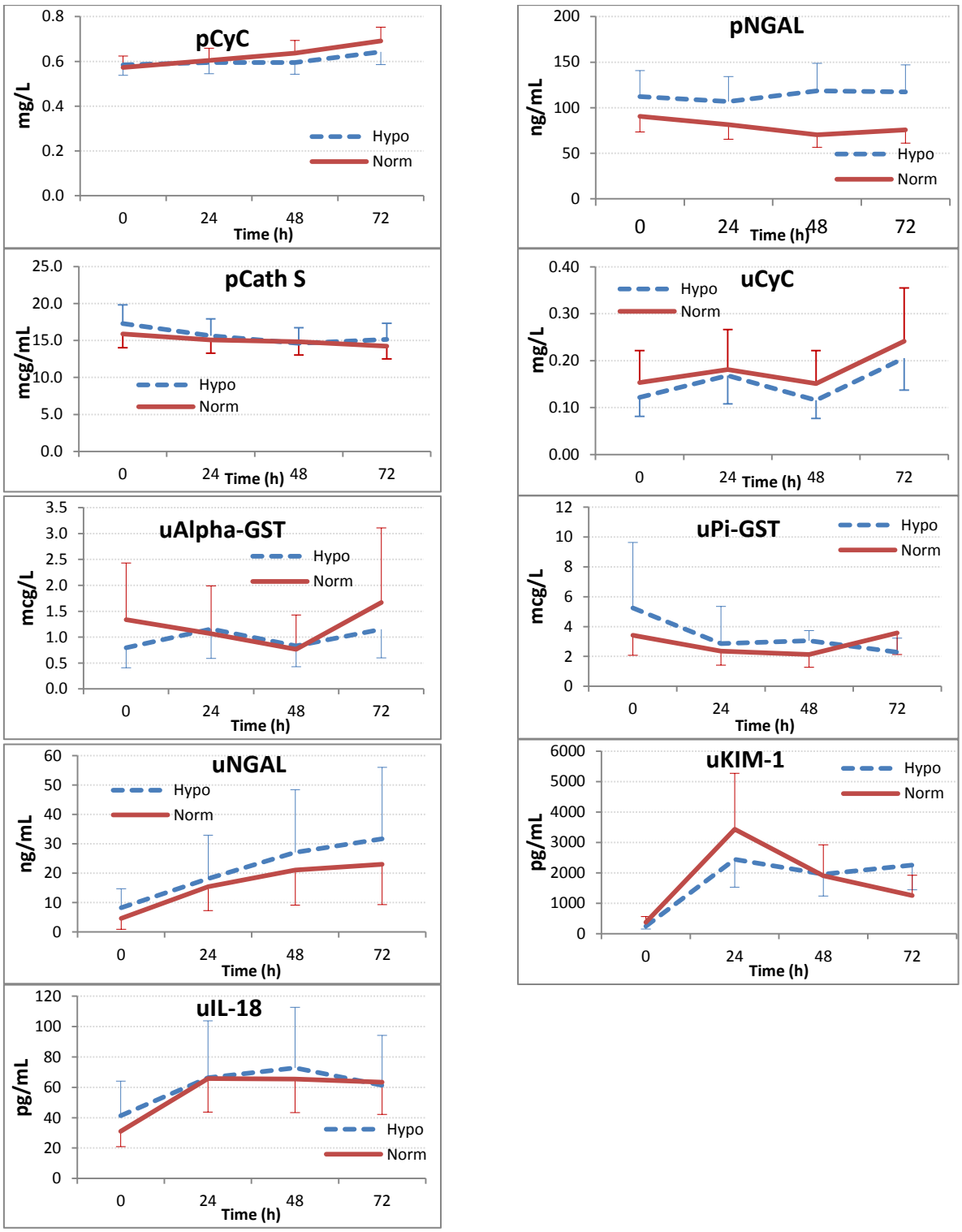
Characteristics	Hypothermia (n=24)	Normothermia(n=27)	p-value
Age	26 (21-41)	35 (26-44)	0.09
Male %	88% (21)	93% (25)	0.54
Randomised pre-hospital %	50% (12)	52% (14)	0.89
Weight	83 ± 17	84 ± 14	0.99
Pre-intubation GCS	4.7 ± 2.3	5.7 ± 1.9	0.09
Motor Score from pre-intubation GCS	1 (1-4)	4 (1-5)	0.05
Hypotensive pre-hospital %	17% (4)	0% (0)	0.05
Hypoxic pre-hospital %	17% (4)	8% (2)	0.41
Fluid volume given pre-hospital	2242 ± 10662	2019 ± 1318	0.51
First Systolic BP in ED	145 ± 32	144 ± 26	0.95
Pupils equal & reactive in ED %	75% (18)	67% (18)	0.51
Both pupils Non-reactive in ED %	13% (3)	4% (1)	0.33
WCC closest to randomisation	17.9 ± 8.7	14.1 ± 5.8	0.07
INR closest to randomisation	1.1 ± 0.5	1.2 ± 0.2	0.43
APTT closest to randomisation	27 ± 12	29 ± 10	0.44
Platelets closest to randomisation	224 ± 54	181 ± 44	0.003
Haemoglobin closest to randomisation	128 ± 21	124 ± 28	0.50
Glucose closest to randomization	7.8 ± 4.3	8.4 ± 5.2	0.66
Mean MAP for 12h post-randomisation	89 ± 10	84 ± 10	0.13
Mean HR for 12h post-randomisation	80 ± 17	88 ± 16	0.11
Mass lesion evacuation performed %	17% (4)	37% (10)	0.10
Craniectomy performed %	13% (3)	19% (5)	0.56
Bifrontal decompressive craniectomy %	0% (0)	11% (3)	0.24
ICP Monitor inserted %	83% (20)	93% (25)	0.31
Mean temperature in first 24h	35 ± 2	37 ± 1	<0.0001
Mean temperature in first 72h	34± 2	37 ± 1	<0.0001
Injury severity score	25 ± 10	30 ± 10	0.07
Median ICH index (day 1-7)	5.4 (1.9-12.1)	2.2 (0-9.7)	0.22
Median CPP index (day 1-7)	8.3 (4.5-12.8)	7.0 (1.5-11.4)	0.46

No. days Noradrenaline > 15mcg/min	0 (0-3)	1.5 (0-2)	0.47
No. days Noradrenaline given	5 (1-6)	3 (1-6)	0.48
Volume hypertonic saline, day 1-7	100 (0-385)	20 (0-313)	0.65
Total Thiopentone given (grams)	3.3 ± 7.2	1.4 ± 5.4	0.29
Volume of Albumin, day 1-7 (mL)	125 ± 368	37 ± 133	0.25
Volume of Colloids, day 1-7 (mL)	91 ± 447	107 ± 309	0.88
Volume of Crystalloids, day 1-7 (mL)	15985 ± 5585	14803 ± 6007	0.47
Volume of FFP, day 1-7 (mL)	175 ± 544	329 ± 499	0.30
Fluid Input, day 1-7 (mL)	23694 ± 8841	23404 ± 9366	0.91
Fluid Output, day 1-7 (mL)	18444 ± 7736	17918 ± 7467	0.81
Fluid balance, day 1-7 (mL)	5250 ± 4437	5487 ± 4885	0.86
Highest APTT	38 ± 8	36 ± 5	0.32
Highest INR	1.4 ± 0.2	1.4 ± 0.2	0.70
Highest Potassium	4.8 ± 0.4	4.6 ± 0.4	0.09
Lowest Potassium	3.3 ± 0.4	3.3 ± 0.3	0.90
Highest White Cell Count	19.8 ± 8.8	16.8 ± 6.6	0.17
Lowest White Cell Count	10.1 ± 6.6	9.5 ± 6.0	0.70
Lowest Platelets	143 (113-188)	117 (102-131)	0.03
Earliest post trauma creatinine	78 ± 18	71 ± 19	0.24
Average Serum Creatinine: days 1 to 7	68 ± 15	66 ± 15	0.68
Peak serum creatinine: days 1 to 7	86 ± 24	77 ± 19	0.16
AKI-free days (pts with GFR↓ > 25%)	5.5 (4-6)	6 (5-7)	0.12
AKI-free days (pts with GFR↓ > 50%)	7 (6-7)	7 (6-7)	0.53

Values are reported as mean ± standard deviation, median (interquartile range), or % (n). GCS=Glasgow Coma Score, BP= blood pressure, ED=Emergency department, WCC=White cell count, INR=International normalized ratio: measure of the extrinsic pathway of coagulation, APTT= activated partial thromboplastin time, MAP=mean arterial blood pressure, HR=heart rate, ICP=intracranial pressure; ISS=Injury severity score: derived from scores from the Abbreviated Injury Scale; ICH index=Intracranial Hypertension Index: the number of end-hourly measures of intracranial pressure of more than 20mmHg divided by the total number of measurements, multiplied by 100; CPP index=cerebral perfusion pressure index: the number of observations of cerebral perfusion pressure of less than 60mmHg divided by the total number of measurements, multiplied by 100; No.=number, GFR↓=decrease in GFR.

The concentration of each biomarker at baseline, then 24, 48 and 72 hours after injury was calculated and reported as geometric means. In Figure 19 the graphs of biomarker concentration over time are plotted for the hypothermia versus normothermia groups. In the plasma NGAL graph patients receiving hypothermia have a clearly differentiated higher concentration of plasma NGAL compared to the normothermic controls (p=0.007). The difference between groups is most pronounced at 48 hours after injury. There is a significant difference in urine KIM-1 concentration between groups over time (p=0.03). The peak for urine KIM-1 is clearly at 24 hours. IL-18 remains raised up to 48 hours. There is a steady rise in urine NGAL and plasma CyC concentration over the 72 hours post injury for both groups.

Figure 19 **Graphs of biomarker concentrations at 0, 24, 48 and 72 hours from injury for the Hypothermia versus Normothermia groups**



p= plasma, u=urine, CyC=cystatin C, NGAL= neutrophil gelatinase-associated lipocalin, Alpha-GST= Alpha-Glutathione S-transferase, Pi-GST= Pi-GST, IL-18= interleukin-18, KIM-1=Kidney Injury Molecule-1

The concentration of each biomarker at each timepoint was calculated and is compared in Table 58 for the 24, 48 and 72 hour timepoints. The change in concentration of each biomarker from baseline to 24 hours was also compared between cooled and normothermic groups (Table 59). At 48 hours after injury the concentration of plasma NGAL was significantly higher in the cooled versus normothermic group ($p= 0.001$, Table 58). At 72 hours after injury the concentration of plasma NGAL and urine KIM-1 were significantly higher in the cooled versus normothermic group ($p= 0.02$ and 0.03 respectively, Table 58).

Table 58 Comparison of biomarker concentration between groups at 24, 48 and 72 hours after injury in ascending order of p-value

24 HOURS			
Biomarker	**Hypothermia (n=24)**	**Normothermia (n=27)**	**p-value**
pNGAL	87 (71-174)	86 (50-119)	0.11
uKIM-1	2537 (1326-4454)	2974 (2359-5735)	0.13
pCath	16 (13-20)	16 (12-19)	0.45
pCyC	0.60 (0.53-0.64)	0.56 (0.53-0.69)	0.50
uCyC	0.16 (0.08-0.33)	0.18 (0.14-0.30)	0.63
uIL-18	76(30-137)	65 (36-117)	0.69
uPi-GST	2.5 (1.6-5.4)	2.5 (1.3-5.9)	0.69
uNGAL	15 (6-41)	16 (7-32)	0.77
uAlpha-GST	0.3 (0.3-3.4)	0.6 (0.3-2.8)	0.98
48 HOURS			
Biomarker	**Hypothermia (n=24)**	**Normothermia (n=27)**	**p-value**
pNGAL	130 (63-190)	67 (50-95)	0.001
uKIM-1	3084 (1608-3941)	1917 (1331-3327)	0.17
pCyC	0.59 (0.52-0.7)	0.62 (0.54-0.71)	0.26
uCyC	0.14 (0.03-0.24)	0.21 (0.09-0.27)	0.33
uNGAL	25 (12-78)	22 (12-42)	0.40
uIL-18	82 (41-121)	64 (33-96)	0.55
uAlpha-GST	0.3 (0.3-2.0)	0.6 (0.3-1.6)	0.64
uPi-GST	2.8 (1.8-5.8)	1.8 (1.4-3.9	0.11
pCath	14.8 (11.5-17.0)	15.6 (13.1-17.4)	0.90
72 HOURS			
Biomarker	**Hypothermia (n=24)**	**Normothermia (n=27)**	**p-value**
pNGAL	127 (50-179)	65 (50-82)	0.02
uKIM-1	2183 (1174-5223)	1206 (772-2237)	0.03
pCyC	0.62 (0.56-0.71)	0.64 (0.60-0.73)	0.24
uAlpha-GST	1.0 (0.3-2.3)	2.1 (0.3-4.9)	0.33
uPi-GST	2.55 (0.5-6)	5.15 (1.65-7.3)	0.26
uNGAL	30.61 (14.16-110.53)	17.97 (10.12-62.55)	0.36
uCyC	0.17 (0.13-0.35)	0.23 (0.15-0.36)	0.46
pCath	14.9 (12.1-19.0)	14.3 (11.9-18.5)	0.58
uIL-18	54 (37-117)	76 (29-135)	1.00

Values are reported as median (interquartile range). p=plasma, u=urine, NGAL=neutrophil gelatinase-associated lipocalin (ng/mL), CyC=cystatin C (mg/L), IL-18=interleukin 18 (pg/mL), KIM-1=Kidney Injury Molecule-1 (pg/mL), Cath S=Cathepsin S (mcg/mL), Alpha-GST=Alpha-glutathione S-transferase (mcg/L)

There was no significant difference in change in concentration in any of the biomarkers from baseline to 24 hours between cooled and normothermic groups.

Table 59 Comparison of the change in biomarker concentration between groups from baseline to 24 hours after injury

Biomarker	Hypothermia (n=24)	Normothermia (n=27)	p-value
uCyC	0.06 (0.01-0.11)	0.01 (-0.03-0.10)	0.22
pCath	-1.6 (-3.6-0.1)	-1.6 (-2.8-0.6)	0.37
uAlpha-GST	0 (-2.2-0.3)	0 (-2.3-1.5)	0.39
uKIM-1	2491 (1191-3613)	2389 (1317-4854)	0.50
pCyC	0.02 (-0.05-0.07)	0.03 (-0.03-0.08)	0.58
uPi-GST	-1 (-7-1)	-1 (-5-3)	0.60
uNGAL	5 (-7-25)	7 (1-23)	0.90
pNGAL	1 (-24-19)	-11 (-37-12)	0.91
uIL-18	17(1-101)	19 (-2-76)	0.95

Values are reported as median (interquartile range). p=plasma, u=urine, NGAL=neutrophil gelatinase-associated lipocalin (ng/mL), CyC=cystatin C (mg/L), IL-18=interleukin 18 (pg/mL), KIM-1=Kidney Injury Molecule-1 (pg/mL), Cath S=Cathepsin S (mcg/mL), Alpha-GST=Alpha-glutathione S-transferase (mcg/L)

5 Discussion

5.1 Overview of the Discussion

We have established that AKI, the consensus term that refers to a continuum of all classes of kidney injury, is relatively common in critically ill patients and has a significant impact on patient outcomes even in milder degrees of AKI (section 1.2). The late detection of AKI as a result of the use of conventional methods of classification (creatinine and urine output) has hindered renal research. Studies of potential treatments for AKI have been largely unsuccessful, which may be due in part to patients being classified with AKI too late for therapies to be effective. As a result, the investigation of alternative early renal biomarkers and treatments for AKI has become a priority in renal research.

Erythropoietin and hypothermia had been identified as two potential therapies for AKI and the conduction of the EPO-TBI and POLAR trials investigating these therapies for patients with TBI presented a unique opportunity to concurrently assess their effect on the kidneys in patients with TBI. Furthermore, it was an opportunity to evaluate several novel renal biomarkers in detecting AKI and in their response to therapy. In previous chapters we have examined in detail the mechanisms of action of the two treatments and the plausibility of and evidence surrounding their prophylactic use for brain and kidney injury. The biomarkers under study have been explored and described and the characteristics of patients with traumatic brain injury have also been visited, allowing us to bring together all these elements in the analysis and interpretation of the study results.

In the following discussion, key findings of the Renal substudies of the EPO-TBI and POLAR trials are first presented, then examined in the light of existing evidence to identify the implications of these findings. The strengths and limitations of the Renal substudies are identified and discussed, then future research that may result from this investigation is outlined. Finally we present the conclusions we have arrived at after navigating through this process.

5.2 Key Results

5.2.1 AKI

Pooled EPO/POLAR-AKI: The occurrence of AKI was greater than expected (50.3%) in the pooled traumatic brain injury (TBI) cohort compared to previous studies[94,138] and most patients (91%) were classified by urine output alone.

EPO-AKI: There is a suggestion of a negative effect of EPO on the kidneys; serum creatinine concentration was higher in patients receiving EPO versus placebo albeit not clinically significant, and more EPO patients developed AKI than placebo patients although the difference was not statistically significant. The suggestion of a negative effect of EPO is supported by an increase in the concentration of all urine renal biomarkers for patients in the EPO group while their concentration decreased or remained static in the placebo group.

In the EPO-AKI analysis a higher creatinine was associated with a shorter clotting time in linear modelling with log creatinine.

POLAR–AKI: More cooled patients developed AKI than normothermic controls (80% v. 51%, p=0.012) and they had fewer AKI-free days than control pts (p=0.04). Creatinine concentration in cooled patients dips and rebounds in response to cooling and rewarming respectively.

5.2.2 Biomarkers

Pooled-Biomarkers: In the graphs from baseline to 24 hours (Figure 16) there was a visibly higher concentration of all biomarkers (except the GST markers) in patients with any degree of AKI compared to those with no AKI. At 24 hours the concentration of each biomarker is lower for the 'no AKI' group than the AKI group.

Plasma CyC and Urine NGAL displayed a moderate ability to detect a reduction in kidney function at 48 hours ($p<0.01$, AUC 0.70; p=0.04, AUC 0.65 respectively) and urine IL-18 at 72 hours (p=0.04, AUC 0.65).

Most patients are classified with AKI using urine output criteria and the difference between AKI versus no-AKI groups is confirmed by a lower serum creatinine in patients with no AKI versus AKI over the first 4 study days (Figure 17).

EPO-Biomarkers: The overall rise in urine biomarker concentration in response to EPO suggests a negative effect of this medication locally on the kidneys or systemically. Plasma NGAL also showed a difference between groups over time (p=0.01).

POLAR-Biomarkers: There was little difference between cooled and control groups for most biomarkers in this study, with the exception of plasma NGAL and KIM-1 that displayed some significant differences.

5.3 Comparison to Literature and Implications of findings

5.3.1 AKI analyses

5.3.1.1 Pooled EPO/POLAR-AKI

The occurrence of AKI in patients with traumatic brain injury (TBI) as found in previous retrospective studies using creatinine criteria alone was around 9 to 11% using any degree of RIFLE AKI[94,138]. In a further recent retrospective study of severe TBI patients (n=136), a higher proportion (23%) were classified as having AKI by using AKIN criteria including urine output during their hospitalisation[139]. The Renal substudies of the EPO-TBI and POLAR trials are the first prospective studies to investigate AKI in patients with traumatic brain injury. Data from patients in these trials were pooled (including treated and control patients) to provide more power to assess the occurrence, severity and duration of AKI in the combined traumatic brain injury population. The occurrence of AKI in the combined EPO-TBI / POLAR cohort using any degree of RIFLE AKI (including urine output classification criteria), was notably higher than previous results from retrospective studies (50.3%), with the vast majority (91%) of patients with AKI classified by urine output alone.

The incidence in AKI is higher in the 2 studies above where urine output criteria are incorporated to classify AKI. The urine output criteria which are common to several AKI classification systems[91,125,126] identify a higher proportion of patients with AKI compared to serum creatinine criteria alone. This is consistent with Bagshaw's findings that the incidence of AKI is underestimated using serum creatinine criteria alone[138], and with Macedo and colleagues, who found that urine output criteria classified significantly more patients with AKI than creatinine criteria[140]. Furthermore, Macedo et al not only found that more patients were AKI-classified using urine output, but that patients classified in any AKI oliguria category had longer ICU stay and a higher risk of mortality compared to non-oliguric patients and this reached significance with prolonged oliguria[140]. In their systematic review[97], Ricci and colleagues recognise that the risk for

mortality is higher in samples classified by serum creatinine alone but support the use of the composite criteria (use of both urine output and creatinine) to classify AKI because its predictive value was found to be more 'statistically stable' in a dedicated comparison performed by Cruz et al[141]. We are not able to support the AKI classification by urine output using associations with other adverse outcomes due to the 'parent' trial management committees' requirement to confine our analysis to renal outcomes. However, urine output appears to be a valid criteria for classifying AKI, although further investigation in this area is warranted. We will publish results on associations between urine output-classified AKI and mortality, length of stay and other adverse outcomes once the 'parent' trials are published.

Our finding of a consistent difference between serum creatinine concentration (although not clinically significant) in patients with AKI v. no AKI over the first 4 study days (Figure 17) in a sample predominantly classified by urine output (91%) suggests that urine output may identify a subclinical level of AKI (undetectable by serum creatinine criteria) that affects outcomes. The fact that there was a visibly higher concentration of all renal biomarkers (except the GST markers) in patients with some degree of AKI compared to those with no AKI (Figure 16) in the same largely urine output-classified sample, is further support of urine output as a marker for clinical and subclinical AKI[142,143]. Several studies have demonstrated an increased sensitivity of the urine output criteria to diagnose AKI as we have found[97,144-146]. However, although our results hint that urine output is an effective marker of AKI, results on AKI-associated mortality of the effect of adding urine output to serum creatinine criteria vary between studies[97,141,147] with no clear answer yet, and further enquiry is required.

Serum creatinine was shown to be an unreliable marker for AKI in patients with traumatic brain injury due to its unexpected rise soon after traumatic injury[94] and its apparent reduction in response to cooling (a treatment quite commonly used in patients with traumatic brain injury). Therefore, finding an alternative biomarker(s) for AKI has become particularly relevant for this patient population. Urine output is a more permissive alternative, and the concentration of the biomarkers; plasma cystatin C and urine NGAL, was significantly higher in patients with AKI compared to those with no AKI at 48 hours after dose/injury, indicating these as potentially useful biomarkers for AKI in this population. In a recent TBI study, urine NGAL concentration at admission was found to identify patients who later developed AKI, and the concentration

remained raised at 24 and 48 hours, further supporting the diagnostic utility of this biomarker for AKI in patients with TBI. Given the heterogenous nature of AKI, it is useful that of the two biomarkers found to differentiate between patients with AKI versus no AKI in our study, one is a marker of GFR (cystatin C) and the other a marker of injury (NGAL). These two biomarkers could potentially be used together or in a 'panel' (similar to troponin for myocardial infarction) to detect AKI.

The notable difference in incidence of AKI between the retrospective studies and our prospective study may be partly due to the methods used to identify TBI patients in retrospective studies (such as diagnostic coding) which could identify a somewhat different patient group. Patient characteristics particular to each study population and treating centre can also contribute to variability in study results.

5.3.1.2 EPO-AKI

There is the hint of a negative effect of EPO on the kidneys when trends are taken into account: a) the higher serum creatinine concentration in patients receiving EPO versus placebo was statistically significant albeit not clinically significant, b) the graphs of creatinine and GFR over time show some separation between the EPO and placebo groups in the first week with a higher creatinine in patients receiving EPO, c) more EPO patients developed AKI than placebo patients although the difference was far from statistically significant, and d) the concentration of all urine biomarkers for patients in the EPO group increased from baseline to 24 hours while their concentration decreased or remained static in the placebo group (Figure 18).

The separation between groups in the graphs of creatinine and estimated GFR in the first week after injury (Figures 1 and 2) may be explained by the fact that all patients received the first dose of study drug, whereas fewer (61%) received the second dose on day 8 and only 40% received the third dose on day 15. Graphs show some separation up to day 8 only. Beyond this point, the fact that fewer patients received dose may not allow detection of a difference in renal function between groups. Where there is separation, a higher serum creatinine in those receiving EPO is unexpected.

There are no published studies of the effect of EPO in patients with traumatic brain injury. A trial investigating whether EPO could reduce the need for blood transfusion unexpectedly found that EPO may reduce mortality in trauma patients[148]. Many animal studies support a protective effect of EPO for kidney tissue[102,103,106,108]and brain tissue[149]

following injury to these organs and there is some clinical evidence for the tissue protective effect of EPO after spinal cord ischaemia-reperfusion injury[150] and for the kidneys after cardiac surgery[112]. However, EPO has not proven to be protective after myocardial infarction[151-153], no renoprotective effect of EPO was found in a recent study of ICU patients at risk for AKI[114], nor was there evidence of benefit in patients following complex valvular heart surgery more recently[156]. Inconsistency in these findings could be related to differences in design and methods used; most of these studies had samples < 100 patients. Notwithstanding, it is possible that EPO is not protective of renal tissue in this patient group. The side effect of increased blood viscosity associated with EPO which increases the risk of thrombotic complications in any organ including the kidneys, may (partly) explain the suggestion in the evidence of a negative impact of EPO on renal function in these patients.

In univariable linear regression models with log creatinine, a higher INR (international normalized ratio) was associated with a lower serum creatinine. EPO tends to increase coagulability so a higher INR may identify the placebo group which, according to the graph, has a lower serum creatinine. The administration of platelets was also associated with a lower serum creatinine in linear regression; this variable may also identify the 'less coagulable' placebo group which has a lower serum creatinine. Greater weight was associated with a higher creatinine which may reflect the general association of morbid obesity with adverse outcomes.

5.3.1.3 POLAR-AKI

The effect of hypothermia on renal function in patients with TBI has never been the focus of investigation in a prospective clinical study[154]. In their report of major complications, Qiu and colleagues found that more TBI patients treated with mild hypothermia soon after injury developed 'renal malfunction' than normothermic controls[156], however, this was not statistically significant (3 patients v. 1 patient [n=80], p=0.27). A pilot study to assess whether mild hypothermia reduces the incidence of contrast-induced nephropathy in patients with pre-existing renal injury found an incidence of 10% in cooled patients compared to 40% in historical controls[120]. Animal studies support a protective effect of hypothermia for kidney tissue following injury[115,116] however, there is mixed evidence for the benefit of hypothermia for the kidneys after cardiac arrest or cardiac surgery[119,122]. The inconsistency in findings may be related to differences in patient groups, design and methods.

In the POLAR cooling trial in TBI patients the occurrence and duration of AKI were significantly greater in patients in the cooled group versus normothermic controls. As the vast majority of patients were classified with AKI using the urine output criteria (41/46, 89%), the difference in AKI between groups cannot be greatly influenced by the unusual response in creatinine to cooling (Figure 3). Furthermore, it is worth commenting that the cooled group, some of whom might have been expected to experience cold-induced diuresis during cooling, was nonetheless the group with a higher incidence of oliguria.

In the graph of creatinine over time for the cooled versus control groups (Figure 3), the creatinine concentration in the hypothermia group seems to dip in response to cooling (cooling lasts for 72 hours as per protocol) and rebound in response to rewarming, then remains notably higher in the cooled group. Moreover, a progressive increase in creatinine associated with rewarming was confirmed (Figure 5). It is well-known that to reduce the negative side effects associated with rewarming, patients should be rewarmed in a very gradual and controlled manner[9]. It is possible that rewarming was not performed 'ideally' in the cooled patients, and the negative side effects of rewarming have contributed to the resulting higher occurrence of AKI in this group. This scenario gives rise to the following questions which are beyond the scope of this thesis:

a) Is it feasible to achieve sufficiently strict adherence to the study rewarming guidelines (increase temperature by 0.17ºC per hour) in a busy ICU, caring for an acutely ill, possibly unstable patient with the equipment and human resources available, or is the degree of vigilance and the fine-tuning required to achieve 'safe' rewarming unattainable in the current system?
b) If favourable neurological results were achieved, would it be reasonable to tolerate a degree of kidney damage?

The final results of the EPO-TBI and POLAR trials will determine the answers to these questions.

The curious dip in creatinine would appear to be a physiological response to cooling. Being a by-product of muscle metabolism, it stands to reason that in the presence of cooling which produces an overall reduction in metabolic processes, creatinine production would be reduced. In response to rewarming, the subsequent increase in creatinine would similarly appear logical. While this response in creatinine concentration

is plausible and logical, we could find no previous work to confirm this and this finding may be the first evidence to document this phenomenon. Interestingly, there is no such reduction observed in the rest of the biomarkers measured. Several of these are urine inflammatory markers that rise from baseline to 24 hours reflecting the different processes involved in their release (Figure 19 and section 2.3).

In univariate linear regression analysis with log creatinine a higher potassium is associated with a higher serum creatinine; which may be explained by the shift of potassium out of the cell with rapid rewarming which is in turn associated with other negative effects of rewarming[9]. A high INR and APTT tend to identify cooled patients and are associated with a higher creatinine. Variables that may identify sicker patients such as pre-hospital hypoxia, duration of noradrenaline, intracranial hypertension index, and the need for a bifrontal decompressive craniectomy were all not unexpectedly associated with higher serum creatinine. The association of the administration of certain fluids with higher creatinine is consistent with recent findings that liberal fluid management may have a negative effect on renal function[157].

5.3.2 Biomarker analyses

5.3.2.1 Use of raw/absolute biomarker concentration

In this study we have used raw urine biomarker concentration without correcting for urine creatinine.

Urine creatinine output has been considered to be generally constant[158], indeed investigators have used urine creatinine concentration with biomarkers in normalised ratios to correct for changes in urine flow[131,159-162]. The assumptions in this approach are that urine creatinine excretion is constant over time and similar between individuals and that biomarker excretion is directly proportional between individuals. However, in critical illness post injury when renal function can rapidly change, and patient heterogeneity makes comparisons between individuals inappropriate, the assumption of a constant urine creatinine excretion is incorrect[158]. Creatinine production and excretion vary between individuals according to age, gender and medications amongst other things, and may constantly change in the setting of injury and critical illness which apply to this population. Creatinine secretion in the proximal tubules and tubular back-leak of creatinine also differ between individuals, further breaking the above assumptions.

Given the flawed assumptions involved in normalising biomarker concentration to urine creatinine concentration, timed urine collection over 4 to 6 hours with urine samples taken from the complete batch, has been suggested to provide an estimate of biomarker excretion rate[158]. This method requires that the biomarkers measured are known to be stable over the time period of collection, which is unknown for several novel biomarkers. There are also practical limitations to timed collections, and for the Renal Substudies of the EPO-TBI and POLAR trials it was considered that timed urine collections on 4 days would be burdensome for site staff and could have a negative effect on the uptake of the parent trials.

Therefore, we were not able to perform timed urine collections, even though this might be considered the ideal method of reporting urine biomarker concentrations in this population. After weighing up the options, and given the limitations of normalising biomarker concentration to urine creatinine, we determined to analyse the raw/absolute concentrations of urine biomarkers alone despite limitations associated with this method due to differences in urinary flow rate. This was considered a reasonable strategy despite the limitations.

The overall lack of strength in findings from the biomarkers means that it is generally only possible to comment on trends noted and occasional noteworthy results.

5.3.2.2 Pooled EPO/POLAR-Biomarkers

There has been one study investigating novel biomarkers for AKI in patients with TBI to the best of our knowledge. Li et al. (n=54) found urine NGAL to be an effective diagnostic marker of AKI (in 24% of the sample who were classified using AKIN criteria) in patients with TBI (AUC=0.88). Urine NGAL was notably raised on admission and remained higher compared to patients with no AKI at 24 and 48 hours after TBI. However, most studies of novel renal biomarkers are of cardiac surgical patients and have focussed on biomarker levels up to 24 hours[134,163-165]. The timepoints we have used (0, 24, 48 and 72 hours post dose/injury) were chosen not only to assess the effect of EPO and cooling on the kidneys but also to assess the biomarker response to EPO and cooling over time which might help to elucidate mechanisms of action of these therapies.

Plasma CyC and urine NGAL were significantly associated with AKI at 48 hours. Plasma CyC is considered a better measure of GFR than serum creatinine given that its concentration is not affected by as many variables and it has outperformed serum creatinine as a diagnostic marker for AKI in numerous studies[133,166-170]. The difference in plasma CyC concentration between AKI groups in the pooled biomarker cohort at 48 hours is consistent with a sustained rise in CyC over 24 hours seen after cardiac surgery by Haase-Fielitz and colleagues[163], albeit at a higher concentration post cardiac surgery. The lower concentration of plasma CyC in TBI versus cardiac surgical patients suggests that patients with TBI may develop a lower grade of AKI similar to post-contrast injury. Given that TBI patients are a relatively young (mean age 33), previously well population the development of lower grade AKI seems coherent.

Urine NGAL in adults undergoing cardiac surgery, which like TBI generally involves a known time of insult, appears to peak at about 3 hours post cardiac surgery then decreases; remaining raised at 24 hours[165]. Urine NGAL concentration, as for many other biomarkers, has not been measured beyond 24 hours in adults. In the pooled EPO-TBI/POLAR data urine NGAL rises from up to 24 hours, peaks at 48 hours and is declining at 72 hours (Figure 8). The 'Risk' category graph (Figure 8) suggests that there is no urine NGAL peak prior to 24 hours after baseline. This indicates a different pattern of urine NGAL release in TBI versus cardiac surgical patients consistent with different mechanisms involved in the development of AKI, and heterogeneity of injury involved in TBI. Alternatively, a different pattern of NGAL release could be related to the fact that baseline bloods in TBI patients were taken anywhere from 3 to 24 hours after injury due to differences in EPO-TBI and POLAR trial inclusion criteria and the unprogrammed nature of TBI. The late collection of the baseline specimens after injury would decrease the likelihood of capturing an early peak at the first timepoint. The concentration of urine NGAL in our brain-injured population is comparable to that in patients who develop contrast-induced nephropathy after coronary angiography[171] indicating that patients with TBI may develop a lower grade of AKI.

Lower grade AKI appears probable in these patients. One of the main reasons for investigating new renal biomarkers is to detect AKI earlier than serum creatinine. Diagnosis at 48 and 72 hours post injury is later than desirable and may not be useful unless the development of AKI in these patients is somewhat delayed after traumatic injury.

The biomarkers that respond to AKI (despite lack of statistical significance) in this population suggest ischaemic (KIM-1, IL-18) and inflammatory (IL-18, NGAL) involvement, and the presence of oxidative stress (NGAL) in the development of AKI[134].

When interpreting results in this study we should keep in mind that the effect of 2 treatments in 2 trials could also be influencing biomarker concentration and creating background 'noise'.

5.3.2.3 EPO-Biomarkers

The overall suggestion in the urine biomarker response to EPO versus placebo, of a negative effect of EPO on the kidneys has been mentioned earlier (section 5.3.1.2). Significant differences in biomarker concentration between EPO and placebo groups were found for urine NGAL and KIM-1, and plasma NGAL.

NGAL: It is unexpected that urine NGAL peaks in the EPO graph and dips in the placebo graph at 24hours after dose given that NGAL is a marker of injury and EPO has anti-inflammatory, anti-apoptotic properties. Amongst its other properties, NGAL is thought to act as a growth factor which is supported by the fact that the bone marrow (site of erythropoiesis) is the tissue with the highest levels of NGAL expression under normal conditions[172,173]. EPO is a potent stimulator of endothelial progenitor cells which appear to be involved in endothelial recovery including tubular cell proliferation after injury, and the formation of new blood vessels in ischaemic areas[174]. With the new growth and angiogenesis that EPO produces it is possible that the production of NGAL is increased and may explain the peak in urine NGAL at 24 hours. This could also explain the different response between groups for plasma NGAL.

KIM-1: The significant difference in response between EPO and placebo groups for KIM-1 suggests that EPO could have a negative effect on renal function; KIM-1 is up-regulated in proximal tubular cells after ischaemic or nephrotoxic injury[54].

Cystatin C: Plasma CyC concentration slowly increases over the 72 hours post injury in EPO and placebo groups consistent with findings of raised CyC level in the CSF of adults[175] or infants[176] after TBI. CyC inhibits the production of cathepsin S which is associated with inflammatory activity[177], which is present after a TBI. The absence of an increase in cathepsin S after TBI fits with the response and inhibitory action of plasma

CyC. The increase in urinary CyC in EPO versus placebo patients is consistent with the overall rise in urinary biomarkers in the EPO group and with the greater number of AKI-free days in the placebo group. Alternatively, the new growth and angiogenesis stimulated by EPO may also contribute to the increased release of CyC which is produced by all nucleated cells.

Alpha and Pi GST: In a study of EPO given to mice with TBI-induced acute lung injury, EPO up-regulated the activity of glutathione S-transferase[178], which is consistent with an initial increase in alpha- and pi-GST in the EPO graphs for these biomarkers after dose. The induction of Alpha1-GST (an antioxidant and detoxifying enzyme) via the Nrf2 (nuclear factor erythroid2-related factor 2) pathway also appeared to be protective against secondary brain injury in mice with TBI[177].

IL-18: In rats with ischaemic stroke given EPO, the concentration of pro-inflammatory cytokine, IL-18, in brain tissue was lower in the group receiving EPO, and brain infarct size was smaller which is consistent with the tissue-protective properties of EPO[135]. In a study of humans after TBI, IL-18 was elevated in their CSF[180]. The graph of urine IL-18 shows higher concentrations in patients receiving EPO versus placebo and the level remains raised from 24 to 48 hours. This is unexpected given the anti-inflammatory properties of EPO.

There is a suggestion in the overall biomarker response to EPO versus placebo that EPO may have a negative effect which may involve renal function (Figure 18). Whether the tubular cell proliferation and angiogenesis that EPO produces contribute to the unexpected rise in biomarkers for AKI in patients receiving EPO is uncertain.

5.3.2.4 POLAR-Biomarkers

There was generally little difference in renal biomarker concentration between hypothermia versus normothermia groups which is unexpected considering the significantly higher incidence of AKI in the cooled versus control group. Cooling may have a 'dampening' effect on the concentration of some biomarkers by reducing metabolic rate.

Plasma NGAL however, displayed a marked difference in concentration between groups. After the physical insult which has caused a TBI, NGAL expression may be increased systemically and perhaps locally in the kidneys. The graph for plasma NGAL

in Figure 19 indicates a higher concentration in cooled patients compared to normothermic patients which reflects the greater frequency of AKI in the cooled versus normothermic group. Tubular injury results in disruption to megalin receptor function through which NGAL habitually transports iron into the cells, leading to the subsequent increase in plasma NGAL[134]. This could ensure the continuation of a mildly elevated plasma NGAL concentration. Urine NGAL does not display a significant difference between the groups but does rise more acutely in the cooled versus control group. A study of NGAL and thermal stresses suggested that on exposure to extreme cold stress NGAL may be upregulated as a protective measure to prevent cold ischaemia injury[181] which might help to explain the response to cooling.

The concentration of urine KIM-1 was also significantly different between groups over time, with a very acute increase at 24 hours in the control group compared to the cooled group. However, the occurrence of AKI is higher in the cooled group compared to the control group which is unexpected. This may be related to a 'dampening' effect of cooling on KIM-1 release by a reduction in metabolic rate or the result could be due to chance.

5.4 Strengths and Limitations

The strength of this study lies in the prospective nature of patient recruitment and data collection allowing a degree of quality control and consistent, accurate identification of TBI patients. The limitations of classification methods relying on traditional markers, creatinine and urine output, are evident in this population; there is no true baseline or peak creatinine, making classification problematic. We have used an adaptation of the RIFLE classification system to categorise AKI that accounts for the typically young TBI population (mean age 33). However, in the end 91% of patients were classified using urine output criteria which are common to all widely used AKI classification systems, thereby virtually cancelling AKI classification method out as a limitation. Furthermore, by showing that alternative validated methods produce similar results (Tables 4, 7 and 11) we substantiate our choice of classification method.

The sample size of the Renal substudies was relatively small and for EPO-Biomarkers in particular was smaller than desired (n=29) due to slow recruitment at the sites taking biomarker specimens. This should be taken into account when interpreting results.

In the pooled analyses, EPO/POLAR-AKI and EPO/POLAR-Biomarkers, in the interests of increasing power we have included all patients rather than just using the controls. This means that in the background the treatments; EPO and cooling, may have some bearing on results. The small sample size (n=70) for the POLAR Renal substudy increases the potential for differences and bias between groups which is evident in the differences in patient characteristics in Table 10. The group receiving hypothermia has more patients with non-reactive pupils and a lower motor score than the normothermic group, however the normothermic group has more patients requiring a decompressive craniectomy. Whether these differences affect the results is uncertain.

The sample size for EPO-Biomarkers is small (n=29), with 14-15 patients per group which means we have very little power to detect significant results (note the large confidence intervals on the graphs, Figure 18). However the number of biomarkers used and the fact that there is a consistent trend in the direction of the evidence in certain areas adds weight to this evidence (e.g. an increase in biomarker concentration in response to EPO suggesting a negative effect on the kidneys albeit subclinical).

We have used raw urine biomarker concentration without correcting for urine creatinine. Although correction for urine creatinine is advocated to correct for changes in urine flow, the assumptions that urine excretion is constant over time and similar between individuals is flawed and moreso in critically ill patients.

The measurement of biomarkers at baseline then at 24 hour intervals up to 72hours means that we do not get a picture of the early response (within 0-24 hours) of the biomarkers to traumatic injury / EPO/ hypothermia. Although there has been little investigation of renal biomarkers in this population, the pattern of biomarker concentration in response to injury has been documented, particularly for cardiac-associated AKI. While the biomarker response to traumatic injury in this population may differ to other settings, part of our main aim was to assess the biomarker response to EPO / hypothermia over time and the timeframe required to detect a response was uncertain. Furthermore, the collection of several novel biomarkers over a prolonged period had not been performed and could provide useful information. The timepoints selected were considered feasible and the most appropriate for our purpose.

The absence of a gold standard to confirm AKI classification complicates the analysis. The gold standard for AKI would technically be a renal biopsy but this invasive procedure is rarely warranted. Serum creatinine has its limitations, moreso in TBI patients as described, and we could not use associations with non-renal outcomes to

support AKI classification in compliance with management committee requests. Instead we have used inferences in the available data to support AKI classification.

5.5 Future Research

The final results of the Renal substudies of the EPO-TBI and POLAR trials may answer some of the questions raised in this thesis and in addition to this the need for further research in several areas has been identified.

Pooled EPO-TBI/POLAR AKI: Our results highlight the need for further investigation into the use of urine output; the most readily available and accessible renal biomarker, as a general biomarker of AKI, and more specifically its utility in this population that has demonstrated such a high rate of AKI detected by urine output alone. The specificity of urine output as a marker of AKI and the most appropriate cutoffs for urine output to define stages of AKI requires further research. In the final substudy data, investigation of the association of urine output-classified AKI with adverse outcomes would shed light on the appropriateness of its use for AKI classification in these patients. If the impact of AKI on this population is clinically significant, further investigation of a) early detection of AKI with renal biomarkers, and b) implementation of potential strategies to minimise the effect of AKI in these patients, may be warranted. It is worth noting that urine output-classified AKI in this population may include subclinical AKI.

EPO-AKI: The evidence from the EPO-TBI Renal substudy in this thesis hints that EPO may have a negative effect on renal function. This requires confirmation or clarification when the substudy data is complete. There is some indication in our results that the concentration of certain renal biomarkers changes in response to EPO. Whether this is related to an effect of EPO on renal function is uncertain. Depending on the final Renal substudy results, further evaluation of key biomarkers in response to EPO and / or renal function may be warranted.

POLAR-AKI: The response of serum creatinine to cooling has not been documented in the literature to our understanding and warrants confirmatory investigation. In addition, for patients undergoing cooling, further investigation of the renal biomarkers that may not be rendered ineffective by cooling could be warranted (plasma NGAL and urine KIM-1). Depending on the final results of the POLAR trial as to whether cooling provides benefit to patients with TBI, further investigation of optimal timing and

method of rewarming may be warranted. However, if we do not have the equipment and staffing capacity to achieve safe rewarming then further investigation in this area may be futile.

Pooled EPO/POLAR-Biomarkers: Plasma cystatin C and urine NGAL were the biomarkers that showed the earliest statistically significant difference between AKI versus no AKI groups at 48 hours. Considering the difficulties identified in classifying AKI in patients with TBI (early rise in creatinine post trauma and decrease in creatinine with cooling), further investigation of alternative diagnostic markers should include these biomarkers. Additional markers that may be worthy of inclusion in future research are urine KIM-1 that showed a significant difference between AKI groups over time and IL-18 that showed a statistically significant difference between groups at 72 hours. Importantly for future investigation, it appears that a lower grade of AKI may predominate in this patient group.

EPO-Biomarkers: The renal biomarker response to EPO suggests a potentially negative effect of EPO that may involve renal function. Depending on the final result of the Renal substudy of EPO-TBI, further investigation of EPO in TBI or trauma patients could be warranted. The effect of EPO on renal function in such studies should also be assessed. Furthermore, it is worth evaluating the effect of EPO on renal function in EPO studies on other patient groups. Biomarkers that showed significant differences between EPO/placebo groups and that may be advisable to use in such studies were urine NGAL, KIM-1, cystatin C and plasma NGAL.

POLAR-Biomarkers: Plasma NGAL and KIM-1 were the only biomarkers that showed a significant difference between groups and may be useful to evaluate cooling interventions in future.

5.6 Conclusions

The occurrence of Acute Kidney Injury (any degree or stage) in the pooled EPO-TBI / POLAR data (50%) is much greater than previously thought, highlighting the need for further investigation into causes, its impact on outcomes, and potential kidney protective measures for traumatic brain injury patients classified with Acute Kidney Injury (AKI).

Serum creatinine was shown to be an unreliable marker for AKI in patients with traumatic brain injury[94]. Consequently, finding alternative methods for classification of

AKI in these patients becomes even more relevant; plasma cystatin C and urine NGAL showed moderate potential for this role.

An unexpected number of patients with AKI were classified by the RIFLE urine output criteria (91%) which is consistent with previous findings of a greater sensitivity of urine output for AKI.

Erythropoietin does not appear to protect the kidneys as we might have expected. In fact, trends hint that erythropoietin may have a slightly negative impact on patients which may involve renal function. The significant response in urine KIM-1 and urine NGAL concentration to erythropoietin suggests a negative effect of erythropoietin for renal function.

Hypothermia was associated with a significantly higher frequency of AKI than normothermia which may be related to the negative side effects of rewarming.

The concentration of plasma cathepsin S was unaffected by AKI, erythropoietin or hypothermia.

Alpha- and Pi-glutathione S transferase showed no significant or interpretable response to AKI, erythropoietin or hypothermia.

Plasma cystatin C rose gradually over 72 hours and its concentration was unaffected by erythropoietin or cooling. However, it may detect patients with AKI at 48 hours after baseline.

Urine cystatin C concentration showed no difference between groups for AKI or hypothermia. Its concentration rose gradually in patients given erythropoietin and was higher than those given placebo at 72 hours after injury ($p=0.06$).

Plasma NGAL did not predict AKI. However, it was significantly raised in cooled patients consistent with a greater occurrence of AKI in this group versus controls.

Urine interleukin-18 showed no significant response to erythropoietin or cooling compared to controls.

The use of several renal biomarkers provided useful information to the study and enhanced the ability to interprete findings.

In summary, AKI is common in patients with traumatic brain injury, therefore its impact on outcomes requires further investigation. Serum creatinine, the most conventional and commonly used biomarker in AKI classification, is an unreliable marker of AKI in this patient group. Plasma cystatin C and urine NGAL were the biomarkers that showed most potential as alternatives and could be further evaluated in patients with TBI. Hypothermia is associated with a higher occurrence of AKI, and there is a suggestion in combined results (requiring further investigation) that erythropoietin may be associated with a negative effect in patients with traumatic brain injury.

Appendices

1. **EPO-TBI trial protocol**
2. **EPO-TBI trial hard-copy case report form (CRF)**
3. **EPO-TBI Person Responsible information and consent form template for sites: generic**
4. **EPO-TBI Person Responsible information and consent form with biomarker specimen information (superseded)**
5. **POLAR trial protocol**
6. **POLAR trial hard-copy case report form (CRF)**
7. **Journal article from which normalised GFR values are taken (see tables 1a and 1b):**

   Wetzels JF, Kiemeney LA, Swinkels DW, et al. Age- and gender-specific reference values of estimated GFR in Caucasians: the Nijmegen Biomedical Study. Kidney Int 2007; 72:632-637

Appendix 1

EPO-TBI trial protocol

ErythroPOietin in Traumatic Brain Injury

(EPO – TBI)

A randomised, placebo-controlled trial of erythropoietin in ICU patients with traumatic brain injury

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THIS STUDY IS ENDORSED BY THE AUSTRALIA AND NEW ZEALAND INTENSIVE CARE SOCIETY CLINICAL TRIALS GROUP (ANZICS CTG).

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1. MANAGEMENT COMMITTEE AUTHORISATION PAGE

We the management committee have read the attached protocol and authorise it as the official protocol for the study entitled:

A randomised, placebo-controlled trial of erythropoietin in ICU patients with traumatic brain injury.

Erythropoietin in Traumatic Brain Injury (EPO-TBI).

Chief Investigator Rinaldo Bellomo		**Date**	18 March 2011
Management Committee Jamie Cooper		**Date**	18 March 2011
Management Committee Craig French		**Date**	18 March 2011
Management Committee Alistair Nichol		**Date**	18 March 2011
Management Committee Alison Street		**Date**	18 March 2011
Management Committee Jeffrey Presneill		**Date**	18 March 2011
Management Committee Ville Pettilä		**Date**	18 March 2011
Management Committee Shirley Vallance		**Date**	18 March 2011
Management Committee Lorraine Little		**Date**	18 March 2011
Management Committee Anne Mak		**Date**	18 March 2011
Management Committee Judy Wills		**Date**	18 March 2011

2. INVESTIGATOR PROTOCOL AGREEMENT SIGNATURE PAGE

I have received and read the Erythropoietin in Traumatic Brain Injury study protocol Version 4 Amendment 2 dated 18 March 2011 and agree to conduct the study in compliance with:

The attached protocol (subject to amendments).

The NHMRC National Statement on Ethical Conduct in Human Research (2007); and

The Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with Therapeutic Goods Administration comments.

The New Zealand Interim Good Clinical Research Practice Guidelines (Volume 2 1998 and Volume 3 2000).

The Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95).

Name of Participating Site: _____

Signature of Investigator: _____ **Date:** _____

Printed Name: _____

3. STUDY ADMINISTRATION STRUCTURE

3.1 Coordinating Centre & Data Management Centre

3.1.1 Responsibilities

Responsible for all aspects of study management including:

- Management of study budget and liaison with funding bodies
- Final protocol
- Case Report Form design
- Database development, maintenance and administration
- Data management
- Protocol training of principal investigators and research coordinators
- Protocol training of site pharmacists and ultrasound technicians
- Preparation and arrangement of investigator payments
- Management of regulatory affairs (TGA etc)
- Management of study set up including assistance with HREC applications
- Randomisation via study website
- Monitoring and close-out site visits
- Organisation of investigator meetings
- Liaison with independent Data and Safety Monitoring Committee
- Data analysis and collaboration on publications

3.1.2 Staff

Professor Jamie Cooper	Director, ANZIC-RC
Professor Rinaldo Bellomo	Principal Investigator, ANZIC-RC
Ms Siouxzy Morrison	Executive Officer, ANZIC-RC
Ms Gillian Syres	Research Manager, ANZIC-RC
Assoc Professor Alistair Nichol	Associate Professor, ANZIC-RC
Assoc Professor Michael Bailey	Senior Statistician, ANZIC-RC
Ms Lorraine Little	Research Fellow, ANZIC-RC
Ms Belinda Howe	Research Fellow, ANZIC-RC
Dr Heather Waddy	ANZIC-RC Affiliate, Monash University
Assoc Professor Chris Reid	Head, Centre for Cardiovascular Research & Education in Therapeutics (CCRET), Monash University
Mr Carl Costolloe	Systems Development Manager, CCRET, Monash University
Ms Ramya Jaganathan	Analyst Programmer, CCRET, Monash University
Ms Phillippa Loane	Case Report Form Coordinator, CCRET, Monash University

3.1.3 Meetings

As required.

3.2 Management Committee

3.2.1 Responsibilities

Responsible for overseeing all aspects of the study management including:

- Liaison with coordinating centre staff, steering committee and ANZICS CTG
- Funding applications, negotiations and communications
- Reports to funding bodies
- Study budget
- Development and approval of final protocol and study materials
- Development and approval of data management systems
- General study management issues

3.2.2 Members

Assoc Professor Craig French	Inaugural Chairman Director of Research, Western Health
Assoc Professor Alistair Nichol	Co-Chairman Associate Professor, ANZIC-RC
Professor Rinaldo Bellomo	Principal Investigator, ANZIC-RC Director of Research, Austin Hospital
Professor Jamie Cooper	Principal Investigator, ANZIC-RC Director of Research, The Alfred Hospital ICU
Assoc Professor Alison Street	Head, Haemostasis / Thrombosis Unit & Haemophilia Centre, The Alfred
Assoc Professor Jeffrey Presneill	Intensivist, Mater Health Services
Ms Shirley Vallance	Clinical Research Manager, The Alfred ICU
Assoc Professor Ville Pettilä	Associate Professor in Intensive Care Medicine, Helsinki University Central Hospital
Ms Lorraine Little	Research Fellow, ANZIC-RC
Ms Anne Mak	Manager, Clinical Trials Pharmacy Services, The Alfred
Ms Judy Wills	Chief Ultrasound Technologist, The Alfred

3.2.3 Meetings

Teleconference as required.

3.3 Steering Committee

3.3.1 Responsibilities

- Overseeing and advisory role
- Data analysis, collaboration and approval of study publications

3.3.2 Members

Management Committee (as above)

Associate Investigators

Assoc Professor Michael Bailey	Senior Statistician, Department of Epidemiology and Preventative Medicine, Monash University
Professor Jeffrey Rosenfeld	Neurosurgeon, The Alfred
Professor John McNeill	Head, Department of Epidemiology and Preventative Medicine, Monash University
Assoc Professor Cristina Morganti-Kossmann	Associate Director, Basic Research, National Trauma Research Institute and Monash University

Site Associate Investigators

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Ms Catherine Simmonds	Research Coordinator, Auckland City Hospital ICU
Dr Seton Henderson	Clinical Director, Christchurch Hospital ICU
Ms Jan Mehrtens	Research Coordinator, Christchurch Hospital ICU
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Ms Miranda Hardie	ICU Research Coordinator, John Hunter Hospital ICU
Dr Samir Haddad	Director, Trauma ICU, King Fahad National Guard Hospital
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Ms Stephanie O'Connor	ICU Research Manager, Royal Adelaide Hospital ICU
Dr David Cooper	Intensivist, Royal Hobart Hospital
Mr Richard McAllister	Research Coordinator, Royal Hobart Hospital ICU
Professor Simon Finfer	Senior Staff Specialist, Royal North Shore Hospital ICU
Mr Simon Bird	Research Coordinator, Royal North Shore Hospital ICU
Professor Geoffrey Dobb	Clinical Professor, Royal Perth Hospital ICU
Ms Jenny Chamberlain	Research Coordinator, Royal Perth Hospital ICU

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Ms Claire Reynolds	ICU Research Coordinator, St Vincent's Hospital Sydney
Dr Imogen Mitchell	Intensivist, The Canberra Hospital
Ms Elise Taylor	Research Coordinator, The Canberra Hospital ICU
Assoc Prof Nerina Harley	Director ICU, The Royal Melbourne Hospital
Ms Deborah Barge	Research Coordinator, The Royal Melbourne Hospital ICU
Dr Geoff Gordon	Director of ICU, The Townsville Hospital
Ms Leonie Jones	Clinical Nurse – Research, The Townsville Hospital ICU
Dr Richard Dinsdale	Intensivist, Wellington Regional Hospital ICU
Ms Diane Mackle	Study Coordinator, Wellington Regional Hospital ICU
Ms Lynn Andrews	Study Coordinator, Wellington Regional Hospital ICU
Dr Vineet Nayyar	Senior Staff Specialist, Westmead Hospital ICU
Ms Christina Skelly	Research Coordinator, Westmead Hospital ICU

3.3.3 Meetings

Bi-annual teleconferences/meetings

3.4 Data and Safety Monitoring Committee (DSMC)

3.4.1 Responsibilities

The Data and Safety Monitoring Committee (DSMC) is responsible for assuring the EPO-TBI study management committee that study participants are not exposed to unnecessary or unreasonable risks and that the study is being conducted according to high scientific and ethical standards. Specifically, the DSMC will:

Assess the performance of the trial with respect to subject recruitment, retention and follow-up, protocol adherence, and data quality and completeness, in order to ensure the likelihood of successful and timely trial completion.

Monitor interim data regarding the safety and efficacy of the study regimens, so that the trial will be concluded as soon as there is convincing evidence of the treatment effects.

Review abstract and publications of main findings prior to submission to ensure the study is being reported appropriately.

Review and consider any protocol modifications or ancillary studies proposed by the study investigators after the main trial begins to ensure that these do not negatively impact on the main trial. Protocol modifications will be considered in the context of their potential impact on scientific integrity and subject safety.

Advise the trial management committee as to whether a protocol should continue as scheduled or undergo a modification due to a finding from the monitoring process.

Maintain a written record of all meetings, recommendations and decisions.

3.4.2 Members

Chair

Professor Deborah Cook, MSc (McMaster), Critical Care Medicine (Stanford), MD (McMaster) FRCPC DABIM; Professor, Department of Medicine, Clinical Epidemiology & Biostatistics; Joint Member, Dept of Clinical Epidemiology & Biostatistics; Academic Chair, Critical Care Medicine, McMaster University; Director, Clinical Effectiveness Outcomes Unit, St. Joseph's Hospital; Past Chair, Canadian Critical Care Clinical Trials Group; Co-Chair, Critical Research Interest Group.

Board Members

Professor Mark Crowther M.D., MSc, FRCPC; Professor and Division Director, Hematology and Thromboembolism (McMaster University); Director of Laboratory Hematology (Hamilton Regional Laboratory Medicine Program) Head of Service, Hematology (St. Joseph's Healthcare, Hamilton); Senior Clinical Research Advisor (Father Sean O'Sullivan Research Centre).

Christian Brun-Buisson MD (Paris, France), Professor of Medicine (Intensive Care) at University Paris-Est Créteil, & INSERM U 657, Institut Pasteur, Paris; Director, Medical ICU (Service de Réanimation Médicale, Centre Hospitalier Universitaire Henri Mondor), AP-HP & Université Paris Est, 94000 Créteil; Chair, Infection Control Program (Assistance Publique Hopitaux de Paris).

Dr François Lauzier MD, M.D.(Université de Sherbrooke), M.Sc. (Université de Sherbrooke), Critical Care Medicine (McMaster University), FRCPC; Assistant Professor of Medicine, Critical Care Division, Departments of Medicine and Anesthesiology, Université Laval, Axe traumatologie - urgence - soins intensifs, Centre de recherche du CHA (Hôpital de l'Enfant-Jésus), Québec City.

3.4.3 Meetings

Meetings will be scheduled prior to trial commencement; and at two pre-specified interim analyses scheduled following recruitment of one-third (202) and two-thirds (404) of the target trial size; with additional meetings as determined by the DSMC at their absolute discretion.

3.5 Clinical Centres

3.5.1 Responsibilities

- Overall management of study at own site in line with the study protocol
- Patient recruitment
- Data collection and data transfer
- Management of data queries
- Liaison with local HREC
- Adherence to local HREC guidelines and reporting requirements
- Adverse event reporting to HREC and ANZIC-RC, in accordance with the study protocol

3.6 Contact Details

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3.6.2 Coordinating Centre

Coordinating centre and serious adverse event contacts

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3.6.4 Brain Injury Markers in Serum and CSF Sub-Study Contact

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4. LIST OF ABBREVIATIONS

AE	Adverse event
AIS	Abbreviated Injury Score
AKI	Acute kidney injury
ANZ	Australia and New Zealand
ANZIC-RC	Australian and New Zealand Intensive Care Research Centre
ANZICS	Australian and New Zealand Intensive Care Society
ATBIS	Australasian Traumatic Brain Injury Study
CARM	Centre for Adverse Reactions Monitoring
CCRET	Centre of Cardiovascular Research and Education in Therapeutics
CPP	Cerebral Perfusion Pressure
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CT	Computerized Tomography
CTG	Clinical Trials Group
CVA	Cerebrovascular Accident
DEPM	Department of Epidemiology and Preventive Medicine
DSMC	Data and Safety Monitoring Committee
DVT	Deep Vein Thrombosis
EPO	Erythropoietin
EQ-5D	Euroqol 5D
GOSE	Glasgow Outcome Scale - Extended
Hb	Haemoglobin
HREC	Human Research Ethics Committee
ICP	Intracranial Pressure
ICU	Intensive Care Unit
INR	International Normalised Ratio
ISS	Injury Severity Score
IU	International Units
KSA	Kingdom of Saudi Arabia
MARC	Medicines Adverse Reactions Committee
MI	Myocardial Infarction
mL	Millilitres
NHMRC	National Health and Medical Research Council
NOK	Next of Kin

NS	National Statement on the Ethical Conduct of Research in Humans
PE	Pulmonary Embolism
QOL	Quality of life
SAE	Serious adverse event
SFDA	Saudi Food and Drug Authority
SF-12	Medical Outcomes Study Short Form 12 version 1
TBI	Traumatic brain injury
TGA	Therapeutic Goods Administration
ULN	Upper Limit of Normal
VNI	Victorian Neurotrauma Initiative
VTE	Venous thromboembolism

5. SYNOPSIS

5.1 Background

Traumatic brain injury (TBI) is a devastating condition which affects close to 1,000 people each year in Australia, causes extensive long-term disability and suffering, and costs the country close to 1 billion dollars per year. Disability follows from primary and secondary brain injury. Attenuation of secondary brain injury (decreasing the additional injury due to the inflammatory, excitotoxic and apoptotic response to trauma) is possible. Erythropoietin (EPO) has recently emerged as a very promising agent to attenuate such injury. EPO has beneficial effects independent of its effect on erythropoiesis, which is relevant to patients with moderate to severe TBI. They include anti-apoptotic activity and protective neurological effects in the presence of hypoxia and ischaemia. EPO protects against brain damage in animal models of TBI. Administration of EPO *up to 24 hours after* a TBI is protective and improves functional neurological outcomes in an animal model. EPO has not been prospectively studied in patients with TBI, but it dramatically improved functional neurological outcome after subarachnoid haemorrhage (a different form of acute brain injury, with some elements in common with TBI). A large multi centre randomised controlled trial[1] found that while EPO did not reduce transfusion rates in trauma patients (including TBI) it significantly decreased 29 day mortality compared to placebo (3.5% vs. 6.6%). However, in this study it was also noted that the patients who received EPO also had increased clinically detected thrombosis. Routine Doppler ultrasound screening detects many more proximal deep venous thromboses (DVT) than clinical diagnosis alone. In addition to neuroprotective effects, EPO has also been shown to protect kidney tissue following injury in many animal studies[2,3,4,5]. The incidence of acute kidney injury (AKI) in TBI may conservatively approximate 30% (refer to Section 11.2.1 EPO-AKI). AKI increases the risk of aggravating cerebral edema[6], and is associated with increased mortality[7] and prolonged length of stay. Accordingly, we will also investigate the renoprotective effect of EPO in patients with TBI. We plan to conduct a trial of EPO in ICU patients with moderate to severe TBI.

5.2 Aim

The primary aim of the study is to determine the efficacy of EPO compared to placebo in improving neurological function in patients six months after moderate or severe TBI.

5.3 Objectives

5.3.1 Primary Outcome:

Combined proportion of unfavourable neurological outcomes at 6 months: severe disability (defined as GOSE scores 2-4) or death (GOSE score 1).

5.3.2 Secondary Outcomes:

a) Probability of an equal or greater GOSE level at 6 months compared to the probability of a lesser GOSE level, using a proportional odds model

b) Proportion of surviving patients with unfavourable neurological outcome (GOSE 2-4) at 6 months

c) Quality of life assessment (SF-12 and EQ-5D) at 6 months

d) Mortality at 6 months

e) Rate of proximal deep venous thrombosis detected during screening by compression Doppler ultrasound

f) Proportion of patients with composite thrombotic vascular events (DVT, PE, myocardial infarction, cardiac arrest and cerebrovascular events) at 6 months

g) Cost effectiveness analysis at 6 months (based on EQ-5D)

5.4 Design

Prospective, multi-centre, double blind, phase III, randomised controlled trial.

5.5 Inclusion Criteria

Patients with moderate (GCS 9-12) or severe (GCS 3-8) TBI admitted to the ICU who are i) ≥ 15 to ≤ 65 years of age, ii) ≤ 24 hours since primary traumatic injury, iii) expected to stay ≥ 48 hours, iv) have a haemoglobin not exceeding the upper limit of the applicable normal (ULN) reference range in clinical use at the treating institution and v) have written informed consent from legal surrogate.

5.6 Exclusion Criteria

i) GCS = 3 and fixed dilated pupils; ii) history of DVT, PE or other thromboembolic event; iii) a chronic hypercoagulable disorder including any known malignancy; iv) treatment with EPO in the last 30 days; v) first dose of study drug unable to be given within 24 hours of primary injury; vi) pregnancy or lactation or 3 months post partum; vii) uncontrolled hypertension; viii) acute myocardial infarct in the past 12 months; ix) past history of epilepsy with seizures in the past 3 months; x) expected to die imminently; xi) inability to perform lower limb ultrasounds; xii) known sensitivity to mammalian cell derived products; xiii) hypersensitivity to the active substance or to any of the additives; xiv) pure red cell aplasia; xv) end stage renal failure (receives chronic dialysis); xvi) severe pre-existing physical or mental disability or severe co morbidity that may interfere with the assessment of outcome; xvii) spinal cord injury; xviii) treatment with any investigational drug within 30 days before enrolment; and xix) the treating physician believes it is not in the best interest of the patient to be randomised to this trial.

5.7 Methods

Patients will be randomised in the ICU, stratified by site and the severity of TBI according to GCS 9-12 and 3-8 (pre-intubation GCS will be used for stratification), and assigned to Epoetin alfa 40,000 IU or placebo in a 1:1 ratio, weekly for up to 3 weeks during the ICU stay. If not contraindicated, pharmaceutical and mechanical venous thrombo-embolism prophylaxis will be prescribed. Compression Doppler ultrasound examinations to monitor patients for the development of proximal DVTs will be performed prior to the first EPO/placebo dose if possible, or at least within 48 hours of the first EPO/placebo dose administration, then twice in each week immediately following each EPO/placebo dose (i.e. up to 7 examinations if the full course of 3 EPO/placebo doses were administered). Refer to Section 10.2.6 Doppler Ultrasound.

5.8 Power Calculations

Based on an unfavourable neurological outcome (death and severe disability) rate of 50% in Australia for moderate and severe TBI, a 574 patient study (two equally sized groups of 287 subjects) will have approximately 90% power to detect a 28% relative risk reduction (50% vs. 36%) [and approximately 80% power to detect a 24 % relative risk reduction (50% vs. 38%)] in 6 month unfavourable neurological outcomes at a two-sided alpha of 0.05. It will also have an 80% power to detect a 9% absolute risk increase in proximal DVT from an assumed baseline rate of 18% (50% Relative Risk Increase) at a one-sided alpha of 0.05. A sample size of 606 patients will allow for a withdrawal and loss to follow up rate of up to 5%.

5.9 Benefits

The mean lifetime medical cost of each survivor with severe disability from TBI in Australia exceeds $3 million AUD. The total lifetime cost for caring for the 250 new survivors of TBI with severe disability each year is close to one billion Australian dollars. Thus, even a small increase in the number of TBI victims who are able to live independently, instead of being permanently disabled, would yield major human and economic gains.

6. BACKGROUND AND RATIONALE

6.1 Clinical and Biological Rationale

Moderate and severe traumatic brain injury is a major public health problem

Traumatic brain injury (TBI) is a leading cause of mortality and long-term disability, particularly affecting young people. In the Australasian Traumatic Brain Injury Study (ATBIS)[8], over six months, we identified 485 patients admitted to the intensive care unit (ICU) with moderate or severe TBI in Australia and New Zealand (ANZ). Their 180-day mortality was 27.4%. Importantly, 50.6% had an `unfavourable` neurological outcome (severe disability or death)[8]. This finding was confirmed in the cohort of patients with TBI included in our recent Saline versus Albumin resuscitation trial (SAFE) [9,10]. Of 415 patients with moderate or severe TBI, 28.9% died and 48.4% had an unfavourable neurological outcome at 24 months[10]. These results demonstrate the mortality and neurological morbidity associated with moderate or severe TBI in ANZ and show that such TBI affects 1,000 people every year. With current best therapies, half the patients with TBI are never capable of living independently in the community. The human and financial costs of supporting these survivors are substantial, as the disabling effects of TBI persist for many years[11,12]. The mean lifetime medical cost of each survivor with severe disability from TBI in Australia exceeds $3 million AUD[13]. The total lifetime cost for caring for the 250 new survivors of TBI with severe disability each year is close to one billion Australian dollars. Thus, even a small increase in the number of TBI victims who are able to live independently, instead of being permanently disabled, would yield major human and economic gains.

The concept of secondary brain injury

The best strategy to decrease the burden of TBI is prevention. A number of measures are constantly being applied (helmets, campaigns against drunk driving or speeding, random breath testing, seat-belt legislation, air-bags, safer cars) to achieve this goal. These measures have decreased mortality but TBI patient numbers have continued to increase. Complementary to these measures, treatment can be directed to decreasing morbidity after primary injury[14]. This goal is biologically plausible and potentially achievable. A large body of experimental and human research shows that, after brain trauma (primary injury), several biochemical pathways are activated leading to secondary brain injury[15]. These pathways include inflammation, oxidative stress, increased vascular permeability and the release of chemicals that are toxic to brain cells (excitotoxic damage)[15]. These secondary events contribute greatly to increasing brain cell death, stroke size, and overall brain injury[16]. Since there are no specific drugs or operations that decrease secondary brain injury, the current management of TBI is supportive, and seeks to prevent or rapidly treat specific complications which further worsen secondary injury (hypotension, hypoxia, brain swelling, increased intracranial pressure[1] and infection)[17]. There has been an intense effort to find other specific agents or novel therapeutic interventions that can attenuate TBI. However, until recently, such trials failed to yield a benefit[18,19] which led to the pessimistic view that this biological potential for improved brain protection might not be realised. However, in a landmark study published in the New England Journal Medicine (NEJM) in 2007 we demonstrated for the first time, that the choice of fluid resuscitation can influence secondary brain injury and improve survival and long term neurological function[10]. These findings

provide proof of concept that specific therapies and interventions may be identified which will decrease secondary brain injury and improve neurological outcome. They provide renewed impetus for the identification of additional protective interventions. One such intervention, which holds much promise, is the administration of erythropoietin (EPO).

EPO as a treatment for traumatic brain injury

EPO is a glycoprotein hormone with pleiotropic cytokine-like effects[20] (***Figure 1***).

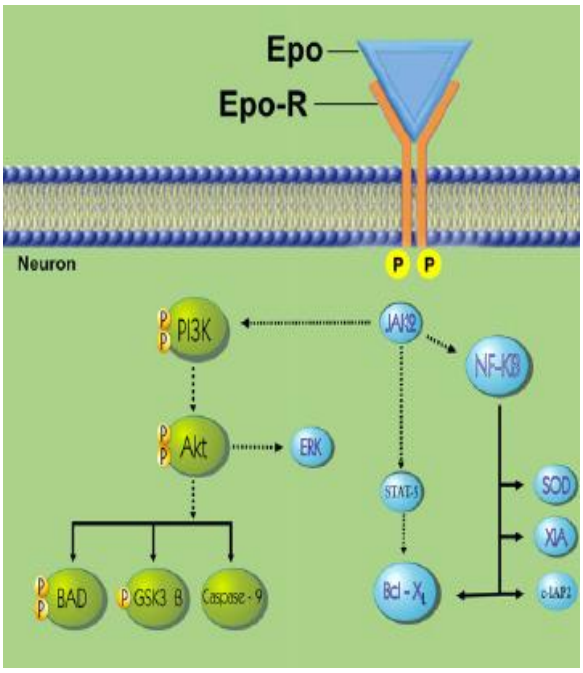


Figure 1.EPO's molecular mechanism of action. EPO binds to its receptor and activates phosphatidylinositol-3-kinase (PI3-K) and Janus-tyrosine-kinase-2 (JAK2). Activation of JAK2 leads to activation of nuclear factor kappa B (NF-kB) which up-regulates the inhibitor of apoptosis genes XIAP and c-IAP2 which inactivate capases, superoxide dismutase (SOD), and the mitochondrial anti-apoptotic protein Bcl-$_{XL}$. Furthermore, JAK2 phosphorylates signal transducers and activators of transcription-5(STAT-5) which stimulates Bcl-$_{XL}$. PI3-K activates the survival pathway of extracellular signal-regulated kinase (ERK) and blocks others associated with cell death [BCLXL/BCL2-associated death promoter (BAD), glycogen synthatase kinase 3B (GSK3B) and capase-9].

EPO is approved for the treatment of anaemia in patients receiving chronic dialysis, patients who have had major surgery, and those undergoing treatment for cancer. Recently EPO has been suggested as a potential treatment for patients with TBI. This is because EPO has numerous potentially beneficial biological effects independent of its effects on erythropoiesis. Some of these effects are especially relevant to critically ill patients with TBI.

- EPO has anti-apoptotic activity and may protect cells from injury due to hypoxemia and ischemia as is typically seen after TBI[15,20,21]. EPO may protect the acutely injured brain by mechanisms such as augmenting neurovascular unit repair, reducing post-traumatic brain oedema, reducing infarct size, decreasing apoptosis and attenuating inflammation[16,22,23].
- EPO is protective against biological insults to other organs, such as hepatic ischemic / reperfusion injury, spinal cord injury and cardiac injury.
- EPO protects against brain damage and improves functional recovery after experimental TBI[16]. In addition EPO *24 hours after* TBI is protective in experimental models and improves functional neurological outcomes[23].
- EPO improved functional neurological outcomes in a randomised controlled trial when administered to patients with subarachnoid haemorrhage[24]. Furthermore, in a double blind proof of concept study, EPO improved functional outcomes of patients after acute ischemic stroke[22]. EPO reduced neurocognitive dysfunction in a double blind randomised controlled trial in patients undergoing coronary artery bypass graft surgery[25].
- EPO significantly decreased 29 day mortality compared to placebo (3.5% vs. 6.6%) in a pre-planned subgroup analysis of trauma patients (including many with TBI) from a large intensive care unit (ICU) randomised controlled trial (***Figure 2***)[26]. This occurred despite a lack of effect on transfusion requirements and confirmed previous retrospective sub-

group findings of decreased mortality in ICU trauma patients treated with EPO (4.8% vs. 10.4%)[26].

- EPO has been shown to protect kidney function in animal models. The incidence of AKI in TBI is significant (refer to Section 11.2.1 EPO-Acute Kidney Injury (AKI)), and AKI is associated with adverse outcomes and increases the risk of aggravating cerebral edema[6] The renoprotective effects of EPO could therefore provide considerable benefits for TBI patients.

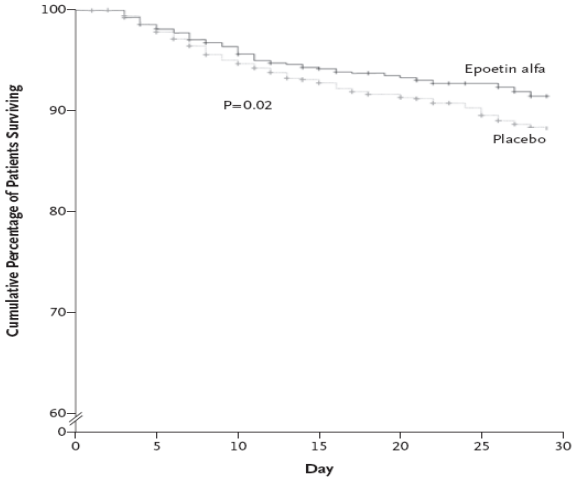


Figure 2. Kaplan–Meier Estimates of Mortality through Day 29 for all Patients Receiving Epoetin Alfa versus Placebo.

The Kaplan–Meier estimates for the trauma patients' subgroup were similar to those shown in the figure, with a logrank test P value of 0.04.

Taken together, these findings indicate that EPO may be an effective treatment in patients with TBI. However, a suitably powered randomised controlled trial is required prior to practice change, especially when incorporating a new therapy with potential complications in critically ill patients. This is particularly true when specific concerns exist.

Specific concerns with using EPO in critically ill TBI patients

Concerns regarding thrombosis: Critically ill patients have an increased risk of venous thromboembolism (VTE) [27]. Most thrombi are asymptomatic and confined to the deep veins of the calf. However, 20-30% of untreated calf vein thrombi extend proximally into the thigh, where, if untreated, they pose a 40 to 50% risk of pulmonary embolism (PE)[28] with a mortality rate of about 25%.

Techniques for detecting deep venous thrombosis: Concern about undiagnosed VTE in the medical-surgical ICU setting is underscored by studies showing that up to 88% of DVTs found by surveillance ultrasound screening were undetected on physical examination[29].

Concerns about the effects of EPO on the rates of thrombosis: Recent studies and clinical trials reported an increased rate of thrombosis with EPO[30,31]. This was mainly observed in patient groups which targeted higher than conventional levels of haemoglobin (>120g/L)[30,31]. Australian ICU clinical practice usually targets haemoglobin levels of 70-100g/L, and rarely exceeds 120g/L[31].

The TRUE effect of EPO on the rate of thrombosis in critically ill patients is unknown: Previous studies which assessed the effects of EPO in the critically ill demonstrated an increased incidence of thrombosis (16.5% vs. 11.5%; hazard ratio 1.41; p=0.008)[1]. However, detection of thrombosis was based on clinical evaluation which understates the true rate[32]. In critically ill trauma patients unsuspected PE is a common post mortem diagnosis. No excess mortality or excess venous thrombosis could be detected in a recent systematic review in patients with chronic heart failure[33].

Concerns regarding the use of VTE prophylaxis in TBI patients: The administration of effective VTE prophylaxis is particularly relevant to the critically ill patient receiving EPO, as the risk of thrombosis can be mitigated by prophylactic anticoagulation[1]. However, we have recently demonstrated that many critically ill patients in Australia do not receive

pharmacological VTE prophylaxis due to concerns regarding bleeding[34]. This is particularly true for TBI[35]. Therefore, patients with TBI are at particular risk of thrombosis, which could be markedly increased by EPO.

Change in clinical practice despite lack of necessary safety information: It has been suggested that EPO should be used in all trauma patients in the ICU[36]. Despite a lack of clear evidence of efficacy or safety, international clinical practice is changing and EPO is currently being administered to critically ill patients. While we demonstrated in our observational study that Australian intensivists do not yet routinely use EPO[8], American physicians are increasingly using EPO for off-label indications[37], such as trauma. These findings have prompted recent editorials[37,38] warning against such practice and have prompted a US Congress investigation into the direct marketing of EPO to physicians. It is imperative we determine both the effectiveness and the safety of EPO in TBI patients before such off-label prescription occurs in Australia.

Current trials of EPO in TBI are not objectively assessing risk of thrombosis: There are currently two active registered randomised controlled trials of EPO in TBI (NCT00375869, NCT00313716). Only one, however, (NCT00313716) will measure a patient centred outcome (Glasgow Outcome Score). Unfortunately, this trial is markedly underpowered (n=200) to detect a neurological benefit of plausible size. Neither one of these studies will objectively assess the effects of EPO on DVT.

A prospective randomised trial powered to detect a clinically plausible improvement in neurological function is clearly required. This trial should accurately determine the effects of EPO on the rates of thrombosis, a sentiment reflected in numerous articles in high impact journals[36,39,40].

Why a multi-centre trial of EPO in TBI should be performed in Australia

There is worldwide interest in the evaluation of EPO in TBI and Australia is uniquely placed to perform such evaluation. In particular, the standard of care of severe TBI patients in Australia is very high. For example, our recent epidemiological survey has shown that Australian ICU and ED specialists are more likely than European specialists to insert invasive monitoring (arterial and central venous catheters) in patients with severe TBI[8,14]. If EPO were shown to be beneficial in a setting where care levels are already high, it would likely change practice not only in Australia but also worldwide. In addition, EPO has recently come off patent. This suggests that the future cost of EPO will substantially decrease, thus facilitating its worldwide use. At the same time, incentives for industry to perform studies such as the one proposed here will diminish.

6.2 Background Summary

TBI is a catastrophic illness with extraordinary human and financial costs. There is substantial experimental evidence, a plausible biological rationale, and supportive clinical evidence from clinical trials to suggest a possible beneficial effect of EPO in TBI. Clinical practice is changing in the USA, prematurely leading to a loss of equipoise despite inconclusive data. There is concern that the use of EPO may increase thrombosis in critically ill patients and there are no trials with adequate methodology to study *both* efficacy and risks of EPO in TBI patients. The significance and cost of TBI, the possible benefits, the growing off-label use, the concern about side effects of EPO, and the lack of direct evidence of benefit make a trial of the highest quality essential to guide future clinical practice.

6.3 Significance

This trial will determine whether EPO therapy is effective and safe in TBI. If EPO is proven to improve neurological outcomes and is introduced clinically, given the high disability rate and high mortality rate, at least 150 patients may have significantly improved neurological outcomes per year in our country by the widespread application of EPO. To change only a

few of these patients from an unfavourable to a favourable neurological outcome would alone result in lifetime dollar savings. This study, aimed at objectively resolving the uncertainty about EPO in ICU patients with moderate and severe TBI in Australia, is of the highest priority.

7. OBJECTIVES

7.1 Aim

We plan to conduct a stratified, prospective, multi-centre, randomised, double-blind, placebo-controlled, phase III trial in ICU patients with moderate (GCS 9-12) or severe (GCS 3-8) TBI to determine whether EPO improves neurological function 6 months after injury.

While determining the efficacy of EPO, we plan to systematically monitor the effect of EPO on proximal DVT rates in ICU patients with moderate (GCS 9-12) or severe (GCS 3-8) TBI.

7.2 Hypothesis

In patients with moderate (GCS 9-12) or severe (3-8) TBI, EPO therapy improves long-term neurological function assessed 6 months after injury.

8. STUDY OUTCOME MEASURES

8.1 Primary Outcome

Proportion of unfavourable neurological outcomes at 6 months: severe disability (defined as GOSE 2-4), or death (GOSE score 1).

8.2 Secondary Outcome

a) Probability of an equal or greater GOSE level at 6 months compared to the probability of a lesser GOSE level, using a proportional odds model

b) Proportion of surviving patients with unfavourable neurological outcome (GOSE 2-4) at 6 months

c) Quality of life assessment (SF-12 and EQ-5D) at 6 months

d) Mortality at 6 months

e) Rate of proximal deep venous thrombosis detected during screening by compression Doppler ultrasound[41]

f) Proportion of patients with composite thrombotic vascular events (DVT, PE, myocardial infarction, cardiac arrest and cerebrovascular events) at 6 months

g) Cost-effectiveness analysis at 6 months based on EQ-5D.

9. OVERALL STUDY DESIGN

9.1 Study Population

9.1.1 Patient Recruitment

606 patients presenting to the ICU with moderate or severe TBI will be recruited into the study from the participating study centres in ANZ over a 2.5 year period.

The 6 month prospective observational study[8] conducted by ANZICS CTG of 635 patients admitted with TBI, determined that 167 patients were i) >18 years of age, ii) had moderate or severe TBI and iii) a haemoglobin <120g/L on the first ICU day and would therefore be eligible for enrolment. Assuming an overall loss to recruitment and refusal of consent rate of 27%, we would still recruit 248 patients / year*, allowing the 18 participating trauma sites in ANZ to complete recruitment of 606 patients in 2.5 years.

*Only one other multi-centre trial will potentially concurrently recruit critically ill patients with TBI in Australia. POLAR is a pre-hospital study investigating hypothermia in critically ill severe TBI patients in 7 sites in ANZ. The EPO-TBI recruitment numbers have been restricted to hospitals not participating in POLAR together with patients not eligible for recruitment into POLAR. Therefore our forecast of 2.5 years is conservative.

However, the DECRA trial (multi centre prospective randomised trial of early decompressive craniectomy in patients with severe traumatic brain injury) is still active and nearing completion. It is anticipated that the DECRA trial will achieve the recruitment target by April 2010. Therefore the recruitment period for EPO-TBI has been revised to 3.5 years (refer to 9.2 Co-enrolment and Competing TBI Studies).

Patients must be within the ICU with a non penetrating moderate (GCS 9-12) or severe (GCS 3-8) TBI less than 24 hours post primary injury to be eligible for enrolment. Specifically, patients will be eligible for enrolment if they are in the ICU and can receive the first dose of study drug within 24 hours post primary injury. Inter-hospital transfers may also be eligible provided the time-lines for randomisation can be met and all of the inclusion criteria and none of the exclusion criteria apply.

9.1.2 Inclusion Criteria

Patients with non-penetrating moderate (GCS 9-12) or severe (GCS 3-8) TBI admitted to the ICU who:

a) Are ≥ 15 to ≤ 65 years of age

b) Are < 24 hours since primary traumatic injury

c) Are expected to stay ≥ 48 hours

d) Have a haemoglobin not exceeding the upper limit of the applicable normal (ULN) reference range in clinical use at the treating institution

e) Have written informed consent from legal surrogate

9.1.3 Exclusion Criteria

Patients will be excluded from the study if any of the following criteria apply:

a) GCS = 3 and fixed dilated pupils

b) History of DVT, PE or other thromboembolic event

c) A chronic hypercoagulable disorder, including known malignancy

d) Treatment with EPO in the last 30 days

e) First dose of study drug unable to be given within 24 hours of primary injury

f) Pregnancy or lactation or 3 months post partum

g) Uncontrolled hypertension (systolic blood pressure of >200 mm Hg or diastolic blood pressure of >110 mm Hg)

h) Acute myocardial infarct within the past 12 months (Refer to APPENDIX 1 - Criteria for Acute Myocardial Infarction[42])

i) Past history of epilepsy with seizures in past 3 months

j) Expected to die imminently (< 24 hours)

k) Inability to perform lower limb ultrasounds

l) Known sensitivity to mammalian cell derived products

m) Hypersensitivity to the active substance or to any of the additives

n) Pure red cell aplasia (PRCA)

o) End stage renal failure (receives chronic dialysis)

p) Severe pre-existing physical or mental disability or severe co-morbidity that may interfere with the assessment of outcome

q) Spinal cord injury

r) Treatment with any investigational drug within 30 days before enrolment

s) The treating physician believes it is not in the best interest of the patient to be randomised to this trial

9.2 Co-enrolment and Competing TBI Studies

In accordance with ANZICS CTG endorsed study guidelines, until DECRA study is completed it will have enrolment priority over EPO-TBI.

TBI patients eligible for both DECRA and EPO-TBI with a GCS 3-12 who have a clear DECRA exclusion will be eligible for EPO-TBI.

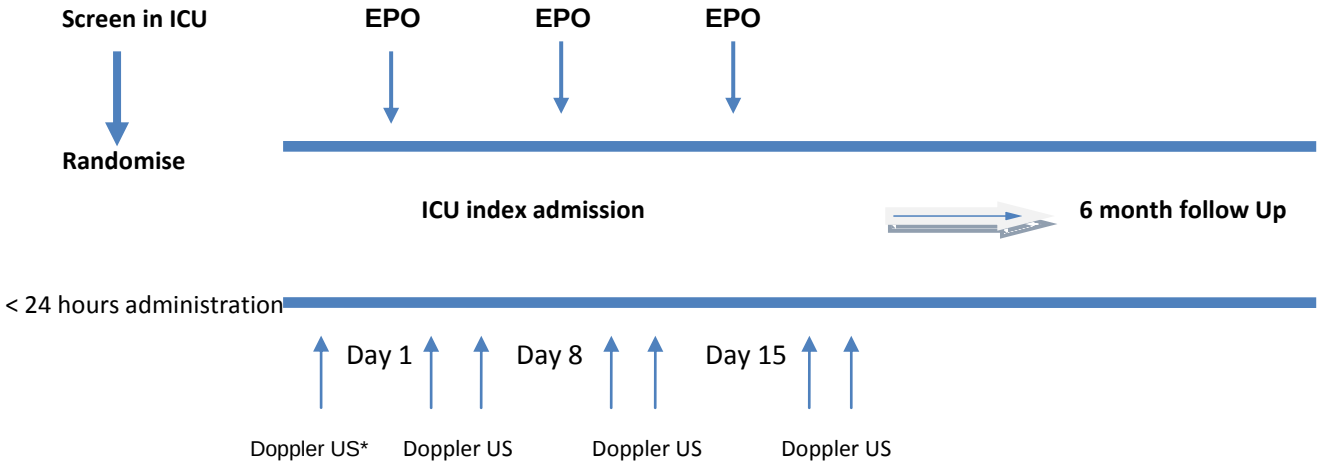
Co-enrolment into the CHEST study is not permitted. Enrolment into other TBI studies is not permitted and enrolment into other randomised controlled trials is strongly discouraged.

9.3 Concomitant Treatment

The ICU team will have full, independent, control of patient management however we will request that TBI standardised clinical practice guidelines are followed. Refer to APPENDIX 2 - Head Injury Management Guidelines

10. STUDY PROCEDURES

10.1 Summary of Study Treatment and Follow up Schedule



**The baseline ultrasound may be performed before or at least within 48 hours after the first dose administration.*

EPO = epoetin alfa 40,000 IU or placebo

10.2 Investigational Product

10.2.1 Study Drug

Study drug is Epoetin alfa 40,000 IU 1 mL in pre-filled syringe, manufactured by Janssen-Cilag Pty Limited or placebo, Sodium Chloride 0.9%. Study drug will be administered by subcutaneous injection.

Epoetin alfa 40,000 IU pre-filled syringe will be purchased by the site pharmacist for the purposes of the study. ANZIC-RC will reimburse the acquisition cost of Epoetin alfa.

Supply of placebo, Sodium Chloride 0.9% 10 mL ampoule will be provided by the site pharmacy.

A Pharmacy Manual will be provided.

10.2.2 Drug Accountability

Drug accountability is the responsibility of the Principal Investigator delegated to the study pharmacist at each site.

Study drug may only be administered to patients enrolled in this study and in accordance with this protocol.

The site pharmacist must seek approval from the ANZIC-RC unblinded project officer before placing the Epoetin alfa drug order.

A drug accountability log is to be kept to record the study drug:

a) receipt

b) dispensed to each patient

c) date of dispensing

Any expired Epoetin alfa 40,000 IU can be destroyed on site after documentation on the Inventory Log.

Expired drug can be destroyed according to local standard practice.

All records and drug accountability log should be made available for inspection by the unblinded project officer.

10.2.3 Dispensing

Following patient randomisation by the site investigator or designee, Pharmacy will dispense the study drug in a TAMPERPROOF sealed box to blind the treatment assignment. The sealed opaque box will have the following labelling:

Clinical Trial Protocol: EPO-TBI

Site HREC Project Number

For UNBLINDED Study Nurse Use Only

Patient Name and Hospital Identification Number

Intensive Care Unit

Date of dispensing

Refrigerate Do not freeze.

The TAMPERPROOF sealed box can only be opened by the site's designated Unblinded Study Nurse. Pharmacy must be notified immediately if the TAMPERPROOF seal is broken or if the box is not opened by the designated Unblinded Study Nurse.

For patients who receive the active treatment:

The sealed box will consist of one Eprex 40,000 IU pre-filled syringe labelled with direction for use "Inject 1ml SUBCUTANEOUSLY over at least 1 minute"

For patients who receive the placebo treatment:

The sealed box will consist of one Sodium Chloride 0.9% 10 mL ampoule labelled with direction for use "Inject 1ml SUBCUTANEOUSLY over at least 1 minute".

On dosing day, Unblinded Study Nurse will administer the study drug to the patient in the absence of any study personnel, ICU and hospital staff.

After dose administration the Unblinded Study Nurse will discard the used study drug and used syringe into a sharp container. The Unblinded Study Nurse must check the sharp container is properly closed to ensure that there will be no access to the used study drug and used syringe.

Study drug will be dispensed on Day 8 and Day 15 if the patient remains in the ICU, if the pre-medication Hb is < 120g/L and if permanent withholding criteria have not been met.

10.2.4 Storage and Handling

Epoetin alfa 40,000 IU will be stored in a secure area refrigerated at 2 – 8 degrees Celsius according to usual hospital procedures.

Sodium Chloride 0.9% 10 mL ampoules will be stored in a restricted area at room temperature.

A temperature log with daily minimum and maximum temperature readings will be kept by the site pharmacist to monitor the temperature of the refrigerator where Epoetin alfa is stored

and the room temperature where the Sodium Chloride 0.9% 10 mL ampoules are stored. Any temperature excursion should be reported immediately to the unblinded project officer.

10.2.5 Administration

Patients will undergo concealed random allocation to either a subcutaneous injection of Epoetin alfa 40,000 IU or placebo, Sodium Chloride 0.9% (1:1 ratio).

For patients randomised to the treatment arm, the unblinded nurse will administer the full contents of the Eprex 40,000 IU pre-filled syringe to the patient.

For patients randomised to the placebo arm, unblinded dosing nurses will draw up 1 mL from the Sodium Chloride 0.9% 10 mL ampoule.

Unblinded dosing nurses will administer the study drug discreetly by pulling the screens around the patient bed area when the dose is administered. The study drug can only be checked with a second unblinded nurse if applicable.

Unblinded dosing nurses cannot discuss study drug treatment with research staff or other members of the ICU or hospital staff except the unblinded pharmacist.

Unblinded dosing nurses will be independent of the study and independent of direct clinical care.

The first dose will be given within 24 hours of the estimated time of traumatic brain injury then weekly for up to 2 more doses (on days 8 and 15). Study drug will only be administered to patients during the ICU admission. If the patient is discharged to the general ward (before Day 8 or before Day 15) they will not receive any further doses of study medication.

Study drug should be given < 24 hours post traumatic brain injury then on Day 8 and Day 15 at 1200 hours ± 4 hours whilst the patient is in the ICU.

The Hb threshold at baseline (prior to the first dose) is different to the second and third doses as follows:

- First dose Hb threshold is: not exceeding the upper limit of the applicable normal (ULN) reference range in clinical use at the treating institution
- Second dose Hb threshold is: < 120 g/L
- Third dose Hb threshold is: < 120 g/L

Day 8 and Day 15 doses may be given ± one day of dosing day so the study medication is prepared in pharmacy business hours.

The weekly dose will not be administered if the patient's pre-medication haemoglobin is ≥ 120g/L. Study drug will be withheld for that dose. The patient will be assessed on the next scheduled dosing day. If the Hb is < 120 g/L the dose may be given if the patient is still in the ICU and has not met the withholding criteria.

Patients readmitted to ICU during the same hospital stay will not received any further doses of study drug.

The study drug should, ideally, be injected at a subcutaneous site distinct from that of other medications (i.e. insulin, heparin, LMWH etc).

The control intervention represents standard care in Australia and New Zealand for ICU TBI patients.

10.2.6 Compression Doppler Ultrasound

Bilateral compression Doppler ultrasound of the lower extremities to monitor for proximal DVT will be performed at baseline (before the first dose if possible or at least within 48 hours

after the first dose administration) then twice weekly after each dose of study medication for 3 weeks or up to ICU discharge whichever occurs first. Up to 6 scheduled (X2 weekly) ultrasounds only will be performed if the patient is an inpatient in the ICU for longer than 3 weeks. No further scheduled (X2 weekly) ultrasounds are required beyond Study Day 21.

Bilateral compression Doppler ultrasounds will be performed on the general ward if the patient has been transferred after a dose of study drug and before a scheduled ultrasound has been performed. The reason for this is that at least one bilateral compression Doppler ultrasound will be performed after each dose of study drug for safety reasons.

The site treating clinicians outside the intensive care unit (known variously by names such as Parent Unit, Admitting Unit, or Attending Medical Service) are required to notify the site researchers of any occurrences of VTE as soon as it is diagnosed.

Additional bilateral Doppler ultrasounds will be performed on suspicion of DVT or PE. Refer to Appendix 4 and Appendix 5 for management guidelines.

A Doppler Ultrasound Manual of Operations will be provided.

10.3 Screening Log

The screen log is designed to monitor patient recruitment at the participating site.

A screening log will be maintained at each participating site by the research coordinator to document patients evaluated for enrolment. The log will provide a record of all patients assessed for eligibility, either randomised in to the study or deemed ineligible for the study. When a patient is considered to be ineligible, the reason(s) will be noted on the log. The information will be entered into a web based log before the first Monday of each month. The log will also be used to assess patient recruitment targets.

10.4 Randomisation

Randomisation will be performed by the site investigator or research coordinator via a tried and tested web based system, including block randomisation at each site. Treatment allocation will be stratified by site and also by severity of head injury i.e. initial Glasgow Coma Score (GCS) 9-12 (moderate) or GCS 3-8 (severe). The pre-intubation GCS will be used for stratification.

After meeting eligibility criteria and obtaining informed consent, patients will be randomised in a 1:1 ratio to receive either Epoetin alfa 40,000 IU or placebo, Sodium Chloride 0.9% 1 mL.

10.5 Blinding

The trial will be conducted as a double-blind study. All site personnel and ANZIC-RC staff will be blinded to treatment allocation except the site pharmacist, site unblinded dosing nurses and the ANZIC-RC unblinded project officer and the CCRET staff administering the web randomisation process.

The site pharmacist will be responsible for preparation of the study drug for each participant in such a way that investigators and staff remain blinded to the study drug being administered.

10.6 Breaking the Blind

In the unlikely event of an emergency where the appropriate treatment of the patient requires knowledge of the study drug, the principal investigator at each site may unblind the treatment assignment by contacting ANZIC-RC.

In all such cases, the adverse event necessitating the emergency unblinding will be reported as a serious adverse event in accordance with the procedures indicated in Section 16.3 Serious Adverse Events.

Unblinding should be discussed with the Chief Investigator Professor Rinaldo Bellomo on +61 (0)3 9496 5992 or the Project Manager, Lorraine Little on +61 (0)437 207 413. However, unbinding the treatment assignment is to be avoided because clinical management will involve treatment of symptoms.

Refer to the study procedure manual for details about emergency unblinding.

10.7 Permanent Withholding Criteria

Study medication will be permanently withheld for any of the following reasons:

a) Development of proximal DVT

b) Development of pulmonary embolism

c) Any other thrombotic event

d) Acute myocardial infarction

e) Cardiac arrest or ventricular fibrillation

f) Cerebrovascular accident

g) Any serious adverse event or protocol deviation where, in the attending physician's opinion, the patient should not receive any further doses of study drug

h) Consent has been withdrawn or consent to continue has not been granted

All patients who are not receiving study drug will continue to be followed for clinical outcomes and receive X2 weekly proximal compression Doppler ultrasound testing except for those situations where consent is withdrawn.

10.8 Table of Events

Assessments / Procedures					Treatment during ICU Admission[1]					Follow up 6 months	
	Screening	Baseline	Daily[2] ICU Admission	Daily Hospital Admission	X 2 Weekly	Day 1	Day 8	Day 15	Hospital Discharge	Research Coordinator	Central Assessor
Inclusion & Exclusion Criteria	X										
Informed Consent	X										
Serum Pregnancy Test[3]	X										
Randomisation[4]		X									
Demographics		X									
VTE Risk Factors		X									
Anticoagulant medication		X									
APACHE II[5]		X									
GCS[6]	X	X									
Injury Severity Score[7]		X									
CT Marshall Score[8]		X									
Vital Signs[9]		X									
Haematology / coagulation[10]	X	X	X								
Renal function data[11]		X	X								
EPO-Biomarkers[12]		X	X								
Brain Injury Markers in Serum & CSF[13]			X								
Study Drug Administration[14]						X	X	X			
Permanent Withholding Criteria[15]			X			X	X	X			
Blood Transfusion			X								
Head Injury Management and Intervention			X								
Doppler Ultrasound[16]		X			X						
Study Outcomes[17]			X	X						X	
Serious Adverse Event[18]			X	X						X	
Protocol Deviations			X			X	X	X			
Adverse Events			X	X						X	
Survival Status									X	X	
Health Economic Evaluation Data[19]										X	
Withdrawal			X	X					X	X	X
GOSE[20]											X
EQ-5D Questionnaire[21]											X
SF-12 Questionnaire[21]											X

N.B. Study Day 1 is from the time of randomisation to end of ICU chart. Patients may be dosed on Day 2 if the first dose can be given within 24 hours of the brain injury occurring. It is not necessary to alter the scheduled weekly doses from Days 8 and 15 if the patient is dosed on Day 2.

1. Study drug and X2 weekly Doppler ultrasounds will be performed whilst the patient is in ICU. When the patient is discharged to the general ward study drug and the X2 weekly ultrasounds will cease. However at least one ultrasound should be done after each dose. For example, if a dose of study drug is administered on Day 8 then the patient is discharged to the ward the next day, a follow up ultrasound should be performed 3 – 5 days after the dose. Refer to the Doppler Ultrasound Manual of Operations for detailed information.

2. Daily data will be collected up to Day 21 then X2 weekly or up to ICU discharge whichever occurs first.

3. All women of child bearing potential will have a serum beta human chorionic gonadotropin (β-HCG) or urine pregnancy test performed prior to randomisation. Women who are more than 12 months post-menopausal or have had hysterectomy and/or bilateral oopherectomy will not require a pregnancy test.

4. Randomisation will be performed by the site investigator or research coordinator. Treatment will be stratified by site and severity of head injury i.e. GCS 9 – 12 (moderate) and GCS 3 – 8 (severe).

5. The APACHE II score data will be collected from time of injury up to randomisation. The APACHE II worksheet is provided in APPENDIX 3 - APACHE II Calculation Worksheet for manual calculation.

6. The pre-intubation, closest to randomisation GCS will be collected at screening for stratification. The motor score from the screening GCS will be collected for baseline trauma scoring.

7. The ISS and maximal AIS scores for each body region can be collected from the site Trauma Registry. AIS version 2005 will be used.

8. A CD copy of the initial CT scan will be sent to the Coordinating Centre for Marshall Scoring assessment by an independent neuro-radiologist. The CD of the CT scan is required for the interim analyses and within 8 weeks of completion of recruitment.

9. Vital signs pre-randomisation: the first SBP on admission; lowest SBP; the last unassisted respiratory rate the worst SaO_2; and pupillary response to closest to randomisation are required for either trauma scoring or prognostic indication.

10. Hb at screening will be assessed for eligibility. Baseline platelet count, Hb, APPT and INR closest to randomisation.

11. Renal function data: First available post-trauma serum creatinine and patient weight (estimated or measured). Daily creatinine and daily dialysis data will be collected up to Day 21 of the *hospital* stay#; urine output to determine renal function will be collected for the first 7 days in ICU or up to ICU discharge whichever occurs first.

    # Serum creatinine and dialysis data will be collected up to Day 21 during the hospital admission so this data is required even if the patient is discharged to the general ward before Day 21.

12. ***The Alfred and The Royal Melbourne hospitals only*** will participate in the EPO-Biomarker part of the protocol. Blood and urine samples will be collected before the first dose is administered then collected at 24, 48 & 72 hours after the first dose for **the first 50 patients only**. Refer to Section 11.2.2 Methods.

13. ***The Alfred Hospital only*** will participate in the Brain Injury Markers in the Serum and CSF Sub-study. Blood samples will be collected from all patients with moderate and severe brain injury, whereas CSF will be collected only when available from the severe patient group (with an EVD in situ implanted prior to the drug administration). Samples will be collect daily for 5 days or up to removal of the EVD.

14. Study drug will be administered on days 1 (or day 2 - within 24 hours of the brain injury occurring), 8 ± 1 & 15 ± 1 during the ICU admission. The first dose following randomisation should be administered within 24 hours of the estimated time of injury. Doses on days 8 and 15 will be administered at 12:00 hours ± 4 hours. If the dose on Day 8 or Day 15 is not given because of a protocol deviation (the dose was erroneously omitted) then the dose can be given the next day with no alteration to the dosing or ultrasound scheduling. For the second and third doses of study drug only ifthe pre-medication Hb is ≥120 g/L the dose will be **withheld for that dose only** (the dose cannot be given the next day). The Hb will be assessed on the day of the next scheduled dose (if applicable) with a view to administer study drug if the Hb is <120 g/L.

15. The permanent withholding criteria is outlined in Section 10.7 Permanent Withholding Criteria

16. Baseline Doppler Ultrasound will be performed prior to the first dose if possible or at least within 48 hours after the first dose administration. X2 weekly Doppler ultrasounds will be performed up to Day 21 or ICU discharge whichever occurs first. Up to 7 ultrasounds are required depending on length of ICU stay. After Study Day 21 scheduled (X2 weekly) ultrasounds are not required. Ultrasounds will be performed upon clinical suspicion of proximal DVT and/or PE in additional to the scheduled (X2 weekly) ultrasounds in ICU or on the general ward if deemed necessary by the treating physician. A positive proximal DVT result will be reported as a DVT outcome on the Study Outcomes Form. ***The Treating Unit are required to contact the ICU research staff as soon as possible if the patient develops a thrombotic event following discharge from the ICU.***

17. Study Outcomes are: proximal DVT; PE; other thrombotic events; myocardial infarction; stroke; cardiac arrest or ventricular fibrillation. These events will be reported on the Daily Assessment form or as a Serious Adverse Event.

18. SAEs will be reported according to the protocol 16.3 Serious Adverse Events. SAES will be collected up to 6 months after randomisation. All serious adverse events should be reported on an SAE form according to Section16.4 Reporting.

19. Data for cost effectiveness analysis will be collected for all patients and for compensable patients in Victoria and New Zealand.

20. The GOSE will be conducted by a central outcome assessor. The GOSE will be presented, in a telephone interview, to the proxy and the patient (if the patient is well enough).

21. Quality of Life questionnaires EQ-5D and SF-12 will be conducted by a central assessor. The questionnaires will be presented, in a telephone interview, to the proxy and patient (if the patient is well enough to participate). The research coordinator is requested to mail the 3 questionnaires to the patient (if applicable) and proxy before the telephone interview.

11. RENAL ASSESSMENT

11.1 Clarification of Renal Assessment

The renal assessment is composed of 2 parts. The first part, EPO – Acute Kidney Injury (AKI), involves all 606 participants from all sites. The second part of the renal assessment involving renal biomarkers will proceed in **two centres only** for logistical reasons; these centres are The Alfred and The Royal Melbourne hospitals in Melbourne, Victoria. All sites should refer to Section 11.2.1 EPO-AKI for the renal assessment protocol relevant to their centre. The Alfred and The Royal Melbourne hospitals should, **in addition**, refer to Section 11.2.2 EPO-Biomarkers for additional information to the renal assessment protocol pertinent to their centres only.

11.2 Renal Assessment Protocols

11.2.1 EPO-AKI

This is an assessment of the effects of erythropoietin on renal function in patients with TBI to be conducted on all patients in the EPO-TBI trial.

Aims

EPO has been shown in many experimental studies to protect the kidneys from injury[2,3,4,5]. AKI is a complication of TBI, which makes its management more complex and costly, and exposes TBI patients to a greater risk of dialysis-related cerebral oedema. We plan to investigate changes in renal function and biomarkers in patients with TBI in this renal assessment of the EPO-TBI trial.

The specific aim of EPO-AKI is to determine whether in patients with TBI, EPO therapy reduces the risk for, severity of, and duration of AKI, compared to placebo.

Background

AKI as classified by the RIFLE criteria (acronym for Risk, Injury, Failure, Loss, End-stage)[43] may occur in approximately 9% of TBI patients in Australia (Risk, Injury and Failure categories) according to results from a recent unpublished preliminary study[44]. Trauma patients compose almost 1 in 10 of those admitted to Australian ICUs and over 42% have TBI[45]. AKI increases the risk of aggravating cerebral edema[6], and is associated with increased mortality[7] and prolonged length of stay[46]. Severe cases require costly treatments and can result in prolonged kidney dysfunction and delayed recovery, escalating the human and financial costs of TBI[47]. Treatment of AKI with dialysis is associated with significant fluctuations in osmolarity which expose the patient to an increased risk of cerebral oedema[6]. Accordingly, we consider it a priority to investigate treatments which simultaneously may protect the brain and kidney after neurotrauma.

In addition to neuroprotective effects, EPO has also been shown to protect kidney tissue in many animal studies following ischemia-reperfusion injury, contrast-induced nephropathy, and in endotoxin or septic models[2-5,48,49]. The neurological and renal cytoprotective effects of EPO appear related to promotion of neovascularisation and tissue regeneration, limitation of apoptosis, and anti inflammatory action[50]. Several trials are currently investigating the effect of EPO on incidence of AKI in cardiac surgical patients; on kidney graft function after kidney transplant; and in ICU patients with AKI; in smaller cohorts[51,52]. Results of a small clinical pilot trial on cardiac surgical patients indicated a reduction in the incidence of AKI, and improved postoperative renal function in patients receiving EPO [53]. However, there has been no such investigation in patients

with TBI. The renal assessment presents a unique opportunity to fill this gap at minimal cost.

Methods

Inclusion and exclusion criteria for the renal assessment will be the same as for EPO-TBI. Degree of AKI post trauma will be categorised using the GFR and urine output criteria of the RIFLE classification system[43]. Maximal change in serum creatinine from baseline in the first 7 days post-trauma will be used and urine output data will be collected for 7 days while in ICU. Outcome criteria 'Loss of kidney function' and 'End stage renal failure' will not be used. The RIFLE criteria have been validated in critically ill populations[7,54,55]. Creatinine collection will continue for 21 days to allow assessment of treatment / post treatment effect, and duration of AKI (Risk/Injury/Failure). The patient's weight will be estimated, if not known, to calculate urine output data in ml/kg/hr. Data will also be collected on the need for Renal Replacement Therapy / Dialysis while in hospital and days when this is received as it affects creatinine level and will be assessed as an outcome.

Power Calculations and Sample Size

The EPO-TBI trial recruitment target is 606 patients in total.

With a minimum of 287 subjects per group this study will have an 80% power to show a difference in a continuously normally distributed outcome (Percent decline in eGFR from baseline to worst eGFR up to day 7 OR maximal change in creatinine from baseline to day 7), equivalent to 24% of one standard deviation with a 2-sided p-value of 0.05. Given one standard deviation is equivalent to about 24% across the range of data, this difference could be thought to equate to a difference of approximately 6%. Similarly, with 287 subjects per trial group receiving EPO/ placebo, EPO-AKI will have an approximately 86% power to show a change in proportion from 9% to 3% with a two sided p-value of 0.05. We estimate that 9% of TBI patients will develop AKI (Risk/Injury/Failure) based on a preliminary study.

Outcomes

<u>Primary outcomes</u>: Severity of AKI measured as increase in serum creatinine / change in eGFR from baseline to peak value in the first 7 days post-trauma.

<u>Secondary outcomes:</u>

- Cumulative proportion in Risk/Injury/Failure RIFLE categories in those receiving EPO compared to the control group. AKI will be classified using maximal change in serum creatinine from baseline in the first 7 days post-trauma; and urine output data collected for 7 days or until ICU discharge (whichever occurs first).
- Duration of AKI measured as AKI free days (number of days not in Risk/Injury/Failure categories) at 21 days after randomisation.

Analysis of Results

Data will be analysed using Intercooled Stata, version 9 and above (Statacorp, Tx, USA). Continuous outcomes will be assessed for normality and log-transformed where appropriate. Univariate analysis will be conducted using appropriate tests. Multivariate analysis will be performed adjusting for baseline imbalances and known covariates. Where sufficient data exists multiple logistic regression will be used for AKI as a binary outcome (No-AKI versus Risk/Injury/Failure). Multivariate analysis on log-transformed biomarkers will be performed using multiple linear regression, while time to event data will be analysed using Cox regression and displayed with Kaplan Meier curves. A two sided p-value of 0.05 will be considered statistically significant.

Significance

EPO-AKI will provide unique prospective information on the incidence, severity and duration of AKI in patients with TBI. It also presents a unique opportunity to investigate the benefit of EPO (a potential kidney protective therapy for AKI) to decrease the occurrence, severity and duration of AKI in TBI patients at minimum cost.

11.2.2 EPO-Biomarkers

This is a more detailed assessment of the effects of EPO on renal biomarkers in patients with TBI to be conducted only at The Alfred and The Royal Melbourne hospitals in Melbourne, Victoria.

Aims

The specific aim of EPO-Biomarkers is to determine whether in patients with TBI:

a) EPO therapy reduces the release of biomarkers NGAL, cystatin-C and L-FABP compared to their release in those receiving placebo
b) The release of these biomarkers predicts the occurrence, severity, duration of and recovery from AKI

Background

Levels of NGAL, cystatin C and L-FABP rise early in AKI compared to creatinine[56-58]. No study has analysed these biomarkers in patients with TBI, or studied their response to therapy over a period of up to 72 hours. If their value as early renal biomarkers is confirmed in patients with TBI and other populations, they may change the definition, diagnosis, prognosis and timing of therapy for AKI in TBI. The EPO-TBI trial provides a unique opportunity to analyse the biomarker response to EPO which could elucidate mechanisms involved in the body's response to this therapy.

Methods

Inclusion and exclusion criteria for EPO-Biomarkers will be the same as for EPO-TBI. Urine and blood specimens will be collected at baseline (after randomisation and before the first dose of study drug) and at 24, 48 and 72 hours after first dose of EPO/placebo, from the first 50 participants recruited between The Alfred and The Royal Melbourne hospitals. Renal biomarker levels in some samples will be measured with point-of-care assays and standardised clinical laboratory platforms, and remaining samples will be processed, freeze-stored and later transported for measurement to Monash Medical Centre laboratory (cystatin C) and Dr Yasushi Takigawa CIMC Co. laboratories, Tokyo, Japan (L-FABP). Investigation into novel biomarkers is at an explosive stage, and other compounds may in a short space of time emerge as solid predictors of renal function. In view of this, two samples of urine and plasma for all 4 time-points from each participant will be stored and kept for future measurement of other biomarkers.

Power Calculations and Sample Size

With 50 subjects from the EPO-TBI trial, EPO-Biomarkers will have an 80% power to detect a correlation coefficient of $r = 0.4$ between continuous variables on any given day with a two-sided p-value of 0.05. Whilst the addition of repeat measures data will increase the power to detect relationships between continuous variables, as the within patient correlation between data points is unknown, it is impossible to calculate the exact increase in power.

By considering a change in values between days 1 and 3, with 25 subjects per group EPO-Biomarkers will have an 80% power to detect a difference between groups in continuously normally distributed variables, equivalent to 80% of one standard deviation with a two-sided p-value of 0.05. As additional repeat measures will once again increase the power of the study, this is a conservative estimate.

Outcomes

Primary outcomes: Levels of biomarkers NGAL, cystatin C and L-FABP in those receiving EPO compared to the control group; and comparison of urine and serum samples.

Secondary outcomes: Relationships between biomarkers and change in eGFR, serum creatinine, RIFLE classes Risk/Injury/Failure, and survival.

Analysis of Results

Data will be analysed using Intercooled Stata, version 9 and later (Statacorp, Tx, USA) and assessed for normality and log-transformed where appropriate. We anticipate that biomarkers (NGAL, cystatin C, L-FABP) will be well approximated by a log-normal distribution. Multivariate analysis will be performed adjusting for baseline imbalances and known covariates using repeat measures analysis of variance. We will determine changes in response over time between treated / untreated groups by fitting an interaction between treatment and time. Relationships between biomarkers and change in serum creatinine, RIFLE classes Risk/Injury/Failure, and survival, will be determined using logistic regression and reported using area under the curve for receiver operating characteristic curves.

Significance

EPO-Biomarkers will provide unique information on the pattern of renal biomarker release in response to EPO and in AKI, in patients with TBI. The EPO-TBI trial provides a unique opportunity to investigate potential kidney protective therapies and new diagnostic approaches to AKI in TBI at minimum cost.

12. BRAIN INJURY MARKERS IN SERUM AND CSF SUB-STUDY

Hypothesis

In patients with moderate and severe TBI, administration of EPO will improve neurological outcome and reduce the release of markers of brain injury in cerebrospinal fluid (CSF) and blood serum.

This sub-study will be conducted at **The Alfred Hospital** only, in the basic research laboratory at the National Trauma Research Institute in collaboration with clinicians managing the Australian multicentre randomised clinical trial, Erythropoietin in Traumatic Brain Injury (EPO-TBI) through the Australian and New Zealand Intensive Care-Research Centre (ANZIC-RC) at the School of Public Health, Monash University, and also in collaboration with the Australian New Zealand Intensive Care Society-Clinical Trials Group (ANZICS-CTG).

Background

We have shown previously that classical brain injury protein markers correlate with brain lesion size, and neurological outcome in TBI patients[59]. In the stroke and schizophrenia clinical trials, EPO administration was associated with improvement of outcome and a decrease in serum S-100 levels[22].

In this sub-study we propose to monitor the release of multiple injury markers: S-100 reflecting glial activation, NSE indicative of neuronal damage and MBP a marker of myelin damage, which are measured in CSF and serum of EPO and placebo treated patients which are then correlated with neurological outcome scores using the 6 month GOSE.

Methods

Inclusion and exclusion criteria for the Brain Injury Markers in Serum and CSF Sub-study will be the same as for EPO-TBI. A total of 80 TBI patients with moderate and severe brain injury will be recruited at the Alfred for this sub-study of the EPO-TBI trial. The analysis will be performed on two groups of patients: 40 receiving EPO and 40 receiving placebo. Equal group size is guaranteed by stratification by site, severity of TBI, and a small block allocation schedule.

The injury markers S-100, NSE and MBP are measured in serum and CSF (if available) using commercial ELISA kits beginning for 5 days after TBI as described previously by our group[59].

Blood samples will be collected daily from all patients with moderate and severe brain injury, whereas CSF will be collected from the drainage bags of those patients who have had an external ventricular drain inserted as part of standard care.

CSF (up to 10 ml) is drained when ICP is above 20 mmHg and collected daily into drainage bags kept at 4°C to minimise protein degradation. Drainage bags are changed every day (09:00 am) until catheter removal. CSF samples are spun at 2000g and aliquots stored at -70°C. Blood samples (10 ml) are collected at the same time and processed as described for CSF. Samples will be collect daily for 5 days or up to removal of the external ventricular drain.

Patients' neurological outcome is assessed at 6 months following TBI using the GOSE and will be provided by the clinicians involved in the EPO-TBI trial.

Power Calculations and Sample Size

Our preliminary data based on MBP measurements showed a r=0.4542 of Spearman Rank Correlation Coefficient between concentration of MBP in serum and GOSE. Based on this, a study of 40 patients will have >80% power with r=0.45 and a two sided p-value of 0.05.

Outcomes

The purpose of these experiments is to explore the ability of EPO treatment to improve neurological deficit coinciding with the attenuation markers of brain damage in TBI patients. We will assess potential correlations between the levels of such markers and the neurological outcome scores based on the GOSE assessed at 6 months post-TBI. The utility of having these measurements may reveal applicable in future clinical trials as prognostic tool of long term neurological outcome.

13. ETHICS

13.1 Guiding Principles

This study is to be performed in accordance with the ethical principles of the Declaration of Helsinki (June 1964 and amended 1975, 1983, 1989, 1996, 2000, 2008 and Note of Clarification 2002 and 2004), ICH GCP Notes for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with Therapeutic Goods Administration comments, NHMRC National Statement on Ethical Conduct in Research Involving Humans (March 2007); the New Zealand Interim Good Clinical Research Practice Guidelines (Volume 2 1998 and Volume 3 2000) and ICH GCP Notes for Guidance on Good Clinical Practice (CPMP/ICH/135/95).

13.2 Ethical Issues of the Study

The three ethical considerations in this study are:

- The enrolment of participants who are unable to consent for themselves, as discussed in Section 12.5 Informed Consent
- Confidentiality of patient data outside the participating institution
- FDA temporary hold on neuroprotective trials in 2009

Informed consent

Patients with moderate or severe TBI will not have capacity to provide informed consent. The patient's legal surrogate will be approached to provide consent for the patient.

Confidentiality of patient data outside the participating institution

Outcome data will be collected from patients in ANZ by a trained central assessor from Monash University at the 6 month follow up. The GOSE, QoL questionnaires and data for cost effectiveness analysis will be collected. The process of forwarding patient details to a central assessor at Monash University for the purpose of follow up data collection has proven successful in other ANZICS CTG studies; SAFE TBI[10] and the NICE[60] trials.

FDA temporary hold

The FDA had a temporary hold on the use of erythropoietin in neuroprotection trials in 2009 based on the data from an adult German stroke trial of EPO neuroprotection (ClinicalTrials.gov NCT00604630) suggesting excess mortality in patients receiving EPO. After investigation FDA approved the amendment to Dr Robertson's protocol for the lower dose of EPO (40,000 units given weekly for up to 3 doses) on 25 February 2009 (Dr Robertson, personal communication). The reasons for the decision were: 1. Subjects in the German stroke study received tissue plasminogen activator (tPA) as part of the standard management for ischemic stroke. 2) the excess deaths occurred with patients treated with tPA, 3) the age of the stroke and TBI patients is different 4) large clinical trials in critically ill patients do not report excess mortality with EPO according to the recent meta-analysis[40], and 5) the DSMB on Review of Dr Robertson' s TBI Study on Sept. 10, 2008 had no safety concerns. Therefore it is evident that no safety concerns regarding EPO with TBI patients have been raised.

13.3 Ethics Committee Approval

Each participating site will submit this protocol and any other relevant study documentation to the responsible local or national constituted Human Research and Ethics Committee (or equivalent). Approval of the protocol, plans for obtaining consent, and related documents will be obtained prior to the start of the study at each site.

It is the principal investigator's responsibility to ensure that all conditions for approval of the study are met and that amendments to the protocol or serious adverse events are also reported to the HREC (or equivalent) as required by that committee.

This protocol will also be submitted to the Guardianship Board or similar where this is necessary for NOK (legal surrogate/ legal guardian) to consent for the patient to take part in the study. It is the responsibility of the site principal investigator to obtain approval from the relevant body prior to obtaining third-party consent to take part in this study.

13.4 Confidentiality of Patient Data

Patients will be randomised via a secure website and will be allocated a unique study number. The site research coordinator will compile an enrolment log including the patient's name, date of birth, hospital identification number, unique study number and date and time of randomisation. Subsequent data will be identified by the unique study

number only. The enrolment log and study data will be kept separately. Follow up details of the patient and their family will be collected including name, address and contact telephone numbers. The contact details will be forwarded to the central assessor in the Department of Epidemiology and Preventative Medicine at Monash University. The central assessor at Monash will perform the follow up GOSE, QoL survey at 6 months in ANZ and collect data for cost effectiveness analysis at 6 months from compensable patients in Victoria (Australia) and New Zealand. A central assessor at the King Fahad National Guard Hospital will perform the follow up GOSE and QoL survey from patients enrolled at that hospital. All data collected in the follow up assessments will be identified by the unique study number only. Study data will be entered into a password protected database managed by the CCRET (Monash University) and ANZIC-RC. No identifying data will be entered into the database. The contact details and study data will be kept in a locked office at the study site and in the central assessor's locked office at Monash University and King Fahad National Guard Hospital.

13.5 Informed Consent

The NHMRC National Statement on the Ethical Conduct of Research in Humans (March 2007) acknowledges in Chapter 4.4 that research involving patients who are heavily dependent on medical care, such as the patients in this study, is necessary to assess and improve the efficacy and safety of interventions used in their treatment. Patients with moderate or severe head injury will not be able to provide informed consent.

Consent will be obtained from the participant's parent or guardian, or legal surrogate or organisation authorised by law (NS 4.4.10). The criteria of who may give consent for a patient to take part in medical research (and the term with which they may be described) varies at participating sites. For the purposes of this protocol, the descriptor 'legal surrogate' describes the person who is legally allowed to give consent for the patient.

The legal surrogate will be invited to consent for the patient after a verbal presentation, the person responsible information and consent form has been read and there has been time for thought, reflection, questions and consultation with others. If the legal surrogate provides consent for the patient to take part in the study, they will be asked to sign the consent form.

In cases where the legal surrogate cannot attend the hospital to sign the consent form within the time constraints of the study, consent for patient participation in the study may be obtained over the telephone in accordance with local HREC guidelines. Telephone consent requires two people, one of whom must be a member of the research staff, to witness the consent statement from the legal surrogate. The telephone conversation must be documented in the patient's medical record and signed by both witnesses to the consent consultation. As soon as the legal surrogate is able to attend the hospital they will be asked to sign a consent form and note that telephone consent was already provided.

In cases where the legal surrogate cannot be determined or contacted, the patient may be enrolled without prior consent, in accordance with the local HREC approval and legislation (as per NS 4.4.13). The patient will be enrolled with a waiver of consent (or Procedural Authorisation in Victoria) and consent obtained from the legal surrogate as soon as possible. Procedural Authorisation is not applicable to participant under 18 years of age.

The legal surrogate will be able to withdraw their consent for the patient to participate in the study at any time without any reduction in quality of care, and if they choose to withdraw the patient, permission will be asked to use the data collected up to that time.

Patients who recover sufficiently to consent for themselves will be asked to consent to continue in the study or offered the chance to withdraw (as per NS 4.4.14). If the patient

chooses to withdraw from the study, they will be asked for permission to use their data up to the time of withdrawal.

All interaction between research staff and potential or actual participants and their legal surrogate will take into consideration the stress or emotional factors associated with critical illness and ensure that the dependency of potential participants and their relatives on medical personnel providing treatment does not compromise the freedom of decision making to participate (as per 4.4.11).

14. DATA MANAGEMENT

14.1 Data Collection Methods

All data will be collected by trained staff at each study site using a paper source document developed by the coordinating centre. Data will then be entered into a web database designed by the CCRET. Data queries will be automatically generated via the electronic data collection database.

Randomised patients will be followed up to death or 6 months post-randomisation. Data collection will be restricted primarily to those variables necessary to define clinical patient characteristics including: baseline demographics, primary diagnoses, physiological parameters, diagnostic interventions, therapeutic interventions and documentation of deaths and other serious adverse events.

Patients and/or their legal surrogate will be asked to provide three possible points of contact (home and close family contact details) to the research staff prior to discharge. Full protocol data will be collected in all patients including those excluded at any stage. Patients (or a proxy – generally a close family member) who are alive at 6 months after randomisation will be interviewed by a trained assessor from Monash University or King Fahad National Guard Hospital (for patients in KSA). The central assessor will use a standardized structured telephone questionnaire[61] to measure the eight-grade GOSE[62], QOL assessments EQ-5D[63] and SF-12[64]. The central assessor at Monash will collect data for cost effectiveness analysis for compensable patients in Victoria (Australia) and New Zealand. Neurological outcomes will then be defined as favourable (GOSE 5 to 8; moderate disability and good recovery) or unfavourable (GOSE 1 to 4; death and severe disability). Patients subsequently withdrawn for any reason (i.e. DVT, PE, MI, CVA, Cardiac Arrest, or other SAE) or did not receive study drug will be followed up, according to the study follow-up schedule and analysed on an intention-to-treat principle.

14.2 Data Variables Collected

Data collection will include:

Baseline

- Patient identifier(s)
- Baseline demographics
- VTE risk factors
- Concomitant anticoagulant medications
- APACHE II
- Glasgow Coma Score
- Injury Severity Score
- CT brain (Marshall score[65])
- Vital signs

- Haematology and coagulation
- Baseline renal function data
- Baseline compression Doppler ultrasound of the lower extremities

Dosing Days (1, 8 & 15)

- Administration of study drug
- Drug accountability

Daily Data

- Blood transfusion
- Brain injury management interventions
- Hb, platelet count, INR, APPT and creatinine
- VTE management
- Urine output (first 7 days during ICU stay)
- Serum creatinine and dialysis data for 21 days
- Study outcome events
- Adverse events
- Serious adverse events

DVT Surveillance

- Compression Doppler ultrasound X2 weekly during ICU admission

Hospital Discharge

- Mechanical ventilation days
- Length of stay
- Discharge destination
- Vital status at ICU and hospital discharge
- Treatment limitations/withdrawal
- Serious adverse events

6 Month Follow Up

- Vital status
- Serious adverse events

Outcome Data

- 6 month GOSE
- EQ-5D
- SF12

Health Economic Evaluation Data

- Clinical costings from compensable patients (Victoria and New Zealand)
- Length of rehabilitation stay
- Level of supported care

- Length of supported care stay

14.3 Data Management

14.3.1 Monitoring

The study will be monitored by a representative of ANZIC-RC. A site initiation teleconference or visit will be conducted before site activation; at least 2 routine monitoring visits will be conducted during the recruitment period; and a close out visit. Email and telephone communication will supplement site visits.

A monitoring report will be prepared following each visit and reviewed by the management committee. A follow up letter will be sent to the principal investigator and research coordinator at the site and will be filed in the site investigator file.

Medical records, any other relevant source documents and the site investigator files must be made available to the ANZIC-RC representative for these monitoring visits during the course of the study and at the completion of the study as needed.

A designated ANZIC-RC project officer will conduct the pharmacy monitoring and examination of pharmacy records and all communications and reports will be kept separate on a password computer to maintain the blind.

The aims of monitoring visits are to:

a) Check the accuracy of the data base by performing source data verification of the electronic CRF against the original source documents
b) Check for protocol deviations and report these to the chief investigator as necessary
c) Review primary and secondary outcome data available for each patient
d) Confirm the consent procedures approved by the site's HREC have been followed and view each original signed consent form
e) Check data security and access
f) Review all serious adverse events (SAEs) and follow up all reported SAEs
g) Review investigator site files for completeness and accuracy
h) Assist the study staff with any queries or problems they may have in relation to the study

14.3.2 Protocol Deviations

A protocol deviation is an unanticipated or unintentional departure from the expected conduct of an approved study that is not consistent with the current research protocol or consent document. A protocol deviation may be an omission, addition or change in any procedure described in the protocol.

Given that the investigator is responsible for patient safety and care he/she may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to trial patients without prior HREC approval. The implemented deviation or change must be reported in a protocol deviation form. The deviation must be reported via the study website by the principal investigator and reported to ANZICS-RC and HREC (if applicable).

15. STATISTICAL CONSIDERATIONS

15.1 Power Calculations and Sample Size

Efficacy of EPO to improve neurological function in TBI patients: Our prospective observational studies (n=900)[8,10] demonstrated unfavourable neurological outcome (death and severe disability) rates of 50% in Australian patients with moderate and severe TBI.

The potential size of the beneficial effect of EPO in TBI has been estimated at a 30% relative risk reduction (RRR) (15% absolute risk reduction, ARR) in unfavourable neurological outcomes at 6 months. This effect would be clinically important and is a conservative estimate based on:

- The recent demonstration of a 68% increase in favourable neurological outcomes at hospital discharge in patients after subarachnoid haemorrhage treated with EPO compared to placebo[24].
- The recent demonstration of a 36% increase in good outcomes (57% vs. 42%, $p<0.05$) at day 30 in acute ischemic stroke patients treated with EPO versus placebo[22].

Effect of EPO on the rate of thrombosis in TBI patients: The rate of proximal DVT formation in critically ill patients receiving pharmacological prophylaxis is 15%[66]. The thrombosis risk is very high in trauma patients and we estimate that the proximal DVT rate would be at least 18% in moderate or severe TBI, where the administration of pharmacological prophylaxis is frequently delayed due to bleeding concerns[35]. This conservative baseline DVT rate estimate is supported by the finding that 20% of patients with severe TBI, without prophylaxis develop DVTs[67]. A clinically relevant increase in proximal DVT rate with EPO therapy has been estimated as a 50% relative risk increase (9% absolute risk increase) in proximal DVT rates while actively screening patients in ICU. This increase would be clinically important and is plausible based on:

- Corwin found a clinically detected (i.e. no routine surveillance tests) 41% increase in thrombotic events among critically ill patients receiving EPO compared to placebo (16.5% vs. 11.5%; hazard ratio 1.41; p=0.008)[1].
- A sub-group exploration analysis of this study[18], which reported a 58% increased rate of clinically detected thrombotic events (again no screening for DVT) in the EPO group compared to placebo, in patients not receiving heparin VTE prophylaxis (20.3% vs. 12.8%; hazard ratio 1.58; 95%; p=0.008)[1].

Study sample size: Given the above considerations, a trial of 574 patients will have a 90% power to detect a 14% absolute risk reduction (50% vs. 36%) [a 28% relative risk reduction], and an 80% power to detect a 24% relative risk reduction (50% vs. 38%) in 6 month unfavourable neurological outcomes (two sided alpha 0.05).

In addition a trial of 574 patients will also have an 80% power to detect a 9% Absolute Risk Increase in proximal DVT rates (50% relative risk increase, conservatively based on previous studies using insensitive diagnostic methods[1], the relatively high rates relating to trauma, and the frequent inability to administer pharmacological VTE prophylaxis in TBI), at a one sided alpha of 0.05. Such one-sided efficacy calculations are applicable, as the primary outcome can alternatively be regarded as assessable by a “non-superiority in the rate of DVT” design, with the same power and significance level. It is assumed that a decrease of any magnitude in the rate of DVTs with EPO would not pose any hazard to the patient. Allowing for a close to 5% withdrawal and loss to long term follow up rate (previous ANZICS-CTG loss to follow up rate and withdrawal of consent rate closer to

1%), we will recruit 606 patients and conduct an adequately powered intention to treat analysis. Should strong evidence of efficacy of EPO be identified, such a difference in neurological outcomes would be highly clinically significant and would likely lead to a widespread change in management of patients with TBI in Australia and internationally.

15.2 Minimising Bias

Blinding of drug: In addition to concealed random allocation all site personnel and ANZIC-RC staff will be blinded to treatment allocation except the site pharmacist; site unblinded dosing nurses; the ANZIC-RC unblinded project officer and the CCRET staff administering the web randomisation process. A designated pharmacist at each site will dispense the study drug. Study drug will be dispensed in a tamperproof sealed container to maintain the blind. The pharmacist will keep an accurate log of all study drug doses given to all patients.

Haemoglobin levels: Previous large multi-centre trials demonstrated that EPO in the acute critically ill patient population does not elevate haemoglobin levels to clinically detectable levels above placebo[1]. Therefore, the blood haemoglobin concentration will not lead to unblinding.

Data Quality: Several procedures to ensure data quality and protocol standardisation will also help to minimise bias. These include: i) a full day investigator meeting will be held for all principal investigators and lead research coordinators prior to study commencement to ensure consistency in procedures; ii) a site initiation teleconference or visit will be conducted prior to site activation by ANZIC-RC to provide protocol training for all site staff including the unblinded dosing nurses; iii) training will be provided for the study pharmacists at the site initiation conference or visit and a detailed Pharmacy Manual will be provided; iv) training sessions will be held for the site coordinating ultrasonographer at the site initiation teleconference or visit and as outlined in APPENDIX 4 - Assessment of Deep Venous Thrombosis (DVT); v) a detailed EPO-TBI Operations Manual will be provided for the investigators and research coordinators; vi) the project manager will conduct at least one monitoring visit at each site during the recruitment period to verify source data and provide re-training as necessary; vii) a designated study unblinded project officer will be responsible for monitoring pharmacy records to verify correct treatment allocation; viii) Monash University CCRET will maintain the electronic database

Analysis of the outcome of excluded patients due to other trials etc. according to the CONSORT guidelines.

15.3 Minimising Risks to Safety of Participants

Critical illness predisposes to thrombosis: Patients in EPO-TBI may be at risk of minor or major thrombosis due to both their critical illness and perhaps due to EPO therapy.

Unblinding of CT and Doppler ultrasound results: To ensure appropriate treatment; participants must be monitored closely and investigation results must be known to the treating clinical staff. As patient safety is paramount, the results of CT and Doppler ultrasound scans will be available to treating staff. However, clinical staff will be blinded as to the patient's treatment allocation.

15.4 Analysis of Results

Independent senior statisticians at Monash University Department of Epidemiology and Preventive Medicine will perform data analysis on an intention-to-treat basis. Baseline and outcome variables will be compared using Chi-square tests for equal proportion, Student's t-test for normally distributed outcomes and Wilcoxon rank-sum tests otherwise. Multivariate models adjusting for baseline imbalances and known covariates will be

performed using logistic regression. A p-value of 0.05 will be considered to be statistically significant.

15.5 Interim Analysis

A group sequential statistical approach will be used to perform two equally-spaced interim analyses (at 33% and 66% of total recruitment) to assess accumulated safety data (differential proportions of DVT and total mortality). The Haybittle-Peto criterion (|Zk|>=3) for early stopping will be applied at these first and second analyses. Assuming no early stopping, the final analyses at full recruitment will be little affected by these interim analyses (final critical value |Zk|>=1.975 rather than 1.960).

16. SAFETY

16.1 Data and Safety Monitoring Committee

An independent Data and Safety Monitoring Committee (DSMC), comprising experts in clinical trials, biostatistics, neurosurgery and intensive care medicine will be established before patient enrolment to review all trial protocols.

The DSMC will conduct two planned interim safety analyses, at one-third (~202) and two-thirds (~404) of the specified overall (606) patient recruitment target. The DSMC will assess at both of these interim analyses at least two outcomes, namely (i) the differential proportion of all-cause mortality between the two treatment groups (a safety assessment of the trial primary outcome), and similarly (ii) the differential proportion of patients with proximal deep venous thromboses (DVT) detected by screened compression Doppler ultrasound (a safety assessment of the trial secondary outcome part [e]).

The DSMC will assess as well the differential cumulative “serious adverse events” reports received to each interim analysis time point. Consistent with the advice of Cook et al[68], adverse events already defined and reported as study outcomes (DVT, PE, other thrombotic events, MI, stroke, cardiac arrest or ventricular fibrillation) will not be labelled and reported a second time as serious adverse events.

The DSMC will provide assistance in the preparation of any Adverse Drug Reaction Reports to the Therapeutic Goods Administration (TGA) in Australia, the Centre for Adverse Reactions Monitoring (CARM) under the guidance of the Medicines Adverse Reactions Committee (MARC) in New Zealand and the Saudi Food and Drug Authority (SFDA) in the Kingdom of Saudi Arabia. If it becomes necessary to unblind a study participant in order to make an expedited report to the TGA or CARM or SFDA, the DSMC will prepare the report.

The DSMC may, in its absolute discretion, request assessment of any other trial data at any time.

16.2 Adverse Events

Adverse events (AEs) are defined as any untoward medical occurrence in a patient or clinical investigation subject administered an investigational intervention and which does not necessarily have to have a causal relationship with this treatment (adapted from the *Note for Guidance on Clinical Safety Data Management: Definitions and Standards for Expedited Reporting (CPMP/ICH/377/95* July 2000).

It is recognised that the patient population with TBI will experience a number of common aberrations in laboratory values, signs and symptoms due to the severity of the underlying disease and the impact of standard therapies. These will not necessarily constitute an adverse event unless they require significant intervention or are considered to be of concern in the investigator’s clinical judgement.

In all cases, the condition or disease underlying the symptom, sign or laboratory value should be reported e.g. renal failure rather than hyperkalaemia, and agitation rather than self-extubation.

16.3 Serious Adverse Events

Definition of a SAE

SAEs are defined in accordance with the Note for Guidance on Clinical Safety Data Management: Definitions and Standards for Expedited Reporting (CPMP/ICH/377/95) (July 2000) as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event which may require intervention to prevent one of the previously listed outcomes

16.4 Reporting

Adverse event and serious adverse event reporting

Adverse events and serious adverse events will be recorded on an online separate case report form.

SAEs which occur from the time of commencement of study treatment to 6 months after the first dose will be reported to the coordinating centre (ANZIC-RC) via the online SAE form. SAEs should be reported to the ANZIC-RC within 24 hours of study staff becoming aware of the event. Minimum information to report will include:

- Patient initials and study number
- Nature of the event
- Commencement and cessation of the event
- An investigator's opinion of the relationship between study involvement and the event (unrelated, possibly, probably or definitely related).
- Whether treatment was required for the event and what treatment was administered.

Web address: **https://epo-tbi.anzicrc.monash.org**

Telephone Numbers:

ANZIC-RC: + 61 3 9903 0513

ANZIC-RC: +61 437 207 413

Chief Investigator: + 61 3 9496 5992

SAEs must be reported online via the study website. The event and report may be discussed with the ANZIC-RC staff or chief investigator if necessary.

Coordinating centre staff will be responsible for following-up SAEs to ensure all details are available. This may involve follow-up within a limited time frame (8-15 calendar days) if the event meets criteria for expedited reporting.

SAEs which are both unexpected and have a causal relationship with the study drug will be reported as Adverse Drug Reactions by the DSMC and Coordinating Centre to the TGA, CARM and the SFDA. These reports will then be forwarded to all sites for reporting to their HRECs as required.

It is the responsibility of each site to inform their HREC of all SAEs which occur at their site, in accordance with local requirements.

Consistent with the advice of Cook et al[68], adverse events already defined and reported as study outcomes (DVT, PE, other thrombotic events, MI, stroke, cardiac arrest or ventricular fibrillation) will not be labelled and reported a second time as serious adverse events.

17. FUNDING

The EPO-TBI study is funded by project grants from the National Health and Medical Research Council (NHMRC - Project grant no. 545902) and the Victorian Neurotrauma Initiative (VNI). The ANZIC-RC will supply infrastructure and administrative support.

18. PUBLICATION

The study will be conducted in the name of the EPO-TBI investigators, the ANZIC-RC and the ANZICS CTG. The central project coordination and data management will be provided by the ANZIC-RC at Monash University, Melbourne. The principal publication from the study will be in the name of the EPO-TBI Investigators with full credit assigned to all collaborating investigators, research coordinators and institutions. Where an individuals' name is required for publication it will be that of the writing committee, with the chair of the writing committee listed first and subsequent authors listed alphabetically.

Funding bodies will be acknowledged in the publication.

19. RESEARCH TIMELINES

Timeframe Indicator	Milestone
January 2008	ANZICS CTG endorsement
March 2008	NHMRC funding application submitted
October 2008	NHMRC funding application approved
January 2009	Commence study organisation
April 2009	Protocol finalised
April 2009	The Alfred HREC submission
September 2009	Participating sites finalised
Sept/Oct 2009	Participating sites HREC applications
December 2009	Investigator Meeting
February 2010	Participating sites HREC approval
May 2010	Study recruitment commences
July 2011	1st interim analysis
September 2012	2nd interim analysis
November 2013	Patient recruitment completed
May 2014	6 month follow up of 100% completed
June 2014	Query resolution and data cleaning completed
June 2014	Database lock
July 2014	Primary analysis completed
August 2014	Manuscript completed

20. REFERENCES

1 Corwin HL, Gettinger A, Fabian TC, et al. Efficacy and safety of epoetin alfa in critically ill patients. N Engl J Med 2007; 357:965-976

2 Kiris I, Kapan S, Kilbas A, et al. The protective effect of erythropoietin on renal injury induced by abdominal aortic-ischemia-reperfusion in rats. Journal of Surgical Research 2008; 149:206-213

3 Goldfarb M, Rosenberger C, Ahuva S, et al. A role for erythropoietin in the attenuation of radiocontrast-induced acute renal failure in rats. Renal Failure 2006; 28:345-350

4 Simon F, Scheuerle A, Calzia E, et al. Erythropoietin during porcine aortic balloon occlusion-induced ischemia/reperfusion injury. Critical Care Medicine 2008; 36:2143-2150

5 Spandou E, Tsouchnikas I, Karkavelas G, et al. Erythropoietin attenuates renal injury in experimental acute renal failure ischaemic/reperfusion model. Nephrology Dialysis Transplantation 2006; 21:330-336

6 Davenport A. Renal replacement therapy in the patient with acute brain injury. American Journal of Kidney Diseases 2001; 37:457-466

7 Bagshaw SM, George C, Dinu I, et al. A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. Nephrology Dialysis Transplantation 2008; 23:1203-1210

8 Myburgh J, Cooper DJ, Finfer S, et al. Epidemiology and 12 month outcomes from traumatic brain injury in Australia and New Zealand. Journal of Trauma 2008; in press, accepted July 2006

9 The_SAFE_Study_Investigators. A comparison of albumin and saline for fluid resuscitation in the intensive care unit. N Engl J Med 2004:2247-2256

10 The_SAFE_Study_Investigators. Saline or albumin for fluid resuscitation in patients with traumatic brain injury. N Engl J Med 2007:874-884

11 Patel HC, Bouamra O, Woodford M, et al. Trends in head injury outcome from 1989 to 2003 and the effect of neurosurgical care: an observational study.[see comment][erratum appears in Lancet. 2006 Mar 11;367(9513):816]. Lancet 2005; 366:1538-1544

12 NIH_Consensus. Development Panel on Rehabilitation of Persons with Traumatic Brain Injury. Journal of the American Medical Association 1999:974-983

13 Kearsley D. Cost of medical care in traumatic brain injury in Victoria, Transport Accident Commission (TAC). Personal communication 2007

14 Murray GD, Teasdale GM, Braakman R, et al. The European Brain Injury Consortium survey of head injuries. Acta Neurochir (Wien) 1999; 141:223-236

15 Bramlett HM, Dietrich WD. Pathophysiology of cerebral ischemia and brain trauma: similarities and differences. J Cereb Blood Flow Metab 2004; 24:133-150

16 Yatsiv I, Grigoriadis N, Simeonidou C, et al. Erythropoietin is neuroprotective, improves functional recovery, and reduces neuronal apoptosis and inflammation in a rodent model of experimental closed head injury. FASEB J 2005; 19:1701-1703

17 Guidelines for the management of severe traumatic brain injury. J Neurotrauma 2007; 24 Suppl 1:S1-106

18 Edwards P, Arango M, Balica L, et al. Final results of MRC CRASH, a randomised placebo-controlled trial of intravenous corticosteroid in adults with head injury-outcomes at 6 months. Lancet 2005; 365:1957-1959

19 Maas AI, Murray G, Henney H, 3rd, et al. Efficacy and safety of dexanabinol in severe traumatic brain injury: results of a phase III randomised, placebo-controlled, clinical trial. Lancet Neurology 2006; 5:38-45

20 Lykissas MG, Korompilias AV, Vekris MD, et al. The role of erythropoietin in central and peripheral nerve injury. Clin Neurol Neurosurg 2007; 109:639-644

21 Coleman T, Brines M. Science review: recombinant human erythropoietin in critical illness: a role beyond anemia? Crit Care 2004; 8:337-341
22 Ehrenreich H, Hasselblatt M, Dembowski C, et al. Erythropoietin therapy for acute stroke is both safe and beneficial. Mol Med 2002; 8:495-505
23 Lu D, Mahmood A, Qu C, et al. Erythropoietin enhances neurogenesis and restores spatial memory in rats after traumatic brain injury. J Neurotrauma 2005; 22:1011-1017
24 Tseng M-Y. Richards H; Czosnya MH, P.J; Pickard, J.L; Kirkpatrick, P.J. Systemic erythropoietin therapy reduces impaired cerebral autoregulation after aneurysm SAH. Intracranial pressure 2007
25 Haljan GMB, A; King, M; Haigh, J; Culleton, B; Faris, P; Zygun, D. The erythropoietin neuroprotective effect: assessment in coronary artery bypass graft surgery (TENPEAKS) - a randomized, double-blind, placebo-controlled, proof of concept clinical trial. Crit Care 2006; 10:227
26 Corwin HL, Gettinger A, Pearl RG, et al. Efficacy of recombinant human erythropoietin in critically ill patients: a randomized controlled trial.[see comment]. JAMA 2002; 288:2827-2835
27 Geerts WH, Pineo GF, Heit JA, et al. Prevention of venous thromboembolism: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004; 126:338S-400S
28 Kakkar VV, Howe CT, Flanc C, et al. Natural history of postoperative deep-vein thrombosis. Lancet 1969; 2:230-232
29 Hirsch DR, Ingenito EP, Goldhaber SZ. Prevalence of deep venous thrombosis among patients in medical intensive care. Journal of the American Medical Association 1995; 274:335-337
30 Bohlius J, Wilson J, Seidenfeld J, et al. Erythropoietin or darbepoetin for patients with cancer. Cochrane Database Syst Rev 2006; 3:CD003407
31 Singh AK, Szczech L, Tang KL, et al. Correction of anemia with epoetin alfa in chronic kidney disease. N Engl J Med 2006; 355:2085-2098
32 Crowther MA, Cook DJ, Griffith LE, et al. Deep venous thrombosis: clinically silent in the intensive care unit. J Crit Care 2005; 20:334-340
33 Van der Meer P, Groenveld H, Januzzi JL, et al. Erythropoietin Treatment in Patients with Chronic Heart Failure: a meta-analysis. Heart 2009
34 Robertson MC, Cade J, Cooper J, Higgins A, McIsaac C, McArthur C, Nichol A, Presneill J, Syres G, Webb S. A point prevalance survey of venous thromboembolism prophylaxis management in critically ill patients in Australia and New Zealand. Anaesthesia and Critical Care 2007; 33:79
35 Scales D, Riva-Cambrin J, Le T, et al. Prophylaxis Against Venous Thromboembolism (VTE) in Neuro-Intensive Care Patients: Survey of Canadian Practice. 2007; Proceeding of the American Thoracic Society:A597
36 Corwin HL. Erythropoietin use in critically ill patients: forest and trees. CMAJ 2007; 177:747-749
37 Hebert PC, Stanbrook M. Indication creep: physician beware. Canadian Medical Association Journal 2007; 177:697, 699
38 Tonks A. Too much of a good thing. British Medical Journal 2007; 334:978-980
39 Cook D, Crowther M, Cook D, et al. Targeting anemia with erythropoietin during critical illness.[comment]. New England Journal of Medicine 2007; 357:1037-1039
40 Zarychanski R, Turgeon AF, McIntyre L, et al. Erythropoietin-receptor agonists in critically ill patients: a meta-analysis of randomized controlled trials. CMAJ 2007; 177:725-734
41 Cook D, Rocker G, Meade M, et al. Prophylaxis of Thromboembolism in Critical Care (PROTECT) Trial: a pilot study. Journal of Critical Care 2005; 20:364-372
42 Thygesen K, Alpert JS, White HD. Universal definition of myocardial infarction. J Am Coll Cardiol 2007; 50:2173-2195

43 Bellomo R, Ronco C, Kellum JA, et al. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Critical Care 2004; 8:R204-R212
44 Moore EM, Bellomo R, Nichol AD, et al. The incidence of acute kidney injury in patients with traumatic brain injury. Unpublished. 2010
45 Bagshaw SM, George C, Gibney RTN, et al. A Multi-Centre Evaluation of Early Acute Kidney Injury in Critically Ill Trauma Patients for the ANZICS Database Management Committee. Unpublished study.
46 Chertow GM, Burdick E, Honour M, et al. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. Journal of the American Society of Nephrology 2005; 16:3365-3370
47 Department of Human Services - Victorian Maintenance renal dialysis program [page on the Internet] [updated 2008 Oct 2; cited 2009 May 10]. Available from: http://www.health.vic.gov.au/renaldialysis/index.htm
48 Johnson DW, Pat B, Vesey DA, et al. Delayed administration of darbepoetin or erythropoietin protects against ischemic acute renal injury and failure. Kidney International 2006; 69:1806-1813
49 Yokomaku Y, Sugimoto T, Kume S, et al. Asialoerythropoietin prevents contrast-induced nephropathy. Journal of the American Society of Nephrology 2008; 19:321-328
50 Yatsiv I, Grigoriadis N, Simeonidou C, et al. Erythropoietin is neuroprotective, improves functional recovery, and reduces neuronal apoptosis and inflammation in a rodent model of experimental closed head injury. FASEB Journal 2005; 19:1701-1703
51 Australian New Zealand Clinical Trials Registry [site on the Internet] [cited 2009 Jun 30]. Available from: http://www.anzctr.org.au.
52 ClinicalTrials.gov. A service of the U.S. National Institutes of Health [site on the Internet] [cited 2009 May 17]. Available from: http://clinicaltrials.gov/ct2/results?term=erythropoietin+AND+acute+kidney+injury
53 Song YR, Lee T, You SJ, et al. Prevention of acute kidney injury by erythropoietin in patients undergoing coronary artery bypass grafting: a pilot study. American Journal of Nephrology 2009; 30:253-260
54 Hoste EA, Clermont G, Kersten A, et al. RIFLE criteria for acute kidney injury are associated with hospital mortality in critically ill patients: a cohort analysis. Critical Care 2006; 10
55 Ostermann M, Chang RW, Ostermann M, et al. Acute kidney injury in the intensive care unit according to RIFLE.[see comment]. Critical Care Medicine 2007; 35:1837-1843; quiz 1852
56 Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, et al. NGAL (neutrophil gelatinase-associated lipocalin) and cystatin C: are they good predictors of contrast nephropathy after percutaneous coronary interventions in patients with stable angina and normal serum creatinine? International Journal of Cardiology 2008; 127:290-291
57 Devarajan P. Emerging biomarkers of Acute kidney injury. In: Ronco C, Bellomo R, Kellum JA, eds. Acute Kidney Injury: Basel:Karger, 2007; 203-213
58 Nakamura T, Sugaya T, Node K, et al. Urinary excretion of liver-type fatty acid-binding protein in contrast medium-induced nephropathy. American Journal of Kidney Diseases 2006; 47:439-444
59 Pleines UE, Morganti-Kossmann MC, Rancan M, et al. S-100 beta reflects the extent of injury and outcome, whereas neuronal specific enolase is a better indicator of neuroinflammation in patients with severe traumatic brain injury. Journal of Neurotrauma 2001; 18:491-498
60 Finfer S, Chittock DR, Su SY, et al. Intensive versus conventional glucose control in critically ill patients. N Engl J Med 2009; 360:1283-1297

61 Wilson JT, Pettigrew LE, Teasdale GM. Structured interviews for the Glasgow Outcome Scale and the extended Glasgow Outcome Scale: guidelines for their use. J Neurotrauma 1998; 15:573-585
62 Teasdale GM, Pettigrew LE, Wilson JT, et al. Analyzing outcome of treatment of severe head injury: a review and update on advancing the use of the Glasgow Outcome Scale. J Neurotrauma 1998; 15:587-597
63 EuroQol. A standardised instrument for use as a measure of health outcome. 1990
64 Ware J J, Kosinski M, Keller SD. A 12-item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Medical Care 1996; 34:220-233
65 Marshall LF MS, Klauber Mr, et al. . The diagnosis of head injury requires a classification based on computed axial tomography. J Neurotrauma 1992; 9 Suppl 1:S287-292
66 Geerts WH, Jay RM, Code KI, et al. A comparison of low-dose heparin with low-molecular-weight heparin as prophylaxis against venous thromboembolism after major trauma. N Engl J Med 1996; 335:701-707
67 Kaufman HH, Satterwhite T, McConnell BJ, et al. Deep vein thrombosis and pulmonary embolism in head injured patients. Angiology 1983; 34:627-638
68 Deborah Cook MD, Francois Lauzier MD, Marcelo G Rocha MD, Mary Jane Sayles RN, Simon Finfer MD. Serious adverse events in academic critical care research. CMAJ 2008; 178:1181-1184

APPENDIX 1 - CRITERIA FOR ACUTE MYOCARDIAL INFARCTION

The term myocardial infarction should be used when there is evidence of myocardial necrosis in a clinical setting consistent with myocardial ischaemia. Under these conditions any one of the following criteria meets the diagnosis for myocardial infarction:

Detection of rise and/or fall of cardiac biomarkers (preferably troponin) with at least one value above the 99th percentile of the upper reference limit together with evidence of myocardial ischaemia with at least one of the following:

- Symptoms of ischaemia;
- ECG changes indicative of new ischaemia (new ST-T changes or new left bundle branch block [LBBB]);
- Development of pathological Q waves in the ECG;
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.
- Sudden, unexpected cardiac death, involving cardiac arrest, often with symptoms suggestive of myocardial ischaemia, and accompanied by presumably new ST elevation, or new LBBB, and/or evidence of fresh thrombus by coronary angiography and/or at autopsy, but death occurring before blood samples could be obtained, or at a time before the appearance of cardiac biomarkers in the blood.
- For percutaneous coronary interventions (PCI) in patients with normal baseline troponin values, elevations of cardiac biomarkers above the 99th percentile URL are indicative of peri-procedural myocardial necrosis. By convention, increases of biomarkers greater than 3 x 99th percentile URL have been designated as defining PCI-related myocardial infarction. A subtype related to a documented stent thrombosis is recognized.
- For coronary artery bypass grafting (CABG) in patients with normal baseline troponin values, elevations of cardiac biomarkers above the 99th percentile URL are indicative of peri-procedural myocardial necrosis. By convention, increases of biomarkers greater than 5 x 99th percentile URL plus either new pathological Q waves or new LBBB, or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium have been designated as defining CABG-related myocardial infarction.
- Pathological findings of an acute myocardial infarction

APPENDIX 2 - HEAD INJURY MANAGEMENT GUIDELINES

All interventions are ultimately at the discretion of the treating clinician.

LINES/FLUIDS

- Arterial line and central line in place
- CVP target 12 mmHg
- Crystalloid preferred resuscitation fluid, albumin preferably avoided.

VENTILATION/OXYGENATION

- S_aO_2 > 95%;
- P_aO_2> 90 mmHg;
- P_aCO_2 35-40 mmHg
- Transfusion threshold Hb 70- 100 g/L

PATIENT POSITION

- Position 15-30 degrees head up, avoiding venous obstruction at the neck
- Position flat if shock is prominent and this improves CPP

SEDATION

- Sedate as per clinicians choice
- Neuromuscular blockade as per clinicians choice (preferably reserved for difficult ICP control)

GLYCAEMIC CONTROL

- Maintain BSL 6-10 mmol/L

SERUM SODIUM

- Serum Na target 140-150 mmol/L

ICP MONITORING and CPP CONTROL

- Monitor ICP - EVD preferred, *Codman* parenchymal catheters acceptable
- ICP target < 20 mmHg
- CPP > 60 mmHg
- Refer to ICP Control Management Guidelines below

SEIZURE PROPHYLAXIS

- As per clinicians choice

VENOUS THROMBO-EMBOLISM PROPHYLAXIS

- Anti-embolism compression stockings and lower limb pneumatic compression devices should be used whenever clinically possible
- Timing and type of pharmaceutical VTE prophylaxis determined after 2nd CT brain scan and discussion with neurosurgical team
- Generally Enoxaparin 40mg subcutaneously is preferred
- An IVC filter may be considered if Enoxaparin contra-indicated or ineffective, or large thrombus detected
- DVT prophylaxis should be prescribed before discharge to the general ward
- VTE surveillance will continue on the general ward by clinical observation (standard care).
- Refer to APPENDIX 4 - Assessment of Deep Venous Thrombosis (DVT) and APPENDIX 5 - Pulmonary Embolism Management Guidelines for DVT and PE Guidelines

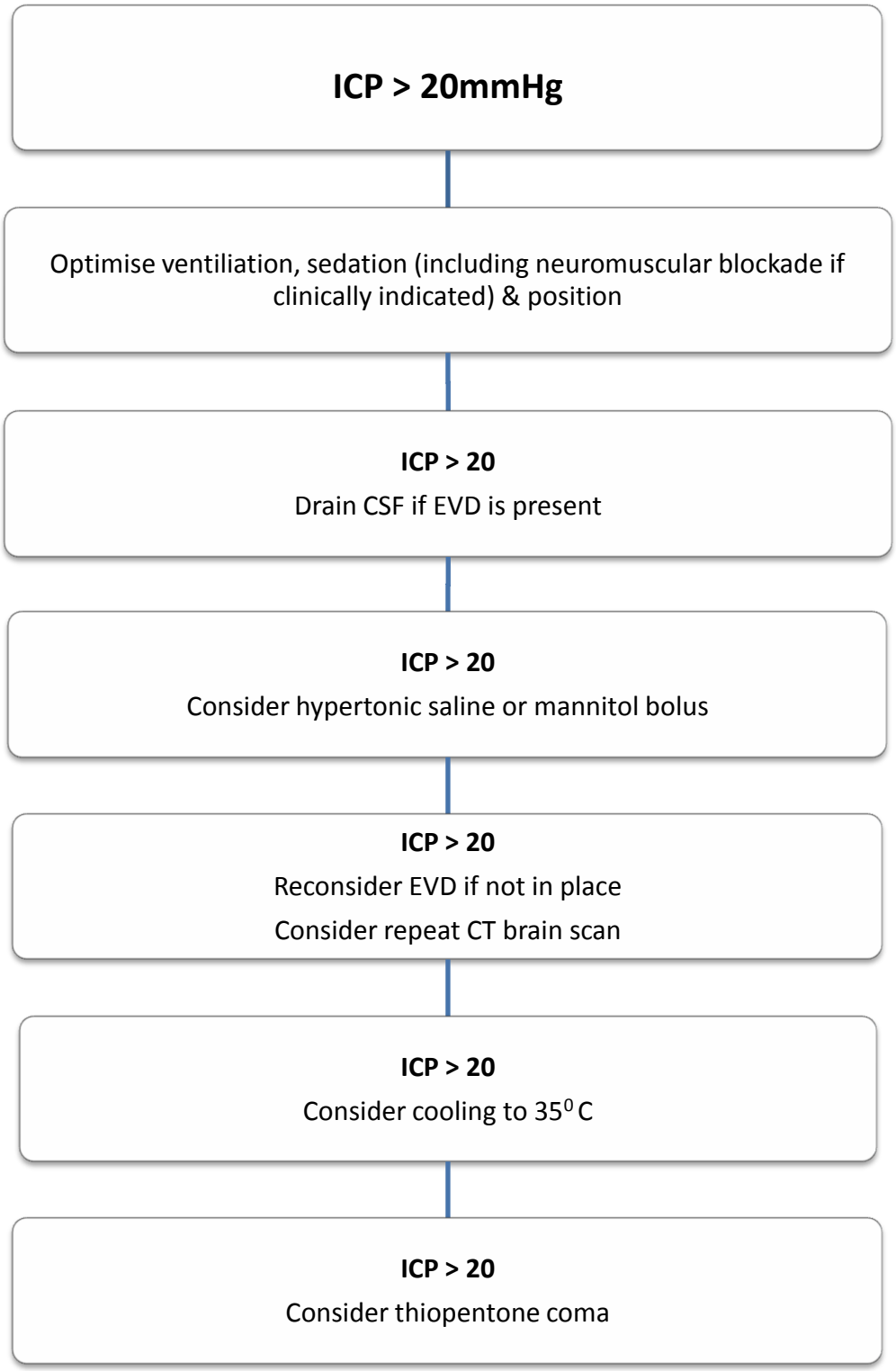
STRESS ULCER PROPHYLAXIS

- As per clinicians choice

NUTRITIONAL SUPPORT

- Aim to commence enteral nutrition within 24 hrs and achieve full nutrition within 72 hrs

ICP CONTROL MANAGEMENT GUIDELINES



APPENDIX 3 - APACHE II CALCULATION WORKSHEET

The APACHE II score is derived from 3 scoring systems: **Part A** – Acute Physiology Score, **Part B** – Age Points, **Part C** – Chronic Health Points

Part A - Acute Physiology Score

For each of the 12 physiological variables, select the most deranged value from the time of injury up to (but not including) the time of randomisation. Enter the value in the right hand column.

For exact non-integer data that is not found in any of the given ranges, round the figure up or down to the nearest whole number. Eg, 44 years and 3 months is rounded down to ≤ 44 years and assigned 0 points; a calculated MAP of 129.7 is rounded up to 130 and assigned 3 points. For integers of xx.5 always round upwards. This is an arbitrary decision but must be followed for every patient to ensure consistency.

1. **Temperature** – this should be a core temperature measurement (rectal, tympanic, oesophageal or via PAC). Where this is not possible, add 0.5^{0}C to the oral or axillary temperature
2. If mean arterial pressure (**MAP**) is not calculated by monitoring equipment, use the manual sphygmomanometer recording of systolic (SBP) and diastolic blood pressure (DBP) to obtain MAP using this equation MAP = [(DBP x 2) + SBP]/3
3. If the patient has an atrial arrhythmia, measure the ventricular response rate (R waves) only to record the heart rate.
4. *A – aD*O_2 is the difference between the calculated alveolar oxygen tension and the arterial oxygen tension. The alveolar oxygen tension is calculated by this equation: *A*O_2 = 713 x *Fi*O_2 – $PaCO_2$ x 1.25. The *Fi*O_2 here is expressed as a proportion of a unit. e.g. 100% *Fi*O_2 = 1 and 60% equals 0.6. If the *FI*O_2 (inhaled oxygen concentration) is greater than 50%, record the most deranged value for the *A – aD*O_2. If the *FI*O_2 is less than 50% record only the *Pa*O2 (arterial oxygen pressure). All measurements are in mmHg.

   A – aDO_2 calculation is [(FiO2 (713)-(PaCO2/0.8)]-PaO2
5. If ABGs have not been performed, choose the most deranged value for the serum venous bicarbonate (HCO_3) in place of the **arterial pH**
6. To obtain a score for the Glasgow Coma Scale (**GCS**) uses the GCS worksheet provided and subtract the GCS score from 15 to arrive at a score on the APACHE worksheet.

Whenever possible, make an attempt to obtain a score for each physiological variable. If one of the 12 variables is not available, assign 0 points and make a note of this absence on the APACHE II worksheet. The assumption being made is that a test or measurement was not ordered because the status of the patient did not warrant investigation, rather than the data was missing.

To complete **Part B** – assign points to the age range that the patient fits in to. eg, a 48 year old patient would be assigned 2 points.

To complete **Part C** – first decide if the patient meets any of the criteria provided on the worksheet for a history of severe organ insufficiency or immunocompromised. If there is no history, assign 0 points. If there is a history, assign points depending on whether the patient is a non-operative emergency admission or an emergency post-operative admission

Finally, add the points recorded for each of the 3 parts. The minimum score is 0 and the maximum score is 71. Keep the completed APACHE II worksheet in the patient CRF worksheet folder for this patient. It may be used for quality assurance measures. You will therefore need to print your hospital ID, Patient Initials and Patient Study Number on the APACHE worksheet.

BASELINE DATA	APACHE II Severity Of Disease Classification	Hospital ID: |__|__|__| Patient Study Number |__|__|__|__|__|__|

A. ACUTE PHYSIOLOGY SCORE (APS)										APS Scores
PHYSIOLOGIC VARIABLE	High Abnormal Range					Low Abnormal Range				
	+ 4	+ 3	+ 2	+ 1	0	+1	+2	+3	+4	
Temperature – rectal (^{o} C)	≥ 41	39 – 40.9		38.5 – 38.9	36 – 38.4	34 - 35.9	32 – 33.9	30 – 31.9	≤ 29.9	
Mean arterial pressure – mmHg	≥ 160	130 - 159	110 – 129		70 - 109		50 - 69		≤ 49	
Heart rate (ventricular response)	≥ 180	140 – 179	110 – 139		70 - 109		55 - 69	40 - 54	≤ 39	
Respiratory rate (non-ventilated or ventilated)	≥ 50	35 – 49		25 - 34	12 - 24	10 - 11	6 - 9		≤ 5	
Oxygenation: A - aDO_2 or PaO_2(mmHg) a. if FIO_2 ≥ 0.5 record A - aDO_2	> 500	350 – 499	200 – 349		< 200					
b. if FIO_2 < 0.5 record only PaO_2					PO_2 -> 70	PO_2 61 - 71		PO_2 55-60	PO_2 < 55	
Arterial pH	≥ 7.7	7.6 – 7.69		7.5 – 7.59	7.33 – 7.49		7.25 – 7.32	7.15 – 7.24	< 7.15	
Serum sodium (mMol/L)	≥ 180	160 – 179	155 – 159	150 - 154	130 - 149		120 - 129	111 - 119	≤ 110	
Serum potassium (mMol/L)	≥ 7	6 – 6.9		5.5 – 5.9	3.5 – 5.4	3 – 3.4	2.5 – 2.9		≤ 2.5	
Serum creatinine (mMol/L) (double point score for acute renal failure)	≥ 0.300	0.171-0.299	0.121-0.17		0.05-0.12		< 0.05			
Haematocrit (%)	≥ 60		50 – 59.9	46 – 49.9	30 – 45.9		20 – 29.9		< 20	
White blood count (total/mm^3) (in 1,000s)	≥ 40		20 – 39.9	15 – 19.9	3 – 14.9		1 – 2.9		< 1	
Glasgow Coma Score (GCS) (Score = 15 minus actual GCS)										
Serum HCO_3 (venous – mMol/L) (**Only use this if no ABGs available**)	≥52	41 – 51.9		32 – 40.9	22 – 31.9		18 – 21.9	15 – 17.9	<15	

. AGE POINTS		
Assign points to age as follows:	**Age (yrs)**	**Points**
	☐ ≤ 44	**0**
	☐ 45–54	**2**
	☐ 55–64	**3**
	☐ 65–74	**5**
	☐ ≥ 75	**6**

C. CHRONIC HEALTH POINTS			
If patient has history of severe organ system insufficiency or is immune–compromised, assign points as follows:	**Points**	**DEFINITIONS:** Organ insufficiency or immuno-compromised state must have been evident **prior** to this hospital admission and conform to the following criteria:	
		LIVER	Biopsy proven cirrhosis & documented portal hypertension (PH); episodes of upper GI bleeding due to PH; or prior episodes of hepatic failure/encephalopathy/coma
☐ **a**. for non-operative or emergency post-operative patients	**5**	**RENAL**	Receiving chronic dialysis
		CARDIOVASCULAR	New York Heart Association Class IV
		RESPIRATORY	Chronic restrictive, obstructive or vascular disease resulting in severe exercise restriction (i.e unable to climb stairs, perform household duties); or documented chronic hypoxia hypercapnia, 2° polycythemia, severe pulmonary hypertension (>40mmHg) or respiratory dependency
☐ **b**. for elective post-operative patients	**2**	**IMMUNOCOMPROMISED**	Patient has received therapy that suppresses resistance to infection, eg. immuno-suppression, chemotherapy, radiotherapy, long term or recent high dose steroids, or has a disease sufficiently advanced to suppress resistance to infection (eg leukaemia, lymphoma, AIDS)

APACHE II SCORE - a sum of:	**A.** APS points =____	**B.** Age points =____	**C.** Chronic Health points = ____	**Sum of A + B + C =** _____ (0 to 71)

*For **intubated** patients use verbal scoring column allocated*

Best Verbal Response

5	Orientated
4	Confused
3	Inappropriate
2	Incomprehensible
1	No Response

"Verbal " Intubated

5	Orientated
3	In Between
1	No Response

Best Motor Response

6	Obeys
5	Localises
4	Flexion – Withd.
3	Flexion – Decort.
2	Extension
1	No Response

Best Eye Opening

4	Spontaneous
3	To Command
2	To Pain
1	No Response

INSPIRED OXYGEN CONVERSION CHART

NASAL CANNULAE

1 LPM = 24%

2 LPM = 28%

3 LPM = 32%

4 LPM=36%

HUDSON OXYGEN MASK

5 - 6 LM = 40%

6 - 7 LPM = 50%

7 - 8 LPM = 60%

HIGH CONCENTRATION MASK

8 - 12 LPM = 60 – 80%

PARTIAL REBREATHING BAG

8 - 12 LPM = 60-80%

NON-REBREATHING BAG

8 - 12 LPM = 90-99%

APPENDIX 4 - ASSESSMENT OF DEEP VENOUS THROMBOSIS (DVT)

A diagnosis of DVT on ultrasound will require non-compressibility of one or more deep venous segments. A proximal leg ultrasound examination will be performed at 1cm intervals. This will include the conventional 6 sites which will be explicitly recorded 1) the trifurcation of the deep calf veins, 2) distal popliteal, 3) proximal popliteal, 4) distal femoral, 5) mid femoral and 6) common femoral. To minimize systematic and random error in ultrasound technique and interpretation, and ensure a standardised approach, we will provide a VTE Operations Manual and the Chief EPO-TBI Ultrasound Technologist will conduct protocol training via phone or teleconference for study ultrasonographers. After protocol training, the Chief Ultrasound Technologist will conduct ultrasound reliability studies with site staff who are not certified, accredited sonographers to determine agreement between the two parties blinded to each other's reading.

APPENDIX 5 - PULMONARY EMBOLISM MANAGEMENT GUIDELINES

The ICU clinical team will investigate and manage patients as they believe clinically appropriate at any time that a diagnosis of pulmonary embolism (PE) is suspected. This protocol appendix provides diagnostic guidelines for a suspected PE, with these guidelines to be use where clinically possible and appropriate.

1. Perform a bilateral lower limb compression Doppler ultrasound if this has not been performed already. A critically ill patient with a clinical suspicion of PE and a newly positive lower limb ultrasound showing evidence of DVT will be recorded as having “DVT” and “PE” for the purposes of this research study.

2. Perform a helical (spiral) chest computed tomography (CT) scan in all possible patients with clinically suspected PE, who do not have contraindications to intravenous contrast and who are sufficiently stable for transport to the radiology suite. Consistent with the treating clinician’s judgement, other therapy may include intravenous fluid, sodium bicarbonate, and/or N-acetylcysteine. For the purposes of this research study, PE will be deemed to have occurred in the presence of a radiological demonstrated intraluminal filling defect in the main, lobar or segmental branches of a pulmonary artery. Wherever possible, non-diagnostic spiral CTs will be resolved by reference to one or more of the following alternative diagnostic methods: ventilation-perfusion lung scanning, serial compression ultrasound, or pulmonary angiography, depending on the specific situation.

Less than 1% of ICU decedents undergo autopsy today; however, any post mortem findings in EPO-TBI will be used to support or clarify diagnoses made by the above means.

APPENDIX 6 –GLASGOW OUTCOME SCALE EXTENDED

The questions can be answered by the patient or close relative, friend or by rehabilitation/nursing staff if appropriate

RESPONDENT:

Patient alone ☐

Relative or friend or carer alone ☐

Patient and relative, friend or carer together ☐

Patient lost to follow up ☐

The injured person is:

Out of hospital ☐

In hospital, rehabilitation centre or residential care ☐

What is the most important factor in recovery?

Effects of head injury ☐

Effects of illness or injury to another part of the body ☐

A mixture of these ☐

Please answer by ticking one box ☐, which best answers, the question.

Is the injured person able to obey simple commands, or say any words?

Yes ☐ No ☐ (2-Vegetative State)

Before the injury was the injured person able to look after themselves at home?

Yes ☐ No ☐

As a result of the injury does the injured person now need someone to help look after them at home? For a no answer they should be able to look after themselves at home (safely) for 24 hrs if necessary. They should not require prompting or reminding and should be capable of being left alone overnight. (Please tick ☐ one box)

Do not need frequent help or supervision in the home ☐

Need some help in the home, but can look after themselves for at least 8 hours if necessary ☐ (4- Upper SD)

Could not look after themselves for 8 hours during the day ☐ (3-Lower SD)

Need help in the home, but not because of the injury ☐

Before the injury was the injured person able to buy things at shops without help?

Yes ☐ No ☐

As a result of the injury does the injured person now need help to buy things at shops?
(Please tick ☐ one box)

Do not need help to shop ☐

Can plan what to buy, take care of money themselves, behave appropriately in public

Need help to get to the shops but once there can do own shopping ☐

Need help to shop ☐ (4- Upper SD)

Need help to shop but not because of the injury ☐

Before the injury was the injured person able to travel without help

Yes ☐ No ☐

As a result of the injury does the injured person now need help to travel? Either driving a car, catching public transport or catching a taxi

(Please tick ☐ one box)

Do not need help to travel ☐

Need help to travel ☐ (4- Upper SD)

Need help to travel but not because of the injury ☐

Employment before the injury: (please tick ☐ one box)

Working	☐	Looking for work	☐
Looking after family	☐	Studying as a student	☐
Retired	☐	None of these	☐

As a result of the injury has there been a change in the injured person's ability to work? (Or to study; or to look after your family) **(Please tick ☐ one box)**

Still do the same work ☐

Still work, but at a reduced level (eg: change from full time to part time or change in level of responsibility) ☐ (6- Upper MD)

Am unable to work, or only able to work in sheltered workshop ☐ (5- Lower MD)

Ability to work has changed but not because of the injury ☐

10. Before the injury did the injured person take part in social and leisure activities outside home (at least once a week?)

Examples include going out with or visiting friends, going to the cinema or live performances, attending sporting events or participating in sporting events, attending religious ceremonies or services

Yes ☐ No ☐

As a result of the injury has there been a change in the ability of the injured person to take part in social or leisure activities outside the home?

(Please tick ☐ one box)

Take part about as often as before ☐

Take part a bit less but at least half as often ☐ (7 – Lower GR)

Take part much less, less than half as often ☐ (6- Upper MD)

Do not take part at all ☐ (5- Lower MD)

Ability to take part has changed but not because of the injury ☐

Before the injury did the injured person have any problems getting on with friends & family?

Yes ☐ No ☐

As a result of the injury does the injured person have any emotional or behavioural problems which cause difficulties in relationships with friends or family?

Examples include quick temper, irritability, mood swings, depression, anxiety, insensitivity to others, rigid thinking, unreasonable or childish behaviour (please tick ☐ one box)

Things are still much the same ☐

There are occasional problems (less than one a week) ☐ (7 –Lower GR)

There are frequent problems (once a week or more) ☐ (6- Upper MD)

There are constant problems (problems every day) ☐ (5- Lower MD)

There are problems for some other reason, not because of the injury ☐

Are there currently any other problems resulting from the injury?

Problems sometimes reported after head injury: headaches, dizziness or balance problems, tiredness or sleeping disturbances, sensitivity to noise or light, slower speed of thinking, trouble with memory, concentration problems (please tick ☐ one box)

No current problems ☐

Some problems, ☐ (7 – Lower GR)

Some problems for other reasons, not because of the head Injury ☐

Before the injury were similar problems present?

(Please tick ☐ one box)

No problems or they were minor problems ☐

Similar problems before ☐

Comments? (Add extra page if required)

GLASGOW OUTCOME SCALE – EXTENDED (TICK APPROPRIATE SCORE)		
DEAD	1	
VEGETATIVE STATE (VS)	2	
LOWER SEVERE DISABILITY (Lower SD)	3	
UPPER SEVERE DISABILITY (Upper SD)	4	
LOWER MODERATE DISABILITY (Lower MD)	5	
UPPER MODERATE DISABILITY (Upper MD)	6	
LOWER GOOD RECOVERY (Lower GR)	7	
UPPER GOOD RECOVERY (Upper GR)	8	

GLASGOW OUTCOME SCORE (TICK APPROPRIATE SCORE)		
DEAD	1	
VEGETATIVE STATE (VS)	2	
SEVERE DISABILITY (SD)	3	
MODERATE DISABILITY (MD)	4	
GOOD RECOVERY (GR)	5	

PATIENT LOST TO FOLLOW UP (TICK IF APPROPRIATE)	

APPENDIX 7 - EQ-5D

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By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking around	❑	please tick
I have some problems in walking around	❑	one box
I am confined to bed	❑	

Personal Care

I have no problems with personal care	❑	please tick
I have some problems washing or dressing myself	❑	one box
I am unable to wash or dress myself	❑	

Usual Activities *(e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities	❑	please tick
I have some problems with performing my usual activities	❑	one box
I am unable to perform my usual activities	❑	

Pain/Discomfort

I have no pain or discomfort	❑	please tick
I have moderate pain or discomfort	❑	one box
I have extreme pain or discomfort	❑	

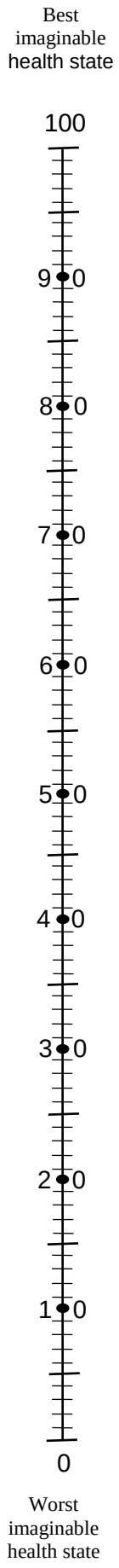
Anxiety/Depression

I am not anxious or depressed	❑	please tick
I am moderately anxious or depressed	❑	one box
I am extremely anxious or depressed	❑	

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad you health state is today.

Your own health state today



APPENDIX 8 - SF-12

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(IQOLA SF-12v1 Standard, Australia (English))

INSTRUCTIONS: This questionnaire asks for your views about your health, how yow you feel and how well you are able to do your usual activities.

Please answer every question by marking one box. If you are unsure about how to answer please give the best answer you can.

1. In general, would you say your health is:

☐	☐	☐	☐	☐
Excellent	**Very good**	**Good**	**Fair**	**Poor**

The following questions are about your activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

2. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling or playing golf

Yes	**Yes**	**No**
Limited a lot	**Limited a little**	**Not limited at all**
☐	☐	☐

3. Climbing **several** flights of stairs

Yes	**Yes**	**No**
Limited a lot	**Limited a little**	**Not limited at all**
☐	☐	☐

During the **past 4 weeks**. Have you had any of the following problems with your work or other regular daily activities **as a result of your physical health?**

4. **Accomplished less** than you would like

Yes	**No**
☐	☐

5. Were limited in the **kind** of work or other activities

Yes	**No**
☐	☐

During the **past 4 weeks** have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)

6. **Accomplished less** than you would like

Yes	No
☐	☐

7. Didn't do work or other activities as **carefully** as usual

Yes	No
☐	☐

8. During the **past 4 weeks,** how much did **pain** interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐	☐	☐	☐	☐

These questions are about how you feel and how things have been with you **during the past 4 weeks.** For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the **past 4 weeks**

9. Have you felt calm and peaceful?

All of the time	Most of the time	A good bit of the time	A little of the time	None of the time
☐	☐	☐	☐	☐

10. Did you have a lot of energy?

All of the time	Most of the time	A good bit of the time	A little of the time	None of the time
☐	☐	☐	☐	☐

11. Have you felt down?

All of the time	Most of the time	A good bit of the time	A little of the time	None of the time
☐	☐	☐	☐	☐

12. During the **past 4 weeks** how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting friends, relatives, etc)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐	☐	☐	☐	☐

Appendix 2

EPO-TBI trial hard-copy case report form

FORM 1
Randomisation

Patient Demographics

1.1 Initials: ☐☐☐ 1.2 Gender: ☐ Male ☐ Female 1.3 Date of birth: ☐☐ / ☐☐☐ / ☐☐☐☐ (dd / mmm / yyyy)

Inclusion Criteria - If NO is ticked to any of the criteria 1.4 to 1.9, the patient is NOT eligible

1.4.1 ICU patient with moderate or severe non-penetrating traumatic brain injury (GCS < 13): ☐ Yes ☐ No

1.4.2 GCS: ☐☐ (preintubation / closest to randomisation) 1.4.3 Motor score from GCS: ☐

1.5 ≥ 15 to ≤ 65 years: ☐ Yes ☐ No

1.6 < 24 hours since non-penetrating traumatic brain injury: ☐ Yes ☐ No

1.7 Expected to stay in ICU for ≥ 48 hours: ☐ Yes ☐ No

1.8.1 Hb not exceeding ULN: ☐ Yes ☐ No

1.8.2 Haemoglobin: ☐☐☐ g/L (closest to randomisation)

1.9 The patients legal surrogate has granted consent: ☐ Yes ☐ No

Exclusion Criteria - If YES or POSITIVE is ticked to any of the criteria 1.10 to 1.30, the patient is NOT eligible

1.10 GCS = 3 and fixed dilated pupils: ☐ Yes ☐ No

1.11 The patient is expected to die imminently (< 24 hours): ☐ Yes ☐ No

1.12 Pregnancy test results: ☐ N/A ☐ Positive ☐ Negative

1.13 Inability to perform lower limb ultrasounds: ☐ Yes ☐ No

1.14 The first dose of study drug cannot be given within 24 hours of brain injury occurring: ☐ Yes ☐ No

1.15 The patient has sustained a spinal cord injury: ☐ Yes ☐ No

1.16 History of DVT, PE or other thromboembolic event: ☐ Yes ☐ No

1.17 The patient is lactating or 3 months post partum: ☐ N/A ☐ Yes ☐ No

1.18 The patient has chronic hypercoagulable disorder, including known malignancy: ☐ Yes ☐ No

1.19 The patient has a history of myocardial infarction within the past 12 months: ☐ Yes ☐ No

1.20 The patient has uncontrolled hypertension (systolic blood pressure > 200 mmHg or diastolic blood pressure > 110 mmHg): ☐ Yes ☐ No

1.21 The patient has a past history of epilepsy with seizures in the past 3 months: ☐ Yes ☐ No

1.22 The patient has end stage renal failure (receives chronic dialysis): ☐ Yes ☐ No

1.23 Known sensitivity to mammalian cell derived products: ☐ Yes ☐ No

1.24 The patient has a known hypersensitivity to the active substance or to any of the additives: ☐ Yes ☐ No

1.25 The patient has pure red cell aplasia (PRCA): ☐ Yes ☐ No

~~1.26 The patient is receiving haematinic agents (any iron containing medication for the treatment of iron deficiency anaemia): ☐ Yes ☐ No~~

1.27 The patient was treated with EPO in the past 30 days: ☐ Yes ☐ No

1.28 The patient has severe pre-existing physical or mental disability or severe co-morbidity that may interfere with the assessment of outcome: ☐ Yes ☐ No

1.29 Treatment with any investigational drug within 30 days of enrolment: ☐ Yes ☐ No

1.30 The treating physician believes it is not in the best interest of the patient to be randomised to this trial: ☐ Yes ☐ No

Randomisation Result

1.31 Patient study number: ☐☐☐☐☐☐ 1.32 Date and time of randomisation: ☐☐ / ☐☐☐ / ☐☐☐☐ (dd / mmm / yyyy) ☐☐ : ☐☐ (HH : mm)

FORM 2 Baseline

Patient Study Number _____

Patient Initials _____

Patient Information

2.1 Date and estimated time of brain injury: __ / ___ / ____ __ : __
dd mmm yyyy HH mm

2.2 Mechanism of injury:
- ☐ Motor vehicle
- ☐ Motorcycle
- ☐ Bicycle
- ☐ Other
- ☐ Pedestrian
- ☐ Fall / Jump
- ☐ Hit by object

2.3 Date of hospital admission: __ / ___ / ____
dd mmm yyyy

2.4 Date and time of ICU admission: __ / ___ / ____ __ : __
dd mmm yyyy HH mm

2.5 Patient weight: ___ kg

VTE Risk Factors

2.6 Tick all risk factors that apply:
- ☐ Family history of VTE (blood relative)
- ☐ Pelvic fracture
- ☐ Femoral fracture
- ☐ Oestrogen (oral contraceptive or hormone replacement)
- ☐ Significant immobility in past 3 months
- ☐ Current smoker
- ☐ None known

2.7 Blood product administration prior to randomisation:
- ☐ Any red cells
- ☐ Any platelets
- ☐ Any FFP
- ☐ Any other clotting product, please specify: _____
- ☐ None

Medications Prior to Hospital Admission

2.8 Tick all anticoagulants that apply:
- ☐ Unfractionated heparin
- ☐ LMWH
- ☐ Warfarin
- ☐ Aspirin
- ☐ Other, please specify: _____
- ☐ None

Haematology - closest to randomisation

2.9 INR: __ . __ ☐ Not available

2.10 APTT: ___ . __ sec ☐ Not available

2.11 Platelets: ___ $x10^9/L$ ☐ Not available

FORM 2 Baseline

Patient Study Number ☐☐☐☐☐☐

Patient Initials ☐☐☐

APACHE II Score

2.12 APACHE II score prior to randomisation: ☐☐ (use an APACHE worksheet to derive the score - refer to data dictionary)

Renal Function Assessment - refer to Data Dictionary

2.13.1 First available post-trauma serum creatinine: ☐☐☐☐ micromol/L ☐ Not available

2.13.2 Date and time post-trauma serum creatinine collected: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy HH mm

~~2.14.1 Total urine output from time of urinary catheter insertion up to randomisation: (Only enter if urine collected for 6 hours or more)~~ ☐☐☐☐ ~~mL~~ ☐ ~~Not collected for ≥ 6 hours~~

~~2.14.2 Number of hours urine collected for since catheter inserted:~~ ☐☐ ~~hr~~

Vital Signs

2.15 Systolic blood pressure, first on admission to hospital ED: (For Revised Trauma Score) ☐☐☐ mmHg

2.16 Last unassisted respiratory rate: (For Revised Trauma Score) ☐☐ bpm ☐ Not available

2.17 Lowest systolic blood pressure, pre randomisation: ☐☐☐ mmHg

2.18 Worst SaO_2, pre randomisation: ☐☐ %

2.19 Pupillary response: (closest to randomisation)
- ☐ PEARL
- ☐ Dilated and both non-reactive
- ☐ Both non-reactive
- ☐ One non-reactive
- ☐ Unknown

FORM 3
Study Drug Record

Patient Study Number _____

Patient Initials _____

Study Drug Administration

3.1 Date of first dose: __ __ / __ __ __ / __ __ __ __
dd mmm yyyy

3.2.1 Was the dose given: ☐ Yes - ***if YES*** 3.2.2 Time of first dose administration: __ __ : __ __
HH mm

☐ No - ***if NO*** 3.2.3 Reason:

- ☐ Hb > ULN
- ☐ Proximal leg DVT
- ☐ PE
- ☐ CVA
- ☐ MI
- ☐ Cardiac arrest or V/Fib
- ☐ Other thrombotic event
- ☐ Palliated
- ☐ Consent withdrawn
- ☐ Discharged from ICU
- ☐ Deceased
- ☐ Withdrawn by physician
- ☐ AE
- ☐ SAE
- ☐ Protocol deviation

3.3 Date of second dose: __ __ / __ __ __ / __ __ __ __
dd mmm yyyy

3.4.1 Was the dose given: ☐ Yes - ***if YES*** 3.4.2 Time of second dose administration: __ __ : __ __
HH mm

☐ No - ***if NO*** 3.4.3 Reason:

- ☐ Hb≥120 g/L
- ☐ Proximal leg DVT
- ☐ PE
- ☐ CVA
- ☐ MI
- ☐ Cardiac arrest or V/Fib
- ☐ Other thrombotic event
- ☐ Palliated
- ☐ Consent withdrawn
- ☐ Discharged from ICU
- ☐ Deceased
- ☐ Withdrawn by physician
- ☐ AE
- ☐ SAE
- ☐ Protocol deviation

3.5 Date of third dose: __ __ / __ __ __ / __ __ __ __
dd mmm yyyy

3.6.1 Was the dose given: ☐ Yes - ***if YES*** 3.6.2 Time of third dose administration: __ __ : __ __
HH mm

☐ No - ***if NO*** 3.6.3 Reason:

- ☐ Hb≥120 g/L
- ☐ Proximal leg DVT
- ☐ PE
- ☐ CVA
- ☐ MI
- ☐ Cardiac arrest or V/Fib
- ☐ Other thrombotic event
- ☐ Palliated
- ☐ Consent withdrawn
- ☐ Discharged from ICU
- ☐ Deceased
- ☐ Withdrawn by physician
- ☐ AE
- ☐ SAE
- ☐ Protocol deviation

FORM 4
Daily Assessment

Patient Study Number

Patient Initials

Notes:
1. **Complete this form each day during index ICU admission up to Day 21 or ICU discharge, whichever occurs first.**
2. **After Day 21, complete this form twice weekly on the same days as the Doppler Ultrasound scheduled days. E.g. Mon/Tue and Thurs/Fri up to ICU discharge.**

4.1 Study day: ☐☐ 4.2 Date: ☐☐ / ☐☐☐ / ☐☐☐☐ (dd / mmm / yyyy)

Blood Transfusion

4.3.1 Blood products administration today:
- ☐ Any red cells - *if YES* 4.3.2 Volume of red cells: ☐☐☐☐☐☐ m/L
- ☐ Any platelets
- ☐ Any FFP
- ☐ Any other clotting product, please specify:_____
- ☐ None

Haematology - refer to Data Dictionary

4.4 Haemoglobin: ☐☐☐ g/L ☐ Not measured

4.5 Platelet count: ☐☐☐ x10^9/L ☐ Not measured

4.6 INR: ☐.☐ ☐ Not measured

4.7 APTT: ☐☐☐.☐ sec ☐ Not measured

ICP Monitoring

4.8 ICP is in situ: ☐ Yes - *if YES, answer Qu 4.9 - 4.19*
☐ No - *if NO, go to Qu 4.20.1*

4.9 CSF venting today: ☐ Yes ☐ No

Intracranial Hypertension Index

4.10 Number of end hours ICP > 20 mmHg: ☐☐ ☐ Not applicable

4.11 Total number of end hours ICP documented: ☐☐ ☐ Not applicable

Cerebral Perfusion Pressure Index

4.12 Number of end hours of CPP ≤ 60 mmHg: ☐☐ ☐ Not applicable

4.13 Total number of end hours CPP documented: ☐☐ ☐ Not applicable

ICP Management

4.14.1 Was the patient actively cooled today: ☐ Yes - *if YES* Number of hypothermia hours: 4.14.2 ☐☐ hr
☐ No

4.15 Episodes of $PaCO_2$ ≤ 33 mmHg: ☐ Yes ☐ No

4.16.1 Total volume Hypertonic saline administered: ☐☐☐☐☐ m/L 4.16.2 Concentration of Hypertonic saline: ☐☐.☐ %

4.16.3 Total volume Hypertonic saline administered: ☐☐☐☐☐ m/L 4.16.4 Concentration of Hypertonic saline: ☐☐.☐ %

4.17.1 Total volume of Mannitol administered: ☐☐☐☐☐ m/L 4.17.2 Concentration of Mannitol: ☐☐.☐ %

4.17.3 Total volume of Mannitol administered: ☐☐☐☐☐ m/L 4.17.4 Concentration of Mannitol: ☐☐.☐ %

4.18 Total amount of Thiopentone administered: ☐☐☐.☐☐ g

4.19 Total amount of Phenobarbitone administered: ☐☐☐.☐☐ g

Brain Tissue Oxygen Monitoring

4.20.1 Is a LICOX monitor in situ: ☐ Yes - *if YES* 4.20.2 Number of hours $PBrO_2$ < 20 mmHg: ☐☐ hr
☐ No

FORM 4
Daily Assessment

Patient Study Number _____

Patient Initials _____

VTE Management

4.21.1 Mechanical prophylaxis today:
- ☐ Antiembolic stockings - *if YES,* **4.21.2** ☐ Right leg ☐ Left leg
- ☐ Sequential pneumatic compression device - *if YES,* **4.21.3** ☐ Right leg ☐ Left leg
- ☐ Vena cava filter
- ☐ None

4.22 Pharmacological prophylaxis today:
- ☐ Unfractionated heparin
- ☐ LMWH
- ☐ Warfarin
- ☐ Other, please specify: _____
- ☐ None

Doppler Ultrasound Today

4.23.1 Compression Doppler ultrasound done today: ☐ Yes - *if YES,* **4.23.2** Reason for ultrasound:
- ☐ Baseline
- ☐ Twice weekly screening
- ☐ Clinical suspicion of DVT
- ☐ Clinical follow up of diagnosis of DVT
- ☐ Clinical suspicion of PE
- ☐ Other, please specify: _____

☐ No

Study Outcome Event

4.24.1 What was diagnosed today: (tick all that apply)
- ☐ Proximal leg DVT - *if YES* **4.24.2** Is a femoral CVC in the same leg as the DVT? ☐ Yes ☐ No
- ☐ Pulmonary embolus - *if YES* **4.24.3** Diagnosis based on:
  - ☐ Helical pulmonary CT scan
  - ☐ Ventilation-perfusion lung scan
  - ☐ Pulmonary angiogram
  - ☐ Echocardiography
  - ☐ Clinical assessment only
  - ☐ Other, please specify: _____
- ☐ Other thrombotic event - *if YES* **4.24.4** Location:
  - ☐ Upper extremity thrombus
  - ☐ Mesenteric venous thrombosis
  - ☐ Vena cava thrombosis
  - ☐ Other, please specify: _____
- ☐ Myocardial infarction
- ☐ Embolic stroke
- ☐ Haemorrhagic stroke
- ☐ Cardiac arrest or ventricular fibrillation
- ☐ None

4.25 Treatment today for study outcome event: (tick all that apply)
- ☐ Unfractionated heparin
- ☐ LMWH
- ☐ Warfarin
- ☐ Vena cava filter
- ☐ Thrombectomy
- ☐ Thrombolysis
- ☐ Other, please specify: _____
- ☐ None

Urine Output Assessment

Notes:
1. **Tick the most deranged value in the 24 hour period**
2. **Refer to Data Dictionary for reference tables**
3. **Collect urine output daily for the first 7 days of ICU admission only or up to ICU discharge, whichever occurs first**

4.26 Urine output assessment:
- ☐ None (does not fit any of the categories below)
- ☐ Average UO < 0.5 ml/kg/hr over 6 hours
- ☐ Average UO < 0.5 ml/kg/hr over 12 hours
- ☐ Average UO < 0.3 ml/kg/hr over 24 hours
- ☐ Anuria for 12 hours

FORM 5 Daily Renal Assessment

Patient Study Number

Patient Initials

Notes: Collect renal data daily up to Day 21 of HOSPITAL admission or up to hospital discharge, whichever occurs first

Renal Function Data - record highest value, if bloods done more than once on the day

	Study Day	Serum Creatinine micromol/L	Renal replacement therapy today
5.1	01	☐ Not measured	☐ Yes ☐ No
5.2	02	☐ Not measured	☐ Yes ☐ No
5.3	03	☐ Not measured	☐ Yes ☐ No
5.4	04	☐ Not measured	☐ Yes ☐ No
5.5	05	☐ Not measured	☐ Yes ☐ No
5.6	06	☐ Not measured	☐ Yes ☐ No
5.7	07	☐ Not measured	☐ Yes ☐ No
5.8	08	☐ Not measured	☐ Yes ☐ No
5.9	09	☐ Not measured	☐ Yes ☐ No
5.10	10	☐ Not measured	☐ Yes ☐ No
5.11	11	☐ Not measured	☐ Yes ☐ No
5.12	12	☐ Not measured	☐ Yes ☐ No
5.13	13	☐ Not measured	☐ Yes ☐ No
5.14	14	☐ Not measured	☐ Yes ☐ No
5.15	15	☐ Not measured	☐ Yes ☐ No
5.16	16	☐ Not measured	☐ Yes ☐ No
5.17	17	☐ Not measured	☐ Yes ☐ No
5.18	18	☐ Not measured	☐ Yes ☐ No
5.19	19	☐ Not measured	☐ Yes ☐ No
5.20	20	☐ Not measured	☐ Yes ☐ No
5.21	21	☐ Not measured	☐ Yes ☐ No

FORM 6 Neurosurgical Procedures

Patient Study Number _____

Patient Initials _____

Neurosurgical Interventions - during hospital admission

Mass Lesion

6.1.1 Was a mass lesion evacuated: ☐ Yes - *if YES,* ☐ No

6.1.2 Date: __ __ / __ __ __ / __ __ __ __ (dd / mmm / yyyy)

6.1.3 Date: __ __ / __ __ __ / __ __ __ __ (dd / mmm / yyyy)

6.1.4 Date: __ __ / __ __ __ / __ __ __ __ (dd / mmm / yyyy)

Craniectomy

6.2.1 Was a craniectomy performed: ☐ Yes - *if YES,* ☐ No

6.2.2 Date: __ __ / __ __ __ / __ __ __ __ (dd / mmm / yyyy)

6.2.3 Date: __ __ / __ __ __ / __ __ __ __ (dd / mmm / yyyy)

6.2.4 Date: __ __ / __ __ __ / __ __ __ __ (dd / mmm / yyyy)

Bifrontal Decompressive Craniectomy

6.3.1 Was a bifrontal decompressive craniectomy performed: ☐ Yes - *if YES,* ☐ No

6.3.2 Date: __ __ / __ __ __ / __ __ __ __ (dd / mmm / yyyy)

Intracranial Monitoring Device - during hospital admission

	Type of monitor (code)	Date and time inserted (dd / mmm / yyyy HH:mm)	Date and time removed (dd / mmm / yyyy HH:mm)
6.4.1			
6.4.2			
6.4.3			
6.4.4			
6.4.5			
6.4.6			
6.4.7			

INTRACRANIAL PRESSURE MONITOR CODES

Type of Monitor: I = Intraparenchymal
E = EVD
L = Licox

FORM 7
Hospital Discharge

Patient Study Number

Patient Initials

Discharge Summary

7.1.1 Date and time of ICU discharge: __ __ / __ __ __ / __ __ __ __ __ __ : __ __
dd mmm yyyy HH mm

7.1.2 ICU outcome: ☐ Alive ☐ Deceased

7.2 Number of ventilation days: __ __ __ days

7.2a Screening compression Doppler ultrasound performed after ICU discharge: ☐ Yes ☐ No

- if YES, 7.2b Date of ultrasound: __ __ / __ __ __ / __ __ __ __
dd mmm yyyy

7.2c Was proximal DVT diagnosed: ☐ Yes ☐ No

7.3 Date of hospital discharge: __ __ / __ __ __ / __ __ __ __
dd mmm yyyy

7.4.1 Patient discharged to:
- ☐ Other acute hospital
- ☐ Rehabilitation
- ☐ Long term care facility
- ☐ Home
- ☐ Died *- if YES,* 7.4.2 Process of death:
  - ☐ Brain death
  - ☐ Death with therapy withdrawn for severe cerebral damage
  - ☐ Death with therapy withdrawn for non-cerebral reasons (e.g. MOF)
  - ☐ Death with maximal support for severe cerebral damage
  - ☐ Death with maximal support for non-cerebral reasons (e.g. MOF)

Trauma Scores - from Trauma Registry

Injury Severity Score (ISS)

7.5 ISS: __ __

Maximal Abbreviated Injury Score (AIS) for each body region

7.6 Head/Neck: ☐ 7.8 Chest: ☐ 7.10 Extremeties or pelvic girdle: ☐

7.7 Face: ☐ 7.9 Abdominal or pelvic contents: ☐ 7.11 External: ☐

ICU Readmission

7.12 Was the patient readmitted to ICU during this hospital admission? ☐ Yes *- if YES, continue below*
☐ No *- if NO, go to Qu 7.19.1*

7.13 Date and time of ICU readmission: __ __ / __ __ __ / __ __ __ __ __ __ : __ __
dd mmm yyyy HH mm

7.14 Date and time of ICU discharge: __ __ / __ __ __ / __ __ __ __ __ __ : __ __
dd mmm yyyy HH mm

7.15 Number of ventilation days: __ __ __ days

7.16 Date and time of ICU readmission: __ __ / __ __ __ / __ __ __ __ __ __ : __ __
dd mmm yyyy HH mm

7.17 Date and time of ICU discharge: __ __ / __ __ __ / __ __ __ __ __ __ : __ __
dd mmm yyyy HH mm

7.18 Number of ventilation days: __ __ __ days

FORM 7
Hospital Discharge

Patient Study Number _____

Patient Initials _____

Compensable Patients

7.19.1 Is this patient a compensable patient? (e.g. TAC or ACC etc) ☐ Yes ☐ No

- if YES

7.19.2 **VICTORIAN PATIENTS**: TAC Claim Number: ☐☐ / ☐☐☐☐☐

7.19.3 **NEW ZEALAND PATIENTS**: ACC Claim Number: ☐☐☐☐☐☐☐☐☐

Consent

Choose all that apply:

7.20.1 Prior telephone consent from person responsible: ☐ *- if YES* 7.20.2 Date and time consent obtained: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy hours minutes

7.21.1 Prior written consent from person responsible (after telephone consent): ☐ *- if YES* 7.21.2 Date and time consent obtained: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy hours minutes

7.22.1 Prior consent/assent from person responsible: ☐ *- if YES* 7.22.2 Date and time consent obtained: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy hours minutes

7.23.1 Delayed consent from patient: ☐ *- if YES* 7.23.2 Date consent obtained: ☐☐ / ☐☐☐ / ☐☐☐☐
dd mmm yyyy

Victorian Sites Only

7.24 Procedural Authorisation obtained: ☐

7.25.1 Delayed consent from person responsible after procedural authorisation: ☐ *- if YES* 7.25.2 Date consent obtained: ☐☐ / ☐☐☐ / ☐☐☐☐
dd mmm yyyy

Withdrawal

7.26.1 Reason for withdrawal:
- ☐ Person responsible withdrew consent
- ☐ Patient withdrew consent
- ☐ Patient was palliated
- ☐ Patient withdrawn by physician

} *if YES* 7.26.2 Can data be used? ☐ Yes ☐ No

7.27 Date of withdrawal: ☐☐ / ☐☐☐ / ☐☐☐☐
dd mmm yyyy

FORM 8
6 Month Follow Up

Patient Study Number _____

Patient Initials _____

Follow up completed by Research Coordinator

6 Month Follow Up

8.1 Date of follow up: __ __ / __ __ __ / __ __ __ __
dd mmm yyyy

8.2.1 Is the patient alive at 6 months? ☐ Yes *- if YES* 8.2.2 Patient's current location:
- ☐ Other acute hospital
- ☐ Rehabilitation
- ☐ Long term care facility
- ☐ Home

☐ No *- if YES, complete Form 10 Serious Adverse Event*

8.3.1 Patient and proxy contact details sent to central assessor for GOSE & QoL follow up: ☐ Yes

☐ No *- if NO* 8.3.2 Reason:
- ☐ Patient withdrew consent
- ☐ Person responsible withdrew consent
- ☐ Patient is lost to follow up

Level of Care Details After Hospital Discharge

		Date of admission	Date of discharge / current date (if not discharged)
8.4	Rehabilitation centre:	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)
8.5	Transitional living centre:	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)
8.6	Readmission to acute hospital:	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)
8.7	Long term low level care facility: (e.g. supported accommodation centre)	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)
8.8	Long term high level care facility: (e.g. nursing home)	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)
8.9	Other (specify): _____	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)	__ __ / __ __ __ / __ __ __ __ (dd mmm yyyy)

Serious Adverse Event - refer to Data Dictionary

8.10 Did the patient experience an SAE after hospital discharge up to 6 months: ☐ Yes *- if YES, complete Form 10 Serious Adverse Event*

☐ No

FORM 9
Adverse Events

Patient Study Number

Patient Initials

Adverse Events

	Event	AE Onset Date dd / mmm / yyyy	Action taken code	Outcome code	Relationship to treatment code	AE Resolution Date (if AE persisting or outcome unknown, leave blank) dd / mmm / yyyy
9.1		/ /				/ /
9.2		/ /				/ /
9.3		/ /				/ /
9.4		/ /				/ /
9.5		/ /				/ /
9.6		/ /				/ /
9.7		/ /				/ /
9.8		/ /				/ /

LIST OF ADVERSE EVENTS

- Seizures
- Hypertensive crisis
- Allergic reaction
- Other, please specify

ADVERSE EVENT CODES:

Action taken:
1 = None
2 = Treatment modified or temporarily discontinued
3 = Treatment permanently discontinued

Outcome:
1 = Unknown / Lost to follow-up
2 = Unresolved
3 = Resolved
4 = Resolved with sequelae

Relationship to treatment:
1 = Not related
2 = Possibly
3 = Probably
4 = Definitely

FORM 10 Serious Adverse Event

Patient Study Number

Patient Initials

Notes:

1. SAE's are events that are: - unexpected in the TBI population or
   - in the opinion of the investigator are related to study treatment
2. Report all deaths, regardless of causality, as an SAE
3. Contact the Project Manager if you wish to discuss reporting
4. Report SAE's within 24 hours of the event becoming known
5. After ICU discharge and up to 6 months, Study Outcome events will be reported as an SAE on this form
6. Enter SAE data into electronic database via study website, do not fax

Serious Adverse Event Details

10.1 Type of report: ☐ Initial report ☐ Follow up report ☐ Final report

10.2 SAE diagnosis:

10.3 SAE type: ☐ Death ☐ Prolongation of hospitalisation or re-hospitalisation ☐ Life threatening ☐ Permanently disabling ☐ Congenital anomaly ☐ Medically important

10.4 SAE description:

10.5 Suspected relationship of SAE to treatment: ☐ Not related ☐ Possibly ☐ Probably ☐ Definitely

10.6 SAE onset date: dd / mmm / yyyy

10.7 Date and time of last dose of study drug: dd / mmm / yyyy HH : mm

10.8 As a result of the SAE study drug was: ☐ Permanently discontinued ☐ Temporarily discontinued ☐ No action

10.9 Treatment of SAE:

10.10 Outcome: ☐ Unknown / Lost to follow-up ☐ Unresolved ☐ Resolved *- if YES,*

10.11.1 SAE resolution date: dd / mmm / yyyy

10.11.2 Was there sequelae? ☐ Yes, please specify: ☐ No

☐ Death *- if YES,*

10.12.1 Death date: dd / mmm / yyyy

10.12.2 Autopsy done: ☐ Yes ☐ No

10.12.3 Report status: ☐ Report pending ☐ Report attached

10.13 Location of death: ☐ ICU ☐ Rehabilitation ☐ Ward ☐ Long term care facility ☐ Other acute hospital ☐ Home

FORM 11
Protocol Deviation

Patient Study Number _____

Patient Initials _____

Protocol Deviation Details

11.1 Date of deviation: __ __ / __ __ __ / __ __ __ __
dd mmm yyyy

11.2 Protocol deviation:

- ☐ Study drug given when Hb > ULN (1st dose)
- ☐ Study drug given when Hb ≥ 120 g/L (2nd or 3rd dose)
- ☐ Study drug not given and patient has not met withholding criteria
- ☐ Study drug given and permanent withholding criteria met
- ☐ Study drug not given on correct dosing day
- ☐ Baseline compression Doppler ultrasound not done before or at least within 48 hours of first dose
- ☐ Twice weekly screening compression Doppler ultrasound not performed
- ☐ Other, specify: _____

11.3 Reason for deviation: _____

11.4 Consequences of deviation:

- ☐ None
- ☐ Study drug permanently withheld
- ☐ Study drug withheld / missed dose
- ☐ Resulted in SAE ***- complete Form 10 Serious Adverse Event***
- ☐ Resulted in AE ***- complete Form 9 Adverse Events***

Appendix 3

EPO-TBI Person Responsible information and consent form template for sites: generic

Person Responsible Information and Consent Form

Full Project Title: A randomised, placebo-controlled trial of erythropoietin in ICU patients with traumatic brain injury

Erythro**PO**ietin in **T**raumatic **B**rain **I**njury "**EPO – TBI**"

Principal Investigator:
Associate Investigators:

Project number: **Version number:** **Date:**

1. Introduction

You are a person responsible for a patient who has a moderate or severe traumatic brain injury (TBI). {Name state} law allows the person responsible for a patient to consent to the patient taking part in medical research where the patient is unable to provide consent for themselves. Accordingly, we are asking for you to consent to the participation of your relative or near friend (the patient) in a research project that is testing a new treatment for TBI. The new treatment is a medication called erythropoietin.

This Person Responsible Information and Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if the patient should take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not the patient should take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish for the patient to take part, they don't have to. The patient will receive the best possible care whether they take part or not.

If you decide that you want the patient to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- understand what you have read;
- consent to the patient taking part in the research project;
- consent to the patient having the tests and treatments that are described;
- consent to the use of the patient's personal and health information as described.

You will be given a copy of this Person Responsible Information and Consent Form to keep.

2. What is the purpose of this research?

Many people who have a traumatic brain injury (TBI)—usually from a blow to the head such as in a vehicle collision or in a fall do not survive or, if they do, suffer from long-term

disability. Previous studies have shown that about 1,000 people in Australia and New Zealand suffer a moderate or severe TBI every year. With current best available treatment and therapies many of these patients sustain loss of brain function and long term disability in varying degrees.

When a patient sustains a traumatic brain injury there are two phases to the injury. First, the head-impact causes immediate damage to the brain. The secondary injury, which can evolve over hours or weeks, is a very complicated process. It involves many, linked, changes to the cells, brain chemistry, tissues or blood vessels that can destroy brain tissue. The treatment of brain injury focuses on trying to minimize the secondary injury and there is much research being done to try to find treatments that will prevent it.

Erythropoietin (EPO) has recently emerged as a drug that may help reduce secondary injury and improve brain function. It has been found to offer some protection to the brain when brain cells are deprived of their normal oxygen supply causing cells to die or be impaired.

The aim of this study is to determine if EPO reduces secondary brain injury and helps patients make a better recovery after traumatic brain injury. We also plan to monitor the effect of EPO on the rate of deep vein thrombosis (DVT - blood clots in the large veins in lower extremity) in patients with moderate or severe TBI in the intensive care unit (ICU).

EPO has been approved for use for other purposes in Australia for some years. It is used for the treatment of anaemia (low levels of red cells in the blood) in patients on long term kidney dialysis, patients who have had major surgery and those undergoing treatment for cancer. However, it is not approved for use in TBI patients. Therefore, EPO is an experimental treatment for TBI which means that it must be tested to see if it is an effective treatment for moderate or severe TBI.

A large study of ICU patients found that while EPO improved survival it was also noted that the patients who received EPO had increased thrombosis (blood clots). However, other studies show that if EPO is given to patients with a haemoglobin concentration (blood count) of less than 120 g/L (lower limit of normal) and at dose of 40,000 units or lower there should not be such an increased risk for thrombosis.

This study is a double-blind, randomised controlled trial. Patients participating in the trial will receive either EPO (40,000 units) or placebo (dummy drug that looks like EPO) weekly for up to 3 doses.

The study is double-blind because we need to work out whether or not the study drug is effective. To do this we need to be able to compare two groups of people by randomly assigning them to receive either the active study drug or placebo. Double-blind means that neither you, the patient nor the study staff will know what treatment is given although it will be known by the hospital pharmacist.

A randomised control trial means that treatment is allocated randomly (by chance like flipping a coin) via a computer program. The study staff cannot influence or predict what treatment is assigned to the participants.

This study is funded by the National Health and Medical Research Council and the Victorian Neurotrauma Initiative (VNI- part of the Victorian Government) and has been designed by investigators from the Australian and New Zealand Intensive Care Society Clinical Trials Group (ANZICS CTG). The study is managed by the Australian and New Zealand Intensive Care Research Centre (ANZIC-RC) at Monash University.

We plan to conduct the study at 18 hospitals in Australia and New Zealand and enrol 606 patients over 3.5 years. At *{insert name of hospital}* we plan to enrol *{insert # of estimated patients}* patients.

3. What does participation in this research involve?

The patient is eligible for the study because they have sustained a moderate or severe traumatic head injury.

If the patient is a woman of child bearing potential a blood sample (10 mls or 2 teaspoons) will be collected to see if they are pregnant if this has not already been performed.

If you consent for the patient to participate, they will be randomised to receive a subcutaneous (SC) injection (a small injection under the skin) of either EPO 40,000 units (1 ml in volume) or placebo (1 ml in volume) within 24 hrs after the initial brain injury. The injection is given in the upper arm, thigh or abdomen (tummy). The patient will have a 1 in 2 or 50% chance of receiving EPO. The injection will be given again on days 8 and 15 if the patient stays in the ICU over this time. When the patient is discharged to the general ward no further doses will be given.

Blood samples (15 mls or 3 teaspoons) will be collected for routine tests each day while the patient is in the ICU. The blood samples are part of the patient's standard care and will be done even if the patient is not in the study.

The patient will be closely monitored for blood clots in the legs. Preventative treatment will be implemented as soon as possible after ICU admission. Support stockings or compression pads wrapped around the legs will be prescribed, and when bleeding after the injury has settled, blood thinning medication. These treatments described are part of the standard TBI care. An ultrasound scan of the upper legs will be done before or at least within 48 hours of the first dose administration then twice weekly whilst the patient is in the ICU so that if any blood clots develop they will be detected early and treated immediately. The ultrasound is a test that is done at the bedside where a small flat scanner (like a computer mouse) is placed on the thigh with a sticky gel and the veins are visualized on a computer screen.

If the patient stays in ICU and receives all the study drug doses (3 doses) and scheduled leg ultrasounds (7 scans) they will be on study for 21 days, but they will not stay in the study if they are well enough to be discharged to the ward prior to this.

The ICU doctor will monitor the patient for any signs or complications of blood clots as part of standard care of the TBI patient.

Information about the patient's injury and management during their hospital stay will be collected from their medical record. The patient will also be interviewed 6 months after the injury to help determine the level of brain function and recovery. A discussion may also be held with you and the rehabilitation staff involved with the patient's care to help us assess the patient's recovery. You will receive a phone call shortly before 6 months from research staff who will arrange a convenient time for a telephone interview.

The patient will not be paid for their participation in this research.

4. Kidney function monitoring

ICU patients with TBI are at risk of developing acute kidney injury. Acute kidney injury increases the risk of brain swelling which may inhibit brain recovery and prolong hospital stay.

EPO has been shown in many experimental studies to protect the kidneys from injury. In addition to brain protection effects, EPO has also been shown to protect the kidneys when kidney cells are deprived of their normal oxygen supply causing cells to die or be impaired.

Therefore, we plan to monitor the patient's kidney function by collecting information about the urine output, and information from the laboratory tests which is part of standard care in the ICU.

Kidney function monitoring involves the collection of information only.

5. What will happen to my test samples?

The only additional blood sample required for the study is the pregnancy test for women of child bearing potential. Samples will be tested and stored at *{insert name of hospital}* Hospital in the laboratory and destroyed after 7 days or after the test has been conducted.

6. What are the possible benefits?

We cannot guarantee or promise that the patient will receive any benefits from this research but possible benefits may include a better recovery and quality of life for patients with moderate or severe TBI.

7. What are the possible risks?

Medical treatments often cause side effects. The patient may have none, some or all of the effects listed below, and they may be mild, moderate or severe. The ICU doctors will be monitoring the patient for side effects. However, if the patient has any of these side effects, or they are worried about them, the doctor will tell you.

Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long lasting or permanent. If a severe side effect or reaction occurs, the ICU doctor may need to stop the study drug. The patient will be monitored closely for any side effects. The ICU doctor will discuss the best way of managing any side effects with you and the patient.

The side effects of EPO are dose-dependent increase in the blood pressure which means the blood pressure returns to normal when it is discontinued, diarrhoea, nausea, vomiting, headache and flu-like symptoms. In other patient populations these adverse events have been less than 5% and comparable with patients who received the placebo.

All patients in ICU have an increased risk (approx. 10%) of clot formation in the blood vessels (called thrombosis or embolism). On top of this EPO may pose an additional risk for thrombosis although this is uncertain. A recent study revealed no more thrombosis with EPO compared to placebo. Although it is extremely rare, EPO may also cause arterial thrombosis and heart attack. For these reasons frequent ultrasounds and ECGs are performed.

Allergic reactions to EPO include rash, itching, anaphylactic reaction (allergic shock) and accumulation of fluid around the eyes.

Having an injection may cause some discomfort or bruising (0.2% of patients). If this happens, it can be easily treated.

The study may involve unknown or unforseen risks.

8. What if new information arises during this research project?

During the research project, new information about the risks and benefits of the project may become known to the researchers. If this occurs, you will be told about this new information and the doctor will discuss whether this new information affects the patient.

9. Can the patient have other treatments during this research project?

It is important to tell the patient's doctor and the research staff about any treatments or medications the patient may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell the doctor about any changes to these during the patient's participation in the research.

10. Are there alternatives to participation?

Participation in this research is not the only option. If the patient does not participate in this study, they will receive standard treatment for moderate or severe TBI. Discuss these

options with the doctor before deciding whether or not the patient can take part in this research project.

11. Does the patient have to take part in this research project?

Participation in any research project is voluntary. If you do not wish the patient to take part they don't have to. If you decide that the patient can take part and later change your mind, you are free to withdraw the patient from the project at any stage.

Your decision whether the patient can take part or not to take part, or to take part and then withdraw, will not affect the patient's routine treatment, their relationship with those treating them or their relationship with *{insert name of hospital}* Hospital.

12. What if I withdraw from this research project?

If you decide to withdraw the patient from the project, please notify a member of the research team before you withdraw them. This notice will allow that person or the research supervisor to inform you if there are any health risks or special requirements for the patient that are linked to withdrawing.

If you decide to withdraw the patient from the project, the researchers would like to keep the personal and health information that has been collected. This is to help them make sure that the results of the research can be measured properly. If you do not want them to do this, you must tell the researchers before you withdraw the patient.

13. Could this research project be stopped unexpectedly?

This research project may be stopped for a variety of reasons. These may include reasons such as:

- Unacceptable side effects;
- The drug/treatment being shown not to be effective; and
- The drug/treatment being shown to work and not need further testing.

14. What will happen when the patient's participation in this research project ends?

Study medication will only be given for up to 3 doses. The standard care provided will continue when the study ends.

15. How will I be informed of the results of this research project?

Once the project has been completed, a summary of the results will be available from the investigator on request.

16. What else do I need to know?

- **What will happen to information about me?**

Any information obtained in connection with this research project that can identify the patient will remain confidential and will only be used for the purpose of this research project. It will only be disclosed with your permission, except as required by law.

Information about the patient may be obtained from their health records held at this, and other, health services for the purposes of this research.

The patient's health records and any information obtained during the study are subject to inspection (for the purpose of verifying the procedures and the data) by the relevant authorities and authorised representatives of the Sponsor, ANZIC-RC, this organisation, *{insert name of hospital}* Hospital, or as required by law. By signing the consent section, you authorise release of, or access to, this confidential information to the relevant study personnel and regulatory authorities as noted above.

The follow up questionnaires conducted at 6 months after enrolment will be carried out by a researcher from ANZIC-RC at Monash University so that the information obtained from the questionnaires will be consistent. For the questionnaires to be conducted centrally, your name and contact details and the patient's name and contact details will be sent to the Monash researcher. Contact details will be address and phone number. The names and contact details collected from you and the patient will be kept in a locked filing cabinet in a secure area of the School of Public Health and Epidemiology at Monash University and will be destroyed by shredding when the questionnaires are complete. By signing this consent form you consent to your name and contact details and the patient's name and contact details being held by the central researcher at Monash University for the purposes of the study.

In any publication and/or presentation, information will be provided in such a way that cannot identify the patient, except with your permission. No identifying material will be used in any reports of this study.

Records for the study will be kept in a secure filing cabinet in a secure office. A database with study information will be generated and this will be kept in a computer that is password protected. Records relating to the study will be kept indefinitely at this site.

It is desirable that the patient's local doctor is advised about participation in this research project. By signing the consent section, you agree to the patient's local doctor being notified about participation in this research project.

Information about participation in this research project may be recorded in the patient's health records.

- **How can I access my information?**

In accordance with relevant Australian and/or state privacy and other relevant laws, the patient has the right to access the information collected and stored by the researchers about them. The patient also has the right to request that any information with which they disagree be corrected. Please contact one of the researchers named at the end of this document if the patient would like to access their information.

- **What happens if I am injured as a result of participating in this research project?**

If the patient suffers an injury as a result of their participation in this research project, please contact the research staff. Hospital care and treatment will be provided by the public health care system (Medicare) at no cost to them if they are eligible for Medicare benefits and elect to be treated as a public patient.

- **Is this research project approved?**

The ethical aspects of this research project have been approved by the Human Research Ethics Committee of *{insert name of hospital}* Hospital.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research* (2007) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies.

17. Consent

I have read, or have had read to me in a language I understand, this document and I understand the purposes, procedures and risks of this research project as described within it.

I am the Person Responsible for [*participant's name*]_____. I consent

to the participation of [*participant's name*]_____ in the research project named above, according to the conditions outlined in this document.

I believe that the carrying out of the procedure is not contrary to the best interests of

[*participant's name*]_____.

I give permission for [*participant's name*]'s_____ doctors, other health professionals, hospitals or laboratories outside this hospital to release information to *{insert hospital name}*

Hospital concerning [*participant's name*]'s_____ disease and treatment that is needed for this project. I understand that such information will remain confidential.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I understand that I will be given a copy of this document to keep.

Participant's name (printed) ……

Name of Person Responsible (printed) ……

Relationship to participant: ……

Signature Date

Witness to signature (printed) ……

Signature Date

Declaration by researcher*: I have given a verbal explanation of the research project, its procedures and risks and I believe that the person named above as the Person Responsible has understood that explanation.

Researcher's name (printed) ……

Signature Date

** A senior member of the research team must provide the explanation and provision of information concerning the research project.*

Note: All parties signing the consent section must date their own signature.

18. Who can I contact?

The person you may need to contact will depend on the nature of your query. Therefore, please note the following:

For further information or appointments:

If you want any further information concerning this project or if the patient has any medical problems which may be related to their involvement in the project (for example, any side effects), you can contact the principal researcher on *{phone #}* or any of the following people:

{Name of Research Coordinator}	Study Coordinator	*{Phone #}*
{Name of Investigator}	Associate Investigator	*{Phone #}*
{Name of Investigator}	Associate Investigator	*{Phone #}*

For complaints:

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about the patient being a research participant in general, then you may contact:

{*Insert name of ethics contact person*}

Appendix 4

EPO-TBI Person Responsible information and consent form with biomarker specimen information (superseded)

Person Responsible Information and Consent Form

Full Project Title: **A randomised, placebo-controlled trial of erythropoietin in ICU patients with traumatic brain injury**

Erythro**PO**ietin in **T**raumatic **B**rain **I**njury "**EPO – TBI**"

Protocol: **ANZICS-RC/RB002**

Site: **The Royal Melbourne Hospital**

Version: **3** Dated: **31 May 2010**

Principal Investigator: **Associate Professor Nerina Harley**

Associate Investigator**: Associate Professor Christopher MacIsaac**

1. Introduction

As the Person Responsible for the patient _____ you are invited to consider the patient's participation in this research project. Victorian law allows the person responsible for a patient to consent to the patient taking part in medical research where the patient is unable to provide consent for themselves. The patient is invited to participate in this study because they have a moderate or severe traumatic brain injury (TBI). This research project is testing a new treatment for TBI. The new treatment is a medication called erythropoietin.

This Person Responsible Information and Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if the patient should take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not the patient should take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish for the patient to take part, they don't have to. The patient will receive the best possible care whether they take part or not.

If you decide that you want the patient to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- understand what you have read;
- consent to the patient taking part in the research project;

- consent to the patient having the tests and treatments that are described;

You will be given a copy of this Person Responsible Information and Consent Form to keep.

If at a later time, whilst still participating in the study the patient becomes able to give consent for him/herself, they will be given a Participant Information and Consent (Continuation of Consent) Form and will be asked for their consent.

2. What is the purpose of this research?

When a patient sustains a traumatic brain injury there are two phases to the injury. First, the head-impact causes immediate damage to the brain. The secondary injury, which can evolve over hours or weeks, is a very complicated process. It results from damage caused by inflammation and changes to the brain chemistry, tissues or blood vessels that can destroy brain tissue. The treatment of brain injury focuses on trying to minimize the secondary injury.

Erythropoietin (EPO) is a drug that may help reduce secondary injury and improve brain function. It has been found to offer some protection to the brain when brain cells are deprived of their normal oxygen supply causing cells to die or be impaired.

A large study of Intensive Care Unit (ICU) patients found that EPO improved survival in trauma patients. However it was also noted that the patients who received EPO had increased thrombosis (blood clots). Other studies show that if EPO is given to patients with a low blood cell count and at dose of 40,000 units or lower there should not be such an increased risk for thrombosis.

The aim of this study is to determine if EPO reduces secondary brain injury and helps patients make a better recovery after traumatic brain injury. We also plan to monitor the effect of EPO on the rate of deep vein thrombosis (DVT - blood clots in the large veins in the legs or lower extremities) in these patients.

EPO has been approved by the Therapeutic Goods Administration of Australia for its use for other purposes for some years. It is used for the treatment of anaemia (low levels of red cells in the blood) in patients on long-term kidney dialysis; patients who have had major surgery; and those undergoing treatment for cancer. However, it is not approved for the treatment of TBI. And therefore its use in this study is considered to be experimental.

We plan to conduct the study at 19 hospitals in Australia, New Zealand and The Kingdom of Saudi Arabia, and enrol 606 patients over 3.5 years. At The Royal Melbourne Hospital we plan to enrol 40 patients.

This study is funded by the National Health and Medical Research Council and the Victorian Neurotrauma Initiative (VNI- part of the Victorian Government) and has been designed by investigators from the Australian and New Zealand Intensive Care Society Clinical Trials Group (ANZICS CTG). The study is managed by the Australian and New Zealand Intensive Care Research Centre (ANZIC-RC) at Monash University.

3. What does participation in this research involve?

Participation in this study consists of a pre treatment period, treatment period and a follow up period.

Pre Treatment Period:

- If the patient is a woman of child bearing potential a blood sample of 10 mls (2 teaspoons) will be collected to see if they are pregnant if this has not already been performed. If the patient is pregnant they will not be able to participate in the study.
- The patient will be assigned by a method of chance, (like the flip of a coin), to one of two groups. This is called randomisation. One group will receive the study treatment EPO, 40,000 units and the other group will receive a placebo. A placebo is a solution that looks like the study treatment but which does not contain any active ingredients. The patient will have a 1 in 2 (50%) chance of receiving EPO. Neither the patient nor the study staff will know which group the patient is in. This is called blinding. However if this information is needed for medical reasons, the study doctor can find out which treatment group the patient is in.

Randomisation, the use of a placebo and blinding are standard research methods used to ensure that the results of the study are true and correct.

Treatment Period:

- The patient will be randomised to receive a subcutaneous (SC) injection (a small injection under the skin) of either EPO 40,000 units (1 ml in volume) or placebo (1 ml in volume) within 24 hrs after the initial brain injury. The injection is given in the upper arm, thigh or abdomen (tummy). The injection will be given again on days 8 and 15 if the patient stays in the ICU over this time. When the patient is discharged to the general ward no further doses will be given.
- We will collect the results of standard care blood tests.
- The patient will be closely monitored for blood clots in the legs, as is standard practice in the ICU. An ultrasound scan of the upper legs will be done before or after the first dose of EPO is given and then twice weekly whilst the patient is in the ICU so that if any blood clots develop they will be detected early and treated immediately. The ultrasound is a test that is done at the bedside where a small flat scanner (like a computer mouse) is placed on the thigh with a sticky gel and the veins are seen on a computer screen.
- If the patient stays in ICU and receives all the study drug doses (3 doses) and scheduled leg ultrasounds (7 scans) they will be on the study for 21 days, but they will not stay in the study if they are well enough to be discharged to the ward prior to this.

- Information about the patient's injury and management during their hospital stay will be collected from their medical record.

Follow up Period

You will receive a phone call shortly before 6 months from a member of the research staff who will arrange a convenient time for a telephone interview.

A copy of the interview questions will be posted to the patient so that they can have an indication of what will be asked during the discussion.

The telephone interview will take approximately 15 minutes. Its purpose is to help determine the patient's level of brain function and recovery.

We may also ask you and the rehabilitation staff involved, some questions about the patient's care to help us assess the patient's recovery.

De- identified information relating to the cost of care will also be collected from the Transport Accident Commission for participants who were injured in a transport-related accident.

The patient will not be paid for their participation in this research.

4. Kidney function monitoring

ICU patients with TBI are at risk of developing acute kidney injury. Acute kidney injury increases the risk of brain swelling which may inhibit brain recovery and prolong hospital stay. Therefore in addition, the first 25 patients enrolled at this site will have blood and urine tests performed to measure specific kidney biomarkers (unique proteins found in blood and urine). Biomarkers tell us the degree of kidney injury that has occurred.

Blood samples of 10mls (2 teaspoons) and urine samples of 10mls will be collected before the first dose of study drug is administered and at 24, 48 and 72 hours after study drug administration.

5. What will happen to the patient's test samples?

An additional blood sample is required for the study for the pregnancy test for women of child bearing potential. Samples will be tested and stored at The Royal Melbourne Hospital in the laboratory and destroyed after 7 days or after the test had been conducted.

Blood and urine samples collected for kidney biomarker testing are in addition to standard care and will be tested at Cincinnati Children's Hospital Medical Centre pathology laboratory, Cincinnati, Monash Medical Centre in Melbourne and CIMC Co. laboratories, Tokyo, Japan. The patient's samples and any paperwork accompanying the samples will be labelled with a unique study code, not their name, address or hospital number. The blood and urine samples will be destroyed at the end of the study.

This type of testing is done to further our knowledge about how the study medication works and it will not produce the type of results that will have any useful meaning that would affect the patient's health or treatment. Therefore the patient will not be informed of the results of the tests.

A small portion (approximately 1 ml or less than one teaspoon) of the 4 blood and urine samples collected from each patient will be stored indefinitely at the National

Trauma Research Institute at the Alfred Hospital for future testing if a new biomarker is developed in the future which will tell us about kidney injury in TBI patients.

By consenting for the patient to take part in this study, you also consent to the collection, storage and use of tissue samples as specified above.

6. What are the possible benefits?

We cannot guarantee or promise that the patient will receive any benefits from this research however; possible benefits may include a better recovery and quality of life for patients with moderate or severe TBI.

7. What are the possible risks?

Medical treatments often cause side effects. The patient may have none, some or all of the effects listed below, and they may be mild, moderate or severe. The ICU doctors will be monitoring the patient for side effects. However, if the patient has any of these side effects, or they are worried about them, the doctor will tell you.

Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long lasting or permanent. If a severe side effect or reaction occurs, the ICU doctor may need to stop the study drug. The patient will be monitored closely for any side effects. The ICU doctor will discuss the best way of managing any side effects with you and the patient.

The side effects of EPO are dose-dependent increase in the blood pressure, which means the blood pressure returns to normal when it is discontinued, diarrhoea, nausea, vomiting, headache and flu-like symptoms. In other patient groups these side effects have been less than 5% (5 in 100).

All patients in ICU have an increased risk (approx. 10% (10 in 100)) of clot formation in the blood vessels (called thrombosis or embolism). On top of this EPO may pose an additional risk for thrombosis although this is uncertain. A recent study revealed no more thrombosis with EPO compared to placebo. Although it is extremely rare, EPO may also cause a clot in an artery and heart attack. For these reasons frequent ultrasounds and monitoring are performed.

EPO can cause chronic anaemia (a decrease in the normal number of red blood cells in the blood). This is an extremely rare side effect.

Allergic reactions to EPO include rash, itching, anaphylactic reaction (allergic shock) and accumulation of fluid around the eyes.

Having an injection may cause some discomfort or bruising. If this happens, it can be easily treated.

There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell the doctor if the patient develops any new or unusual symptoms.

8. What if new information arises during this research project?

During the research project, new information about the risks and benefits of the project may become known to the researchers. If this occurs, you will be told about

this new information and the doctor will discuss whether this new information affects the patient.

9. Can the patient have other treatments during this research project?

Participants in this study will continue to receive all standard care treatment for their condition.

It is important to tell the patient's doctor and the research staff about any treatments or medications the patient may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell the doctor about any changes to these during the patient's participation in the research.

10. Are there alternatives to participation?

The patient does not have to participate in this study to receive any medical care they may require. Participation in this research is not the only option. If the patient does not participate in this study, they will receive standard treatment for moderate or severe TBI. The study doctor will discuss these options with you before you decide whether to consent to the patient's participation in this research project.

11. Does the patient have to take part in this research project?

Participation in any research project is voluntary. You can decline to consent to the patient taking part in the study. If you decide to consent to the patient taking part and later change your mind, you are free to withdraw the patient from the project at any stage.

Your decision whether to consent for the patient to take part or not, or to take part and then withdraw, will not affect the patient's routine treatment, your relationship with those treating the patient or your or the patient's relationship with The Royal Melbourne Hospital.

12. What if I withdraw the patient from this research project?

If you decide to withdraw the patient from the project, please notify a member of the research team before you withdraw them. This notice will allow that person or the research supervisor to inform you if there are any health risks or special requirements for the patient that are linked to withdrawing.

If you decide to withdraw the patient from the project, the researchers would like to keep the personal and health information that has been collected. This is to help them make sure that the results of the research can be measured properly. If you do not want them to do this, you must tell the researchers before you withdraw the patient.

13. Could this research project be stopped unexpectedly?

This research project may be stopped for a variety of reasons. These may include reasons such as:

- Unacceptable side effects;
- The drug/treatment being shown not to be effective; and

- The drug/treatment being shown to work and not need further testing.

14. What will happen when the patient's participation in this research project ends?

The standard care provided will continue when the study ends.

15. How will I be informed of the results of this research project?

Once the project has been completed, a plain English summary of the results will be available from the investigator on request.

16. What else do I need to know?

- **What will happen to the patient's information?**

Any information obtained in connection with this research project that can identify the patient will remain confidential and will only be used for the purpose of this research project. It will only be disclosed with your permission, except as required by law.

Information about the patient may be obtained from their health records held at this, and other, health services for the purposes of this research.

The patient's health records and any information obtained during the study are subject to inspection (for the purpose of verifying the procedures and the data) by the relevant authorities and authorised representatives of the Sponsor, ANZIC-RC, this organisation, The Royal Melbourne Hospital ethics committee, or as required by law. By signing the consent section, you authorise release of, or access to, this confidential information to the relevant study personnel and regulatory authorities as noted above.

The follow up questionnaires conducted at 6 months after enrolment, will be carried out by a researcher from ANZIC-RC at Monash University so that the information obtained from the questionnaires will be consistent. For the questionnaires to be conducted centrally, your name and contact details and the patient's name and contact details will be sent to the Monash researcher. Contact details will be address and phone number. The names and contact details collected from you and the patient will be kept in a locked filing cabinet in a secure area of the School of Public Health and Epidemiology at Monash University and will be destroyed by shredding when the questionnaires are complete. By signing this consent form you consent to your name and contact details and the patient's name and contact details being held by the central researcher at Monash University for the purposes of the study.

In any publication and/or presentation of the results of the study, information will be provided in such a way that cannot identify the patient, except with your permission.

Any information collected and stored by the study doctor and staff at this site, which can be used to identify the patient will be kept in a secure filing cabinet in a secure office.

A database with study information will be generated and this will be kept in a computer that is password protected. A unique study number will be assigned to the patient's study data. The information collected for the trial and entered in to the database does not include the patient's name, address, or other information that may directly identify them.

When the study has finished, the data collected by the study doctor and staff will be kept for at least 15 years in a secure archiving facility away from the hospital. Only the study doctor and staff have access to these archives. At the end of the 15-year period, the data will be destroyed. .

It is desirable that the patient's local doctor is advised about participation in this research project. By signing the consent section, you agree to the patient's local doctor being notified about participation in this research project.

Information about participation in this research project will be recorded in the patient's health records.

- **How can I access the patient's information?**

In accordance with relevant Australian and/or state privacy and other relevant laws, the patient has the right to access the information collected and stored by the researchers about them. The patient also has the right to request that any information with which they disagree be corrected. Please contact one of the researchers named at the end of this document if the patient would like to access their information.

- **What happens if the patient is injured as a result of participating in this research project?**

If the patient suffers an injury as a result of their participation in this research project, hospital care and treatment will be provided by the public health care system at no cost if they/you elect for them to be treated as a public patient.

- **Is this research project approved?**

The ethical aspects of this research project have been approved by the Melbourne Health Human Research and Ethics Committee.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research* (2007) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies.

17. Consent

I am the Person Responsible for _____.

I have read, or have had read to me in a language I understand, this document and I understand the purposes, procedures and risks of this research project as described within it.

I believe that the carrying out of the procedure is not contrary to the best interests of

_____.

I consent to the participation of_____ in the research project named above, according to the conditions outlined in this document.

I have had an opportunity to ask questions and I am satisfied with the answers I have received. I understand that I will be given a copy of this document to keep.

Name of Person Responsible (printed) _____

Signature Date

Witness (Required when a Person Responsible cannot read this document for him/herself except where an interpreter is used.)

Name of Witness (printed)_____

Signature Date

Interpreter (Required when this document is read to the participant in a language other than English)

Name of Interpreter (printed) _____

Signature Date

Declaration by Principal or Associate Investigator *: I have given a verbal explanation of the research project, its procedures and risks and I believe that the person named above as the Person Responsible has understood that explanation.

Investigator's name (printed) ……

Signature Date

**. Note: All parties signing the consent section must date their own signature.*

18. Who can I contact?

The person you may need to contact will depend on the nature of your query. Therefore, please note the following:

For further information:

If you want any further information concerning this project or if the patient has any medical problems which may be related to their involvement in the project (for example, any side effects), you can contact the Principal Researcher on 9342 7441 or any of the following people:

Name: Ms Deborah Barge/ Ms Tania Caf

Position: Study Coordinators

Telephone: 9342 7000 ask for pager 671

Name: Assoc Prof Christopher MacIsaac

Position: Associate Investigator

Telephone: 9342 7441

For complaints:

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about the patient being a research participant in general, then you may contact:

Name: Dr. Angela Watt

Position: Manager, Melbourne Health Human Research Ethics Committee

Telephone: 9342 7550

Appendix 5

POLAR trial protocol

The POLAR-RCT

The Prophylactic hypOthermia trial to Lessen trAumatic bRain injury-Randomised Controlled Trial

(NCT 00987688 &ACTRN12609000764235)

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Assoc/Professor Stephen Bernard
Professor Peter Cameron
Professor Andrew Forbes
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Ms Lynne Murray
Assoc/Professor Alistair Nichol
Ms Lynette Newby
Assoc/Professor Jeffrey Presneill
Dr Stephen Rashford
Professor Jeffrey Rosenfeld
Dr Tony Smith
Mr Michael Stephenson
Mr Tony Trapani
Assoc/Professor Tony Walker
Assoc/Professor Steve Webb

Coordinating Centre: The Australian & New Zealand Intensive Care Research Centre
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Fax: +61 3 9903 0071
mailto:anzicrc@med.monash.edu.au

This study is endorsed by the Australian & New Zealand Intensive Care Society Clinical Trials Group (ANZICS CTG)

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1. MANAGEMENT COMMITTEE AUTHORISATION PAGE

We the management committee have read the attached protocol and authorize it as the official protocol for the study entitled *The prophylactic hypothermia trial to lessen traumatic brain injury – randomised controlled trial. (POLAR RCT) Version4 9th November 2010 (ACTRN12605000009617)*

Chief Investigator Professor Jamie Cooper		**Date**	9th November 2010
Management Committee Assoc/Professor Michael Bailey		**Date**	9th November 2010
Management Committee Assoc/Professor Stephen Bernard		**Date**	9th November 2010
Management Committee Professor Peter Cameron		**Date**	9th November 2010
Management Committee Professor Andrew Forbes		**Date**	9th November 2010
Management Committee Dr Colin McArthur		**Date**	9th November 2010
Management Committee Ms Lynne Murray		**Date**	9th November 2010
Management Committee Ms Lynette Newby		**Date**	9th November 2010
Management Committee Assoc/Professor Alistair Nichol		**Date**	9th November 2010
Management Committee		**Date**	9th November 2010

Assoc/Professor Jeffrey Presneill

Management Committee **Date** 9th November 2010

Dr Stephen Rashford

Management Committee **Date** 9th November 2010

Professor Jeffrey Rosenfeld

Management Committee **Date** 9th November 2010

Dr Tony Smith

Management Committee **Date** 9th November 2010

Mr Michael Stephenson

Management Committee **Date** 9th November 2010

Mr Tony Trapani

Management Committee **Date** 9th November 2010

Assoc/Professor Tony Walker

Management Committee **Date** 9th November 2010

Assoc/Professor Steve Webb

2. INVESTIGATOR PROTOCOL AGREEMENT SIGNATURE PAGE

I have received and read the *The prophylactic hypothermia trial to lessen traumatic brain injury – randomised controlled trial (POLAR RCT) (ACTRN12605000009617) study protocol Version 4 9th November 2010* and agree to conduct the study in accordance with:

- The attached protocol (subject to amendments);
- In accordance with the NHMRC National Statement on Ethical Conduct in Human Research (2007); and
- The Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with Therapeutic Goods Administration comments.
- The New Zealand Interim Good Clinical Research Practice Guidelines (Volume 2: 1998 and Volume 3: 2000)

Name of Participating Site: _____

Signature of Investigator: _____ **Date:** _____

Printed Name: _____

3. STUDY ADMINISTRATION STRUCTURE

3.1 Coordinating Centre & Data Management Centre

Australian and New Zealand Intensive Care Research Centre (ANZIC-RC) and Department of Epidemiology and Preventive Medicine (DEPM) Monash University, Victoria, Australia

3.1.1 Responsibilities

- overall management of the study including assistance with HREC applications
- management of study budget and liaison with funding bodies
- protocol and case report form (CRF) design and production
- database design and management
- protocol training of research coordinators and POLAR study team
- protocol training of ambulance paramedics
- preparation and arrangement of investigator payments
- study set-up
- randomisation
- coordination of data entry and feedback of data enquiries
- monitoring and close-out site visits
- organisation of investigator meetings
- serious adverse event notification
- data analysis and collaboration on publications

3.1.2 Staff

• Prof Jamie Cooper	Principal Investigator, ANZIC-RC
• Ms Siouxzy Morrison	Executive Officer, ANZIC-RC
• Ms Gillian Syres	Research Manager, ANZIC-RC
• A/Prof Alistair Nichol	Senior lecturer, ANZIC-RC
• Prof Andrew Forbes	Head Biostatistics Unit, DEPM, Monash University
• A/Prof. Chris Reid	Head, Monash Centre of Cardiovascular Research and Education in Therapeutics (CCRET), Monash University
• Mr Tony Trapani	Research Fellow, ANZIC-RC
• A/Prof Michael Bailey	Biostatistician, ANZIC-RC
• Ms Philippa Loane	Case report form (CRF) coordinator, CCRET, Monash University
• Mr Carl Costolloe	Database developer, CCRET, Monash University

3.1.3 Meetings:

- As required

3.2 Executive Committee

3.2.1 Responsibilities

- Day to Day study management
- Liaise with Management Committee and sites

3.2.2 Members

• Professor Jamie Cooper	Principal investigator ANZIC-RC
• A/Professor Alistair Nichol	A/Professsor, ANZIC-RC
• A/ Professor Stephen Bernard	Medical Advisor, Ambulance Victoria
• Mr Tony Trapani	Research Fellow, ANZIC-RC
• Ms Lynne Murray	Adjunct Senior Research Fellow, ANZIC-RC

3.2.3 Meetings

- Weekly and as required

3.3MANAGEMENT COMMITTEE

3.3.1 Responsibilities

Overseeing all aspects of the study management including

- liaison with coordinating centre staff
- liaison with steering committee
- liaison with ANZICS CTG
- liaison with ambulance services
- overseeing funding applications
- overseeing disbursement & administration of funds
- ensuring fiscal responsibilities are maintained
- development and approval of final protocol & study materials
- development and approval of data collection tools and methods
- general study management issues

3.3.2 Members

• A/Professor Michael Bailey	Biostatistician ANZIC-RC
• A/ Professor Stephen Bernard	Medical Advisor, Ambulance Victoria
• Professor Jamie Cooper	Principal investigator ANZIC-RC
• Professor Peter Cameron	Head of Emergency Trauma Research Group, DEPM, Monash University
• Professor Andrew Forbes	Head Biostatistics Unit, DEPM, Monash University
• Dr Colin McArthur	Director Critical Care Medicine, Auckland City Hospital
• Ms Lynne Murray	Adjunct Senior Research Fellow, ANZIC-RC
• Ms Lynette Newby	Research Coordinator, DCCM, Auckland City

	Hospital
• A/Professor Alistair Nichol	Senior Lecturer, ANZIC-RC
• A/ Professor Jeffrey Presneill	Staff Specialist, Intensive Care Unit, Mater Hospital
• Dr Stephen Rashford	Medical Director, Queensland Ambulance Service
• Professor Jeffrey Rosenfeld	Neurosurgeon, The Alfred
• Dr Tony Smith	Medical Advisor, St John Northern Region, NZ Ambulance Service
• Mr Michael Stephenson	MICA Group Manager, Ambulance Victoria
• Mr Tony Trapani	Research Fellow, ANZIC-RC
• A/Professor Tony Walker	General Manager Quality & Education Services, Ambulance Victoria
• A/Professor Steve Webb	Intensive Care Consultant, Royal Perth Hospital

3.4 Steering Committee

3.4.1 Responsibilities

- Oversight and advisory role
- Data analysis, collaboration and approval of study publications

3.4.2 Members

- Management committee (as above)
- State/Country Ambulance Service investigators
- Associate investigators*

*Local representatives to be appointed once sites confirmed (suggested1 Intensive Care Physician, 1 Neurosurgeon, 1 Emergency Dept Physician, 1 Research Coordinator per site)

3.5 Contact Details

3.5.1 Chief investigator

Chief Investigator

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Fax: +61 3 90763780

J.cooper@alfred.org.au

3.5.2 Coordinating centre

ANZIC-RC

Department of Epidemiology and Preventive Medicine, School of Public Health and Preventive Medicine

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Research Fellow

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4. ABBREVIATIONS

AE	Adverse event
AKI	Acute Kidney Injury
ANZ	Australia and New Zealand
ANZIC-RC	Australian and New Zealand Intensive Care Research Centre
ANZICS	Australian and New Zealand Intensive Care Society
ARC	Australian Research Council
ARR	Absolute risk reduction
ATBIS	Australasian Traumatic Brain Injury Study
BTF	Brain trauma foundation
CCM	Critical Care Medicine
CCRET	Centre of Cardiovascular Research and Education in Therapeutics
CI	Confidence interval
CPP	Cerebral Perfusion Pressure
CRF	Case report form
CSF	Cerebrospinal fluid
CTG	Clinical Trials Group
CVP	Central venous pressure
DEPM	Department of Epidemiology and Preventive Medicine
DSMC	Data Safety Monitoring Committee
ED	Emergency Department
ETT	Endotracheal tube
HDEC	Human Disability Ethics Committee
HREC	Human Research Ethics Committee
HTS	Hypertonic saline
ICP	Intracranial Pressure
ICU	Intensive Care Unit
IMV	Invasive mechanical ventilation
IV	Intravenous
NEJM	New England Journal of Medicine
NHMRC	National Health and Medical Research Council
NNT	Number needed to treat
NOK	Next of kin
QOL	Quality of life
NS	National statement
RGC	Research Governance Committee
RCT	Randomised controlled trial
RRI	Relative risk increase
RRR	Relative risk reduction
RSI	Rapid sequence intubation
SAE	Serious adverse event
SF	Short form
TBI	Traumatic brain injury

5. LAY DESCRIPTION

Traumatic brain injury (TBI) is a leading cause of death and long term disability, particularly in young adults. Studies from Australia have shown that approximately half of those with severe traumatic brain injury will be severely disabled or dead 6 months post injury. Given the young age of many patients with severe TBI and the long term prevalence of major disability, the economic and more importantly the social cost to the community is very high.

Pre-hospital and hospital management of patients with severe brain injury focuses on prevention of additional injury due primarily to lack of oxygen and insufficient blood pressure. This includes optimising sedation & ventilation, maintaining the fluid balance and draining Cerebrospinal Fluid (CSF) & performing surgery where appropriate. In recent years there has been a research focus on specific pharmacologic interventions however to date there has been no treatment that has been associated with improvement of neurological outcomes.

One treatment that shows promise is the application of hypothermia (cooling). This treatment is commonly used in Australia to decrease brain injury in patients with brain injury following out-of-hospital cardiac arrest. Cooling is thought to protect the brain using a number of mechanisms. There have been a number of animal studies that have looked at how cooling is protective and also some clinical research that suggests some benefit. However at the current time there is insufficient evidence to provide enough proof that cooling should be used routinely for patients with brain injury and like all treatments there can be some risks and side effects.

The POLAR trial has been developed to investigate whether early cooling of patients with severe traumatic brain injury is associated with better outcomes. It is a randomised controlled trial, which is a type of trial that provides the highest quality of evidence.

Unconscious patients will be assessed by ambulance paramedics and if found to be suitable for the trial they will receive cooling by an infusion of chilled salty fluid (Intervention group) or they will be treated in the normal manner (Control group). Allocation to the two groups occurs randomly like flipping a coin. On arrival to hospital, cooled patients will be checked to make sure it is safe to continue cooling and if so they will be cooled by a vest containing cooled water. Control group patients will have their temperature maintained at 37^0C using cooling vests if required for fever prevention. The cooled group will have their temperature lowered to 33^0C and will be kept cool for at least 3 days. After 3 days their temperature will be slowly raised. If they have any issues with control of brain swelling they will be recooled to a temperature that helps maintain control of the swelling. Some patients may also be enrolled into the trial when they are admitted to hospital. All patients will have data collected during the pre-hospital stage and during the hospital admission. The patient's recovery will be measured at 6 months post the injury.

6. SYNOPSIS

6.1 Background

Traumatic brain injury (TBI) is a devastating condition which affects close to 1,000 people each year in Australia, causes extensive disability and suffering, and costs the country close to 1 billion dollars per year. There is a clear scientific rationale for the use of early prophylactic hypothermia as a treatment following TBI, including laboratory studies, positive clinical trials and meta-analyses. A single multi-centre clinical trial by Clifton et al found no improvement in outcomes in patients treated with prophylactic hypothermia.[1] This trial had important methodological limitations. These limitations, together with new evidence in current meta-analyses have led to prophylactic hypothermia for the first time being suggested as a possible therapy in international guidelines, but not as a "standard of care". Meta-analyses should be hypothesis generating and should not alone lead to practice change. Therefore, few clinicians are likely to implement prophylactic hypothermia based on the lingering questions of efficacy, logistics, and potentially increased cost. A large, multi-centre randomised controlled trial in Australia and New Zealand with the capacity to demonstrate clinical benefit is urgently required.

6.2 Design

- Prospective randomised controlled multi-centre trial of early and sustained prophylactic hypothermia in 500 patients with severe TBI.

6.3 Aim

The primary aim of the study is to determine whether early and sustained prophylactic hypothermia, compared to standard Australian and New Zealand `normothermic` care, is associated with an increased proportion of favourable neurological outcomes six months after severe TBI. The null hypothesis is that there is no difference in the proportions of favourable neurological outcomes between patients assigned to either prophylactic hypothermia or standard normothermic care.

6.4 Patient population

- Patients with severe blunt traumatic brain injury who do not have bleeding or other contraindications to cooling.

6.5 Methods

Eligible patients will be randomised in the pre-hospital setting or on admission to the Emergency Department. POLAR study trained paramedics and physicians will screen patients in the pre-hospital setting. Eligible patients will be randomised if they fulfil the inclusion criteria with no pre-hospital exclusion criteria. Those randomised to the normothermia group will follow standard care. For those randomised to the "cooling arm", pre-hospital prophylactic hypothermia will be induced by exposure and by infusing up to 2 litres intravenous cold (4°C) 0.9% sodium chloride aiming for a core temperature of 35°C during transport. In the emergency department the "cooling arm" patients will be assessed to exclude significant bleeding and, once significant bleeding has been excluded, surface cooling vests/wraps will be applied to reach the target core temperature of 33°C. The patient will be then maintained at this temperature for a further 72 hours. Patients with significant bleeding will have cooling withheld until it is safe to decrease the temperature to the target core temperature of 33°C. Patients who have not been randomised pre-hospital will be re-screened in the ED. Eligible patients will be randomised if they fulfil the inclusion criteria with no ED exclusion criteria. Hypothermia will be induced by administration of up to 2L intravenous ice-cold (4°C) 0.9% sodium chloride followed by application of the surface cooling vests/wraps to achieve the target core temperature of 33°C. Patients allocated to standard `normothermic` care will be maintained at a core temperature of 37°C ± 0.5°C.

6.6 Outcome measures

The primary outcome measure is the proportion of favourable neurological outcomes (Glasgow Outcome Score Extended: GOSE 5 to 8) at six months following injury. Secondary outcome measures include, quality of life, mortality and incidence of adverse events.

7. BACKGROUND & RATIONALE

7.1 Clinical & biological rationale

Traumatic brain injury (TBI) is a leading cause of death and long term disability, particularly in young adults[2]. In a recent analysis of an international database of 2664 patients with severe TBI, mortality was 28%[3]. Of great societal importance, most of the patients who survive TBI have permanent neurological disability. At 6-months, only 52% of these patients had favourable neurological outcomes (good recovery or moderate disability) and were able to live independently and 48% had unfavourable neurological outcomes (dead, vegetative state or severe disability). Given the young age of many patients with severe TBI and the long term prevalence of major disability, the economic and social cost to the community is very high.

Australia and New Zealand Perspective

The ANZICS-CTG identified TBI as a key research area where interventional trials might improve survival and functional outcomes and has conducted key preliminary studies. The prospective Australasian Traumatic Brain Injury Study (ATBIS) was undertaken over a 6-month period during 2001 to determine the prevalence, management strategies and outcomes of TBI patients admitted to intensive care units (ICUs) in Australia and New Zealand (ANZ)[4]. There were 363 patients with severe TBI (defined initial Glasgow Coma Score (GCS) ≤ 8) recruited from 16 centres. The twelve-month mortality in patients with severe TBI was 35.1% and the rate of favourable neurological outcomes at 12 months was 48.5%. The ANZICS-CTG then conducted a detailed analysis of the TBI patients in the SAFE study[5] (saline vs. albumin fluid evaluation[6]). The 318 patients with severe TBI had a 32% mortality rate and 46% had favourable neurological outcomes at 24 months. Finally, an interim analysis (n=150) of a pre-hospital trial evaluating paramedic rapid sequence intubation in severe TBI (Rapid Sequence Induction (RSI[7] trial, ACTRN12605000177651)), found a mortality rate of 35% and 59% favourable neurological outcomes at 6 months (personal communication, CI-B).

Clearly, these findings confirm that, despite well resourced and integrated health systems, severe TBI in Australia and New Zealand continues to carry a high rate of mortality and long term disability. The mean lifetime medical and rehabilitation cost of each TBI survivor with severe disability in Australia currently exceeds $3 million[8]. The lifetime cost of caring for the 150 Australian victims of TBI who survive with significant disability each year amounts to nearly a billion dollars. Even a small increase in the number of TBI patients who have favourable rather than unfavourable neurological outcomes, would yield major human and economic savings.

Prophylactic hypothermia: a treatment for severe TBI

Current management of TBI is supportive and focuses on prevention and treatment of cerebral hypoxia using sedation, intravenous fluids, monitoring and manipulation of oxygen, intra-cranial pressure (ICP) and cerebral perfusion pressure (CPP)[9]. Despite decades of research investigating novel pharmacological therapies for patients with TBI, clinical trials of novel therapies to date have not demonstrated substantial improvements in outcomes[10-12]. However, the recent landmark paper in the New England Journal of Medicine (NEJM)[5] reported that the choice of fluid therapy after primary brain injury may positively influence long term neurological outcomes by decreasing secondary brain injury. This paper restored optimism that it is feasible to decrease secondary brain injury and improve long term outcomes.

Currently, a therapy with great potential to reduce neurological damage and improve outcome after severe TBI is the application of early prophylactic hypothermia[13]. This therapy involves the rapid reduction of core body temperature after injury to 33°C, to attenuate brain injury. This treatment is commonly used in Australia and New Zealand to decrease brain injury in patients with severe neurological injury following out-of-hospital cardiac arrest[13].

There is extensive scientific rationale supporting early prophylactic hypothermia as a treatment following TBI including extensive laboratory data, supportive clinical trials and meta-analyses.

Laboratory studies of hypothermia in TBI

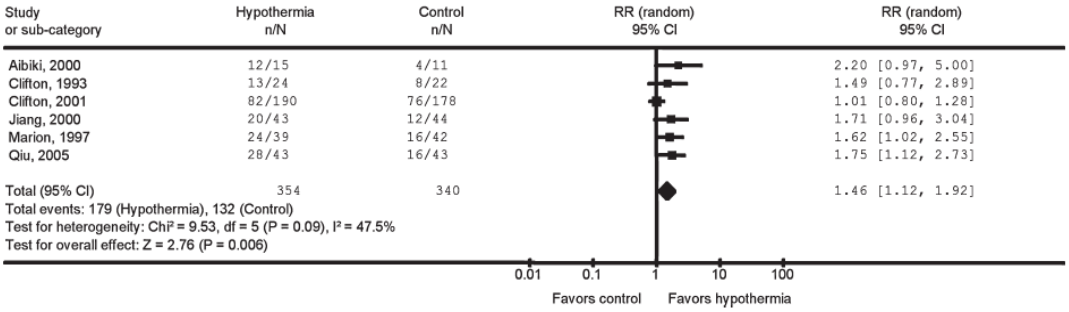
Many experimental studies have confirmed that moderate hypothermia confers protection against ischaemic and non-ischaemic brain hypoxia[14]. Many post-traumatic adverse events that occur in the

injured brain at a cellular and molecular level are highly temperature-sensitive and are a good target for induced hypothermia. Hypothermia has demonstrated powerful neuroprotective effects in experimental models and influences multiple biochemical cascades that are set in motion after TBI (secondary brain injury)[14]. These mechanisms are multifactorial and include: reduction in brain metabolic rate, positive effects on cerebral blood flow, blockade of excitotoxic mechanisms, calcium antagonism, decrease in oedema, modulation of the inflammatory response and modulation of apoptosis[14]. Furthermore, experimental models also suggest that a 'therapeutic window' exists where the induction of hypothermia soon after the primary TBI may provide optimal neurological protection leading to improved outcomes[15-18].

Clinical studies of hypothermia in TBI

Over the past two decades, clinical trials of prophylactic hypothermia in TBI have been conducted and supported a likely benefit[13]. These include single centre trials[19-23] and a single multi-centre trial[1]. Furthermore, a meta-analysis of the highest quality clinical trials of hypothermia in severe TBI was conducted in 2007[24] by the Brain Trauma Foundation (BTF), the recognised international medical body which promulgates evidence-based guidelines for the management of TBI. This meta-analysis reported a significant increase in long term favourable neurological outcomes (Relative Risk (RR) 1.46, 95% confidence interval (95% CI) 1.12 to 1.92, p=0.006, figure 1) with no significant decrease in mortality (RR 0.76, 95% CI 0.05 to 1.05, p=0.18) in patients treated with prophylactic hypothermia compared to normothermia.

Figure 1 [9]



The only published multi-centre hypothermia trial (Clifton, 2001[1]) reported no improvement in outcomes in patients treated with prophylactic hypothermia. This trial was well conducted but had key methodological limitations.

First, there was an average delay of 8.4 hours in attaining the target temperature [1]. This delay was largely due to inferior cooling technology and this may have confounded detection of a protective effect. The subgroup of patients in this trial who were initially hypothermic and then randomised to hypothermia had a significant long-term neurological advantage compared to normothermia[1, 25]. Future trials should induce hypothermia as early as possible and ideally at the location of the traumatic event. There is considerable Australian and international experience with paramedic-initiated early hypothermia for cardiac arrest, and this experience can be applied to severe TBI patients [26, 27].

Second, in severe TBI the generalised swelling of brain tissue frequently persists for over 48 hours. It is therefore likely that therapeutic levels of hypothermia lasting for only 48 hours (in the Clifton trial [1]) were insufficient. This assertion is supported by the BTF meta-analysis finding that trials of prophylactic hypothermia longer than 48 hours were associated with significantly reduced mortality (RR 0.51; 95% CI 0.34 to 0.78)[9]. Therapeutic levels of hypothermia should be induced in excess of 48 hours, which has been well tolerated in patients with severe TBI [28].

Third, the Clifton trial withdrew hypothermia on a time-based trigger rather than according to a physiological trigger [1]. Rewarming of patients despite increases in intracranial pressure is not considered optimal clinical practice [29]. Hypothermia should be withdrawn using a physiological trigger (i.e. ICP) and over a prolonged period to avoid uncontrolled intracranial hypertension [29].

Fourth, the Clifton trial induced and maintained hypothermia using cooling blankets and bags of ice. This practice is associated with difficulty achieving a constant patient temperature and problems targeting gradual re-warming. The recent development of electronically controlled surface (skin) cooling pads through which cold water is channelled has revolutionised the controlled induction, maintenance and ultimate emergence from hypothermia.

Finally, the lack of a 'run in' period, lack of protocolised brain specific care, the inclusion of low-enrolment centres and the large inter-centre variance in the Clifton trial[1] may have further confounded the detection of a protective effect. Interestingly, when the problematic Clifton trial was

excluded from the BTF meta-analysis, a pooled estimate demonstrated a significant reduction of mortality (RR 0.64; 95%CI 0.46 to 0.89)[9].

Potential adverse effects and complications of hypothermia

Despite a temperature of 33ºC being perhaps the ideal balance of therapeutic effect and potential adverse side-effects [13], it is important to consider the potential complications, specifically coagulopathy and infection. Hypothermia may prolong clotting times and alter platelet function and this could lead to increased blood loss. However, this may not significantly increase bleeding in the context of severe TBI [30]. Future trials of prophylactic hypothermia such as this one should include a strategy to minimise potential risks of bleeding. For example, induction of mild hypothermia (minimum temperature 35°C) in the field to optimise brain protection, while limiting the risk of coagulopathy prior to a robust assessment of bleeding in the Emergency Department (ED). Meta-analyses reported increased pneumonia in patients cooled for prolonged periods [31]. While it has previously been demonstrated in a small study that this increased rate of infection did not increase mortality[28], any future trial should incorporate optimal standard of care measures to reduce infectious complications.

Hypothermia decreases clearance of drugs that are cleared by the liver. These include propofol, midazolam and morphine which will accumulate over time unless doses are reduced [40].

The cooling phase of hypothermia is associated with hypokalaemia and hypomagnesaemia. The rewarming phase is associated with hyperkalaemia and hypermagnesimia[40].

The requirement for a multi-centre phase III trial of prophylactic hypothermia in severe TBI

Methodological shortcomings of the Clifton trial, together with limited evidence in recent meta-analyses[9] has led to prophylactic hypothermia for the first time being suggested as a possible therapy in the international BTF guidelines, but not a “standard of care”. Meta-analyses are best considered as hypothesis generating and should never form the basis for changes in clinical practice. Therefore, despite this recommendation, few if any clinicians are likely to implement prophylactic hypothermia owing to lingering concerns of efficacy, logistics, and increased costs. A large, multi-centre randomised controlled trial with the capacity to demonstrate benefit is required.

Currently, 2 multi-centre phase III trials of prophylactic hypothermia in severe TBI are underway (USA; NCT00178711, Japan; NCT00134472). However, there are a number of reasons why an Australian multi-centre phase III trial of prophylactic hypothermia in severe TBI is urgently required. First, these trials may not succeed, being underpowered to detect a plausible improvement in patient-centred outcomes, and by repeating previous methodological concerns such as insufficient duration of hypothermia, time based triggers for rewarming and the lack of a national hospital / ICU research network. Second, there are substantial differences in organisational and training structures of pre-hospital care and transport, emergency departments and ICUs between Australia and other countries [32]. Finally, the standard of emergency and ICU care in Australia and New Zealand is high. The ATBIS survey found that Australian and New Zealand TBI patients are more likely than European patients to receive invasive monitoring [4]. Therefore, if prophylactic hypothermia was shown to be beneficial in a setting where care levels are high, it would likely change practice not only in Australia and New Zealand but also internationally.

7.2 Significance

If prophylactic hypothermia is found to improve neurological outcomes, given the high disability rate, at least 200 patients per year will have significantly improved neurological outcomes in Australia/New Zealand by the widespread application of prophylactic hypothermia. If standard ‘normothermic’ care is shown to be equivalent, or even superior to prophylactic hypothermia, patients will be able to continue to receive less invasive and less expensive care and the features of Australian/New Zealand standard care can be used to inform practice worldwide.

8. OBJECTIVES

8.1 Aim

The primary aim of the study is to determine whether early and sustained prophylactic hypothermia, compared to standard `normothermic` care, is associated with an increased proportion of favourable neurological outcomes six months after severe TBI.

8.2 Null Hypothesis

That there is no difference in the proportion of favourable neurological outcomes six months after severe traumatic brain injury in patients treated with early and sustained hypothermia, compared to standard normothermic management.

9. STUDY OUTCOME MEASURES

9.1 Primary outcome

The proportion of favourable neurological outcomes (Glasgow Outcome Score Extended: GOSE 5 to 8) at six months following injury

9.2 Secondary outcome

- Probability of an equal or greater GOSE level at 6 months compared to the probability of a lesser GOSE level, using a proportional odds model
- Probability of an equal or greater GOSE level at 6 months compared to the probability of a lesser GOSE level,using the “sliding dichotomy” method
- Quality of life assessments (QOL)
    - EQ5D
    - SF12
- Proportion of favourable (GOSE 5-8) neurological outcomes in survivors at six months following injury
- Mortality (all cause) at
    - six months
    - hospital discharge
- Incidence of adverse events, specifically:
    - Bleeding
    - Infection.
- Health economic evaluation
- Test for interaction between time to reach 33C and dichotomised GOSE scores in the hypothermic group
- Test for interaction between the effect of cooling on neurological function at 6 months and the presence of surgically evacuated mass lesions.

10. OVERALL STUDY DESIGN

10.1 Study population

Patients with severe blunt traumatic brain injury.

10.2 Pre-hospital Inclusion Criteria

- Blunt trauma with clinical diagnosis of severe TBI and GCS <9
- Estimated age ≥ 18 and < 60 years of age
- The patient is intubated or intubation is imminent

10.3 Pre-hospital Exclusion Criteria

- Clinical diagnosis of drug or alcohol intoxication as predominant cause of coma
- Randomisation unable to be performed within 3 hrs of estimated time of injury

- Estimated transport time to study hospital >2.5hrs
- Able to be intubated without drugs
- Systolic BP <90mmHg
- Heart rate > 120bpm
- Cardiac arrest at the scene or in transit
- GCS=3 + un-reactive pupils
- Penetrating neck/torso injury
- Known or obvious pregnancy
- Receiving hospital is not a study site
- Evidence of current anti-coagulant treatment
- Known to be carer dependent due to a pre-existing neurological condition

10.4 Emergency Dept Inclusion Criteria

- Blunt trauma with clinical diagnosis of severe TBI and GCS <9
- Estimated age ≥ 18 and < 60 years of age
- The patient is intubated or intubation is imminent

10.5 Emergency Dept Exclusion Criteria

- Clinical diagnosis of drug or alcohol intoxication as predominant cause of coma
- Randomisation unable to be performed within 3 hrs of estimated time of injury
- Able to be intubated without drugs
- Persistent Systolic BP <90mmHg
- GCS=3 + un-reactive pupils
- Cardiac arrest at the scene or in transit
- Clinically significant bleeding likely to require haemostatic intervention, for example:
    - Bleeding into the chest, abdomen or retro-peritoneum likely to require surgery +/- embolisation
    - Pelvic fracture likely to require surgery +/- embolisation
    - More than two long bone fractures requiring operative fixation
- Penetrating neck/torso injury
- Positive urine or blood pregnancy test
- Evidence of current anti-coagulant treatment
- Known to be carer dependent due to a pre-existing neurological condition
- In the treating clinician's opinion, "cooling" is not in the patient's best interest

10.6 Co-enrolment

Co-enrolment of participants in other randomised controlled trials will not be permitted.

11. PARTICIPATING STUDY SITES

- Alfred Hospital, Melbourne, Australia
- Royal Melbourne Hospital, Melbourne, Australia
- Princess Alexandra Hospital, Brisbane, Australia

- Royal Perth Hospital, Perth, Australia
- Sir Charles Gairdner Hospital, Perth, Australia
- Auckland City Hospital New Zealand

12. STUDY PROCEDURES

12.1 Pre-hospital assessment of patient for study suitability

Patients will be assessed by study trained and affiliated ambulance paramedics &/or physicians. If they fulfil the inclusion criteria and have no exclusion criteria they will be randomised by opening the next opaque envelope in the randomisation series kept in each participating ambulance. The envelopes will contain a wristband to be placed on the patient & a label which designates the study number and treatment allocation. A small number of details will be completed on the wristband and label (Patient's initials, Date and time of randomisation). The label will be placed in the patient's hospital medical record on hospital arrival. The ambulance paramedics/physicians will also document the randomisation of the patient on the ambulance case report form. The wristband and label in the patient's medical record will alert hospital staff to the patient's enrolment in the study.

12.2 Pre-Hospital Standard study care (normothermia - control group)

Patients randomised to the standard care arm, will be transferred to the nearest participating centre with no exposure and no cold fluids during transport. If the patients core temperature is <36.5^{o}C the patient will be transported covered by blankets in a heated vehicle in accordance with usual clinical practice.

12.3 Pre-hospital study intervention- Induction of hypothermia

The prophylactic hypothermia protocol will be commenced at the scene. Hypothermia will be induced pre-hospital by exposure and by intravenous (IV) infusion of ice-cold (4°C) 0.9% sodium chloride (similar to the RICH[26] and other[27] trials) to achieve a target core temperature of 35°C during transport.

To **avoid** fluid overload the volume of cold fluid administered will be dependent on intubation status

Randomised **after intubation** – give **1000m**l cold normal saline (100mls/min)

Randomised **before intubation** – give **2000ml** cold normal saline (100mls/min)

If the patient's temperature is <35°C the patient will not receive cold fluid however they will be transported to hospital with a light covering.

12.4 Patient review on ED arrival

On arrival in the emergency department the patients randomised to the "cooling arm" will be assessed for:

Clinically significant bleeding including but not limited to:

- Bleeding into the chest, abdomen or retro-peritoneum likely to require surgery +/- embolisation
- Pelvic fracture likely to require surgery +/- embolisation
- More than two long bone fractures requiring operative fixation

If in the treating clinician's opinion there is clinically significant bleeding (or a high risk of clinically significant bleeding), further cooling will be **withheld** and the patient maintained at a core temperature of 35-37 ^{o}C. Hypothermia will be re-instituted as soon as possible following successful control of bleeding.

If hypothermia **cannot be reinstituted** within **48hrs** of the injury the patient will be **withdrawn** from the "cooling arm" protocol and will follow standard management.

12.5 .Drug and/or alcohol affected patients with normal CT brain

Patients who are clinically significantly drug or alcohol affected with a normal brain (or minor injury only) on CT imaging, will have the study intervention of active cooling withheld until they can be

clinically assessed. Whilst waiting for drugs and alcohol to clear the patient will be kept sedated and at 35°C. Sedation will be weaned and a clinical assessment performed at the clinician's discretion. If during clinical assessment the patient shivers significantly they may be warmed to a core temperature of 36°C.

The patient will have the study intervention of cooling **withdrawn** and will receive standard management if:

- They localise or obey when clinically assessed, and or
- In the treating clinician's opinion they do not have a severe TBI, and or

In the treating clinician's opinion they do not require an ICU admission. Data will be collected and the follow up assessment performed on all patients who have the study intervention withdrawn.

If the patient's motor score is withdrawal or worse (GCS(m)≤4) they will be re-sedated, have the surface cooling vests/wraps applied and will continue in the "cooling arm".

12.6 Hospital assessment of patient for study suitability

Patients who have not been enrolled in the study pre-hospital will be assessed for study suitability on arrival in the ED. If they fulfil the inclusion criteria and have no exclusion criteria they will be randomised by opening the next opaque envelope in the randomisation series kept in the ED. The envelopes will contain a wristband to be placed on the patient & a label which designates the study number and treatment allocation. A small number of details will be completed on the wristband and label (Patient's initials, Date and time of randomisation). The label will be placed in the patient's hospital medical record. The wristband and label in the patient's medical record will alert hospital staff to the patient's enrolment in the study.

12.7 ED study intervention – Induction of hypothermia

The prophylactic hypothermia protocol will commence immediately following randomisation. Hypothermia will be induced by exposure and intravenous infusion (IV) of ice-cold (4°C) 0.9% sodium chloride (similar to the RICH[26] and other[27] trials) to achieve a target core temperature of 35°C.

To **avoid** fluid overload the volume of cold fluid administered will be dependent on intubation status

Randomised **after intubation** – give **1000m**l cold normal saline (100mls/min)

Randomised **before intubation** – give **2000ml** cold normal saline (100mls/min)

12.8 Temperature control system

Temperature control of the "cooling arm" patients will be achieved using a temperature control console & surface temperature control vests/leg wraps/blankets which facilitate thermal transfer. The vests/leg wraps/blankets will be applied by nursing staff to approximately 40% of the body surface area. Temperature control will be initiated in the ED after the patient has been reviewed.

12.9 Standard study care (normothermia – control arm group)

The core temperature of 36.5-37.5°C will be targeted in the control group patients. Active warming with blankets and other standard measures may be required to achieve the target temperature. Febrile patients (defined as temperature >38 °C will be treated by paracetamol if clinically suitable, and by the application of temperature control vests/wraps/blankets with a target core temperature of 37 (±0.5 °C)

12.10 Study intervention (hypothermia – cooling arm group)

The temperature control vest/wraps/blankets will be applied as soon as possible with a target core temperature of 33 °C to be achieved by rapid cooling.

12.10.1 Withholding (temporary cessation) hypothermia

If a patient allocated to hypothermia develops clinically significant bleeding at any time, cooling will be withheld and they will be rewarmed (to 35°C -37°C core temperature). Patients in whom any such bleeding is controlled may resume the hypothermic protocol (to 33°C) within 48 hours of the initial injury. Patients with bleeding controlled but requiring surgery will have hypothermia withheld and will

be rewarmed to 35°C - 37°C. They will be reviewed for significant bleeding following the surgery and will resume the hypothermia protocol as soon as possible if there are no bleeding concerns.

12.10.2 Withdrawal (permanent cessation) of hypothermia

Patients with

- Significant bleeding which prevents initiation of cooling within 48hrs post injury
- Positive urine or blood pregnancy test
- Normal CT Brain scan & patient localising or obeying after decreased sedation
- In the treating clinician's opinion, the patient does not require an ICU admission
- In the treating clinician's opinion, hypothermia is not in the patient's best interest

Will have cooling **permanently withdrawn** and will follow the standard care control group protocol.

Data will be collected and the follow up assessment performed on all patients who have the study intervention withdrawn.

12.10.3 Rewarming protocol

72 hours after randomisation, the hypothermic patient will be assessed for suitability for controlled, active, gradual rewarming (0.17°C per hour) to 37°C. It is imperative that rewarming occurs slowly. The recommended maximum rate of rewarming using the Gaymer Meditherm III is 0.5°C every 3 hours. It is imperative that core temperature is utilised. (See "Appendix F: Cooling and Rewarming Guidelines").

Prior to commencing rewarming asses the patient's ICP, haemodynamic and electrolyte status (esp. potassium and magnesium which may alter during rewarming). Correct any abnormalities. Rewarming should only commence if the ICP is <20mmHg and stable. If at any time during the rewarming process there is a sustained rise in ICP (>20mmHg for > 5mins) the patient will be re-cooled to a temperature that controls the ICP (minimum core temperature 33°C). The patient will remain at this temperature until the clinician determines it is appropriate to recommence warming of the patient. Assessment for further rewarming will occur daily or earlier at the clinicians' discretion. Transient rises in ICP due to suctioning, turning and coughing may not require cessation of rewarming.

Patients will be carefully monitored to ensure that hypotension is promptly treated with an initial small fluid bolus and/or by vasopressors. If the patient continues to be haemodynamically unstable rewarming will cease until stability is achieved.

Patient's electrolyte status (esp. potassium and magnesium) should be monitored and electrolyte levels corrected during rewarming.

During rewarming there is an increased risk of shivering (esp. between 34°C to 35°C). As shivering greatly increases metabolic demand it is vital that shivering is monitored and managed quickly and aggressively. (See Appendix F: cooling and rewarming Guidelines)

The maximum protocolised period of hypothermia will be 7 days.

Once a patient has completed re-warming and reached 37°C, they will be maintained at normothermia (below 38°C) using the surface cooling pads up to a maximum of 7 days or until the patient is discharged from ICU if prior to 7 days.

In the control group pyrexia above 38°C should be avoided, for up to 96 hours post randomisation. After 96 hours post randomisation patients should be managed as per unit protocol. (Surface cooling systems may be utilised for control patients)

12.11 TBI management & ICU management

Patients enrolled in both the hypothermia and normothermia groups will be managed according to current international evidence based guidelines[9] including insertion of an ICP monitor unless de-sedation and clinical assessment is planned or insertion is contraindicated (note: ICP monitor should not be removed in the hypothermia group until rewarming has been completed). This protocolised care (as previously used by ANZICS CTG TBI network in DECRA and HTS[11]trials) includes a stepped regimen for both the management of increased intra-cranial pressure (ICP) (>20mmHg) and severely lowered cerebral perfusion pressure (CPP) (<60mmHg), and targets for fluid management.

ICU management guidelines to ensure optimal standard of care and reduction of infectious complications have been developed.

See Appendix D

12.11.1 Fluid management

Fluid overload may contribute to cerebral oedema. Therefore after resuscitation during the first 24hours, positive daily fluid balances > 500mls should be avoided. In the absence of hypovolaemia, periods of relative hypotension should be managed primarily with vasopressors (adrenaline/noradrenaline).

12.12 CT Brain scans

On conclusion of recruitment the admission CT Brain scan will be assessed by a Neurosurgeon or Neuroradiologist blinded to the treatment group using the Marshall Grading system[33].(Deidentified CT brain scans will be forwarded to the coordinating centre for assessment).

12.13 Blinding

To ensure appropriate treatment, participants must be monitored closely and investigation results known to the treating clinical staff. As patient safety is paramount, and a key patient vital sign (temperature) is due to the treatment itself, it is not possible to blind clinical staff as to the patient's treatment allocation. Bias will be minimised by concealed treatment allocation prior to randomisation, by protocolised treatment in both groups, and by assessment of the primary outcome by centralised, blinded and trained research staff (as accomplished successfully in recent studies including SAFE[6], SAFE-TBI [5], HTS[4, 11], and ATBIS[4]). The primary outcome measure is robust and subject to minimal ascertainment bias.

12.14 Contamination

Contamination will be minimised by using:

- Standardised and protocolised patient management in both groups by paramedics and emergency department staff
- Clearly distinct temperature goals.

Patients in the normothermia group who develop a strong clinical indication for induced hypothermia, such as for the therapy of refractory intracranial hypertension not responsive to other measures, will be permitted to receive a therapeutic reduction in core temperature below 37°C, down to a minimum of 35 ^{o}C.

12.15 Study pilot/run-in phase

A study pilot/run in phase will be conducted at the lead site. This will involve enrolling 4 patients in the ED. The 4 pilot/run in phase patients will be allocated to receive hypothermia. The pilot/run in phase will allow the co-ordinating centre to optimise and refine the protocol paying particular attention to inclusion/exclusion criteria, safety assessment on hospital admission and administration of the cooling, rewarming protocol and resolving any unforseen logistical issues.

A study run-in phase will also be conducted at all other sites. The first two patients at each site will be allocated to hypothermia in the ED. The purpose of this run-in period is to allow the co-ordinating centre to check site adherence to the prescribed protocol to ensure minimum site variance in management and to allow site feedback before commencing the study.

12.16 Randomisation

Randomisation will be performed by the paramedics/physicians using a closed envelope system including block randomisation (RSI[7] and RICH[26] trials). Treatment allocation will be stratified by 2 strata:

- a four level variable of Victoria / Queensland / Western Australia / New Zealand
- a two level variable of pre-hospital enrolment or emergency department enrolment

Eligible patients will be randomised to normothermia (standard care) or prophylactic hypothermia in a 1:1 ratio.

12.17 Table of events

See Appendix A

13. ETHICS

13.1 Guiding principles

This study will be performed in accordance with the ethical principles of the Declaration of Helsinki (June 1964 and amended 1975, 1983, 1989, 1996, 2000, 2008 and Note of Clarification 2002 and 2004), ICH GCP Notes for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with Therapeutic Goods Administration comments and NHMRC National Statement on Ethical Conduct in Research Involving Humans (March 2007).

13.2 Ethical issues of the study

The two ethical considerations in this study are:

- Emergency research & consent
- The risk/benefit ratio of the study treatment.

13.2.1 Emergency research and consent

Some research must necessarily be performed on patients in emergency (definition is unexpected) situations when they are unable to provide consent, as with all other categories of patients. The principle of justice requires this to be the case. This study constitutes emergency research. Principle 29 of the Declaration of Helsinki acknowledges that some clinical research will involve patients who are physically incapable of giving consent "Research on individuals from whom it is not physically possible to obtain consent, including proxy or advance consent, should be done only if the physical or mental condition that prevents obtaining informed consent is a necessary characteristic of the research population"

To be eligible for recruitment in this study patients will be unconscious (GCS<9) and consequently will be unable to consent to participation. The study intervention must be instigated as soon as possible by the paramedics/physicians in the field or ED; thus consent/assent cannot be sought from the participant's next of kin (NOK)/person responsible. The National Statement (NS) on Ethical Conduct in Research Involving Humans (March 2007) acknowledges in section 4.4 that research involving patients who are heavily dependent on medical care in emergency circumstances, such as the patients in this study, may proceed when the participants ability to give consent is non existent[34]. This requires approval from the appropriate ethical body to waiver or defer consent (section 7.3).

13.2.2 Risk /benefit ratio of study treatment

As discussed previously in section 7.1 current literature supports a likely benefit from the use of prophylactic hypothermia in severe TBI12. These include single centre trials[19-23] and a single multi-centre trial[1]. Furthermore, a meta-analysis of the highest quality clinical trials of hypothermia in severe TBI was conducted in 2007[9] by the Brain Trauma Foundation (BTF), the recognised international medical body which promulgates evidence-based guidelines for the management of TBI. This meta-analysis reported a significant increase in long term favourable neurological outcomes (RR 1.46, 95% CI 1.12 to 1.92, p=0.006, figure 1) with no significant decrease in mortality (RR 0.76, 95%CI 0.05 to 1.05, p=0.18) in patients treated with prophylactic hypothermia compared to normothermia. The only published multi-centre hypothermia trial in adults (Clifton, 2001[1]) reported no improvement in outcomes in patients treated with prophylactic hypothermia. This trial was well conducted but had methodological limitations discussed previously in section 7.1.

As with most treatment interventions there are some potential associated risks. Patients with severe TBI are at increased risk for ventilator-associated pneumonia (VAP)[35] and it is thought that the risk may potentially increase with hypothermia[36]. The risk of VAP and other infections in this study will be minimised by utilising guidelines for optimal standards of care to reduce infectious complications.

Coagulopathy can also occur with cooling although to date none of the large clinical trials in patients with TBI has reported significantly increased risk of bleeding associated with hypothermia[37].

However to minimise potential risks of bleeding, induction of mild hypothermia (minimum core temperature 35oC) in the field will be utilised to optimise brain protection, while limiting the risk of coagulopathy prior to a robust assessment of bleeding in the ED. Once admitted to hospital the participant will be reviewed to ensure that there is no significant bleeding and that it is safe to continue to receive the study intervention. Patients randomised in the field to the hypothermia group with haemodynamic instability presumed secondary to bleeding will be rewarmed until bleeding is controlled. Patients in whom bleeding is clearly controlled (i.e. correction of surgically-isolated lesions), will later resume the hypothermic protocol.

Over time low electrolyte levels may develop in the induction phase of cooling due to increased renal excretion and intracellular shift [38]. Electrolytes are measured regularly as part of standard intensive care and abnormalities can be treated accordingly. Potassium levels may rise during the rewarming phase as potassium that was transferred into the cell in the induction phase is released[38]. Controlled slow rewarming will give the kidneys time to excrete any excess potassium. Hypovolemia can occur due to “cold diuresis” during the induction phase[38]. Haemodynamic parameters will be monitored carefully and if instability develops a fluid challenge test would be warranted.

Cardiac arrhythmias have been linked to cooling however there is no hypothermia-induced risk for severe arrhythmias unless core temperature decreases to <30oC[39]. Bradycardia may occur when the core temperature drops below 35.5oC. The bradycardia may not require treatment however, bradycardia (<50 beats/min) associated with hypotension may respond to a low dose chronotropic agent (eg. Adrenaline). Temperature will be tightly controlled at 33°C ie; above the temperature range known to cause harmful arrhythmias. Patients will have constant cardiac monitoring as part of standard intensive care.

Hypothermia can simultaneously decrease insulin sensitivity & reduce insulin secretion by pancreatic islet cells. Patients treated with hypothermia will be at a higher than average risk for developing hyperglycaemia[39]. Patients have blood glucose tests performed regularly and control of hyperglycaemia forms a part of regular intensive care.

There is an increased risk of feeding intolerance in patients with traumatic brain injury[40]. The risk may increase with hypothermia as cooling may impair bowel function and delay gastric emptying. Feeding rates and tolerance will need to be carefully monitored and appropriate measures instituted if feeding intolerance develops.

Hypothermia decreases drug clearance and may lead to drug accumulation. Sedation is utilised during hypothermia induction to minimise shivering. Once 34°C has been reached consideration should be given to ceasing or dose reducing propofol and opiates. Fentanyl may also be considered as an alternative to morphine for pain control. Barbiturates can be considered for ICP control as required.

Hypothermia can also alter the movement of electrolytes such as potassium and magnesium, during the cooling and rewarming phases. Electrolyte levels will be monitored.

13.3 Ethics committee approval

In Australia, this protocol will be submitted to a Human Research and Ethics Committee constituted according to the NHMRC National Statement on Ethical Conduct in Research Involving Humans (March 2007) for each institution. In New Zealand, this protocol will be submitted to the appropriate Health and Disability Ethics Committee, accredited by the Health Research Council and constituted in accordance with the Operational Standard for Ethics Committees March 2006. Ethical clearance from the appropriate legal body will be required from any participating sites in other countries. Approval will also be sought using the appropriate legislative requirements for each state and country. Approval of the protocol, plans for obtaining consent, and related documents will be obtained prior to the start of the study at each site.

It is the investigator’s responsibility to ensure that all conditions for approval of the study are met and that amendments to the protocol or serious adverse events are also reported to the HREC (or equivalent) as required by that committee.

13.4 Confidentiality of patient data

Patients will be randomised either pre-hospital or in ED and will receive a study number. The Research Coordinator will compile a study enrolment log which will link the study number to the patient name. Subsequent data will be identified by the study number. The enrolment log and study data will be kept separately. Follow up details of the patient and their family will be collected including name, address and contact telephone numbers. The contact details will be forwarded to the co-

ordinating centre. The co-ordinating site will perform the follow up assessments to ensure consistency and accuracy. All data collected in the follow up assessment will be identified by the study code. The follow up contact details and study data will be kept separately. Study data will be entered into a password protected database managed by the CCRET (Monash University). No identifying data will be entered into the database. The contact details and study data will be kept in a locked office at both the study site and coordinating site.

13.5 Informed consent

Unconscious patients with severe head injury will not be able to provide informed consent. This trial will therefore use deferral of consent, in accordance with section 4.4 of the NS [34]. This approach has proven successful for 5 previous NHMRC funded randomised trials (including TBI and pre-hospital trials) - (SAFE[6], RSI[7], RICH[26], out of hospital cardiac arrest[41] and HTS[5] trials).

As soon as is reasonably possible and appropriate the patient's NOK/person responsible will be informed of the patient's inclusion in the research and will be informed of the option to withdraw the patient from it without any reduction in quality of care (NS 4.4.14).[34] If the NOK/person responsible chooses to withdraw their consent for continued participation they will be asked for permission to use the data collected up to that time.

Patients who recover sufficient cognition to understand the explanation of the study will be asked to consent to continue in the study or be offered the chance to withdraw. If the patient chooses to withdraw from the study, they will be asked for permission to use their data up to the time of withdrawal (as per 4.4.14)[34].

All interaction between research staff and participants and their relatives will take into consideration the stress or emotional factors associated with critical illness and ensure that the dependency of potential participants and their relatives on medical personnel providing treatment does not compromise their actual or perceived freedom of decision making to participate (as per 4.4.11)[34].

14. DATA MANAGEMENT

14.1 Data collection methods

All data will be collected by trained staff at each study site using a paper source document developed by the coordinating centre. Data will then be entered into a website designed by the CCRET. Data queries will be automatically generated via the website.

Randomised patients will be followed up to death or six months post-randomisation. Data collection will be restricted primarily to those variables necessary to define clinical patient characteristics including: baseline demographics, primary diagnoses, physiological parameters, diagnostic interventions, therapeutic interventions and documentation of deaths and other serious adverse events.

Patients and/or their legal surrogates will be asked to provide 3 possible points of contact (home and close family contact details) to the research staff prior to discharge. For patients discharged alive from ICU, follow-up will be restricted to information concerning duration of hospital stay and vital status at hospital discharge and six months. Full protocol data will be collected in all patients including those excluded at any stage. In addition, patients who are alive at 6 months after randomization (or a proxy – generally a close family member) will be interviewed by a single, trained and blinded assessor. This blinded assessor will use a standardized structured telephone questionnaire[42] to measure the eight-grade GOSE [43], and a QOL assessment EQ5D[44] & SF12®[45]. Neurological outcomes will then be defined as favourable (GOSE 5 to 8; moderate disability and good recovery) or unfavourable (GOSE 1 to 4; death and severe disability). Patients allocated to the prophylactic hypothermia group in whom hypothermia is subsequently withdrawn for any reason (i.e. bleeding) will be followed up, according to the study follow-up schedule and analysed on an intention-to-treat principle.

Health economic data related to utilisation of medical services post hospital discharge will be collected. The transport accident commission and accident compensation corporation will assist with provision of health economic data.

14.2 Data variables collected

Pre-hospital

- Patient identifier(s)

- Baseline demographics
- Incident details
- Pre-hospital vital signs, Glasgow coma scale & temperature
- Date & timing of cooling intervention
- Type and volume of fluid therapy

Hospital admission

- Vital signs, Glasgow coma scale
- Pregnancy test (female)
- Date & time of surface temperature control vests/leg wrap application
- Haematological and biochemical parameters
- Hourly core temperature
- Type and volume of fluid therapy including blood
- Bleeding incidents
- Brain injury management interventions
- CT Brain scan (Marshall score[33])
- Injury severity score

Intensive Care Admission

- Vital signs, Glasgow coma scale
- Haematological and biochemical parameters
- Hourly core temperature
- Type and volume of fluid therapy including blood
- Urine output/fluid balance
- ICU interventions
- Brain injury management interventions
- Feeding; delivery method
- Feeding tolerance
- Infection surveillance
- Bleeding incidents

Hospital discharge

- Length of mechanical ventilation
- Length of stay
- Discharge destination
- Vital status and hospital discharge
- Treatment limitations/withdrawal

Outcome data

- 6 month GOSE
- EQ5D
- SF12®

Health Economic evaluation data

- Clinical costing
- Duration of rehabilitation stay

- Duration of nursing home stay
- Level of home care

General data collection

- Withdrawal from study protocol

14.3 Data management

Data management will be performed by the CCRET. Streamlined data collection instruments and procedures will be developed using experience from our previous TBI trials (HTS[11], RSI[7], ATBIS[4], SAFE-TBI[5]).

14.4 Monitoring

Prior to study commencement, a start-up meeting will be held for study staff. During the study, the project manager will make one monitoring visit following the study run-in phase and at least one other monitoring visit to each site during the recruitment period. The purpose of these visits is to ensure the study is conducted according to the protocol, all applicable guidelines and regulations, and to perform source data verification.

A monitoring report will be prepared following each visit and reviewed by the management committee. A copy of the report will be sent to the principal investigator and study coordinator at the site and will be filed in the site investigator file.

Medical records, any other relevant source documents and the site investigator files must be made available to the monitor for these monitoring visits during the course of the study and at the completion of the study as needed.

14.4.1 Aims of monitoring visits:

- Check the accuracy of the database by performing source data verification of the CRF against the original source documents.
- Check for protocol violations or deviations and report these to the chief investigator as necessary.
- Review secondary outcome data available for each patient.
- Confirm the consent procedures approved by the site's HREC have been followed and view each original signed consent form.
- Check data security and access.
- Review all serious adverse events (SAEs) and follow up all reported SAEs.
- Review investigator site files for completeness and accuracy.
- Assist the study staff with any queries or problems they may have in relation to the study.

15. STATISTICAL CONSIDERATIONS

15.1 Sample size calculation

Sample size and power calculations (primary outcome)

The recent prospective studies (RSI[7], SAFE-TBI[5] and ATBIS3) found a weighted mean rate of current favourable neurological outcomes of 50% in Australian and New Zealand patients with severe TBI.

A beneficial effect of prophylactic hypothermia in severe TBI of **30% relative risk increase** (RRI) from **50%** to **65%** (15% absolute risk increase (ARI)) in favourable neurological outcome at 6 months following injury would be a clinically relevant and important effect. Based on these figures, a study of fixed size with full compliance and follow-up would require **364** patients (182 in each of two treatment arms) to have an **80%** two-sided power to detect a 1**5% ARI** (from **50%** to **65%)** in favourable neurological outcome at 6 months following injury. This is a plausible and conservative estimate, based on:

A recent meta-analysis[1] in severe traumatic brain injury comparing prophylactic hypothermia to normothermia which demonstrated a 46% improvement of favourable outcomes (RR 1.46, 95% CI 1.12 to 1.92, p=0.006).

A finding of a 50% increase (p=0.02) in favourable outcomes in a sub-group of patients with severe traumatic brain injury <45 years of age who were hypothermic on arrival and subsequently randomised to hypothermia versus normothermia[1, 9].

A 30% RRI in favourable neurological outcomes is approximately two-thirds of the RRI demonstrated elsewhere[11]. If prophylactic hypothermia were proven to be beneficial, such a difference in neurological outcomes would be highly clinically significant (NNT=7) and would lead to a widespread change in management of severe TBI patients in Australia and internationally.

To ensure availability of the required number of evaluable patients, the sample size will be inflated to account for losses to follow-up, withdrawal of hypothermia, due to contraindications and interim data analysis. Allowing an overall proportion of **5%** loss to long term follow up (previous CTG studies and HTS[4] of 1%), expands the sample size to **384** patients. Furthermore, based on pilot data (ATBIS[11]), allowance for a **12%** rate of withdrawal of the hypothermic intervention for patients randomised to hypothermia (8% bleeding, 2% withdrawal of surrogate / physician consent (previous pre-hospital trials (HTS)[46] <1%), and an estimated 2% rate of inappropriate paramedics enrolment (i.e. cardiovascular accident not TBI) increases the sample size to **496** patients. In addition the trivial **0.7%** sample size inflation factor associated with use of two interim Haybittle-Peto analysis adds a requirement for two more subjects. The trial recruitment target is **500** subjects.

Sample size and power calculations (secondary outcomes)

The secondary outcome analyses using proportional odds and the sliding dichotomy are included because although more complex for clinicians to understand, have increased power for outcome assessments in TBI patients. For example using the proportional odds model, this study of 182 evaluable subjects in each of two treatment groups would have a one-sided power of 96% to detect this magnitude of reference improvement as statistically significant with type I error (alpha) = 0.05

15.2 Statistical and analytical plan

Independent statisticians at Monash University will perform an intention-to-treat analysis based on all patients enrolled in the study. Baseline and outcome variables will be compared using Students t-test and Chi-squared test as appropriate. The favourable – unfavourable neurological outcome dichotomy at six months, quality of life assessment, proportion of favourable outcomes in survivors and mortality proportions will also be compared between treatment groups using the stratified Mantel-Haenszel test, and with logistic regression models adjusting for relevant covariates. Time-to-event analyses will be undertaken using non-parametric Kaplan-Meier and semi-parametric Cox proportional hazards regression methods. In addition to the above analyses of binary outcome variables, two further secondary analytic approaches will be applied to generate secondary outcomes. First, a proportional odds cumulative logit model, adjusting for relevant covariates, will be applied to the eight-level vector of 6-month GOSE. Second, the tertiles-of-risk sliding dichotomy approach to the analysis of outcome from severe TBI will be reported[12, 47]. The latter involves a dichotomisation of the 6-month GOSE for unfavourable versus favourable outcome differentiated according to each subject's baseline prognostic risk.

15.3 Interim analyses

There will be no analyses for futility.

Two planned interim safety analyses for potential harm will be performed by the independent data and safety monitoring committee (DSMC) at 25% and 50% patient enrolment, The first (at 125 patients) will examine mortality only with a P value for stopping of P<0.005. The second (at 256 patients) will examine mortality and proportion of favourable neurological outcomes. (P<0.005) The analyses will use the Haybittle-Peto rule applied to mortality data between treatment groups and the conventional 3-standard deviation threshold of a standardised statistic (|Zk=1|≥ 3) calculated from a normal approximation to the discrete binomial difference in mortality proportions. Assuming no early stopping, such a single interim Haybittle-Peto analysis will not, in practice, require adjustment (for repeated significance testing) to the conventional statistical significance level (p<0.05) to be applied at the final analyses of the completed trial.

16. SAFETY

16.1 Data Safety Management Committee

An independent data safety monitoring committee (DSMC), comprising experts in clinical trials, biostatistics, emergency medicine and intensive care has been established. The committee chaired by Associate Professor Jamie Hutchinson (University of Toronto, Canada) will be charged with monitoring total mortality and serious adverse events and reviewing the interim analysis

16.1.1 Adverse events

Adverse events (AEs) are defined as any untoward medical occurrence in a patient or clinical investigation subject administered an investigational intervention and which does not necessarily have to have a causal relationship with this treatment (adapted from the Note for Guidance on Clinical Safety Data Management: Definitions and Standards for Expedited Reporting (CPMP/ICH/377/95 July 2000).

It is recognised that the patient population with severe traumatic brain injury will experience a number of common aberrations in laboratory values, signs and symptoms due to the severity of the underlying injury and the impact of standard therapies. These will not necessarily constitute an adverse event unless they require significant intervention or are considered to be of concern in the investigator's clinical judgement.

In all cases, the condition or disease underlying the symptom, sign or laboratory value should be reported e.g. renal failure rather than hyperkalaemia, and agitation rather than self-extubation.

16.1.2 Serious adverse events

Serious Adverse Events (SAE) are defined in accordance with the Note for Guidance on Clinical Safety Data Management: Definitions and Standards for Expedited Reporting (CPMP/ICH/377/95) (July 2000) as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event which may require intervention to prevent one of the previously listed outcomes.

In this study all SAEs will be reported regardless of suspected causality. However, consistent with the advice of Cook et al (Cook D, Lauzier F, Rocha MG, Sayles MJ, Finfer S. Serious adverse events in academic critical care research. CMAJ 2008; 178:1181-1184), adverse events already defined and reported as study outcomes (total mortality) will not be labelled and reported a second time as serious adverse events.

16.1.3 Reporting

Separate case report forms will be developed to record adverse events and serious adverse events.

SAEs which occur from the time of commencement of study treatment to 7 days post cessation of study treatment will be reported to the coordinating centre (ANZIC-RC) by faxing the supplied SAE form. SAEs should be reported to the ANZIC-RC within 24 hours of study staff becoming aware of the event.

Minimum information to report will include:

- patient initials and study number
- nature of the event
- commencement and cessation of the event

- an investigator's opinion of the relationship between study involvement and the event (unrelated, possibly, probably or definitely related).
- whether treatment was required for the event and what treatment was administered.

Fax number

+ 61 3 9903 0071

Telephone Numbers

ANZIC RC: + 61 3 9903 0513

ANZIC RC: +61 419 155 983

Chief Investigator:

Prof DJ Cooper + 61 3 9076 2838 / +61 419 770 051

SAEs must be reported on the fax form but may also be discussed with the ANZIC RC staff or chief investigator if necessary.

Coordinating centre staff will be responsible for following-up SAEs to ensure all details are available. It is the responsibility of each site to inform their HREC of all SAEs which occur at their site, in accordance with local requirements.

17. FUNDING

The POLAR study is funded by a project grant from the National Health and Medical Research Council (NHMRC) (Project grant no. 545901), & the Victorian Neurotrauma Initiative. The ANZIC-RC will supply infrastructure and administrative support.

18. SUB-STUDIES

18.1 Renal sub study (Appendix I)

The Renal Sub study is composed of 2 parts.

- POLAR-Acute Kidney Injury: Minimal data collection.
- Renal Biomarkers: Measurement of specific renal biomarkers (The Alfred & Royal Melbourne Hospital).

18.2 POLAR BEAR sub study (Appendix J)

The POLAR BEAR sub study will assess the energy expenditure of patients enrolled in the POLAR study (The Alfred & Auckland Hospital)

18.3 The Biomarker and Excito-toxicity POLAR sub study (Appendix K)

The specific aim of this sub-study is to determine the level of biomarkers in patients with TBI following hypothermia or normothermia treatment and evaluate the efficacy in predicting long-term outcome. We hypothesise that treatment with hypothermia after TBI will ameliorate neurological deficit and reduce the secretion of brain injury markers in the blood of TBI patients (The Alfred).

19. AUTHORSHIP & PUBLICATION

The study will be conducted in the name of the POLAR RCT investigators, the ANZIC RC, and the ANZICS CTG. The central project coordination and data management will be provided by the ANZIC-RC at Monash University, Melbourne. The principal publication from the study will be in the name of the POLAR RCT Investigators with full credit assigned to all collaborating investigators, research coordinators and institutions. Where individual names are required for publication these will be that of

the writing committee, with the chair of the writing committee listed first and subsequent authors listed alphabetically.

19.1 Sub study publication

The manuscripts will be submitted for publication after approval by the CTG executive and trial investigators according to CTG & ANZIC-RC policy. The authorship will be under the names of the sub study investigators on behalf of the POLAR investigators.

20. RESEARCH TIMELINES

Time frame indicators	Milestone
January 2008	Australian and New Zealand Intensive Care Society Clinical Trials Group (ANZICS CTG) endorsement obtained
March 2008	NHMRC funding application submitted
October 2008	NHMRC funding application approved
January 2009	Commence study organisation
April 2009	Protocol finalised
April 2009	The Alfred HREC submission
April 2009	Participating sites finalised
June 2009	Monash University HREC submission
September 2009	Participating sites HREC submission
January 2010	Pilot phase commences at The Alfred
February 2010	Ambulance Research Governance Committee approval
February 2010	Study start up meeting
August 2010	Ambulance paramedic training completed
September 2010	Study recruitment commences
August 2011	25% recruitment completed
October 2011	1st interim analysis
June 2012	50% recruitment completed
December 2012	6 mth follow up of 50% recruitment completed
December 2012	2nd interim analysis
February 2014	Patient recruitment completed
August 2014	6mth follow up of 100% recruitment completed
August 2014	Query resolution and data cleaning completed
September 2014	Database lock
October 2014	Complete data analysis
November 2014	Submission of initial manuscript

The time lines will be reviewed at each interim analysis

21. APPENDIX A: TABLE OF EVENTS

	Pre-hospital	ED Admission	ICU Admission	Day 1	Day 2	Day 3	Day 4-6	Day 7	Day8-14	Hospital Discharge	Follow Up 6 months
Inclusion & Exclusion Criteria	✓	✓									
Randomisation	✓	✓									
GCS	✓	✓								✓	
Systolic BP	✓	✓									
RR(pre-intubation)	✓	✓									
Temperature	✓	✓	✓	✓	✓	✓	✓	✓			
Pregnancy test (female)		✓									
Initiation of cooling protocol	✓	✓									
Initiation of standard care protocol	✓	✓									
Safety Assessment		✓									
Continuation of cooling protocol – temperature control device		✓	✓	✓	✓	✓	✓(#)	✓(#)			
Continuation of standard care protocol – temperature control device		✓	✓	✓	✓	✓	✓	✓			
Withhold cooling protocol		✓									
*Withdraw from cooling protocol**		✓									

	Pre-hospital	ED Admission	ICU Admission	Day 1	Day 2	Day 3	Day 4-6	Day 7	Day8-14	Hospital Discharge	Follow Up 6 months
Injury Severity Scoring		✓									
Documentation of Therapeutic interventions (ICP)		✓	✓	✓	✓	✓	✓	✓	✓	✓	
Documentation of vasopressor administration		✓	✓	✓	✓	✓	✓	✓	✓	✓	
VAP Surveillance			✓	✓	✓	✓	✓	✓	✓	✓	
Adverse events		✓	✓	✓	✓	✓	✓	✓	✓		
Serious adverse events		✓	✓	✓	✓	✓	✓	✓	✓		
Survival status										✓	✓
GOSE by independent blinded assessor											✓
EQ5D											✓
SF12											✓
Length of rehabilitation stay											✓
Length of nursing home stay											✓
Level of home care											✓
Clinical costings										✓	✓

(#) Rewarming will be dependent on ICP control

22. APPENDIX B: PRE-HOSPITAL STUDY PROCESS

Assess patient for study suitability

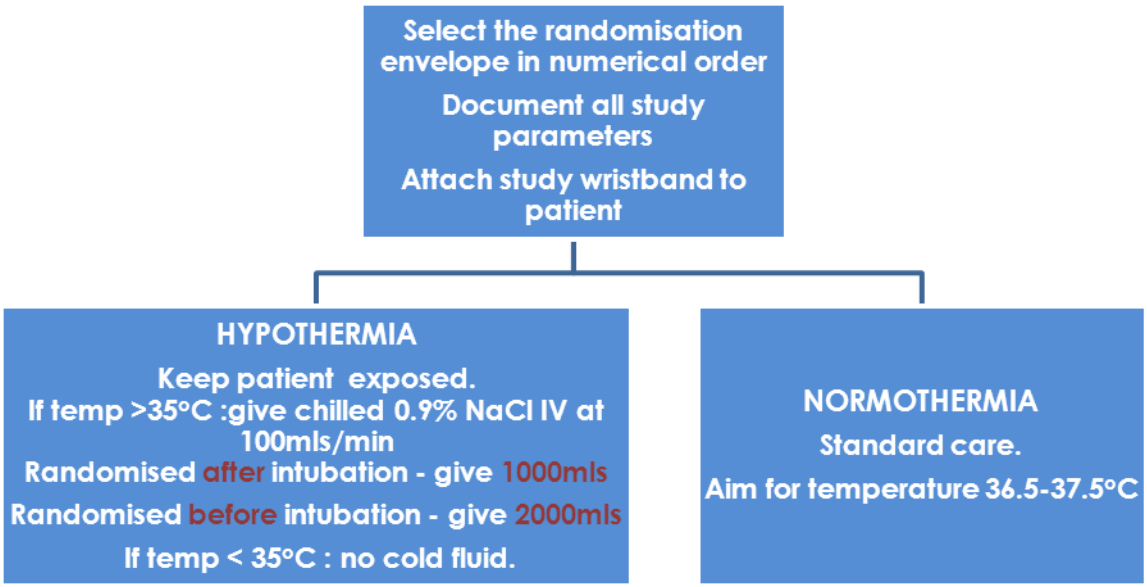
INCLUSION CRITERIA

- Blunt trauma with clinical diagnosis of severe TBI and GCS <9
- Estimated age ≥ 18 and < 60 years of age
- The patient is intubated or intubation is imminent

EXCLUSION CRITERIA

- Clinical diagnosis of drug or alcohol intoxication as predominant cause of coma
- Randomisation unable to be performed within 3 hrs of estimated time of injury
- Estimated transport time to study hospital >2.5hrs
- Able to be intubated without drugs
- Systolic BP <90mmHg
- Heart rate > 120bpm
- Cardiac arrest at scene or in transit
- GCS=3 + un-reactive pupils
- Penetrating neck/torso injury
- Known or obvious pregnancy
- Receiving hospital is not a study site.
- Evidence of current anti-coagulant treatment
- Known to be carer dependent due to a pre-existing neurological condition

If no exclusion criteria proceed to RANDOMISATION



23. APPENDIX C: EMERGENCY DEPARTMENT STUDY PROCESS

PATIENTS RANDOMISED PRE-HOSPITAL

NORMOTHERMIC - CONTROL GROUP:

- Follow standard temperature management, target temperature 37 °C
- Follow brain injury guidelines

HYPOTHERMIC – COOLING GROUP:

The patient will be assessed before progressing to active cooling

TEMPORARILY WITHOLD cooling protocol if:

In the clinician's opinion the patient has **clinically significant bleeding** or **risk of significant bleeding including** but not limited to.

- Bleeding into the chest, abdomen or retro-peritoneum likely to require surgery +/- embolisation
- Pelvic fracture likely to require surgery +/- embolisation
- More than two long bone fractures requiring operative fixation

- Rewarm to 35-37 °C & correct coagulopathy if present.
- Perform any interventions to control bleeding.
- Follow cooling protocol ASAP when bleeding controlled

The patient is **drug** or **alcohol** affected with a **normal CT brain**.

- Hold patient at 35°C while drugs and alcohol clear (can be warmed to 36°C if shivering an issue).
- Reduce sedation.
- Check neurological function

IF NONE OF THE ABOVE PRESENT CONTINUE WITH COOLING PROTOCOL:

- Apply surface temperature control vests/leg wraps/blankets
- Connect to temperature control module

Commence temperature control to target temperature 33 °C

PERMANENTLY WITHDRAW THE PATIENT FROM THE COOLING PROTOCOL & FOLLOW STANDARD MANAGEMENT IF THE FOLLOWING IS PRESENT:

- Significant bleeding which prevents initiation of cooling within **48hrs** post injury
- **Positive** urine or blood **pregnancy** test
- **Normal CT Brain** scan & patient **localising or obeying (GCS(M)≥5)** after decreased sedation
- In the treating clinician's opinion the patient does not require an ICU admission
- In the treating clinician's opinion, cooling is not in the patient's best interest.

PATIENT RANDOMISATION IN ED

Patients not previously randomised will be assessed for study eligibility by ED staff.

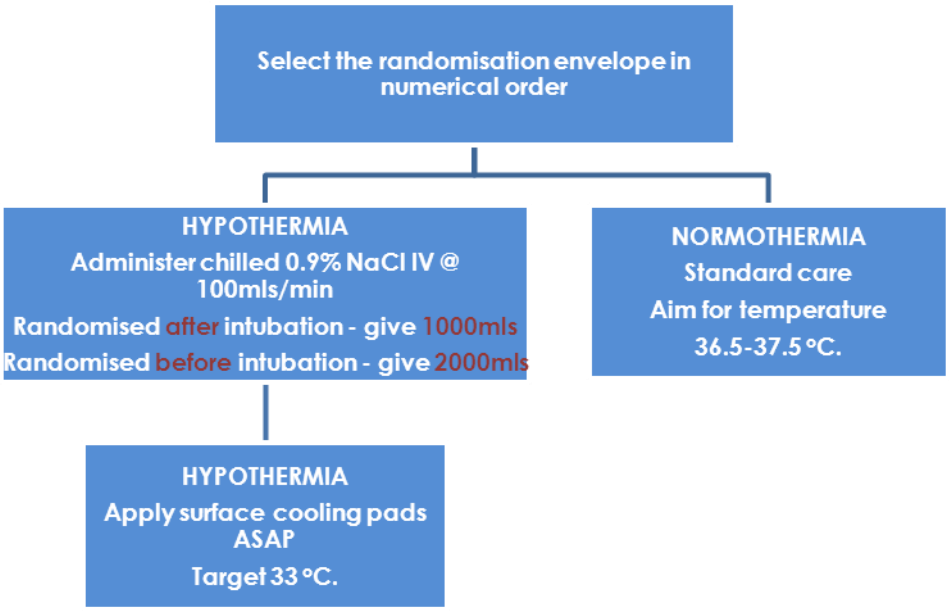
INCLUSION CRITERIA

- Blunt trauma with clinical diagnosis of severe TBI and GCS <9
- Estimated age ≥ 18 and < 60 years of age
- The patient is intubated or intubation is imminent

EXCLUSION CRITERIA

- Clinical diagnosis of drug or alcohol intoxication as predominant cause of coma
- Randomisation unable to be performed within 3 hrs of estimated time of injury
- Able to be intubated without drugs
- Persistent Systolic BP <90mmHg
- Clinically significant bleeding likely to require haemostatic intervention, for example:
    - Bleeding into the chest, abdomen or retro-peritoneum likely to require surgery +/- embolisation
    - Pelvic fracture likely to require surgery +/- embolisation
    - More than two long bone fractures requiring operative fixation
- GCS=3 + un-reactive pupils
- Cardiac arrest at scene or in transit
- Penetrating neck/torso injury
- Positive urine or blood pregnancy test
- Evidence of current anti-coagulant treatment
- Known to be carer dependent due to a pre-existing neurological condition
- In the treating clinician's opinion, cooling is not in the patient's best interest.

If no exclusion criteria proceed to RANDOMISATION



24. APPENDIX D: SEVERE TBI & ICU MANAGEMENT GUIDELINE

LINES/FLUIDS

- Arterial line and central line in place
- CVP target 8-12 mmHg (if CVP transduced)
- Crystalloid preferred resuscitation fluid, avoid albumin
- MAP target >80mmHg until ICP known (then to achieve CPP with minimum MAP of 70mmHg), using fluids and vasopressor (type of vasopressor is clinicians choice) if required
- After initial resuscitation in otherwise stable patients, positive daily fluid balances>500mls should be avoided.

VENTILATION/OXYGENATION

- SpO2 > 95%;
- PaO2> 90 mmHg;
- PaCO2 35-40 mmHg

PATIENT POSITION

- Position 15-30 degrees head up, avoiding venous obstruction in the neck.
- Position flat if shock prominent and this improves CPP

SEDATION (Recommendations only)

- Recommended agent use
  - Benzodiazepine – midazolam 0-15 mgs/hour
  - Opiate – fentanyl 0-100 micrograms/hour
  - Propofol – 0-100 mgs/hour and cease when patient at 34°C
- Neuromuscular blockade as per clinician's choice (preferably reserved for difficult ICP control)

GLYCAEMIC CONTROL

- Maintain BSL 6- 10 mmol/L

SERUM ELECTROLYTES

- Serum Na target 140-150 mmol/L
- Serum Potassium as per unit protocol. Monitor 2 hrly during rewarming
- Serum Magnesium as per unit protocol. Monitor prior to rewarming, mid rewarming and when normothermia is reached.

ICP MONITORING & CPP CONTROL

- Monitor ICP - EVD preferred, Codman parenchymal catheter acceptable
- ICP target <20mmHg
- CPP target >60mmHg
- Do not remove ICP monitor before completion of rewarming

See Appendix E – ICP control

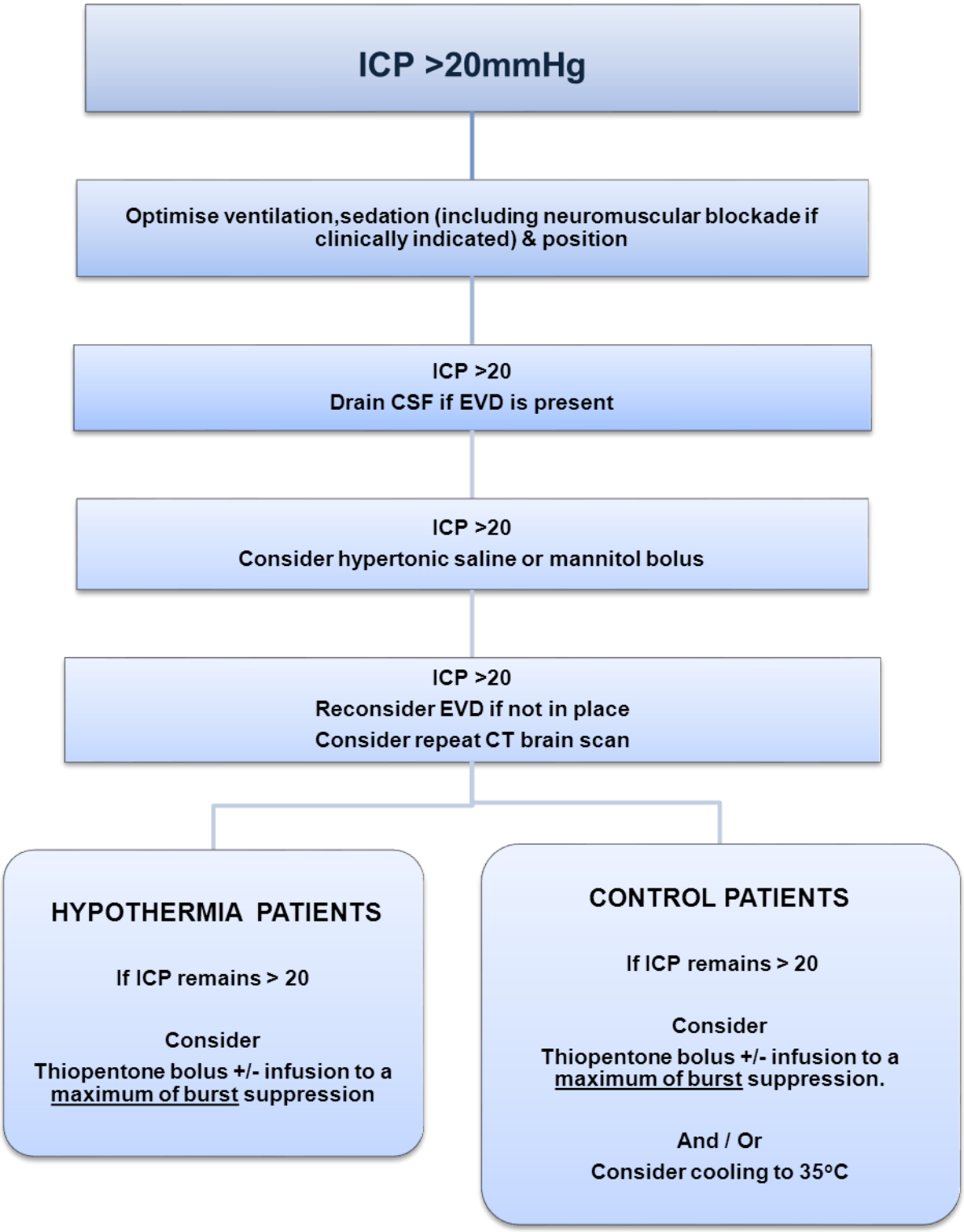
NUTRITIONAL SUPPORT

- Aim to commence enteral nutrition within 24 hrs and to achieve full nutrition within 72hrs

DVT, ULCER, SEIZURE PROPHYLAXIS

- As per clinician's choice

25. APPENDIX E: ICP CONTROL FOR BOTH STUDY GROUPS



26. APPENDIX F: COOLING AND REWARMING GUIDELINES

PURPOSE:

This guideline outlines the management of patients enrolled in the POLAR study who are allocated to therapeutic hypothermia

INDICATION

Patients enrolled in the POLAR study (see Inclusion/Exclusion document)

HYPOTHERMIA MANAGEMENT

Temperature control:

- Commence therapeutic hypothermia as soon as possible following randomisation
- During ambulance transport and in the Emergency Department: Expose the patient to promote environmental cooling. Infuse up to 2000mL ice cold crystalloid (0.9% saline at 4°C) at 100mls/min. Temperature goal during transport is 35°C. (Tympanic temperature)
- IN ED /ICU: Apply surface cooling using MEDITHERM III vests and leg wraps (see MEDITHERM III instructions for use) as soon as possible
- Set target temperature on cooling console to 33°C to institute rapid cooling with the auto rapid (hare) setting
- Turn down the temperature of inspired gas heating to the Non-Invasive Ventilation setting (34°C)
- Monitor core temperature using bladder temperature (preferred) or oesophageal temperature
- This temperature is ideally to be maintained for a period of 72 hours after severe head injury
- A target temperature of 32.5°C to 33.5°C (ideally 33°C) represents the optimal balance between clinical effectiveness and adverse side effects. In addition, at this temperature, there is generally minimal shivering. This generally means that further muscle-relaxants may be withheld.
- Should the core temperature rise to 33.5°C:
    - Check the temperature setting and circulating water temperature on the cooling machine
    - Check that there is cold water flow through the jacket
    - Increase sedation and/or administer further neuromuscular blocking agent as required for shivering. See Management of Shivering in this appendix
- Should the core temperature fall below 32.5°C:
    - Check the temperature setting and circulating water temperature on the cooling machine
    - Check that there is warm water flow through the jacket

Respiratory:

- Adjust ventilator to provide arterial blood gases (not corrected for temperature)
- pO2 >90mmHg
- pCO2 35-40mmHg (Hypothermia decreases CO2 production thus this pCO2 usually requires a decrease in minute volume of about 30% ie: tidal volume of 8ml/kg and a rate of 8bpm)

Cardiovascular:

- In severe traumatic brain injury, the CPP should be maintained at a minimum of 60mmHg
- The MAP target is 80 mmHg if an ICP monitor is not inserted. Increase MAP initially with crystalloid resuscitation fluids (avoid albumin)

- A decreased heart rate (<50 beats/minute) is a physiological effect of hypothermia. Low dose adrenaline may be considered to keep heart rate >50 beats/min if hypotension (absolute or relative). The electrocardiograph may show prolonged QT interval and Osbourne waves
- If an inotrope is required to increase blood pressure, an adrenaline infusion should be commenced. If the dose exceed 5mic/min or the lactate level is increasing, then an infusion of noradrenaline should be used. If a further fluid challenge is required for CVP<12mmHg, infuse a bolus (i.e. 500mL) of 0.9% saline
- Lactate levels may initially be increased by an adrenaline infusion. This hyperlactaemia is not an indication to stop cooling.

Neurological:

- On-going sedation is required to prevent shivering. Options include midazolam/ morphine infusion or propofol infusion.. If shivering occurs despite sedation, a non-depolarising neuromuscular blocking drug may be administered as required.
- Magnesium may decrease shivering threshold, we suggest that magnesium be corrected to upper normal range, prior to cooling (if possible) and before commencing rewarming.
- Calcium infusions are toxic to injured neurones, thus calcium should not be routinely administered to correct asymptomatic hypocalcaemia in patients with neurological injury.

Renal:

- Frequent monitoring and appropriate correction of electrolytes (K+, Mg++, PO4-) is required. In particular, hypokalaemia is usual during cooling, therefore carefully monitor K+ and replace for a potassium level >4.0 mmole/L. During rewarming, the potassium level increases however this generally does not require treatment.

Gastrointestinal:

- Aim to commence enteral nutrition within 24hrs and to achieve full nutrition within 72hrs.
- Commence target feeding at 70% of predicted because of the decreased metabolic rate.
- Gut motility may be decreased during hypothermia and high gastric residual volumes should be expected. Follow site ICU protocol for gastric intolerance.

Endocrine:

- Hyperglycaemia is common during hypothermia due to decreased insulin release and should be treated according to the site ICU Insulin protocol (generally BSL-6-10 mmol/L)

Skin Integrity

- Cooling causes vasoconstriction in the skin and may increase the risk of wound infection

Infection:

- Cooling can decrease the number and function of leucocytes and may potentially be associated with higher infection risk. Clinicians should be alert to the masking of increased temperature and the potential blunting of the leukocyte response as markers of infection
- The diagnosis of pneumonia is difficult during therapeutic hypothermia, since fever is masked and there may be no increase in the white cell count. The use of antibiotics should be considered if a new pulmonary infiltrate develops

Haematology:

- In traumatic brain injury, anticoagulation is not generally used during the first 7 days
- Hypothermia increases clotting times although the measured INR and APTT may appear normal since the samples are warmed prior to analysis
- Prolonged therapeutic hypothermia (>5 days) leads to gradual decreases in the platelet and white cell counts

Rewarming from hypothermia

72 hours post randomisation? #

YES
- (Proceed)

NO
(Do Not Proceed)

 # If cooling commenced late we require a minimum of 48hrs of cooling to have occurred prior to considering rewarming.

ICP Stable and <20mmHg **

YES
- (Proceed)

No (sustained ICP>20mmHg)
- Manage ICP
- Reconsider warming in 12 hours

*** Transient rises in ICP >20mmHg such as during suctioning, turning, moving, etc are not relevant to this dot point.*

Haemodynamic and electrolyte status stable

No

Status Corrected

YES
- (Proceed)

The rewarming rate aim is 0.17C/hr
- Ensure patient in Tortoise Mode (Gaymer Meditherm III)
- 'Set Point Control' to 0.5C above present set temperature.(ie: increase to 33.5C).
- **DO NOT** remove wraps or turn off cooling machine.
- **DO NOT** allow patient to rewarm passively.
- **Constantly monitor patient temperature** and ensure rewarming does not occur faster than required.

If at any time during rewarming there is a sustained ICP instability (ICP >20mmHg) -
- **Despite medical treatment**
- **Transient rises in ICP>20 mmHg such as during suctioning, turning, moving etc. are not relevant.**
- **Halt rewarming procedure and manage ICP instability.**

If required
- **Re cool to a point where ICP stability is maintained.**
- **Reassess in 12 hours for suitability to re-commencement of rewarming procedure.**

Continue to monitor *(ICU Management guidelines-= Appendix D and E)*
- *For alterations in Potassium and Magnesium.*
- *Haemodynamic instability.*
- *ICP instability*
- *Infection (Temperature spikes (>38C) are common post rewarming.)*

Repeat rewarming process increasing Meditherm III "set point control" by 0.5C every 3 hours.

Management of Re-Warming

The rewarming of a patient is a **critical period**. The aim is to reach 37+/-0.5 degrees. It is critical that rewarming occurs **slowly** and in a **controlled** manner. If rewarming occurs too quickly it may cause damage to neural cells.

- Ensure the water level is above the green line in Gaymer Mediterm III.
- Ensure all cooling device connections are open.
- Ensure temperature probe is connected and appropriately connected to the patient
- Choose the **tortoise mode** on the Gaymer Mediterm III and "set point control" temperature at **0.5C degrees above current setting (ie.33.5C)**. The Tortoise mode will allow cooling to occur at 0.17 degrees per hour.
- **DO NOT** remove wraps or turn off machine.
- **DO NOT** allow patient to rewarm passively.
- **After 3 hours increase the "set point control" by another 0.5°C. Continue this increase every 3 hours until patient reaches 37.0°C**
- Rewarming to 37C should take approximately 24 hours.

Continue to OBSERVE patient temperature as rewarming occurs.

<u>If rewarming is occurring too quickly, it may be necessary to increase set point by 0.2 per 2 hours.</u> The slower rewarming rate may increase risk of shivering.

Rewarming Special considerations

- **In urgent situations (uncontrolled bleeding or urgent theatre) a faster rewarming rate may be necessary and should be led by the intensivist, but wherever possible utilise the slower rewarming rate.**
- **When transporting the patient consideration should be given to continuing cooling while the patient is undergoing a procedure or being transported.**
- **Gaymer Meditherm III is able to be utilised in theatre. Consideration should be given to utilising the device during theatre to maintain a patient temperature.**
- **DO NOT PRE COOL PATIENT for expected transport**
- **AVOID sudden cessation of cooling and removal of cooling warps.**

Changes in Potassium and magnesium occur during rewarming.

- Check potassium 2/24hrly
- Check magnesium prior to commencement, half way and at the end of rewarming.
- Haemodynamic instability may occur. Small doses of adrenaline may be required.

Haemodynamic instability may occur during rewarming

- Try and avoid fluid boluses
- Consider increase in inotropes
- Blood if Hb<9
- Hold rewarming until patient stable (clinicians discretion)

ICP instability

- See ICP management guidelines / flowchart

Infection (Temperature spikes (>38.3C) are common post rewarming.)

- Clinicians should treat febrile patients as per the site ICU Guideline for the Management of Fever. A full septic screen is recommended.
- Keep wraps available or on patient for a total of 96 hours.
- Utilise Gaymer Meditherm III to regulate temperature to normothermia if there is a temperature spike.
- The maximum protocolised period of hypothermia will be 7 days. Once a patient has completed the re-warming protocol and reached 37°C, they will be maintained at normothermia (below 38°C) using the surface cooling pads for 96 hours post randomisation. After this time the will be managed according to ward protocol.
- Pyrexia above 38°C should also be avoided in **all patients**, for up to 96 hours post randomisation, by application of surface cooling systems after this time patients should be managed according as per unit protocol.

Management of Shivering

- Shivering is most pronounced at temperatures between 34C and 35.5C but may be seen beyond these points.
- Shivering will significantly increase metabolic demand and increase ICP.
- Review sedation and neuromuscular blockade prior to rewarming
- Shivering must be immediately treated with additional sedation/muscle relaxant.
- Sedation can be weaned if ICP is stable once core temperature is >36.0°C
- It is important to monitor for shivering at all times but it is most important during initial cooling and during rewarming phases as patient temperature passes shiver point.
- As rewarming (which is slow) will take longer than initial cooling (which is rapid), shivering may be prolonged during the slow rewarming phase (0.17C per hour).
- Shivering should be monitored continually and managed immediately and aggressively.

Shivering Assessment

- There are shivering assessment tools that can grade the amount of shivering. In this study we desire no shivering. The Bedside Shivering Assessment Scale (BSAS) can be used to monitor shivering if desired.
- It is important to monitor for shivering at all times but it is most important during initial cooling and during rewarming phases as patient temperature passes shiver point.
- Shivering observations should be done:

  - During induction of hypothermia, maintain continuous surveillance for shivering and conduct a formal shivering assessment hourly until target temperature (33C) is reached.
  - During maintenance of hypothermia continuous surveillance for shivering should continue and a formal shivering assessment should be conducted 2 hourly
  - During rewarming hypothermia, maintain continuous surveillance for shivering and conduct a formal shivering assessment hourly until target temperature (37C) is reached.
- The suggested method to assess for shivering is
  - Observe patient for 2 minutes during which time the jaw, neck, chest, arms and legs should be visually inspected and palpated for shivering.

Pharmacological management

- Manage serum magnesium at upper level of normal
- Consider active skin counter-warming (using warm wraps or hot air blower on hand, feet and face)
- Maximise sedation. Options include midazolam, morphine, fentanyl or propofol.
  - Recommendations (only) are:
    - Benzodiazepine – midazolam 0-15 mgs/hour
    - Opiate – fentanyl 0-100 micrograms/hour
    - Propofol – 0-100 mgs/hour (cease when patient at 34°C)
- If shivering continues despite these agents consideration should be given to adding
  - Pethidine 50mgs/4hrly

  And also consider
  - Clonidine 75micrograms (only if blood pressure allows)

Neuromuscular blockade can also be utilised to manage shivering. Clinicians should consider seizure activity monitoring if NMB are utilise

Cooling phase

Once a patient has reached 34C consideration should be given to decreasing medications that have been utilised to manage shivering. Reduced metabolism and decreased shivering are likely at this temperature.

Rewarming Phase

Once a patient has reached 35.5C consideration should be given to decreasing medications that have been utilised to manage shivering.

27. APPENDIX F: GLASGOW OUTCOME SCALE

6 Month Follow Up

16.1 Date of follow up: ☐☐ / ☐☐☐ / ☐☐☐☐ (dd / mmm / yyyy) ☐ Patient lost to follow up ☐ Patient alive (unscorable)

Extended Glasgow Coma Scale

The questions can be answered by the patient or close, relative, friend or by rehabilitation / nursing staff if appropriate

16.2 Respondent:
- ☐ Patient alone
- ☐ Relative or friend or carer alone
- ☐ Patient and relative, friend or carer together
- ☐ Patient lost to follow up

16.3 The injured person is:
- ☐ Out of hospital
- ☐ In hospital, rehabilitation centre or residential care

16.4 What is the most important factor in recovery?
- ☐ Effects of head injury
- ☐ Effects of illness or injury to another part of the body
- ☐ A mixture of these

Please answer by crossing one box, which best answers the question.

16.5 Is the injured person able to obey simple commands, or say any words? ☐ Yes ☐ No (2 - Vegetative state)

16.6 Before the injury was the injured person able to look after themselves at home? ☐ Yes ☐ No

16.7 As a result of the injury does the injured person now need someone to help look after them at home?
For a no answer they should be able to look after themselves at home (safely) for 24 hrs if necessary. They should not require prompting or reminding and should be capable of being left alone overnight.

Cross one box only:
- ☐ Do not need frequent help or supervision in the home
- ☐ Need some help in the home, but can look after themselves for at least 8 hours if necessary (4 - Upper SD)
- ☐ Could not look after themselves for 8 hours during the day (3 - Lower SD)
- ☐ Need help in the home, but not because of the injury

16.8 Before the injury was the injured person able to buy things at shops without help? ☐ Yes ☐ No

16.9 As a result of the injury does the injured person now need help to buy things at shops?

Cross one box only:
- ☐ Do not need help to shop
  Can plan what to buy, take care of money themselves, behave appropriately in public.
- ☐ Need help to get to the shops but once there can do own shopping
- ☐ Need help to shop (4 - Upper SD)
- ☐ Need help to shop, but not because of the injury

16.10 Before the injury was the injured person able to travel without help? ☐ Yes ☐ No

16.11 As a result of the injury does the injured person now need help to travel?
Either driving a car, catching public transport or catching a taxi.

Cross one box only:
- ☐ Do not need help to travel
- ☐ Need help to travel (4 - Upper SD)
- ☐ Need help to travel, but not because of the injury

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Extended Glasgow Coma Scale *continued*

16.12 Employment before the injury?
- ☐ Working
- ☐ Studying as a student
- ☐ Looking for work
- ☐ Retired
- ☐ Looking after family
- ☐ None of these

16.13 As a result of the injury has there been a change in the injured person's ability to work?
(Or to study; or to look after your family)

Cross one box only:
- ☐ Still do the same work
- ☐ Still work, but at a reduced level (6 - Upper MD)
  (e.g. Change from full time to part time or change in level of responsibility)
- ☐ Am unable to work, or only able to work in sheltered workshop (5 - Lower MD)
- ☐ Ability to work has changed, but not because of the injury

16.14 Before the injury did the injured person take part in social and leisure activities outside home? ☐ Yes ☐ No
At least once a week. Examples include going out with or visiting friends, going to the cinema or live performances, attending sporting events or participating in sporting events, attending religious ceremonies or services

16.15 As a result of the injury has there been a change in the ability of the injured person to take part in social or leisure activities outside the home?

Cross one box only:
- ☐ Take part about as often as before
- ☐ Take part a bit less but at least half as often (7 - Lower GR)
- ☐ Take part much less, less than half as often (6 - Upper MD)
- ☐ Do not take part at all (5 - Lower MD)
- ☐ Ability to take part has changed, but not because of the injury

16.16 Before the injury did the injured person have any problems getting on with friends and family? ☐ Yes ☐ No

16.17 As a result of the injury does the injured person have any emotional or behavioural problems which cause difficulties in relationships with friends or family?
Examples include quick temper, irritability, mood swings, depression, anxiety, insensitivity to others, rigid thinking, unreasonable or childish behaviour.

Cross one box only:
- ☐ Things are still much the same
- ☐ There are occasional problems (less than one a week) (7 - Lower GR)
- ☐ There are frequent problems (once a week or more) (6 - Upper MD)
- ☐ There are constant problems (problems everyday) (5 - Lower MD)
- ☐ There are problems for some other reason, not because of the injury

16.18 Are there currently any other problems resulting from the injury?
Problems sometimes reported after head injury include headaches, dizziness or balance problems, tiredness or sleeping disturbances, sensitivity to noise or light, slower speed of thinking, trouble with memory, concentration problems.

Cross one box only:
- ☐ No current problems
- ☐ Some problems (7 - Lower GR)
- ☐ Some problems for other reasons, not because of the head injury

16.19 Before the injury were similar problems present?
- ☐ No problems or they were minor problems
- ☐ Similar problems before

16.20 Comments: Add extra page if required

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Extended Glasgow Coma Scale *continued*

Glasgow Outcome Scale - Extended

16.21 Tick appropriate score:

- ☐ 1 - Dead
- ☐ 2 - Vegetative state (VS)
- ☐ 3 - Lower severe disability (Lower SD)
- ☐ 4 - Upper severe disability (Upper SD)
- ☐ 5 - Lower moderate disability (Lower MD)
- ☐ 6 - Upper moderate disability (Upper MD)
- ☐ 7 - Lower good recovery (Lower GR)
- ☐ 8 - Upper good recovery (Upper GR)

Glasgow Outcome Score

16.22 Tick appropriate score:

- ☐ 1 - Dead
- ☐ 2 - Vegetative state (VS)
- ☐ 3 - Severe disability (SD)
- ☐ 4 - Moderate disability (MD)
- ☐ 5 - Good recovery (GR)

28. APPENDIX H:EURO QUALITY OF LIFE (EQ5D)

EQ - 5D

By placing a cross in one box in each group below, please indicate which statements best describe your own health state today.

16.23 Mobility:
- ☐ I have no problems in walking around
- ☐ I have some problems in walking around
- ☐ I am confined to bed

16.24 Personal care:
- ☐ I have no problems with personal care
- ☐ I have some problems washing or dressing myself
- ☐ I am unable to wash or dress myself

16.25 Usual activities: (e.g. work, study, housework, family or leisure activities)
- ☐ I have no problems with performing my usual activities
- ☐ I have some problems with performing my usual activities
- ☐ I am unable to perform my usual activities

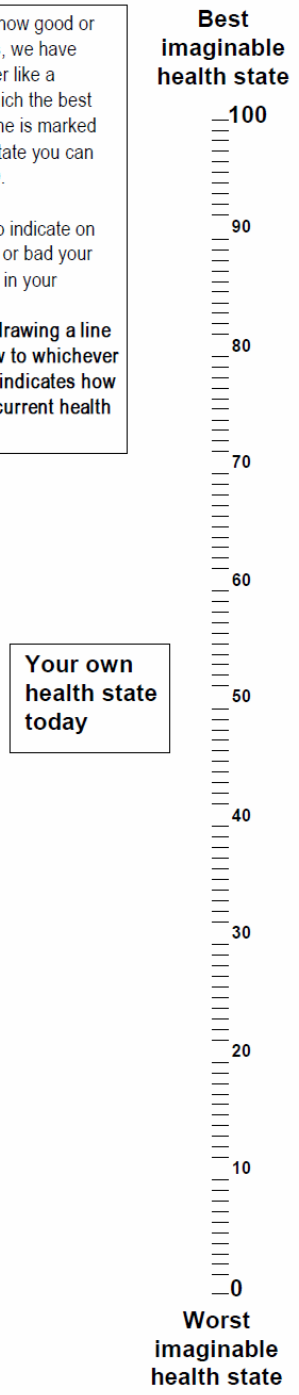
16.26 Pain / discomfort:
- ☐ I have no pain or discomfort
- ☐ I have moderate pain or discomfort
- ☐ I have extreme pain or discomfort

16.27 Anxiety / depression:
- ☐ I am not anxious or depressed
- ☐ I am moderately anxious or depressed
- ☐ I am extremely anxious or depressed

16.28 Health state scale:

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion.
Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your current health state is.



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29. APPENDIX G:ASSESSMENT OF QUALITY OF LIFE (SF-12)

SF - 12

INSTRUCTIONS: *This questionnaire asks for your views about your health, how you feel and how well you are able to do your usual activities. Please answer every question by crossing one box. If you are unsure about how to answer please give the best answer you can.*

16.29 In general, would you say your health is:

☐	☐	☐	☐	☐
Excellent	Very good	Good	Fair	Poor

The following questions ask about your activities you might do during a typical day.

16.30 Does **your health now limit you** in these activities? If so, how much?

	Yes limited a lot	Yes limited a little	No Not limited at all
16.30.1 Moderate activities: (such as moving a table, pushing a vacuum cleaner, bowling or playing golf)	☐	☐	☐
16.30.2 Climbing **several** flights of stairs:	☐	☐	☐

16.31 During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

	Yes	No
16.31.1 **Accomplished less** than you would like:	☐	☐
16.31.2 Were limited in the **kind** of work or other activities:	☐	☐

16.32 During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your emotional problems**? (such as feeling depressed or anxious)

	Yes	No
16.32.1 **Accomplished less** than you would like:	☐	☐
16.32.2 Didn't do work or other activities as **carefully** as usual:	☐	☐

16.33 During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

☐	☐	☐	☐	☐
Not at all	A little bit	Moderately	Quite a bit	Extremely

These questions are about how you feel and how things have been with you ***during the past 4 weeks****. For each question, please give the one answer that comes closest to the way you have been feeling.*

16.34 How much of the time during the **past 4 weeks** ...

	All of the time	Most of the time	A good bit of the time	A little of the time	None of the time
16.34.1 Have you felt calm and peaceful?	☐	☐	☐	☐	☐
16.34.2 Did you have a lot of energy?	☐	☐	☐	☐	☐
17.34.3 Have you felt down?	☐	☐	☐	☐	☐

16.35 During the **past 4 weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities? (like visiting friends, relatives, etc.)

☐	☐	☐	☐	☐
All of the time	Most of the time	Some of the time	A little of the time	None of the time

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30. APPENDIX I: RENAL SUB STUDY

30.1 POLAR-Acute Kidney Injury (AKI)

This is a study of the effects of early and sustained hypothermia on renal function in patients with traumatic brain injury (TBI) to be conducted on all patients in the POLAR trial.

30.1.1 Aims

Acute kidney injury (AKI) is a complication of TBI, which makes its management more complex and costly, and exposes TBI patients to a greater risk of dialysis-related cerebral edema. We plan to investigate changes in renal function and biomarkers in patients with TBI in this substudy of the POLAR trial.

The specific aim of POLAR-AKI is to determine whether in patients with TBI, early and sustained hypothermia reduces the risk for, severity of, and duration of AKI, compared to normothermia.

30.1.2 Background

AKI as classified by the RIFLE criteria (acronym for Risk, Injury, Failure, Loss, End-stage)[48] may occur in approximately 9% of TBI patients in Australia (Risk, Injury and Failure categories).[49] Trauma patients compose almost 1 in 10 of those admitted to Australian ICUs and over 42% have TBI.[50] AKI increases the risk of aggravating cerebral edema,[51] and is associated with increased mortality[52] and prolonged length of stay.[53] Severe cases require costly treatments and can result in prolonged kidney dysfunction and delayed recovery, escalating the human and financial costs of TBI.[54] Treatment of AKI with dialysis is associated with significant fluctuations in osmolarity which expose the patient to an increased risk of cerebral edema.[51] Accordingly, we consider it a priority to investigate treatments which simultaneously may protect the brain and kidney after neurotrauma.

Evidence suggests that mild hypothermia (32-35°C) in the first hours after an ischemic event can prevent or ameliorate permanent injury not only in the brain as for TBI but also in the kidneys.[39] Some mechanisms through which hypothermia may protect the brain and kidneys include: the blockade of excitotoxic mechanisms, modulation of the inflammatory response and modulation of apoptosis.[14] Hypothermia (~33°C) has been shown to reduce the risk of renal failure after induced renal ischemia-reperfusion injury in animals[55, 56]. There has been little clinical investigation of the renal effects of hypothermia and no such study in patients with TBI. The POLAR trial presents a unique opportunity to clinically investigate the influence of hypothermia on kidney function in TBI patients at minimal cost.

30.1.3 Methods

Inclusion and exclusion criteria for the Renal Substudy will be the same as for POLAR. Degree of AKI post trauma will be categorised using the GFR and urine output criteria of the RIFLE classification system[48] and comparisons made. Maximal change in serum creatinine from baseline in the first 7 days post-trauma will be used and urine output data will be collected for 7 days while in ICU. RIFLE outcome criteria 'Loss of kidney function' and 'End stage renal failure' will not be used. The RIFLE criteria have been validated in critically ill populations.[52, 57, 58] Creatinine collection will continue for 10 days to allow assessment of treatment/ post treatment effect, and duration of AKI. The patient's weight will be estimated if not known to calculate urine output data in ml/kg/hr. Data will also be collected on the need for Renal Replacement Therapy / Dialysis while in hospital and days when this is received as it affects creatinine level and will be assessed as an outcome.

30.1.4 Power calculations and sample size

The POLAR trial recruitment target is 500 patients. With 250 subjects per group this study will have an 80% power to show a difference in a continuous normally-distributed outcome, equivalent to 25% of one standard deviation with a 2-sided p-value of 0.05. Given one standard deviation is equivalent to about 24% across a range of data, this difference could be thought to equate to a difference of approximately 6%. With 250 subjects per trial group receiving cooling / normothermia, POLAR-AKI will have an approximately 80% power to show a change in proportion of 6% in those with any degree of AKI (9% v. 3%) with a two sided p-value of 0.05. We estimate that 9% of TBI patients will develop AKI (Risk/Injury/Failure) based on a preliminary study.[49]

30.1.5 Primary outcomes

Severity of AKI (measured as Percent decline in eGFR from baseline to worst eGFR up to day 10 OR increase in serum creatinine from baseline to peak value while in ICU over 10 days)

30.1.6 Secondary outcomes

Cumulative proportion in Risk/Injury/Failure RIFLE categories in those receiving cooling v. normothermia.

Duration of AKI measured as AKI free days (number of days not in Risk/Injury/Failure categories) for 10 days or duration of data collection.

30.1.7 Analysis of results

Data will be analysed using Intercooled Stata, version 9 or later (Statacorp, Tx, USA). Data will initially be assessed for normality and log-transformed where appropriate. Univariate analyses will be conducted using appropriate tests. Multivariate analysis will be performed adjusting for baseline imbalances and known covariates. Where sufficient data exists, multiple logistic regression will be used for AKI as a binary outcome. Multivariate analysis on log-transformed biomarkers will be performed using multiple linear regression, while time to event data will be analysed using Cox regression and displayed with Kaplan Meier curves. A two sided p-value of 0.05 will be considered statistically significant.

30.1.8 Significance

POLAR-AKI will provide unique prospective information on the incidence, severity and duration of AKI in patients with TBI. It also presents a unique opportunity to evaluate the benefit of early and sustained hypothermia (a potential kidney protective therapy for AKI) to decrease the occurrence, severity and duration of AKI in TBI patients. The POLAR trial provides a unique opportunity to investigate a kidney protective therapy for AKI in TBI at minimum cost.

30.2 POLAR-Renal Biomarkers

This is a study of the effects of early and sustained hypothermia on renal biomarkers in patients with TBI to be conducted only at The Alfred and Royal Melbourne Hospitals in Melbourne, Victoria.

30.2.1 Aims

The specific aims of POLAR-Biomarkers are to determine whether in patients with TBI:

a) early and sustained hypothermia reduces the release of biomarkers NGAL, cystatin C and L-FABP compared to their release in those receiving normothermia.

b) the release of these biomarkers predicts the occurrence, severity, duration of and recovery from AKI.

30.2.2 Background

Levels of NGAL, cystatin C and L-FABP rise early in AKI compared to creatinine.62-64 No study has analysed these biomarkers in patients with TBI, or studied their response to therapy over a period of up to 72hours. If their value as early renal biomarkers is confirmed in patients with TBI and other populations, they may change the definition, diagnosis, prognosis and timing of therapy for AKI in TBI. The POLAR trial provides a unique opportunity to analyse the biomarker response to cooling which could elucidate mechanisms involved in the body's response to this therapy.

30.2.3 Methods

Inclusion and exclusion criteria for POLAR-Biomarkers will be the same as for POLAR. Urine and blood specimens will be collected as soon as practicable after randomisation and at 24, 48 and 72 hours after these first specimens, from the first 50 participants recruited between the Alfred and Royal Melbourne hospitals. Renal biomarker levels in some samples will be measured with point-of-care assays (plasma NGAL) and remaining samples will be processed, freeze-stored and later transported for measurement to Monash Medical Centre laboratory (cystatin C); Dr Yasushi Takigawa, CIMC Co. laboratories, Tokyo, Japan (L-FABP); and Cincinnati Children's Hospital Medical Centre laboratories, Cincinnati, USA (urine NGAL). Investigation into novel biomarkers is at an explosive stage, and other

compounds may in a short space of time emerge as solid predictors of renal function. In view of this, two samples of urine and plasma for all 4 time-points from each participant will be stored and kept for future measurement of other biomarkers.

30.2.4 Power calculations and sample size

With 50 subjects from the POLAR trial, POLAR-Biomarkers will have an 80% power to detect a correlation coefficient of r = 0.4 between continuous variables on any given day with a two-sided p-value of 0.05. Whilst the addition of repeat measures data will increase the power to detect relationships between continuous variables, as the within patient correlation between data points is unknown, it is impossible to calculate the exact increase in power.

By considering a change in values between days 1 and 3, with 25 subjects per group POLAR-Biomarkers will have an 80% power to detect a difference between groups in continuously normally distributed variables, equivalent to 80% of one standard deviation with a two-sided p-value of 0.05. As additional repeat measures will once again increase the power of the study, this is a conservative estimate.

30.2.5 Primary outcomes:

Levels of biomarkers NGAL, cystatin C and L-FABP in those receiving cooling v. normothermia; and comparison of urine and serum samples.

30.2.6 Secondary outcomes:

Relationships between biomarkers and change in eGFR, serum creatinine, RIFLE classes Risk/Injury/Failure, and survival.

30.2.7 Analysis of results

Data will be analysed using Intercooled Stata, version 9 or later (Statacorp, Tx, USA) and assessed for normality and log-transformed where appropriate. We anticipate that biomarkers (NGAL, cystatin C, L-FABP) will be well approximated by a log-normal distribution. Multivariate analysis will be performed adjusting for baseline imbalances and known covariates using repeat measures analysis of variance. We will determine changes in response over time between treated / untreated groups by fitting an interaction between treatment and time. Relationships between biomarkers and change in eGFR, serum creatinine, RIFLE class Risk/Injury/Failure, and survival, will be determined using logistic regression and reported using area under the curve for receiver operating characteristic curves.

30.2.8 Significance

POLAR-Biomarkers will provide unique information on the pattern of renal biomarker release in response to cooling and in AKI, in patients with TBI. The POLAR trial provides a unique opportunity to investigate this potential kidney protective therapy and new diagnostic approaches to AKI in TBI at minimum cost.

30.3 Renal sub study contact details

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31. APPENDIX J: POLAR BASAL ENERGY ASSESSMENT RESEARCH (POLAR BEAR)

This is a study of the effects of early and sustained hypothermia in TBI patients on resting energy requirements with TBI to be conducted at The Alfred and Auckland City Hospital.

31.1 Background & Rationale

Traumatic Brain Injury (TBI), defined as an external insult to the head resulting in damage to the brain, is one of the leading causes of hospitalisation, death and disability worldwide, particularly in young adults[2]. In 2004-2005 there were over 22,000 hospitalisations in Australia for TBI, many of whom required a period in intensive care due to the severity of their injury and the consequences resulting from it[59]. In a recent analysis of an international database of 2664 patients with severe TBI, mortality was 28% and only 52% of these patients had favourable neurological outcomes (good recovery or moderate disability) and were able to live independently at 6 months [3]. Given the young age of many patients with severe TBI and the long term prevalence of major disability, the economic and social cost to the community is very high.

Current management of TBI is supportive and focuses on multiple interventions for the prevention and treatment of secondary brain injury hypoxia including sedation, intravenous fluids, monitoring and manipulation of oxygen, intra-cranial pressure (ICP) and cerebral perfusion pressure (CPP)[9]. Despite decades of research investigating novel therapies for patients with TBI, recent clinical trials have not demonstrated substantial improvements in outcome [10, 12, 60]. One intervention with great potential to reduce neurological damage and improve outcome after severe TBI is the application of early prophylactic hypothermia [13]. This therapy involves the rapid reduction of core body temperature to 33°C and is commonly used in Australia to treat patients with severe neurological injury following out-of-hospital cardiac arrest [13].

There is a compelling scientific rationale to further investigate whether early prophylactic hypothermia is efficacious in TBI. On the background of extensive laboratory data, clinical trials over the past two decades have supported a likely benefit [19-23]. Furthermore, a 2007 meta-analysis of the highest quality clinical trials of hypothermia in severe TBI [61] reported a significant increase in long term favourable neurological outcomes (relative risk (RR) 1.46, p=0.006) with no significant decrease in mortality (RR 0.76, 95% confidence interval (CI) 0.05 to 1.05, p=0.18) in patients treated with prophylactic hypothermia compared to normothermia.

The only published multi-centre hypothermia trial [1] reported no improvement in outcomes in patients treated with prophylactic hypothermia. Whilst the trial was well conducted, it had key methodological limitations including a prolonged time until commencement of hypothermia (an average of 8 hours) and a short duration of hypothermia (48 hours). In addition, the trial induced and maintained hypothermia using cooling blankets and bags of ice, a practice which is renowned for difficulty in achieving constant patient temperature and targeting gradual re-warming. The recent development of electronically controlled surface (skin) cooling pads has significantly advanced the controlled induction, maintenance and ultimate emergence from hypothermia.

Members of the ANZICS Clinical Trials Group have designed a large, multi-centre randomised controlled trial to investigate the efficacy of prophylactic hypothermia in patients with TBI. The POLAR (the Prophylactic hypOthermia trial to Lessen trAumaticbRain injury) study will enrol 512 patients and has been designed to address the major limitations of the previous multicentre study by inducing prophylactic hypothermia early (in the ambulance) and maintaining it for a prolonged period (a minimum of 3 up to 7 days) using well controlled surface cooling pads.

Nutrition therapy in the critically ill setting is an essential element of patient care. It has now been clearly demonstrated to reduce complication rates and improve morbidity and mortality [62-64]. Enteral nutrition (EN) is preferred over parenteral nutrition as it is associated with better clinical outcomes [62], especially in patients with trauma [65]. It is also recommended that EN is commenced within 24-48 hours of admission to the ICU in the critically ill [3, 66, 67].

Whilst the route of delivery and timing of nutrition therapy has become increasingly understood, the amount and composition that should be delivered is more difficult to define. The primary goal of providing nutrition therapy during critical illness is to supply adequate macro and micronutrients to meet metabolic demands, which are usually significantly increased in the presence of injury or illness [68]. One of the biggest challenges in administration of nutrition therapy is the prediction of each

patient's true nutritional needs. Assessment of the individual patient's total energy requirements is most commonly performed using mathematical equations which estimate energy requirements using age, gender, height, weight and severity of illness. Such predictive equations were derived many decades ago and are potentially inaccurate in the critically ill population. The most commonly used formula in Australia, the Schofield equation [69], was developed in 1985, and is based on a meta-analysis of 100 studies which included 7000 healthy subjects, almost half of whom were Italian soldiers, and is therefore likely to be significantly unhelpful in an ICU [69-71]. In addition there is considerable variation in energy expenditure depending on the time course of the illness and the interventions that are applied. Therefore it is likely that single predictions are inaccurate for all phases of critical illness.

The gold standard for prediction of energy expenditure is indirect calorimetry, a machine attached to the patient's ventilator circuit, which measures the utilisation of oxygen and production of carbon dioxide to obtain a value for resting energy expenditure [72, 73]. Because the lungs are the sole source of oxygen intake and carbon dioxide excretion, changes in the concentration of these gases reasonably reflect energy metabolism. An indirect calorimetry measurement of only 15 minutes duration can successfully predict energy requirements with less than a 4% error. This allows for quick, accurate and real-time determination of the metabolic response to injury in mechanically ventilated and critically ill individuals [73]. Essentially this technology could eliminate the error associated with a predictive equation and transform current nutrition practices by allowing adjustment of the nutrition regime accordingly [72-74].

A significant concern in the provision of nutrition therapy is that patients are often inadequately nourished and receive significantly less than their predicted requirements, due to a number of patient-related and logistic factors, thereby putting patients at risk of malnutrition [75, 76]. The inverse of this is over-nourishment, however the consequences may not be as serious as prolonged under-nourishment. In a retrospective analysis of 55 mechanically ventilated patients, only 40% of patients received the amount they required when using predictive equations alone, compared to using indirect calorimetry measurements. A quarter of the patients were classified as inadequately nourished by the predictive equation and 35% were over-nourished [77]. In TBI patients, inadequate nourishment in the form of delayed nutritional therapy has been shown to be associated with a prolonged acute phase response and poor outcomes [78]. Inadequate nourishment is a significant problem given the inaccuracy of clinical tools for prediction of energy expenditure in the critically ill, intolerance of EN due to gastrointestinal dysmotility and the regular interruptions of nutrition regimes which are common place in the ICU[75, 76]. We have recently demonstrated in a large international survey of 2850 ICU patients that the average proportion of predicted energy requirements that were actually delivered was 58% [62].

It could be expected that hypothermia would reduce energy expenditure, however there is sparse data in the literature to support this. One study of 10 patients who had brain injury from stroke demonstrated that hypothermia to 33°C for 3 days led to a total energy expenditure fall of between 25 and 29% [79]. There have been 2 small studies in TBI patients suggesting a similar response. The first investigated multiple metabolic parameters in 31 TBI patients who had prophylactic hypothermia and found that resting expenditure decreased by over 35% at 33°C [80]. More recently, a study of 5 TBI patients showed that hypothermia to 32°C led to a total reduction of 30.3% in energy expenditure such that on average a 1°C reduction in temperature led to 5.9% less energy expenditure[81]. Neither of these studies had a control group of normothermic patients to determine whether other interventions may have contributed to these changes.

It therefore seems likely that significant reductions in energy expenditure do occur during hypothermia, but how much and what this might mean remains unknown. It is also unknown what happens during the rewarming phase after a period of prophylactic hypothermia in TBI patients. A study from over 20 years ago showed that rewarming after hypothermia during surgery (for cardiopulmonary bypass) led to increased energy expenditure [82] but this has not been measured in TBI patients. Indeed the metabolic response to all phases of illness and intervention in TBI patients remains poorly understood. Whilst there have been studies in this area, most were conducted 15-20 years ago when the management of TBI patients was often quite different and there have been no studies in an Australasian population.

Understanding energy expenditure in TBI patients has critical implications for setting nutrition therapy targets. Although less nutrition may be required during hypothermia, hypothermia may have adverse effects on the tolerance of nutrition therapy. An understanding of energy expenditure by severe TBI patients, including during hypothermia, is of fundamental importance to nutritional management in TBI. It will be of even greater importance if hypothermia becomes the standard of care. This study will allow us to measure energy expenditure throughout the intensive care period of TBI care. We anticipate that energy expenditure will change considerably over time as injury and treatment varies.

The POLAR study will therefore allow us to study energy expenditure in TBI patients with 2 predominant objectives. The first of these is that we will specifically investigate the effects of hypothermia on energy expenditure and the second is that we will investigate the energy expenditure response over the entire course of ICU treatment of TBI patients. We can also observe in these patients the actual nutrition therapy received and how well this matches both their predicted and measured requirements.

The research questions we therefore aim to address are (1) "how is energy expenditure affected by treatment with prophylactic hypothermia in severe TBI patients", and (2) "what is the daily energy expenditure in severe TBI patients over the course of their illness, whether they receive hypothermia or not"?

31.1.1 Significance

Traumatic Brain Injury is one of the leading causes of hospitalisation, death and disability worldwide, particularly in young adults. Prophylactic hypothermia is a highly promising intervention which may become a standard of care if the POLAR study shows a beneficial effect on neurological outcomes.

Nutrition therapy is another important intervention that is not well understood. Determining the optimal amount of nutrition therapy to deliver to critically ill TBI patients is a significant challenge, especially as treatments such as hypothermia can affect their energy expenditure, and therefore their nutritional requirements.

Discovering how energy expenditure changes both in response to hypothermia and throughout the course of the post-hypothermia period will allow clinicians to accurately determine optimal nutritional goals. Optimising nutrition delivery and maximising its benefits requires accurate assessment of the patient's nutritional needs to avoid the complications of over and under-nourishment.

This study will provide us with this information to enable dietitians to improve nutritional delivery and will be particularly pertinent if hypothermia becomes a standard of care for TBI. The POLAR BEAR study will be the only one of its kind to our knowledge that can provide this information. The nature of this research lends itself to generate further hypotheses and we hope that the POLAR BEAR study will be the first of a series of nutrition and TBI research projects led by the ANZIC-RC, in conjunction with The Alfred Hospital so that we can emerge as a leader in research and best practice for nutrition for the critically ill adult.

31.2 Objectives

31.2.1 Hypothesis

Our primary hypothesis is that TBI patients who receive prophylactic hypothermia will have reduced energy expenditure (measured by indirect calorimetry) of at least 20% during the initial 72 hour period of treatment compared to patients who receive standard care

31.2.1 Objectives

Our primary objective is to assess the daily energy expenditure (as measured by indirect calorimetry) in TBI patients over the first 72 hours after enrolment in the POLAR study so as to compare patients who receive prophylactic hypothermia with those who receive standard care.

Our secondary objective is to assess the daily energy expenditure (as measured by indirect calorimetry) in TBI patients who receive either prophylactic hypothermia or standard care until the end of their period on mechanical ventilation.

Other objectives are:

(1) To assess the daily energy expenditure (as measured by indirect calorimetry) in TBI patients during rewarming after prophylactic hypothermia.

(2) To assess the daily energy expenditure (as measured by indirect calorimetry) in TBI patients in the period after warming from prophylactic hypothermia.

(3) To assess the daily energy expenditure (as measured by indirect calorimetry) in TBI patients who have fever.

(4) To assess the difference in daily energy expenditure between measurements with indirect calorimetry and estimations using the Schofield predictive equation at The Alfred and fixed prescription (cal/kg) at Auckland City in TBI patients.

(5) To assess the amount of energy that is actually delivered by nutrition therapy in TBI patients as a proportion of both estimated and measured energy expenditure.

31.3 Study outcome measures

31.3.1 Primary outcome

The primary outcome for this study will be:

Mean daily energy expenditure as measured by indirect calorimetry over the first 72 hours after enrolment into the POLAR study

31.3.1 Secondary outcome

Secondary outcomes will be:

Mean daily energy expenditure as measured by indirect calorimetry over the first 7 days after enrolment into the POLAR study

Mean daily energy expenditure as measured by indirect calorimetry over defined periods of intervention: (1) period of prophylactic hypothermia; (2) period of rewarming; (3) period of post-hypothermia treatment; and (4) periods of fever (defined as periods where body temperature > 39oC)

Mean daily energy expenditure as estimated by the Schofield equation and fixed prescription as a proportion of mean daily energy expenditure as measured by indirect calorimetry

Actual delivered energy by nutrition therapy as a proportion of mean daily energy expenditure as estimated by the Schofield equation and fixed prescription methods Actual delivered energy by nutrition therapy as a proportion of mean daily energy expenditure as measured by indirect calorimetry

31.4 Study design

This will be a substudy of all patients who are enrolled into the POLAR study in 2 participating centres (Alfred Hospital and Auckland City Hospital) - a prospective, randomised controlled trial.

31.4.1 Inclusion Criteria:

40 consecutive patients admitted to the Alfred Hospital or Auckland City Hospital who are enrolled in the POLAR study and who also meet the following criteria:

Informed consent is obtained for the sub-study

Indirect calorimetry can commence within 24 hours of enrolment

31.4.2 Exclusion criteria:

Intercostal catheter with air leak

Known air leak in ventilation circuit

31.5 Methods:

All patients meeting eligibility criteria at the Alfred Hospital ICU, Melbourne, Australia and Auckland City Hospital, Auckland, New Zealand will be enrolled in the study subject to obtaining informed consent. The patients will follow all trial procedures of the POLAR study.

Indirect calorimetry (using the Quark RMR Nutritional Assessment Device, Cosmed, Rome, Italy at The Alfred Hospital and the Deltatrac II, DatexOhmeda, Madison, USA at Auckland City Hospital) will

be attached to the mechanical ventilator circuit and will be commenced within 24 hours of enrolment into the study.

As per previous validation studies for indirect calorimetry, a steady respiratory and physiological state defined as 5 consecutive minutes where VO2 and VCO2 change by less than 10% will be achieved for 5-10 minutes prior to indirect calorimetry measurements [83]. The first and last 5 minutes of each test will be discarded for accuracy. If deemed necessary measurements may be extended to ensure a steady state is achieved. Detailed information about the patients clinical state will be collected to allow correlation of indirect calorimetry measurements during the time course of prophylactic hyperthermia or normothermia.

Indirect calorimetry measurements will then be performed over a 30 minute time period twice daily at a specified time point (am and pm) until day 7 or until extubation, death or discharge from the ICU. After day 7, indirect calorimetry will be performed over a 30 minute time period once every 2 days until extubation, death or discharge from the ICU.

No other procedures will be necessary and nutritional support will not be influenced by any of these measurements as they will not be made available to clinical staff. Nutrition will be prescribed as per the main POLAR study protocol (described above).

In addition to the main POLAR study data collection and these indirect calorimetry measurements, data will be collected on several other aspects of nutritional therapy prescription and delivery including timing of commencement, route of delivery, amount prescribed, amount delivered, gastric residual volumes, use of promotility drugs, serum albumin, serum glucose and bowel actions. These will only be collected if they are measured as part of routine care.

31.6 Participating study sites

The Alfred Hospital, Melbourne

Auckland City Hospital, Auckland, New Zealand

31.7 Ethics

This study will be performed in accordance with the ethical principles of the Declaration of Helsinki (June 1964 and amended 1975, 1983, 1989, 1996, 2000, 2008 and Note of Clarification 2002 and 2004), ICH GCP Notes for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with Therapeutic Goods Administration comments and NHMRC National Statement on Ethical Conduct in Research Involving Humans (National Statement) (March 2007).

Patients will be enrolled into the study at the same time as for the POLAR study.

The ethical implications of the study are that the patients will have a severe traumatic brain injury and will be incapable of consenting to participate at the time of enrolment. The study intervention (indirect calorimetry) does not pose any additional risks or discomforts to the patient. However, as the indirect calorimetry must be commenced within 24 hours of the patient being enrolled into the POLAR study, there may not be sufficient time for the patient's Person Responsible to be contacted and discuss the study with the researcher without causing additional distress.

The POLAR study will enrol patients utilising the provisions for emergency research included in the National Statement.

Some research must necessarily be performed on patients in 'emergency' (the definition is 'unexpected') situations when they are unable to provide consent, as with all other categories of patients. To be eligible for recruitment in the POLAR study patients will be unconscious (GCS<9) and consequently will be unable to consent to participation. The National Statement acknowledges in section 4.4 that research involving patients who are heavily dependent on medical care in emergency circumstances, such as the patients in this study, may proceed when the participants ability to give consent is nonexistent. This requires approval from the appropriate ethical body to waiver or defer consent (section 7.3).

The Alfred Research and Ethics Committee has approved the POLAR study, including the use of emergency consent. Application for an amendment will be made to the Committee if funding is secured for this study, and the Committee will be asked to approve the inclusion of this POLAR BEAR study using the same consent provisions.

As soon as is reasonably possible and appropriate the patient's Person Responsible will be informed of the patient's inclusion in the research and will be asked to consent to the patients continuation in the study. Patients who recover sufficiently to understand the explanation of the study will also be asked to consent to continue in the study or be offered the chance to withdraw.

31.8 Data management

31.8.1 Data Collection Methods

All data will be collected by trained staff at each study site using a paper source document developed by the coordinating centre. Data will then be entered into a website designed by the CCRET. Data queries will then be automatically generated via the website. Some of the variables will be obtained from the POLAR trial and others are only relevant to the POLAR BEAR sub study and will be collected in addition.

31.9 Data variables collected

Pre hospital

- Patient identifier(s)
- Baseline demographics
- Inclusion and exclusion criteria (POLAR BEAR)
- Pre-hospital vital signs, Glasgow coma scale & temperature
- Date & timing of cooling intervention

Hospital admission

- Vital signs, Glasgow coma scale
- Date & time of surface temperature control vests/leg wrap application
- Biochemical parameters
- Hourly core temperature
- Injury severity score

Intensive Care Admission

- Vital signs, Glasgow coma scale
- Height
- Weight
- Date and time nutrition commenced
- Biochemical parameters
- Hourly core temperature
- Energy and protein requirements as per usual prediction method
- Total daily sedation dose

Nutrition (daily)

- Type of nutrition received and if EN, the route delivered
- Formulation name (EN and/or PN)
- Daily volume, energy and protein prescription (EN and/or PN)
- Actual volume, energy and protein received (EN and/or PN)
- Gastric residual volume total on that study day
- Promotility drug use
- Number of bowel actions

- Indirect calorimetry measurements (twice daily days 1-7 and once every second day from Day 7 until discharge, extubation or death)
- Bed side shivering assessment scale twice daily during indirect calorimetry measurements until cooling is removed or day 7 (whichever comes first)

Hospital discharge

- Length of mechanical ventilation
- Length of stay
- Discharge destination
- Vital status at ICU and hospital discharge
- Treatment limitations/withdrawal

Outcome data

- 6 month GOSE
- EQ5D
- SF12®
- Health Economic evaluation data

Clinical costing

- Duration of rehabilitation stay
- Duration of nursing home stay
- Level of home care`

31.10 Data management

Data management for POLAR BEAR will be conducted by the Project Manager

31.11 Monitoring

Each POLAR site will be monitored once 50% of the total number of required patients has been enrolled into the study

31.12 Statistical considerations

31.12.1 Sample size calculation

Sample size calculations were based on our observational cohort of 478 trauma patients. Based on a mean predicted energy requirement of 2000 calories and a standard deviation of 400 calories this study will require a minimum of 16 subjects per group to have an 80% power to detect a difference in total energy expenditure over the first 72 hours between the standard care and hypothermic patients of 400 calories with a two-sided p-value of 0.05. A difference of this magnitude (20% absolute reduction) has been chosen as a conservative estimate from previous studies [79-81] which showed that hypothermia may reduce energy expenditure by between 25-30%. Allowing a further 20% for non completion, this study will recruit a total of 20 patients per group.

31.12.2 Statistical and analytical plan

Data analysis will be performed by a statistician at the Australian and New Zealand Intensive Care Research Centre (ANZIC-RC). Reports will be issued to the sub-study and the main POLAR study investigators. Appropriate statistical techniques will be used for comparison of normally distributed and non-parametric data.

31.13 Safety

As Per the POLAR trial. There are no additional risks posed by the POLAR BEAR sub-study.

31.14 Funding

POLAR BEAR is supported by a $5000 grant (AuSPEN) for statistical services and data management and $20,000 (The Alfred Foundation) for salary support of the project manager. The ANZIC-RC will provide infrastructure and administrative support.

31.15 Publication

The study will be conducted in the name of the AuSPEN Clinical Research Group, the ANZICS Clinical Trials Group (pending endorsement) and the ANZIC-RC on behalf of the POLAR investigators. Authorship will be under the names of the sub-study investigators on behalf of the POLAR investigators.

31.16 POLAR BEAR contact details

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32. APPENDIX K: BIOMARKER & EXCITOTOXICITY POLAR SUB-STUDY

32.1 Aims

TBI increases the release of the cellular injury markers, S100β, neuron specific enolase (NSE), and myelin basic protein (MBP) into the serum. These markers have been shown to reflect the degree of neurological impairment after injury and have substantial prognostic value of long-term outcome. We plan to investigate the changes in these biomarkers in patients with TBI as a sub-study of the POLAR trial.

The specific aim of this sub-study is to determine the level of biomarkers in patients with TBI following hypothermia or normothermia treatment and evaluate the efficacy in predicting long-term outcome. We hypothesise that treatment with hypothermia after TBI will ameliorate neurological deficit and reduce the secretion of brain injury markers in the blood of TBI patients.

32.2 Background

There has been considerable work on the potential use of brain specific proteins as markers for tissue damage as well as markers of prognostic value for outcome. The identification of markers following TBI in blood serum would be of great benefit for all patients, as this would provide clinicians with additional molecular diagnostic parameters to improve monitoring and potentially the treatment provided to TBI patients.

S100β: S100β is a family member of acidic low molecular weight calcium binding proteins that are found primarily in astrocytes (S100β) within the CNS. We and others have previously found S100β levels to be dramatically increased following TBI[84, 85]. The utility of the use of S100β as a prognostic tool has been reinforced by studies by Petzold et al.[85] showing that the levels of S100β correlate with outcome.

Glial fibrillary acidic protein (GFAP): GFAP represents the major constituent of the cytoskeleton of astrocytes and is found only in glial cells of the CNS[86]. Astrogliosis is a hallmark of astrocytes activation and occurs in both TBI and hypoxic brain damage. In animal models, the increases in GFAP are associated with the degree of astrogliosis[87] and was shown in this model by immunohistochemistry[88].

Neuron specific enolase (NSE): NSE is glycolytic enzyme found in high concentrations in neuroendocrine cells, platelets and neurons. Increased CSF and serum levels of NSE are usually associated with acute neurodegeneration. Raised levels of NSE following TBI have been correlated with contusion size and cerebral inflammation[84], with very high levels being associated with death[89]. Also, following hypoxia-ischemia, plasma NSE correlated with clinical outcome[90].

Mylein basic protein (MBP): In the CNS, 30% of the myelin is composed of myelin basic protein (MBP). Human MBP has been shown to be released into the CSF by the destruction of myelin, and is therefore a marker of demyelination activity. Elevated levels of MBP in CSF have been observed in patients with head trauma and neurological diseases[91].

32.3 Methods

Inclusion and exclusion criteria for the Biomarker Sub-study will be the same as for POLAR. Blood samples (10 ml) will be collected daily for 5 days post-injury in a subset of 80 patients enrolled in POLAR trial which will be divided into 40 treated with hypothermia and 40 untreated control patients. Blood samples will be spun at 2000g and serum frozen at -70°C. Serum samples will be stored and managed in the laboratory at National Trauma Research Institute by our associate investigators. S100β, NSE, and MBP will be measured in serum using commercial ELISA kits (R&D Systems, CanAg). The results will be correlated with patient's neurological outcome scores assessed at 6 month post-injury (GOSE) to allow comparisons between patients treated with hypothermia and control normothermic patients. GOSE will be provided by the clinicians managing the POLAR-RCT.

32.4 Power calculations and sample size

Our preliminary data based on MBP measurements showed a r=0.4542 of Spearman Rank Correlation Coefficient between concentration of MBP in serum and GOSE. Based on this, a study of

40 patients (per group: hypothermia and normothermia) will have >80% power with r=0.45 and a two sided p-value of 0.05.

32.5 Analysis of results

Data will be analysed using GraphPad Prism 5. Continuous outcomes will be assessed for normality and log-transformed where appropriate. Multivariate analysis on normal or log-transformed biomarkers will be performed using multiple linear regression analysis while group comparisons over time using 2-way ANOVA. A p-value of 0.05 will be considered statistically significant.

32.6 Outcomes

The purpose of the experiments of this sub-study is to explore whether improvement of neurological deficit in TBI patients following hypothermia will coincide with attenuated release of brain injury markers in blood serum as compared to TBI patients kept under normothermic conditions. Therefore differences in the concentration of serum markers will be assessed between the treatment groups and correlated with the neurological GOSE scores. If our hypothesis will prove correct these markers may become applicable in future clinical trials as prognostic tool of long term neurological outcome.

32.7 Contact details

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33. APPENDIX L: POLAR TEG

Aims:

We plan to investigate changes in coagulation and in particular platelet function in patients with TBI in this sub study of the POLAR trial.

Background:

Therapeutic Hypothermia and POLAR.

Therapeutic hypothermia is used in patients with severe TBI to improve outcomes, both as a prophylactic therapy to reduce secondary brain injury, and also as one of only 3 possible "second tier" therapies for refractory rises in intracranial pressure. We are, at present, testing hypothermia in a rigorous NHMRC funded randomised trial (POLAR - ACTRN 12605000009617).

One concern with this therapy is the effect of hypothermia on coagulation. Most haematologists agree that hypothermia adversely affects coagulation status and studies in animals and in vitro confirm this [37, 40, 92]. Accordingly, trauma patients are often hypothermic on arrival at an emergency department and are routinely aggressively rewarmed during emergency resuscitation using sophisticated massive transfusion devices.

Strangely, little clinical work has been done to inform us of the direct effect of hypothermia in the severe TBI population. Review articles[93] state that hypothermia to 35C is generally safe and that 33C is not usually associated with bleeding complications in TBI patients however, there is little to support these contentions in the original research literature.

Hypothermia impairs coagulation by inhibiting temperature dependent enzymes involved in the coagulation cascade [94, 95]. Hypothermia also causes platelet dysfunction [96, 97], but the temperature at which these elements become *clinically relevant* and or predominant has not been studied in trauma patients and is completely unknown.

There have been no reports of increased bleeding in patients suffering severe TBI and managed with hypothermia [1, 98, 99], but coagulation was not studied in detail and has generally been de-emphasised by hypothermia advocates. Haemostasis in *cardiac arrest* patients treated with hypothermia has been studied by Spiel et al, and it has been concluded that the potential bleeding complications of therapeutic hypothermia are "minimal" [100]. However and obviously, cardiac arrest patients do not have the same traumatic tissue injuries *at risk of bleeding* as trauma, and specifically TBI patients.

There has been little clinical investigation of the effects of hypothermia on coagulation and no such study of this type in patients with severe TBI. The POLAR trial presents a unique opportunity to clinically investigate the influence of hypothermia on TBI patients at minimal cost. POLAR gives us an opportunity to study the effects of hypothermia on coagulation in severe TBI patients. As this work has never been done in TBI patients especially those receiving hypothermia, it will have direct clinical applicability.

Laboratory Testing

The most common routine laboratory-based coagulation tests International Normalised Ratio (INR), Activated Partial Thromboplastin Time (APTT) and platelet numbers are used to inform clinicians of the patients' coagulation status [101] but may be poor indicators of *in vivo* coagulation in hypothermic

patients [101]. These samples test plasma (blood minus cells) rather than whole blood which excludes the contribution of the cellular elements to the in vivo coagulation process [102-104]. The assays are also performed at a standard temperature of 37C (regardless of the patient's actual temperature) which likely underestimates the effects of hypothermia on coagulation [101]. Thromboelastography (TEG) has been previously used in bleeding cardiac surgical patients to measure coagulation deficits and we will use this technology in *TBI patients [105]*. It can be performed at the patient bedside (Point-of-Care) and also at the patient's temperature. We can therefore, for the first time, get an clearer picture of the effect of patient temperature on coagulation in the patient group of concern[106], [107] .

Significance

There has been little clinical investigation of the effects of hypothermia on coagulation and no such study of this type in patients with severe TBI. POLAR gives us an opportunity to study the effects of hypothermia on coagulation in severe TBI patients. As this work has never been done in TBI patients especially those receiving hypothermia, it will have direct clinical applicability.

Objectives

Hypothesis

TBI patients who receive prophylactic hypothermia will have alterations in their coagulation status as measured by the TEG.

Primary Outcome

The coagulation status of hypothermic POLAR TBI patients will be compared to that of the normothermic control group POLAR TBI patients.

Secondary Outcome

Hypothermic patient's coagulation status will be compared with the same patient's blood tested at normothermia.

Study Design

An observational sub study of patients enrolled in the POLAR RCT.

Recruiting Site

The Alfred Hospital Melbourne

Inclusion criteria

- Enrolment in the POLAR study

Exclusion Criteria

- Death is imminent
- Patient on Heparin or Clexane
- Inability to cool POLAR "hypothermia" patient

Ethics and consent

The Alfred Research and Ethics Committee has approved the POLAR study, including the use of emergency consent. Application for an amendment will be made to the Committee to approve the inclusion of this POLAR TEG study using the same consent provisions. The plain language and information form will include details about the sub study including the study design, methodology and significance.

Research plan and Methods

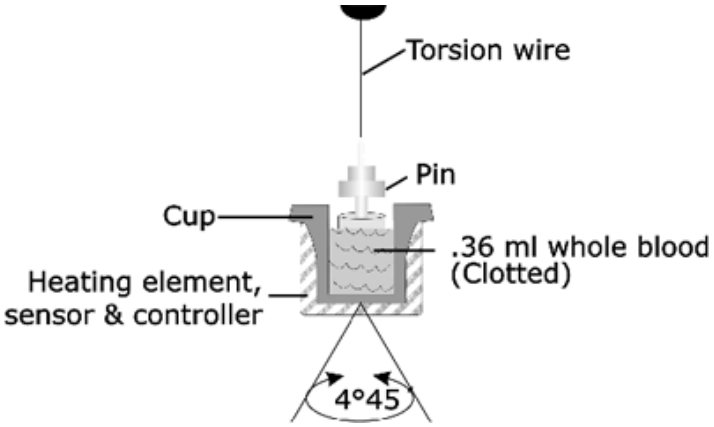
Twenty patients will be tested in this sub-study (10 control / 10 hypothermic). Wherever possible eligible patients will be enrolled consecutively, however logistical constraints may preclude this.

Demographic data and baseline data will be gathered from patient notes and the coagulation profile will be drawn from routine sampling of patients. Patients will undergo TEG analysis as per TEG schedule and TEG analysis methodology. Patients will exit this observational study upon completion of the final blood sample.

TEG analyser technology

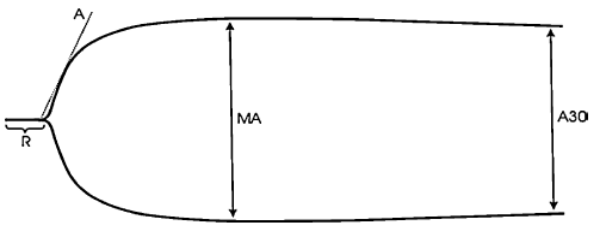
The TEG analyzer has a sample cup that oscillates back and forth constantly at a set speed through an arc of 4"45'. Each rotation lasts ten seconds. A whole blood sample of 360 ul is placed into the cup, and a stationary pin attached to a torsion wire is immersed into the blood. When the first fibrin forms, it begins to bind the cup and pin, causing the pin to oscillate in phase with the clot. The acceleration of the movement of the pin is a function of the kinetics of clot development.

The torque of the rotating cup is transmitted to the immersed pin only after fibrin-platelet bonding has linked the cup and pin together. The strength of these fibrin-platelet bonds affects the magnitude of the pin motion, such that strong clots move the pin directly in phase with the cup motion. Thus, the magnitude of the output is directly related to the strength of the formed clot. As the clot retracts or lyses, these bonds are broken and the transfer of cup motion is diminished. The rotation movement of the pin is converted by a mechanical-electrical transducer to an electrical signal which can be monitored by a computer.



The resulting hemostasis profile is a measure of the time it takes for the first fibrin strand to be formed, the kinetics of clot formation, the strength of the clot (in shear elasticity units of dyn/cm2) and dissolution of clot.

Normal TEG trace



R time *– reaction time - the initiation phase of coagulation / clotting time (clotting factors)*

A angle *– rate of clot formation/strengthening (speed of fibrin build up and crosslinking)*

MA *- maximum amplitude – reflects the ultimate strength of the clot (fibrinogen, platelet count and function).*

A30 *– amplitude at 30 mins - reflects clot stability/breakdown (fibrinolysis)*

TEG schedule

Sample 1

Hypothermia patients will have TEG blood samples taken while at 33C and prior to rewarming (48 to 72hrs hours post POLAR randomisation). Samples will be analysed using the TEG at both 33C and 37C (see TEG analysis methodology). At each temperature (33C and 37C) standard and functional fibrinogen assays will be performed. Standard and functional fibrinogen at 33C will be tested followed by standard and functional fibrinogen at 37C. The second analysis will require the patient to have a fresh sample taken.

Control patients will have blood taken 48 to 72hrs hours post POLAR randomisation and analysed at 37C using the TEG. Standard and functional fibrinogen assays will be performed at 37C (see TEG analysis methodology).

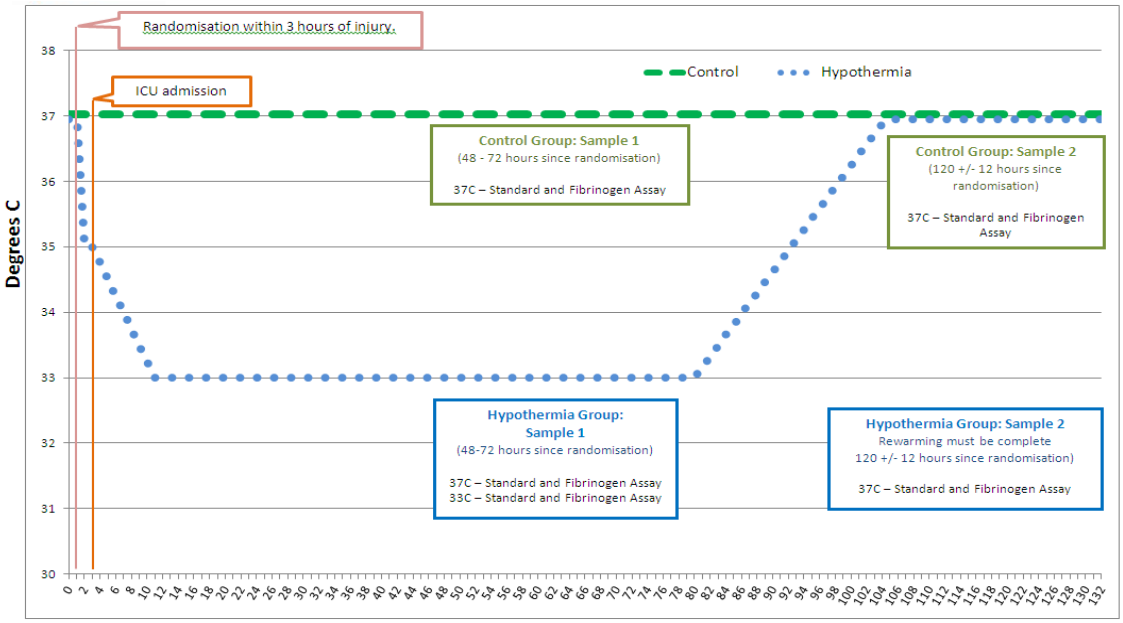
Sample 2

Hypothermia patients will have blood taken 120 hours +/- 12hrs post randomisation once they have reached 37C. Samples will be analysed using the TEG at 37C. Standard and Fibrinogen TEG Assay will be performed (see TEG analysis methodology).

Control patients will have blood taken 120 +/-12hrs post randomisation and analysed at 37C using the TEG. Standard and fibrinogen TEG Assay will be performed (see TEG analysis methodology).

Routine laboratory testing includes International Normalised Ratio (INR), Activated Partial Thromboplastin (APTT) & Platelet count.

		Hypothermic therapy patient	**Standard therapy patient**
Sample 1	48 and 72hrs hours post POLAR randomisation	Standard and Fibrinogen assay at 33C followed by, Standard and Fibrinogen assay at 37C	Standard and Fibrinogen assay at 37C only.
Sample 2	120 +/-12hrs post POLAR randomisation	Standard and Fibrinogen assay at 37C only.	Standard and Fibrinogen assay at 37C only.



TEG analysis methodology:

Samples for analysis will be drawn from the arterial catheter and must be used within 4 minutes.

The collection of samples will, wherever possible, coincide with routine blood collection to minimise fluid and blood loss to the patient. A 10ml aliquot will be collected and discarded to eliminate the dilutional effect of saline in the arterial line (standard practice). The sample for testing is then collected.

Standard Assay

- Set temperature of analyser
- Load plain test pin and cup
- Collect 1ml of blood from arterial line (follow unit standard practice)
- Deliver 1ml aliquot into kaolin vial
- Mix carefully by inversion (not pill rolling as this may warm the sample).
- Pipette 360µl of kaolin mixture into loaded plain test cup
- Commence computer program

Fibrinogen Assay

- Set temperature of analyser
- Load plain test pin and cup
- Collect 0.5ml of blood from arterial line (follow unit standard practice)
- Deliver 0.5ml aliquot into functional fibrinogen assay vial
- Mix carefully by inversion (not pill rolling as this may warm the sample).
- Pipette 360µl of kaolin mixture into loaded plain test cup
- Commence computer program

Analysis shall be performed with the computerised thromboelastograph coagulation analyser (Haemoscope Corp., Skokie, IL). The coagulation profile is displayed on a computer running TEG analytic software which is connected to the analyser via an A/D interface box. The values of normal controls are displayed enabling comparison.

Quality Control

Routine quality control should be performed prior to patient testing if it has not been performed in the last 7 days.

Data variables

- Gender
- Date of Birth
- Injury time
- Blood pressure / pulse / Respiratory Rate / Blood sugar
- Fluid and Blood component Input and output
- Glasgow coma scale
- Pupil reaction
- Temperature
- Date & timing of cooling intervention (commenced and ceased)
- APACHE II
- AIS Head (max)
- CT brain scan – Marshall score
- INR
- APTT
- Platelet count
- TEG
    - R time
    - A angle
    - MA
    - A30
- Bleeding incidents
- Medications (pre injury and during ICU stay)

Data analysis and statistics

Both conventional (INR and APTT) and TEG related (platelet function and fibrinogen) indices of coagulation will be compared between treatment and control groups at two time points 1) between 48 and 72hrs hours post POLAR randomisation, 2) 120 hours (+/- 12hrs) post randomisation. These indices will also be compared in the same samples at both 33C and 37C, in the treatment group patients All data will be analysed using SAS version 9.2 (SAS Institute Cary, NC, USA). Data will be assessed for normality and log-transformed where appropriate. Comparisons within and between groups will be performed using mixed linear modelling accounting for repeat measures with individual patients treated as random effects. Should any indices have insufficient symmetry to satisfy the assumption of normality, changes from baseline will firstly be considered followed by non-linear modelling if required. A two-sided p-value of 0.05 will be considered to be statistically significant.

Safety

As Per the POLAR trial. There are no additional risks posed by the POLAR TEG sub-study. The results of the study will be forwarded to the DSMC.

Funding

Funding for the sub-study has been obtained from the Institute for Safety, Compensation and Recovery Research.

Publication

The study will be conducted in the name of the ANZIC-RC on behalf of the POLAR investigators. Authorship will be under the names of the sub-study investigators on behalf of the POLAR investigators. Publication of results will be embargoed until publication of the main study results unless specific permission for early publication is given by the POLAR management committee.

34. BIBLIOGRAPHY

1. Clifton, G.L., et al., *Lack of effect of induction of hypothermia after acute brain injury.* N Engl J Med, 2001. **344**(8): p. 556-63.
2. Langlois, J.A., W. Rutland-Brown, and M.M. Wald, *The epidemiology and impact of traumatic brain injury: a brief overview.* J Head Trauma Rehabil, 2006. **21**(5): p. 375-8.
3. Hukkelhoven, C.W., et al., *Patient age and outcome following severe traumatic brain injury: an analysis of 5600 patients.* J Neurosurg, 2003. **99**(4): p. 666-73.
4. Myburgh, J., et al., *Epidemiology and 12 month outcomes from traumatic brain injury in Australia and New Zealand.* Journal of Trauma, 2008. **in press, accepted July 2006**.
5. The_SAFE_Study_Investigators, *Saline or albumin for fluid resuscitation in patients with traumatic brain injury.* N Engl J Med, 2007. **357**(9): p. 874-84.
6. The_SAFE_Study_Investigators, *A comparison of albumin and saline for fluid resuscitation in the intensive care unit.* N Engl J Med, 2004. **350**(22): p. 2247-56.
7. Bernard, S.A., *The effects on outcome at 6 months of pre-hospital rapid sequence intubation compared with no intubation in patients with severe head injury* Australian New Zealand Clinical Trials Registry, 2005. **ACTRN12605000177651**
8. Kearsley, D., *Cost of medical care in traumatic brain injury in Victoria, Transport Accident Commission (TAC).* Personal communication, 2007.
9. *Guidelines for the management of severe traumatic brain injury.* J Neurotrauma, 2007. **24 Suppl 1**: p. S1-106.
10. Edwards, P., et al., *Final results of MRC CRASH, a randomised placebo-controlled trial of intravenous corticosteroid in adults with head injury-outcomes at 6 months.* Lancet, 2005. **365**(9475): p. 1957-9.
11. Cooper, D.J., et al., *Prehospital hypertonic saline resuscitation of patients with hypotension and severe traumatic brain injury: a randomized controlled trial.* Journal of the American Medical Association, 2004. **291**(11): p. 1350-7.
12. Maas, A.I., et al., *Efficacy and safety of dexanabinol in severe traumatic brain injury: results of a phase III randomised, placebo-controlled, clinical trial.* Lancet Neurol, 2006. **5**(1): p. 38-45.
13. Bernard, S.A. and M. Buist, *Induced hypothermia in critical care medicine: a review.* Crit Care Med, 2003. **31**(7): p. 2041-51.
14. Sahuquillo, J. and A. Vilalta, *Cooling the injured brain: how does moderate hypothermia influence the pathophysiology of traumatic brain injury.* Curr Pharm Des, 2007. **13**(22): p. 2310-22.
15. Clifton, G.L., et al., *Marked protection by moderate hypothermia after experimental traumatic brain injury.* J Cereb Blood Flow Metab, 1991. **11**(1): p. 114-21.
16. Clark, R.S., et al., *Mild posttraumatic hypothermia reduces mortality after severe controlled cortical impact in rats.* J Cereb Blood Flow Metab, 1996. **16**(2): p. 253-61.
17. Koizumi, H. and J.T. Povlishock, *Posttraumatic hypothermia in the treatment of axonal damage in an animal model of traumatic axonal injury.* J Neurosurg, 1998. **89**(2): p. 303-9.
18. Markgraf, C.G., G.L. Clifton, and M.R. Moody, *Treatment window for hypothermia in brain injury.* J Neurosurg, 2001. **95**(6): p. 979-83.
19. Clifton, G.L., et al., *A phase II study of moderate hypothermia in severe brain injury.* J Neurotrauma, 1993. **10**(3): p. 263-71; discussion 273.
20. Marion, D.W., et al., *Treatment of traumatic brain injury with moderate hypothermia.* N Engl J Med, 1997. **336**(8): p. 540-6.
21. Qiu, W.S., et al., *Therapeutic effect of mild hypothermia on severe traumatic head injury.* Chin J Traumatol, 2005. **8**(1): p. 27-32.
22. Jiang, J., M. Yu, and C. Zhu, *Effect of long-term mild hypothermia therapy in patients with severe traumatic brain injury: 1-year follow-up review of 87 cases.* J Neurosurg, 2000. **93**(4): p. 546-9.
23. Zhi, D., S. Zhang, and X. Lin, *Study on therapeutic mechanism and clinical effect of mild hypothermia in patients with severe head injury.* Surg Neurol, 2003. **59**(5): p. 381-5.
24. *Guidelines for the management of severe traumatic brain injury. III. Prophylactic hypothermia.* J Neurotrauma, 2007. **24 Suppl 1**: p. S21-5.
25. Clifton, G.L., et al., *Hypothermia on admission in patients with severe brain injury.* J Neurotrauma, 2002. **19**(3): p. 293-301.
26. Bernard, S.A., *The effect on outcome at hospital discharge of paramedic cooling compared with hospital cooling after resuscitation from cardiac arrest.* Australian New Zealand Clinical Trials Registry, 2005. **ACTRN12605000179639**.

27. Kim, F., et al., *Pilot randomized clinical trial of prehospital induction of mild hypothermia in out-of-hospital cardiac arrest patients with a rapid infusion of 4 degrees C normal saline.* Circulation, 2007. **115**(24): p. 3064-70.
28. Bernard, S.A., C.J.B. Mac, and M. Buist, *Experience with prolonged induced hypothermia in severe head injury.* Crit Care, 1999. **3**(6): p. 167-172.
29. Narayan, R.K., et al., *Clinical trials in head injury.* J Neurotrauma, 2002. **19**(5): p. 503-57.
30. Tokutomi, T., et al., *Effect of hypothermia on serum electrolyte, inflammation, coagulation, and nutritional parameters in patients with severe traumatic brain injury.* Neurocrit Care, 2004. **1**(2): p. 171-82.
31. Alderson, P., C. Gadkary, and D.F. Signorini, *Therapeutic hypothermia for head injury.* Cochrane Database Syst Rev, 2004(4): p. CD001048.
32. Bellomo, R., P.J. Stow, and G.K. Hart, *Why is there such a difference in outcome between Australian intensive care units and others?* Curr Opin Anaesthesiol, 2007. **20**(2): p. 100-5.
33. Marshall, L.F., et al., *The diagnosis of head injury requires a classification based on computed axial tomography.* J Neurotrauma, 1992. **9 Suppl 1**: p. S287-92.
34. *National Health and Medical Research Council and the Australian Research Council. National Statement on ethical conduct in human research.* . 2007.
35. Zygun, D.A., et al., *Ventilator-associated pneumonia in severe traumatic brain injury.* Neurocritical Care, 2006. **5**(2): p. 108-14.
36. Sydenham, E., I. Roberts, and P. Alderson, *Hypothermia for traumatic head injury.* Cochrane Database Syst Rev, 2009(2): p. CD001048.
37. Watts, D.D., et al., *Hypothermic coagulopathy in trauma: effect of varying levels of hypothermia on enzyme speed, platelet function, and fibrinolytic activity.* Journal of Trauma-Injury Infection & Critical Care, 1998. **44**(5): p. 846-54.
38. Polderman, K.H., S.M. Peerdeman, and A.R. Girbes, *Hypophosphatemia and hypomagnesemia induced by cooling in patients with severe head injury.[see comment].* Journal of Neurosurgery, 2001. **94**(5): p. 697-705.
39. Polderman, K.H., *Induced hypothermia and fever control for prevention and treatment of neurological injuries.* Lancet, 2008. **371**(9628): p. 1955-69.
40. Polderman, K.H., et al., *Therapeutic hypothermia and controlled normothermia in the intensive care unit: practical considerations, side effects, and cooling methods.[see comment].* Critical Care Medicine, 2009. **37**(3): p. 1101-20.
41. Bernard, S.A., et al., *Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia.* N Engl J Med, 2002. **346**(8): p. 557-63.
42. Wilson, J.T., L.E. Pettigrew, and G.M. Teasdale, *Structured interviews for the Glasgow Outcome Scale and the extended Glasgow Outcome Scale: guidelines for their use.* J Neurotrauma, 1998. **15**(8): p. 573-85.
43. Teasdale, G.M., et al., *Analyzing outcome of treatment of severe head injury: a review and update on advancing the use of the Glasgow Outcome Scale.* J Neurotrauma, 1998. **15**(8): p. 587-97.
44. EuroQol group. *EQ-5D A standardised instrument for use as a measure of health outcome*. 1990; Available from: http://www.euroqol.org/eq-5d/what-is-eq-5d.html.
45. Ware, J., Jr., M. Kosinski, and S.D. Keller, *A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity.* Medical Care, 1996. **34**(3): p. 220-33.
46. National Health and Medical Research Council and the Australian Research Council, *National Statement on ethical conduct in human research.* 2007.
47. Murray, G.D., et al., *Design and analysis of phase III trials with ordered outcome scales: the concept of the sliding dichotomy.* J Neurotrauma, 2005. **22**(5): p. 511-7.
48. Bellomo, R., et al., *Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group.* Critical Care, 2004. **8**(4): p. R204-R212.
49. Moore, E.M., et al., *The incidence of acute kidney injury in patients with traumatic brain injury.* Renal Failure, 2010. **32**(9).
50. Bagshaw, S.M., et al., *A Multi-Centre Evaluation of Early Acute Kidney Injury in Critically Ill Trauma Patients for the ANZICS Database Management Committee. Unpublished study.*
51. Davenport, A., *Renal replacement therapy in the patient with acute brain injury.* American Journal of Kidney Diseases, 2001. **37**(3): p. 457-66.
52. Bagshaw, S.M., et al., *A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients.* Nephrology Dialysis Transplantation, 2008. **23**(4): p. 1203-10.
53. Chertow, G.M., et al., *Acute kidney injury, mortality, length of stay, and costs in hospitalized patients.* Journal of the American Society of Nephrology, 2005. **16**(11): p. 3365-70.
54. Department of Human Sevices - Victorian Maintenance renal dialysis program [page on the Internet] [updated 2008 Oct 2; cited 2009 May 10], Available from: http://www.health.vic.gov.au/renaldialysis/index.htm.

55. Delbridge, M.S., et al., *The effect of body temperature in a rat model of renal ischemia-reperfusion injury.* Transplantation Proceedings, 2007. **39**(10): p. 2983-5.
56. Zager, R.A., et al., *Degree and time sequence of hypothermic protection against experimental ischemic acute renal failure.* Circulation Research, 1989. **65**(5): p. 1263-9.
57. Hoste, E.A.J., et al., *RIFLE criteria for acute kidney injury are associated with hospital mortality in critically ill patients: a cohort analysis.* Critical Care 2006. **10**(3): p. R73.
58. Ostermann, M. and R.W.S. Chang, *Acute kidney injury in the intensive care unit according to RIFLE.* Critical Care Medicine, 2007. **35**(8): p. 1837-1843.
59. Helps Y, H.G.H., *Hospital Separations due to traumatic brain injury , Australia 2004-05*, in *Injury Research and Statistics Series No. 45*. 2008, AIHW: Adelaide. p. 1-140.
60. Cooper, D.J., et al., *Prehospital hypertonic saline resuscitation of patients with hypotension and severe traumatic brain injury: a randomized controlled trial.* Jama, 2004. **291**(11): p. 1350-7.
61. Brain Trauma, F., et al., *Guidelines for the management of severe traumatic brain injury. XII. Nutrition.[erratum appears in J Neurotrauma. 2008 Mar;25(3):276-8 Note: Multiple author names added].* Journal of Neurotrauma, 2007. **24 Suppl 1**: p. S77-82.
62. Heyland, D.K., et al., *Canadian clinical practice guidelines for nutrition support in mechanically ventilated, critically ill adult patients.[see comment].* Jpen: Journal of Parenteral & Enteral Nutrition, 2003. **27**(5): p. 355-73.
63. Scurlock, C. and J.I. Mechanick, *Early nutrition support in the intensive care unit: a US perspective.* Current Opinion in Clinical Nutrition & Metabolic Care, 2008. **11**(2): p. 152-5.
64. Alberda, C., et al., *The relationship between nutritional intake and clinical outcomes in critically ill patients: results of an international multicenter observational study.* Intensive Care Med, 2009. **35**(10): p. 1728-37.
65. Kudsk, K.A., et al., *Enteral versus parenteral feeding. Effects on septic morbidity after blunt and penetrating abdominal trauma.* Ann Surg, 1992. **215**(5): p. 503-11; discussion 511-3.
66. Doig, G.S., et al., *Early enteral nutrition, provided within 24 h of injury or intensive care unit admission, significantly reduces mortality in critically ill patients: a meta-analysis of randomised controlled trials.* Intensive Care Med, 2009. **35**(12): p. 2018-27.
67. Marik, P.E. and G.P. Zaloga, *Early enteral nutrition in acutely ill patients: a systematic review.* Crit Care Med, 2001. **29**(12): p. 2264-70.
68. Pepe, J.L. and C.A. Barba, *The metabolic response to acute traumatic brain injury and implications for nutritional support.* Journal of Head Trauma Rehabilitation, 1999. **14**(5): p. 462-74.
69. Ferrie S, W., M, *Back to Basics: Estimating energy requirements for adult hospital patients.* Nutrition & Dietetics, 2007. **64**: p. 192-199.
70. Green, A.J., P. Smith, and K. Whelan, *Estimating resting energy expenditure in patients requiring nutritional support: a survey of dietetic practice.* European Journal of Clinical Nutrition, 2008. **62**(1): p. 150-3.
71. Reeves, M.M. and S. Capra, *Predicting energy requirements in the clinical setting: are current methods evidence based?* Nutrition Reviews, 2003. **61**(4): p. 143-51.
72. Battezzati, A. and R. Vigano, *Indirect calorimetry and nutritional problems in clinical practice.* Acta Diabetologica, 2001. **38**(1): p. 1-5.
73. da Rocha, E.E., V.G. Alves, and R.B. da Fonseca, *Indirect calorimetry: methodology, instruments and clinical application.* Current Opinion in Clinical Nutrition & Metabolic Care, 2006. **9**(3): p. 247-56.
74. Dempsey, D.T., et al., *Energy expenditure in acute trauma to the head with and without barbiturate therapy.* Surgery, Gynecology & Obstetrics, 1985. **160**(2): p. 128-34.
75. Berger, M.M. and R.L. Chiolero, *Hypocaloric feeding: pros and cons.* Current Opinion in Critical Care, 2007. **13**(2): p. 180-6.
76. Stapleton, R.D., N. Jones, and D.K. Heyland, *Feeding critically ill patients: what is the optimal amount of energy?* Critical Care Medicine, 2007. **35**(9 Suppl): p. S535-40.
77. Alberda, C., et al., *Energy requirements in critically ill patients: how close are our estimates?* Nutr Clin Pract, 2002. **17**(1): p. 38-42.
78. Foley N, M.S., Pikul J, Salter K, Teasell R, *Hypermetabolism following Moderate to Severe Traumatic Acute Brain Injury: A Systematic Review.* Journal of Neurotrauma, 2008(25): p. 1415-1431.
79. Bardutzky, J., et al., *Energy expenditure in ischemic stroke patients treated with moderate hypothermia.* Intensive Care Med, 2004. **30**(1): p. 151-4.
80. Tokutomi, T., et al., *Optimal temperature for the management of severe traumatic brain injury: effect of hypothermia on intracranial pressure, systemic and intracranial hemodynamics, and metabolism.* Neurosurgery, 2003. **52**(1): p. 102-11; discussion 111-2.

81. Saur, J., et al., *Modification of the Harris-Benedict equation to predict the energy requirements of critically ill patients during mild therapeutic hypothermia.* In Vivo, 2008. **22**(1): p. 143-6.
82. Chiara, O., et al., *Hypermetabolic response after hypothermic cardiopulmonary bypass.* Crit Care Med, 1987. **15**(11): p. 995-1000.
83. McClave, S.A., et al., *Achievement of steady state optimizes results when performing indirect calorimetry.* JPEN J Parenter Enteral Nutr, 2003. **27**(1): p. 16-20.
84. Pleines, U.E., et al., *S-100 beta reflects the extent of injury and outcome, whereas neuronal specific enolase is a better indicator of neuroinflammation in patients with severe traumatic brain injury.* J Neurotrauma, 2001. **18**(5): p. 491-8.
85. Petzold, A., et al., *Role of serum S100B as an early predictor of high intracranial pressure and mortality in brain injury: a pilot study.* Crit Care Med, 2002. **30**(12): p. 2705-10.
86. Eng, L.F., et al., *An acidic protein isolated from fibrous astrocytes.* Brain Res, 1971. **28**(2): p. 351-4.
87. O'Callaghan, J.P., *Quantitative features of reactive gliosis following toxicant-induced damage of the CNS.* Ann N Y Acad Sci, 1993. **679**: p. 195-210.
88. Csuka, E., et al., *IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alpha, TGF-beta1 and blood-brain barrier function.* J Neuroimmunol, 1999. **101**(2): p. 211-21.
89. Vazquez, M.D., et al., *Creatine kinase BB and neuron-specific enolase in cerebrospinal fluid in the diagnosis of brain insult.* Am J Forensic Med Pathol, 1995. **16**(3): p. 210-4.
90. Schaarschmidt, H., H.W. Prange, and H. Reiber, *Neuron-specific enolase concentrations in blood as a prognostic parameter in cerebrovascular diseases.* Stroke, 1994. **25**(3): p. 558-65.
91. Berger, R.P., et al., *Identification of inflicted traumatic brain injury in well-appearing infants using serum and cerebrospinal markers: a possible screening tool.* Pediatrics, 2006. **117**(2): p. 325-32.
92. Dirkmann, D., et al., *Hypothermia and acidosis synergistically impair coagulation in human whole blood.* Anesthesia and analgesia, 2008. **106**(6): p. 1627-32.
93. Polderman, K.H., *Mechanisms of action, physiological effects, and comp0lications of hypothermia.* Crit Care Med, 2009. **37**(7): p. s186-s202.
94. Reed RL, B.A., Hudson JD, et al, *Hypothermia and blood coagulation: Dissociation between enzyme activity and clotting factor levels.* Circ Shock, 1990. **160**: p. 515-518.
95. Rohrer, M.J. and A.M. Natale, *Effect of hypothermia on the coagulation cascade.* Critical care medicine, 1992. **20**(10): p. 1402-5.
96. Valeri, C.R., et al., *Hypothermia-induced reversible platelet dysfunction.* Annals of surgery, 1987. **205**(2): p. 175-81.
97. Michelson, A.D., et al., *Reversible inhibition of human platelet activation by hypothermia in vivo and in vitro.* Thrombosis and haemostasis, 1994. **71**(5): p. 633-40.
98. Clifton, G.L., et al., *Very early hypothermia induction in patients with severe brain injury (the National Acute Brain Injury Study: Hypothermia II): a randomised trial.* Lancet neurology, 2011. **10**(2): p. 131-9.
99. Hutchison, J.S., et al., *Hypothermia Therapy after Traumatic Brain Injury in Children.* N Engl J Med, 2008. **358**(23): p. 2447-2456.
100. Spiel, A.O., et al., *Hemostasis in cardiac arrest patients treated with mild hypothermia initiated by cold fluids.* Resuscitation, 2009. **80**(7): p. 762-5.
101. Whitten CW, G.P., *Thromboelastography(R): Past, Present, and Future.* Anesthesiology 2000. **92**(5): p. 1226.
102. Reed RL, J.T., Hudson JD, et al, *The disparity between hypothermic coagulopathy and clotting studies.* J Trauma, 1992. **33**: p. 465-470.
103. Valeri CR, M.H., Cassidy G, et al, *Effects of temeprature on bleeding time and clotting time in normal male and female volunteers.* Crit Care Med, 1995. **1995**(23): p. 698-704.
104. Felfernig, M., et al., *Effects of temperature on partial thromboplastin time in heparinized plasma in vitro.* European journal of anaesthesiology, 2001. **18**(7): p. 467-70.
105. Westbrook, A.J., et al., *Protocol based on thromboelastograph (TEG) out-performs physician preference using laboratory coagulation tests to guide blood replacement during and after cardiac surgery: a pilot study.* Heart, lung & circulation, 2009. **18**(4): p. 277-88.
106. Ganter, M.T. and C.K. Hofer, *Coagulation monitoring: Current techniques and clinical use of viscoelastic point-of-care coagulation devices.* Anesthesia and analgesia, 2008. **106**(5): p. 1366-1375.
107. Jackson, G.N.B., K.J. Ashpole, and S.M. Yentis, *The TEG((R)) vs the ROTEM((R)) thromboelastography/thromboelastometry systems.* Anaesthesia, 2009. **64**(2): p. 212-215.

Appendix 6

POLAR trial hard-copy case report form (CRF)

anzic research centre

POLAR Study

FORM 1 Demographic Data

polar

Please enter randomisation and patient demographic details into the electronic CRF as soon as possible after randomisation

Randomisation

1.0 Pilot study: ☐ Yes ☐ No

1.1 POLAR study number: ☐☐☐☐☐ 1.2 Patient initials: ☐☐☐ 1.3 Hospital code: ☐☐☐

1.4 Date and time of randomisation: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐
dd mmm yyyy HH mm

1.5 Patient randomised to:
☐ Hypothermia (cooling group)
☐ Normothermia (control group)

1.6 Where was the patient randomised?
☐ Pre-hospital
☐ Emergency Department

Patient Demographics

1.7 Gender:
☐ Male
☐ Female

1.8 Date of birth: ☐☐/☐☐☐/☐☐☐☐
dd mmm yyyy

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POLAR Study
FORM 2 At Scene

POLAR Study Number ☐☐☐☐☐
Patient Initials ☐☐☐

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Injury Details

2.1 Date and time of injury: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐
dd mmm yyyy HH mm

2.2 Date and time of ambulance arrival: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐
dd mmm yyyy HH mm

2.3 Mechanism of injury:
- ☐ Motor vehicle
- ☐ Bicycle
- ☐ Pedestrian
- ☐ Hit by object
- ☐ Motorcycle
- ☐ Other
- ☐ Fall / Jump

At Scene Data

2.4 Temperature: ☐☐.☐ °C ☐ Unknown

2.5 Systolic BP: ☐☐☐ mmHg ☐ Unknown

2.6 Respiratory rate: ☐☐ bpm ☐ Unknown
(pre-intubation)

2.7 Heart rate: ☐☐☐ bpm ☐ Unknown

2.8 GCS: ☐☐ ☐ Unknown
(pre-intubation)

2.9 Motor score from the above GCS: ☐ ☐ Unknown

2.10 Pupillary response:
- ☐ Both reacting
- ☐ None reacting
- ☐ One reacting
- ☐ Unknown

2.11 Hypotensive pre-hospital: ☐ Yes ☐ No ☐ Unknown
(sysBP < 90 mmHg)

2.12 Hypoxic pre-hospital: ☐ Yes ☐ No ☐ Unknown
(SaO_2 < 90%)

Fluid Delivery - pre-hospital

2.13 Total volume of fluid administered pre-hospital: ☐☐☐☐ mls
(including cooled fluid)

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POLAR Study
FORM 3 Hospital Admission

POLAR Study Number ☐☐☐☐☐

Patient Initials ☐☐☐

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Hospital Admission Data

3.1 Date and time of hospital admission: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐
(dd mmm yyyy HH mm)

3.2 Patient weight: ☐☐☐ kg

3.3 Blood alcohol level: ☐☐☐ mmol/L ☐ Not performed

3.4 WCC: ☐☐☐.☐ x10⁹/L ☐ Not performed

3.5 INR: ☐☐.☐ ☐ Not performed

3.6 APTT: ☐☐☐ secs ☐ Not performed

3.7 Platelets: ☐☐☐ x10⁹/L ☐ Not performed

3.8 Haemoglobin: ☐☐☐ g/L ☐ Not performed

3.9 Glucose: ☐☐.☐ mmol/L ☐ Not performed

3.10 GCS: (pre-intubation) ☐☐ ☐ Not performed

3.11 Motor score from 3.10 GCS: ☐ ☐ Not performed

3.12 Pupillary response:
- ☐ Both reacting
- ☐ None reacting
- ☐ One reacting
- ☐ Unknown

3.13 Systolic BP: ☐☐☐ mmHg ☐ Not performed

3.14 Respiratory rate: ☐☐ ☐ Not performed

3.15 Hypotensive (Emergency Department): ☐ Yes ☐ No ☐ Unknown
(sysBP < 90 mmHg)

3.16 Hypoxic (Emergency Department): ☐ Yes ☐ No ☐ Unknown
(SaO_2 < 90%)

Fluid Delivery

3.17 Total volume of cold fluid delivered: ☐☐☐☐ mls
(Pre-hospital and Emergency Department)

Renal Sub Study

3.18.1 Serum creatinine: ☐☐☐ µmol/L ☐ Not performed

3.18.2 Date and time serum creatinine sample taken: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐
(dd mmm yyyy HH mm)

3.19 Does the patient have end stage renal failure? ☐ Yes ☐ No

Female Patients

3.20.1 Pregnancy test performed:
- ☐ Positive
- ☐ Negative
- ☐ Not performed

- if POSITIVE 3.20.2 Was the patient withdrawn from the cooling protocol?
- ☐ Yes
- ☐ No

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POLAR Study
FORM 4 Study Intervention

POLAR Study Number ☐☐☐☐☐

Patient Initials ☐☐☐

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Cooling Group Data (complete if randomised to hypothermia)

4.1 Was surface cooling instigated at any time: ☐ Yes ☐ No *- if NO, go to Qu 4.5*

4.2 Temperature prior to application of surface cooling: ☐☐.☐ °C

4.3 Date and time of surface cooling applied: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐
dd mmm yyyy HH mm

4.4 Date and time target temperature first reached: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐
(Target temperature 33°C)
dd mmm yyyy HH mm

☐ Target temperature not reached

Withdrawing Cooling

4.5.1 Was cooling permanently withdrawn: ☐ Yes *- if YES,* ☐ No

4.5.2 Date and time cooling withdrawn: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐
dd mmm yyyy HH mm

4.5.3 Reason cooling withdrawn:
(Choose all that apply)

- ☐ Pregnancy
- ☐ Patient does not have severe TBI
- ☐ Clinically significant bleeding
- ☐ SAE
- ☐ Physician decision
- ☐ Miscommunication
- ☐ Other (specify) _____

Rewarming

4.6 Date and time of commencement of first rewarming: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐
dd mmm yyyy HH mm

4.7 Date and time normothermia was reached: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐
(Target temperature 37±0.5 °C)
dd mmm yyyy HH mm

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POLAR Study
FORM 5 Control Group

POLAR Study Number ☐☐☐☐☐
Patient Initials ☐☐☐

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Complete if patient is randomised to normothermia, standard care

Control Group Data

5.1 Identify if the **control** group patient:
- ☐ Does not have a severe TBI
- ☐ Required urgent surgery for bleeding (within the first 72 hrs post randomisation)

5.2.1 Did the patient receive surface cooling at any time?
- ☐ Yes - ***if YES,*** 5.2.2 Surface cooling for:
  - ☐ Pyrexia
  - ☐ ICP control - ***if YES,*** 5.2.3 Target temperature for ICP control: ☐☐.☐ °C
  - ☐ Other
- ☐ No

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POLAR Study
FORM 6 Neurosurgical Interventions

POLAR Study Number ☐☐☐☐☐

Patient Initials ☐☐☐

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Mass Lesion

6.1.1 Was a mass lesion evacuated: ☐ Yes - *if YES,* ☐ No

6.1.2 Type of lesion evacuated: ☐ SDH ☐ EDH ☐ ICH / Contusion

6.1.3 Date and time: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy HH mm

6.1.4 Type of lesion evacuated: ☐ SDH ☐ EDH ☐ ICH / Contusion

6.1.5 Date and time: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy HH mm

(If there is more than 2 mass lesions evacuated please notify Project Manager)

Unilateral Decompressive Craniectomy

6.2.1 Was a craniectomy performed: ☐ Yes - *if YES,* ☐ No

6.2.2 Date and time: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy HH mm

6.2.3 Date and time: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy HH mm

6.2.4 Date and time: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy HH mm

Bifrontal Decompressive Craniectomy

6.3.1 Was a bifrontal decompressive craniectomy performed: ☐ Yes - *if YES,* ☐ No

6.3.2 Date and time: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy HH mm

ICP Monitoring

6.4 ICP monitor inserted: ☐ Yes - ***if YES, complete Form 7***
☐ No - ***if NO, go to Form 8***

6.5 Type of ICP monitor: ☐ Intraparenchymal
☐ EVD

Brain Tissue Oxygen Monitoring

6.6 Was a Brain Tissue Oxygen monitor inserted: ☐ Yes
☐ No

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POLAR Study
FORM 7 ICP Measurement & Management

POLAR Study Number ☐☐☐☐☐

Patient Initials ☐☐☐

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ICP Measurement

7.1 Date and time monitor was inserted: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy HH mm

7.2 Date and time monitor was removed: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy HH mm

Intracranial Hypertension Index

7.3 Number of end hours ICP > 20 mmHg: ☐☐☐ hrs

7.4 Total number of end hours ICP documented: ☐☐☐ hrs

Cerebral Hypoperfusion Pressure Index

7.5 Number of end hours CPP ≤ 60 mmHg: ☐☐☐ hrs

7.6 Total number of end hours CPP documented: ☐☐☐ hrs

ICP Management

7.7 Total volume Hypertonic saline administered: ☐☐☐☐ mls

7.8 Concentration of Hypertonic saline: ☐☐ . ☐ %

7.9 Total volume of Mannitol administered: ☐☐☐☐ mls

7.10 Concentration of Mannitol: ☐☐ . ☐ %

7.11 Total dose of Thiopentone administered: ☐☐☐ . ☐ gms

7.12 Total dose of Phenobarbitone administered: ☐☐☐ . ☐ gms

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POLAR Study
FORM 8 Temperature Monitoring

POLAR Study Number

Patient Initials

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Hourly Temperature

Monitored for the first 72 hours post randomisation

8.1 - 8.72

Hour	Temperature °C	Hour	Temperature °C	Hour	Temperature °C
1	.	25	.	49	.
2	.	26	.	50	.
3	.	27	.	51	.
4	.	28	.	52	.
5	.	29	.	53	.
6	.	30	.	54	.
7	.	31	.	55	.
8	.	32	.	56	.
9	.	33	.	57	.
10	.	34	.	58	.
11	.	35	.	59	.
12	.	36	.	60	.
13	.	37	.	61	.
14	.	38	.	62	.
15	.	39	.	63	.
16	.	40	.	64	.
17	.	41	.	65	.
18	.	42	.	66	.
19	.	43	.	67	.
20	.	44	.	68	.
21	.	45	.	69	.
22	.	46	.	70	.
23	.	47	.	71	.
24	.	48	.	72	.

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POLAR Study
FORM 9 Hours 1 - 12 Post Randomisation

POLAR Study Number

Patient Initials

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Hourly Data Collection

	9.1 Heart rate bpm	9.2 MAP mmHg
Hour 1		
Hour 2		
Hour 3		
Hour 4		
Hour 5		
Hour 6		
Hour 7		
Hour 8		
Hour 9		
Hour 10		
Hour 11		
Hour 12		

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POLAR Study

FORM 10 Daily Data

POLAR Study Number

Patient Initials

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Daily Data Collection

	10.1 Date (dd / mm / yyyy)	10.2 Mean temperature (°C)	10.3.1 ICP > 20 mmHg	10.3.2 Number of end hour episodes	10.4.1 CPP ≤ 60 mmHg	10.4.2 Number of end hour episodes	10.5.1 EVD in situ	10.5.2 CSF venting	10.6.1 Brain tissue $PBrO_2$ < 20 mmHg	10.6.2 Number of end hour episodes	10.7.1 Noradrenaline administered	10.7.2 Noradrenaline > 15 mcg/min
Day 1	/ /		Yes → / No / N/A		Yes → / No / N/A		Yes → / No	Yes / No	Yes → / No / N/A		Yes → / No	Yes / No
Day 2	/ /		Yes → / No / N/A		Yes → / No / N/A		Yes → / No	Yes / No	Yes → / No / N/A		Yes → / No	Yes / No
Day 3	/ /		Yes → / No / N/A		Yes → / No / N/A		Yes → / No	Yes / No	Yes → / No / N/A		Yes → / No	Yes / No
Day 4	/ /	.	Yes → / No / N/A		Yes → / No / N/A		Yes → / No	Yes / No	Yes → / No / N/A		Yes → / No	Yes / No
Day 5	/ /	.	Yes → / No / N/A		Yes → / No / N/A		Yes → / No	Yes / No	Yes → / No / N/A		Yes → / No	Yes / No
Day 6	/ /	.	Yes → / No / N/A		Yes → / No / N/A		Yes → / No	Yes / No	Yes → / No / N/A		Yes → / No	Yes / No
Day 7	/ /	.	Yes → / No / N/A		Yes → / No / N/A		Yes → / No	Yes / No	Yes → / No / N/A		Yes → / No	Yes / No
Day 8	/ /	.	Yes → / No / N/A		Yes → / No / N/A		Yes → / No	Yes / No	Yes → / No / N/A		Yes → / No	Yes / No
Day 9	/ /	.	Yes → / No / N/A		Yes → / No / N/A		Yes → / No	Yes / No	Yes → / No / N/A		Yes → / No	Yes / No
Day 10	/ /	.	Yes → / No / N/A		Yes → / No / N/A		Yes → / No	Yes / No	Yes → / No / N/A		Yes → / No	Yes / No

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POLAR Study
FORM 10 Daily Data

POLAR Study Number ☐☐☐☐☐
Patient Initials ☐☐☐

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Daily Data Collection *continued*

	10.8.1 Adrenaline administered	10.8.2 Adrenaline > 5 mcg/min	10.9 Other vasopressor administered	10.10 Renal replacement or dialysis	10.11 Volume of crystalloids administered (mls)	10.12 Volume of 4% albumin administered (mls)	10.13 Volume of colloids administered (mls)	10.14 Volume of packed red blood cells administered (mls)	10.15 Volume of FFP administered (mls)	10.16 Total fluid volume administered (mls)
Day 1	☐ Yes → ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐☐
Day 2	☐ Yes → ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐☐
Day 3	☐ Yes → ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐☐
Day 4	☐ Yes → ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐☐
Day 5	☐ Yes → ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐☐
Day 6	☐ Yes → ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐☐
Day 7	☐ Yes → ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐☐
Day 8	☐ Yes → ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐☐
Day 9	☐ Yes → ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐☐
Day 10	☐ Yes → ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐☐

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POLAR Study
FORM 10 Daily Data

POLAR Study Number

Patient Initials

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Daily Data Collection *continued*

	10.17 Delivery of feeds	10.18 Naso gastric input (mls)	10.19 Naso gastric output (mls)	10.20 Total TPN (mls)	10.21 Average UO < 0.5 ml/kg/hr over 6 hrs	10.22 Average UO < 0.5 ml/kg/hr over 12 hrs	10.23 Average UO < 0.3 ml/kg/hr over 24 hrs	10.24 Anuria for ≥ 12 hrs	10.25 Total fluid output (mls)	10.26 $PaCO_2$ ≤ 33 mmHg	10.27 Most abnormal WCC ($x10^9$/L)	10.28 Highest INR
Day 1	☐ Enteric ☐ Jejunal ☐ Parenteral	☐ N/A	☐ N/A	☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A		☐ Yes ☐ No	.	.
Day 2	☐ Enteric ☐ Jejunal ☐ Parenteral	☐ N/A	☐ N/A	☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A		☐ Yes ☐ No	.	.
Day 3	☐ Enteric ☐ Jejunal ☐ Parenteral	☐ N/A	☐ N/A	☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A		☐ Yes ☐ No	.	.
Day 4	☐ Enteric ☐ Jejunal ☐ Parenteral	☐ N/A	☐ N/A	☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A		☐ Yes ☐ No	.	.
Day 5	☐ Enteric ☐ Jejunal ☐ Parenteral	☐ N/A	☐ N/A	☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A		☐ Yes ☐ No	.	.
Day 6	☐ Enteric ☐ Jejunal ☐ Parenteral	☐ N/A	☐ N/A	☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A		☐ Yes ☐ No	.	.
Day 7	☐ Enteric ☐ Jejunal ☐ Parenteral	☐ N/A	☐ N/A	☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A		☐ Yes ☐ No	.	.
Day 8	☐ Enteric ☐ Jejunal ☐ Parenteral	☐ N/A	☐ N/A	☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A		☐ Yes ☐ No	.	.
Day 9	☐ Enteric ☐ Jejunal ☐ Parenteral	☐ N/A	☐ N/A	☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A		☐ Yes ☐ No	.	.
Day 10	☐ Enteric ☐ Jejunal ☐ Parenteral	☐ N/A	☐ N/A	☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A		☐ Yes ☐ No	.	.

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POLAR Study
FORM 10 Daily Data

POLAR Study Number ☐☐☐☐☐
Patient Initials ☐☐☐

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Daily Data Collection						Hypothermia Group Patients ONLY *continued*					
	10.29 Highest APTT (secs)	10.30 Lowest platelets ($x10^9$/L)	10.31 Serum creatinine (µmol/L)	10.32 Lowest K+ for day (mmol/L)	10.33 Highest K+ for day (mmol/L)	10.34.1 Did the patient receive surface cooling?	10.34.2 Was rewarming commenced?	10.34.3 Was rewarming paused?	10.34.4 Reason rewarming was paused: (choose all that apply)		10.34.5 Was patient recooled?
Day 1	☐☐☐	☐☐☐	☐☐☐	☐☐.☐	☐☐.☐	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Raised ICP ☐ Hypotension	☐ Arrhythmia ☐ Other (specify): _____	☐ Yes ☐ No
Day 2	☐☐☐	☐☐☐	☐☐☐	☐☐.☐	☐☐.☐	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Raised ICP ☐ Hypotension	☐ Arrhythmia ☐ Other (specify): _____	☐ Yes ☐ No
Day 3	☐☐☐	☐☐☐	☐☐☐	☐☐.☐	☐☐.☐	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Raised ICP ☐ Hypotension	☐ Arrhythmia ☐ Other (specify): _____	☐ Yes ☐ No
Day 4	☐☐☐	☐☐☐	☐☐☐	☐☐.☐	☐☐.☐	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Raised ICP ☐ Hypotension	☐ Arrhythmia ☐ Other (specify): _____	☐ Yes ☐ No
Day 5	☐☐☐	☐☐☐	☐☐☐	☐☐.☐	☐☐.☐	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Raised ICP ☐ Hypotension	☐ Arrhythmia ☐ Other (specify): _____	☐ Yes ☐ No
Day 6	☐☐☐	☐☐☐	☐☐☐	☐☐.☐	☐☐.☐	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Raised ICP ☐ Hypotension	☐ Arrhythmia ☐ Other (specify): _____	☐ Yes ☐ No
Day 7	☐☐☐	☐☐☐	☐☐☐	☐☐.☐	☐☐.☐	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Raised ICP ☐ Hypotension	☐ Arrhythmia ☐ Other (specify): _____	☐ Yes ☐ No
Day 8	☐☐☐	☐☐☐	☐☐☐	☐☐.☐	☐☐.☐	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Raised ICP ☐ Hypotension	☐ Arrhythmia ☐ Other (specify): _____	☐ Yes ☐ No
Day 9	☐☐☐	☐☐☐	☐☐☐	☐☐.☐	☐☐.☐	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Raised ICP ☐ Hypotension	☐ Arrhythmia ☐ Other (specify): _____	☐ Yes ☐ No
Day 10	☐☐☐	☐☐☐	☐☐☐	☐☐.☐	☐☐.☐	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Yes → ☐ No	☐ Raised ICP ☐ Hypotension	☐ Arrhythmia ☐ Other (specify): _____	☐ Yes ☐ No

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POLAR Study
FORM 11 Hospital Stay

POLAR Study Number ☐☐☐☐☐

Patient Initials ☐☐☐

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ICU Admission

11.1 Date and time of ICU admission: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

11.2 Date and time of ICU discharge: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

ICU Readmission

11.3 Was the patient readmitted to ICU? ☐ Yes - ***if YES, continue below***

☐ No - ***if NO, skip to Qu 11.8***

Please record the date and time of readmission and date and time of discharge for each ICU readmission that occurred.

11.4 Date and time of ICU readmission: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

11.5 Date and time of ICU discharge: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

11.6 Date and time of ICU readmission: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

11.7 Date and time of ICU discharge: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

ICU Outcome

11.8 ICU outcome at final ICU admission: ☐ Alive ☐ Deceased

HDU Admission

11.9 Date and time of HDU admission: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

11.10 Date and time of HDU discharge: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

HDU Readmission

11.11 Was the patient readmitted to HDU? ☐ Yes - ***if YES, continue below***

☐ No - ***if NO, skip to Qu 11.16***

Please record the date and time of readmission and date and time of discharge for each HDU readmission that occurred.

11.12 Date and time of HDU readmission: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

11.13 Date and time of HDU discharge: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

11.14 Date and time of HDU readmission: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

11.15 Date and time of HDU discharge: ☐☐/☐☐☐/☐☐☐☐ ☐☐:☐☐ (dd mmm yyyy HH mm)

HDU Outcome

11.16 HDU outcome at final HDU admission: ☐ Alive ☐ Deceased

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POLAR Study

FORM 11 Hospital Stay

POLAR Study Number ☐☐☐☐☐

Patient Initials ☐☐☐

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Hospital Stay Details

11.17 Total number of ventilation hours: ☐☐☐ hours

11.18 Total number of days of RRT: ☐☐☐ days

11.19 Total number of days of ECMO treatment: ☐☐☐ days

11.20 Date and time of hospital discharge: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy HH mm

11.21 Hospital outcome: ☐ Alive ☐ Deceased

Compensable Patients

11.22.1 Is this patient a compensable patient? (e.g. TAC or ACC etc) ☐ Yes ☐ No

- if YES

11.22.2 VICTORIAN PATIENTS: TAC Claim Number: ☐☐ / ☐☐☐☐☐

11.22.3 NEW ZEALAND PATIENTS: ACC Claim Number: ☐☐☐☐☐☐☐☐☐

Deceased Patients

11.23 Date of death: ☐☐ / ☐☐☐ / ☐☐☐☐
dd mmm yyyy

11.24 Process of death:
- ☐ Brain death
- ☐ Death predominantly due to cerebral reasons
- ☐ Death predominantly due to non-cerebral reasons
- ☐ Unknown

Injury Data

11.25 Maximum body region AIS
(AIS version 2005 - 2008 update)

11.25.1 Head/Neck: ☐

11.25.2 Face: ☐

11.25.3 Chest: ☐

11.25.4 Abdominal or pelvic contents: ☐

11.25.5 Extremities or pelvic girdle: ☐

11.25.6 External: ☐

11.26 ISS: ☐☐
(AIS version 2005 - 2008 update)

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POLAR Study
FORM 12 Adverse Events

POLAR Study Number ☐☐☐☐☐
Patient Initials ☐☐☐

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Serious Adverse Events

12.1 Did an SAE occur? ☐ Yes ☐ No
- if YES, Complete Form 13 Serious Adverse Event (See SAE flow chart for instructions)

Arrhythmias

12.2.1 Bradycardia: (< 40 bpm) ☐ Yes *- if YES* 12.2.2 Number of events: ☐☐
☐ No

12.3.1 Ventricular tachycardia: ☐ Yes *- if YES* 12.3.2 Number of events: ☐☐
☐ No

12.4.1 Ventricular fibrillation: ☐ Yes *- if YES* 12.4.2 Number of events: ☐☐
☐ No

12.5.1 Asystolic or PEA cardiac arrest: ☐ Yes *- if YES* 12.5.2 Number of events: ☐☐
☐ No

Hypotension

12.6.1 MAP <60 mmHg: ☐ Yes *- if YES* Number of events: 12.6.2 Induction of cooling: ☐☐ 12.6.4 Rewarming: ☐☐
☐ No
12.6.3 Maintenance of cooling: ☐☐ 12.6.5 Normothermia: ☐☐

Infections

12.7.1 Pneumonia: ☐ Yes *- if YES* 12.7.2 Number of events: ☐☐
☐ No

12.8.1 Proven bacteraemia: ☐ Yes *- if YES* 12.8.2 Number of events: ☐☐
☐ No

12.9.1 Cerebral abscess: ☐ Yes *- if YES* 12.9.2 Number of events: ☐☐
☐ No

12.10.1 CNS infection: ☐ Yes *- if YES* 12.10.2 Number of events: ☐☐
☐ No

12.11.1 Neurosurgical wound infection: ☐ Yes *- if YES* 12.11.2 Number of events: ☐☐
☐ No

12.12.1 Other infection (specify): ☐ Yes *- if YES* 12.12.2 Number of events: ☐☐
_____ ☐ No

Bleeding

12.13.1 New intracerebral: ☐ Yes *- if YES* 12.13.2 Number of events: ☐☐
☐ No

12.14.1 New significant bleeding: ☐ Yes *- if YES* 12.14.2 Number of events: ☐☐
☐ No

Intractable ICP

12.15.1 Intractable ICP: ☐ Yes *- if YES* 12.15.2 Number of events: ☐☐
☐ No

Increase in cerebral bleed

12.16 Increase in cerebral bleed ☐ Yes ☐ No

If 'increase in cerebral bleed' is 'Yes' please sent CT Report.
You can add small comments below.

Comments

Other Adverse Events

12.17 List: 1) _____ 2) _____

3) _____ 4) _____

5) _____ 6) _____

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POLAR Study
FORM 13
Serious Adverse Event

POLAR Study Number ☐☐☐☐☐

Patient Initials ☐☐☐

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Instructions: 1. Follow the SAE flow chart to decide if the AE requires reporting
2. All deaths should be reported as an SAE
3. Contact the Project Manager if you wish to discuss reporting

Serious Adverse Event Details

13.1 Type of report: ☐ Initial report ☐ Follow up report ☐ Final report
(One report may be both an Initial and Final report OR a Follow up and Final Report)

13.2 SAE diagnosis: ☐

13.3 SAE type:
- ☐ Death
- ☐ Life threatening
- ☐ Congenital anomaly
- ☐ Prolongation of hospitalisation or re-hospitalisation
- ☐ Permanently disabling
- ☐ Medically important

13.4 SAE description: (Including any treatments)

☐

13.5 Suspected relationship of SAE to trial: ☐ Not related ☐ Unlikely ☐ Possible ☐ Probable ☐ Definitely

13.6 SAE onset date: ☐☐/☐☐☐/☐☐☐☐ (dd / mmm / yyyy)

13.7 SAE resolution date: ☐☐/☐☐☐/☐☐☐☐ (dd / mmm / yyyy)

13.8.1 Outcome:
- ☐ Unknown
- ☐ Unresolved
- ☐ Resolved
- ☐ Death - *if YES* 13.8.2 Death date: ☐☐/☐☐☐/☐☐☐☐ (dd / mmm / yyyy)

13.8.3 Cause of death: ☐

13.9 As a result of the SAE:
- ☐ No action was taken
- ☐ Study intervention temporarily withheld
- ☐ Study intervention permanently discontinued

FAX all unexpected SAE's to Tony Trapani +61 3 9521 5304

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POLAR Study

FORM 14 Consent

POLAR Study Number ☐☐☐☐☐

Patient Initials ☐☐☐

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Consent Details

14.1.1 Delayed consent/assent obtained from patient's next of kin or legal surrogate: ☐ Yes ☐ No - *if YES* 14.1.2 Date consent obtained: ☐☐ / ☐☐☐ / ☐☐☐☐ (dd / mmm / yyyy)

14.2.1 Delayed consent obtained from the patient: ☐ Yes ☐ No - *if YES* 14.2.2 Date consent obtained: ☐☐ / ☐☐☐ / ☐☐☐☐ (dd / mmm / yyyy)

14.3 Patient died prior to obtaining consent but data collection consent obtained: (EC approval)
☐ Yes
☐ No
☐ N/A

If all of the above consents are answered NO, continue below.

14.4 Reason consent not obtained:

☐ Informed consent not obtained due to next of kin/surrogate refusal but data can be used

☐ Informed consent not obtained due to next of kin/surrogate refusal, data cannot be used

☐ Informed consent not obtained due to patient refusal but data can be used

☐ Informed consent not obtained due to patient refusal, data cannot be used

☐ Patient discharged prior to consent being obtained and lost to follow-up

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POLAR Study
FORM 15 6 Month Follow Up by Site

POLAR Study Number ☐☐☐☐☐

Patient Initials ☐☐☐

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6 Month Follow Up

15.1.1 Is the patient alive at 6 months? ☐ Yes - *if YES* 15.1.2 Patient's current location:
- ☐ Home without additional care
- ☐ Home with additional care
- ☐ Other acute hospital
- ☐ Rehabilitation
- ☐ Long term care facility (Hospice/Hostel)
- ☐ Nursing home
- ☐ Other (specify): _____

☐ No - *if NO, Complete Form 13 Serious Adverse Event*

15.2 Date of follow up: ☐☐ / ☐☐☐ / ☐☐☐☐ (dd / mmm / yyyy)

15.3.1 Patient and proxy contact details sent to central assessor for GOSE & QoL follow up: ☐ Yes

☐ No - *if NO* 15.3.2 Reason:
- ☐ Patient withdrew consent
- ☐ Person responsible / legal surrogate withdrew consent
- ☐ Patient is lost to follow up

Level of Care Details After Hospital Discharge

		Date of admission (dd / mmm / yyyy)	Date of discharge / current date (if not discharged) (dd / mmm / yyyy)
15.4.1 Rehabilitation centre:	☐ Yes - *if YES* ☐ No ☐ Unknown	15.4.2	15.4.3
15.5.1 Transitional living centre:	☐ Yes - *if YES* ☐ No ☐ Unknown	15.5.2	15.5.3
15.6.1 Readmission to acute hospital:	☐ Yes - *if YES* ☐ No ☐ Unknown	15.6.2	15.6.3
15.7.1 Long term low level care facility: (e.g. supported accommodation centre)	☐ Yes - *if YES* ☐ No ☐ Unknown	15.7.2	15.7.3
15.8.1 Long term high level care facility: (e.g. nursing home)	☐ Yes - *if YES* ☐ No ☐ Unknown	15.8.2	15.8.3
15.9.1 Other (specify): _____	☐ Yes - *if YES* ☐ No ☐ Unknown	15.9.2	15.9.3

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POLAR Study
FORM 17 Protocol Variation

POLAR Study Number ☐☐☐☐☐

Patient Initials ☐☐☐

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Incorrect randomisation pre-hospital (tick the incorrect criteria & provide a brief explanation if possible)

17.1 Date of first protocol variation: ☐☐ / ☐☐☐ / ☐☐☐☐
dd mmm yyyy

Inclusion Criteria

		Pre Hospital	Emergency
17.2.1	Blunt trauma with clinical diagnosis of severe TBI and GCS < 9:	☐	☐
17.2.2	Estimated age ≥ 18 and < 60 years of age:	☐	☐
17.2.3	The patient is intubated or intubation is imminent:	☐	☐

Exclusion Criteria

		Pre Hospital	Emergency
17.3.1	Clinical diagnosis of drug or alcohol intoxication as predominant cause of coma:	☐	☐
17.3.2	Randomisation unable to be performed within 3 hrs of estimated time of injury:	☐	☐
17.3.3	Estimated transport time to study hospital > 2.5 hrs:	☐	☐
17.3.4	Able to be intubated without drugs:	☐	☐
17.3.5	Systolic BP < 90 mmHg:	☐	☐
17.3.6	Heart rate > 120 bpm:	☐	☐
17.3.7	Cardiac arrest at the scene or in transit:	☐	☐
17.3.8	GCS = 3 + un-reactive pupils:	☐	☐
17.3.9	Penetrating neck/torso injury:	☐	☐
17.3.10	Known or obvious pregnancy:	☐	☐
17.3.11	Positive urine or blood pregnancy test:	☐	☐
17.3.12	Receiving hospital is not a study site:	☐	☐
17.3.13	Evidence of current anti-coagulant treatment:	☐	☐
17.3.14	Known to be carer dependent due to pre-existing neurological condition:	☐	☐
17.3.15	Clinically significant bleeding likely to require haemostatic intervention:	☐	☐
17.3.16	In the treating clinician's opinion, "cooling" is not in the patient's best interest:	☐	☐

Additional Details, if required

17.4.1 Hypothermia patient (not cooled to 33 ± 0.5 °C): ☐ Yes ☐ No

17.4.2 Brief description:

17.5.1 Hypothermia patient (temperature > 36.5 °C in first 72 hours): ☐ Yes ☐ No

17.5.2 Brief description:

17.6.1 Hypothermia patient (cooling withdrawn prematurely): ☐ Yes ☐ No

17.6.2 Brief description:

17.7.1 Hypothermia patient (rewarmed too quickly (< 15 hrs)): ☐ Yes ☐ No

17.7.2 Brief description:

17.8.1 Control patient (cooled to < 35 °C): ☐ Yes ☐ No

17.8.2 Brief description:

17.9.1 (Both patient groups) ICP/EVD monitor not used or removed prior to rewarming: ☐ Yes ☐ No

17.9.2 Brief description:

Please ensure Note to File has been completed if required.
AE or SAE form may also be required

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POLAR Study
FORM 18 CT Brain Scan

POLAR Study Number ☐☐☐☐☐
Patient Initials ☐☐☐

polar

CT Brain Scan

18.1 Date and time of scan: ☐☐ / ☐☐☐ / ☐☐☐☐ ☐☐ : ☐☐
dd mmm yyyy HH mm

Focal Lesion & Haemorrhage

18.2.1 Subdural: ☐ Yes - *if YES* 18.2.2 Estimated volume: ☐☐☐ mls
☐ No

18.3.1 Extradural: ☐ Yes - *if YES* 18.3.2 Estimated volume: ☐☐☐ mls
☐ No

18.4.1 Contusion: ☐ Yes - *if YES* 18.4.2 Estimated volume: ☐☐☐ mls
☐ No

18.5.1 Intracerebral haemorrhage: ☐ Yes - *if YES* 18.5.2 Estimated volume: ☐☐☐ mls
☐ No

18.6 Subarachnoid haemorrhage: ☐ Yes
☐ No

18.7 Intraventricular haemorrhage: ☐ Yes
☐ No

18.8 Petechial haemorrhages: ☐ Yes
☐ No

Mass Effect / Pressure

18.9.1 Midline shift: ☐ Yes - *if YES* 18.9.2 Shift: ☐☐ mm
☐ No

18.10 Basal cisterns: ☐ Normal ☐ Compressed ☐ Absent

18.11

CATEGORY	DESCRIPTION
☐ DIFFUSE INJURY I	No visible pathology on CT
☐ DIFFUSE INJURY II	Cisterns present and/or lesions present Shift 0 - 5 mm No high or mixed density lesions > 25 ml
☐ DIFFUSE INJURY III	Cisterns compressed or absent Shift 0 - 5 mm No high or mixed density lesions > 25 ml
☐ DIFFUSE INJURY IV	Shift > 5 mm No high or mixed density lesions > 25 ml
☐ EVACUATED MASS LESION	Any surgically evacuated lesion
☐ NON-EVACUATED MASS LESION	High or mixed density lesion > 25 ml Not surgically evacuated

18.12 Scored from: ☐ CT scan ☐ Report

Appendix 7

Journal article from which normalised GFR values are taken (see tables 1a and 1b):

Wetzels JF, Kiemeney LA, Swinkels DW, et al. Age- and gender-specific reference values of estimated GFR in Caucasians: the Nijmegen Biomedical Study. Kidney Int 2007; 72:632-637

http://www.kidney-international.org

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see commentary on page 534

Age- and gender-specific reference values of estimated GFR in Caucasians: The Nijmegen Biomedical Study

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The Nijmegen Biomedical Study is a population-based cross-sectional study conducted in the eastern part of the Netherlands. As part of the overall study, we provide reference values of estimated glomerular filtration rate (GFR) for this Caucasian population without expressed risk. Age-stratified, randomly selected inhabitants received a postal questionnaire on lifestyle and medical history. In a large subset of the responders, serum creatinine was measured. The GFR was then measured using the abbreviated Modification of Diet in Renal Disease (MDRD) formula. To limit possible bias, serum creatinine was calibrated against measurements performed in the original MDRD laboratory. The study cohort included 2823 male and 3274 female Caucasian persons aged 18–90 years. A reference population of apparently healthy subjects was selected by excluding persons with known hypertension, diabetes, cardiovascular- or renal diseases. This healthy study cohort included 1660 male subjects and 2072 female subjects, of which 869 of both genders were 65 years or older. The median GFR was 85 ml/min/1.73 m^2 in 30–to 34-year-old men and 83 ml/min/1.73 m^2 in similar aged women. In these healthy persons, GFR declined approximately 0.4 ml/min/year. Our study provides age- and gender-specific reference values of GFR in a population of Caucasian persons without identifiable risk.

Kidney International (2007) **72,** 632–637; doi:10.1038/sj.ki.5002374; published online 13 June 2007

KEYWORDS: population-based study; GFR; kidney function; MDRD

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In recent years, chronic kidney disease (CKD) has received increasing attention.[1] Patients with CKD are at increased risk for cardiovascular morbidity and mortality.[2] Furthermore, patients with CKD may progress to end-stage renal disease.[3] As early treatment of patients with CKD may reduce cardiovascular risk and delay the onset of end-stage renal disease, it is important to identify patients with CKD in an early stage.[4] To increase the awareness of CKD and improve treatment of patients with CKD, the Kidney Disease Outcomes Quality Initiative (K/DOQI) from the US National Kidney Foundation has developed guidelines for the diagnosis and classification of CKD.[5] K/DOQI has proposed to classify CKD in five stages according to the absence or presence of albuminuria and the level of the glomerular filtration rate (GFR). The guidelines propose that patients with GFR <60 ml/min/1.73 m^2 are at risk and need to be evaluated and properly treated.

Classification of patients with CKD thus necessitates accurate assessment of GFR. For many years, serum creatinine has been used in routine clinical practice as a marker of GFR. It is well recognized that serum creatinine is not an accurate marker of GFR.[6] Serum creatinine is dependent on muscle mass and the relation with GFR is influenced by age, gender, and body weight. Therefore, formulas have been developed for the estimation of GFR. The K/DOQI guidelines advocate to use the recently developed 'Modification of Diet in Renal Disease (MDRD)'-formula for the calculation of GFR. This formula was derived from the MDRD study, which included patients with renal disease, in whom GFR was measured with an accurate, invasive technique using subcutaneous administration of Iothalamate.[7] This formula provides a good estimate of GFR, in particular, in the GFR range <60 ml/min/1.73 m^2.[8,9] It is important to realize that for correct reporting of MDRD-GFR attention must be given to calibration of the creatinine assay against the creatinine assay that was originally used in the original MDRD laboratory.[10] The importance hereof was recently stipulated by a working group.[11]

Many laboratories have adopted this formula and have started automated reporting of calculated MDRD-GFR.[12] It was recently shown that automated reporting of GFR indeed leads to earlier identification of patients with CKD.[13] However, reference values of estimated GFR are virtually lacking. Lack of reference values for estimated GFR hampers the interpretation of reported MDRD-GFR by medical laboratories. Therefore, we have estimated GFR using the MDRD formula for participants of the Nijmegen Biomedical Study. We have calibrated the creatinine assay against the MDRD laboratory. Our data provide reference values of estimated GFR for a Caucasian population.

RESULTS

Age- and gender-specific reference values of GFR

Values of estimated GFR per 5-year age groups are given for men and women in Tables 1a and 1b and 2a and 2b. In Table 1a and 1b, data are given for the disease-free population. Reference values for this 'healthy' population are depicted in Figures 1 and 2. The data in Table 2a and 2b are based on the results obtained in the population with reported comorbidities.

Stages of CKD

We have used the definitions of the K/DOQI. These definitions require information on proteinuria or hematuria to classify persons as CDK stage 1 or 2. Thus, our data only allow correct classification of persons in stage 3 and above. Figure 3 depicts the prevalence of CKD stage 3–5 (GFR $<60\,ml/min/1.73\,m^2$) in our population of disease-free subjects. As expected, the prevalence increases with age, reaching 42% in men and 44% in women of age 85 and higher.

DISCUSSION

Our data provide reference values for MDRD-GFR using data of an apparently healthy Caucasian population. We have used the abbreviated MDRD equation that is nowadays most regularly used. The MDRD formula provides the best estimate of GFR, particularly in the range of GFR $<60\,ml/min/1.73\,m^2$.[8,9] Awareness of CKD has increased in the past decade. Especially, the publication of guidelines by the K/DOQI from the National Kidney Foundation has fostered interest in CKD worldwide.[5]

Table 1a | Estimated GFR in non-diseased caucasian males of the Nijmegen Biomedical Study

Age (years)	*N*	Mean ± s.d.	Range	P5	P25	P50	P75	P95
18–24	94	100 ± 13	72–137	77	90	99	109	121
25–29	96	93 ± 13	67–125	74	82	90	102	117
30–34	118	86 ± 13	63–133	68	77	85	93	107
35–39	125	85 ± 14	61–118	65	74	85	95	110
40–44	143	84 ± 13	54–124	66	76	83	92	106
45–49	160	83 ± 13	50–123	63	73	82	91	105
50–54	143	79 ± 12	46–120	60	71	78	87	97
55–59	158	76 ± 13	27–118	58	68	75	84	98
60–64	149	75 ± 15	48–199	59	67	73	83	95
65–69	154	75 ± 14	51–165	56	66	74	82	97
70–74	102	71 ± 12	38–102	54	64	70	79	92
75–79	112	70 ± 13	41–110	45	62	70	79	91
80–84	73	67 ± 15	41–129	43	58	69	77	87
> 85	33	62 ± 16	34–101	35	47	65	72	92

GFR, glomerular filtration rate.
Values are given as means (s.d.), ranges and 5th, 25th, 50th, 75th, and 95th percentile.

Table 1b | Estimated GFR in non-diseased females of the Nijmegen Biomedical Study

Age (years)	*N*	Mean ± s.d.	Range	P5	P25	P50	P75	P95
18–24	187	91 ± 15	58–186	72	80	90	99	112
25–29	159	85 ± 13	55–140	63	76	83	93	107
30–34	171	85 ± 15	53–153	63	74	83	93	113
35–39	193	79 ± 13	55–165	63	72	76	85	102
40–44	195	77 ± 12	48–117	58	67	77	84	100
45–49	227	74 ± 10	47–109	56	67	74	81	91
50–54	191	73 ± 13	51–152	56	64	71	79	93
55–59	174	70 ± 12	48–149	53	63	69	76	89
60–64	180	68 ± 12	41–148	50	61	68	75	84
65–69	156	66 ± 10	44–102	52	60	65	71	85
70–74	95	66 ± 11	40–96	49	58	64	73	85
75–79	77	62 ± 11	37–100	45	54	61	69	82
80–84	40	64 ± 14	46–114	46	56	62	73	88
> 85	27	59 ± 14	30–87	36	48	61	69	78

GFR, glomerular filtration rate.
Values are given as means (s.d.), ranges and 5th, 25th, 50th, 75th, and 95th percentile.

Table 2a | Estimated GFR in caucasian males with reported comorbidity of the Nijmegen Biomedical Study

Age (years)	*N*	Mean ± s.d.	Range	P5	P25	P50	P75	P95
18–24	1	—	—	—	—	77	—	—
25–29	8	—	15–128	—	—	86	—	—
30–34	4	—	74–107	—	—	98	—	—
35–39	15	86 ± 12	69–106	69	78	81	98	106
40–44	29	81 ± 17	13–118	63	75	83	88	98
45–49	39	78 ± 14	53–135	54	70	77	85	102
50–54	66	76 ± 14	50–107	52	66	75	86	97
55–59	100	76 ± 13	30–108	58	68	75	86	98
60–64	150	71 ± 14	42–106	50	61	71	80	98
65–69	182	68 ± 15	6–112	44	59	69	78	89
70–74	194	66 ± 15	25–112	40	58	66	76	91
75–79	180	62 ± 15	23–105	36	53	62	73	84
80–84	150	60 ± 16	15–102	31	48	61	72	84
> 85	45	56 ± 16	11–87	29	48	55	64	84

GFR, glomerular filtration rate.
Values are given as means (s.d.), ranges and 5th, 25th, 50th, 75th, and 95th percentile. For age classes with $N < 10$ only medians and range is given.

Table 2b | Estimated GFR in caucasian females with reported comorbidity of the Nijmegen Biomedical Study

Age (years)	N	Mean ± s.d.	Range	P5	P25	P50	P75	P95
18–24	7	—	84–142	—	—	102	—	—
25–29	15	81 ± 9	65–101	65	73	83	85	101
30–34	33	79 ± 16	39–127	60	72	78	86	111
35–39	50	77 ± 12	51–113	58	68	76	83	92
40–44	43	79 ± 17	57–159	64	70	75	86	102
45–49	60	70 ± 15	5–90	52	64	70	78	87
50–54	101	72 ± 11	42–100	56	63	71	80	93
55–59	106	70 ± 12	28–98	51	62	70	77	91
60–64	141	67 ± 12	27–98	48	61	67	75	86
65–69	154	63 ± 14	23–111	42	53	63	72	88
70–74	166	61 ± 12	16–94	43	53	62	70	79
75–79	131	59 ± 14	26–97	36	50	59	69	86
80–84	100	56 ± 15	26–91	29	46	55	65	82
> 85	95	55 ± 15	21–95	29	45	53	64	82

GFR, glomerular filtration rate.
Values are given as means (s.d.), ranges and 5th, 25th, 50th, 75th, and 95th percentile. For age classes with $N < 10$ only median and range are given.

The K/DOQI guidelines have proposed a classification scheme, and defined five stages of CKD according to the presence of proteinuria and the level of GFR. Patients with GFR < 60 ml/min/1.73 m^2 (stages 3 and higher) are considered to be at high risk for cardiovascular morbidity, mortality, and end-stage renal disease and should receive special attention, directed at the identification and treatment of cardiovascular risk factors. Many reports have recently been published describing the prevalence of CKD in various populations.[14–16]

However, these figures must be interpreted with caution. First, most studies have calculated GFR using uncalibrated creatinine assays.[14–16] The importance of proper calibration of the creatinine assays has recently been stressed.[17,18] The impact of the differences in creatinine assays is illustrated in a recent study. Clase *et al.*[16] calculated the prevalence of CKD in a non-diabetic US population using data from National Health and Nutrition Examination Survey III. Using uncalibrated creatinine, they noted a 26.7% prevalence of CKD stages 3–5 in 60- to 69-year-old Caucasian men. This figure dropped to 7.4% when using calibrated creatinine.[19,20] Most studies published in recent years are flawed by the use of improper creatinine values.

Secondly, K/DOQI guidelines do not take age in account when classifying patients. As GFR decreases with age, the number of persons with CKD stages 3–5 (diseased) increases with age, as shown by many investigators and as illustrated in Figure 3. However, our data indicate that the 60 ml/min/1.73 m^2 cannot be used to define a diseased population. Our reference values illustrate that MDRD-GFR decreases with age. A GFR of 60 ml/min/1.73 m^2 is within the normal reference range for men > 60 years and women > 50 years. Our data suggest that recommendations to define kidney disease must be changed. In a recent study, the use of a threshold value of 60 ml/min/1.73 m^2 independent of age was also questioned.[21] Using a database of the VA system in the US containing creatinine data of predominantly male persons, it was shown that a GFR of 50–59 ml/min/1.73 m^2 was associated with an increased mortality risk only in younger persons (age 18–54 years). For older persons, GFR

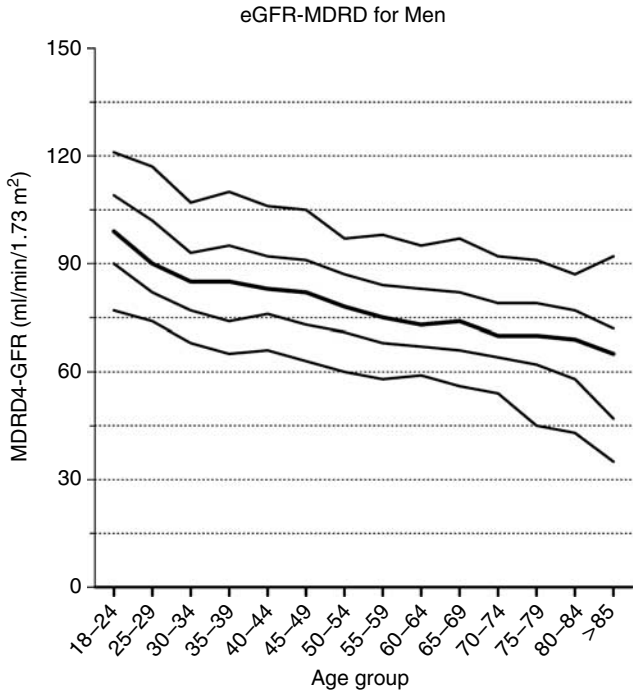

Figure 1 | Reference values of estimated GFR for non-diseased Caucasian males. Median values and 5, 25, 75, and 95 percentiles are shown for persons grouped in 5 years age classes.

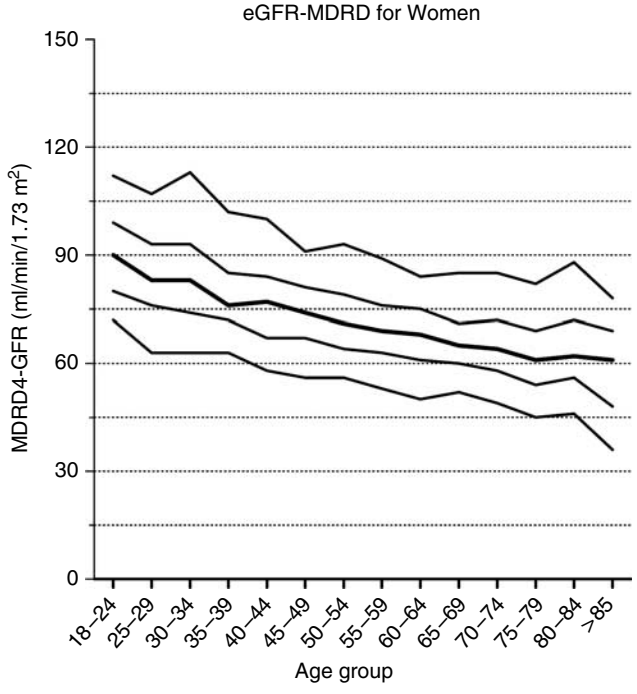

Figure 2 | Reference values of estimated GFR for non-diseased Caucasian females. Median values and 5, 25, 75, and 95 percentiles are shown for persons grouped in 5 years age classes.

values below 50 ml/min/1.73 m^2 (age 55–74) or 40 ml/min/1.73 m^2 proved better thresholds. These figures compare nicely with our reference values, clearly falling below the 5% percentile.

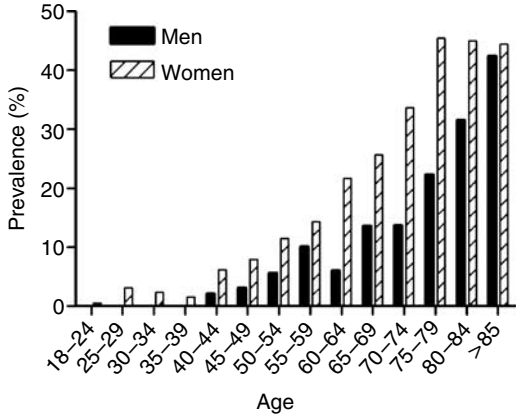

Figure 3 | Prevalence of CKD stages 3–5 (GFR <60 ml/min/1.73 m^2) according to age in the non-diseased Caucasian Nijmegen Biomedical Study population. Black bars represent men and open bars women.

Admittedly, there is debate on the use of formulas for estimating GFR. Newer formulas have been developed, however, their performance compared with the MDRD formula has been questioned. In a recent study Hallan *et al.*[22] showed that the MDRD formula (using calibrated creatinine) gives nearly unbiased estimates of GFR, whereas all other formula had a much larger negative bias especially in the elderly.

Our study has several limitations. We have used a questionnaire to ascertain the health status of the participants. Thus, persons who are unaware of a underlying comorbidity may be incorrectly classified as 'healthy'. Participants were also asked if they had seen their family physician or a hospital specialist within 3 or 12 months before the study, respectively. Fifty-three percent of the study participants denied any contact with such health-care professionals. Limiting the analysis to this subgroup had no major effect: only for the age groups >80 years (with few remaining persons), the fifth percentile was higher at values of 45 ml/min/1.73 m^2. We have measured serum creatinine at one time point only. Correct classification of CKD stage 3–5 requires decreased GFR values for a period of at least three months. Therefore, we may have overestimated the true prevalence of CKD stages 3–5 in our population. As our healthy study participants had not used medication or contacted a physician in the 3 months before the study, the likelihood of major classification errors is small. Of note, our data do not necessarily reflect true GFR. The seemingly lower estimated GFR in women may thus be the consequence of the well-known underestimation of true GFR by the MDRD formula in women.[8,23] Lastly, our data cannot be applied to other, non-Caucasian populations.

In conclusion, we provide age- and sex-specific values of estimated GFR using the MDRD formula with proper calibration of creatinine. These values should allow a better interpretation of reported GFR data of individual patients. As such, the data should help in guiding treatment of the individual patients.

MATERIALS AND METHODS

Sample design

Details of the Nijmegen Biomedical Study have been described before.[24] In brief, the Nijmegen Biomedical Study is a population-based cross-sectional study conducted by the Radboud University Nijmegen Medical Centre. Approval to conduct the study was obtained from the Institutional Review Board. Nijmegen is a town in the eastern part of The Netherlands with 156 000 inhabitants, approximately 87% of Caucasian descent. Age and sex stratified randomly selected adult (age 18 years and older) inhabitants of Nijmegen ($n = 22\,452$) received an invitation to fill out a postal questionnaire on lifestyle and medical history. The following questions were used to collect data on pre-existent renal and vascular disease:

'Have you ever been diagnosed by a physician with any of the underlying diseases: myocardial infarction, stroke or cerebrovascular disease, diabetes, hypertension, or any kidney disease'. In addition, specific information was gathered on the use of any drug therapy in the last 6 months.

The response to the questionnaire was 41.7% ($n = 9371$). In addition, 68.9% of the responders donated 2×8.5 ml blood ($n = 6455$). No physical examination was carried out. Serum creatinine was measured in these samples. We limited the analysis for this paper to the 6097 Caucasian participants with valid data, of whom 2823 were male and 3272 female. Of this population, 2365 reported an underlying condition (hypertension $n = 1032$, diabetes $n = 358$, myocardial infarction $n = 362$, stroke $n = 127$, kidney disease $n = 145$, or the use of diuretic, antihypertensive, or antirheumatic drugs $n = 347$). The remaining 3732 participants (1660 male, 2072 female) were defined as the disease-free population.

Laboratory methods

Serum creatinine was measured by a kinetic alkaline picrate method on an Aeroset auto-analyser of Abbott. In view of the importance of interlaboratory and methodological differences in the creatinine assays on results of estimated GFR creatinine data obtained by the Jaffe method were calibrated against the Roche enzymatic creatinine assay. We also have sent 40 serum samples to the Cleveland Clinic Laboratory, which is the original MDRD laboratory. The Cleveland Clinic Laboratory has measured serum creatinine using a Beckman-modified kinetic alkaline picrate reaction.

Calculations

We observed the following relationship between the Roche enzymatic creatinine assay and the Jaffe alkaline picrate assay, yielding the following equation: y (Nijmegen enzymatic) $= 1.266 \times$ (Nijmegen Jaffe) -29.

Comparison between creatinine measured in our laboratory and in the Cleveland Clinic Laboratory revealed y (creatinine Cleveland) $= 1.021 \times$ (Nijmegen enzymatic) $+ 11$.

For calculation of GFR, we used serum creatinine values calibrated to the original MDRD laboratory values.

GFR was calculated using the abbreviated MDRD formula: $186 \times (\text{serum creatinine (in } \mu\text{mol/l)}/88.4)^{-1.154} \times (\text{age (in years)})^{-0.203} \times 0.742$ (if female).

Patients were classified in stages of GFR according to the classification of CKD as defined by the K/DOQI guidelines. Notably, as we have no information on urinary protein excretion, we only provide data on the prevalence of CKD stages 3–5.

We calculated means with standard deviation and 5th, 25th, 50th, 75th, and 95th percentile using STATA software (Version 9.1).

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REFERENCES

1. Snyder S, Pendergraph B. Detection and evaluation of chronic kidney disease. *Am Fam Physician* 2005; **72**: 1723–1732.
2. Sarnak MJ, Levey AS. Cardiovascular disease and chronic renal disease: a new paradigm. *Am J Kidney Dis* 2000; **35**: S117–S131.
3. Ishani A, Grandits GA, Grimm RH *et al.* Association of single measurements of dipstick proteinuria, estimated glomerular filtration rate, and hematocrit with 25-year incidence of end-stage renal disease in the multiple risk factor intervention trial. *J Am Soc Nephrol* 2006; **17**: 1444–1452.
4. Levey AS, Coresh J, Balk E *et al.* National Kidney Foundation Practice Guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med* 2003; **139**: 137–147.
5. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; **39**: S1–S246.
6. Manjunath G, Sarnak MJ, Levey AS. Prediction equations to estimate glomerular filtration rate: an update. *CONH* 2001; **10**: 785–792.
7. Levey AS, Bosch JP, Breyer Lewis J *et al.* A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med* 1999; **130**: 461–470.
8. Froissart M, Rossert J, Jacquot C *et al.* Predictive performance of the Modification of Diet in Renal Disease and Cockcroft-Gault equations for estimating renal function. *J Am Soc Nephrol* 2005; **16**: 763–773.
9. Poggio ED, Wang X, Greene T *et al.* Performance of the Modification of Diet in renal Disease and Cockcroft-Gault equations in the estimation of GFR in helath and in chronic kidney disease. *J Am Soc Nephrol* 2005; **16**: 459–466.
10. Murthy K, Stevens LA, Stark PC, Levey AS. Variation in the serum creatinine assay calibration: a practical application to glomerular filtration rate estimation. *Kidney Int* 2005; **68**: 1884–1887.
11. Myers GL, Miller WG, Coresh J *et al.* Recommendations for improving serum creatinine measurement: a report from the laboratory working group of the National Kidney Disease Education Program. *Clin Chem* 2006; **52**: 5–18.
12. The Australian Creatinine Consensus Working Group. Chronic Kidney Disease and automatic reporting of estimated GFR: a position statement. *Med J Aust* 2005; **183**: 138–141.
13. Akbari A, Swedko PJ, Clark HD *et al.* Detection of chronic kidney disease with laboratory reporting of estimated glomerular filtration rate and an educational program. *Arch Intern Med* 2004; **164**: 1788–1792.
14. Cirillo M, Laurenzi M, Mancini M *et al.* Low glomerular filtration rate in the population: prevalence, associated disorders, and awareness. *Kidney Int* 2006; **70**: 800–806.
15. Viktorsdottir O, Palsson R, Andresdottir MB *et al.* Prevalence of chronic kidney disease based on estimated glomerular filtration rate and proteinuria in Icelandic adults. *Nephrol Dial Transplant* 2005; **20**: 1799–1807.
16. Clase CM, Garg AX, Kiberd BA. Prevalence of low glomerular filtration rate in nondiabetic Americans: third National Health and Nutrition Examination Survey (NHANES). *J Am Soc Nephrol* 2002; **13**: 1349.
17. Hallan S, Asberg A, Lindberg M, Johnsen H. Validation of the Modification of Diet In Renal Disease formula for estimating GFR with special emphasis on calibration of the serum creatinine assay. *Am J Kidney Dis* 2004; **44**: 84–93.
18. Coresh J, Astor BC, McQuillan G *et al.* Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. *Am J Kidney Dis* 2002; **39**: 920–929.
19. Clase CM, Garg AX, Kiberd BA. Estimating the prevalence of low glomerular filtration rate requires attention to the creatinine assay calibration. *J Am Soc Nephrol* 2002; **13**: 2812–2816.

20. Coresh J, Eknoyan G, Levey AS. Estimating the prevalence of low glomerular filtration rate requires attention to the creatinine assay calibration. *J Am Soc Nephrol* 2002; **13**: 2811–2812.
21. O'Hare AM, Bertenthal D, Covinsky KE *et al.* Mortality risk stratification in chronic kidney disease: one size for all ages? *J Am Soc Nephrol* 2006; **17**: 853.
22. Hallan S, Astor B, Lydersen S. Estimating glomerular filtration rate in the general population: the second Health Survey of Nord-Trondelag (HUNT II). *Nephrol Dial Transplant* 2006; **21**: 1525–1533.
23. Cirillo M, Anastasio P, de Santo NG. Relationship of gender, age, and body mass index to errors in predicted kidney function. *Nephrol Dial Transplant* 2005; **20**: 1791–1798.
24. Hoogendoorn EH, Hermus AR, de Vegt F *et al.* Thyroid function and prevalence of anti-thyroperoxidase antibodies in a population with borderline sufficient iodine intake: influences of age and sex. *Clin Chem* 2006; **52**: 104–111.

References

1 Asemota AO, George BP, Bowman SM, et al. Causes and Trends in Traumatic Brain Injury for United States Adolescents. J Neurotrauma 2012

2 Myburgh JA, Cooper DJ, Finfer SR, et al. Epidemiology and 12-month outcomes from traumatic brain injury in Australia and New Zealand. J Trauma 2008; 64:854-862

3 Maas AI, Stocchetti N, Bullock R. Moderate and severe traumatic brain injury in adults. Lancet Neurology 2008; 7:728-741

4 Thurman D. Epidemiology and economics of head trauma. In: Miler L, Hayes R, eds. Head trauma: basic, preclinical, and clinical
directions. New York: Wiley and Sons, 2001; 1193-1202

5 Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. Lancet 1974; 2:81-84

6 Parikh S, Koch M, Narayan RK. Traumatic brain injury. Int Anesthesiol Clin 2007; 45:119-135

7 Maas AI, Hukkelhoven CW, Marshall LF, et al. Prediction of outcome in traumatic brain injury with computed tomographic characteristics: a comparison between the computed tomographic classification and combinations of computed tomographic predictors. Neurosurgery 2005; 57:1173-1182; discussion 1173-1182

8 Marshall LF, Marshall SB, Klauber MR, et al. The diagnosis of head injury requires a classification based on computed axial tomography. J Neurotrauma 1992; 9 Suppl 1:S287-292

9 Moore EM, Nichol AD, Bernard SA, et al. Therapeutic hypothermia: Benefits, mechanisms and potential clinical applications in neurological, cardiac and kidney injury. Injury 2011; 42:843-854

10 Auer RN. Non-pharmacologic (physiologic) neuroprotection in the treatment of brain ischemia. Ann N Y Acad Sci 2001; 939:271-282

11 Globus MY, Alonso O, Dietrich WD, et al. Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. J Neurochem 1995; 65:1704-1711

12 Leker RR, Shohami E, Leker RR, et al. Cerebral ischemia and trauma-different etiologies yet similar mechanisms: neuroprotective opportunities. Brain Research - Brain Research Reviews 2002; 39:55-73

13 Luo CL, Li BX, Li QQ, et al. Autophagy is involved in traumatic brain injury-induced cell death and contributes to functional outcome deficits in mice. Neuroscience 2011; 184:54-63

14 Morganti-Kossmann MC, Rancan M, Stahel PF, et al. Inflammatory response in acute traumatic brain injury: a double-edged sword. Current Opinion in Critical Care 2002; 8:101-105

15 Mazzeo AT, Beat A, Singh A, et al. The role of mitochondrial transition pore, and its modulation, in traumatic brain injury and delayed neurodegeneration after TBI. Exp Neurol 2009; 218:363-370

16 Sullivan PG, Rabchevsky AG, Hicks RR, et al. Dose-response curve and optimal dosing regimen of cyclosporin A after traumatic brain injury in rats. Neuroscience 2000; 101:289-295

17 Sullivan PG, Rabchevsky AG, Waldmeier PC, et al. Mitochondrial permeability transition in CNS trauma: cause or effect of neuronal cell death? J Neurosci Res 2005; 79:231-239

18 Sullivan PG, Thompson M, Scheff SW. Continuous infusion of cyclosporin A postinjury significantly ameliorates cortical damage following traumatic brain injury. Exp Neurol 2000; 161:631-637
19 Dirksen MT, Laarman GJ, Simoons ML, et al. Reperfusion injury in humans: a review of clinical trials on reperfusion injury inhibitory strategies. Cardiovasc Res 2007; 74:343-355
20 Friberg H, Wieloch T. Mitochondrial permeability transition in acute neurodegeneration. Biochimie 2002; 84:241-250
21 Vespa P, Bergsneider M, Hattori N, et al. Metabolic crisis without brain ischemia is common after traumatic brain injury: a combined microdialysis and positron emission tomography study. J Cereb Blood Flow Metab 2005; 25:763-774
22 National Collaborating Centre for Acute Care (UK). Head Injury: Triage, Assessment, Investigation and Early Management of Head Injury in Infants, Children and Adults. 2007
23 Study Group on Head Injury of the Italian Society for Neurosurgery. Guidelines for minor head injured patients' management in adult age. The Study Group on Head Injury of the Italian Society for Neurosurgery. J Neurosurg Sci 1996; 40:11-15
24 Vos PE, Battistin L, Birbamer G, et al. EFNS guideline on mild traumatic brain injury: report of an EFNS task force. Eur J Neurol 2002; 9:207-219
25 Huisman TA, Schwamm LH, Schaefer PW, et al. Diffusion tensor imaging as potential biomarker of white matter injury in diffuse axonal injury. AJNR Am J Neuroradiol 2004; 25:370-376
26 Maas AI, Dearden M, Teasdale GM, et al. EBIC-guidelines for management of severe head injury in adults. European Brain Injury Consortium. Acta Neurochir (Wien) 1997; 139:286-294
27 McConeghy KW, Hatton J, Hughes L, et al. A review of neuroprotection pharmacology and therapies in patients with acute traumatic brain injury. CNS Drugs 2012; 26:613-636
28 Temkin NR, Anderson GD, Winn HR, et al. Magnesium sulfate for neuroprotection after traumatic brain injury: a randomised controlled trial. Lancet Neurology 2007; 6:29-38
29 Shakur H, Andrews P, Asser T, et al. The BRAIN TRIAL: a randomised, placebo controlled trial of a Bradykinin B2 receptor antagonist (Anatibant) in patients with traumatic brain injury. Trials 2009; 10:109
30 Simmon VF. Response to: The BRAIN TRIAL: a randomised, placebo controlled trial of a Bradykinin B2 receptor antagonist (Anatibant) in patients with traumatic brain injury. Trials 2009; 10:110; author reply 111
31 Morris GF, Bullock R, Marshall SB, et al. Failure of the competitive N-methyl-D-aspartate antagonist Selfotel (CGS 19755) in the treatment of severe head injury: results of two phase III clinical trials. The Selfotel Investigators. J Neurosurg 1999; 91:737-743
32 Maas AI, Murray G, Henney H, 3rd, et al. Efficacy and safety of dexanabinol in severe traumatic brain injury: results of a phase III randomised, placebo-controlled, clinical trial. Lancet Neurology 2006; 5:38-45
33 Langham J, Goldfrad C, Teasdale G, et al. Calcium channel blockers for acute traumatic brain injury. Cochrane Database of Systematic Reviews 2003:CD000565
34 Lei J, Gao G, Jiang J. Acute traumatic brain injury: is current management evidence based? An empirical analysis of systematic reviews. J Neurotrauma 2012
35 Ker K, Blackhall K. Beta-2 receptor antagonists for acute traumatic brain injury. Cochrane Database of Systematic Reviews 2008:CD006686
36 Perel P, Al-Shahi Salman R, Kawahara T, et al. CRASH-2 (Clinical Randomisation of an Antifibrinolytic in Significant Haemorrhage) intracranial bleeding study: the effect of

tranexamic acid in traumatic brain injury--a nested randomised, placebo-controlled trial. Health Technol Assess 2012; 16:iii-xii, 1-54
37 Ma J, Huang S, Qin S, et al. Progesterone for acute traumatic brain injury. Cochrane Database of Systematic Reviews 2012; 10:CD008409
38 Wright DW, Kellermann AL, Hertzberg VS, et al. ProTECT: a randomized clinical trial of progesterone for acute traumatic brain injury. Ann Emerg Med 2007; 49:391-402, 402 e391-392
39 Xiao G, Wei J, Yan W, et al. Improved outcomes from the administration of progesterone for patients with acute severe traumatic brain injury: a randomized controlled trial. Critical Care (London, England) 2008; 12:R61
40 Hatton J, Rosbolt B, Empey P, et al. Dosing and safety of cyclosporine in patients with severe brain injury. J Neurosurg 2008; 109:699-707
41 Mazzeo AT, Alves OL, Gilman CB, et al. Brain metabolic and hemodynamic effects of cyclosporin A after human severe traumatic brain injury: a microdialysis study. Acta Neurochir (Wien) 2008; 150:1019-1031; discussion 1031
42 Mazzeo AT, Brophy GM, Gilman CB, et al. Safety and tolerability of cyclosporin a in severe traumatic brain injury patients: results from a prospective randomized trial. J Neurotrauma 2009; 26:2195-2206
43 Sullivan PG, Sebastian AH, Hall ED. Therapeutic window analysis of the neuroprotective effects of cyclosporine A after traumatic brain injury. J Neurotrauma 2011; 28:311-318
44 Cooper DJ, Rosenfeld JV, Murray L, et al. Decompressive craniectomy in diffuse traumatic brain injury. N Engl J Med 2011; 364:1493-1502
45 Melnikov VY, Ecder T, Fantuzzi G, et al. Impaired IL-18 processing protects caspase-1-deficient mice from ischemic acute renal failure. J Clin Invest 2001; 107:1145-1152
46 Melnikov VY, Faubel S, Siegmund B, et al. Neutrophil-independent mechanisms of caspase-1- and IL-18-mediated ischemic acute tubular necrosis in mice. J Clin Invest 2002; 110:1083-1091
47 Parikh CR, Jani A, Melnikov VY, et al. Urinary interleukin-18 is a marker of human acute tubular necrosis. Am J Kidney Dis 2004; 43:405-414
48 Parikh CR, Abraham E, Ancukiewicz M, et al. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. J Am Soc Nephrol 2005; 16:3046-3052
49 Parikh CR, Jani A, Mishra J, et al. Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation. American Journal of Transplantation 2006; 6:1639-1645
50 Parikh CR, Mishra J, Thiessen-Philbrook H, et al. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. Kidney Int 2006; 70:199-203
51 Washburn KK, Zappitelli M, Arikan AA, et al. Urinary interleukin-18 is an acute kidney injury biomarker in critically ill children. Nephrology Dialysis Transplantation 2008; 23:566-572
52 Haase M, Bellomo R, Story D, et al. Urinary interleukin-18 does not predict acute kidney injury after adult cardiac surgery: a prospective observational cohort study. Critical Care 2008; 12:R96
53 Adademir T, Ak K, Aljodi M, et al. The effects of pulsatile cardiopulmonary bypass on acute kidney injury. Int J Artif Organs 2012; 35:511-519
54 Vaidya VS, Ramirez V, Ichimura T, et al. Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. American Journal of Physiology - Renal Physiology 2006; 290:F517-529
55 Bonventre JV, Bonventre JV. Kidney injury molecule-1 (KIM-1): a urinary biomarker and much more. Nephrology Dialysis Transplantation 2009; 24:3265-3268

56 Ichimura T, Asseldonk EJ, Humphreys BD, et al. Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. J Clin Invest 2008; 118:1657-1668
57 Vaidya VS, Waikar SS, Ferguson MA, et al. Urinary biomarkers for sensitive and specific detection of acute kidney injury in humans. Clin Transl Sci 2008; 1:200-208
58 Prozialeck WC, Vaidya VS, Liu J, et al. Kidney injury molecule-1 is an early biomarker of cadmium nephrotoxicity. Kidney Int 2007; 72:985-993
59 van Timmeren MM, Bakker SJ, Vaidya VS, et al. Tubular kidney injury molecule-1 in protein-overload nephropathy. American Journal of Physiology - Renal Physiology 2006; 291:F456-464
60 Vinken P, Starckx S, Barale-Thomas E, et al. Tissue Kim-1 and urinary clusterin as early indicators of cisplatin-induced acute kidney injury in rats. Toxicol Pathol 2012; 40:1049-1062
61 Wu CT, Weng TI, Chen LP, et al. Involvement of caspase-12-dependent apoptotic pathway in ionic radiocontrast urografin-induced renal tubular cell injury. Toxicol Appl Pharmacol 2013; 266:167-175
62 Nejat M, Pickering JW, Devarajan P, et al. Some biomarkers of acute kidney injury are increased in pre-renal acute injury. Kidney Int 2012; 81:1254-1262
63 Han WK, Bailly V, Abichandani R, et al. Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury.[see comment]. Kidney Int 2002; 62:237-244
64 Zhang PL, Rothblum LI, Han WK, et al. Kidney injury molecule-1 expression in transplant biopsies is a sensitive measure of cell injury. Kidney Int 2008; 73:608-614
65 Koyner JL, Vaidya VS, Bennett MR, et al. Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. Clinical Journal of The American Society of Nephrology: CJASN 2010; 5:2154-2165
66 Peralta CA, Katz R, Bonventre JV, et al. Associations of urinary levels of kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) with kidney function decline in the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Kidney Dis 2012; 60:904-911
67 Endre ZH, Pickering JW. Acute kidney injury clinical trial design: old problems, new strategies. Pediatr Nephrol 2013; 28:207-217
68 Vaidya VS, Ozer JS, Dieterle F, et al. Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. Nat Biotechnol 2010; 28:478-485
69 Boyer TD. The glutathione S-transferases: an update. Hepatology 1989; 9:486-496
70 Sheehan D, Meade G, Foley VM, et al. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 2001; 360:1-16
71 Pai MP, Norenberg JP, Telepak RA, et al. Assessment of effective renal plasma flow, enzymuria, and cytokine release in healthy volunteers receiving a single dose of amphotericin B desoxycholate.[Erratum appears in Antimicrob Agents Chemother. 2005 Oct;49(10):4427]. Antimicrob Agents Chemother 2005; 49:3784-3788
72 Polak WP, Kosieradzki M, Kwiatkowski A, et al. Activity of glutathione S-transferases in the urine of kidney transplant recipients during the first week after transplantation. Ann Transplant 1999; 4:42-45
73 Sundberg AG, Appelkvist EL, Backman L, et al. Urinary pi-class glutathione transferase as an indicator of tubular damage in the human kidney. Nephron 1994; 67:308-316
74 Yang Y, Sharma R, Zimniak P, et al. Role of alpha class glutathione S-transferases as antioxidant enzymes in rodent tissues. Toxicol Appl Pharmacol 2002; 182:105-115

75 Vaubourdolle M, Chazouilleres O, Briaud I, et al. Plasma alpha-glutathione S-transferase assessed as a marker of liver damage in patients with chronic hepatitis C. Clin Chem 1995; 41:1716-1719
76 Yavuz I, Asgun FH, Bolcal C, et al. Importance of urinary measurement of glutathione S-transferase in renal dysfunction patients after on- and off-pump coronary artery bypass surgery. Thorac Cardiovasc Surg 2009; 57:125-129
77 Shi GP, Villadangos JA, Dranoff G, et al. Cathepsin S required for normal MHC class II peptide loading and germinal center development. Immunity; 10:197-206
78 de Nooijer R, Bot I, von der Thusen JH, et al. Leukocyte cathepsin S is a potent regulator of both cell and matrix turnover in advanced atherosclerosis. Arteriosclerosis, Thrombosis & Vascular Biology 2009; 29:188-194
79 Wang B, Sun J, Kitamoto S, et al. Cathepsin S controls angiogenesis and tumor growth via matrix-derived angiogenic factors. J Biol Chem 2006; 281:6020-6029
80 Liu J, Ma L, Yang J, et al. Increased serum cathepsin S in patients with atherosclerosis and diabetes. Atherosclerosis 2006; 186:411-419
81 Naour N, Rouault C, Fellahi S, et al. Cathepsins in human obesity: changes in energy balance predominantly affect cathepsin s in adipose tissue and in circulation. J Clin Endocrinol Metab 2010; 95:1861-1868
82 Taleb S, Cancello R, Poitou C, et al. Weight loss reduces adipose tissue cathepsin S and its circulating levels in morbidly obese women. J Clin Endocrinol Metab 2006; 91:1042-1047
83 Lindstedt L, Lee M, Oorni K, et al. Cathepsins F and S block HDL3-induced cholesterol efflux from macrophage foam cells. Biochem Biophys Res Commun 2003; 312:1019-1024
84 Liu J, Sukhova GK, Sun JS, et al. Lysosomal cysteine proteases in atherosclerosis. Arteriosclerosis, Thrombosis & Vascular Biology 2004; 24:1359-1366
85 Sukhova GK, Zhang Y, Pan JH, et al. Deficiency of cathepsin S reduces atherosclerosis in LDL receptor-deficient mice. J Clin Invest 2003; 111:897-906
86 Fernandez PL, Farre X, Nadal A, et al. Expression of cathepsins B and S in the progression of prostate carcinoma. Int J Cancer 2001; 95:51-55
87 Flannery T, Gibson D, Mirakhur M, et al. The clinical significance of cathepsin S expression in human astrocytomas. Am J Pathol 2003; 163:175-182
88 Kos J, Sekirnik A, Kopitar G, et al. Cathepsin S in tumours, regional lymph nodes and sera of patients with lung cancer: relation to prognosis. Br J Cancer 2001; 85:1193-1200
89 Jobs E, Ingelsson E, Riserus U, et al. Association between serum cathepsin S and mortality in older adults. JAMA 2011; 306:1113-1121
90 Arnlov J, Arnlov J. Cathepsin S as a biomarker: where are we now and what are the future challenges? Biomarkers in Medicine 2012; 6:9-11
91 Bellomo R, Ronco C, Kellum JA, et al. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Critical Care 2004; 8:R204-R212
92 Bagshaw SM, George C, Dinu I, et al. A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. Nephrology Dialysis Transplantation 2008; 23:1203-1210
93 Ostermann M, Chang RWS. Acute kidney injury in the intensive care unit according to RIFLE. Crit Care Med 2007; 35:1837-1843
94 Moore EM, Bellomo R, Nichol AD, et al. The incidence of acute kidney injury in patients with traumatic brain injury. Ren Fail 2010; 32:1060-1065

95 Li N, Zhao WG, Zhang WF. Acute kidney injury in patients with severe traumatic brain injury: implementation of the acute kidney injury network stage system. Neurocritical Care 2011; 14:377-381
96 Davenport A. Renal replacement therapy in the patient with acute brain injury. Am J Kidney Dis 2001; 37:457-466
97 Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. Kidney Int 2008; 73:538-546
98 Chertow GM, Burdick E, Honour M, et al. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol 2005; 16:3365-3370
99 Moore EM, Simpson JA, Tobin AE, et al. Preoperative eGFR and RIFLE-classified postoperative AKI predict length of stay post coronary bypass surgery in an Australian setting. Anaesth Intensive Care 2010; 38:113-121
100 Ricci Z, Ronco C. Year in review: Critical Care 2004 - nephrology. Critical Care (London, England) 2005; 9:523-527
101 Goldfarb M, Rosenberger C, Ahuva S, et al. A role for erythropoietin in the attenuation of radiocontrast-induced acute renal failure in rats. Ren Fail 2006; 28:345-350
102 Johnson DW, Pat B, Vesey DA, et al. Delayed administration of darbepoetin or erythropoietin protects against ischemic acute renal injury and failure. Kidney Int 2006; 69:1806-1813
103 Kiris I, Kapan S, Kilbas A, et al. The protective effect of erythropoietin on renal injury induced by abdominal aortic-ischemia-reperfusion in rats. J Surg Res 2008; 149:206-213
104 Mitra A, Bansal S, Wang W, et al. Erythropoietin ameliorates renal dysfunction during endotoxaemia. Nephrology Dialysis Transplantation 2007; 22:2349-2353
105 Simon F, Scheuerle A, Calzia E, et al. Erythropoietin during porcine aortic balloon occlusion-induced ischemia/reperfusion injury. Crit Care Med 2008; 36:2143-2150
106 Spandou E, Tsouchnikas I, Karkavelas G, et al. Erythropoietin attenuates renal injury in experimental acute renal failure ischaemic/reperfusion model. Nephrology Dialysis Transplantation 2006; 21:330-336
107 Yokomaku Y, Sugimoto T, Kume S, et al. Asialoerythropoietin prevents contrast-induced nephropathy. J Am Soc Nephrol 2008; 19:321-328
108 Nijboer WN, Ottens PJ, van Dijk A, et al. Donor pretreatment with carbamylated erythropoietin in a brain death model reduces inflammation more effectively than erythropoietin while preserving renal function. Crit Care Med 2010; 38:1155-1161
109 Yatsiv I, Grigoriadis N, Simeonidou C, et al. Erythropoietin is neuroprotective, improves functional recovery, and reduces neuronal apoptosis and inflammation in a rodent model of experimental closed head injury. FASEB J 2005; 19:1701-1703
110 Australian New Zealand Clinical Trials Registry [site on the Internet] [cited 2009 Jun 30]. Available from: http://www.anzctr.org.au.
111 ClinicalTrials.gov. A service of the U.S. National Institutes of Health [site on the Internet] [cited 2009 May 17]. Available from: http://clinicaltrials.gov/ct2/results?term=erythropoietin+AND+acute+kidney+injury
112 Song YR, Lee T, You SJ, et al. Prevention of acute kidney injury by erythropoietin in patients undergoing coronary artery bypass grafting: a pilot study. Am J Nephrol 2009; 30:253-260
113 de Seigneux S, Ponte B, Weiss L, et al. Epoetin administrated after cardiac surgery: effects on renal function and inflammation in a randomized controlled study. BMC Nephrol 2012; 13:132

114 Endre ZH, Walker RJ, Pickering JW, et al. Early intervention with erythropoietin does not affect the outcome of acute kidney injury (the EARLYARF trial). Kidney Int 2010; 77:1020-1030
115 Delbridge MS, Shrestha BM, Raftery AT, et al. The effect of body temperature in a rat model of renal ischemia-reperfusion injury. Transplant Proc 2007; 39:2983-2985
116 Zager RA, Gmur DJ, Bredl CR, et al. Degree and time sequence of hypothermic protection against experimental ischemic acute renal failure. Circ Res 1989; 65:1263-1269
117 Heyman SN, Rosenberger C, Rosen S. Critical Assessment of Animal Models of Acute Renal Failure. In: Ronco C, Bellomo R, Kellum JA, eds. Critical Care Nephrology. New York: Elsevier, 2009
118 Sahuquillo J, Vilalta A. Cooling the injured brain: how does moderate hypothermia influence the pathophysiology of traumatic brain injury. Curr Pharm Des 2007; 13:2310-2322
119 Boodhwani M, Rubens FD, Wozny D, et al. Effects of mild hypothermia and rewarming on renal function after coronary artery bypass grafting. Ann Thorac Surg 2009; 87:489-495
120 Polderman KH. Induced hypothermia and fever control for prevention and treatment of neurological injuries. Lancet 2008; 371:1955-1969
121 Zeiner A, Holzer M, Sterz F, et al. Mild resuscitative hypothermia to improve neurological outcome after cardiac arrest. A clinical feasibility trial. Hypothermia After Cardiac Arrest (HACA) Study Group. Stroke 2000; 31:86-94
122 Zeiner A, Sunder-Plassmann G, Sterz F, et al. The effect of mild therapeutic hypothermia on renal function after cardiopulmonary resuscitation in men. Resuscitation 2004; 60:253-261
123 Ricci Z, Ronco C. Year in review 2007: Critical Care - nephrology. Critical Care (London, England) 2008; 12:230
124 Wetzels JF, Kiemeney LA, Swinkels DW, et al. Age- and gender-specific reference values of estimated GFR in Caucasians: the Nijmegen Biomedical Study. Kidney Int 2007; 72:632-637
125 Mehta RL, Kellum JA, Shah SV, et al. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Critical Care (London, England) 2007; 11:R31
126 KDIGO Clinical Practice Guideline for Acute Kidney Injury. Kidney International - Supplement 2012; 2:1-138
127 Johnson DW, Jones GR, Mathew TH, et al. Chronic kidney disease and automatic reporting of estimated glomerular filtration rate: new developments and revised recommendations. Med J Aust. 2012; 197:224-5
128 Bagshaw SM, Bennett M, Haase M, et al. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. Intensive Care Med; 36:452-461
129 Endre ZH, Westhuyzen J. Early detection of acute kidney injury: emerging new biomarkers.[erratum appears in Nephrology (Carlton). 2008 Jun;13(3):268]. Nephrology 2008; 13:91-98
130 Gok MA, Pelsers M, Glatz JF, et al. Comparison of perfusate activities of glutathione S-transferase, alanine aminopeptidase and fatty acid binding protein in the assessment of non-heart-beating donor kidneys. Ann Clin Biochem 2003; 40:252-258
131 Hall IE, Koyner JL, Doshi MD, et al. Urine cystatin C as a biomarker of proximal tubular function immediately after kidney transplantation. Am J Nephrol 2011; 33:407-413
132 Hall IE, Yarlagadda SG, Coca SG, et al. IL-18 and urinary NGAL predict dialysis and graft recovery after kidney transplantation. J Am Soc Nephrol 2010; 21:189-197

133 Herget-Rosenthal S, Marggraf G, Husing J, et al. Early detection of acute renal failure by serum cystatin C. Kidney Int 2004; 66:1115-1122
134 Moore E, Bellomo R, Nichol A. Biomarkers of acute kidney injury in anesthesia, intensive care and major surgery: from the bench to clinical research to clinical practice. Minerva Anestesiol 2010; 76:425-440
135 Moore EM, Bellomo R, Nichol AD. Erythropoietin as a novel brain and kidney protective agent. Anaesth Intensive Care 2011; 39:356-372
136 Polderman KH, Peerdeman SM, Girbes AR. Hypophosphatemia and hypomagnesemia induced by cooling in patients with severe head injury. J Neurosurg 2001; 94:697-705
137 Makris K, Markou N, Evodia E, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) as an early marker of acute kidney injury in critically ill multiple trauma patients. Clin Chem Lab Med 2009; 47:79-82
138 Bagshaw SM, George C, Gibney RTN, et al. A multi-center evaluation of early acute kidney injury in critically ill trauma patients. Ren Fail 2008; 30:581-589
139 Li N, Zhao W-G, Zhang W-F. Acute kidney injury in patients with severe traumatic brain injury: implementation of the acute kidney injury network stage system. Neurocritical Care 2011; 14:377-381
140 Macedo E, Malhotra R, Claure-Del Granado R, et al. Defining urine output criterion for acute kidney injury in critically ill patients. Nephrology Dialysis Transplantation 2011; 26:509-515
141 Cruz DN, Bolgan I, Perazella MA, et al. North East Italian Prospective Hospital Renal Outcome Survey on Acute Kidney Injury (NEiPHROS-AKI): targeting the problem with the RIFLE Criteria. Clinical Journal of The American Society of Nephrology: CJASN 2007; 2:418-425
142 Clerico A, Galli C, Fortunato A, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as biomarker of acute kidney injury: a review of the laboratory characteristics and clinical evidences. Clin Chem Lab Med 2012; 50:1505-1517
143 Haase M, Devarajan P, Haase-Fielitz A, et al. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. J Am Coll Cardiol 2011; 57:1752-1761
144 Hoste EA, Clermont G, Kersten A, et al. RIFLE criteria for acute kidney injury are associated with hospital mortality in cirtically ill patients: a cohort analysis. Critical Care 2006; 10
145 Hoste EAJ, Kellum JA. Acute kidney injury: epidemiology and diagnostic criteria. Current Opinion in Critical Care 2006; 12:531-537
146 Joannidis M, Metnitz B, Bauer P, et al. Acute kidney injury in critically ill patients classified by AKIN versus RIFLE using the SAPS 3 database. Intensive Care Med 2009; 35:1692-1702
147 Haase M, Bellomo R, Matalanis G, et al. A comparison of the RIFLE and Acute Kidney Injury Network classifications for cardiac surgery-associated acute kidney injury: a prospective cohort study. J Thorac Cardiovasc Surg 2009; 138:1370-1376
148 Corwin HL, Gettinger A, Fabian TC, et al. Efficacy and safety of epoetin alfa in critically ill patients.[see comment]. N Engl J Med 2007; 357:965-976
149 Xiong Y, Chopp M, Lee C-P. Erythropoietin improves brain mitochondrial function in rats after traumatic brain injury. Neurol Res 2009; 31:496-502
150 Xiong M, Chen S, Yu H, et al. Neuroprotection of erythropoietin and methylprednisolone against spinal cord ischemia-reperfusion injury. Journal of Huazhong University of Science and Technology Medical Sciences 2011; 31:652-656
151 Ludman AJ, Yellon DM, Hasleton J, et al. Effect of erythropoietin as an adjunct to primary percutaneous coronary intervention: a randomised controlled clinical

trial.[Erratum appears in Heart. 2011 Nov;97(21):1812 Note: Mutharangu, Vivek [corrected to Muthurangu, Vivek]]. Heart 2011; 97:1560-1565
152 Reveal Investigators, Najjar SS, Rao SV, et al. Intravenous erythropoietin in patients with ST-segment elevation myocardial infarction: REVEAL: a randomized controlled trial. JAMA 2011; 305:1863-1872
153 Kim JH, Shim JK, Song JW, et al. Effect of erythropoietin on the incidence of acute kidney injury following complex valvular heart surgery: a double blind, randomized clinical trial of efficacy and safety. Crit Care. 2013;17:R254. [Epub ahead of print]
154 Prunier F, Biere L, Gilard M, et al. Single high-dose erythropoietin administration immediately after reperfusion in patients with ST-segment elevation myocardial infarction: results of the erythropoietin in myocardial infarction trial. Am Heart J 2011; 163:200-207.e201
155 Susantitaphong P, Alfayez M, Cohen-Bucay A, et al. Therapeutic hypothermia and prevention of acute kidney injury: a meta-analysis of randomized controlled trials. Resuscitation; 83:159-167
156 Qiu W, Zhang Y, Sheng H, et al. Effects of therapeutic mild hypothermia on patients with severe traumatic brain injury after craniotomy. J Crit Care 2007; 22:229-235
157 Liu KD, Thompson BT, Ancukiewicz M, et al. Acute kidney injury in patients with acute lung injury: impact of fluid accumulation on classification of acute kidney injury and associated outcomes. Crit Care Med 2011; 39:2665-2671
158 Waikar SS, Sabbisetti VS, Bonventre JV. Normalization of urinary biomarkers to creatinine during changes in glomerular filtration rate. Kidney Int 2010; 78:486-494
159 Askenazi DJ, Koralkar R, Levitan EB, et al. Baseline values of candidate urine acute kidney injury biomarkers vary by gestational age in premature infants. Pediatr Res 2011; 70:302-306
160 Jeong T-D, Kim S, Lee W, et al. Neutrophil gelatinase-associated lipocalin as an early biomarker of acute kidney injury in liver transplantation. Clin Transplant 2012; 26:775-781
161 Sola-Del Valle DA, Mohan S, Cheng J-T, et al. Urinary NGAL is a useful clinical biomarker of HIV-associated nephropathy. Nephrology Dialysis Transplantation 2011; 26:2387-2390
162 Wagener G, Minhaz M, Mattis FA, et al. Urinary neutrophil gelatinase-associated lipocalin as a marker of acute kidney injury after orthotopic liver transplantation. Nephrology Dialysis Transplantation 2011; 26:1717-1723
163 Haase-Fielitz A, Bellomo R, Devarajan P, et al. Novel and conventional serum biomarkers predicting acute kidney injury in adult cardiac surgery--a prospective cohort study. Crit Care Med 2009; 37:553-560
164 Koyner JL, Bennett MR, Worcester EM, et al. Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery.[see comment]. Kidney Int 2008; 74:1059-1069
165 Wagener G, Jan M, Kim M, et al. Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. Anesthesiology 2006; 105:485-491
166 Delanaye P, Lambermont B, Chapelle JP, et al. Plasmatic cystatin C for the estimation of glomerular filtration rate in intensive care units. Intensive Care Med 2004; 30:980-983
167 Herget-Rosenthal S, Poppen D, Husing J, et al. Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. Clin Chem 2004; 50:552-558
168 Herrero-Morin JD, Malaga S, Fernandez N, et al. Cystatin C and beta2-microglobulin: markers of glomerular filtration in critically ill children.[see comment]. Critical Care (London, England) 2007; 11:R59

169 Royakkers AANM, van Suijlen JDE, Hofstra LS, et al. Serum cystatin C-A useful endogenous marker of renal function in intensive care unit patients at risk for or with acute renal failure? Curr Med Chem 2007; 14:2314-2317
170 Villa P, Jimenez M, Soriano M-C, et al. Serum cystatin C concentration as a marker of acute renal dysfunction in critically ill patients.[see comment]. Critical Care (London, England) 2005; 9:R139-143
171 Ling W, Zhaohui N, Ben H, et al. Urinary IL-18 and NGAL as early predictive biomarkers in contrast-induced nephropathy after coronary angiography. Nephron 2008; 108:c176-181
172 Kell DB. Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. BMC Medical Genomics 2009; 2
173 Schmidt-Ott KM, Mori K, Li JY, et al. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 2007; 18:407-413
174 Bahlmann FH, Fliser D. Erythropoietin and renoprotection. Curr Opin Nephrol Hypertens 2009; 18:15-20
175 Hanrieder J, Wetterhall M, Enblad P, et al. Temporally resolved differential proteomic analysis of human ventricular CSF for monitoring traumatic brain injury biomarker candidates. J Neurosci Methods 2009; 177:469-478
176 Gao W-M, Chadha MS, Berger RP, et al. A gel-based proteomic comparison of human cerebrospinal fluid between inflicted and non-inflicted pediatric traumatic brain injury. J Neurotrauma 2007; 24:43-53
177 Shi GP, Villadangos JA, Dranoff G, et al. Cathepsin S required for normal MHC class II peptide loading and germinal center development. Immunity 1999; 10:197-206
178 Jin W, Wu J, Wang H, et al. Erythropoietin administration modulates pulmonary Nrf2 signaling pathway after traumatic brain injury in mice. Journal of Trauma-Injury Infection & Critical Care; 71:680-686
179 Jin W, Wang H, Yan W, et al. Role of Nrf2 in protection against traumatic brain injury in mice. J Neurotrauma 2009; 26:131-139
180 Yatsiv I, Morganti-Kossmann MC, Perez D, et al. Elevated intracranial IL-18 in humans and mice after traumatic brain injury and evidence of neuroprotective effects of IL-18-binding protein after experimental closed head injury. J Cereb Blood Flow Metab 2002; 22:971-978
181 Roudkenar MH, Halabian R, Roushandeh AM, et al. Lipocalin 2 regulation by thermal stresses: protective role of Lcn2/NGAL against cold and heat stresses. Exp Cell Res 2009; 315:3140-3151