CLINICAL ASSOCIATIONS AND PROGNOSTIC SIGNIFICANCE OF MITOTIC RATE IN PRIMARY CUTANEOUS MELANOMA

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Abstract

Melanoma is a significant cause of morbidity and mortality in Australia. Whilst recent developments in targeted therapies have offered exciting breakthroughs in the treatment of advanced disease, survival rate remains grim for those afflicted with the systemic spread of melanoma. For local cutaneous disease, a patient's initial prognosis is primarily determined by the stage which the melanoma is first diagnosed, reflected largely but not solely by its Breslow thickness. Attempts are being made to better understand both the clinical and histologic heterogeneity of melanoma, since it is recognized that some subtypes of melanoma behave more aggressively than others.

This thesis aims to examine the distinct clinical and histopathologic features of aggressive primary cutaneous melanomas, as a means to aid clinicians with timely detection of at-risk individuals. It also aims to extend upon our current knowledge of melanoma prognostication. In order to pursue these aims, two original studies were undertaken; both focused on mitotic rate, a histologic feature used to quantify the degree of tumour cell proliferation and has emerged in recent years as an important prognosticator of clinical outcomes. The first study of this thesis seeks to elucidate patient and tumour characteristics associated with mitotically active melanoma. The predictive value of mitotic rate for melanoma survival is explored in the second study.

Acknowledgements

First and foremost, I would like to thank my MPhil supervisors, Professor Rory Wolfe, Associate Professor John W Kelly and Dr Martin Haskett. Professor Rory Wolfe has been unwavering in his support throughout all aspects of my research study, in particularly offering invaluable guidance in biostatistics. Both Associate Professor John Kelly and Dr Martin Haskett have been remarkable mentors and role models instrumental in shaping not only my academic career, but also my clinical career in medicine and above all in dermatology. It is both my honour and privilege to have worked under the tutelage of these three great leaders.

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Finally, I would like to thank my parents, David Shen and Phoebe Fei, for their unyielding support and loving encouragement. Leaving an oppressive communist regimen, I am forever indebted to them for the sacrifices they have made in starting a new life in a democratic society built on meritocracy, and in so doing, presented me with opportunities that they never had.

Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma, except where due reference is made in the text of the thesis.

To the best of my knowledge, this thesis contains no material previously published or written by another person except where due reference is made in the text of the thesis.

Signed

Dated

Monash University

Declaration for thesis based or partially based on conjointly published or unpublished work

General Declaration

In accordance with Monash University Doctorate Regulation 17.2 Doctor of Philosophy and Research Master's 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 1 original paper published in a peer-reviewed journal and 1 unpublished publication. The core theme of this thesis is the demographic, clinical and histopathologic determinants of aggressive primary melanoma. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the School of Public Health and Preventive Medicine under the supervision of Professor Rory Wolfe, Associate Professor John Kelly and Dr Martin Haskett.

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 3 to 4, my contribution to the work involved the following:

Thesis chapter	Publication title	Publication status	Nature and extent of candidate's contribution
3	Characteristics and associations of high-mitotic-rate melanoma	Published	Study design, data analysis and interpretation, manuscript development and preparation
4	Mitotic rate as a prognostic indicator for melanoma survival: the influence of different modes of analysis	Completed, to be submitted	Study design, data analysis and interpretation, manuscript development and preparation

I have renumbered sections of the submitted or published papers in order to generate consistent presentation within the thesis.

Signed:

Date:

Chapter 1: General Introduction

Outline of Chapter 1

This introductory chapter presents an overview of the epidemiology of melanoma and its etiology, diagnosis and treatment. In order to understand the role of mitotic rate in melanoma prognosis, this chapter presents the relevant background to the established and emerging prognostic markers for primary invasive cutaneous melanoma. The overarching research questions of the work presented in this thesis are thereby outlined.

1.1 Epidemiology

1.1.1 Overall Incidence

Melanoma is a significant contributor to the burden of cancer in Australia. The "sunburnt country" has the highest incidence of skin cancer in the world[1], with a lifetime risk for invasive melanoma approximating 1 in 15 for men and 1 in 24 for women before age 85 years[2]. Over the past decade, melanoma has consistently been ranked amongst the top five most common cancers diagnosed in Australia and amongst the top ten most common cause of cancer death[3].

1.1.2 Age at diagnosis

Over half of new cases of melanoma are diagnosed in people aged 60 years and above. It is the commonest cancer diagnosed in individuals between 19 and 25 years of age[2]. The median age at diagnosis of melanoma according to data published by the Australian Institute of Health and Welfare in 2011 was 64 years in males and 59 years in females[2]. There has been no significant change in the median age at diagnosis over the last decade.

1.1.3 Gender variation

Melanoma incidence is known to differ between genders. Despite the finding from overseas that greater ultraviolet radiation exposure and solarium use may be contributing to the increasing incidence of melanoma in women[4], melanoma incidence is still rising at a faster rate in males compared with females in Australia. In 1982, 28.1 per 100,000 men and 26.3 per 100, 000 women were diagnosed with melanoma. In 2009, incidence rose to 61.7 per 100,000 in men and 40.0 per 100, 000 in women[2].

1.1.4 Tumour pathology

Most diagnoses of melanoma are made at early stages of tumour development. Based on data from the New South Wales (NSW) cancer registry, localized primary melanoma contributed to 88% of new cases of melanoma diagnosed between 1983 and 2000. Just over half (53%) of cases diagnosed were thin (<1mm) melanomas whilst 10% fell into the ≥3mm thickness category[5]. This is comparable to incidences in Victoria, where 63% of all new cases diagnosed during 1989 to 2004 were <1mm in thickness, whilst only 11% were found to be ≥3mm in thickness[6].

The most common histopathologic subtype of melanoma in NSW in 2003, barring unspecified cases (49%), was superficial spreading melanoma (32%). Nodular melanoma was diagnosed in 9% of cases[5]. A population-based study comprising of cases reported to the Victorian Cancer Registry (VCR) from years 1989 to 2004, revealed similar findings, with superficial spreading melanoma responsible for 56% of cases whilst nodular melanoma contributed to 14% of invasive melanomas[6]. Both studies found that thin tumours (<1mm) were most commonly observed in superficial spreading and lentigo maligna melanomas (74% and 83% respectively), whereas thick tumours (≥3mm) were most frequent in nodular melanomas (40% and 53% respectively)[5,6]. Section 1.3.2 of this chapter discusses in further detail the main distinct melanoma histologic subtypes, their morphological features, clinical behavior and prognostic value.

1.1.5 Tumour Sites

The anatomic site varies according to gender. In men, over half of tumours are localized on the trunk, with 39% on the back; in women, 42% are localized on the lower extremity, with 24% on the lower leg, followed by 25% on the trunk. Cutaneous melanoma localized to the

head and neck region and the upper extremity are nearly equivalent in both sexes[7,8]. This pattern of site distribution has been consistent amongst most industrial nations[9-12].

The site-specific incidence of melanoma also varies according to age. The incidence of melanoma localized on the trunk and on the lower extremity decreases with advancing age, whereas a significant increase of melanoma localized to head and neck is found in older patients. Moreover, melanomas developing at different body sites are associated with distinct patterns of sun exposure. Melanomas of the head and neck are associated with cumulative patterns of sun exposure, whereas truncal melanomas are associated with intermittent sun exposure[13-15]. Distinct histologic melanoma subtypes have been noted to have predilection for varying anatomic locations. Data from the VCR indicated superficial spreading melanomas occurred predominantly on the extremities (57%) whilst nodular melanomas showed a predilection for the head and neck (25%) and lower extremities (28%)[16].

1.1.6 Trends in incidence

A continuous increase of cutaneous melanoma incidence rates has been observed during the last four decades in many countries with fair-skinned Caucasian populations[17]. Whilst varying between countries, the annual increase of melanoma incidence has been estimated at between 3-7%, thus representing a doubling of rates every 10 to 20 years[18-22].

Melanoma incidence in Australia has increased steadily in the past few decades and remains the highest globally (Figure 1). In 1982, the age-standardized incidence rate per 100,000 people was 26.8; from 2002 to 2009, annual incidence increased to 49.8 per 100,000 people with a male to female ratio of 1.5:1[2]. There are estimated over 11,000 new cases of melanoma diagnosed in Australia annually[23].

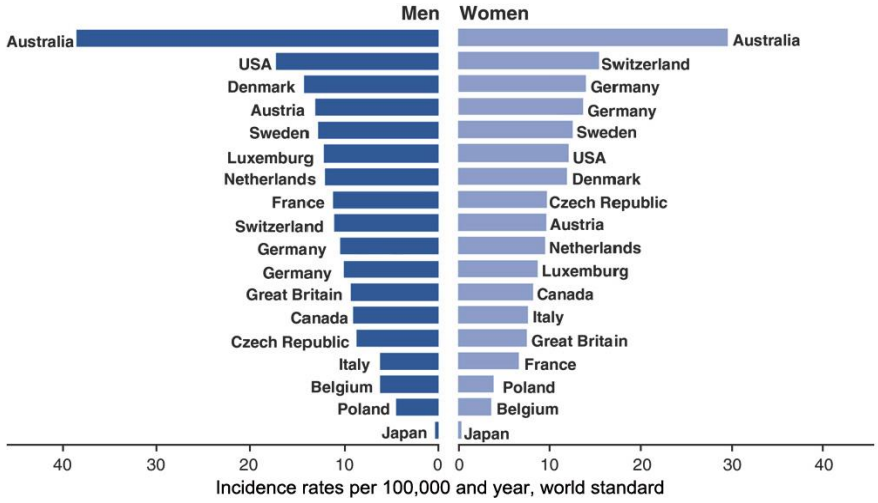

Figure 1: Melanoma incidence rates worldwide from 17 countries in 2002. USA, United States of America. Adapted from Garbe, C. ,& Leiter, U. (2009). Melanoma epidemiology and trends. *Clinics in Dermatology*, *27*(1), 3-9.

1.1.7 Overall mortality

Despite more vigilant screening and early detection initiatives, melanoma mortality has not appreciably declined. Whilst making up only 2.3% of skin cancer diagnoses in Australia, melanoma is responsible for 75% of skin cancer deaths. From 1968 to 2007, melanoma mortality annually increased by 1.4%. In 2007, there were 1279 deaths from cutaneous melanoma (864 men and 415 women), accounting for 3.2% of all cancer deaths in Australia[2,24]. Age-specific mortality rates from 2001-2007 are summarized in Table 1.

Table 1: Age specific mortality rates (Year 2001-2007)*

	Mortality rate (per 100,000 population)		
Age	Men	Women	Persons
≤29	0.2	0.2	0.2
30-39	1.8	1.1	1.4
40-49	3.8	2.1	3.0
50-59	6.9	3.5	5.2
60-69	19.1	7.9	13.5
70-79	40.4	14.5	26.6
≥80	73.6	27.5	44.3

* Adapted from the Australian Institute of Health and Welfare (AIHW). Australian Cancer Incidence and Mortality (ACIM) books <http://www.aihw.gov.au/cancer/datacubes/acim_books_2007.cfm> accessed 2nd January 2013.

1.1.8 Trends in mortality and survival

Survival from melanoma has remained high, although survival trends have been inconsistent over time. Until the 2000s, melanoma survival in Australia increased significantly between every time period: 5-year survival grew in the period 1982-1987 from 86% to 91% in 1994-1999, after which there has been no appreciable change in survival rate[3]. In Victoria, the 5-year survival rate for those diagnosed with primary invasive melanoma between 2006 to 2010 was 90%[25]. Table 2 summarizes the recent melanoma survival rates in Victoria.

1.1.9 Survival trends by gender

Despite the promising trend of steadily improving overall survival rates over the years, mortality nevertheless increased in Australian men with cutaneous melanoma between 1968 and 2007. Females are found to have higher survival than males: 5-year survival was 94% for females compared with 89% for males[3] (Figure 2). The question remains as to what factors potentially contribute to poorer survival in men; be it delayed diagnosis, poorer treatment, or the propensity for men to develop more aggressive primary invasive melanomas than women.

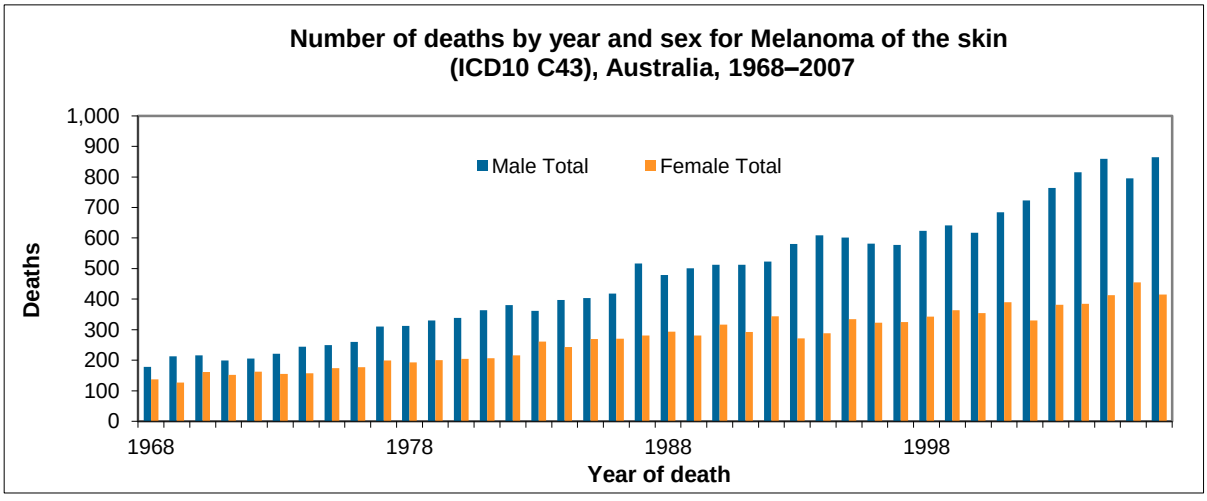

Figure 2: Number of deaths by year and sex for cutaneous melanoma, Australia, 1968-2007. Source: AIHW Australian Cancer Database (2007)

Table 2: Summary of relative survival from melanoma of the skin, Victoria, 1986-1990 to 2006-2010.				
Years after diagnosis	**Number of deaths**	**Survival**	**95% confidence interval**	
1	594	97	(96, 97)	
2	465	95	(94, 95)	
3	391	93	(92, 93)	
4	342	91	(91, 92)	
5	284	90	(89, 91)	
By subgroup	**Number of Deaths**	**5-year survival**	**95% confidence interval**	**p-value**
All cases	2076	90	(89, 91)	
Sex				
Male	1393	87	(86, 88)	<0.01
Female	683	93	(92, 94)	
Age at diagnosis				
0-44	129	94	(93, 95)	<0.01
45-54	140	93	(92, 94)	
55-64	258	92	(90, 93)	
65-74	383	90	(88, 91)	
75+	1166	83	(80, 85)	
Region of residence				
Melbourne	1365	90	(89, 91)	0.72
Rest of Victoria	711	90	(88, 91)	
Integrated cancer services region				
Southern	614	90	(89, 92)	0.34
Western and Central	252	89	(87, 91)	
North Eastern	499	91	(89, 92)	
Barwon	167	92	(89, 94)	
Grampians	98	91	(87, 94)	
Loddon-Mallee	162	90	(87, 92)	
Hume	134	89	(86, 92)	
Gippsland	150	87	(83, 90)	
Thickness				
≤1mm	526	100	(100, 101)	<0.01
1-2mm	319	89	(87, 91)	
2.1-4mm	366	74	(71, 78)	
>4mm	394	50	(46, 55)	
Selected periods				
1986-1990	947	85	(84, 87)	<0.01
1991-1995	1109	89	(88, 90)	
1996-2000	1405	91	(90, 92)	
2001-2005	1586	91	(90, 92)	
2006-2010	2076	90	(89, 91)	

Source: Cancer Survival Victoria, The Victorian Cancer Registry, 2012.

1.1.10 Survival trends by age

Rates of melanoma survival decrease with advancing age. Data from the VCR indicated that older age at diagnosis was associated with poorer survival, with estimates falling from 94% in persons under 45 years to 83% for persons over 75 years at diagnosis[25].

1.1.11 Survival trends by tumour thickness

Five-year survival for locally advanced tumours (>4mm) was 55% compared with almost 100% survival for melanomas detected at an early local stage (<1mm), indicating that the mortality in this group was the same as for the general population when adjusted for age and gender[3]. This significant gradient in survival in tumour thickness supports the importance of early detection.

1.1.12 Prevalence

Cutaneous invasive melanoma is consistently amongst the top five most prevalent cancers in Australia as a result of its high incidence and good overall survival. At the end of 2007, there were more than 136,000 people in Australia who were diagnosed with melanoma in the previous 26 years, including almost 45,800 diagnosed in the previous 5 years, representing a 26-year prevalence of 640 people per 100,000 population or 0.6% of the total Australian population[3]. Table 3 summarizes the incidence, mortality and prevalence for melanoma in Australia between years 1982 to 2010.

Table 3: Summary of new cases, deaths and prevalence for melanoma of the skin, Australia, 1982-2010.

Sex	New cases in 1982 to 2007	Subsequent deaths to 2010	5-year prevalence as at end of 2007	26-year prevalence	
				No. As at end of 2007	Rate per 100, 000 population
Males	103, 734	40, 402	25, 740	70, 654	669.1
Females	84, 040	22, 893	20, 013	65, 362	612.1
Persons	187, 774	63, 295	45, 753	136, 016	640.4

Source: AIHW Australian Cancer Database (2007).

1.2 Aetiology

The cause for melanoma development is complex and incompletely understood. However, it is widely regarded that the transformation of melanocytes to tumour cells likely involves interaction between genetic susceptibility and environmental insults. Both tumour development de novo or malignant transformation from a pre-existing benign naevus are thus thought to result from the accumulation of sequential cellular alterations, including activation of oncogenes and suppression of cellular repair mechanisms[26,27].

1.2.1 Pathogenesis and tumour progression

Primary melanoma is a malignancy of neural crest derived melanocytes. These specialized pigmented cells are predominantly present in the basal layer of the epidermis, but also found in the eyes, ears, gastrointestinal tract, leptomeninges, and oral and genital mucous membranes. The normal function of melanocytes is to produce and transfer a dark pigment called melanin to keratinocytes, which are also found in the epidermis. The transferred melanin is concentrated in the perinuclear space of keratinocytes and protects the nucleus from damage due to ultraviolet (UV) radiation[28].

Three distinct pathogenic steps have been proposed in melanoma tumour suppression. In an early-stage tumour, the melanoma displays only radial growth and is confined to the epidermis. Upon progression, it develops into microinvasive melanoma, in which microscopic extensions invade the superficial papillary dermis. Locally advanced melanomas may progress to the vertical growth phase, which is characterized by invasive growth with involvement deep into the dermis. In this stage of growth, the melanoma is believed to have gained the potential to metastasize[29].

1.2.2 Genetic mechanisms

A wide array of genes has been implicated in melanoma development; the discussion of which is beyond the scope of this thesis. It is thought that melanoma development and progression arise from complex aberrations in cell proliferation signaling. The mitogen-activated protein kinase (MAPK) pathway and the phosphoinostide 3-kinase (PI3K)/protein kinase B (AKT) pathway are two signaling pathways that have been actively studied. Mutations in proto-oncogenes C-KIT, BRAF and NRAS are deemed critical events in unregulated MAPK and PI3K/AKT activation and are the targets of current mutation-specific therapies for disseminated disease. BRAF, which has been found in up to 60% of cutaneous melanomas, is the most common genetic mutation found in melanoma. NRAS mutations occur in 20% of melanomas. Other studied genes include the p16, alternate reading frame (ARF), and cyclin-dependent kinase 4. These tumour suppressor genes have significance in both sporadic and germline mutations in primary and metastatic melanoma[29-33].

1.2.3 Risk factors

Along with ongoing genetic research on melanoma tumorigenesis, numerous other risk factors centering on patient demographics, phenotypic markers, environmental exposures and historical features have been identified, and are henceforth described in greater detail. In terms of patient characteristics, age, personal and family history confer significant risk for tumour development along with naevus count and Fitzpatrick skin phototype. Solar field damage is deemed the main exogenous risk factor[34-36].

a) Endogenous risk factors

Age poses one of the strongest risks for melanoma. A 70-year-old Australian man is eight times more likely to develop melanoma in the next decade (2.5%), and a 70-year-old women three times more likely (1.0%) than is a 30-year-old man or woman (0.3%)[2,23].

Certain phenotypic markers have been shown to increase melanoma risk. Meta-analyses of studies undertaken mainly in populations of European origin have shown that light versus dark skin colour, red-blond versus black hair, and blue versus dark brown eyes, confer risk increases of about two-fold. Similarly, a person with a Fitzpatrick skin phototype I (burn easily, never tan), is about twice as susceptible to melanoma as one with skin phototype IV (always tan, never burn), as are the most heavily freckled versus those without freckles[36]. Patients with high naevus counts (>100 naevi, whole body) had seven times (6.89, 95% CI, 4-63-10.3) the risk of people with low naevus count (<15 naevi) in a meta-analysis[34]. The number of naevi is found to positively correlate with UV exposure and has been used as surrogate measurement of UV-induced cutaneous damage[37]. Patients with more than one atypical naevus have an increased relative risk for melanoma of 3.63 (95% CI, 2.85-4.62)[38]. In a more recent pooled analysis of 15 case control studies, the presence of numerous naevi, large naevi and clinically atypical naevi were found to confer increased risk for melanoma and this risk varied little by geographic latitude[39].

Retrospective large-scale studies in several cancer registries have shown that a history of previous melanoma is a potent predictor of future melanoma. The risk is highest in the first one to two years after diagnosis and may be partly accounted for by more vigilant surveillance[34]. A history of non-melanoma skin cancer or pre-malignant lesions such as solar keratoses confers relative risks of around fourfold when examined by meta-analysis[34].

b) Exogenous risk factors

Solar radiation is thought to be the main exogenous driver of melanoma incidence at the population level, with significant contributions made by lifetime exposure, an intermittent pattern of exposure and exposure in childhood and adolescence. The relative risks for the highest categories of exposure, compared with the lowest, are >1.5. However, these low relative risks may be due, at least in part, to discordance in the measures of exposure[34,35].

1.3 Detection, diagnosis and classification of melanoma

The detection of malignant melanoma relies upon timely clinical recognition followed by histological confirmation of diagnosis.

1.3.1 Clinical presentation

Early clinical recognition of melanoma is essential in the successful treatment of melanoma. Pigmented cutaneous lesions can be initially evaluated using the ABCDE acronym (asymmetry, border irregularity, colour variegation, diameter ≥ 6mm and evolution). Not all melanomas present with all five features; it is the combination of the different ABCDE parameters that makes a cutaneous lesion a suspect for malignancy. Current screening practices aimed at improving the detection of high-risk patients have emphasized the "E" for "evolution" criterion of the ABCDE acronym[40,41]. Thus a cutaneous lesion that is changing in morphology over time should arouse clinical suspicion of malignant change.

1.3.2 Melanoma subtypes

Four major subtypes of melanoma have been described: superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, and acral lentiginous melanoma. The less common melanoma subtypes include desmoplastic melanoma, naevoid melanoma, clear cell

sarcoma and solitary dermal melanoma. The utility of these melanoma subtypes lies in their distinct macroscopic and microscopic features, which may both aid diagnosis and provide prognostic information.

1.3.3 Superficial spreading melanoma (SSM)

SSM is the most common melanoma subtype, accounting for over half of all melanoma diagnoses[42]. The name is derived from a prolonged radial growth phase before invasive vertical growth commences. SSM has a predilection for sun-exposed areas. Intermittent sun exposure, a high naevus count and past family history of melanoma, have been associated with the development of SSM[43]. Its clinical features typically respect the ABCDE rule, appearing variegated in pigmentation with a sharply marginated and irregular border. SSM is characterized histologically by pagetoid spreading of atypical melanocytes in the epidermis[44].

1.3.4 Lentigo maligna melanoma (LMM)

LMM commonly occurs in the elderly with sun-damaged skin and like SSM, has a predilection for sun-exposed sites[45]. Clinically, LMM commonly presents as a gradually enlarging and variably pigmented patch. Transformation is typically slow and it may take many years before invasive growth becomes apparent[46]. Like SSM, this melanoma subtype shares clinical features respecting the ABCDE rule of melanoma identification. Histologically, atypical melanocytes proliferate in a lentiginous manner in sun-damaged skin that usually exhibits epidermal and dermal atrophy and solar elastosis[47].

1.3.5 Nodular melanoma (NM)

NM comprises approximately one third of diagnosed cases of melanoma[42] and are characterized by an early onset of the vertical growth phase[48]. They appear more often in men

than women and in contrast to SSM and LMM, may not conform morphologically to the ABCDE rule and can more easily evade clinical detection[49]. Histologically, there is dermal melanocytic proliferation extending vertically up to the epidermis[44,45].

1.3.6 Acral lentiginous melanoma (ALM)

ALM is primarily observed on the palms, soles, subungal regions and in the oral mucosa. Accounting for less than 5% of all melanomas, this subtype is the predominant form of melanoma in non-Caucasian populations[50]. Clinically, an ALM usually begins as a variably coloured macule that develops irregular borders and variegation in colour as it expands in size with time. Histologically, early ALM shows diffuse lentiginous proliferation of atypical melanocytes along the basal layer[51].

1.3.7 Desmoplastic melanoma (DM)

DM is a rare melanoma subtype composed of spindle cells surrounded by abundant collagen[52]. Appearance of DM is variable and like NM, typically defies the ABCDE identification scheme. Local control of DM remains problematic due mainly to its proclivity for neurotropism. A systematic review of 14 studies involving DM demonstrated local recurrence rates ranging from 7% to 56%. This is compared with a rate of 3% for other melanoma subtypes. Conversely, nodal and distant metastases, ranging from 7% to 44%, are lower than other forms of cutaneous melanoma[53]. More recent studies have subclassified DM into pure (≥90% desmoplasia) and mixed subgroups as means to delineate its unique clinical behavior. However, subclassification of DM into its histologic subgroups is fraught with interobserver variability and debate persists as to its clinical utility[54,55].

1.4 Management of Melanoma

Management of melanoma is guided by the stage of the tumour at initial diagnosis. Surgical excision remains the definitive treatment for localized disease.

1.4.1 Staging

Staging of melanoma is based on the tumour-node-metastasis (TNM) staging criteria. The most recent TNM categories described by the American Joint Committee on Cancer (AJCC) consider histopathologic factors such as primary tumour thickness, presence of ulceration and rate of mitosis to predict the prognosis and determine the appropriate treatment for localized cutaneous melanoma. Based on several recent survival studies[69,121,123,124], mitosis has replaced Clark level as a stage modifier for T1 melanoma, with a mitosis of $\geq 1/mm^2$ upgrading the staging of a T1 tumour from T1a to T1b. The N designation signifies the degree of regional lymph node involvement subcategorized by microscopic and macroscopic disease, and the M category defines the presence and location of distant metastatic disease. Table 4 summarizes the current AJCC staging system for melanoma[63].

Table 4: TNM melanoma classification		
T	**Thickness (mm)**	**Ulceration Status/Mitoses**
Tis	NA	
T1	≤1.00	T1a: Without ulceration and mitosis <1/mm^2 T1b: With ulceration or mitoses ≥ 1/mm^2
T2	1.01-200	T2a: Without ulceration T2b: With ulceration
T3	2.01-4.00	T3a: Without ulceration T3b: With ulceration
T4	>4.00	T4a: Without ulceration T4b: With ulceration
N	**Number of Metastatic Nodes**	**Nodal Metastatic Burden**
N0	0	NA
N1	1	N1a: micrometastasis[1] N1b: macrometastasis[2]
N2	2-3	2a: micrometastasis 2b: macrometastasis 3c: In-transit metastases/satellites without metastatic nodes
N3	4+ metastatic nodes, or matted nodes, or in-transit metastases/satellites with metastatic nodes	
M	**Site**	**Serum LDH**
M0	No distant metastases	NA
M1a	Distant skin, subcutaneous, of nodal metastases	Normal
M1b	Lung metastases	Normal
M1c	All other visceral metastases Any distant metastasis	Normal Elevated

[1] Micrometastases are diagnosed after sentinel lymph node biopsy
[2] Macrometastases are defined as clinically detectable nodal metastases confirmed pathologically
Adapted from Balch CM, Gershenwald JE, Soong SJ et al. Final version of 2009 AJCC melanoma staging and classification. J ClinOncol 2009; 27: 6199-206.

1.4.2 Treatment

Wide local excision is considered definitive treatment for primary invasive melanoma, although the recommended surgical margin varies in the medical literature. The current Cancer Council of Australia guidelines recommend surgical excision margins up to 2 cm for primary invasive melanoma. For lesions less than 1mm in thickness, a margin of 1cm is recommended. For lesions 1 to 4mm in thickness, a margin of 1-2cm is recommended. For

lesions greater than 4mm in thickness, a 2-cm margin of resection is generally considered adequate. For melanoma in situ, a surgical margin of 5mm is deemed adequate[56].

Sentinel lymph node biopsy (SLNB) is an invasive test that can be utilized in melanoma staging and results from SLNB can confer prognostic information. It is recommended when it may alter the estimated survival rate sufficiently to affect a patient's decision making, and as such, SLNB are most commonly offered to patients with melanomas that are greater than 1mm in Breslow thickness. The survival benefit of regional lymphadenectomy following positive SLNB remains equivocal and its utility both as an investigative tool and therapeutic intervention for patients with primary invasive melanoma is the subject of ongoing debate[57,58].

Therapeutic options for metastatic melanoma have widened in recent years although treatment resistance remains problematic. Traditional chemotherapeutic agents and adjuvant therapy including interferon and radiotherapy confer limited benefit on overall prognosis. Whilst a detailed consideration of the current armamentarium of therapeutic options for metastatic melanoma is beyond the scope of this thesis, it is worthwhile to mention that new drug therapies, including drugs that target melanoma specific receptors and drugs with immunomodulatory functions, are currently in development.

1.5 Prognostic factors

Following the recognition of the Clark levels of invasion and the Breslow thickness in 1969[59] and 1970[60], respectively, new insights have evolved as a result of large-scale population-based analyses on the multitude of clinical and histopathologic variables deemed significant in predicting the clinical behavior of melanoma. Many of the prognostic factors are closely interrelated. For localized melanoma, the dominant predictors of survival remain tumour

thickness and ulceration. Factors such as age, sex, anatomic location, and satellite/in-transit lesions are also recognized as important prognosticators. Evidence is emerging for the possible prognostic value of lymphovascular invasion, tumour regression, perineural invasion, pre-existing naevus and tumour-infiltrating lymphocytes. The significance of mitotic rate is a primary focus of this thesis and will be discussed separately in the Literature Review.

1.5.1 Patient age

Various studies have reported age to be an independent prognostic factor for melanoma survival, with increasing age associated with poor clinical outcomes[5]. In a study of over 17,000 patients, each 10-year increase in age was associated with a decline in both 5-and 10-year survival rates. Patients younger than 30 years of age had a 5-year survival rate of 87% compared to 71% and 60% for those in their 70s and 80s or more, respectively[61]. A smaller study that included 488 patients with primary cutaneous melanoma showed an 84% 10-year survival rate for patients less than 65 years of age compared with 57% for those 65 years of age or greater[62].

1.5.2 Gender

Many studies report that women have a better prognosis compared to men, even in patients with nodal metastasis. Gender follows age in importance in the 2008 AJCC analysis of localized melanoma prognostic factors[63]. Using 68,495 invasive melanoma cases diagnosed from 1992 to 2005 in the Surveillance Epidemiology and End Results (SEER), the risk of death for women was lower than for men (HR 0.76, 95% CI, 0.71-0.81)[64]. In another study investigating gender's influence in cutaneous melanoma, 11,774 melanoma cases were analyzed for survival and disease progression by gender. This study found that women compared with men had a higher melanoma-specific survival (HR 0.62, 95% CI, 0.56-0.70)[65].

They also had significantly lower risks of progression, lymph node metastasis, and visceral metastasis. However, the influence of gender can be confounded by differences in thickness, ulceration, and anatomic site of the melanoma; women have been shown to have thinner lesions with less frequent ulceration compared with men.

1.5.3 Tumour site

A number of studies have reported a correlation between prognosis and anatomic location, noting that lesions of the extremities have a better prognosis than head, neck and truncal melanomas[62,66, 67]. In the absence of metastatic disease and for all ranges of tumour thickness, a 10-year survival rate of 90% was observed when the primary melanoma was on the extremities compared to 70% for axial melanoma[62]. In comparison to melanomas find on the extremities, axial lesions were also more likely to be ulcerated, to have positive lymph nodes, and to be classified as a nodular melanoma. The same study also found 5- and 10-year survival rates for patients with head and neck melanoma of 83% and 76%, respectively, compared with 92% and 89%, respectively, for melanomas at other sites ($P<0.001$).

1.5.4 Tumour thickness

As defined by Dr Alexander Breslow, the Breslow depth of the melanoma, or its tumour thickness, is the number of millimetres measured from the top of granular layer to the most deeply invasive tumor cell[60]. Breslow depth has been validated as an independent prognostic factor[61,62,68,69]. In the 1997 version of the AJCC melanoma staging system, the cutoff point between a T1 and T2 melanoma was defined as 0.75mm[70]. A subsequent revision of the AJCC system in 2002 stratified thickness into four categories, ≤1.0mm (T1), 1.01-2.0mm (T2), 2.01-4.0mm (T3), and >4.0mm (T4)[71]. T1 melanomas are termed “thin”, T2 and 3 melanomas “intermediate” and T4 melanomas “thick”. Ten-year survival rate of patients with

T1 melanomas without other adverse prognostic factors has been reported as 92%, compared with 50% in patients with T4 melanomas[63].

1.5.5 Presence of ulceration

Ulceration is defined as a full-thickness epidermal defect in the absence of trauma or a recent surgical procedure[72]. The incidence of ulceration rises with increasing tumour thickness, ranging from 6%-12.5% for thin (≤1mm) melanomas to 63%-72.5% for thick (>4.0mm) lesions[61,72]. Ulceration has been deemed an important independent prognostic feature of a primary melanoma, second in significance to tumour thickness[61,73]. Thus the presence of ulceration raises a primary melanoma to the next T category's risk level[63].

1.5.6 Clark level of invasion

The Clark level describes the anatomic parts of the skin affected by the melanoma and indicates the depth to which it has invaded. Clark level I defines intraepidermal melanoma with an intact basement membrane; level II signifies the melanoma is in the papillary dermis; level III denotes a tumour that fills and expands the papillary dermis; level IV represents invasion into the reticular dermis, and level V defines melanoma encroaching into the subcutaneous fat[59]. The significance of Clark level in melanoma prognostication has been equivocal. Although five-year survival for Clark level II melanomas has been reported as 95%, falling to 55% for Clark level V melanomas[68], these results were confounded by tumour location, thickness and ulceration. Several recent studies have shown that Clark level is not an independent predictor of outcome, even in thin melanomas, when age, tumour location, tumour thickness, ulceration and mitotic rate are taken into account[74,75]. Currently, Clark level remains part of the AJCC staging system for patients with thin melanoma[76] and is used

when mitotic rate has not been evaluated, such that in the absence of known mitotic rate, the designation of Clark level of IV or V will upgrade a thin tumor from T1a to T1b.

1.5.7 Other melanoma histologic prognostic factors

In addition to the AJCC melanoma staging criteria, other clinical and histologic prognostic factors have been found to influence outcome. Although the evidence is inconclusive in relation to their independent prognostic value they may assist clinical decision-making. The prognostic information derived from histologic features is summarized in Table 5.

a) Lymphovascular invasion

Lymphovascular involvement denotes invasion of tumour cells into the microvasculature in the dermis by either abutting or penetrating the endothelium and lodging within the vessel lumen. Lymphovascular invasion have been shown to significantly increase the risk of relapse, lymph node involvement, distant metastases, and death[77-81]. In thick melanomas the presence of lymphovascular involvement by tumour cells is associated with a 5-year overall survival rate of 25% compared to 50% without lymphovascular involvement[82].

b) Microsatellitosis

Microsatellites are discrete tumour nests that are separated from the main body of the tumour by normal reticular dermal collagen or subcutaneous fat[83]. Microsatellitosis was found to increase from 4.6% in tumours less than 1.5mm thick to 65% in those greater than 4mm. In the former group, 5-year survival rate for patients with tumours showing microsatellites was 36% compared to 89% for those with no microsatellites[83]. In tumours greater than 1.5mm thick, microsatellites are associated with a significant increase in the frequency of regional lymph node metastasis.

c) Regression

Regression indicates the replacement of tumour tissue with fibrosis, degenerated malignant melanoma cells, lymphocytic proliferation, and the formation of telangiectatic vessels[84]. The role of regression in determining survival is unclear due to inherent inconsistencies in the interpretation of these features by dermatopathologists. One study showed regression to correlate with adverse clinical outcomes[85], whereas another found that regression seen in thin melanoma confers reduced metastatic risk[86].

d) Tumour-infiltrating lymphocytes

Tumour-infiltrating lymphocytes are believed to represent the response of the immune system to melanoma cells. This response is usually gauged by the level of lymphocytic infiltrate present at the leading edge of tumour invasion. It can be categorized by dermatopathologists as brisk, non-brisk, or absent[87] and survival rates have been noted to decline with increasing degree of tumour infiltrating lymphocytes[88].

e) Neurotropism

Neurotropism describes the finding of melanoma cells within neural structures (intraneural) or surrounding nerves (perineural) or finding cells exhibiting neural or nerve sheath differentiation. Neural involvement is associated with an increased risk of local recurrence, although association with metastatic disease is unclear[89]. As aforementioned, the desmoplastic melanoma histologic subtype has show a predilection for neurotropism[53,90].

f) Pre-existing naevus

The presence of a benign melanocytic naevus situated within a melanoma has been reported to range from 10% to over 50% of cases, with the variation potentially due to different criteria being applied to identify naevus and melanoma cells[91-94]. This finding is often assumed to mean that the melanoma has arisen from melanocytes within the naevus and these melanomas are termed "naevus associated melanoma" or "melanoma with pre existing naevus". Melanomas without this finding are typically termed "de novo melanoma". A fair skin complexion and a history of sunburn are significant associations of naevus-associated melanoma when compared with de novo melanoma[95]. Naevus associated melanoma is predominantly seen with the superficial spreading melanoma histologic subtype[96] and has been associated with a favourable prognosis[101], although this statistical advantage in survival was lost when taking into account tumour thickness[97].

Table 5: Melanoma histologic features with prognostic value found in the dermatopathology report

Histologic feature	Description	Potential impact on prognosis
Histologic Subtype	SSM LMM NM DM ALM	LMM and SSM have been found to have a better prognosis than NM and ALM, but when controlling for tumour thickness, studies have not consistently found a significant difference between subtypes. DM has the propensity for neurotropism and local recurrence.
Tumour thickness	Measure of the melanoma from the top of granular layer down to the lowest tumour cell in millimetres	Consistently proven as the dominant independent prognostic indicator in primary invasive cutaneous melanoma.
Ulceration	Full-thickness epidermal defect in the absence of trauma or recent surgical procedure	Widely recognized as a significant independent prognostic indicator, second only to tumour thickness.
Clark level of invasion	A description of the anatomic involvement of the melanoma within the cutaneous and subcutaneous structures	Equivocal prognostic value although Clark level of invasion is still used in the staging of T1 melanoma by the AJCC melanoma staging system when the mitotic rate is unknown.
Microsatellitosis	Microsatellites are discrete tumour nests greater than 0.05 millimetres in diameter that are separated from the primary tumour by normal reticular dermal collagen or subcutaneous fat	Significantly increases the risk of regional lymph node spread.
Lymphovascular invasion	Presence of tumour cells within a vessel lumen	Potential marker for haematologic and lymphatic spread of melanoma cells, but there is a high potential for misinterpretation in the presence of torturous vessels; Prognostic power overlaps with angiotropism.
Tumour infiltrating lymphocytes	Brisk: diffuse infiltrate of lymphocytes throughout the dermal tumour cells or the presence of lymphocytes along 90% of the circumference of the lesion base Non-brisk: focal infiltrate of lymphocytes Absent: no lymphocytes are admixed with melanoma cells	Presence of a host inflammatory response is generally associated with a better prognosis but prognostic power is limited because of the inconsistency in controlling for other prognostic features in previous studies.
Regression	Thought to be caused by interaction between the host immune response and the tumour cells in which there is partial or complete absence of tumour cells in both the dermis and epidermis found within a melanoma	Unclear prognostic value because of inconsistencies in definition and measurement and lack of control of other histologic variables.
Neurotropism	Neoplastic infiltration of nerve fibres, recognized as either intraneural or perineural invasion.	Found to increase risk for local recurrence with an unclear role in metastatic disease, but there is limited data reported in the literature.
Pre-existing naevus	The presence of remnants of melanocytic naevi in melanoma.	Predominantly is seen with SSM and is thought to be associated with improved clinical outcome.

SSM, superficial spreading melanoma; LMM, lentigo maligna melanoma; NM, nodular melanoma; DM, desmoplastic melanoma; ALM, acral lentiginous melanoma; AJCC, American Joint Cancer Committee; Adapted from Payette MJ, Katz M, Grant-Kels JM. Melanoma prognostic factors found in the dermatopathology report. J ClinDermatol 2009; 27-74.

1.6 Mitotic rate

A histologic feature of primary invasive melanoma, mitotic rate (MR) is gaining recognition in recent years as a marker of tumour proliferative potential. The following discusses the biological relationship of tumour mitotic rate with tumour cell development, the histological assessment of tumour mitotic rate, the clinical associations of mitotic rate and the implications for melanoma prognostication in the context of the current AJCC melanoma staging system.

1.6.1 Cell mitosis biology

Mitosis is the process by which a parent cell divides into two identical daughter cells through first replication and then separation of genetic information. Crucial for cellular growth and repair, the process of mitosis is complex, with the sequence of events divided into stages corresponding to the completion of one set of events and the beginning of the next. These stages are prophase, prometaphase, metaphase, anaphase and telophase. The primary result of mitosis is the transfer of the parent cell's genome into two daughter cells. These two cells are identical to the original parent cell[102].

The process by which mitosis takes place is regulated by the genetic make-up of the cell but should these regulatory mechanisms fail, cell division can proceed unchecked, resulting in excessive and uncontrolled cellular growth and proliferation and ultimately tumour development, be it clinically benign or malignant. The presence of mitotic figures in any primary tumour shows that the cells are dividing and that the tumour is actively growing. The number of mitotic figures may therefore correlate with the growth and metastatic potential of the tumour.

1.6.2 The study of mitotic rate in melanoma prognosis

A major barrier to the study of MR in primary melanomas is its infrequent inclusion in routine histopathologic reporting. Even in a geographic area with a high melanoma incidence, such as

Queensland, Australia, fewer than 50% of pathology reports on primary melanomas documented MR in a recent study assessing the completeness of histopathologic reporting of melanoma[98]. Similarly, in another recently published study undertaken at the H. Lee Moffitt Cancer Center in Florida, 47% of outside pathology reports for patients with thin (≤1mm) or in situ melanoma failed to report MR[99].

Reasons for MR not being consistently examined in many series range from omission due to its perceived insignificance to lack of consensus about the method of recording mitoses. Previously, the method used to determine MR was based on recommendations of the 1972 International Pigment Cell Conference for the classification of malignant melanoma. The average number of mitoses in at least 10 high power fields (HPF) over the entire component of the lesion was obtained and then expressed as the number of mitoses per 5 HPF. For small melanomas, where the dermal component was <10 HPF, the entire melanoma was assessed.

Confounding the matter is that MR may be analyzed either as a continuous or as a categorical variable. As both forms of considering this variable have been used in univariate and multivariate studies alike, discordance in results among various studies have eventuated[66,73,74,100,101]. This is further considered in Chapter 4.

For all these reasons, a standardized assessment of mitotic rate is now recommended as a required component of pathology reporting by the AJCC, with results expressed as mitoses per millimetre squared (/mm^2). To be accepted as a mitotic figure, finger-like extensions of chromatin, extending from a condensed chromatin mass, must be unequivocally present, and have to correspond to either a metaphase or a telophase figure. The method used to count MR is as follows: all hematoxylin and eosin 5μm thick histologic sections of each specimen are evaluated to determine the location of the tumour in which the number of mitotic figures is typically greatest. Generally, mitotic activity is more pronounced

at the invading front (generally the deep border) of the tumour. The number of mitotic figures, beginning in the area with the greatest number of mitoses, is extended to adjacent contiguous fields until a 1-mm^2 area is reached[103,104]. This method of recording mitoses has provided an acceptably reproducible approach[66,73].

Conversion of measurement of mitotic rate in mitoses per mm^2, to measurements in HPF (or vice versa), which approximates to a ratio of 1:5, can be highly variable[105] and introduces difficulty when comparing studies utilizing different methods of measurement.

1.6.3 Introduction of mitotic rate to the AJCC melanoma staging system

The poor survival of patients who had mitotically active melanomas was first reported by Allen and Spitz in 1953[106]. Since then, MR has emerged as an independent prognostic marker of melanoma survival. Detailed analysis of data on primary tumour MR recorded in the AJCC Melanoma Staging Database, which contains information from multiple cancer cooperative groups, has supported previous findings that there is a significant correlation between increasing MR and declining survival. The 2009 AJCC classification introduced a number of significant modifications to its 2002 classification, including MR of at least 1 mitosis/mm^2 replacing Clark level of invasion as the primary criterion for defining T1b category[63,103] (Table 6). Tumour thickness, MR, and ulceration are thus designated as the primary independent prognostic factors in defining the local tumour burden based on the results of this multivariate study (Table 7). Combining these three components, 5- and 10- year survival rates were 97% and 93% respectively in patients with T1a melanoma and 53% and 39% respectively in patients with T4b melanoma ($P<0.0001$)[63,69]. The 5-year survival ranged from 97% with less than 1 mitosis/mm^2 to 60% with greater than 20 mitoses/mm^2 for stage 1 and 2 disease (Table 8).

However, through the examination of multiple MR thresholds, a mitotic count ≥ 1/mm^2 was shown to have the most significant correlation with patient survival. The 10-year survival rate declines to 87% in T1b patients whose Breslow thickness is 0.5 to 1.0mm compared with a 93% survival rate in T1a patients whose melanoma is of the same Breslow depth. Hence, although tumour thickness serves as the primary prognostic factor, MR and ulceration is believed to further stratify Breslow thickness as means to improve its prognostic accuracy[103].

When comparing the survival rates reported in the 2002 and 2009 AJCC staging manuals, similar 5-year survival rates were found in all categories, including the T1b category, despite the change in category criteria (Table 9). Whilst prognosis for patients worsens with increasing MR, the current AJCC staging implies that the predictive role of MR in melanoma survival remains limited to those with T1 melanoma. Tumour thickness and ulceration continue to serve as the dominant prognostic variables for T2-4 disease.

Most recently, the value of MR has been extended to stratifying the risk of sentinel lymph node positivity in patients with thin melanomas (≤1mm). Whilst sentinel lymph node biopsy (SLNB) is generally not recommended for melanomas ≤0.75mm thick, current recommendations from the National Comprehensive Cancer Network (NCCN) highlight the possible designation of MR as a high-risk feature in 0.76-1.00mm thick melanomas along with tumour ulceration, hence in this group of patients, SLNB may be considered in the appropriate clinical setting[107].

Table 6: Differences between the sixth edition (2002) and the seventh edition (2009) of the melanoma staging system

Factor	Sixth Edition Criteria	Recommended Seventh Edition Criteria	Comments
Thickness	Primary determinant of T staging	Same	Thresholds of 1.0, 2.0 and 4.0mm
Clark level of invasion	Used only for defining T1 melanomas	Same	Used as a default criterion only if mitotic rate cannot be determined
Ulceration	Included as a secondary determinant of T and N staging	Same	Signifies a locally advanced lesion; dominant prognostic factor for grouping stages I, II and III
Mitotic rate per square millimetre	Not used	Used for categorizing T1 melanoma	Mitosis $\geq 1/mm^2$ used as primary criterion for defining T1b melanoma
Satellite metastases	In N category	Same	Merged with in-transit lesions
Immunohistochemical detection of nodal metastases	Not included	Included	Must include at least 1 melanoma-associated marker unless diagnosis cellular morphology is present
0.2mm threshold of defined N+	Implied	No lower threshold of staging N+ disease	Isolated tumour cells or tumour deposits<0.1, meeting the criteria for histologic or immunohistochemical detection of melanoma should be scored as N+
Number of nodal metastases	Primary determinant of N staging	Same	Thresholds of 1, 2-3, 4+ nodes
Metastatic volume	Included as a second determinant of N staging	Same	Clinically occult (microscopic) nodes are diagnosed at sentinel node biopsy whilst clinically apparent (macroscopic) nodes are diagnosed by palpation or imaging studies or by finding of gross extracapsular extension in a clinically occult node
Lung metastases	Separate category as M1b	Same	Has a somewhat better prognosis than other visceral metastases
Elevated serum LDH	Included as a second determinant of M staging	Same	Recommend a second confirmatory LDH level if elevated
Clinical versus pathologic staging	Sentinel node results incorporated into definition of pathologic staging	Same	Large variability in outcome between clinical and pathologic staging

LDH, lactate dehydrogenase

Adapted from Balch CM, Gershenwald JE, Soong SJ, et al. Melanoma of the skin. In: Edge SB, Byrd DR, Compton CC, et al, editors. AJCC staging manual. 7th edition. New York; Springer 2010. P.325-44.

Table 7: Multivariate Cox regression analysis of prognostic factors in 10,233 patients with localized cutaneous melanoma (Stage I and II)

Variable	Chi-Square (1 df)	P	HR	95% CI
Tumour thickness	84.6	<0.0001	1.25	1.19-1.31
Mitotic rate	79.1	<0.0001	1.26	1.20-1.32
Ulceration	47.2	<0.0001	1.56	1.38-1.78
Age	40.8	<0.0001	1.16	1.11-1.22
Gender	32.4	<0.0001	0.70	0.62-0.79
Site	29.1	<0.0001	1.38	1.23-1.54
Clark level	8.2	0.0041	1.15	1.04-1.26

CI, confidence interval; HR, hazard ratio.
Adapted from Balch CM, Gershenwald JE, Soong SJ, et al. AJCC staging manual. 7th edition. New York: Springer; 2010. P.325-44.

Table 8: 2008 AJCC melanoma staging database on mitotic rate and survival

Number of Mitoses/mm^2	N	Survival rate (%)± SE	
		5 years	10 years
0<1	3312	97.3±0.4	92.7±0.7
1<2	2117	92.0±0.7	84.2±1.2
2<5	3254	86.9±0.7	75.4±1.2
5<11	2049	78.1±1.1	68.0±1.8
11<20	673	69.5±2.2	57.6±2.7
≥20	259	59.4±3.9	47.6±5.0
Total	11,664[1]		

[1]Included patients with mitoses, tumour thickness, and follow-up information available.
Adapted from Balch CM, Gershenwald JE, Soong SJ, et al. Melanoma of the skin. In: Edge SB, Byrd DR, Compton CC, et al, editors. AJCC staging manual. 7th edition. New York: Springer 2010. P.325-44.

Table 9: Comparison of the 2002 and 2009 AJCC melanoma staging database T classification survival rates in patients with localized melanoma (stage I and II)

Stage	T classification	2002 AJCC 5-year survival	2009 AJCC 5-year survival
IA	T1a	95%, n = 4510	97%, n = 9452
IB	T1b[1]	91%, n = 1380	94%, n = 2389
	T2a	89%, n = 3285	92%, n = 6429
IIA	T2b	77%, n = 958	82%, n = 1517
	T3a	79%, n = 1717	79%, n = 3127
IIB	T3b	63%, n = 1523	68%, n = 2164
	T4a	67%, n = 563	71%, n = 1064
IIC	T4b	45%, n = 978	53%, n = 1397

[1] 2002 T1b criteria used Clark level of invasion, whereas the 2009 T1b criteria uses mitotic rate.
Data from Balch CM, Gershenwald JE, Soong SJ, et al. Melanoma of the skin. In: Edge SB, Byrd DR, Compton CC, et al, editors. AJCC staging manual. 7th edition. New York: Springer; 2010. P.325-44; and Balch CM, Atkins MB, JE, et al. Melanoma of the skin. In: Green FL, Page DL, Fleming ID, et al, editors. AJCC staging manual. 6th edition. New York: Springer; 2002. P.209-20.

1.7 Research questions and outline of thesis

As MR is increasingly recognized as a biologic marker of melanoma aggressivity, this may carry implications for the way in which we detect and assess patients. The relative scarcity of research into mitotic rate in the current literature served as the impetus for the current thesis, which presents two separate analyses aimed at illuminating the importance of mitotic rate in the care of patients with primary cutaneous invasive melanoma. The specific questions to be addressed are:

(i) What are the distinct clinical and histopathologic characteristics of patients with high mitotic rate melanoma?

(ii) Does MR relate to melanoma-specific mortality in the Australian population?

Following an overview of the current literature on the associations and prognostic significance of mitotic rate in Chapter 2, the principal studies addressing the above two research questions are presented in Chapters 3 and 4 respectively. Relevant subsections including the aims, methodologies and results of each study are detailed within the content of each chapter, structured in the form of an original research publication in accordance with the stipulated format of the peer-reviewed journal to which it was or will be submitted. Finally, Chapter 5 summarises the main findings of the thesis, provides a critical appraisal of such findings within the context of previously reported data and proposes suggestions for ways to further our understanding of tumour aggressivity and prognostic prediction inpatients with primary cutaneous melanoma.

Chapter 2: Review of Literature

Outline of Chapter 2

This chapter firstly describes the clinicopathologic factors associated with the development of aggressive primary melanoma. Secondly, a formal review of the existing evidence in the current literature of the prognostic significance of mitotic rate for melanoma survival will be presented.

2.1 Characteristics and associations of aggressive primary melanoma

Beyond the well-established risk factors of melanoma development[34-35], attention has turned to gaining a better understanding of the pathogenesis and characteristics of melanoma associated with poor clinical outcome and is responsible for driving melanoma mortality rates. In identifying the potential patient risk groups, studies have thus focused on the clinical and pathologic factors associated with thick and rapidly growing melanomas. The classification of melanoma by histologic subtype has also been recognized as having potential prognostic value, with nodular melanoma thought to behave more aggressively than other melanoma subtypes. What has surfaced from the studies that have addressed this question thus far is a distinct set of phenotypic, clinical and pathological features shared by aggressive forms of primary cutaneous melanoma, which is listed in Table 10.

Table 10: Reported associations of aggressive primary cutaneous invasive melanoma

Patient characteristics
Male
Elderly
History of cumulative sun exposure
Low naevi and freckle count
Residing alone
Tumour presentation
Atypical clinical features (symmetry, border regularity)
Location on the head and neck
Rapid growth rate
Amelanosis
Histopathologic features
Increasing thickness
Presence of ulceration
Increasing mitotic rate
Nodular subtype

2.1.1 Associations of thick melanoma

Prognosis for melanomas defined as thin (≤1mm) is excellent as they are generally cured with surgery whereas long-term survival for thick tumours (> 4mm) is appreciably poorer. Several studies in Australia and elsewhere[16, 108-111] have sought to delineate the particular characteristics of advanced-stage melanoma at diagnosis. One of these studies was by Chamberlain et al, which examined the clinical associations of thick melanoma based on two groups of patients with invasive melanoma – those derived from the 1998 Victorian Cancer Registry and those treated at the Victorian Melanoma Service between October 1, 1994 and April 31, 1999. In total, 1809 patients were included in the study. Melanomas were categorized by thickness into thin (≤1mm), intermediate (>1-3mm) and thick (>3mm) and compared according to patient age, sex, tumour type and site. Patients at risk of developing thick melanoma were identified as older men, those presenting with head and neck lesions and phenotypically had fewer naevi[16].

More recently, Grange et al investigated the clinical and sociodemographic factors associated with thick melanoma based on a retrospective, population-based study in France using a survey of cancer registries and questionnaires to practitioners[112]. Among 898 melanomas, 149 (16.6%) were categorized as thick lesions (≥3mm). By multivariate analysis, factors independently associated with thick melanomas were the nodular and acrolentiginous subtypes; location on the head and neck and lower limb; older age, male sex and individuals living alone. Moreover, immediate clinical recognition by dermatologists was found to be poorer for thick melanomas than thin tumours (77.1% versus 88.8%; p-value = 0.01)[112].

2.1.2 Associations of rapidly growing melanoma

Liu et al in 2006[113] investigated the spectrum of growth rates in melanomas and identified the characteristics and associations of rapidly growing tumours. Rate of growth in primary invasive

melanoma was calculated as the ratio of Breslow thickness to time to melanoma development. One third of the melanomas grew ≥0.5mm per month. The median monthly growth rate was 0.12mm for superficial spreading melanomas, 0.13mm for lentigo maligna melanomas, and 0.49mm for nodular melanomas. Rapidly growing tumours occurred more commonly in males, older individuals and those with fewer melanocytic naevi and freckles. Predominantly of the nodular subtype, these lesions were found to possess atypical morphological features that may delay detection, including having symmetry, amelanosis and regularity in border.

2.1.3 Associations and significance of nodular melanoma

Nodular melanoma (NM) has gained recognition as a melanoma subtype distinct in its clinical behavior from other subtypes, due to a potential for rapidly developing depth of invasion and atypical clinical morphology that can confound diagnostic accuracy[10]. After undertaking a comparison of their clinical and histopathologic features at a single institution over three decades, Warycha et al in 2008 contended that nodular melanoma is associated with more aggressive histologic features than superficial spreading melanoma and its rate of immediate clinical detection, unlike the latter, did not improve over time[114]. In this study, a total of 1684 patients diagnosed with 1734 melanomas were prospectively enrolled. Of these, 1143 patients (69% SSM, 11% NM, 23% other) were diagnosed between 2002 and 2007. Superficial spreading melanomas were diagnosed as thinner lesions over time with a low incidence of histologic ulceration, whereas there was no significant change in the median tumour thickness or ulceration status of nodular melanomas during the same time period. The median age at diagnosis of nodular melanoma, however, did significantly increase over time (51 years to 63 years, $P<0.01$). With findings suggesting that improvements have been made in the early detection of superficial spreading melanoma but not nodular melanoma, the investigators, like several others[113, 115], thus

advocated for a greater emphasis on "E" for "evolution" criterion of the ABCDE rule as means to improve clinical detection of the nodular melanoma subtype[114].

More importantly, nodular melanoma has been implicated as a main contributor to melanoma deaths and its incidence is continuing to rise in Australia despite more vigilant public health preventive strategies. A recently published Victorian study comprising of 5775 cases of primary invasive cutaneous melanoma reported to the Victorian Cancer Registry during 1989, 1994, 1999 and 2004 revealed that despite nodular melanoma only accounting for 14% of cases of melanoma diagnoses, it was responsible for 43% of melanoma-specific deaths. In contrast, whilst superficial spreading melanoma accounted for the majority of cases (56%), it contributed to fewer (30%) deaths[6].

Evidence for the burden of nodular melanoma however, has proven to be inconsistent across different studies. Two previous published studies examined the contribution of tumour type to melanoma deaths using the Surveillance, Epidemiology and End Results (SEER) cancer registry data[116, 117]. Both studies however, had high proportions of melanoma of indeterminate subtype. Of all melanoma deaths, Criscione and Weinstock reported 18% caused by NM, 17% by SSM and 54% by melanoma of indeterminate subtype. Data from the study of Shaikh et al revealed that 19% of deaths were caused by NM, whilst 24% of deaths were by SSM, and 49% by melanoma of indeterminate subtype[117].

2.1.4 Associations of mitotic rate (MR)

The clinical and histopathologic associations of high MR melanoma have yet to be addressed as a primary focus of any particular study in the current medical literature, although this has been alluded to in several studies. Rate of tumour growth was observed to be a clinical surrogate of

MR[113]. As aforementioned in Chapter 1, Section 1.6.1, the presence of many mitotic figures in any primary tumour is indicative of active cell division. This is likely to correspond clinically with more rapid tumour rate of growth and greater propensity for distant spread.

A previous observation was made of a close correlation existing between MR and primary tumour thickness, as well as MR and ulceration. A degree of mitotic activity was observed in most patients with melanomas that were greater than 2.5mm in thickness, but activity was uncommon in those with primary tumours less than 1mm thick. Likewise, in patients with ulcerated melanomas from the same thickness groups, the mean number of mitoses increased to 2.3/mm^2 and 10.7/mm^2 respectively[69].

2.2 Prognostic significance of mitotic rate (MR)

Allen and Spitz first described in 1953 the poor prognosis in patients with high MR primary melanomas[107], with the observation that the survival of patients "varied significantly with the abundance or dearth of mitotic figures". Nearly half a century later, MR began to be identified as having independent prognostic value. Whilst evidence began to emerge of the robust predictive value of MR, culminating in its inclusion in the current AJCC melanoma staging system[103], there is, however, no consistent approach in the statistical analysis of MR in multivariate models.

For this thesis, a review of the English-language medical literature relating to the prognostic significance of MR in primary invasive melanoma was conducted using PubMed and Ovid searches. Multivariate analyses of mitotic rate as a predictor of disease-specific or overall survival in primary cutaneous melanoma were included in this review. A variety of separate and combined search terms including "mitotic rate", "melanoma", "prognosis", "survival" and "multivariate" were used. What follows is a discussion of the findings of this systematic review,

as summarized in Table 11, and a critical analysis of the existing evidence base, highlighting the varied analytic approaches with which mitotic rate has been examined in the context of established prognostic factors.

2.2.1 Mitotic rate as a predictor of survival

In 1989, Vollmer[73] was amongst the first to highlight the possible independent prognostic value of MR from the summary of 31 multivariate studies that included MR, out of which 14 showed it carried independent prognostic significance. Vollmer did note, however, that many of the studies utilized inconsistent methodological approaches that prevented fair and meaningful comparative analyses. In the same year, Clark et al examined the prognostic value of 23 clinicopathologic variables amongst 386 cases of Stage 1 primary melanoma and determined six variables that had independent prognostic significance. Out of the six, mitotic rate (/mm^2) had the greatest predictive value for survival, surpassing tumour infiltrating lymphocytes, tumour thickness, anatomic site, histologic regression and patient gender [66]. Subsequently, there have been ten multivariate analyses examining MR as an independent prognostic factor[66,69, 118-126]. All 11 multivariate studies are summarized in Table 11 and further discussed in Chapter 4.

MacKie et al in 1995[118], in a retrospective cohort study of 289 patients with primary cutaneous malignant melanomas diagnosed in Scotland between 1979 and 1986, identified four distinct subgroups of males and females with ulcerated or non-ulcerated lesions. Prognosis was shown to be markedly different across subgroups of the melanoma population, even to the extent that essential prognostic factors were not the same in the distinct subgroups[118]. Mitotic rate, expressed in HPF and investigated categorically, only retained prognostic value for females with ulcerated melanoma, along with tumour thickness and anatomical site of the primary tumour.

In a study of 691 cases of primary invasive melanoma, Ostmeier et al in 1999[119] examined MR as a categorical variable (<3, ≥3 mitoses/mm^2) in the multivariate cox regression analysis with metastasis as the end-point. Ulceration lost its prognostic significance when MR was included in the analysis, a finding that was later reproduced by Barnhill et al in 2005[122] when MR was again examined categorically.

Massi et al, in 2000[120], based on a retrospective cohort study of 140 patients, evaluated the clinical outcome of patients with >3mm thick cutaneous melanoma and tested the prognostic value of a series of clinicopathological parameters on disease-free and cause-specific survival. In the multivariate analysis, tumour thickness, infiltrating invasive front, presence of ulceration and mitoses/mm^2 remained significant independent predictors of poor clinical outcome[120]. A year later, Schmid-Wendtner et al[126], based on a retrospective study of 2715 patients treated for one invasive cutaneous melanoma between 1968 and 1992, evaluated the clinical utility of the modified prognostic index, defined as the product of square tumour thickness and number of mitoses/mm^2 and asserted its usefulness for defining a subgroup of patients who are at risk of developing metastases[126].

An subsequent attempt by Retsas et al to validate the 2002 AJCC proposal for the introduction of ulceration of the primary cutaneous melanoma as an independent prognostic factor in a retrospective cohort study of 1284 patients instead revealed mitotic rate superseding ulceration in predictive value[121]. Patients with a histological diagnosis of malignant melanoma at all primary anatomical sites and stages were registered consecutively with the Melanoma Unit at the Charing Cross Hospital in a prospectively maintained electronic database. Clinic-pathological variables known for their prognostic significance were studied. Univariate and multivariate analyses revealed MR as a predictor for survival second only to thickness in predicting overall survival[121].

Subsequently, Azzola et al[123] and Francken et al[124] again provided evidence for the predictive value of MR for melanoma survival based on separate cohorts from the Sydney Melanoma Unit registry.

A large multi-centre study[69] investigating the survival impact of MR compared with other clinical and pathologic features in patients with AJCC stages I and II melanoma also noted the independent prognostic value of MR for melanoma survival. The study supported previous findings that in patients with a localized primary melanoma, a high tumour MR reflects more aggressive tumour behavior that is associated with poorer survival[69].

From the eleven recent multivariate studies identified in the systemic review, there was one study in which MR failed to reach statistical significance as prognosticator of clinical outcomes. A study of 832 patients with primary invasive melanoma from the New York University Melanoma Cooperative Group (NYU-MCG) database examined MR as a continuous variable in prognosticating 10-year survival[125]. With a mean follow-up duration of 9.3 years, univariate analysis showed that survival declined with increasing mitotic rate. The 10-year survival was 91.4% if no mitoses/mm^2 were found, 78.2% for 2 mitoses/mm^2 and 68.8% for 3 or more mitoses/mm^2. When subjected to multivariate analysis against thickness, ulceration, age, sex and site, the number of mitoses did not reach statistical significance in predicting survival amongst patients in the NYU-MCG database[125].

Table 11: Summary of recent multivariate studies on mitotic rate as a predictor of survival

Studies	No. of patients	Mean follow-up (years)	Factors studied	Method of analysis of MR	End-point	Significance of MR
Thompson et al, 2011	10,233	Not specified	7 (age, sex, site thickness, MR, ulceration, Clark level)	Categorical (MR = 0, 0<1, 1<2, 2<5, 5<10, 10<20, ≥20/mm^2)	10-year survival	<0.001
Barnhill et al, 2005	473	> 5	5 (age, thickness, TMR, ulceration, solar keratosis)	Categorical (MR = 0, 1-6, >6/mm^2)	5-year survival	<0.01
Francken et al, 2004	1211	4.5 (censored cases, median) 13.8 (uncensored cases, median)	6 (AJCC Stage, site, age, MR, Clark level, sex)	Continuous analysis with categorical coding (MR = ≤2, >2-8, >8/mm^2)†	10-year survival	0.008
Azzola et al, 2003	3661	4.3 (censored cases)	7 (age, sex, site, thickness, MR, ulceration, Clark level of invasion)	Continuous analysis with categorical coding (Method A: MR = 0, 1-4, 5-10, ≥11/mm^2; Method B: MR = 0-1, 2-4, ≥5/mm^2)†	10-year survival	<0.0001
Retsas et al, 2002	1284	5.5	9 (thickness, MR, age, ulceration, sex, prognostic index, Clark level, bleeding from primary lesion, site)	Continuous	10-year survival	0.005
Schmid-Wendtner et al, 2001	2715	7.5	8 (thickness, MR, sex, site, Clark level of invasion, tumour type, age, prognostic index)	Method A: Categorical (MR≤4, >4.0-8.8, 8.9≤14.5, >14,5) Method B: PI Method C: Modified PI	Metastasis	<0.05 (categorical) <0.0001 (PI, modified PI)
Massi et al, 2000	275	8.6	7 (thickness, ulceration, MR, macroscopic thickness, tumour invasion front, tumour type, lymphovascular invasion)	Continuous and categorical (MR = <10, ≥10/mm$^{2)}$	10-year survival	0.004 (continuous) 0.005 (categorical)
Ostmeier et al, 1999	691	7.0 (median)	5 (thickness, ulceration, MR, site, age, sex)	Categorical (MR = <3, ≥3/mm^2)	Metastasis	0.0235
MacKie et al, 1995	289	5.2	8 (thickness, ulceration, MR, Clark level, tumour type, age, sex, site)	Categorical (MR = <1, 1-5, >5/10HPF)	5-year survival	0.02
Vossaert et al, 1992	832	9.3	6 (thickness, site, ulceration, MR, age, gender)	Continuous	10-year survival	0.1064
Clark et al, 1989	286	13.9	23 (tumour type, Clark level, microsatellitosis, multiple primary tumours, regression, thickness, ulceration, vascular invasion, angiogenesis, MR, multiple cell populations, nodular growth, plasma cells, predominant cell type, tumour	Categorical (MR = 0, 0.1-6.0, >6.0)	8-year survival	OR for survival (MR=0; OR = 11.7, MR=0.1-6.0; OR = 3.5; MR >6.0, OR =1)

	infiltrating lymphocytes, lymph node dissection, age, sex, site, prophylactic regional lymph node dissection, adjuvant therapy, year of wide excision)	

† Mitoses/mm^2 were approximated from mitoses/5HPF (high power field), MR, mitotic rate; AJCC, American Joint Cancer Committee; PI, prognostic index (tumour thickness/mm x mitoses/mm^2), modified prognostic index (tumour thickness/mm^2 x mitoses/mm^2), OR, odds ratio

2.2.2 Mitotic rate as a predictor of sentinel lymph node positivity

Lymphatic mapping and sentinel lymphadenectomy (LM/SL) provide prognostic information for patients with localized cutaneous melanoma, although its clinical utility is still under investigation. The predictive role of MR for sentinel lymph node (SLN) positivity, although not a primary focus of this thesis, is discussed for completeness and recent studies examining its role is summarized in Table 12.

Kesmodel et al, based on a retrospective study of 181 patients who underwent LM/SL from January 1996 through January 2004, identified MR as a significant predictor of SLN positivity by multivariate analysis, with the finding that MR > 0/mm^2 is a significant predictor of SLN positivity. Sondak et al[128] and Kruper et al[129] also demonstrated the utility of MR in stratifying risk for SLN positivity. Conversely, Roach et al, based on a study of patients with ≥1mm thick melanoma, showed that MR was only weakly prognostic of SLN status[130]. Whilst SLNB is conventionally offered to patients with intermediate thickness melanomas irrespective of MR, the most current NCCN guidelines have recognized the potential predictive value of MR for SLNB positivity in a subgroup of patients with thin melanomas, that is, those with 0.76 -1.00mm thick tumours that are ulcerated and have a MR ≥1[107].

Table 12: Summary of recent multivariate studies on mitotic rate as a predictor of SLN positivity				
Studies	**No. of patients**	**Factors studied**	**Method of analysis of mitotic rate**	**Independent predictor**
Roach et al, 2010	551	9 (MR, thickness, ulceration, age, sex, Clark level of invasion, regression, site, lymphovascular invasion)	Categorical (MR=<6, ≥6/mm^2)	No
Kruper et al, 2006	791	9 (sex, site, age, thickness, Clark level of invasion, tumour infiltrating lymphocytes, MR, ulceration, regression)	Categorical (MR=0, 0.1-5, >5)	Yes
Kesmodel et al, 2005	227	7 (age, sex, site, MR, thickness, Clark level of invasion, ulceration)	Binary (0, >0)	Yes
Sondak et al, 2004	419	12 (age, sex, site, lymphovascuar invasion, regression, vertical growth phase, satellitosis, ulceration, neurotropism, periadnexal extension, thickness)	Categorical (MR<1, 1-5, >5)	Yes
Wagner et al, 2000	275	6 (thickness, Clark level of invasion, MR, regression, ulceration, vertical growth)	≤2, 3-5, ≥6/HPF	Yes
SLN, sentinel lymph node; CI, confidence interval; MR, mitotic rate; HPF, high power field				

2.3 Aims of this study

With the recognition of MR as a significant predictor of survival, the rationale for this dissertation is two-fold: to firstly explore the characteristics of patients with high mitotic rate melanoma and secondly to re-examine the clinical importance of MR and the implications this has on our care of patients with primary cutaneous invasive melanoma.

The first study, presented in Chapter 3, addresses a question that has as yet not been comprehensively examined in the current literature. Based on a well-characterized cohort of Australian patients who received definitive treatment at a tertiary referral centre, we explored the phenotypic, clinical and histopathologic associations of high MR melanoma.

The second study, presented in Chapter 4, primarily aims to add to the current literature on the prognostic utility of MR in relation to melanoma survival. Linkage of the patient cohort on the Victorian Melanoma registry with a population-based cancer registry was performed. The secondary aim was to explore the various methods of analyzing the independent prognostic significance of MR in order to achieve a fair and reproducible analytic approach.

Chapter 3: Paper 1

3.1 Characteristics and associations of high-mitotic-rate melanoma

This chapter addresses the question of which individuals are at risk of developing aggressive melanoma identified by greater mitotic rate. This work provides the background to the exploration of the correlation between mitotic rate and patient survival that will be presented in Chapter 4. This chapter incorporates one publication examining the clinical, histopathologic and demographic characteristics and associations of high mitotic rate melanoma. Ethics approval for this study was attained from the Monash University Human Ethics Committee (see appendix).

1. Shen et al., Characteristics and associations of high mitotic rate melanoma. JAMA Dermatology 2014 Oct; 150 (10):1048-55.

Declarations for Thesis Chapter 3

In the case of Chapter 3, the nature and extent of my contribution to the work was the following:

Manuscript: Characteristics and associations of high-mitotic-rate melanoma

Nature of contribution	**Extent of contribution**
Data collection, study design, data analysis and interpretation, manuscript development and preparation	75%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Wolfe	Study design, data analysis and interpretation, manuscript development and preparation
McLean	Study design, manuscript editing
Haskett	Study design, manuscript editing
Kelly	Study design, data interpretation, manuscript development and preparation

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work

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TITLE

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ABSTRACT

Introduction:

Mitotic rate is now recognized as having independent prognostic significance in melanoma survival. However, its clinicopathologic associations have not been the focus of any previous study.

Objective:

To identify a set of patient and tumor characteristics associated with high mitotic rate melanoma, with the aim of facilitating the earlier detection of aggressive primary invasive melanoma.

Design:

Cross-sectional study of patients reviewed by the Victorian Melanoma Service between January 2006 and December2011.

Setting:

Analysis of data from a single-institution public hospital-based multidisciplinary melanoma clinic in Victoria, Australia.

Patients:

The Victorian Melanoma Service reviewed 2397 cases during the study period and 1441 patients diagnosed with 1500 primary invasive melanomas were included.

Main outcome measures:

Mitotic rate was measured as a count per mm^2 and analyzed as ordered categories (0, <1, 1 and <2, 2, 3-4, 5-9 and ≥10) according to patient demographics, phenotypic markers, historical data, tumor presentation and histopathologic features.

Results:

Melanomas with higher mitotic rates were more likely to occur in men (OR [odds ratio] = 1.5, 95% CI [confidence interval] = 1.3-1.8), elderly patients (age ≥ 70, OR =2.1, 95% CI = 1.7-

2.8) and those with a history of solar keratosis. These melanomas occurred more frequently on the head and neck (OR = 1.4, 95% CI = 1.0-1.9), and presented more often as amelanotic (OR = 1.9, 95% CI = 1.4-2.5) and rapidly growing lesions (≥2mm/month, OR=12.5, 95% CI = 8.4 - 18.5). An association was seen with the nodular melanoma subtype (vs. superficial spreading, OR = 2.5, 95% CI = 1.8-3.4), tumor thickness (≤1mm, OR =1.0 (reference), 1.01-4mm, OR=4.5, >4mm, OR=12.6) and ulceration (OR = 2.0, 95% CI= 1.5-2.7). These histopathologic features, along with amelanosis and rate of growth, remained as significant associations with high mitotic rate in the overall multivariate analysis.

Conclusion:

High mitotic rate primary cutaneous melanoma is associated with aggressive histologic features and atypical clinical presentation. It has a predilection for the head and neck region and is more likely to be seen in elderly men with a history of cumulative solar damage, who present clinically with rapid tempo disease.

INTRODUCTION

Mitotic rate, a quantifiable marker of tumor cellular proliferation, has been closely correlated with survival with some studies demonstrating independent prognostic significance[1-11]. For thin tumors ≤1mm, the mitotic rate is now a criterion alongside ulceration for defining T1b melanoma[4].

Previous studies of aggressive melanoma have focused on the characteristics and associations of thick melanoma. Thick melanomas have been shown to have a strong association with the nodular subtype[12-17], to grow rapidly and to occur more frequently in elderly men and in individuals with fewer nevi and fewer freckles[18,19]. However the existing literature has scarce data regarding the clinical presentation and associations of high mitotic rate melanoma to

assist in identifying those at risk for poor prognosis. The aim of this study was to delineate the clinical, phenotypic and histological associations of high mitotic rate melanoma.

METHODS

Patients and Data Collection

Institutional ethics approval was obtained for a retrospective review of all patients at the Victorian Melanoma Service (VMS) from January 2006 to December 2011. All patients whose primary invasive melanoma was histologically reviewed and mitotic rate assessed at the VMS were included in the study. Two expert dermatopathologists reviewed the original histopathology sections for each case.

The following data were collected prospectively for each subject: demographic information (age, sex, ethnicity), phenotypic markers (eye color, hair color, skin prototype), historical features (number of blistering sunburn, previous melanoma, solar keratosis, family history of a first degree relative with melanoma) and tumor presentation (amelanosis, body site and time from the initial observation of the lesion to confirmed diagnosis). Clinical examination of all patients undertaken by a dermatology resident provided count of total nevi, clinically determined dysplastic nevi, freckles and solar lenities. The count of total number of melanocytic nevi was grouped (<20, 20-50, >50-100, >100-200, >200). Dysplastic nevi were counted exactly. Freckles and solar lentigines were recorded as few, moderate, or many. For the purpose of this study, tumor site was classified as head and neck, trunk, upper extremities, and lower extremities. We used a previously described historical estimation of rate of growth of a tumor that was derived by dividing its Breslow thickness in millimeter(s) by the time from the initial observation of the change in the lesion to histological diagnosis in month(s) [19,20]. Amelanotic tumors were those that appeared to the patient to be without pigmentation.

Mitotic rate was determined histologically by first examining the entire tumor looking for mitotic figures. The area with the greatest density of mitotic activity was used as the focus and the number of mitoses in a surrounding area of $1mm^2$ was counted. Mitotic rate was reported by the dermatopathologist either as a discrete integer or as <1 and <2 per mm^2.

Tumor type was classified histologically as superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo maligna melanoma (LMM), acral lentiginous melanoma (ALM) or desmoplastic melanoma (DM) according to the classification systems of Clark et al and McGovern et al. Less common tumor types were grouped as 'other'. Other histologic features assessed were Breslow thickness, ulceration, Clark level of invasion, lymphovascular invasion, microsatellitosis, tumor infiltrating lymphocytes, regression, neurotropism and pre-existing nevus.

Statistical Analysis

Mitotic rate followed a skewed distribution and was categorized for analysis as 0, <1, 1 and <2, 2, 3-4, 5-9 and ≥10. This ensured reasonable numbers of patients in each category. The association of mitotic rate with each of the clinical and histopathologic parameters was summarized with non-parametric Spearman rank correlation coefficients. Ordinal logistic regression was used to examine univariate and multivariate associations between mitotic rate and other variables. Odds ratios from this analysis can be interpreted as increased odds of a higher category of mitotic rate regardless of where the scale is dichotomized. This interpretation relies on the assumption of proportional odds, which was tested for the final multivariate model. Statistical analyses were performed using SPSS statistical software (SPSS version 19.0; SPSS Inc., Chicago, Ill) and a p-value <0.05 was considered statistically significant.

RESULTS

2397 cases presented to the VMS during the study period, from which, 1441 patients diagnosed with 1500 primary melanomas were included in the analysis. Of the 1500 melanomas, 813 (54%) occurred in males (mean age 60 years) and 687 occurred in females (mean age 55 years).

Description of mitotic rate

The mitotic rate ranged from 0 to 75 mitoses/mm^2. 41.7% of the melanomas had a mitotic rate of less than 1/mm^2; 28.8% had a mitotic rate of between 1 and 2 mitoses/mm^2 and the remaining 29.5% were melanomas with mitoses ≥3/mm^2. Mean mitotic rate was 2.8/mm^2 (standard deviation = 4.9, interquartile range 2.5/mm^2)

Associations of patient characteristics with mitotic rate

Univariate analysis demonstrated strong associations between older age (≥70 years, OR = 2.1, 95% CI = 1.7-2.8), male gender (OR = 1.5, 95% CI = 1.3-1.8) and increasing mitotic rate (Table 2). The ratio of men to women in the ≥ 10/mm^2 mitotic rate group was 2.2:1 with 45% of these patients aged ≥ 70 years. The presence of solar keratosis was also associated with higher mitotic rate (OR = 1.3, 95% CI = 1.1-1.6). Conversely, a history of blistering sunburns and a significant family history of melanoma were associated with lower mitotic activity ($p = 0.02$ and $p < 0.0005$ respectively). Age (≥70 years, OR = 1.9, $p<0.0005$), male sex (OR = 1.4, $p=0.001$) and family history (OR = 0.7, $p=0.002$) remained as significant associations with mitotic rate on multivariate analyses of patient characteristics (Table 1).

Associations of clinical presentation with mitotic rate

Univariate analysis revealed that head and neck location ($p=0.005$), amelanosis ($p<0.0005$) and increasing rate of growth ($p<0.0005$) had strong associations with high mitotic rate. 50% of melanomas with mitotic rate $\geq 5/mm^2$ were amelanotic. Initial detection by the patient rather than the doctor or others was associated with higher mitotic rate ($p<0.0005$). Amelanosis and rate of growth remained as independent associations of increasing mitotic rate in the multivariate analysis of clinical presenting features ($p<0.0005$) (Table 2).

Associations of histopathologic features with mitotic rate

By univariate analysis, all histopathologic factors with the exception of tumor infiltrating lymphocytes and desmoplasia were significantly associated with mitotic rate. Patients in the higher mitotic rate groups had thicker melanomas with greater proportions being ulcerated. Over half (53.8%) of cases in which mitotic rate was $\geq 10/mm^2$ were ulcerated, in contrast with only 6% in the $0/mm^2$ mitotic rate group (Table 5). Nodular melanoma was associated with a higher mitotic rate, with a ratio of 2.3:1 with superficial spreading melanoma in the $\geq 10/mm^2$ group. Superficial spreading melanoma accounted for the majority of the low mitotic rate melanomas (73% in the $0/mm^2$ category). Similarly, features of regression (OR=0.4, 95% CI = 0.4-0.6, $p<0.0005$) and the presence of pre-existing dysplastic nevi (OR = 0.2, 95% CI = 0.1-0.3, $p<0.0005$) were associated with lower mitotic activity.

Nodular tumor type, thickness, ulceration and Clark level of invasion remained as significant predictors of high mitotic rate in the multivariate analyses of tumor and histopathologic factors. An inverse correlation remained with pre-existing dysplastic nevus (Table 3).

Overall multivariate analyses

The independent associations of mitotic rate from the 3 multivariate models were entered into a final multivariate ordinal logistic regression model. This included both patient and tumor characteristics: age, sex, amelanosis, rate of growth; and histopathologic parameters: tumor type, thickness, ulceration, Clark level of invasion and pre-existing nevus.

Nodular tumor type (OR=2.3, 95% CI=1.6-3.3, p<0.0005), ulceration (OR=2.0, 95% CI=1.5-2.8, p<0.0005), Clark level of invasion (Level V, OR = 6.2, 95% CI = 3.1-12.2, p<0.0005) and thickness (>4mm, OR=8.4, 95% CI=4.7-15.1, p<0.0005) retained strong positive associations with mitotic rate whilst an inverse relationship persisted with pre-existing dysplastic nevus (OR=0.4, 95% CI=0.2-0.6, p<0.0005). Rate of growth (p = 0.01) and amelanosis (p = 0.05) also independently correlated with higher mitotic rate, although to a lesser extent than the histopathologic variables. The age and sex associations that were observed in earlier analyses did not appear strong in this analysis and so their relationships with higher mitotic rate could be explained by other parameters in the analysis (Table 4).

The proportional odds assumption was rejected for the model overall, however the large sample size translated to high power for this proportional odds test. Tests of the same assumption in the univariate analyses indicated that it was satisfied for most variables with the two exceptions of thickness and tumor level which may have more complex associations with mitotic rate than those described by the multivariate odds ratios.

DISCUSSION

Mitotic rate, a quantifiable measure of tumor growth, has been shown to correlate with melanoma survival[4, 5]. Disease-free survival has been found to decline with increasing mitotic rate, with in-transit, nodal and distant recurrences occurring more commonly in patients with

high mitotic rate (≥5 mitoses/mm^2) melanoma[21]. In conjunction with Breslow thickness and ulceration, mitotic rate comprises the current AJCC staging system for localized melanoma[5]. However, whilst the presence of many mitotic figures has been recognized as a poor prognostic feature, the clinicopathologic characteristics of patients with high mitotic rate melanoma have yet to be formally elucidated.

This study has identified that patients with higher mitotic rate tumors were more likely to be older, to be male, to have a history of significant solar field damage, to present with rapidly growing primary melanoma that was more likely to be located on the head and neck. Melanomas with very high mitotic activity (≥ 10 mitoses/mm^2) were predominantly thick and ulcerated nodular tumor subtypes. Conversely, the superficial spreading melanoma subtype, features of regression and the presence of pre-existing nevi were found to be characteristic of lesions with sparse mitotic activity. These melanomas were significantly thinner than their more mitotically active counterparts and tended to occur more commonly in patients with previous blistering sunburns and a significant family history of a first degree relative with melanoma. This accords with previous observations of associations between these historical factors and thin tumors[22, 23].

Studies investigating patients at the greatest risk of mortality from primary invasive melanoma have focused mainly on those presenting with thick lesions. There is a recognized association between tumor thickness, male sex and older age [13,14,18]. In a retrospective survey of 1124 patients, Hersey et al found that 68% of patients with thick melanoma (≥3mm) were men and 75% were aged greater than 70 years. The head and neck region was the most common site for thick melanoma[13], although Hanrahan et al later observed that tumor site was not related to thickness when nodular tumor type was taken into account[17].

As a phenotypic marker of cumulative sun damage, solar keratosis is considered a risk factor for melanoma[24,25] and its association with melanoma occurring on the head and neck and in men has been recognised[18,25]. In this study, the presence of solar keratosis was shown to be associated with high mitotic rate, although this relationship was lost when taking into account age and sex. Nodular melanoma, which demonstrated higher mitotic activity than any other tumor type in this current study, has been more strongly linked to solar keratosis than superficial spreading melanoma[26].

Despite the likelihood of being amelanotic, high mitotic rate melanomas were most commonly first discovered by patients themselves. For instance, 62.1% of melanomas with a mitotic rate of $\geq 5/mm^2$, were first detected by patients themselves. This is possibly a result of their rapid growth rate, which, as a clinical measure of tumor proliferation, has been reported previously to closely correlate with mitotic rate[19]. The same study also described an association between rapid tumor growth and atypical tumor morphology including amelanosis, symmetry, elevation and border regularity[19],which are recognized features of nodular melanoma[27]. Whilst tumor subtype was chosen as a histological variable for the purposes of this study, it is important to recognize that each tumor subtype has characteristic morphological features that are helpful in their clinical detection. The presenting features of nodular melanoma may pose a challenge in this regard due to its symmetrically elevated, often amelanotic appearance that does not fit the classic "ugly duckling" radial growth phase melanoma as defined by the ABCDE rule[28] (Fig 1).

The previous observation that thick and ulcerated melanomas are mitotically active[7,29] was consistent with the current findings. However, it must be noted that desmoplastic melanoma,

which was found to be comparable in thickness to nodular melanoma, exhibited low mitotic activity. This may serve to explain, to a degree, the distinct clinical behavior suggested for this rare melanoma subtype that is characterized by its propensity for local recurrence but a lower incidence of distant spread relative to other forms of cutaneous melanoma[30,31].

As this is the first formal description of the clinicopathologic associations of high mitotic rate melanoma, results from this single-center study merit replication elsewhere to not only confirm generalizability but also to further explore the potential implications for our detection and treatment of at-risk patients, whom, in this study, were found to have a distinct phenotypic and historical profile. Mitotically active melanomas were more often seen in older men with chronic solar field damage. These tumors have a predilection for the head and neck and can present with nodular morphology and amelanosis. Such atypical clinical features may pose a challenge to timely detection, thus a high index of suspicion is warranted when the patient reports a history of morphological change and rapid growth.

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LEGENDS

Figure 1: A patient with high mitotic rate melanoma

A male patient in his 80's presenting with a rapidly growing amelanotic nodular melanoma on the scalp. At diagnosis, it had a Breslow thickness of 7.4mm and a mitotic rate of >90/mm^2.

TABLES

Table 1: Patient characteristics and mitotic rate

Table 2: Clinical presentation and mitotic rate

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Table 4: Overall multivariate analyses

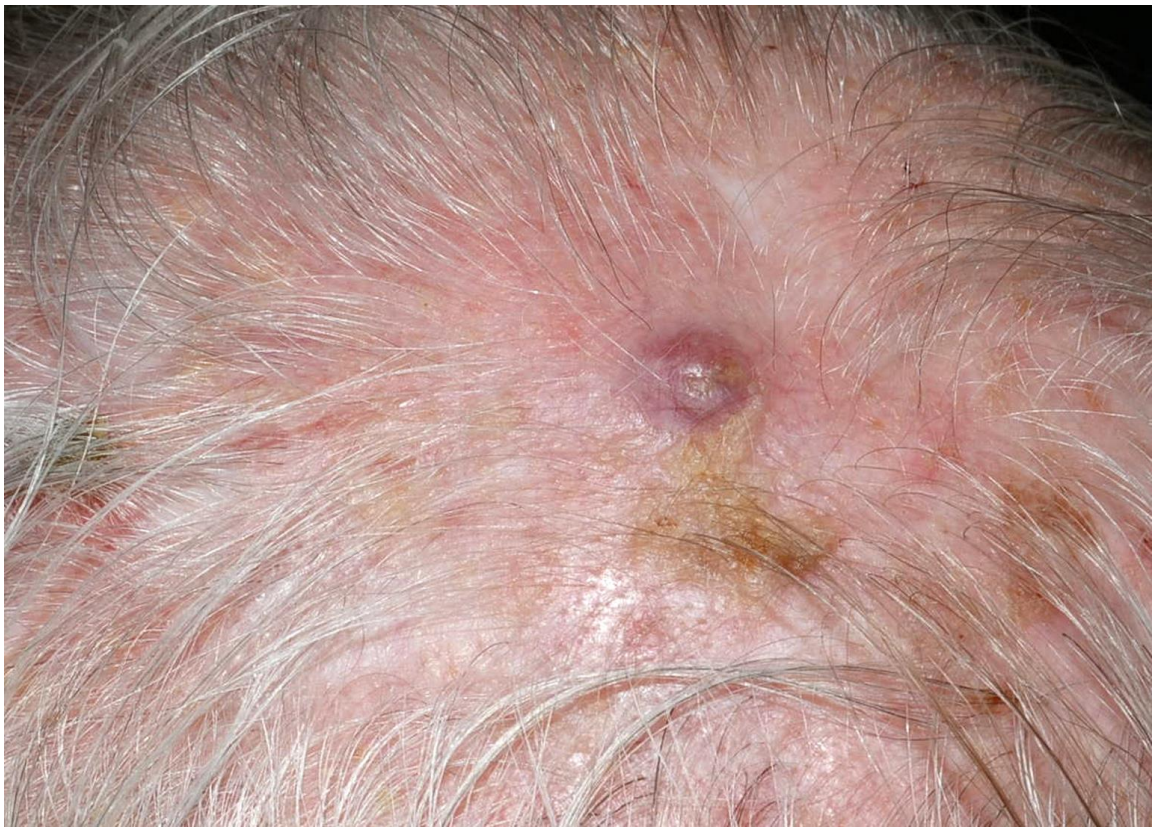


Figure 1: A patient with high mitotic rate melanoma

A male patient in his 80's presenting with a rapidly growing amelanotic nodular melanoma on the scalp. At diagnosis, it had a Breslow thickness of 7.4mm and a mitotic rate of >90/mm^2.

Table 1 Patient Characteristics and Mitotic Rate

Characteristics	Melanomas, No. †	Mitoses/ mm^2		Univariate Analyses		Multivariate Analyses	
		Mean (SD),	Median	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Age							
<50	469	2.0 (3.4)	1.0	1.0	<0.0005	1.0	<0.0005
50<70	623	2.7 (5.0)	1.0	1.2 (1.0-1.5)		1.2 (0.9-1.5)	
≥70	408	4.0 (6.1)	2.0	2.1 (1.7-2.8)		1.9 (1.4-2.5)	
Sex							
M	813	3.3 (5.1)	1.0	1.5 (1.3-1.8)	<0.0005	1.4 (1.2-1.7)	0.001
F	687	2.3 (4.7)	1.0	1.0		1.0	
Eye colour							
Blue	792	2.9 (4.6)	1.0	1.2 (0.9-1.6)	0.71	-	-
Green	167	3.0 (5.1)	1.0	1.1 (0.7-1.5)			
Hazel	261	2.8 (6.2)	1.0	1.2 (0.8-1.6)			
Brown	187	2.5 (3.9)	1.0	1.0			
Hair colour							
Blond	298	2.3 (3.7)	1.0	0.8 (0.5-1.3)	0.42	-	-
Red	147	2.7 (5.1)	1.0	0.8 (0.5-1.3)			
Light Brown	402	3.0 (4.8)	1.0	1.0 (0.6-1.5)			
Brown	473	3.1 (5.6)	1.0	1.0 (6.9-1.6)			
Black	85	2.8 (4.2)	1.0	1.0			
Skin phototype							
I	340	2.6 (4.3)	1.0	0.5 (0.2-1.1)	0.20	-	-
II	569	2.8 (4.8)	1.0	0.5 (0.2-1.2)			
III	425	2.9 (4.4)	1.0	0.6 (0.3-1.3)			
IV	91	3.2 (8.4)	1.0	0.5 (0.2-1.2)			
V	16	3.7 (4.7)	2.0	1.0			
Total nevi							
<20	303	3.1 (5.7)	1.0	1.1 (0.4-3.0)	0.10	-	-
20-50	382	2.6 (4.5)	1.0	0.9 (0.3-2.4)			
50-100	276	3.1 (5.1)	1.0	1.1 (0.4-3.0)			
100-200	211	2.3 (4.4)	1.0	0.8 (0.3-2.1)			

200-500	99	2.5 (4.8)	1.0	0.8 (0.3-2.1)			
>500	10	1.7 (1.7)	1.0	1.0			
Freckles							
Few	470	2.6 (5.1)	1.0	1.0 (0.8-1.3)	0.40	-	-
Moderate	254	2.2 (4.1)	1.0	0.9 (0.6-1.1)			
Many	373	2.9 (4.8)	1.0	1			
Solar lentigines							
Few	469	2.5 (5.0)	1.0	1.0(0.8-1.2)	0.91	-	-
Moderate	215	2.6 (3.8)	1.0	1.0 (0.7-1.3)			
Many	403	2.9 (4.8)	1.0	1.0			
Dysplastic nevi							
Yes	283	2.7 (5.1)	1.0	1.0 (0.8-1.2)	0.85	-	-
No	950	2.6 (4.2)	1.0	1.0			
Blistering sunburns							
None	543	2.9 (4.1)	1.0	1.0	0.02	1.0	0.07
1-5	628	2.8 (5.7)	1.0	0.7 (0.6-0.9)		0.8 (0.6-1.0)	
6-10	121	2.7 (4.5)	1.0	0.7 (0.5-1.1)		0.8 (0.5-1.1)	
10-20	76	1.8 (2.8)	1.0	0.6 (0.4-0.9)		0.6 (0.4-0.9)	
>20	60	2.7 (4.4)	1.0	0.8 (0.5-1.3)		0.8 (0.5-1.4)	
Previous melanoma							
Yes	161	3.2 (5.3)	1.0	1.1 (0.8-1.5)	0.53	-	-
No	1339	2.8 (4.9)	1.0	1.0			
1st degree relatives with melanoma							
Yes	294	2.1 (3.8)	0.5	0.6 (0.5-0.8)	<0.0005	0.7 (0.6-0.9)	0.002
No	1206	3.0 (5.2)	1.0	1.0		1.0	

Abbreviations: SD, standard deviation; CI, confidence interval

† Variation in total numbers in each category is due to unobtainable data

Table 2 Clinical Presentation and Mitotic Rate

Characteristics	Melanomas, No. †	Mitoses/ mm^2		Univariate Analyses		Multivariate Analyses	
		Mean (SD),	Median	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Who first suspected							
Patient	745	3.1 (4.6)	1.0	1.7 (1.0-2.8)	<0.0005	1.8 (1.0-3.6)	0.07
Relative	95	3.1 (8.4)	1.0	1.2 (0.7-2.2)		1.3 (0.6-2.8)	
Partner	168	2.5 (4.3)	1.0	1.2 (0.7-2.1)		1.6 (0.8-3.3)	
Doctor	365	2.3 (4.6)	0.5	0.9 (0.5-1.6)		1.1 (0.6-2.2)	
Other	47	1.7 (2.2)	1.0	1.0		1.0	
Amelanosis					<0.0005		<0.0005
Yes	198	5.1 (7.9)	3.0	2.9 (2.2-3.7)		1.9 (1.4-2.5)	
No	1302	2.5 (4.2)	1.0	1.0		1.0	
Location					0.005		0.07
Head and Neck	369	3.8 (5.5)	1.0	1.5 (1.1-2.0)		1.4 (1.0-1.9)	
Lower extremities	301	2.4 (5.1)	1.0	1.0 (0.8-1.4)		1.1 (0.8-1.6)	
Trunk	451	2.5 (4.7)	1.0	1.0 (0.8-1.3)		1.0 (0.7-1.3)	
Upper extremities	371	2.6 (4.1)	1.0	1.0		1.0	
Rate of growth (mm/month)							
<0.2	448	1.3 (2.5)	0.5	1.0	<0.0005	1.0	<0.0005
0.2<0.5	228	2.2 (3.2)	1.0	2.1 (1.6-2.8)		2.0 (1.5-2.6)	
0.5 <2	316	3.8 (4.7)	2.0	4.3 (3.3-5.6)		4.2 (3.2-5.5)	
≥ 2	125	7.4 (7.6)	5.0	14.6 (10.0-21.2)		12.5 (8.4-18.5)	

Abbreviations: SD, standard deviation; CI, confidence interval

† Variation in total numbers in each category is due to unobtainable data

Table 3 Histopathologic features and mitotic rate

Characteristics	Melanomas, No. †	Mitoses/ mm^2		Univariate Analyses		Multivariate Analyses	
		Mean (SD),	Median	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Tumor type							
SSM	901	1.9 (4.2)	1.0	1.0	<0.0005	1.0	<0.0005
NM	247	6.8 (6.5)	5.0	9.1 (7.0-11.8)		2.5 (1.8-3.4)	
LMM	191	2.1 (3.9)	0.5	0.8 (0.6-1.1)		0.8 (0.6-1.1)	
DM	68	2.9 (4.7)	1.0	1.7 (1.1-2.6)		0.2 (0.1-0.3)	
ALM	35	2.6 (3.4)	1.0	1.7 (0.9-3.0)		0.9 (0.5-1.9)	
Other*	58	2.0 (3.1)	1.0	1.3 (0.8-2.1)		0.5 (0.3-1.0)	
Tumour thickness, mm							
≤ 1	708	0.6 (1.3)	0.5	1.0	<0.0005	1.0	<0.0005
1.01– 4	614	3.7 (4.2)	2.0	11.8 (9.4-14.9)		4.5 (3.2-6.1)	
> 4	178	8.6 (9.1)	6.0	47. 6 (33.5-67.6)		12.6 (7.5-21.1)	
Ulceration							
Yes	272	6.9 (7.8)	4.5	7.8 (6.1-10.0)	<0.0005	2.0 (1.5-2.7)	<0.0005
No	1200	1.9 (3.4)	1.0	1.0		1.0	
Clark Level							
II	380	0.4 (1.3)	0	1.0	<0.0005	1.0	<0.0005
III	328	2.1 (4.1)	1.0	4.6 (3.5-6.1)		3.1 (2.2-4.3)	
IV	671	3.8 (4.6)	2.0	15.4 (11.8-20.4)		4.6 (3.1-6.6)	
V	120	6.9 (9.4)	4.0	36.1 (24.2-53.9)		8.3 (4.4-15.5)	
Microsatellitosis							
Yes	29	7.4 (5.5)	6.0	7.5 (3.9-14.2)	<0.0005	1.8 (0.7-4.5)	0.099
No	1331	2.8 (4.6)	1.0	1.0		1.0	
Lymphovascular invasion					<0.0005		0.598
Yes	64	5.6 (5.7)	4.0	4.4 (2.9-6.7)		1.2 (0.7-1.9)	
No	1414	2.7 (4.9)	1.0	1.0		1.0	

Tumour infiltrating							
lymphocytes					0.218		
Yes	824	2.8 (4.6)	1.0	1.1 (0.9-1.4)		-	-
No	466	2.9 (4.7)	1.0	1.0			
Regression							
Yes	359	2.0 (4.2)	0.5	0.4 (0.4-0.6)	<0.0005	0.9 (0.7-1.2)	0.490
No	1081	3.1 (4.8)	1.0	1.0		1.0	
Desmoplasia							
Yes	78	3.5 (5.4)	1.0	1.5 (1.0-2.1)	0.063	-	-
No	1258	2.8 (4.6)	1.0	1.0			
Neurotropism							
Yes	50	4.5 (6.1)	2.0	2.4 (1.5-3.8)	<0.0005	1.5 (0.8-2.9)	0.228
No	1387	2.8 (4.6)	1.0	1.0		1.0	
Pre-existing nevus							
Yes							
Dysplastic	96	0.5 (0.9)	0	0.2 (0.1-0.3)	<0.0005	0.3 (0.2-0.5)	<0.0005
Non-dysplastic	271	1.8 (3.1)	1.0	0.6 (0.5-0.7)		0.7 (0.5-0.9)	
No	1014	3.3 (5.5)	1.0	1.0		1.0	

Abbreviations: SD, standard deviation; CI, confidence interval, SSM, superficial spreading melanoma; NM, nodular melanoma, LMM, lentigo maligna melanoma; DM, desmoplastic melanoma; ALM, acral lentiginous melanoma

† Variation in total numbers in each category is due to unobtainable data

* Other tumour types were grouped together because of low numbers

Table 4 Overall multivariate analyses

	Mitotic rate, per mm^2							**Odds ratio**	**P value**
	0	**<1**	**1<2**	**2**	**3-4**	**5-9**	**≥ 10**	**(95% CI)**	
Total n = 1500	364	261	297	135	156	155	132		
Age, years									
<50	131 (36)	90 (34.5)	106 (35.7)	41 (30.4)	42 (26.9)	35 (22.6)	24 (18.2)	1.0	0.40
50<70	146 (40.1)	127 (48.7)	127 (42.8)	59 (43.7)	52 (33.3)	64 (41.3)	48 (36.4)	0.9 (0.7-1.2)	
≥70	87 (23.9)	44 (16.9)	64 (21.5)	35 (25.9)	62 (39.7)	56 (36.1)	60 (45.5)	1.1 (0.8-1.6)	
Mean (SD), years	56.0 (16.4)	54.9 (15.1)	55.6 (16.4)	56.8 (17.6)	61.4 (16.6)	61.7 (16.4)	64.8 (15.2)		
Sex									
Male	174 (47.8)	133 (51.0)	151 (50.8)	80 (59.3)	94 (60.3)	90 (58.1)	91 (68.9)	1.2 (0.9-1.5)	0.14
Female	190 (52.2)	128 (49.0)	146 (49.2)	55 (40.7)	62 (39.7)	65 (41.9)	41 (31.1)	1.0	
Amelanosis									
Yes	23 (6.3)	21 (8.0)	29 (9.8)	24 (17.8)	29 (18.6)	40 (25.8)	32 (24.2)	1.4 (1.0-1.9)	0.05
No	341 (93.7)	240 (92)	268 (90.2)	111 (82.2)	127 (81.4)	115 (74.2)	100 (75.8)	1.0	
Rate of growth, mm/month									
<0.2	148 (62.2)	108 (54.3)	102 (45.7)	24 (24.0)	32 (24.6)	23 (18.3)	11 (10.9)	1.0	0.001
0.2<0.5	46 (19.3)	45 (22.6)	54 (24.2)	20 (20.0)	25 (19.2)	23 (18.3)	15 (14.9)	1.3 (1.0-1.8)	
0.5 <2	35 (14.7)	44 (22.1)	60 (26.9)	40 (40.0)	52 (52)	46 (36.5)	39 (38.6)	1.5 (1.1-2.1)	
≥ 2	9 (3.8)	2 (1.0)	7 (3.1)	16 (16.0)	21 (16.2)	34 (27.0)	36 (35.6)	2.6 (1.6-4.2)	
Mean (SD), mm/month	0.04 (0.56)	0.33 (0.43)	0.45 (0.65)	1.11 (1.41)	1.06 (1.42)	1.61 (2.33)	1.79 (1.81)		
Tumor type									

SSM	253 (72.9)	185 (73.1)	207 (71.4)	68 (55.3)	88 (59.1)	65 (43.3)	35 (26.9)	1.0	<0.0005
NM	3 (0.9)	14 (5.5)	32 (11.0)	26 (21.1)	42 (28.2)	57 (38.0)	73 (56.2)	2.3 (1.6-3.3)	
LMM	71 (20.5)	38 (15.0)	28 (9.7)	12 (9.8)	11 (7.4)	17 (11.3)	14 (10.8)	0.8 (0.6-1.2)	
DM	13 (3.7)	10 (4.0)	17 (5.9)	10 (8.1)	6 (4.0)	6 (4.0)	6 (4.6)	0.1 (0.1-0.3)	
ALM	7 (2.0)	6 (2.4)	6 (2.1)	7 (5.7)	2 (1.3)	5 (3.3)	2 (1.5)	0.8 (0.4-1.7)	
Thickness, mm									
≤ 1	301 (82.7)	197 (75.5)	154 (51.9)	26 (19.3)	20 (12.8)	7 (4.5)	3 (2.3)	1.0	<0.0005
1.01– 4	53 (14.6)	60 (23.0)	127 (42.8)	93 (68.9)	107 (68.6)	112 (72.3)	62 (47.0)	3.3 (2.3-4.8)	
> 4	10 (2.7)	4 (1.5)	16 (5.4)	16 (11.9)	29 (18.6)	36 (23.2)	67 (50.8)	8.4(4.7-15.1)	
Mean (SD), mm	0.8 (1.0)	0.9 (1.0)	1.4 (1.5)	2.4 (2.5)	2.7 (2.1)	3.2 (2.3)	4.9 (3.4)		
Ulceration									
Yes	6 (1.7)	20 (7.8)	29 (9.9)	35 (26.7)	46 (30.3)	65 (42.5)	71 (53.8)	2.0 (1.5-2.8)	<0.0005
No	349 (98.3)	237 (92.2)	263 (90.1)	96 (73.3)	106 (69.7)	88 (57.5)	61 (46.2)	1.0	
Clark Level									
II	205 (56.3)	115 (44.1)	51 (17.2)	4 (3.0)	4 (2.6)	0	1 (0.8)	1.0	
III	74 (20.3)	76 (29.1)	92 (31.0)	24 (17.8)	22 (14.1)	20 (12.9)	20 (15.3)	3.1 (2.2-4.5)	
IV	78 (21.4)	65 (24.9)	138 (46.5)	89 (65.9)	112 (71.8)	111 (71.6)	78 (59.5)	4.4 (2.9-6.6)	<0.0005
V	7 (1.9)	5 (1.9)	16 (5.4)	18 (13.3)	18 (11.5)	24 (15.5)	32 (24.4)	6.2(3.1-12.2)	
Pre-existing nevus									
Yes									
Dysplastic	50 (15.5)	22 (8.7)	16 (5.8)	3 (2.5)	5 (3.4)	0	0	0.4 (0.2-0.6)	<0.0005
Non-dysplastic	73 (22.6)	51 (20.2)	69 (25.0)	26 (21.7)	26 (17.9)	15 (10.3)	11 (9.2)	0.7 (0.5-1.0)	
No	200 (61.9)	180 (71.1)	191 (69.2)	91 (75.8)	114 (78.6)	130 (89.7)	108 (90.8)	1.0	

SUPPLEMENTAL TABLE AND FIGURES

Supplemental Table 1: Mitotic rates

Supplemental Figure 1: Box plots of the relationship between mitotic rate and tumor thickness.

Horizontal lines within boxes represent the 25th percentile, median, and 75th percentile values.

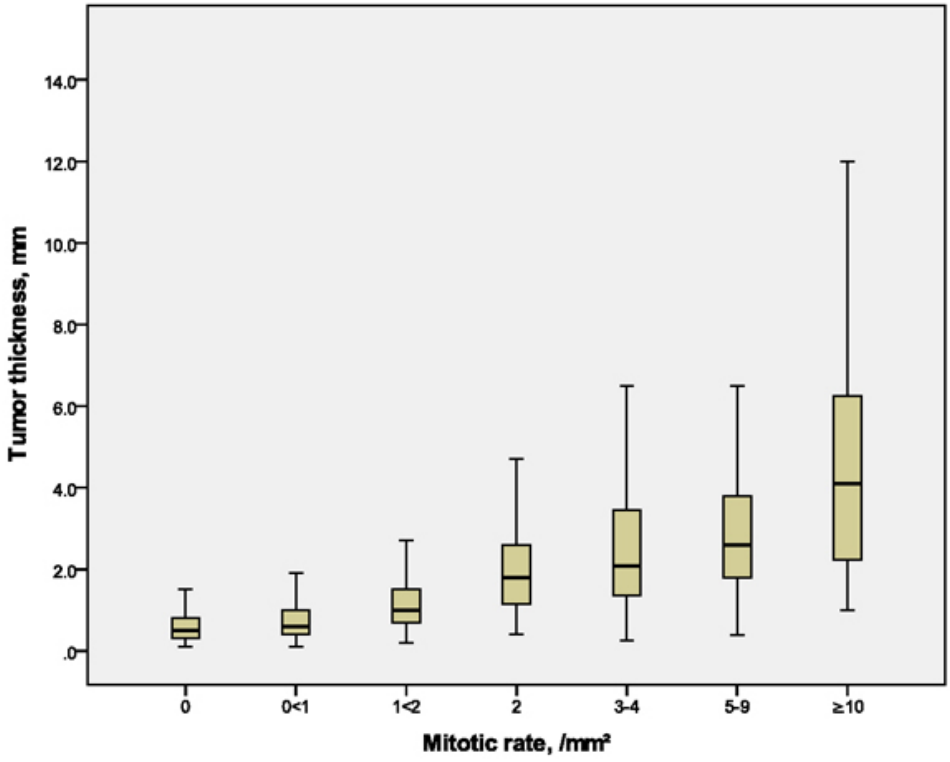
Supplemental Figure 2:Box plots of the relationship between mitotic rate and tumor type.

Horizontal lines within boxes represent the 25th percentile, median, and 75th percentile values.

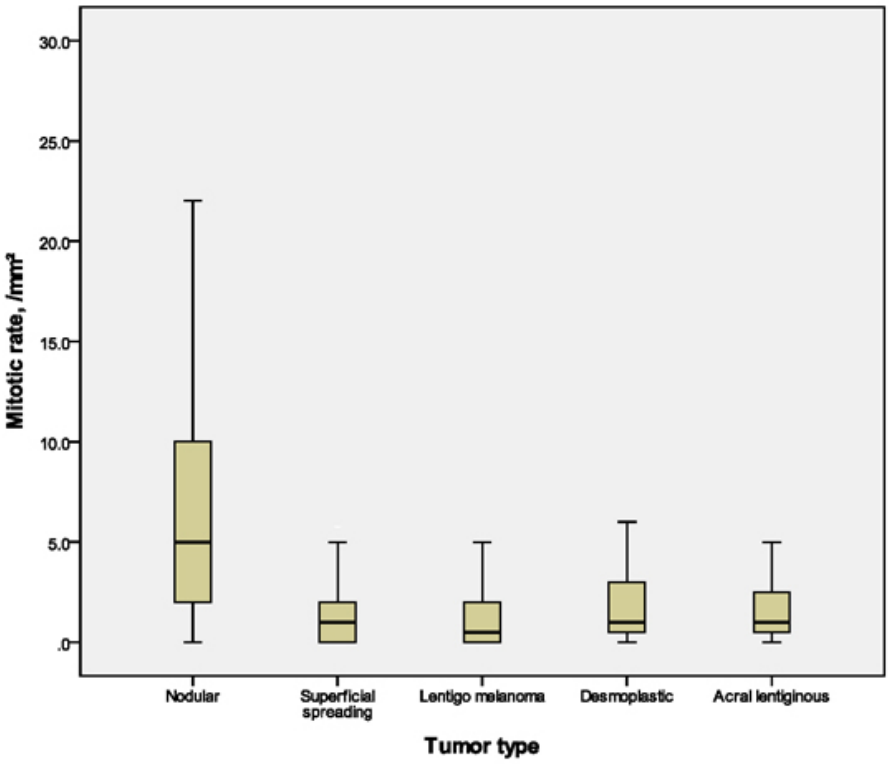
Supplemental Table 1 Mitotic rates

Mitotic rate/mm^2	**Melanomas, n (%)**
Total	1500
0	364 (24.3)
<1	261 (17.4)
1<2	297 (19.8)
2	135 (9.0)
3-4	156 (10.4)
5-9	155 (10.3)
≥ 10	132 (8.8)
Mean (SD)	2.8 (4.9)
Median (IQR)	1.0 (2.5)
Range	0-75

Supplemental Figure 1



Supplemental Figure 2



Chapter 4: Paper 2

4.1 Mitotic rate as a prognostic indicator for melanoma survival: the influence of different modes of analysis

This chapter presents an examination of the prognostic value of mitotic rate in primary invasive cutaneous melanoma. Patients on the Victorian Melanoma Service database were linked with the Victorian Cancer Registry. An analytical issue that emerged in the course of addressing the main aim for this present study was whether mitotic rate, measured as an integer count, should be analysed either as a categorical or continuous variable. Both approaches have been taken in previous studies. We demonstrate that the analytical approach taken can impact on the conclusions drawn from the analysis. Ethics approval was obtained from the Monash University Human Ethics Committee and the Victorian Melanoma Service Participant Information and Consent Document (see Appendix).

Declarations for Thesis Chapter 4

In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Manuscript: Mitotic rate as a prognostic indicator for melanoma survival: the influence of different modes of analysis

Nature of contribution	**Extent of contribution**
Data collection, study design, data analysis and interpretation, manuscript development and preparation	75%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Wolfe	Study design, data analysis and interpretation, manuscript development and preparation
McLean	Study design, manuscript editing
Haskett	Study design, manuscript editing
Kelly	Study design, data interpretation, manuscript development and preparation

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work

Candidate's signature: Date:

Main supervisor's signature: Date:

TITLE

Mitotic rate as a prognostic indicator for melanoma survival: the influence of different modes of analysis

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Key words: mitotic rate, tumor thickness, ulceration, tumor subtype, melanoma, associations

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ABSTRACT

Objective:

To examine the significance of mitotic rate as a prognostic factor in patients with localized cutaneous melanoma.

Design:

Retrospective cohort study of patients treated at the Victorian Melanoma Service (VMS) between January 1st, 2006 and December 31st, 2011.

Setting:

Analysis of data from a single-institution multidisciplinary melanoma clinic in Victoria, Australia linked with mortality data from the Victorian Cancer Registry.

Patients:

1396 patients from the Victorian Melanoma Service, with complete clinical and histologic information were studied.

Main outcome measures:

MR was expressed as mitoses per mm^2 in the dermal part of the tumour in which most mitoses were seen. Factors predicting melanoma-specific survival were separately analyzed in relation to MR using the Cox proportional hazards regression model. In the multivariate analysis, the prognostic significance of MR was determined by using three separate methods for comparative analysis.

Results:

Overall survival times declined as mitotic rate increased: 5-year survival ranged from 94% for patients whose tumours had 0 mitoses/mm^2 to 71% for those with $\geq$ 10 mitoses/mm^2. A significant correlation was seen between tumour thickness and MR ($r = 0.55$). Different modes of analysis used in previous studies were tested on the same data set. With Cox multivariate regression analysis, MR did not reach independent statistical significance in two

of three methods but did so in the third. Ulceration superseded mitotic rate in prognostic importance in all three analyses.

Conclusion:

Although MR as a negative correlate for survival was confirmed, the current study demonstrated the variability of its prognostic power depending upon the method of statistical analysis. A standardized approach is therefore warranted to accurately position MR amongst the hierarchy of prognostic variables for primary invasive melanoma.

INTRODUCTION

The optimal management of patients with cutaneous melanoma requires elucidation of the most important prognostic factors. For this reason, numerous features of primary invasive melanoma have been investigated to identify independent predictors of patient survival(Eldh, Boeryd et al. 1978, McGovern, Shaw et al. 1979, Drzewiecki, Christensen et al. 1980, Van Der Esch, Cascinelli et al. 1981, Worth, Gallagher et al. 1989). Mitotic rate (MR), a quantifiable measure of tumour cell proliferation, did not achieve independent prognostic significance for survival in these earlier studies, which measured this histologic feature in a high power field. This form of assessment has been shown to be less reproducible than the current method of recording mitoses per mm^2, beginning with the region of greatest mitotic activity in the invasive dermal component (Clark, Elder et al. 1989, Vollmer 1989). Application of this new method may have enhanced the prognostic value of MR as demonstrated by several published studies in recent years(Ostmeier, Fuchs et al. 1999, Massi, Borgognoni et al. 2000, Schmid-Wendtner, Baumert et al. 2001, Retsas, Henry et al. 2002, Azzola, Shaw et al. 2003, Barnhill, Katzen et al. 2005, Thompson, Soong et al. 2011), prompting a revision of the AJCC melanoma staging system whereby MR replaced Clark level of invasion as one of the criteria for staging localized primary melanoma (Balch, Gershenwald et al. 2009).

However, there remains inconsistency in the way in which MR has been analyzed as a prognostic variable. Studies have assessed MR as a continuous variable(Vossaert, Silverman et al. 1992, Massi, Borgognoni et al. 2000, Retsas, Henry et al. 2002), as a categorical variable(Ostmeier, Fuchs et al. 1999, Massi, Borgognoni et al. 2000, Schmid-Wendtner, Baumert et al. 2001, Barnhill, Katzen et al. 2005, Thompson, Soong et al. 2011) and as a combination of both(Azzola, Shaw et al. 2003, Francken, Shaw et al. 2004), possibly

influencing the findings. Based on a large cohort of Australian patients treated at a tertiary referral service, the primary objective of this study was to explore the influence in which different statistical analyses of MR may have on its prognostic significance.

METHODS

Extensive clinical and pathologic details of patients treated for primary invasive melanoma between January 1st, 2006 and December 31st, 2011 at the VMS, Alfred Health, Victoria, Australia were recorded at the time patients underwent treatment and subsequently retrieved from the database for the study.

Patients chosen for inclusion in this study had only one primary invasive cutaneous melanoma and were at AJCC Stage I or II, had received their definitive treatment for this at the VMS and for whom information regarding all measured prognostic variables available for analysis were included in the study. Primary lesion pathology was reviewed by two expert histopathologists at the VMS.

Clinical and pathologic parameters as well as follow-up information were extracted from the prospectively maintained VMS database, with confirmation and supplementation from individual medical records. Clinical factors evaluated were the age of the patient (grouped into <50, $50<70$ and ≥ 70 years), gender and site of primary lesion. The anatomic site was designated as head and neck, trunk, upper or lower extremities. Sentinel lymph node biopsy (SLNB) status and long-term follow-up information including local, regional and distant metastasis were also recorded. The SLNB procedure used has been previously described(Thompson 2001). Pathologic details, assessed at the time of first definitive treatment of the patient's primary lesion, included the Breslow thickness, presence or absence

of ulceration, Clark level of invasion and the MR of the primary tumour. MR was expressed as the number of mitoses/mm^2 in the dermal area of the tumour in which most mitoses were noted. This was determined by evaluating all hematoxylin and eosin stained histologic sections of each specimen to find the dermal area of the tumour in which the mitotic figures were the most numerous. The number of mitoses was counted in a 1-mm^2 area, beginning in the field with the greatest numbers of mitoses and counting in successive surrounding fields.

To study melanoma-specific and overall survival, linkage was undertaken with the Victoria Cancer Registry (VCR). The VCR registry provided the last recorded information relating to the clinical status of the subject as of October 31st 2012. Date and cause of death were specified in the registry. Both melanoma-specific and all-cause deaths were used as end-points in this study.

Multivariate analysis of MR was analyzed using three methods. For Method A, MR was expressed as a continuous variable. For Method B, MR was categorized as (MR=0, >0 to <1, 1 to <2, 2, 3-4, 5-9, ≥10/mm^2). Method C followed the approach used by (Azzola, Shaw et al. 2003). The categorization of MR in Method C was coded such that each category took an integer value from 1 to 7 (increasing with increasing MR) and the resulting variable included in analysis as a continuous variable. Other putative prognostic factors were similarly coded. Age was coded in deciles as 1: 1-9, 10-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79 and ≥80. Anatomic site was coded as 1: head and neck, 2: trunk and 3: extremities.

Statistical Analyses

Statistical analysis was performed using the software package SPSS (SPSS version 19.0; SPSS Inc., Chicago, Ill). Clinical and pathological characteristics were analyzed according to

categories of MR. The Kruskal-Wallis statistic was used to test associations between continuous variables and categorical variables. The Pearson chi-square test or the Fischer exact test was used to assess associations between categorical variables, as appropriate.

The time to recurrence was defined as time to the first melanoma-related event (local recurrence, in transit metastasis, regional lymph node recurrence and distant metastasis). Disease-free survival (DFS) from the date of diagnosis of primary cutaneous melanoma to the date of first recurrence, and overall survival (OS) was calculated from the date of diagnosis to the date of death. DFS and OS were censored at the most recent date of contact on the VCR, if patients were alive at the time of last follow-up or at date of death for patients who died without evidence of melanoma recurrence. Melanoma-specific survival curves were generated according to the Kaplan-Meier product-limit method and were compared using the log-rank test and the Cox proportional hazards regression method. The strength of contribution of prognostic factors was measured by the chi-square values from the Wald test of the coefficient(s) for each prognostic factor in the Cox model.

RESULTS

Of the 2397 melanoma cases treated at the VMS between January 1st 2006 to 2011, 1396 patients with one primary cutaneous melanoma were identified, including 737 males (53%) and 656 females (47%). The patients ranged in age from 9 years to 98 years (mean 56.8, standard deviation 16.4). There were 119 melanoma-specific deaths and 140 deaths overall, accounting for 8.5% and 10.0% of the cohort respectively. The mean follow-up duration was 3.5 years (standard deviation 1.8 years, range 6.6 years).

Significant clinical associations with higher mitotic rate melanoma were male gender, older age, head and neck location and amelanosis ($p<0.0001$). Significant histologic associations with higher mitotic rate melanoma were thicker lesions, ulcerated lesions and nodular tumour type ($p<0.0001$) (Table 1).

116 patients underwent SLNB including 59 males (51%). Thirty of these 116 cases (28%) were SLN-positive. SLN-positive tumours had higher mitotic rate than SLN-negative tumours ($p = 0.028$), however this association was no longer significant when tumour thickness was taken into account. Sixteen of the 30 patients underwent complete lymph node dissection, and 14 of these patients (87%) had additional lymph node involvement. Intransit, regional, nodal and distant metastases were more common in the high ($\geq$5 mitoses/mm^2) mitotic rate group. Time to recurrence for all events was less for patients in this group (Local recurrence, $p = 0.008$; Intransit recurrence, $p= 0.009$; Regional nodal recurrence, $p = 0.003$; Distant metastases, $p = 0.01$) (Table 2).

Univariate analysis indicated that increasing mitotic rate was significantly associated with decreased melanoma-specific survival (MSS) (Figure 1). MSS was also decreased with male sex, older age, head and neck primary tumour site, increasing tumour thickness (Figure 2), presence of ulceration (Figure 3) and tumour type. As the number of mitoses per mm^2 increased, survival rates declined, with estimated 5-year survival ranging from 94% for patients with a MR of <1 mitoses/mm^2 to 71% for those with a MR of $\geq$10 mitoses/mm^2 (Table 3). Higher MR correlated with increasing tumour thickness ($r= 0.55$). Mean tumour thickness (3.7 cf 1.5) and mean mitotic rate (7.0 cf 2.0) were both greater in ulcerated tumours. By using two variables – tumour thickness and mitotic rate – the 5-year survival rate ranged from 96% for patients with a tumour thickness $\leq$1.00mm and <1.00 mitoses/mm^2 to

69% for those with a tumour thickness greater than 4.00mm and ≥ 10 mitoses/mm^2 (Table 4). This suggests that adjusting for thickness could explain the univariate association between mitotic rate and survival. Stratification by ulceration further weakened the association between MR and survival.

Cox multivariate regression analysis indicated that increasing tumour thickness, presence of ulceration and older age remained as independent predictors of melanoma-specific survival. When included in these analyses, either as a continuous or a categorical variable, mitotic rate lost its significance (Methods A and B) (Table 5). In contrast, when MR was coded categorically and subsequently analyzed as a continuous variable (Method C), an apparent prognostic value for survival was seen (Table 6). When ulceration was removed from the multivariate analysis, mitotic rate regained its independent prognostic significance in all 3 multivariate analyses.

DISCUSSION

Whilst tumour thickness serves as the single most important prognostic indicator for localized cutaneous malignant melanoma(Sondergaard and Schou 1985, Balch, Buzaid et al. 2000), discussion continues as to whether factors other than thickness may potentially have some additional value for predicting patient outcome. Mitotic rate, a potential histologic correlate for clinical rate of tumour growth, has gained recognition as having independent prognostic value in melanoma survival. Various recent studies, including an analysis of the 2008 AJCC melanoma staging database(Thompson, Soong et al. 2011), revealed a worsening prognosis with increasing mitotic rate (Retsas, Henry et al. 2002, Azzola, Shaw et al. 2003, Francken, Shaw et al. 2004, Barnhill, Katzen et al. 2005).

We re-examined the prognostic value of MR in a well-characterized cohort of melanoma patients, of whom 19.4% had MR≥5 mitoses/mm^2, and followed for an average of 3.5 years. We confirm the declining overall survival rate with increasing rate of tumour mitosis. A shorter disease free interval was shown with higher mitotic rate tumours, suggesting that a rapidly growing primary melanoma may be associated with a faster tempo of disease progression.

Allowance for thickness, ulceration and older age, however, explained the association between MR and primary melanoma survival. Our findings indicate that tumour thickness and ulceration are the two dominant histologic predictors of survival over the first five years after diagnosis of primary melanoma.

Where MR falls within the hierarchy of prognostic variables in primary invasive melanoma has been the subject of previous studies, with some demonstrating MR taking precedence over the ulcerative state of the tumour (Clark et al, 1989, Retsas, Henry et al. 2002, Azzola, Shaw et al. 2003, Barnhill, Katzen et al. 2005). Others, however, have made less robust findings with MR trialing behind ulceration and various histopathologic variables in predicting survival. MR has been analyzed either as a categorical variable or as a continuous variable, and when it has been analyzed as a categorical variable, different studies have chosen different cut-offs. Possibly for these reasons, discordant findings have emerged from the various studies investigating mitotic rate as a prognostic tool in primary cutaneous melanoma.

Reviewing the findings of the existing cohorts was limited by the under-reporting of the relevant descriptive data regarding characterization of the study cohort. Table 7 contains a

review of previous studies on MR as a predictor of survival, grouped in accordance with their method of analysis. All three statistical approaches are potentially open to criticism. Method A assumes by default a linear effect for each increment in mitoses/mm^2 and in any study this assumption would need to examined and tested. Method B relies on arbitrarily defined categories so the choice of categories needs to be justified in each application. These potential limitations of analyzing MR as a continuous or categorical variable may apply to Method C. In a study of 3661 patients, Azzola et al. utilized the method of analyzing MR whereby it was categorically coded prior to analysis as a continuous variable. The study indicated that MR was a highly significant independent prognostic factor, second only to tumour thickness as the most powerful predictor of survival (Azzola, Shaw et al. 2003). Using the same statistical approach, the finding that MR was an important prognostic factor was confirmed in a second study from the same group involving a separate cohort of patients (Francken, Shaw et al. 2004). In our study, MR lacked statistical significance when analyzed as a categorical or continuous variable, however applying Azzola et al.'s method of analysis revealed a markedly more significant predictive value of MR yet this unorthodox approach to analysis is problematic because of the need to assume constancy of effect across arbitrarily defined categories.

The present study also demonstrated a close correlation between tumour thickness, ulceration and MR. This close correlation may account for the fact that one or another of these variables predominates in different prognostic models and data sets. The etiology of tumour ulceration is not well understood but its development may relate to a rapidly proliferating tumour exceeding its own vascular supply, leading to tissue ischaemia and necrosis. In this sense, MR could be considered as the precedent to ulceration.

The utility of MR in establishing metastatic risk in a sentinel lymph node for T1b melanomas is currently uncertain. There has been support for the consideration of SLNB in thin melanomas with high mitotic rate, although the exact cut-off points varied amongst studies(Sondak, Taylor et al. 2004, Kesmodel, Karakousis et al. 2005). In keeping with previous findings, our study demonstrated a correlation with mitotic rate and sentinel lymph node status. In addition, the finding that a high rate (87%) of involvement of non-sentinel nodes on completion lymphadenectomy in association with high MR tumours suggests a possible role for MR in determining the need for completion dissection following a positive SLNB.

An important limitation of the study is the limited duration of follow-up. Within this period of follow-up, ulceration took precedence over MR in predicting survival, although the prognostic power of MR in T1 melanomas may become more apparent with a longer duration of study. In addition, there were very few thin (≤1mm) tumours with high mitotic rates, possibly explaining why MR did not have an effect on survival in this group of patients in our study.

Comparative analyses with population-based studies may be warranted for validation of the current findings as results may be influenced by the characteristics of an older cohort of Australian patients treated at tertiary referral centre. Nonetheless, in order to accurately ascertain the prognostic importance of MR and to undertake fair comparisons between various multivariate studies, this study highlights the imperative for investigators to employ a consistent and standardized statistical approach by which MR is analyzed.

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LEGENDS

Figure 1: Survival curves of 1396 patients with localized, cutaneous melanoma grouped according to 4 categories of tumour mitoses/mm^2.

Figure 2: Survival curves of 1396 patients with localised cutaneous melanoma when tumour thickness was grouped into 4 categories.

Figure 3: Survival curves of 1372 patients with localised, cutaneous melanoma when grouped according to presence of ulceration.

TABLES

Table 1: Clinical and pathological characteristics

Table 2: Clinical outcomes

Table 3: Univariate analyses and 5-year survival of prognostic factors

Table 4: 5-year cause-specific survival (%) by tumour thickness and mitotic rate

Table 5: Cox regression Multivariate analysis of Prognostic factors (Methods A and B) for Melanoma-specific survival (n = 119)

Table 6: Cox regression for 1396 patients for melanoma-specific deaths (Method C)

Table 7: Summary of Multivariate Studies

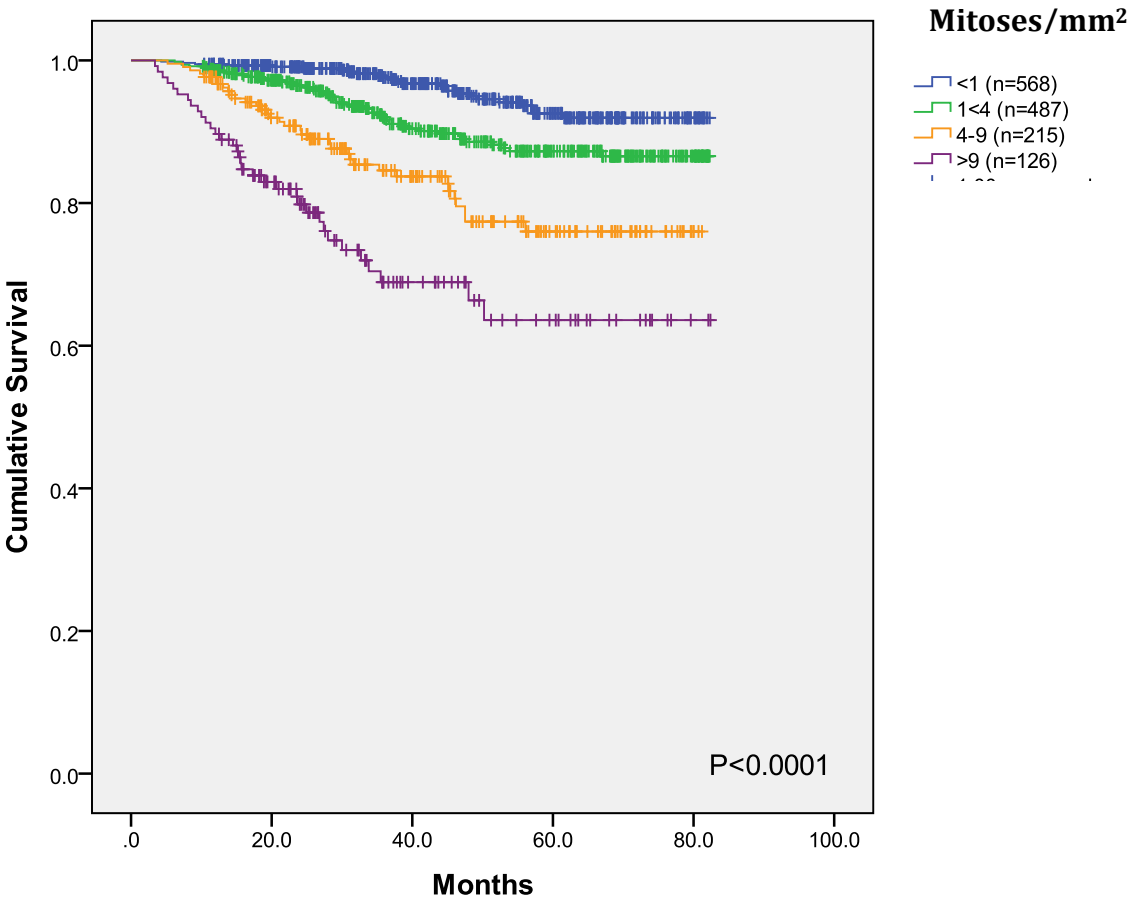

Figure 1: Survival curves of 1396 patients with localized, cutaneous melanoma grouped according to 4 categories of tumour mitoses/mm^2.

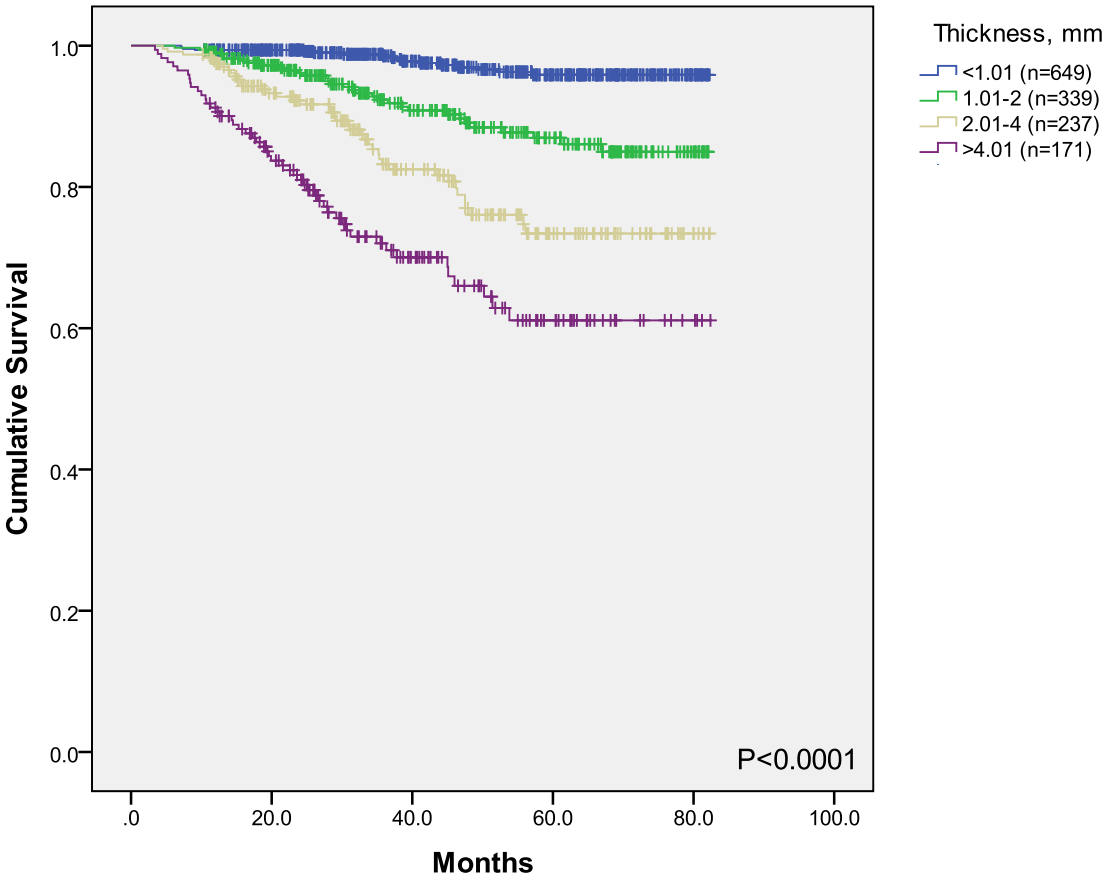

Figure 2: Survival curves of 1396 patients with localised cutaneous melanoma when tumour thickness was grouped into 4 categories.

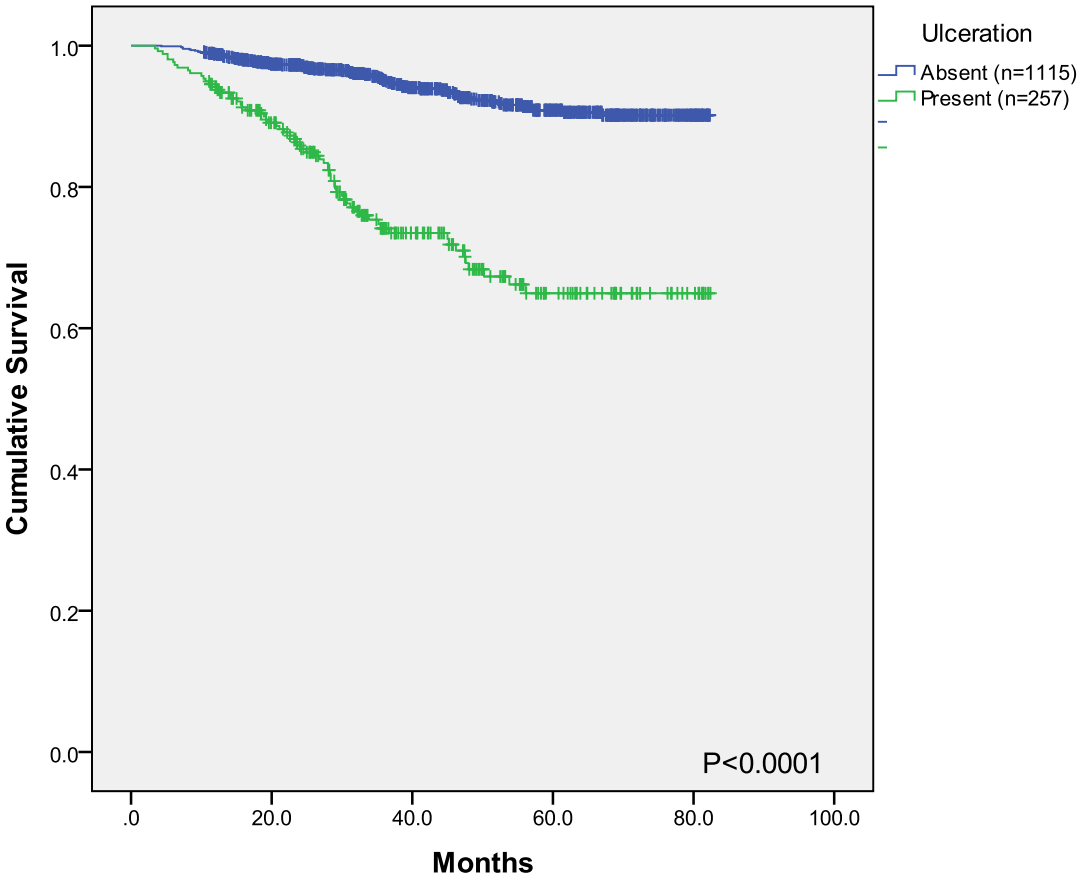

Figure 3: Survival curves of 1372 patients with localised, cutaneous melanoma when grouped according to presence of ulceration.

Table 1: Clinical and pathological characteristics

	Mitotic rate, /mm^2							
	Low		Intermediate			High		
	0	<1	1<2	2	3-4	5-9	≥10	*P*-value
Total n. of patients (n = 1396)(%)	326	242	284	128	145	145	126	
Follow-up, months								
Mean (SD)	50.4 (±20.7)	37.8 (±15.5)	48.3 (±23.2)	43.8 (±20.6)	41.9 (±21.3)	37.8 (±20.8)	32.6 (±19.9)	
Range	7.4-74.9	4.3-81.5	6.2-82.3	8.3-82.0	9.8-82.2	5.2-80.0	3.4-82.4	
Age, y								
Mean	54.7	53.7	55.0	56.4	59.8	60.9	64.6	
Standard deviation	16.4	14.8	16.2	17.7	16.3	16.4	15.1	
Range	20-94	17-85	19-79	19-90	9-92	18-92	20-94	
Age group								
<50	128 (39.3)	89 (36.8)	103 (36.3)	41 (32.0)	43 (29.7)	35 (24.1)	24 (19.0)	<0.0005
50<70	131 (40.2)	119 (49.2)	126 (44.4)	55 (43.0)	51 (35.2)	60 (41.4)	46 (36.5)	
≥70	67 (20.6)	34 (14.0)	55 (19.4)	32 (25.0)	51 (35.2)	50 (34.5)	56 (44.4)	
Sex								
Female	183 (56.1)	119 (49.2)	142 (50)	54 (42.2)	59 (40.7)	61 (42.1)	39 (31.0)	<0.0005
Male	143 (43.9)	123 (50.8)	142 (50)	74 (57.8)	86 (59.3)	84 (57.9)	87 (69.0)	
Primary tumour site								
Head and neck	72 (22.4)	51 (21.1)	61 (21.5)	31 (24.2)	30 (20.7)	46 (31.7)	50 (39.7)	0.002
Upper extremities	76 (23.3)	42 (17.4)	51 (18.0)	19 (14.8)	33 (22.8)	33 (22.8)	22 (17.5)	
Trunk	96 (29.4)	84 (34.7)	85 (29.9)	45 (35.2)	41 (28.3)	32 (22.1)	35 (27.8)	
Lower extremities	81 (24.8)	65 (26.9)	85 (29.9)	33 (25.8)	41 (28.3)	33 (22.8)	19 (15.1)	
Amelanosis								
Yes	22 (6.7)	21 (8.7)	28 (9.9)	23 (18.0)	27 (18.6)	39 (26.9)	32 (25.4)	<0.0005
No	304 (93.3)	221 (91.3)	256 (90.1)	105 (82.0)	118 (81.4)	106 (73.1)	94 (74.6)	
Tumour Type								
SSM	224 (68.7)	175 (72.3)	199 (70.1)	65 (50.8)	84 (57.9)	61 (42.1)	34 (27.0)	<0.0005
NM	3 (0.9)	12 (5.0)	30 (10.6)	26 (20.3)	39 (26.9)	53 (36.6)	69 (54.8)	
LMM	63 (19.3)	33 (13.6)	27 (9.5)	11 (8.6)	10 (6.9)	16 (11.0)	14 (11.1)	
DM	13 (4.0)	9 (3.7)	16 (5.6)	9 (7.0)	2 (1.4)	6 (4.1)	6 (4.8)	
ALM	6 (1.8)	6 (2.5)	6 (2.1)	6 (4.7)	2 (1.4)	5 (3.4)	2 (1.6)	
Other[†]	17 (5.2)	7 (2.9)	6 (2.1)	11 (8.6)	8 (5.5)	4 (2.8)	1 (0.8)	
Tumour thickness, mm								
Mean	0.8	0.9	1.4	2.5	2.7	3.2	4.8	
Standard deviation	1.0	1.0	1.4	2.5	2.2	2.3	3.2	
Range	7.3	8.9	13.4	20.9	15.8	18.7	18.0	
≤ 1.0	266 (81.6)	184 (76.0)	147 (51.8)	23 (18.0)	19 (13.1)	7 (4.8)	3 (2.4)	<0.0005
1.01-2	35 (10.7)	42 (17.4)	95 (33.5)	51 (39.8)	55 (37.9)	40 (27.6)	21 (16.7)	
2.01-4	15 (4.6)	12 (5.0)	27 (9.5)	38 (29.7)	44 (30.3)	64 (44.1)	37 (29.4)	
>4.01	10 (3.1)	4 (1.7)	15 (5.3)	16 (12.5)	27 (18.6)	34 (23.4)	65 (51.6)	
Clark level[†]								
II	178 (54.6)	107 (44.2)	49 (17.3)	3 (2.3)	4 (2.8)	0	1 (0.8)	<0.0005
III	69 (21.2)	74 (30.6)	87 (30.6)	23 (18.0)	21 (14.5)	18 (12.4)	19 (15.2)	
IV	72 (22.2)	57 (23.6)	134 (47.2)	84 (65.6)	106 (73.1)	104 (71.7)	74 (59.2)	
V	7 (2.2)	4 (1.7)	14 (4.9)	18 (14.1)	14 (9.7)	23 (15.9)	31 (24.8)	
Ulceration								
Present	4 (1.2)	18 (7.4)	27 (9.5)	33 (25.8)	45 (31.0)	61 (42.1)	69 (54.8)	<0.0005
Absent	322 (98.8)	224 (92.6)	257 (90.5)	95 (74.2)	100 (69.0)	84 (57.9)	57 (45.2)	

[†] 1 case with indeterminate Clark level excluded from analysis

Table 2: Clinical outcomes

	Mitotic rate, /mm^2							
	Low		Intermediate			High		
	0	<1	1<2	2	3-4	5-9	≥10	*P*-value
Total n. of patients (n = 1396) (%)	326	242	284	128	145	145	126	
SLN status (n=116)								
Negative	5 (71.4)	7 (100)	23 (82.1)	15 (88.2)	16 (59.3)	11 (78.6)	9 (56.3)	0.028
Positive (n= 30)	2 (28.6)	0	5 (17.9)	2 (11.8)	11 (40.7)	3 (21.4)	7 (43.8)	
Local recurrence								
Yes	9 (2.8)	3 (1.2)	6 (2.1)	4 (3.1)	3 (2.1)	6 (4.1)	4 (3.2)	0.39
No	317 (97.2)	239 (98.8)	278 (97.9)	124 (96.9)	142 (97.9)	139 (95.9)	122 (96.8)	
Time to recurrence, months (mean, SD)	37.6 (±18.6)	19.3 (±6.0)	13.3 (±16.8)	11.4 (±10.5)	17.4 (±22.4)	17.0 (±17.3)	11.4 (±3.3)	0.008
Intransit recurrence								
Yes	4 (1.2)	2 (0.8)	4 (1.4)	5 (3.9)	7 (4.8)	11 (7.6)	6 (4.8)	<0.0005
No	322 (98.8)	240 (99.2)	280 (98.6)	123 (96.1)	138 (95.2)	134 (92.4)	119 (95.2)	
Time to recurrence, months (mean, SD)	23.1 (±21.2)	6.7 (±1.9)	4.4 (±5.0)	2.6 (±4.1)	12.4 (±10.1)	6.2 (±5.4)	12.7 (±11.5)	0.009
Regional nodal recurrence								
Yes	9 (2.8)	8 (3.3)	13 (4.6)	15 (11.7)	24 (16.6)	24 (16.6)	19 (15.1)	<0.0005
No	317 (97.2)	234 (96.7)	271 (95.4)	113 (88.3)	121 (83.4)	121 (83.4)	107 (84.9)	
Time to recurrence, months (mean, SD)	24.0 (±14.1)	17.2 (±10.7)	20.6 (±13.3)	10.9 (±9.2)	12.8 (±11.0)	12.4 (±9.7)	9.4 (±10.0)	0.003
Distant metastases								
Yes	10 (3.1)	12 (5.0)	16 (5.6)	15 (11.7)	19 (13.1)	23 (15.9)	30 (23.8)	<0.0005
No	316 (96.9)	230 (95.0)	268 (94.4)	114 (88.3)	126 (86.9)	122 (84.1)	96 (76.2)	
Time to recurrence, months (mean, SD)	26.1 (±17.6)	22.9 (±16.0)	21.1 (±16.4)	18.1 (±16.4)	15.7 (±12.6)	13.1 (±11.1)	10.9 (±11.2)	0.01

SLN, sentinel lymph node, SD, standard deviation

Table 3: Univariate analysis and 5-year Survival of Prognostic factors									
		Death due to melanoma (n = 119)				Death due to all causes (n = 140)			
	N	5-year survival, %	Risk Ratio	95% CI	P-value	5-year survival, %	Risk ratio	95% CI	P-value
Age, years									
<50	463	95	1		<0.0001	95	1		<0.0001
50<70	588	88	2.4	1.3-4.2	0.004	88	2.5	1.4-4.4	0.002
≥70	345	73	5.5	3.1-9.8	<0.0001	67	7.9	4.5-13.8	<0.0001
Sex									
Female	657	91	1			88	1		
Male	739	83	1.9	1.3-2.9	0.001	82	1.8	1.3-2.6	0.001
Primary tumour site									
Upper extremity	277	92	1		0.012	88	1		0.004
Head and neck	342	83	2.3	1.2-4.4	0.008	79	2.0	1.2-3.5	0.009
Trunk	420	84	2.2	1.2-4.1	0.013	84	1.5	0.9-2.6	0.142
Lower extremity	357	91	1.3	0.7-2.6	0.46	90	0.9	0.5-1.6	0.676
Amelanosis									
Yes	192	86	1.2	0.7-2.0	0.46	86	1.5	0.9-2.4	0.083
No	1204	87	1			81	1		
Tumour type									
Superficial spreading	842	90	1		0.004	89	1		<0.0001
Nodular	232	76	2.2	1.4-3.4	0.001	71	2.4	1.5-3.7	<0.0001
Lentigo Maligna	174	89	1.2	0.7-2.3	0.51	87	1.3	0.8-2.4	0.338
Desmoplastic	61	81	2.1	1.0-4.7	0.063	78	2.4	1.2-4.9	0.019
Acral Lentiginous	33	69	3.1	1.2-7.9	0.016	69	2.7	1.1-6.8	0.034
Tumour thickness, mm									
≤1.0	649	96	1		<0.0001	96	1		<0.0001
1.01-2	339	85	4.8	2.4-9.2		85	3.9	2.1-7.1	
2.01-4	237	77	9.1	4.7-17.4		74	7.8	4.3-14.0	
≥4.01	171	68	14.5	7.5-27.8		61	15.4	8.6-27.5	
Mitoses, /mm^2									
0	326	94	1		<0.0001	93	1		<0.0001
<1	242	88	0.9	0.4-2.2	0.87	88	0.7	0.3-1.7	0.50
1<2	284	92	1.2	0.6-2.7	0.57	91	1.3	0.7-2.6	0.44
2	128	82	3.2	1.5-6.9	0.003	81	3.0	1.5-6.1	0.003
3-4	145	81	3.6	1.7-7.6	0.001	79	3.3	1.7-6.5	0.001
5-9	145	79	4.3	2.1-8.8	<0.0001	75	4.0	2.1-7.8	<0.0001
≥10	126	71	6.6	3.3-13.2	<0.0001	63	7.2	3.8-13.6	<0.0001
Clark level									
II	342	96	1		<0.0001	95	1		<0.0001
III	311	92	2.3	0.9-5.7	0.083	90	2.4	1.1-5.4	0.033
IV	631	83	6.3	2.8-13.8	<0.0001	81	5.3	2.6-10.7	<0.0001
V	111	71	13.2	5.5-31.6	<0.0001	63	15.0	6.9-32.7	<0.0001
Ulceration									
Present	257	71	4.6	3.1-6.8	<0.0001	65	5.0	3.4-7.2	<0.0001
Absent	1139	91	1			90	1		

Table 4: 5-year cause-specific survival (%) by tumour thickness and mitotic rate															
Mitotic rate (mitoses/mm^2)		<1.00			1.00-4			5-9			≥10			Overall	
Tumour thickness (mm)	**Total n. of patients**	**%**	**SE**	**n**	**%**	**SE**	**n**	**%**	**SE**	**n**	**%**	**SE**	**n**	**%**	**SE**
0.0-1.0	649	96	0.01	450	97	0.02	189	100	-	7	100	-	3*	97	0.01
1.01-2.0	339	79	0.08	77	85	0.03	201	90	0.06	40	94	0.06	21	82	0.02
2.01-4.0	237	76	0.13	27	78	0.05	109	85	0.05	64	59	0.13	37	70	0.06
>4.00	171	71	0.15	14	76	0.09	58	54	0.11	34	69	0.06	65	35	0.15

SE, standard error
*5-year survival rates cannot be accurately measured in this subgroup due to small patient numbers

Table 5: Cox regression Multivariate analysis of Prognostic factors (Methods A and B) for Melanoma-specific survival (n = 119)

	Wald	Df	Risk ratio	95% CI	P-value
Age					
<50	18.3	2	1		<0.0001
50<70			1.9	1.0-3.6	
≥70			3.5	1.9-6.6	
Sex					
Female	1.9	1	1		0.152
Male			1.4	0.9-2.1	
Primary tumour site					
Upper extremity	10.0	3	1		0.019
Head and neck			1.4	0.7-2.6	
Trunk			0.9	0.5-1.9	
Lower extremity			2.1	1.1-3.9	
Tumour type					
SSM	9.5	4	1		0.052
NM			0.6	0.4-1.0	
LMM			0.7	0.3-1.3	
DM			0.5	0.2-1.1	
ALM			2.1	0.8-5.4	
Tumour thickness, mm					
≤1.0	12.1	3	1		0.004
1.01-2			3.6	1.5-8.4	
2.01-4			5.1	2.0-12.5	
≥4.01			5.7	2.1-15.2	
Mitoses/mm^2					
Method A: continuous variable	2.6	1	1	1.0-1.04	0.13
Method B*:	3.9	6			0.694
0			1	0.4-2.4	
<1			0.8	0.4-1.8	
1<2			0.9	0.4-2.1	
2			1.0	0.4-2.4	
3-4			1.1	0.5-2.5	
5-9			1.6	0.7-3.6	
≥10					
Clark level					
II	4.9	3	1		0.12
III			0.8	0.3-2.4	
IV			1.0	0.4-3.0	
V			2.1	0.6-6.9	
Ulceration					
Present	7.7	1	1.8	1.2-2.8	0.005
Absent			1		

*Method B adjusts for the same set of factors listed in the table

Table 6: Cox regression for 1396 patients for melanoma-specific deaths (Method C)				
Variable	**Wald**	**Df**	**Hazard ratio, 95% CI**	**p-value**
Thickness	4.5	1	1.2 (1.0-1.4)	0.033
Mitotic rate	3.9	1	1.1 (1.0-1.3)	0.048
Age	19.9	1	1.4 (1.2-1.6)	<0.0001
Sex	3.5	1	0.7 (0.5-1.0)	0.06
Ulceration	8.3	1	1.9 (1.2-2.8)	0.004
Tumour type	2.0	1	1.1 (0.96 - 1.31)	0.154
Clark level of invasion	5.0	1	1.4 (1.0-1.96)	0.026
Location	0.037	1	1.0 (0.8-1.3)	0.846

DF: degree of freedom; CI: confidence interval
Thickness was coded as 1:≤1.00mm, 2:1.01-2.00mm, 3:2.01-3.00mm, 4: 3.01-4.00 and 5:4.01-5.00mm, 6:>5.0
Mitotic rate was stratified into 1=0, 2=<1, 3=1<2, 4=2,5=3-4,6=5-9, 7=≥10.
Age was coded as deciles: 0-9, 10-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, ≥80, Ulceration was coded as 0: absent and 1: present. Gender was coded as 0: male and 1: female. Tumour site was coded as 1: head and neck, 2:trunk, 3: extremities. Tumour type was coded as 1: SSM, 2: DM, 3:LMM, 4:NM, 5:ALM, Tumour level was coded as Clark Level II: 1, III: 2, IV: 3, V: 4.

Table 7: Summary of Multivariate Studies					
Study	**N**	**Mean follow-up, years**	**Methods of MR (/mm^2)**	**End-point**	**P-Value**
Current Study	1396	3.5	Method A: Continuous Method B: Categorical Method C: Continuous / categorical	5-year survival	0.13 0.694 0.048
METHOD A					
Retsas et al, 2002	1284	5.5	Continuous	10-year survival	0.005
Massi et al, 2000	275	8.6	Continuous	10-year survival	0.004
Vossaert et al, 1992	832	9.3	Continuous	10-year survival	0.1064
METHOD B					
Thompson et al, 2011	10,233	Not specified	Categorical (0, 0<1, 1<2, 2<5, 5<10, 10<20, ≥20)	10-year survival	<0.001
Barnhill et al, 2005	473	> 5	Categorical (0, 1-6, >6)	5-year survival	<0.01
Schmid-Wendtner et al, 2001	2715	7.5	Categorical (≤4, >4.0-8.8, 8.9≤14.5, >14,5)	Metastasis	<0.05
Massi et al, 2000	275	8.6	Categorical (<10, ≥10)	10-year survival	0.005
Ostmeier et al, 1999	691	7.0 (median)	Categorical (<3, ≥3)	Metastasis	0.0235
Clarke et al, 1989	386	13.9	Categorical (0, 0.1-6, >6)	8-year survival	Not specified; odds ratio for survival: MR=0; OR = 11.7, MR=0.1-6.0; OR = 3.5; MR >6.0, OR =1)
METHOD C					
Francken et al, 2004	1211	13.8	Continuous / categorical (≤2, >2-8, >8)	10-year survival	0.008
Azzola et al, 2003	3661	4.3 (censored cases)	Continuous / categorical (0, 1-4, 5-10, ≥11)	10-year survival	<0.0001

Chapter 5: Discussion

This chapter provides a collective summary of the main findings of the thesis; examines the clinical implications of such findings and offers suggestions for further research. The strengths and limitations of the studies undertaken are also discussed.

5.1 Main findings

This study aimed to examine firstly, the associations of mitotically active melanoma and secondly, the significance of mitotic rate as a predictor of melanoma survival. The use of the Victorian Melanoma Service (VMS) database enabled a comprehensive analysis of clinical, historical and phenotypic characteristics of patients who developed what is regarded as biologically aggressive primary cutaneous melanomas, as typified by their mitotic count. From this database, it was identified that such melanomas have the propensity to occur in elderly men with a history of solar field damage, who presented with rapid tempo disease. These tumours were predominantly found on the head and neck region. Morphologically, high mitotic rate melanomas were likely to present as amelanotic lesions that, despite the lack of pigmentation, tended to first arouse suspicion in the patients themselves. A significant family history of melanoma and past history of blistering sunburns were associated with thin melanomas as exemplified by tumour types with a predominant radial growth phase.

Histopathologically, high mitotic melanoma was associated with tumour thickness, nodular tumour type and other aggressive histologic features including Clark level of invasion, ulceration, lymphovascular invasion, perineural invasion and microsatellitosis. It exhibited an inverse relationship with blistering sunburn, regression and pre-existing naevus. Amelanosis, thickness, nodular tumour type, ulceration and Clark level of invasion remained as independent associations of high mitotic rate melanoma.

In contrast, the prognostic significance of mitotic rate was not as clear to define as its clinicopathologic associations in this study. By univariate analysis, it was evident that survival declined with increasing mitotic rate. In addition, high mitotic rate was predictive of a greater likelihood of loco regional recurrence and distant metastatic disease as well as sentinel lymph node positivity. By multivariate analysis however, the robust nature of mitotic

rate in prognosticating clinical outcome was lost when age, thickness and ulceration were taken into account. What was also demonstrated in the present study was the variation in the independent significance of mitotic rate when different methodological approaches were employed, which produces subtle yet nonetheless noticeable differences in the value of mitotic rate in predicting survival, and hence its clinical utility.

5.2 Addition to the literature

As the prognostic significance of mitotic rate emerged only in recent years, there is a paucity of comprehensive data on the characteristics and associations of high-mitotic-rate melanoma in the current literature. Numerous studies have, however, examined the risk profile of patients with the propensity to develop thick and rapidly growing tumours[16, 111], which, as the current study has demonstrated, hold a close correlation with mitotic rate. There is a recognized association between tumour thickness, male sex and older age. In addition the tendency for thick tumours to occur on the head and neck has been reported. Attention has also turned to the clinicopathologic characteristics of nodular melanoma, a tumour subtype found to be associated with poor prognosis[6]. Nodular melanoma is recognized as a tumour subtype that defies the classic ABCDE rule of melanoma detection, with its propensity to present with atypical clinical features that may lead to delayed diagnosis. Nodular melanoma has been shown to not only been associated with greater tumour thickness but also rapid rate of growth and amelanosis[2]. Patients with phenotypically fewer freckles and naevi but with a history of cumulative sun exposure as exemplified by the presence of solar keratosis have been shown to be more likely to develop de novo rapidly growing tumours which are found to be predominantly nodular in type[1]. In contrast to the nodular tumour type, superficial spreading melanoma has been associated with a slower rate of growth[111]and better prognosis[6].

Previous observations of the characteristics of locally advanced melanoma were confirmed and elaborated upon in this study. Distinct patient characteristics, clinical features and histologic markers of tumour aggressivity were shown to strongly correlate with high mitotic rate. At the same time, patients with mitotically inactive melanomas were identified to be more likely female, with a family history of a first-degree relative with melanoma and a clinical history of previous blistering sunburns. Such patients are more likely to have tumours with a predominant radial growth phase such as that of the superficial spreading and lentigo maligna subtypes and histologically, were likely to be associated with features of regression and pre-existing naevi.

The significance of mitotic rate has been the subject of numerous multivariate studies in recent years[69, 116-124]. Subsequent to reaching a consensus with regards to the measurement of mitotic rate, evidence for its prognostic utility began to emerge. Although with different methodological approaches, some of which may be considered statistically unorthodox[119, 121-2], the mounting evidence for the robust prognostic value of mitotic rate culminated to significant changes made to the staging of melanoma. The most recent AJCC staging system for melanoma replaced Clark level of invasion with mitotic rate as the primary criterion for staging T1b melanoma, along with ulceration.

Whilst the current literature has shown mitotic rate as an important independent prognostic factor in primary invasive melanoma, this present study produced findings contrary to previous assertions. Assigning such significance to mitotic rate may require reconsideration in light of the current findings that utilized multiple methodological approaches to elucidate the independent prognostic significance of this histologic feature of invasive melanoma. Whilst mitotic rate can be considered as a marker of inherent tumour aggressivity, as shown by its association with various clinical end-points, the examination of its significance when taking

into account other established prognostic variables such as tumour thickness and ulceration would appear to require a more standardized method of analysis in order to allow for accurate and fair comparisons between different studies.

5.3 Clinical Implications

The current findings have implications for the clinical care of skin cancer patients. Firstly, awareness of the characteristics of aggressive primary invasive cutaneous melanoma can aid the detection of patients at risk of developing melanomas that carry poor prognosis. Clinicians should be made aware of the potential atypical morphology of aggressive primary melanomas that defies the ABCDE rule. Emphasis should be placed on "E" for evolution. Clinical suspicion should be aroused in a rapidly growing lesion despite having features that are not classically associated with malignant melanocytic transformation such as symmetry, colour homogeneity and border regularity. Furthermore, awareness of the demographic and historical background of patients will aid in early detection, with elderly males with a history of solar field damage falling into this risk group.

Secondly, melanoma prevention should revolve around similar principles. The importance of sun protection and timely skin surveillance should also be recommended. Finally, discussion with the patient regarding prognosis involves elucidation of the importance of tumour thickness and ulceration as providing guidance in overall clinical outcome. Mitotic rate should remain as an integral part of the histopathology report for the diagnosis of a primary invasive melanoma. For prognostication, high mitotic activity may portend aggressive clinical behavior and its clinical utility in stratifying risk for early localised melanoma should be made aware of amongst treating clinicians. In patients with thin tumours (≤1mm), the presence of ulceration and a high mitotic count should alert the clinician to the likelihood of a more worse prognosis than in those with melanoma lacking in these histopathological

features. This information needs to be relayed to the patient when discussing the long-term outcome. Awareness of this should be reflected in our approach to managing this group of patients with high-risk thin melanomas, as it is already beginning to take place in the realm of investigative options such as SLNB[107]. In addition, certain patient characteristics must also be taken into account when prognosticating survival, namely the age and gender of the patient.

5.4 Strengths and limitations of this study

The strengths of this study relate to the characterization of the patient cohort and its methodological approach. The sample size for both studies was adequate in performing the statistical analyses undertaken. A consistent and comprehensive set of descriptive and quantitative data relating to patient characteristics, tumour presentation and histologic information were available and utilized for investigation based on the Victorian Melanoma Service (VMS) Registry. This provided a clinically and histopathologically well-characterized cohort for analysis in both studies.

The quality and consistency of care provided to the patients within the two studies minimized bias. The VMS is a tertiary multidisciplinary referral centre providing comprehensive care for patients diagnosed with melanoma with surgical, oncological and allied health support. Retention of patients during the period of study was excellent. All patients studied underwent definitive treatment at VMS and subsequent recurrence of disease were also treated at VMS through re-referral by practitioners elsewhere.

The assessment of clinical end-points was based on information from a reliable source for the second study. Data linkage with the Victorian Cancer Registry (VCR) provided information relating to ascertainment of death and the cause of death, allowing for reliable analysis of melanoma-specific survival.

In addition, there was consistent measurement of key variables. Mitotic rate was measured in count per mm^2 and cases in which it was expressed as HPF were excluded to minimize differential error. Two histopathologists with expertise in diagnosing melanocytic lesions reviewed the original specimens from outside sources, which served to minimize inter-observer variability.

There were however, limitations inherent within the two studies. Firstly, duration of follow-up for the second study on the prognostic significance of mitotic rate was relatively short in comparison to previous multivariate prognostic studies. Only patients presenting to the VMS after 2005 were studied as MR was measured in HPF prior to this. The second study also analyzed an older cohort of patients, which may serve to bias the findings. Age was revealed as a remarkably potent predictor of melanoma-specific survival in the present study, which deviated somewhat from previous findings. However, comparative analysis of data was difficult due to many studies lacking clear characterization of the cohorts studied[69].

Secondly, as the VMS is a tertiary referral center, there is the possibility of referral bias with patients with more locally advanced disease or with complex medical comordities referred from the community. Whilst the VMS registry contained a comprehensive set of clinical and histopathological data, there were however relevant information which was not included such as the patient's socio-economic status. There may also be recall bias with obtainment of information concerning time when first noticed development or change of lesion to time to diagnosis, a measurement used to calculation of rate of growth in the first study.

Thirdly, whilst the VCR linkage provided accurate information relating to ascertainment of the cause of death, there is however an in-built delay of approximately 2 months in the update

of information with a further delay if the patient were to decease outside of Victoria. The actual cases of deaths may therefore be slightly greater than what was recorded for the study on prognosis.

5.5 Future directions

The notion that the inherent aggressivity of melanoma is determined by a combination of clinicopathologic variables is supported by the current findings. Whilst thickness remains as the most important determinant of clinical outcome in primary melanoma, a wide array of clinical and histologic factors can assist in refining its prognostic accuracy. However, further investigation should be undertaken to broaden our understanding of the associations and significance of aggressive primary melanoma.

Firstly, a consensus needs to be reached regarding a standardized method of analyzing mitotic rate as means to allow for fair and accurate comparisons being made between various studies. Secondly, characterization of the study cohort should also be described for similar purposes. Finally, large population-based studies can serve to validate findings from tertiary databases.

Whilst the overall survival for melanoma continues to improve in Australia, melanoma-specific mortality amongst certain demographic groups is in fact climbing, in particularly in men. Questions therefore still remain as to why this is the case and whether this is a result of factors intrinsic to men or to do with exogenous factors. Suffice to say much still needs to be done to elucidate the pathogenesis and progression of malignant melanoma and to further our understanding of how we can best detect at-risk groups and provide optimal treatment for melanoma patients.

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Appendix 1 Paper 1

Original Investigation

Characteristics and Associations of High-Mitotic-Rate Melanoma

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IMPORTANCE Mitotic rate is now recognized as having independent prognostic significance in melanoma survival. However, its clinicopathologic associations have not been the focus of any previous study.

OBJECTIVE To identify a set of patient and tumor characteristics associated with high-mitotic-rate melanoma with the aim of facilitating the earlier detection of aggressive primary invasive melanoma.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional study of patients from a multidisciplinary melanoma clinic based in a public hospital. A total of 2397 cases from January 2006 to December 2011 were reviewed by the Victorian Melanoma Service, and 1441 patients with 1500 primary invasive melanomas were included in the study.

MAIN OUTCOMES AND MEASURES Mitotic rate was measured as number of mitoses per mm^2 and analyzed as ordered categories (0, <1, 1 and <2, 2, 3-4, 5-9, and ≥10) according to patient demographics, phenotypic markers, historical data, tumor presentation, and histopathologic features.

RESULTS Melanomas with higher mitotic rates were more likely to occur in men (odds ratio [OR], 1.5; 95% CI, 1.3-1.8), patients 70 years or older (OR, 2.1; 95% CI, 1.7-2.8), and those with a history of solar keratosis (OR, 1.3; 95% CI, 1.1-1.6). These melanomas occurred more frequently on the head and neck (OR, 1.4; 95% CI, 1.0-1.9) and presented more often as amelanotic (OR, 1.9; 95% CI, 1.4-2.5) and rapidly growing (≥2 mm/mo) lesions (OR, 12.5; 95% CI, 8.4-18.5). An association was seen with the nodular melanoma subtype (vs superficial spreading [reference]) (OR, 2.5; 95% CI, 1.8-3.4), greater tumor thickness (vs ≤1 mm [reference]) (>1-4 mm: OR, 4.5; 95% CI, 3.2-6.1; >4 mm: OR, 12.6; 95% CI, 7.5-21.1), and ulceration (OR, 2.0; 95% CI, 1.5-2.7). These histopathologic features, along with amelanosis and rate of growth, remained as significant associations with high mitotic rate in the overall multivariate analysis.

CONCLUSIONS AND RELEVANCE High-mitotic-rate primary cutaneous melanoma is associated with aggressive histologic features and atypical clinical presentation. It has a predilection for the head and neck region and is more likely to be seen in elderly men with a history of cumulative solar damage who present clinically with rapidly developing disease.

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Mitotic rate, a quantifiable marker of tumor cellular proliferation, has been closely correlated with survival, some studies demonstrating independent prognostic significance.[1-11] For thin tumors, 1 mm or thinner, the mitotic rate is now a criterion alongside ulceration for defining T1b melanoma.[4]

Previous studies of aggressive melanoma have focused on the characteristics and associations of thick melanoma. Thick melanomas have been shown to have a strong association with the nodular subtype,[12-17] to grow rapidly, and to occur more frequently in elderly men and in individuals with fewer nevi and fewer freckles.[18,19] However, the existing literature has scarce data regarding the clinical presentation and associations of high-mitotic-rate melanoma to assist in identifying those at risk for poor prognosis. The aim of this study was to delineate the clinical, phenotypic, and histologic associations of high-mitotic-rate melanoma.

Methods

Patients and Data Collection

Institutional ethics approval was obtained from the Alfred Hospital for a retrospective review of all patients seen at the Victorian Melanoma Service (VMS) from January 2006 to December 2011. Patient informed consent was waived. All patients whose primary invasive melanoma was histologically reviewed and assessed for mitotic rate at the VMS were included in the study. Two expert dermatopathologists independently reviewed the original histopathology sections for each case.

The following data were collected prospectively for each subject: demographic information (age, sex, ethnicity), phenotypic markers (eye color, hair color, skin prototype), historical features (number of blistering sunburns, previous melanoma, solar keratosis, family history of a first-degree relative with melanoma), and tumor presentation (amelanosis, body site, and time from the initial observation of the lesion to confirmed diagnosis). Clinical examination of all patients undertaken by a dermatology resident provided count of total nevi, clinically determined dysplastic nevi, freckles, and solar lentities. The count of total number of melanocytic nevi was grouped (<20, 20-50, >50-100, >100-200, >200-500, and >500) based on previously observed risk thresholds for melanoma.[20,21] Dysplastic nevi were counted exactly, and freckles and solar lentigines were recorded as few, moderate, or many. For the purpose of this study, tumor site was classified as head and neck, trunk, upper extremities, and lower extremities. We used a previously described historical estimation of rate of growth of a tumor that was derived by dividing its Breslow thickness in millimeters by the time, in months, from the initial observation of the change in the lesion to histologic diagnosis.[19,22] Amelanotic tumors were those that appeared to the patient to be without pigmentation.

Mitotic rate was determined histologically by first examining the entire tumor looking for mitotic figures. The area with the greatest density of mitotic activity was used as the focus, and the number of mitoses in a surrounding area of 1 mm^2 was counted. Mitotic rate was reported by the dermatopathologist either as a discrete integer or as less than 1 and less than 2 per mm^2.

Tumor type was classified histologically as superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, acral lentiginous melanoma, or desmoplastic melanoma. Less common tumor types were grouped as *other*. Other histologic features assessed were Breslow thickness, ulceration, Clark level of invasion, lymphovascular invasion, microsatellitosis, tumor infiltrating lymphocytes, regression, neurotropism, and preexisting nevus.

Statistical Analysis

Mitotic rate followed a skewed distribution and was categorized for analysis (0, <1, 1 and <2, 2, 3-4, 5-9, and ≥10 mitoses/mm^2). These cutoffs were chosen to ensure reasonable numbers of patients in each category. The association of mitotic rate with each of the clinical and histopathologic parameters was summarized with nonparametric Spearman rank correlation coefficients. Ordinal logistic regression was used to examine univariate and multivariate associations between mitotic rate and other variables. Odds ratios (ORs) from this analysis can be interpreted as increased odds of a higher category of mitotic rate regardless of where the scale is dichotomized. This interpretation relies on the assumption of proportional odds, which was tested for the final multivariate model. Nonlinearity of the association between mitotic rate and thickness was explored using fractional polynomials. Statistical analyses were performed using IBM SPSS Statistics version 19.0 (IBM Corporation) and Stata version 13 (StataCorp LP) statistical software packages. $P < .05$ was considered statistically significant.

Results

A total of 2397 patients presented to the VMS during the study period, and 1441 of these, diagnosed as having 1500 primary melanomas, were included in the analysis. Of the 1500 melanomas, 813 (54%) occurred in men (mean age, 60 years), and 687 occurred in women (mean age, 55 years).

Description of Mitotic Rate

The mitotic rate ranged from 0 to 75 mitoses/mm^2 (eTable in the Supplement): 41.7% of the melanomas had a mitotic rate of less than 1/mm^2; 28.8% had a mitotic rate of between 1 and 2 mitoses/mm^2, and the mitotic rate of remaining 29.5% of melanomas was 3 or more mitoses/mm^2. The mean (SD) mitotic rate was 2.8 (4.9) mitoses/mm^2 (interquartile range, 2.5/mm^2).

Associations of Patient Characteristics With Mitotic Rate

Univariate analysis demonstrated strong associations between older age (≥70 years) (OR, 2.1; 95% CI, 1.7-2.8), male (OR, 1.5; 95% CI, 1.3-1.8), and increasing mitotic rate (**Table 1**). The ratio of men to women in the highest mitotic rate group (≥10/mm^2) was 2.2:1, and 45% of these patients were 70 years or older. The presence of solar keratosis was also associated

Table 1. Patient Characteristics and Mitotic Rate

Characteristic	Melanomas, No.[a]	Mitotic Rate, No./mm²		Univariate Analysis		Multivariate Analysis	
		Mean (SD)	Median	OR (95% CI)	P Value	OR (95% CI)	P Value
Age, y							
<50	469	2.0 (3.4)	1.0	1 [Reference]	<.001	1 [Reference]	<.001
50 to <70	623	2.7 (5.0)	1.0	1.2 (1.0-1.5)		1.2 (0.9-1.5)	
≥70	408	4.0 (6.1)	2.0	2.1 (1.7-2.8)		1.9 (1.4-2.5)	
Sex							
Male	813	3.3 (5.1)	1.0	1.5 (1.3-1.8)	<.001	1.4 (1.2-1.7)	.001
Female	687	2.3 (4.7)	1.0	1 [Reference]		1 [Reference]	
Eye color							
Blue	792	2.9 (4.6)	1.0	1.2 (0.9-1.6)	.71	NA	NA
Green	167	3.0 (5.1)	1.0	1.1 (0.7-1.5)			
Hazel	261	2.8 (6.2)	1.0	1.2 (0.8-1.6)			
Brown	187	2.5 (3.9)	1.0	1 [Reference]			
Hair color							
Blond	298	2.3 (3.7)	1.0	0.8 (0.6-1.0)	.39	NA	NA
Red	147	2.7 (5.1)	1.0	0.8 (0.6-1.1)			
Light brown	402	3.0 (4.8)	1.0	1.0 (0.7-1.2)			
Brown	473	3.1 (5.6)	1.0	1 [Reference]			
Black	85	2.8 (4.2)	1.0	1.0 (0.6 -1.5)			
Skin phenotype							
I	340	2.6 (4.3)	1.0	1 [Reference]	.20	NA	NA
II	569	2.8 (4.8)	1.0	1.0 (1.0-1.1)			
III	425	2.9 (4.4)	1.0	1.2 (1.2-1.5)			
IV	91	3.2 (8.4)	1.0	1.9 (1.0-1.1)			
V	16	3.7 (4.7)	2.0	2.0 (0.9-5.0)			
Total nevi							
<20	303	3.1 (5.7)	1.0	1 [Reference]	.08	NA	NA
20-50	382	2.6 (4.5)	1.0	0.8 (0.6-1.0)			
>50-100	276	3.1 (5.1)	1.0	1.0 (0.8-1.3)			
>100-200	211	2.3 (4.4)	1.0	0.7 (0.5-0.9)			
>200-500	99	2.5 (4.8)	1.0	0.7 (0.5-1.0)			
>500	10	1.7 (1.7)	1.0	0.9 (0.3-2.5)			
Freckles							
Few	470	2.6 (5.1)	1.0	1 [Reference]	.41	NA	NA
Moderate	254	2.2 (4.1)	1.0	0.8 (0.6-1.1)			
Many	373	2.9 (4.8)	1.0	1.0 (0.8-1.3)			
Solar lentigines							
Few	469	2.5 (5.0)	1.0	1 [Reference]	.92	NA	NA
Moderate	215	2.6 (3.8)	1.0	1.0 (0.8-1.4)			
Many	403	2.9 (4.8)	1.0	1.1 (0.8-1.3)			
Dysplastic nevi							
Yes	283	2.7 (5.1)	1.0	1.0 (0.8-1.2)	.85	NA	NA
No	950	2.6 (4.2)	1.0	1 [Reference]			
Blistering sunburns							
None	543	2.9 (4.1)	1.0	1 [Reference]	.02	1 [Reference]	.07
1-5	628	2.8 (5.7)	1.0	0.7 (0.6-0.9)		0.8 (0.6-1.0)	
6-10	121	2.7 (4.5)	1.0	0.7 (0.5-1.1)		0.8 (0.5-1.1)	
10-20	76	1.8 (2.8)	1.0	0.6 (0.4-0.9)		0.6 (0.4-0.9)	
>20	60	2.7 (4.4)	1.0	0.8 (0.5-1.3)		0.8 (0.5-1.4)	
Previous melanoma							
Yes	161	3.2 (5.3)	1.0	1.1 (0.8-1.5)	.53	NA	NA
No	1339	2.8 (4.9)	1.0	1 [Reference]			
First-degree relative with melanoma							
Yes	294	2.1 (3.8)	0.5	0.6 (0.5-0.8)	<.001	0.7 (0.6-0.9)	.002
No	1206	3.0 (5.2)	1.0	1 [Reference]		1 [Reference]	

Abbreviations: NA, not applicable; PR, odds ratio.

[a] Variation in total numbers in each category is due to unobtainable data.

with higher mitotic rate (OR, 1.3; 95% CI, 1.1-1.6). Conversely, a history of blistering sunburns and a clinically significant family history of melanoma were associated with lower mitotic activity ($P = .02$ and $P < .001$, respectively). Age 70 years or older (OR, 1.9; $P < .001$), male sex (OR, 1.4; $P = .001$), and family history (OR, 0.7; $P = .002$) remained as significant associations

Table 2. Clinical Presentation and Mitotic Rate

Characteristic	Melanomas, No.[a]	Mitotic Rate, No./mm²		Univariate Analysis		Multivariate Analysis	
		Mean (SD)	Median	OR (95% CI)	P Value	OR (95% CI)	P Value
Who first suspected							
Patient	745	3.1 (4.6)	1.0	1 [Reference]		1 [Reference]	
Relative	95	3.1 (8.4)	1.0	0.7 (0.5-1.1)		0.7 (0.5-1.1)	
Partner	168	2.5 (4.3)	1.0	0.7 (0.5-1.0)	<.001	0.9 (0.6-1.2)	.01
Doctor	365	2.3 (4.6)	0.5	0.5 (0.4-0.7)		0.6 (0.5-0.8)	
Other	47	1.7 (2.2)	1.0	0.6 (0.4-1.0)		0.5 (0.3-1.0)	
Amelanosis							
Yes	198	5.1 (7.9)	3.0	2.9 (2.2-3.7)	<.001	1.9 (1.4-2.5)	<.001
No	1302	2.5 (4.2)	1.0	1 [Reference]		1 [Reference]	
Location							
Head and neck	369	3.8 (5.5)	1.0	1.5 (1.2-1.9)		1.4 (1.1-1.9)	
Upper extremity	301	2.6 (4.1)	1.0	1.0 (0.8-1.3)		1.0 (0.8-1.4)	
Lower extremity	371	2.4 (5.1)	1.0	1.0 (0.8-1.3)	.005	1.2 (0.9-1.6)	.07
Trunk	451	2.5 (4.7)	1.0	1 [Reference]		1 [Reference]	
Rate of growth, mm/mo							
<0.2	448	1.3 (2.5)	0.5	1 [Reference]		1 [Reference]	
0.2 to <0.5	228	2.2 (3.2)	1.0	2.1 (1.6-2.8)		2.0 (1.5-2.6)	
0.5 to <2.0	316	3.8 (4.7)	2.0	4.3 (3.3-5.6)	<.001	4.2 (3.2-5.5)	<.001
≥2.0	125	7.4 (7.6)	5.0	14.6 (10.0-21.2)		12.5 (8.4-18.5)	

Abbreviations: OR, odds ratio.

[a] Variation in total numbers in each category is due to unobtainable data.

with mitotic rate on multivariate analyses of patient characteristics (Table 1).

Associations of Clinical Presentation With Mitotic Rate

Univariate analysis revealed that head and neck location ($P = .005$), amelanosis ($P < .001$), and increasing rate of growth ($P < .001$) had strong associations with high mitotic rate. Half (50%) of melanomas with a mitotic rate of 5 mitoses/mm² or higher were amelanotic. Initial detection by the patient rather than the physician or others was associated with higher mitotic rate ($P < .001$) and remained as an independent association in the multivariate analysis ($P = .007$), along with amelanosis and rate of growth ($P < .001$ for both) (Table 2).

Associations of Histopathologic Features With Mitotic Rate

By univariate analysis, all histopathologic factors except tumor-infiltrating lymphocytes and desmoplasia were significantly associated with mitotic rate. Exploratory analysis indicated that the relationship between thickness and mitotic rate (eFigure 1 in the Supplement) was best characterized by an exponential increase in mitotic rate with increasing thickness, and this relationship was adequately captured by the 3 categories of tumor thickness detailed in Table 3. Patients in the higher mitotic rate groups had thicker melanomas with greater proportions being ulcerated. In cases with a mitotic rate of 10 mitoses/mm² or higher, over half (53.8%) were ulcerated, in contrast with only 6% in the 0 mitoses/mm² group (Table 4). Nodular melanoma was associated with a higher mitotic rate, with a ratio of 2.3:1 compared with superficial spreading melanoma (reference) in the 10 mitoses/mm² or higher group. Superficial spreading melanoma accounted for the majority of the low-mitotic-rate melanomas (73% in the 0/mm² category) (eFigure 2 in the Supplement). Similarly, features of regression (OR, 0.4; 95% CI, 0.4-0.6; $P < .001$) and the presence of preexisting dysplastic nevi (OR, 0.2; 95% CI, 0.1-0.3; $P < .001$) were associated with lower mitotic activity.

Nodular tumor type, thickness, ulceration, and Clark level of invasion remained as significant predictors of high mitotic rate in the multivariate analyses of tumor and histopathologic factors. An inverse correlation remained with preexisting dysplastic nevus (Table 3).

Overall Multivariate Analyses

The independent associations of mitotic rate from the 3 multivariate models were entered into a final multivariate ordinal logistic regression model. This included both patient and tumor characteristics (age, sex, first suspected by whom, amelanosis, rate of growth) and histopathologic parameters (tumor type, thickness, ulceration, Clark level of invasion, and preexisting nevus).

Nodular tumor type (OR, 2.3; 95% CI, 1.6-3.3; $P < .001$), ulceration (OR, 2.0; 95% CI, 1.4-2.8; $P < .001$), Clark level 5 invasion (OR, 5.9; 95% CI, 3.0-11.7; $P < .001$), and thickness greater than 4 mm (OR, 8.8; 95% CI, 4.9-15.9; $P < .001$) retained strong positive associations with mitotic rate, while an inverse relationship persisted with preexisting dysplastic nevus (OR, 0.4; 95% CI, 0.2-0.6; $P = .001$). Rate of growth ($P = .01$) and amelanosis ($P = .03$) independently correlated with higher mitotic

Table 3. Histopathologic Features and Mitotic Rate

Characteristic	Melanomas, No.[a]	Mitotic Rate, No./mm²		Univariate Analysis		Multivariate Analysis	
		Mean (SD)	Median	OR (95% CI)	P Value	OR (95% CI)	P Value
Tumor type							
SSM	901	1.9 (4.2)	1.0	1 [Reference]	<.001	1 [Reference]	<.001
NM	247	6.8 (6.5)	5.0	9.1 (7.0-11.8)		2.5 (1.8-3.4)	
LMM	101	2.1 (3.9)	0.5	0.8 (0.6-1.1)		0.8 (0.6-1.1)	
DM	68	2.9 (4.7)	1.0	1.7 (1.1-2.6)		0.2 (0.1-0.3)	
ALM	35	2.6 (3.4)	1.0	1.7 (0.9-3.0)		0.9 (0.5-1.9)	
Other[b]	58	2.0 (3.1)	1.0	1.3 (0.8-2.1)		0.5 (0.3-1.0)	
Tumor thickness, mm							
≤1	708	0.6 (1.3)	0.5	1 [Reference]	<.001	1 [Reference]	<.001
>1-4	614	3.7 (4.2)	2.0	11.8 (9.4-14.9)		4.5 (3.2-6.1	
>4	178	8.6 (9.1)	6.0	47. 6 (33.5-67.6)		12.6 (7.5-21.1)	
Ulceration							
Yes	272	6.9 (7.8)	4.5	7.8 (6.1-10.0)	<.001	2.0 (1.5-2.7)	<.001
No	1200	1.9 (3.4)	1.0	1 [Reference]		1 [Reference]	
Clark level							
2	380	0.4 (1.3)	0	1 [Reference]	<.001	1 [Reference]	<.001
3	328	2.1 (4.1)	1.0	4.6 (3.5-6.1)		3.1 (2.2-4.3)	
4	671	3.8 (4.6)	2.0	15.4 (11.8-20.4)		4.6 (3.1-6.6)	
5	120	6.9 (9.4)	4.0	36.1 (24.2-53.9)		8.3 (4.4-15.5)	
Microsatellitosis							
Yes	29	7.4 (5.5)	6.0	7.5 (3.9-14.2)	<.001	1.8 (0.7-4.5)	.10
No	1331	2.8 (4.6)	1.0	1 [Reference]		1 [Reference]	
Lymphovascular invasion							
Yes	64	5.6 (5.7)	4.0	4.4 (2.9-6.7)	<.001	1.2 (0.7-1.9)	.60
No	1414	2.7 (4.9)	1.0	1 [Reference]		1 [Reference]	
Tumor-infiltrating lymphocytes							
Yes	824	2.8 (4.6)	1.0	1.1 (0.9-1.4)	.22	NA	NA
No	466	2.9 (4.7)	1.0	1 [Reference]			
Regression							
Yes	359	2.0 (4.2)	0.5	0.4 (0.4-0.6)	<.001	0.9 (0.7-1.2)	.40
No	1081	3.1 (4.8)	1.0	1 [Reference]		1 [Reference]	
Desmoplasia					.06	NA	NA
Yes	78	3.5 (5.4)	1.0	1.5 (1.0-2.1)			
No	1258	2.8 (4.6)	1.0	1 [Reference]			
Neurotropism							
Yes	50	4.5 (6.1)	2.0	2.4 (1.5-3.8)	<.001	1.5 (0.8-2.9)	.23
No	1387	2.8 (4.6)	1.0	1 [Reference]		1 [Reference]	
Preexisting nevus							
Yes							
Dysplastic	96	0.5 (0.9)	0	0.2 (0.1-0.3)	<.001	0.3 (0.2-0.5)	<.001
Nondysplastic	271	1.8 (3.1)	1.0	0.6 (0.5-0.7)		0.7 (0.5-0.9)	
No	1014	3.3 (5.5)	1.0	1 [Reference]		1 [Reference]	

Abbreviations: ALM, acral lentiginous melanoma; DM, desmoplastic melanoma; LMM, lentigo maligna melanoma; NA, not applicable; NM, nodular melanoma; OR, odds ratio; SSM, superficial spreading melanoma.

[a] Variation in total numbers in each category is due to unobtainable data.

[b] Other tumor types were grouped together because of low numbers.

rate, although to a lesser extent than the histopathologic variables. The person by whom malignant growth was first suspected ($P = .03$) also remained a significant association. The age and sex associations that were observed in earlier analyses did not appear strong in this analysis, and so their relationships with higher mitotic rate could be explained by other parameters in the analysis (Table 4).

The proportional odds assumption was rejected for the model overall; however, the large sample size translated to high power for this proportional odds test. Tests of the same assump-

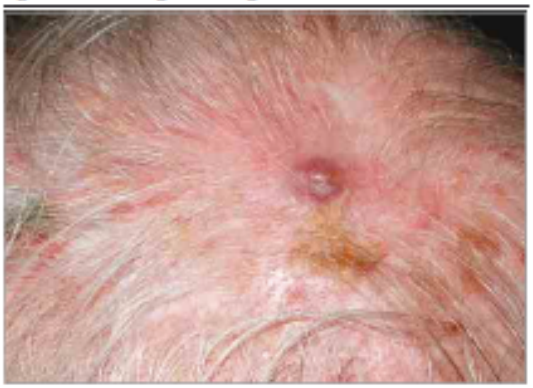
Table 4. Overall Multivariate Analyses[a]

Characteristic	Mitotic Rate, Mean (SD), No./mm²							OR (95% CI)	*P* Value
	0	<1	1 to <2	2	3 to 4	5 to 9	≥10		
Total lesions (n = 1500)	364	261	297	135	156	155	132	NA	NA
Patient age, y									
<50	131 (36.0)	90 (34.5)	106 (35.7)	41 (30.4)	42 (26.9)	35 (22.6)	24 (18.2)	1 [Reference]	.33
50 to <70	146 (40.1)	127 (48.7)	127 (42.8)	59 (43.7)	52 (33.3)	64 (41.3)	48 (36.4)	1.0 (0.7-1.3)	
≥70	87 (23.9)	44 (16.9)	64 (21.5)	35 (25.9)	62 (39.7)	56 (36.1)	60 (45.5)	1.2 (0.9-1.7)	
Mean (SD), y	56.0 (16.4)	54.9 (15.1)	55.6 (16.4)	56.8 (17.6)	61.4 (16.6)	61.7 (16.4)	64.8 (15.2)	NA	
Who first suspected									
Patient	151 (44.2)	116 (46.4)	152 (53.5)	71 (55.5)	87 (58.8)	90 (62.1)	78 (63.4)	1 [Reference]	.03
Relative	19 (5.6)	24 (9.6)	22 (7.7)	5 (3.9)	8 (5.4)	11 (7.6)	6 (4.9)	1.0 (0.6-1.5)	
Partner	40 (11.7)	36 (14.4)	33 (11.6)	16 (12.5)	16 (10.8)	14 (9.7)	13 (10.6)	0.9 (0.6-1.2)	
Physician	119 (34.8)	65 (26.0)	66 (23.2)	33 (25.8)	33 (22.3)	23 (15.9)	26 (21.1)	0.6 (0.5-0.9)	
Other	13 (3.8)	9 (3.6)	11 (3.9)	3 (2.3)	4 (2.7)	7 (4.8)	0	0.5 (0.2-1.0)	
Sex									
Male	174 (47.8)	133 (51.0)	151 (50.8)	80 (59.3)	94 (60.3)	90 (58.1)	91 (68.9)	1.3 (1.0-1.6)	.07
Female	190 (52.2)	128 (49.0)	146 (49.2)	55 (40.7)	62 (39.7)	65 (41.9)	41 (31.1)	1 [Reference]	
Amelanosis									
Yes	23 (6.3)	21 (8.0)	29 (9.8)	24 (17.8)	29 (18.6)	40 (25.8)	32 (24.2)	1.4 (1.0-2.0)	.03
No	341 (93.7)	240 (92)	268 (90.2)	111 (82.2)	127 (81.4)	115 (74.2)	100 (75.8)	1 [Reference]	
Rate of growth, mm/mo									
<0.2	148 (62.2)	108 (54.3)	102 (45.7)	24 (24.0)	32 (24.6)	23 (18.3)	11 (10.9)	1 [Reference]	.001
0.2 to <0.5	46 (19.3)	45 (22.6)	54 (24.2)	20 (20.0)	25 (19.2)	23 (18.3)	15 (14.9)	1.4 (1.1-1.9)	
0.5 to <2	35 (14.7)	44 (22.1)	60 (26.9)	40 (40.0)	52 (52)	46 (36.5)	39 (38.6)	1.6 (1.2-2.2)	
≥2	9 (3.8)	2 (1.0)	7 (3.1)	16 (16.0)	21 (16.2)	34 (27.0)	36 (35.6)	2.6 (1.6-4.3)	
Mean (SD), mm/mo	0.04 (0.56)	0.33 (0.43)	0.45 (0.65)	1.11 (1.41)	1.06 (1.42)	1.61 (2.33)	1.79 (1.81)	NA	
Tumor type									
SSM	253 (72.9)	185 (73.1)	207 (71.4)	68 (55.3)	88 (59.1)	65 (43.3)	35 (26.9)	1 [Reference]	<.001
NM	3 (0.9)	14 (5.5)	32 (11.0)	26 (21.1)	42 (28.2)	57 (38.0)	73 (56.2)	2.3 (1.6-3.3)	
LMM	71 (20.5)	38 (15.0)	28 (9.7)	12 (9.8)	11 (7.4)	17 (11.3)	14 (10.8)	0.9 (0.6-1.3)	
DM	13 (3.7)	10 (4.0)	17 (5.9)	10 (8.1)	6 (4.0)	6 (4.0)	6 (4.6)	0.1 (0.1-0.3)	
ALM	7 (2.0)	6 (2.4)	6 (2.1)	7 (5.7)	2 (1.3)	5 (3.3)	2 (1.5)	0.9 (0.4-1.9)	
Thickness, mm									
≤1	301 (82.7)	197 (75.5)	154 (51.9)	26 (19.3)	20 (12.8)	7 (4.5)	3 (2.3)	1 [Reference]	<.001
>1-4	53 (14.6)	60 (23.0)	127 (42.8)	93 (68.9)	107 (68.6)	112 (72.3)	62 (47.0)	3.3 (2.3-4.9)	
>4	10 (2.7)	4 (1.5)	16 (5.4)	16 (11.9)	29 (18.6)	36 (23.2)	67 (50.8)	8.8 (4.9-15.9)	
Mean (SD), mm	0.8 (1.0)	0.9 (1.0)	1.4 (1.5)	2.4 (2.5)	2.7 (2.1)	3.2 (2.3)	4.9 (3.4)	NA	
Ulceration									
Yes	6 (1.7)	20 (7.8)	29 (9.9)	35 (26.7)	46 (30.3)	65 (42.5)	71 (53.8)	2.0 (1.4-2.8)	<.001
No	349 (98.3)	237 (92.2)	263 (90.1)	96 (73.3)	106 (69.7)	88 (57.5)	61 (46.2)	1 [Reference]	
Clark level									
2	205 (56.3)	115 (44.1)	51 (17.2)	4 (3.0)	4 (2.6)	0	1 (0.8)	1 [Reference]	<.001
3	74 (20.3)	76 (29.1)	92 (31.0)	24 (17.8)	22 (14.1)	20 (12.9)	20 (15.3)	3.2 (2.2-4.7)	
4	78 (21.4)	65 (24.9)	138 (46.5)	89 (65.9)	112 (71.8)	111 (71.6)	78 (59.5)	4.3 (2.8-6.5)	
5	7 (1.9)	5 (1.9)	16 (5.4)	18 (13.3)	18 (11.5)	24 (15.5)	32 (24.4)	5.9 (3.0-11.7)	
Preexisting nevus									
Yes									
Dysplastic	50 (15.5)	22 (8.7)	16 (5.8)	3 (2.5)	5 (3.4)	0	0	0.4 (0.2-0.6)	.001
Nondysplastic	73 (22.6)	51 (20.2)	69 (25.0)	26 (21.7)	26 (17.9)	15 (10.3)	11 (9.2)	0.7 (0.5-1.0)	
No	200 (61.9)	180 (71.1)	191 (69.2)	91 (75.8)	114 (78.6)	130 (89.7)	108 (90.8)	1 [Reference]	

Abbreviations: ALM, acral lentiginous melanoma; DM, desmoplastic melanoma; LMM, lentigo maligna melanoma; NM, nodular melanoma; OR, odds ratio; SSM, superficial spreading melanoma.

[a] Unless otherwise indicated, data are mean (SD) number of melanomas.

Figure. Clinical Photograph of a High-Mitotic-Rate Melanoma Lesion



This man in his 80s presented with a rapidly growing amelanotic nodular melanoma on the scalp. At diagnosis, it had a Breslow thickness of 7.4 mm and a mitotic rate of greater than 90 mitoses/mm^2.

tion in the univariate analyses indicated that it was satisfied for most variables, with the 2 exceptions of thickness and tumor level, which may have more complex associations with mitotic rate than those described by the multivariate ORs. A univariate model with the logarithm-transformed values of thickness captured the exponential relationship, as identified in eFigure 1 in Supplement, but surprisingly also failed the proportional odds test, although this could be explained by evidence of a threshold at the first category of mitotic rate, that is, at the count of 0. This model with log-thickness passed the proportional odds test when restricted to melanomas with mitotic rate greater than 0.

Discussion

Mitotic rate, a quantifiable measure of tumor growth, has been shown to correlate with melanoma survival.[4,5] Disease-free survival has been found to decline with increasing mitotic rate, with in-transit, nodal, and distant recurrences occurring more commonly in patients with high-mitotic-rate melanoma (≥5 mitoses/mm^2).[29] In conjunction with Breslow thickness and ulceration, mitotic rate is a criterion in the current AJCC staging system for localized melanoma.[5] However, while the presence of many mitotic figures has been recognized as a poor prognostic feature, the clinicopathologic characteristics of patients with high-mitotic-rate melanoma have yet to be formally elucidated.

This study has identified that patients with higher mitotic rate tumors were more likely to be older, to be male, to have a history of significant solar field damage, and to present with rapidly growing primary melanoma that was more likely to be located on the head and neck. Melanomas with very high mitotic activity (≥10 mitoses/mm^2) were predominantly thick and ulcerated nodular tumor subtypes. Conversely, the superficial spreading melanoma subtype, features of regression, and the presence of preexisting nevi were found to be characteristic of lesions with sparse mitotic activity. These melanomas were significantly thinner than their more mitotically active counterparts and tended to occur more commonly in patients with previous blistering sunburns and a family history of a first-degree relative with melanoma. This accords with previous observations of associations between these historical factors and thin tumors.[24,25]

Studies investigating patients at the greatest risk of mortality from primary invasive melanoma have focused mainly on those presenting with thick lesions. There is a recognized association between tumor thickness, male sex, and older age.[13,14,18] In a retrospective survey of 1124 patients, Hersey et al[13] found that 68% of patients with thick melanoma (≥3 mm) were men, and 75% were older than 70 years. The head and neck region was the most common site for thick melanoma,[13] although Hanrahan et al[17] later observed that tumor site was not related to thickness when nodular tumor type was taken into account.

As a phenotypic marker of cumulative sun damage, solar keratosis is considered a risk factor for melanoma,[26,27] and its association with melanoma occurring on the head and neck and in men has been recognized.[18,27] In this study, the presence of solar keratosis was shown to be associated with high mitotic rate, although this relationship was lost when age and sex were factored into the analysis. Nodular melanoma, which demonstrated higher mitotic activity than any other tumor type in the current study, has been more strongly linked to solar keratosis than superficial spreading melanoma.[28]

Despite the likelihood of being amelanotic, high mitotic rate melanomas were most commonly first discovered by patients themselves. For instance, 62.1% of melanomas with a mitotic rate of 5/mm^2 or higher were first detected by patients themselves. This is possibly a result of their rapid growth rate, which, as a clinical measure of tumor proliferation, has been reported previously to closely correlate with mitotic rate.[19] The same study also described an association between rapid tumor growth and atypical tumor morphology, including amelanosis, symmetry, elevation, and border regularity,[19] which are recognized features of nodular melanoma.[29] While tumor subtype was chosen as a histologic variable for the purposes of this study, it is important to recognize that each tumor subtype has characteristic morphologic features that are helpful in their clinical detection. The presenting features of nodular melanoma may pose a challenge in this regard owing to its symmetrically elevated, often amelanotic appearance that does not fit the classic "ugly duckling" radial growth phase melanoma as defined by the ABCDE rule (asymmetry, border irregularity, color variegation, diameter >6 mm, and evolving) (**Figure**).[30]

The previous observation that thick and ulcerated melanomas are mitotically active[7,31] was consistent with the current findings. However, it must be noted that desmoplastic melanoma, which was found to be comparable in thickness to nodular melanoma, exhibited low mitotic activity. This may serve to explain, to a degree, the distinct clinical behavior suggested for this rare melanoma subtype that is characterized by its propensity for local recurrence but a lower incidence of distant spread relative to other forms of cutaneous melanoma.[32,33]

Conclusions

To our knowledge, this is the first formal description of the clinicopathologic associations of high-mitotic-rate melanoma. The results from this single-center study merit replication elsewhere to confirm generalizability and to further explore the potential implications for detection and treatment of at-risk patients, who in this study were found to have a distinct phenotypic and historical profile. Mitotically active melanomas were more often seen in older men with chronic solar field damage. These tumors have a predilection for the head and neck and can present with nodular structure and amelanosis. Such atypical clinical features may pose a challenge to timely detection; thus a high index of suspicion is warranted when the patient reports a history of morphologic change and rapid growth.

ARTICLE INFORMATION

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Study concept and design: Shon, McLean, Kelly.
Acquisition, analysis, or interpretation of data: Shon, Wolfe, McLean, Haskett, Kelly.
Drafting of the manuscript: Shon, Kelly.
Critical revision of the manuscript for important intellectual content: Shon, Wolfe, McLean, Haskett, Kelly.
Statistical analysis: Shon, Wolfe.
Obtained funding: Kelly.
Administrative, technical, or material support: Shon, McLean, Kelly.
Study supervision: Wolfe, McLean, Haskett, Kelly.

Conflict of Interest Disclosures: None reported.

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Appendix 2: Ethics approval

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

Date:	**6 July 2012**
Project Number:	**CF12/1800 – 2012000993**
Project Title:	**Clinical Associations and Prognostic significance of high mitotic rate melanomas**
Chief Investigator:	**Assoc Prof John Kelly**
Approved:	**From: 6 July 2012 To: 6 July 2017**

Terms of approval

1. The Chief Investigator is responsible for ensuring that permission letters are obtained, if relevant, and a copy forwarded to MUHREC before any data collection can occur at the specified organisation. **Failure to provide permission letters to MUHREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research.**
2. Approval is only valid whilst you hold a position at Monash University.
3. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. The Explanatory Statement must be on Monash University letterhead and the Monash University complaints clause must contain your project number.
6. **Amendments to the approved project (including changes in personnel):** Requires the submission of a Request for Amendment form to MUHREC and must not begin without written approval from MUHREC. Substantial variations may require a new application.
7. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
8. **Annual reports:** Continued approval of this project is dependent on the submission of an Annual Report. This is determined by the date of your letter of approval.
9. **Final report:** A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
10. **Monitoring:** Projects may be subject to an audit or any other form of monitoring by MUHREC at any time.
11. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

[redacted]

Professor Ben Canny
Chair, MUHREC

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