PREDICTION IN CUTANEOUS MELANOMAS

A MULTICONTINENTAL APPROACH

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PREDICTION IN CUTANEOUS MELANOMAS

A MULTICONTINENTAL APPROACH

Predictie voor het cutaan melanoom

Een multi continentale aanpak

(met een samenvatting in het Nederlands)

Proefschrift

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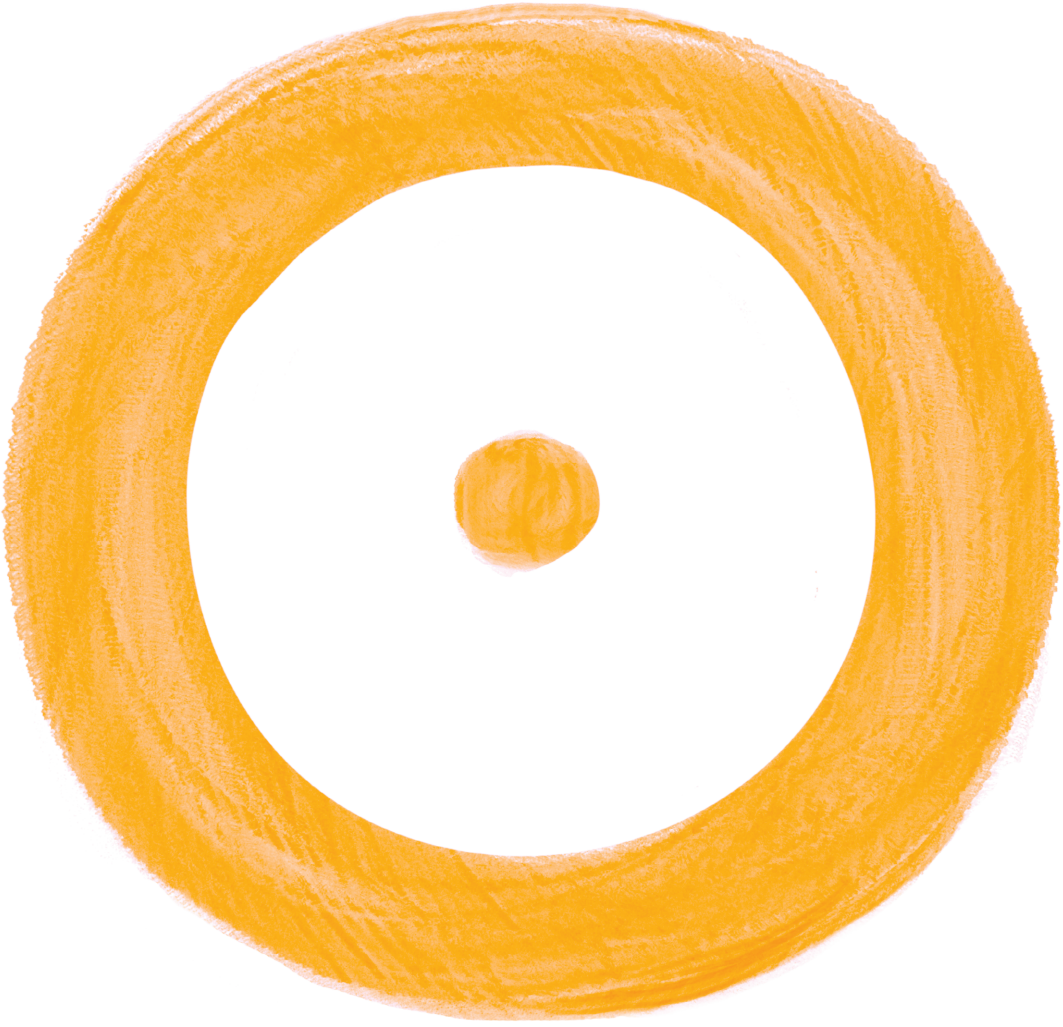

CHAPTER 1

General introduction

GENERAL INTRODUCTION

A brief history of melanoma

The ancient Egyptians regarded the sun as the creator of the universe and the giver of life. The sun was therefore worshipped as a god, named Ra. Today, the sun is still worshipped by many people, although their worship is expressed differently. Even though modern day science will not dispute the role of the sun in the existence of life, it is now very well known that excessive sun exposure is a major preventable contributor to an individual's risk of developing melanoma.[1-4] It seems probable that the first recorded description of melanoma can be found in the writings of the Greek physician Hippocrates of Cos (460-370 B.C.), derived from the Greek *μέλας* (dark) and *ωμα* (tumor).[5] Later, in 1651, it was described in European medical literature as *"fatal black tumors with metastases and black fluid in the body"*, and it took until 1804 before René Laënnec, the inventor of the stethoscope, named melanoma as a distinct disease.[5] The first insightful observations of melanoma have been attributed to the general practitioner William Norris. In 1820, he was the first to suspect a hereditary nature in some patients with melanomas.[6] He was also the first to propose a relationship between nevi and melanomas, and the first to note that *"when the disease appears in several parts of the body, physics will, I fear, uniformly fail, and surgery will be foiled"*. In 1857, he was the first to advocate a wide local excision[7], although William Sampson Handley is now widely considered as the founder of this technique, suggesting to perform a wide local excision of a melanoma with margins of 2 inches (roughly 5cm), along with the removal of lymph nodes.[8] These margins have been adhered to for 50 years, before randomized controlled trials showed the safety of narrower margins of 1cm and 2cm.[9-12]

In 1966 Wallace Clark, a dermatologist and pathologist, was the first to make an effort to introduce a staging system for patients with melanomas.[13] He noted that the histopathologic level of invasion correlated with prognosis, and was able to identify 5 levels of invasion; Clark levels I to V. In 1970, his fellow-pathologist Alexander Breslow refined this subdivision into a more accurate measurement in millimeters, measured from the granular layer of the epidermis to the deepest melanoma cell into the skin.[14] Today, Breslow thickness still forms the basis of the current American Joint Committee on Cancer (AJCC) Staging System, together with ulceration status.[15] These two pathological characteristics combined determine if sentinel node (SN) biopsy is indicated: patients with melanomas ≥0.8mm thick or those with melanomas <0.8mm with ulceration present, are eligible for this procedure.[16,17]

Incidence, risk factors and clinical presentation

In most cases, melanoma presents itself on visibly exposed skin. It is therefore theoretically the most straightforward of all types of cancer to detect. Melanomas arise from the same cells as nevi (moles): melanocytes (pigment cells) that are

situated in the basal layer of the epidermis. This makes melanoma an entity distinct to epithelial skin tumors, such as basal cell carcinomas and squamous cell carcinomas, which arise from keratinocytes (epithelial cells). Is has been estimated that 2 out of 3 Australians will get diagnosed with some form of skin cancer in their life, compared to 1 out of 5 Dutch people.[18,19] Epithelial skin cancers comprise the majority of skin-cancer diagnoses – roughly 90% – and melanoma 10%.[20] Although melanoma is not the most common form of skin cancer, it is responsible for 70-90% of skin-cancer related deaths.[20-23] Melanomas can also develop in mucosal tissue, such as in the nasal cavity, vulva/vagina, esophagus, rectum, and in the retina. This thesis focusses on cutaneous melanoma.

Excessive sun seeking behavior, the use of indoor tanning beds, and unprotected work- and leisure related outdoor activities all contribute to an individual's risk of developing a melanoma.[1-4] The debate on which type of skin cancer (epithelial versus melanocytic) was primarily caused by which type of ultraviolet (UV) exposure (chronic or intermittent) started after Fears' landmark paper in 1977.[24] Fears et al. hypothesized that melanomas were primarily caused by intermittent, excessive UV-exposure, whereas basal cell carcinomas and squamous cell carcinomas were primarily caused by progressive accumulation of UV-light. Today, it seems plausible that both UV-exposure types offer a distinct pathological pathway by which melanomas can develop[25], and that especially sunburns in childhood are a strong determinant of melanoma risk.[2,3,26] Men are more likely to have their melanoma located on the trunk, as opposed to females who are more likely to develop melanoma on the legs.[27,28] A possible explanation for this could be there are gender-related differences in sun exposure.[29,30] It has been clearly demonstrated that UV-exposure plays an important role in the pathophysiology of melanoma.[1-4] This makes overexposure to UV-light the most preventable cause of skin cancer, melanoma being no exception. It has been estimated that 86% of melanomas can be attributed to UV-radiation overexposure.[31] Although more therapeutic options are becoming available for patients with melanomas, prevention is still key. In any case, it is better than cure – and there is room for improvement.

Australia has the highest (age-standardized) incidence rates of melanomas worldwide, closely followed by New Zealand.[32] The Netherlands is accredited the 5th highest incidence rate in the world. Melanoma affects approximately 1 in 20 Australians, and 1 in 50 Dutch people.[19,33] Risk factors for developing a melanoma other than UV-exposure, include the presence of (multiple) nevi, fair skin type, fair hair colour and older age.[2,3] As William Norris observed, genetics can play a role in developing melanoma as well: approximately 10% of patients with cutaneous melanomas have a positive melanoma family history, of which the germline mutation of the *CDKN2A* gene is the most common cause.[34] Mutation carriers have a life-time melanoma risk of approximately 70%, many of them developing melanoma at a younger age while

being at increased risk to develop multiple melanomas.[35,36] In addition, they are at increased risk of developing other tumors such as pancreatic cancer and head and neck cancer.[37-40]

To explain the risk of a nevus becoming a potential melanoma, the ABCD rule was introduced in 1985; an acronym for asymmetry in colour and shape, border irregularity, colour variation (≥2 colours, although melanoma can also be amelanotic) and diameter (≥6mm).[41] Later, in 2004, it was suggested to add an "E", for evolving.[42] Although the majority of people believe one should mainly be aware of pre-existing nevi, only 30% of melanomas are thought to arise from a pre-existing nevus.[43-45] The majority of melanomas thereby arise *de novo*. Most people – with the exception of children with congenital nevi – are born without nevi, and die without nevi. This implicates that there is not only change in melanomas, but also in benign nevi, as they too can develop, change and involute during life. The development of nevi starts in early childhood, and reaches a peak at 40 years of age.[46] The development of new nevi after the age of 40 years is therefore something to be cautious about. Relatively simple knowledge and understanding of the benign behavior of nevi is essential for assessment of nevi in daily dermatologic clinical practice.

Four main subtypes of melanoma can be identified. Superficial spreading melanoma (SSM) is the most common subtype (70%), and usually presents as a flat slow growing lesion.[47] Nodular melanoma accounts for 20% of all melanomas. As the name suggests, it grows as a nodule, which may be pigmented or amelanotic. Clinically, it can easily be confused with other amelanotic tumours, such as basal cell carcinoma or pyogenic granuloma. Nodular melanomas tend to occur more in older males[48] and have a faster growth rate than SSM [49] and appear almost always *de novo*.[50] They do not adhere to the ABCD(E)-rule, rather, an EFG-rule has been suggested (elevated, firm and growing).[51] Lentigo maligna melanoma represents 5-10% of all melanomas and is mostly diagnosed as a large, flat macule on the face in older patients, reflecting the cumulative DNA damage that drives this melanoma subtype.[52,53] Acral lentiginous melanoma, by definition, involves the acral sites (palms and soles). Well-established risk factors for melanoma in general, such as sun exposure and fair skin type, are not applicable to this melanoma subtype.[54] Interestingly, it is the most common type of melanoma in the Asian population, but is rare (1-2%) in American and European populations.[52,53,55]

The sentinel node concept in melanoma

An SN is defined as any lymph node receiving lymphatic drainage directly from the primary tumor site.[56] The SN procedure in melanoma was first described in 1992 by Donald Morton.[57] The rationale behind the SN concept is that of cascadic, lymphatic spread, starting from the SN to the regional nodes and then, after a variable latency period, to distant sites via the bloodstream. Others have opposed

this concept and believe in a simultaneous metastasis model, where melanoma independently spreads through the lymphatic and haematogenous system at the same time.[58] A third concept was proposed in 1991 by Wallace Clark, who believed not all melanomas were able to metastasize.[59] Those who were able to metastasize followed independent dissemination pathways, which holds that melanomas have the potential to 1) metastasize lymphatically without metastasizing haematogenously; 2) metastasize lymphatically with concurrent or subsequent haematogenous metastases; 3) metastasize haematogenously without first metastasizing lymphatic; or 4) in rare cases, metastasize to only one specific organ (the "organ restricted metastatic pathway"). In melanoma, this is most often seen as cutaneous metastases.

Two of the largest conducted international randomized controlled trials on SN biopsy in patients with melanoma, the Multicenter Selective Lymphadenectomy Trial (MSLT)1 and the MSLT-2, are based on the SN concept as proposed by Donald Morton.[60,61] The MSLT-1 clearly showed that SN status provides important prognostic information, and resulted in the worldwide implementation of this technique in melanoma patients.[60] However, the results from the MSLT-1 also showed that SN biopsy has no therapeutic value as it did not influence melanoma-specific survival. Because there were no clinical consequences to a positive SN biopsy at the time, it was only performed to optimally inform patients on their prognosis. This has now changed, as a positive SN biopsy sets the indication for adjuvant systemic therapy.[62] Therefore, the reason to perform a SN biopsy has drastically changed over the years. The enactment of SN biopsies in the Netherlands over a 15-year period is described in Chapter 2. In case of a positive SN biopsy, it was routine to perform a completion lymph node dissection (CLND). The MSLT-2 randomly assigned patients with a positive SN biopsy to immediate CLND, or a nodal observation group with ultrasound.[61] It was shown that immediate CLND did not increase survival among patients with melanoma, and let to the worldwide expulsion of standard immediate CLND after a positive SN biopsy in 2017.

Surgical and systemic treatment

When a melanoma is suspected, a complete excision of the whole lesion with a 2mm margin is performed. This is also referred to as a diagnostic excision. Punch biopsies and shave excisions should be avoided, and the former should only be considered for large lesions located on specific cosmetic or functional sites, like the face or genitals.[63] The reason that punch biopsies should be avoided is not because of potential metastatic spread, but because it complicates histological assessment, no definitive thickness of the melanoma can be established, and it induces sampling error.[64,65] The definitive diagnosis of a melanoma is made by the pathologist. Diagnosing a melanoma rests on several histopathological characteristics, such asymmetry of the lesion, cell atypia, Pagetoid intraepidermal spread and ascension of melanocytic cells, and the presence of (deep) mitoses.[66] Immunohistochemistry (e.g. PRAME)

and molecular diagnostics play an increasing role. In some cases, the combination of clinical appearance and suspicion can attribute to the definitive diagnosis. In case of a melanoma the pathologist measures its Breslow thickness and determines if ulceration is present and an additional, wider excision will be performed. Surgical excision margins that are advocated are 5mm for patients with melanoma *in situ*, 1cm for patients with melanomas ≤2mm thick and 2cm for patients with melanomas >2mm thick.[15,67] In addition, patients with melanomas ≥0.8mm thick or those with melanomas <0.8mm with ulceration present, are eligible for SN biopsy.[16,17] For these patients, it is recommended that the wide excision is planned simultaneously to SN biopsy.[63] At present, overall SN-positivity ranges only from 16% to 27%.[60,68,69] There is thus an urgent need for a more tailored approach to selecting patients for SN biopsy. This has led us to externally validate the Melanoma Institute Australia (MIA) nomogram to predict SN-positivity in patients with melanoma in a European, population-based cohort in Chapter 16.

Today, a positive SN biopsy sets the indication for adjuvant therapy. Although William Norris already noted in 1820 that neither medical nor surgical treatment was effective when melanoma was disseminated, it has taken until the 21st century (2012 in the Netherlands) before adequate treatment options for patients with unresectable stage III or stage IV disease became available and were implemented. This treatment constitutes of immunotherapy (ipilimumab, nivolumab and/or pembrolizumab) or, in case of the presence of a BRAFV600-mutation, targeted therapy (BRAF-MEK-inhibitors). Martincorena and Campbell compared the mutational burden in 20 tumor types, which clearly showed that melanoma had the highest mutational burden of all cancer types, followed by lung cancer.[70] This finding explains why patients with melanoma can be highly susceptible to targeted therapy. However, a known problem is that responses are temporary, as most patients develop resistance within 1 year.[71] Therapeutic options for patients diagnosed with melanoma are rapidly developing now that immunotherapy has emerged as a treatment modality for cancer patients. Durable survival benefits in over 50% of stage IV patients can now be achieved, whereas earlier stage IV diagnosis was considered a death sentence.[72] For stage IV patients, 5-year overall survival (OS) rates of 52% can be reached for patients treated with nivolumab-plus-ipilimumab[73], and 2-year OS rates of 55% for patients treated with pembrolizumab have been objectified.[74] This has revolutionized treatment for patients with melanomas, for which the immunologist James Allison received the Nobel prize in 2018. In patients with stage III melanoma, ipilimumab is also effective compared to placebo (7-year absolute OS difference of 8.7%), but considered too toxic, and was therefore not advised.[75,76] One-year treatment with nivolumab has shown superior results over treatment with ipilimumab in the Checkmate-238 study (this study included stage IIIB-C and stage IV patients).[77] A recent update showed 4-year recurrence-free survival (RFS) rates of 51.7% for the nivolumab-treated patients versus 41.2% for the ipilimumab-treated patients.[78] 4-year OS was 77.9% and 76.6%,

respectively. In the EORTC-1325 study three-year recurrence-free survival (RFS) rates of one-year treatment with pembrolizumab compared to placebo were found to be 63.7% and 44.1%, respectively.[79,80] Targeted therapy was investigated in the COMBI-AD trial, showing 5-year RFS rates of 52% versus 36%, respectively, after 1-year treatment.[81,82] These systemic treatment options have thoroughly changed the prognostic outlook for these patients. Since 2018, systemic therapy has also been approved for patients with stage III disease in the adjuvant setting.[77,79,81] Finally, there seems to be the start of a new era for patients with melanoma.[62] Therefore, the identification of SN metastases nowadays significantly impacts clinical practice, as it implicates worse survival and sets the indication for adjuvant systemic therapies for patients with stage III disease.[15,62] So it is important that SN status is assessed correctly, because patients could be at risk of unjustified exposure to the severe and potentially fatal side-effects of adjuvant systemic treatment in case of a false-positive SN diagnosis.[83] In this thesis, we have systematically re-evaluated 'initially positive' SNs in Chapter 5.

Predictors and survival of melanoma

Once a melanoma has developed, several predictors of survival in patients with melanomas have been identified, the two most consistently important being Breslow thickness and ulceration status of the primary tumor. Together, they form the basis of the AJCC eighth edition system for T-staging of cutaneous melanomas. [15,84,85] To optimally inform a patient on his or her individual prognosis, it is essential to know which clinicopathological characteristics play a role in survival besides Breslow thickness and ulceration status. In this thesis, the association between survival and several individual clinicopathological characteristics is assessed, such as presence of regression (Chapter 8), melanoma subtype (Chapter 9) and sex (Chapter 13). The additional prognostic value of SN status over known predictors such as Breslow thickness and ulceration has been questioned by some who have suggested that if equivalent prognostic information can be derived using variables obtained from standard histological examination, patients could be spared an SN biopsy.[86-88] Surprisingly, evidence-based studies in the melanoma literature assessing the additional prognostic information provided by SN biopsy are sparse. This thesis therefore incorporates Chapter 4, where the additional prognostic value of SN status over basic clinicopathological characteristics in predicting survival is investigated in two large independent melanoma datasets.

Melanomas can be categorized as thin, intermediate and thick, corresponding with ≤1.0mm, 1.1-4.0mm and >4.0mm Breslow thickness, respectively.[15] It has been estimated that 58% to 81% of patients presenting with primary cutaneous melanomas have thin melanomas.[89-93] Overall, their prognosis is very good, with reported 5-year survival rates of 88.6% to 98.8%,[94,95] however, a subset (3-4% of patients [96]) develops recurrent disease. Since patients with thin melanomas constitute such a

high proportion of all patients diagnosed with melanoma, in absolute numbers more people ultimately die from T1 melanomas than from T2, T3 or T4 melanomas.[91,97,98] Literature on patients that have been diagnosed with a thin melanoma and that develop a recurrence is scarce. More information about this selected group of patients is required to optimally inform them on their prognosis, using readily available clinicopathological characteristics. This has led us to develop the nomogram that is presented in Chapter 14.

All the aforementioned characteristics of nevi, of melanomas and of high-risk patients play an important role in raising awareness of melanoma and in optimizing preventative screening strategies. Once a melanoma has developed, it is crucial to diagnose it at the earliest stage possible. In general, the earlier a melanoma can be diagnosed, the lower its Breslow thickness and the better the patient's survival. Dermatologists play a key role in detecting melanoma as early as possible, not only by assessing suspicious nevi macroscopically, but especially because dermatologists are experts in using a dermatoscope. A dermatoscope is a handheld magnifier that not only magnifies, but also enables its user to see through the skin. It allows the user to detect melanomas at an earlier stage, and thus improving survival.[99] Dermoscopy is also vital for short-term monitoring of suspicious lesions. In some cases, an experienced dermatologist can opt for short-term monitoring of a nevus, instead of immediate excision. In these cases, a dermatoscopic image is taken and a patient is generally seen back in 3 months to re-evaluate the lesion dermatoscopically.[100] In case of change in the lesion, the dermatologist will yet decide to excise the lesion. In case of no change, the lesion will be deemed benign and no further treatment is required. The use of dermoscopy for short-term monitoring can lead to excision reductions of 56.4%, sparing time, costs and unnecessary scars.[101]

An important addition to dermoscopy is total body photography, where a set of 24 standardized photographs are taken from a patient.[102] This can be especially helpful in assessing patients with multiple moles, mainly for the reason to (annually) monitor if any new lesions develop, and to monitor change in preexisting nevi. Skin surveillance on a population-based level is not regarded meaningful, because it is not cost-effective. Rather, appropriate selection of high-risk patients is warranted, e.g. by selecting those with increased numbers of atypical nevi, or a strong family history, or presence of a strong melanoma-predisposing mutation.[103,104] Studies have clearly shown that skin surveillance (by digital dermoscopy and total body photography) in these high-risk patients is cost-effective.[105] This is mainly due to the detection of melanoma at an earlier stage, resulting in less extensive treatment, and a lower annual excision rate for suspicious lesions.

AIM OF THIS THESIS

The above makes clear that several improvements in staging and treatment of melanoma patients are warranted. In this thesis, we aimed to: 1) assess the enactment of SN biopsy, the concordance between pathologists and its incremental value to that of other clinicopathological predictors; 2) thoroughly assess several individual clinicopathological variables associated with survival in patients diagnosed with melanomas and 3) develop and validate several nomograms to optimize and personalize the risk of SN-positivity and the risk of recurrence in patients diagnosed with melanomas.

THESIS OUTLINE

Part I **Sentinel node biopsy**
Part I describes the enactment, yield, concordance between pathologists and the incremental value of sentinel node biopsy.

Chapter 2 Assessment of the trend of SN enactment in the Netherlands.

Chapter 3 Discussion on the probability of SN-positivity in relation to Breslow thickness.

Chapter 4 Thorough examination of the additional value of SN biopsy to that of readily available clinicopathological predictors, using data from the Netherlands and Australia.

Chapter 5 Investigation of the histopathological assessment of SN-positivity.

Chapter 6 Analysis of the effect of time interval between melanoma diagnosis and sentinel node biopsy in Dutch and Australian melanoma patients on the SN-positivity rate and survival.

Chapter 7 Analysis of the effect of time interval between melanoma diagnosis and sentinel node biopsy in Dutch and Australian melanoma patients on the size of SN tumour deposits.

Part II **Clinicopathological variables for survival**
Part II details several clinicopathological variables related to survival in patients diagnosed with cutaneous melanoma.

Chapter 8 Investigation of the influence of presence of regression on survival in Dutch and Australian patients with stage I and II melanomas.

Chapter 9 Thorough analysis of the effect of melanoma subtypes on survival.

Chapter 10 Comparison of survival of Dutch patients with a single melanoma to the survival of patients with multiple melanomas.

Chapter 11 Assessment of survival of Dutch and Australian patients with ultra-thick melanomas.

Chapter 12 Comparison of survival of patients with thick melanomas and a negative SN biopsy to the survival of patient with intermediate or thin melanoma and a positive SN biopsy.

Chapter 13 Comparison of survival of male melanoma patients to that of female melanoma patients.

Part III **Nomogram-based predictions: a multicontinental approach**
Part III of this thesis focuses on nomogram-based predictions. Despite current treatment options, melanoma can have a sinister and unpredictable course of disease. In clinical practice it can be unpredictable who will develop metastases, and who will not, especially in patients with thin melanomas. To optimize the generalizability of the results, a multi-continental approach is used.

Chapter 14 Development and validation of a nomogram to predict recurrence in patients with thin melanomas using multicontinental data from the Netherlands and Australia.

Chapter 15 Validation of a European model to predict recurrence in patients with a negative sentinel node biopsy.

Chapter 16 Validation of the online available Australian Melanoma Institute Australia (MIA) model to predict SN-positivity, using nation-wide data from the Netherlands.

In **Chapter 17**, the findings of chapters 2 to 16 are summarized. In **Chapter 18** a general discussion will be presented together with the conclusions of the work. Finally, a Dutch translation of the summary can be found in **Chapter 19**.

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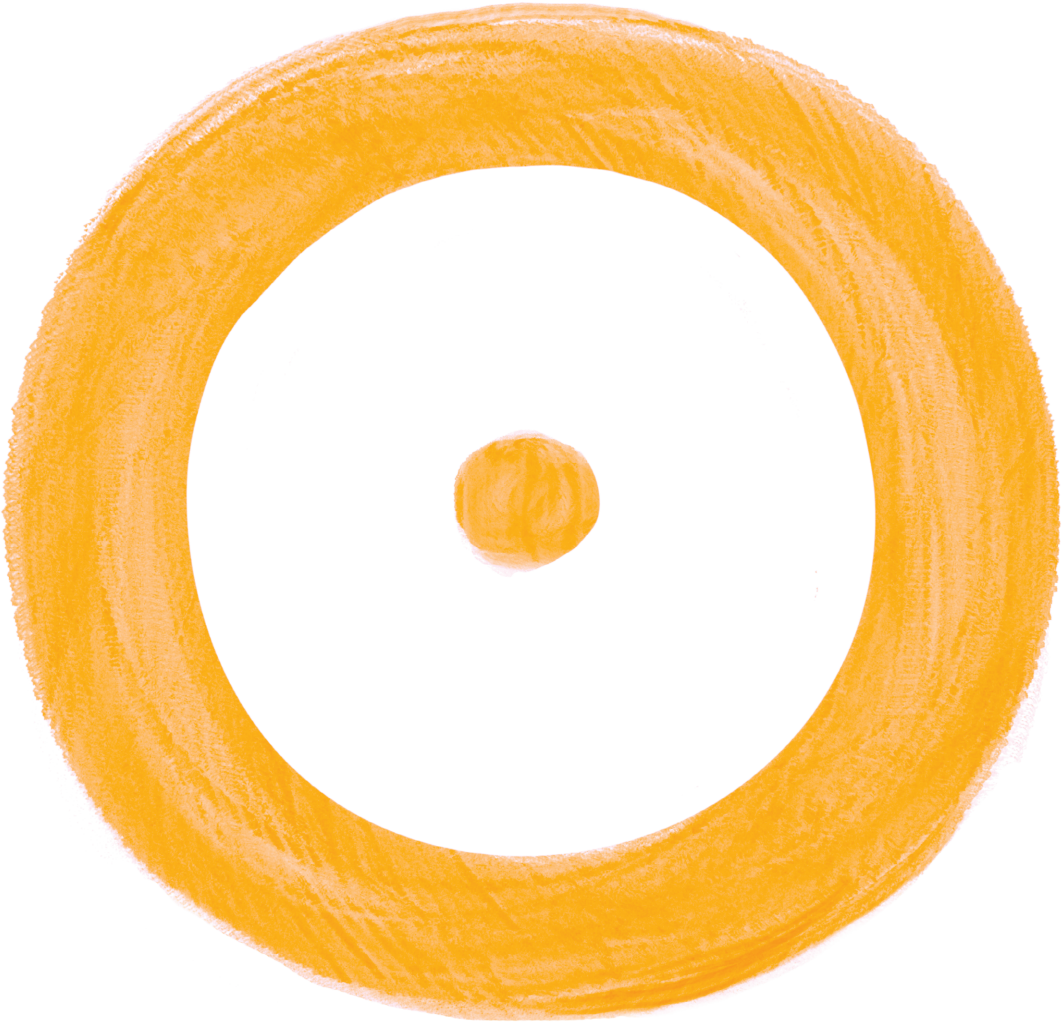
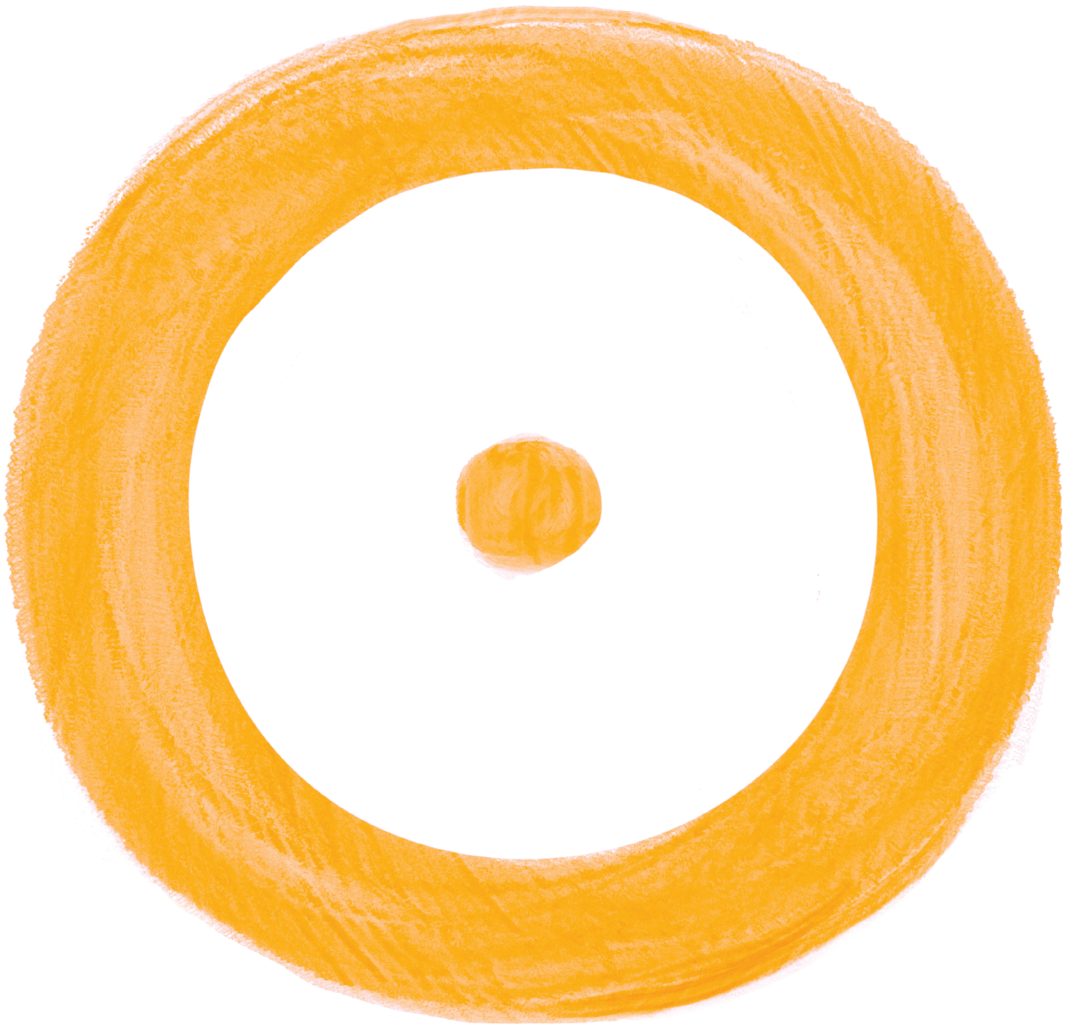


PART I

SENTINEL NODE BIOPSY



CHAPTER 2

Trends in sentinel lymph node biopsy enactment for cutaneous melanoma

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ABSTRACT

Background: Over recent years, sentinel lymph node biopsy (SLNB) recommendations in guidelines for cutaneous melanoma have changed considerably. We aimed to assess trends in enactment of SLNB to evaluate to what extent guidelines were adhered to, and to identify clinical and pathological determinants of (non-)adherence.

Methods: Clinicopathological data from the Dutch nationwide network and registry of histopathology and cytopathology were retrieved from patients diagnosed with primary cutaneous melanoma in the Netherlands between 2003 and 2014. SLNB enactment was analyzed per year. Multivariable regression models were developed to assess the determinants of SLNB enactment.

Results: A total of 51,510 primary cutaneous melanomas in 49,514 patients were diagnosed, of which 24,603 melanomas were eligible for SLNB as they were staged T1b or higher. In practice, only 9761 (39.7%) patients underwent SLNB, with an increasing trend from 39.1% in 2003 to 47.8% in 2014 ($p < 0.001$). A total of 759 (2.9%) of 26,426 patients without SLNB indication underwent SLNB anyway. Variables significantly associated with enactment of SLNB were male sex, younger age, and melanoma on sites other than the head and neck.

Conclusions: Although there was an increasing trend in time in SLNB enactment, enactment of SLNB did not comply well with recommendations in (inter)national guidelines. Female sex, higher age, and melanoma located on the head and neck were associated with non-enactment of SLNB.

INTRODUCTION

Melanoma accounts for the vast majority of skin cancer-related deaths (almost 90%)[1,2] mainly due to regional or distant metastases that have already formed by the time of diagnosis. Reasoning from the concept of a stepwise spread of metastases through locoregional lymph nodes before going into the bloodstream to distant sites, locoregional lymph node dissection was introduced as a therapeutic procedure. However, for many sites of the body (apart from the extremities), the nearest lymph node basin is not obvious, making it often difficult to decide where to perform lymph node dissection and failing to improve prognosis. With the introduction of the sentinel lymph node biopsy (SNLB) procedure to identify the exact location of the first draining lymph node,[3] it became possible to both perform a targeted lymph node dissection aiming to improve the prognosis of SLNB-positive patients, and deny SLNB-negative patients a superfluous surgical procedure. Following its introduction, various guidelines around the world incorporated SNLB indications to select which patients would benefit and which patients would not. For the first time, in 1997, the Dutch national melanoma guidelines mentioned SLNB as 'a promising intervention'.[4] On an international level, the 6th edition of the American Joint Committee on Cancer (AJCC) staging manual (2003–2009) incorporated the SLNB result into the definition of pathological staging.[5] Indications for SLNB in the 6th AJCC were not specifically defined per stage as SLNB was "a standard for staging nodal metastases in patients with clinically uninvolved lymph nodes".[6] Although (inter)national guidelines differed slightly, most agreed that in the 7th AJCC, an indication for SLNB was melanoma with a Breslow thickness (BT) >1.00 mm, with some guidelines also including select patients with a BT ≤1.00 mm (e.g. when other adverse parameters such as ulceration, increased mitotic rate, or young age were present).[7,8,9] Various follow-up studies have shown that SLNB status can provide important prognostic information,[10,11,12,13,14] but, in 2006, the results from the Multicenter Selective Lymphadenectomy Trial (MSLT)-1 showed that SLNB has no therapeutic value as it did not seem to improve melanoma-specific survival.[13] Anticipating this publication, guidelines were adapted to no longer propose routine SLNB, but they advised to enact SLNB to inform selected groups of patients on their prognosis. Furthermore, the MSLT-II trial showed that additional complete lymph node dissection does not increase melanoma-specific survival among patients with sentinel node metastases.[14] The 2005 Dutch national guideline stated SLNB needs to be reserved for patients who want to be informed as optimally as possible, not as a standard diagnostic procedure.[15] In 2007, guidelines were adapted to include the MSLT-1 results, without rectification of the aforementioned advice. Since 2012, Dutch guidelines have recommended SLNB as a prognostic procedure for patients with melanoma stage T1b or higher;[16,17] however, the definition of stage T1 has changed over the years. Although BT cut-offs are equal at 1.00 mm, in the 6th AJCC ulceration was incorporated for the first time, and, in the 7th AJCC, mitoses ≥1/mm2 were added as a second determinant (besides ulceration) for T1a

and T1b melanoma. In the recent 8th edition of the AJCC staging manual, mitoses were again eliminated.[18]

In view of these evolving views on the indications of SLNB as a staging or therapeutic procedure, the changes in the AJCC staging system, and less belief in a stepwise pattern of metastases, enactment of SLNB may well have changed over the years. The aim of this study was therefore to evaluate trends in enactment of SLNB in the Netherlands and analyze clinicopathologic determinants of (non-)adherence to guidelines.

METHODS

Collection of data

Data for this retrospective nationwide study were derived from 'PALGA', the Dutch nationwide network and registry of histopathology and cytopathology, which has prospectively collected all pathology data from all pathology laboratories in the Netherlands since 1987 (http://www.palga.nl).

Study population

For this cohort study, the pathologic reports of all newly diagnosed adult melanoma patients in the Netherlands between 2003 and 2014 were analyzed; for these patients, the 6th AJCC was valid from 2003 to 2009, and the 7th AJCC was valid from 2010 to 2014. Melanoma in situ, Spitzoid tumors of unknown malignant potential (STUMP), melanocytic tumors of unknown malignant potential (MELTUMP), and superficial atypical melanocytic proliferation of uncertain significance (SAMPUS) were excluded, as well as non-cutaneous, desmoplastic melanomas, and melanomas without, or unclear, BT reported. We excluded patients with a positive direct complete lymph node dissection, fine-needle aspiration, or otherwise diagnosed positive lymph nodes within 14 days of diagnosis of the melanoma to ensure patients were free of metastases prior to their melanoma. For the present study, this yielded a dataset of adults with histologically proven invasive, primary, cutaneous melanomas diagnosed between 2003 and 2014 in the Netherlands.

For each patient, clinicopathological variables were extracted from the pathology text files, including date of diagnosis, age, sex, BT (mm), T stage, ulceration (present or absent), type of melanoma (superficial spreading [SSM], nodular [NM], lentigo maligna [LMM], or acral lentiginous [ALM]), body site (head and neck, trunk, arms, or legs) and SLNB enactment (yes or no). As guidelines do not comment on the time between primary excision and SLNB, in a multidisciplinary setting, we decided to include all SLNBs performed within 100 days after initial diagnosis as SLNB, as previously described.[19] Mitoses were included for melanoma for the time period the 7th AJCC was valid, since mitotic rate ≥ 1/mm2 implies SLNB indication. If patients had

more than one primary melanoma, these melanomas were considered separately in the analysis, resulting in total number of melanomas instead of patients. SLNB guideline indication adherence was analyzed per year. All data were encoded and used anonymously. Ethical approval was granted by the board of PALGA.

Statistical analysis

Univariate variables were analyzed using the Chi square test or Mann–Whitney U test, as appropriate. Continuous variables are presented as median with interquartile range (IQR) or mean with standard deviation (SD) for non-normally distributed data and normally distributed data, respectively. Categorical variables are presented as numbers and percentages. To prevent confounding by indication, patients with other lymph node-related procedures, such as complete lymph node dissection or fine-needle aspiration, within 100 days after initial melanoma diagnosis were excluded when calculating SLNB percentages and trends. Trends in time were assessed using a linear-by-linear association test. To account for a possible delay in adoption of the 7th AJCC guideline, we applied the 6th edition of the AJCC staging manual to the time period in our study for which the new 7th AJCC was applicable (2010–2014), leading to an additional analysis that excluded mitotic rate as a criterion. Regression models for melanoma with and without SLNB indication were developed to assess the association of clinicopathological variables (age [continuous], sex, BT [continuous for the model with SLNB indication, categorical for the model without SLNB indication], year [continuous], ulceration, body site, and melanoma subtype) with SLNB use. Variables were entered in a backward, stepwise method, and data were analyzed using SPSS version 21 (IBM Corporation, Armonk, NY, USA). Two-sided p-values < 0.05 were considered significant.

RESULTS

Patients and melanoma incidence trends

Between 2003 and 2014, a total of 51,510 melanomas in 49,514 patients were diagnosed — 47,549 single melanomas and 3961 multiple melanomas. According to AJCC staging, a total of 25,137 (48.8%) melanomas were staged to the 6th AJCC, and 26,373 (51.2%) were staged to the 7th AJCC. The total number of melanomas diagnosed per year increased from 2960 in 2003 to 5807 in 2014, with a median BT of 0.89 (IQR 0.50–1.70). A total of 55.4% of patients were female. Age ranged from 18 to 106 years, with a mean age of 56.98 years (SD 16.00). The trunk was the most common body site, harboring 42.6% of melanomas. Ulceration occurred in 6760 (13.1%) melanomas, and most melanomas were staged T1 (Table 1).

Table 1. Clinical and histopathological characteristics of all primary, cutaneous melanomas in the Netherlands from 2003 to 2014, with and without SLNB indication, stratified for enactment of SLNB.

		Melanoma with SLNB indication (n=24,603) [1]			Melanoma without SLNB indication (n=26,426)		
Characteristic	**All patients, n=51,510**	**No SLNB enacted, n=14,842**	**SLNB enacted, n=9761**	**p-value**	**No SLNB enacted, n=25,667**	**Enacted SLNB, n=759**	**p-value**
Sex [*n* (%)]				<0.001*			0.67
Female	28,524 (55.4)	7919 (53.4)	4913 (50.3)		15,081 (58.8)	440 (58.0)	
Male	22,986 (44.6)	6923 (46.6)	4848 (49.7)		10,586 (41.2)	319 (42.0)	
Age in years mean (SD)							
[*n* (%)]	56.98 (16.00)	62.28 (16.92)	54.98 (14.41)	<0.001*	54.81 (15.36)	50.34 (13.48)	<0.001*
18-35	5042 (9.8)	1011 (6.8)	1001 (10.3)	<0.001*	2890 (11.3)	106 (14.0)	<0.001*
36-55	18,658 (36.2)	4050 (27.3)	3832 (39.3)		10,258 (40.0)	379 (49.9)	
56-75	20,770 (40.3)	6006 (40.5)	4247 (43.5)		10,042 (39.1)	254 (33.5)	
>75	7040 (13.7)	3775 (25.4)	681 (7.0)		2477 (9.7)	20 (2.6)	
Year of diagnosis [*n* (%)]				<0.001*			<0.001*
2003	2960 (5.7)	852 (5.7)	548 (5.6)		1463 (5.7)	66 (8.7)	
2004	3115 (6.0)	907 (6.1)	470 (4.8)		1646 (6.4)	57 (7.5)	
2005	3442 (6.7)	1019 (6.9)	494 (5.1)		1832 (7.1)	58 (7.6)	
2006	3462 (6.7)	1032 (7.0)	524 (5.4)		1816 (7.1)	56 (7.4)	
2007	3762 (7.3)	1056 (7.1)	635 (6.5)		1981 (7.7)	56 (7.4)	
2008	4085 (7.9)	1070 (7.2)	660 (6.8)		2247 (8.8)	67 (8.8)	
2009	4312 (8.4)	1086 (7.3)	768 (7.9)		2311 (9.0)	98 (12.9)	
2010	4693 (9.1)	1410 (9.5)	866 (8.9)		2315 (9.0)	63 (8.3)	
2011	5055 (9.8)	1505 (10.1)	985 (10.1)		2447 (9.5)	69 (9.1)	
2012	5260 (10.2)	1627 (11.0)	1066 (10.9)		2472 (9.6)	51 (6.7)	
2013	5557 (10.8)	1675 (11.3)	1280 (13.1)		2499 (9.7)	58 (7.6)	
2014	5807 (11.3)	1603 (10.8)	1465 (15.0)		2638 (10.3)	60 (7.9)	
Breslow thickness in mm	0.89	1.60	1.85	<0.001*	0.55	0.90	<0.001*
Median (IQR)	(0.50-1.70)	(1.10-3.00)	(1.30-2.90)		(0.40-0.70)	(0.73-1.00)	
[*n* (%)]				<0.001*			NA
0.01-1.00	29,957 (58.2)	3012 (20.3)	513 (5.3)				
1.01-2.00	11,345 (22.0)	6203 (41.8)	5043 (51.7)		25,667 (100.0)	759 (100.0)	
2.01-3.00	4470 (8.7)	2244 (15.1)	2137 (21.9)		NA: T1a or T1nos	NA: T1a or	
3.01-4.00	2247 (4.4)	1188 (8.0)	985 (10.1)			T1nos	
>4.00	3491 (6.8)	2195 (14.8)	1083 (11.1)				

Table 1. (continued).

Characteristic	All patients, n=51,510	Melanoma with SLNB indication (n=24,603) [1]			Melanoma without SLNB indication (n=26,426)		
		No SLNB enacted, n=14,842	SLNB enacted, n=9761	p-value	No SLNB enacted, n=25,667	Enacted SLNB, n=759	p-value
Body site [*n* (%)]				<0.001*			<0.001*
H&N	6412 (12.4)	2794 (18.8)	582 (6.0)		2891 (11.2)	42 (5.6)	
Trunk	21,937 (42.6)	5428 (36.6)	4286 (44.0)		11,703 (45.6)	324 (42.7)	
Arms	7637 (14.8)	2228 (15.0)	1469 (15.1)		3772 (14.7)	121 (15.9)	
Legs	13,953 (27.1)	3925 (26.4)	3159 (32.2)		6495 (25.3)	251 (33.1)	
Missing	1571 (3.0)	467 (3.2)	265 (2.7)		806 (3.1)	21 (2.8)	
Ulceration [*n* (%)]				<0.001*			NA
No	38,463 (74.7)	9405 (63.4)	6539 (67.0)		NA: T1a or T1nos	NA: T1a or T1nos	
Yes	6760 (13.1)	3968 (26.7)	2545 (26.1)				
Missing	6287 (12.2)	1469 (9.9)	677 (6.9)				
Mitosis [*n* (%), 7th AJCC]	*N = 26,373*	*N=7820*	*N=5663*	<0.001*	NA: T1a or T1nos	NA: T1a or T1nos	NA
No	8305 (31.5)	4288 (54.8)	2736 (48.3)				
Yes	7100 (26.9)	382 (4.9)	336 (5.9)				
Missing	10,968 (41.6)	3150 (40.3)	2591 (45.8)				
Subtype [*n* (%)]				<0.001*			<0.001*
SSM	37,163 (72.1)	8807 (59.4)	5991 (61.4)		21,562 (84.0)	618 (81.4)	
NM	6590 (12.8)	3510 (23.7)	2509 (25.7)		330 (1.3)	20 (2.6)	
LMM	2406 (4.7)	620 (4.2)	86 (0.9)		1682 (6.6)	13 (1.7)	
ALM	406 (0.8)	173 (1.2)	162 (1.6)		61 (0.3)	4 (0.5)	
Missing	4945 (9.6)	1732 (11.7)	1013 (10.4)		2032 (7.9)	104 (13.7)	
T-stage [*n* (%)]				<0.001*			NA
T1	29,956 (58.2)	3012 (20.3)	512 (5.2)		NA: T1a or T1nos	NA: T1a or T1nos	
T2	11,334 (22.0)	6206 (41.8)	5043 (51.7)				
T3	6718 (13.0)	3433 (23.1)	3122 (32.0)				
T4	3492 (6.7)	2195 (14.8)	1084 (11.1)				

1 Exclusion of 481 patients with lymph node procedures other than SLNB.
SLNB sentinel lymph node biopsy, SD standard deviation, IQR interquartile range, AJCC American Joint Committee on Cancer, SSM superficial spreading melanoma, LMM lentigo maligna melanoma, ALM acral lentiginous melanoma, NM nodular melanoma, NA not applicable, * indicates statistical significance

Trends in sentinel lymph node biopsy (SLNB)

The trend in time of SLNB enactment increased significantly from 39.1% in 2003 to 47.8% in 2014 (Fig. 1). When stratifying for T stage, we observed a trend for all stages, except T1a melanoma, especially from 2006 onward (Fig. 2). Adjusting the 100-day threshold for SLNB enactment to 200 days did not significantly alter these percentages (data not shown). When accounting for a possible delay to adoption of the 7th AJCC, 56.6% (instead of 47.8%) of SLNB enactments in all eligible patients (≥ T1b) would be reached in 2014, due to 2934 melanomas with mitoses >1/mm2 that would have been staged T1b in the 7th AJCC, and in whom SLNB was not performed, but were classified as T1a in the 6th AJCC (Figs. 1 and 2).

Figure 1. Trend in enacted SLNB (n=9761) in ≥T1b melanoma between 2003 and 2014 in the Netherlands, including the anticipated delay period of the 7th AJCC. Linear-by-linear association: *p<0.001. SLNB sentinel lymph node biopsy, AJCC American Joint Committee on Cancer, MSLT-1 Multicenter Selective Lymphadenectomy Trial-1.

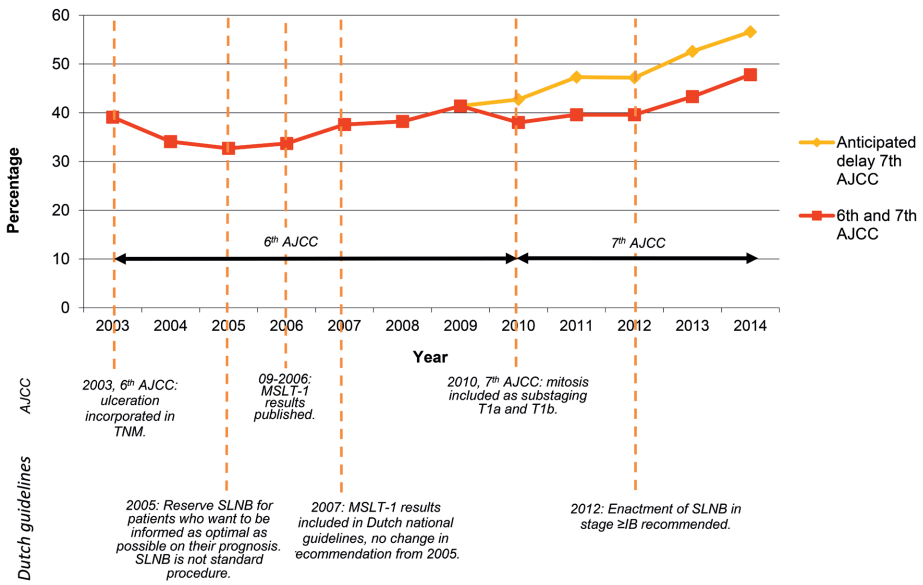
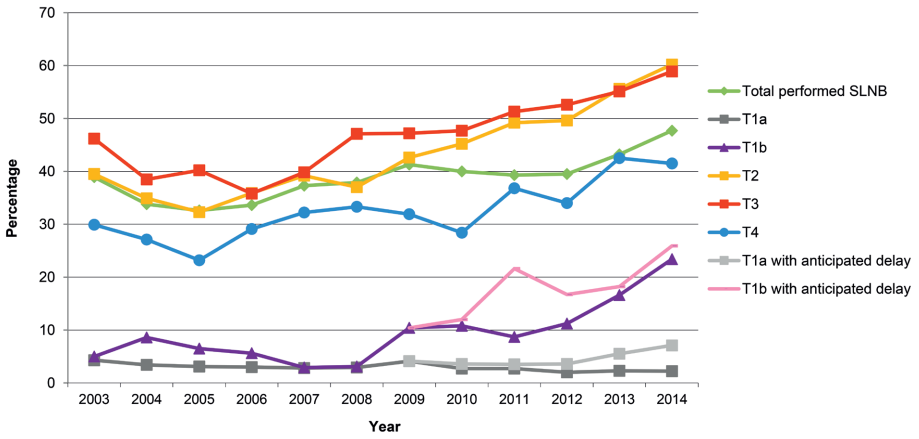


Figure 2. Percentage of enacted SLNB (n=10,520) per stage per year for primary, cutaneous melanoma in the Netherlands, including the anticipated delay period of the 7th AJCC. Linear-by-linear association: *p<0.001. SLNB sentinel lymph node biopsy, AJCC American Joint Committee on Cancer.



SLNB enactment in eligible melanomas according to guidelines

A total of 25,084 melanomas had an indication for SLNB, according to the guidelines at the time, as they were staged T1b or higher. Lymph node procedures other than SLNB were performed in 481 melanomas (1.9%). Excluding this group, a total of 9761 (39.7%) of all 24,603 eligible melanomas underwent SLNB in practice.

According to the evolving guidelines over the year, 14,842 melanomas had an indication for SLNB but were not enacted. This group had different characteristics than the group of melanomas in whom SLNB was enacted according to guidelines. Univariable analysis revealed that melanomas with SLNB indication but with no SLNB enactment had a higher mean age, comprised more females, had lower BT, and were less often ulcerated (Table 1). Multivariable analysis for enactment of SLNB excluded 4886 cases with missing values. All variables, except ulceration, showed a significant association with SLNB enactment. Women had significantly lower odds of receiving SLNB compared with men (odds ratio [OR] 0.78, 95% confidence interval 0.73–0.83), as had older patients and head and neck melanomas. ALMs and NMs were more likely to receive SLNB compared with SSMs (Table 2).

Table 2. Multivariable regression for factors associated with enactment of SLNB for cutaneous, primary melanoma (n = 19,717) in patients with SLNB indication in the Netherlands between 2003 and 2014.

Variable	OR (95% CI)	p-value
Age (per year)	0.97 (0.97-0.98)	<0.001*
Breslow-thickness (per mm)	1.06 (1.04-1.07)	<0.001*
Year (per year)	1.07 (1.06-1.08)	<0.001*
Body site		
H&N	Reference	
Trunk	2.97 (2.65-3.33)	<0.001*
Arms	3.05 (2.68-3.47)	<0.001*
Legs	3.47 (3.08-3.91)	<0.001*
Subtype		
SSM	Reference	
NM	1.27 (1.18-1.37)	<0.001*
LMM	0.64 (0.50-0.82)	<0.001*
ALM	1.39 (1.09-1.76)	0.007*
Sex		
Male	Reference	
Female	0.78 (0.73-0.83)	<0.001*

Ulceration not significant.
SLNB sentinel lymph node biopsy, OR odds ratio, CI confidence interval, SSM superficial spreading melanoma, NM nodular melanoma, LMM lentigo maligna melanoma, ALM acral lentiginous melanoma, * indicate statistical significance

SLNB enactment in non-eligible melanomas according to guidelines

Conversely, a total of 759 (2.9%) of 26,426 patients without SLNB indication underwent SLNB. Of these, 500 (65.9%) had stage T1a and 259 (34.1%) had stage T1NOS. Compared with other non-eligible patients in whom SLNB was not performed, patients with SLNB enactment had a lower mean age of 50.34 versus 54.81 years, a higher median BT of 0.90 versus 0.55, and melanomas that were less often located on the head and neck (all $p < 0.001$). In multivariable regression, 2868 melanomas were excluded because of missing data. Patients who underwent SLNB without indication were more frequently males of younger age, with higher BT and melanoma on sites other than the head and neck (Table 3).

Table 3. Multivariable regression for factors associated with enactment of SLNB for cutaneous, primary melanoma (n = 23,558) in patients without SLNB indication in the Netherlands between 2003 and 2014.

Variable	OR (95% CI)	p-value
Age (per year)	0.98 (0.97-0.99)	<0.001*
Breslow-thickness (in mm)		
0.01-0.24	Reference	
0.25-0.49	1.68 (0.60-4.69)	0.32
0.50-0.74	3.61 (1.33-9.80)	0.01*
0.75-1.00	24.86 (9.27-66.69)	<0.001*
Body site		
Head and neck	Reference	
Trunk	1.70 (1.17-2.47)	0.005*
Arms	2.19 (1.46-3.29)	<0.001*
Legs	2.20 (1.50-3.23)	<0.001*
Sex		
Male	Reference	
Female	0.80 (0.67-0.95)	0.01*

Year and type melanoma not significant. Ulceration not applicable (T1a or T1NOS melanoma).
SLNB sentinel lymph node biopsy, OR odds ratio, CI confidence interval, * indicates statistical significance

DISCUSSION

Over recent years, SLNB recommendations in guidelines for cutaneous melanoma have changed considerably. Current and previous Dutch and international guidelines advise SLNB in melanoma stage IB or higher.[14,20,21,22] Dutch guidelines from 2005 describe SLNB as promising and to reserve it for patients who want to be 'informed as optimally as possible'.[15] In 2007, the results of MSLT-1 were included, without rectification of advice from 2005.9 From 2012, SLNB has been advised in all patients with melanoma stage T1b or higher.[16,17]

We have shown that in the Netherlands, the use of SLNB for melanoma has increased, likely due to these evolving guidelines following the results of landmark studies. Enactment of SLNB increased from 39.1% in 2003 to 47.8% in 2014. SLNB guidelines were apparently not adequately adhered to in the Netherlands as only 39.7% of eligible tumors underwent SLNB. Although an obvious increasing trend has been observed since publication of the Dutch 2012 guidelines, even in more recent years, such as 2014, not even half of the eligible patients in fact underwent SLNB. When accounting for a possible delay to adoption of the 7th AJCC, SLNB enactment rose to 56.6% in 2014; however, there was an apparent 3-year delay from 2010 to 2013 due to patients with mitoses>1/mm2 in whom SLNB was not performed. We found no studies on delays in the adoption of new guidelines in order to compare this finding.

We found female sex, older age, and melanoma in the head and neck region to be associated with non-enactment of SLNB. Huismans et al. assessed factors such as sex, age, socioeconomic status, BT, and hospital type influencing the use of SLNB in the north-eastern part of the Netherlands and found 42% of SLNB enactment in a total of 2413 patients with melanomas with a BT>1 mm;[23] however, compared with other nations, this percentage is low. Bilimoria et al.[24] used US National Cancer Database (NCDB) data (n = 16,598) of stage I and II melanoma patients, between 2004 and 2005, and found a 48.7% enactment rate; Murtha et al.[25] used Surveillance, Epidemiology, and End Results (SEER) data of 13,307 melanoma patients, between 2010 and 2012, with a 59.9% enactment rate; Moreno-Ramirez et al.[26] analyzed 478 melanoma stage T1a–T4b patients in their center, with a 63.2% enactment rate; and Blakely et al.[27] analyzed 865 melanoma patients, between 2005 and 2015, with a 93.2% enactment rate.

In the Netherlands, considerable regional practice variation of 22.5–56.6% was previously reported by Verstijnen et al.[28] in patients with a BT>1 mm. Interesting is the finding that guidelines for SLNB enactment are not adequately adhered to. Explanations for this non-adherence in general can vary greatly, ranging from lack of familiarity to low outcome expectancy or disagreement.[29] For melanoma-specific adherence to guidelines, Kang and Wong and Varey et al.[30,31] showed that for wide local excisions for melanoma, surgeons with a high melanoma caseload (>30) were more likely to perform procedures concordant with the guidelines than those with a lower caseload. Another reason might be that Dutch guidelines have only advised on SLNB since 2005, and waited until 2012 to provide a recommendation, which is still not solid advice. Other than that, we do not have a plausible explanation, other than more defensive versus selective attitudes that may differ per country, with the Netherlands apparently being more selective and with a relatively low adherence rate of 39.7%. In line with this, Cormier et al.[32] used US SEER data and showed almost 10% of stage IA melanomas are overtreated when it comes to lymph node therapy, probably reflecting a more defensive attitude. Another important finding is that for both SLNB indicated and non-indicated melanomas, female patients had significantly lower odds of receiving an SLNB, with an OR of 0.78 and 0.80, respectively. While Huismans et al. and Verstijnen et al. corroborate our findings, with ORs of 0.86 and 0.85, respectively, it is surprising that none of the previously mentioned studies have considered patient sex in their analyses. There are three possible sex-related explanations that may account for our lower OR; (1) female melanoma patients have other characteristics that we did not include in our multivariable model; (2) sex-specific decision making, e.g. when female patients more often decline SLNB, or medical information is perceived differently; or (3) clinician-specific sex bias in approaching and informing female patients. No studies have been conducted in melanoma patients to support any of these explanations, however there is some

general evidence of physician sex-related differences in both decision making and approach to patients.[33,34,35,36]

Another finding supported by previous literature is that head and neck melanomas had the lowest percentage of SLNB enactment.[23,24,25,29] This may be explained by the technical challenge associated with localization, and also as lymphatic drainage can occur to multiple or bilateral sites, with the sentinel lymph node itself being relatively small.[37] Furthermore, our finding that older patients more often refrain from SLNB is also sustained by others.[23,24,25,26,28] An explanation for this could be relevant comorbidities influencing prognosis in older patients, or a more conservative approach in view of a generally lower life expectancy.

Although we assessed multiple factors associated with SLNB use, we did not take into consideration socioeconomic status, race, and regional practice variation, which have been shown to influence SLNB use.[23,24,25,28] Another limitation is that mitosis status was missing in 41.6% of T1 melanomas. As a mitotic rate ≥ 1/mm2 implies SLNB indication in the 7th AJCC, this might have influenced the number of eligible patients for SLNB. As opposed to Verstijnen et al. and Huismans et al., we included ulceration since its presence means SLNB indication for T1 melanoma.[38] Other strengths of our study include our large sample size and generalizability due to the nationwide cohort.

CONCLUSIONS

There was an increasing trend in SLNB enactment for all melanoma stages, except T1a melanoma. Enactment of SLNB did not comply well with recommendations in (inter)national guidelines. Female sex, higher age, and melanoma in the head and neck region were associated with non-enactment of SLNB.

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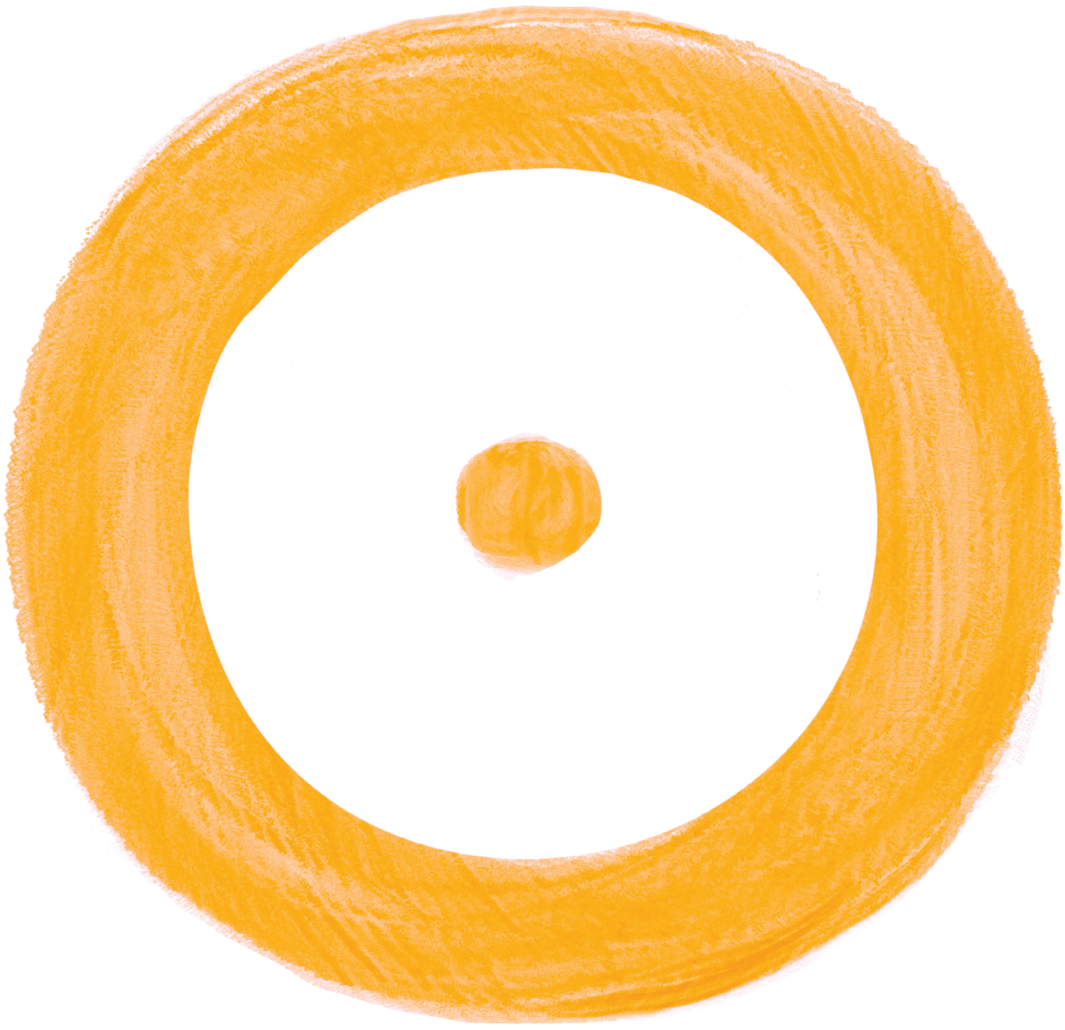

CHAPTER 3

Probability of sentinel lymph node positivity in melanoma

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Despite that guideline recommendations for its indication differ slightly per country, it is believed that sentinel lymph node biopsies (SLNBs) should be considered in melanoma patients with >1 mm Breslow thickness (BT). Some guidelines advise the procedure to be discussed with category T1b patients as well, meaning melanoma ≤0.8 mm BT with ulceration or melanoma 0.8–1.0 mm BT irrespective of ulceration[1]. Since 2012, Dutch guidelines recommend SLNB as a prognostic procedure for patients with melanoma stage T1b and higher. However, it is generally accepted that only 20% of all SLNB in melanoma harbour metastases[2], with wide variation between melanomas with low and high BT. In addition, it is an invasive procedure that can lead to complications such as infection, seroma, and lymphedema, as has been shown by Moody et al. in their recent systemic review[3]. Reasoning from this, this leaves room to optimise the yield of the SLNB. In this study, our aim was to evaluate the total percentage positive SLNBs for melanoma per T-category on a nation-wide level, with special focus on the subset of T1b patients. We obtained data from 'PALGA', the Dutch Nationwide Network and Registry of Histopathology and Cytopathology, yielding a cohort with primary, cutaneous melanoma patients between 2003 and 2014 who underwent SLNB. All patients were reclassified according to the 8th tumour-node-metastasis American Joint Committee on Cancer (AJCC), and SLNB yield was evaluated accordingly. Melanoma category T1b were subdivided into three categories: <0.8 mm with ulceration, 0.8–1.0 mm without ulceration and 0.8–1.0 mm with ulceration. For the current study, the 6th AJCC was valid from 2003 to 2010, which meant no official SLNB indication in the Netherlands. The 7th AJCC was valid from 2010 to 2014 (end of study period), which meant a SLNB indication for all melanoma >1.00 mm or ≤1.00 mm with ulceration or mitotic rate ≥1/mm2 (the latter group categorised as pT1b).

A total of 10,523 melanoma patients were included. Melanoma metastases were found in 2441 (23.2%) of all enacted SLNB. Stratified for T-category and ulceration, the chance of a positive SLNB significantly increased from 8.1% for T1a to 45.3% in T4b (Table 1). In the subset of T1b melanoma, the chance of a positive SLNB was 11.8% in melanoma <0.8 mm BT with ulceration, 9.3% for 0.8–1.0 BT without ulceration and 19.3% for 0.8–1.0 BT with ulceration, with a significant difference between the latter two ($p = 0.015$). Further analysis of category T1a patients showed that the patients in whom SLNB was performed more often had mitoses (45.3% versus 10.2%, $p < 0.001$) and were younger of age (51.57 years versus 55.06 years, $p < 0.001$) compared with the patients in whom SLNB was not performed (data not shown). Possibly, the 8.1% SLNB positivity rate in T1a melanoma patients could be explained by the fact that it has been shown that a mitotic rate ≥1/mm2 and younger age increase the chance of a positive SLNB in thin melanomas: German data by Kretschmer et al., showed a positivity rate of 19.7% in young patients with 0.76–1.0 mm melanoma[4]. It seems SLNB has been performed selectively in this cohort.

Recently SLNB positivity was introduced as a biomarker to select patients for adjuvant treatment[5]. This gives a new dimension to the use of SLNB procedures in melanoma, because now SLNB positivity is not only providing more accurate prognostic information but also has therapeutic consequences. This makes the need to better inform patients on their chances of a positive SLNB even bigger. Instead of performing SLNB in all patients >1.0 mm (and in some <1.0 mm ulcerated melanoma), we strongly believe there is room for a more tailored approach, as we have shown SLNB positive patients comprise a heterogeneous group. Our data can be used for shared decision-making, as insight in the chance of a positive SLNB on an individual level can be weighed against the risk of the procedure, such as complications and narcosis. For example, while there will probably be little disagreement on the value of SLNB in T3 and T4 patients, shared decision-making in T1 and T2 may be easier having the present data at hand.

Table 1. Distribution of stages according to the 8th TNM/AJCC and chances of sentinel lymph node biopsy (SLNB) positivity for all melanoma in the Netherlands from 2003 to 2014.

Stage		Total	# SLNB	# Positive SLNB	Positive SLNB rate (%)
T1	a	18,377	296	24	8.1
	b (all) [a]	8708	1010	105	10.4
	<0.8 with ulceration		*17*	*2*	*11.8*
	0.8-1.0 without ulceration		*835*	*78*	*9.3*
	0.8-1.0 with ulceration		*57*	*11*	*19.3*
	nos	3095	29	1	3.4
T2	a	8375	3899	603	15.5
	b	1543	706	152	21.5
	nos	1246	401	63	15.7
T3	a	3579	1744	535	30.7
	b	2499	1148	421	36.7
	nos	607	210	66	31.4
T4	a	1121	405	164	40.5
	b	2114	618	280	45.3
	nos	246	57	27	47.4
Total		51,510	10,523	2441	23.2

[a] 101 patients were 0.8-1.0 mm Breslow thickness had a missing ulceration status; 14 positive SLNB were found in this group (13.9% positivity rate).

To the best of our knowledge, we present the largest data set available to describe SLNB positivity for cutaneous melanoma patients stratified for T-category and ulceration. Other strengths of our study include its generalisability, because we used nation-wide data instead of single-centre data. A limitation is that, because of the retrospective nature of our study, selection bias might have occurred. As we have discussed, SLNB enactment in T1a melanoma might have had specific reasons (e.g.

presence of mitosis) which could have potentially led to an overestimated percentage of positive SLNBs we reported here.

CONCLUSIONS

The chance of SLNB metastases increases with melanoma stage and when ulceration is present. It was interesting to note the impact of ulceration on SLNB yield in category T1b patients.

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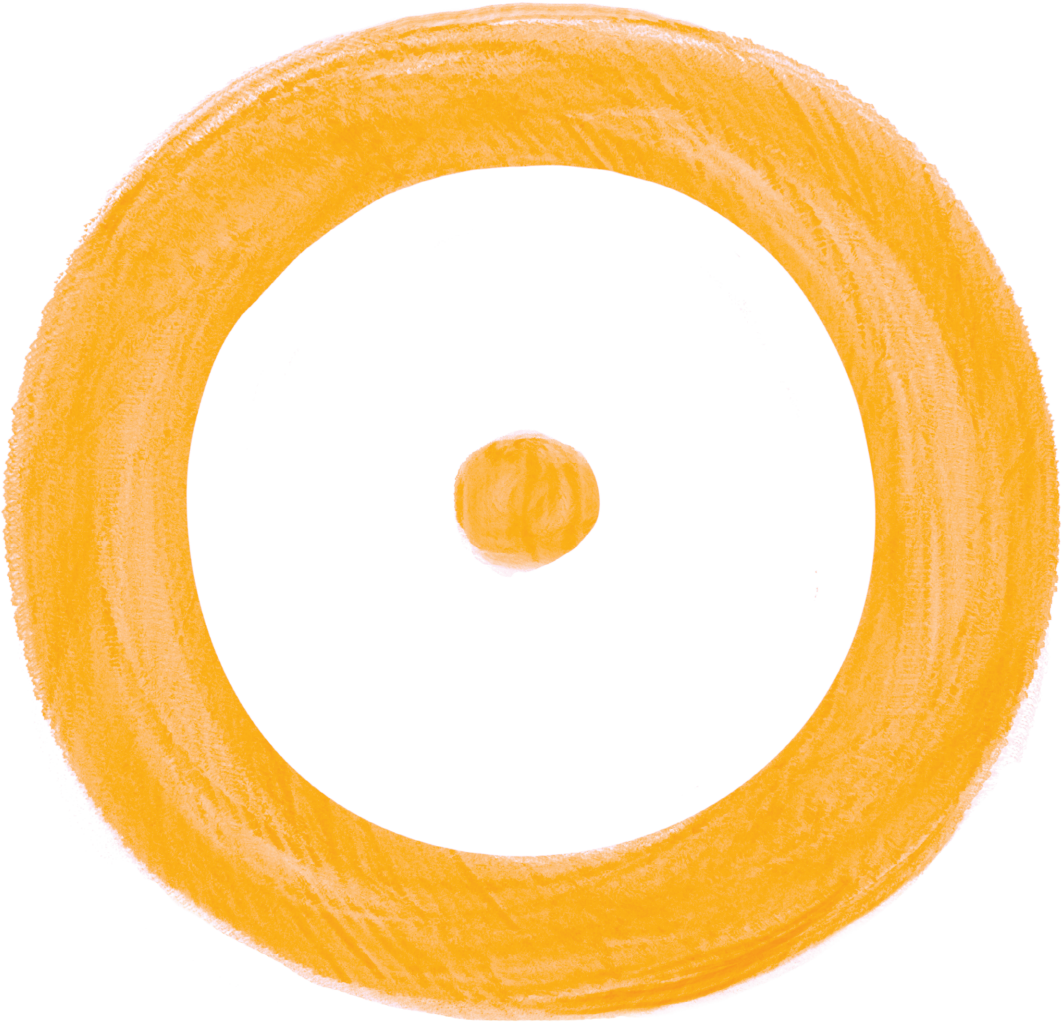

CHAPTER 4

Sentinel node biopsy in patients with melanoma improves the accuracy of staging when added to clinicopathological features of the primary tumor

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ABSTRACT

Background: It has been claimed, without supporting evidence, that knowledge of sentinel node (SN) status does not provide more accurate prognostic information than basic clinicopathological features of a primary cutaneous melanoma. We sought to investigate this claim and to quantify any additional value of SN status in predicting survival outcome.

Methods: Data for a Dutch population-based cohort of melanoma patients (n = 9272) and for a validation cohort from a large Australian melanoma treatment center (n = 5644) were analyzed. Patients were adults diagnosed between 2004 and 2014 with histologically-proven, primary invasive cutaneous melanoma who underwent SN biopsy. Multivariable Cox proportional hazards analyses were carried out in the Dutch cohort to assess recurrence-free survival (RFS), melanoma-specific survival (MSS) and overall survival (OS). The findings were validated using the Australian cohort. Discrimination (Harrell's C-statistic), net benefit using decision curve analysis and net reclassification index (NRI) were calculated.

Results: The Dutch cohort showed an improved C-statistic from 0.74 to 0.78 for OS and from 0.74 to 0.76 for RFS when SN status was included in the model with Breslow thickness, sex, age, site, mitoses, ulceration, regression and melanoma subtype. In the Australian cohort, the C-statistic increased from 0.70 to 0.73 for OS, 0.70 to 0.74 for RFS and 0.72 to 0.76 for MSS. Decision curve analyses showed that the 3-year and 5-year risk of death or recurrence were more accurately classified with a model that included SN status. At 3 years, sensitivity increased by 12% for both OS and RFS in the development cohort, and by 10% and 6% for OS and RFS, respectively, in the validation cohort.

Conclusions: Knowledge of SN status significantly improved the predictive accuracy for RFS, MSS and OS when added to a comprehensive suite of established clinicopathological prognostic factors. However, clinicians and patients must consider the magnitude of the improvement when weighing up the advantages and disadvantages of SN biopsy for melanoma.

INTRODUCTION

Several predictors of survival in patients with cutaneous melanomas have been identified, the two most consistently important being Breslow thickness and ulceration status of the primary tumor. Together, they form the basis of the American Joint Committee on Cancer (AJCC) eighth edition system for T-staging of cutaneous melanomas.[1,2,3] When metastatic melanoma is detected by sentinel node (SN) biopsy, patients are upgraded from stage I or II to stage III, implying a worse survival than SN-negative patients.[4] However, the additional prognostic value of SN status over known predictors such as Breslow thickness and ulceration has been questioned by some who have suggested that if equivalent prognostic information can be derived using parameters obtained from standard histological examination, patients could be spared an SN biopsy.[5, 6, 7, 8] Surprisingly, evidence-based studies in the melanoma literature assessing the additional prognostic information provided by SN biopsy are sparse. We therefore sought to investigate the additional prognostic value of SN status in predicting patient outcomes in two large independent datasets, one population-based (from the Netherlands) and the other from a large melanoma treatment center in Australia [Melanoma Institute Australia (MIA)].

METHODS

Study cohorts

For the development cohort of Dutch melanoma patients, data for all patients in the Netherlands with newly-diagnosed invasive melanoma between January 2004 and December 2014 were obtained from PALGA, the Dutch Pathology Registry. Since 1991, PALGA has been prospectively collecting data from all Dutch pathology laboratories.[9] Follow-up data were obtained from the Netherlands Cancer Registry, which records information on every cancer patient treated in the Netherlands. All data were encoded and used anonymously. Ethical approval was granted by the board of PALGA.

The validation cohort was obtained from the prospectively-maintained research database of MIA, a tertiary referral center that sees and treats around a third of melanoma patients in the state of New South Wales, Australia. A search of the MIA database was carried out to identify all patients diagnosed with invasive melanoma in the same time period as the Dutch cohort. Approval for use of information from the MIA database was obtained from the Sydney Local Health District Ethics Committee.

Study population

The study population consisted of patients who underwent SN biopsy within 100 days of initial diagnosis of a primary cutaneous melanoma. Patients aged <18 years and those with multiple primary invasive melanomas were excluded (i.e. excluding melanoma in-situ). Demographic data collected included date of diagnosis, age, sex, primary tumor anatomical site and recurrence. Recurrence was defined as either cutaneous (local or in-transit), nodal (regional only) or distant metastasis. Pathological data collected included Breslow thickness, melanoma subtype, SN status, presence or absence of ulceration, mitoses and regression. A 'not known' category was created for missing data for regression, mitoses and melanoma subtype.[10] For both datasets, primary outcomes were recurrence-free survival (RFS) and overall survival (OS). Melanoma-specific survival (MSS) was analyzed as an outcome using MIA data only because cause of death was not recorded in the Dutch Cancer Registry. RFS, MSS and OS were calculated from date of diagnosis to date of recurrence, death due to melanoma, or death from any cause, respectively. Patients without recurrence were censored at either their date of death or the last date known alive or 1 January 2018 (the data collection cut-off date), whichever occurred earlier.

Statistical analysis

Categorical variables were summarized using frequency and proportion and continuous variables as median with range. Differences between the two cohorts were assessed using the chi-square test or the Mann–Whitney U test. Statistical analysis was carried out using univariable and multivariable Cox proportional hazard

models on the development cohort. Clinicopathological variables available from standard pathology reports were included as predictors in the analysis. The variables included Breslow thickness, sex, age, anatomical site, mitoses, ulceration, regression and melanoma subtype. Given the large sample size, number of clinical events and the fact that we included basic, readily-available clinicopathological variables, no variable selection procedure was carried out. The proportional hazards assumption was assessed using the Schoenfeld residuals test.

The additional value of SN status was evaluated by comparing the multivariable model without and with SN status for each of the specified survival outcomes. In addition, the incremental effect of SN status was assessed relative to a model that included only Breslow thickness and a model that included both Breslow thickness and ulceration. The quantified incremental value of SN status was estimated using various complementary statistical metrics that included the C-statistic [equivalent to the area under the curve (AUC)], net benefit using decision curve analysis and net reclassification index (NRI).[11] For internal validation (to assess overfitting), C-statistics for the multivariable risk models were generated using a bootstrap method with 1000 replications.[11] External model validation was then carried out for each outcome by applying the estimated regression coefficients from the Dutch cohort (development set) to the MIA cohort (validation set) to re-calculate all three predictive performance measures.[12]

Decision analysis curves

Decision curve analyses were carried out for each survival outcome at fixed time points (3 years and 5 years) to identify patients at high risk for either recurrence or death. The decision curve analysis plots estimated the net benefit at a range of clinically-relevant risk thresholds. The threshold probability was used to determine whether a patient was defined as high risk or low risk, and to model the clinical consequences of true-positive and false-positive results using a clinical net benefit function.[13]

NRI

The NRI was calculated to evaluate improvements in risk predictions at 3 years and 5 years after melanoma diagnosis. NRI reflects the net improvement in the classification of patients with lower and higher predicted survival outcomes from the model (model sensitivity and specificity), based on the addition of new variables (in this case SN biopsy).[14,15] Pre-specified risk thresholds were set at <5%, 5%-10% and >10% for recurrence or death and were calculated at 3 years and 5 years.[14]

All statistical analyses were carried out using R version 3.6.1 (R Core Team, Vienna, Austria). A two-sided p-value of <0.05 was considered statistically significant.

RESULTS

Recruitment

In total, 9272 Dutch patients and 5644 MIA patients were included. Their full clinicopathological details are presented in Table 1. Clinicopathological differences between SN-negative and SN-positive patients for the two cohorts are presented in supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2020.11.015.

Table 1. Patient and melanoma characteristics in the Dutch and MIA datasets.

Characteristic	Dutch data (development) n = 9272	MIA data (validation) n = 5644	*p*-value
Gender (n (%))			
Female	4735 (51.1)	2303 (40.8)	<0.0001
Male	4537 (48.9)	3341 (59.2)	
Age at diagnosis (n (%))			
≤30	513 (5.5)	343 (6.1)	<0.0001
31-50	3114 (33.6)	1549 (27.4)	
51-70	4286 (46.2)	2609 (46.2)	
>70	1359 (14.7)	1143 (20.3)	
Age at diagnosis in years			
Median (range)	55 (18-100)	58 (18-102)	<0.0001
Breslow thickness (mm)			
Median (range)	1.7 (0.2-62.0)	2.0 (0.3-47.0)	<0.0001
Breslow thickness in mm (n (%))			
≤1.0	1165 (12.6)	640 (11.3)	<0.0001
1.1 – 2.0	4440 (47.9)	2375 (42.1)	
2.1 – 4.0	2712 (29.2)	1739 (30.8)	
>4.0	955 (10.3)	866 (15.3)	
Mitoses (n (%))			
No	762 (8.2)	466 (8.3)	<0.0001
Yes	5667 (61.1)	4932 (87.4)	
Not known	2843 (30.7)	246 (4.4)	
Ulceration (n (%))			
No	6460 (74.3)	3798 (71.6)	0.0005
Yes	2229 (25.7)	1503 (28.4)	
Regression (n (%))			
Absent	3086 (33.3)	1743 (30.9)	<0.0001
Present	719 (7.8)	3094 (54.8)	
Not known	5467 (59.0)	807 (14.3)	

Table 1. (continued).

Characteristic	Dutch data (development) n = 9272	MIA data (validation) n = 5644	*p*-value
Number of positive SN (n (%))			
0	7166 (77.3)	4647 (82.4)	<0.0001
1	1489 (16.1)	677 (12.0)	
2	310 (3.3)	205 (3.6)	
≥3	92 (1.0)	115 (2.0)	
Missing (but positive SN)	215 (2.3)	0 (0)	
Primary tumor site (n (%))			
Arm	1393 (15.4)	1048 (18.6)	<0.0001
Head and Neck	561 (6.2)	952 (16.9)	
Leg	3000 (33.3)	1475 (26.1)	
Trunk	4064 (45.1)	2169 (38.4)	
Melanoma subtype (n (%))			
Superficial spreading	5870 (63.3)	2304 (40.8)	<0.0001
Nodular	2154 (23.2)	1805 (32.0)	
Lentigo maligna	85 (0.9)	104 (1.8)	
Acral lentiginous	153 (1.7)	110 (1.9)	
Desmoplastic	48 (0.5)	523 (10.8)	
Not known	962 (10.4)	798 (14.1)	
Median follow-up in years (IQR)	5.1 (3.3-7.7)	3.9 (1.7-7.1)	<0.0001

There is 6% missing for ulceration in both Dutch and MIA data.
There is 3% missing for anatomical site in Dutch data.
There is 0.4% missing for Breslow thickness in MIA data.
IQR, interquartile range; SN, sentinel node

Survival analyses

Both univariable analyses (supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2020.11.015) and multivariable Cox regression analyses (Table 2) showed that SN-positive patients had a substantially worse prognosis than SN-negative patients. On multivariable Cox analysis, the hazard ratios (HR) associated with SN-positivity were 2.7 [95% confidence interval (CI) 2.4-3.0] for OS and 2.9 (95% CI 2.6-3.2) for RFS in the Dutch (development) cohort. There was no violation of the proportional hazards assumption for any of the variables analyzed.

Table 2. Multivariable Cox regression analyses for OS and RFS in the Dutch development cohort.

		OS		RFS	
		Multivariable		Multivariable	
Variable	Class	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
SN status	Positive vs Negative	2.70 (2.42-3.00)	<0.0001	2.91 (2.63-3.23)	<0.0001
Breslow thickness	Per mm	1.07 (1.06-1.08)	<0.0001	1.06 (1.05-1.08)	<0.0001
Gender	Male vs Female	1.43 (1.28-1.60)	<0.0001	1.24 (1.12-1.38)	<0.0001
Age at diagnosis	Per year	1.03 (1.03-1.04)	<0.0001	1.01 (1.01-1.02)	<0.0001
Primary tumor site	Trunk vs Head and Neck	0.71 (0.59-0.86)	<0.0001	0.66 (0.54-0.80)	<0.0001
	Arm vs Head and Neck	0.60 (0.48-0.76)		0.46 (0.37-0.58)	
	Leg vs Head and Neck	0.57 (0.47-0.71)		0.73 (0.60-0.90)	
Mitosis	Yes vs No	1.19 (0.91-1.56)	0.3131	1.76 (1.34-2.30)	<0.0001
	Not known vs No	1.13 (0.86-1.49)		1.41 (1.06-1.87)	
Ulceration	Yes vs No	2.16 (1.94-2.41)	<0.0001	2.13 (1.92-2.36)	<0.0001
Regression	Present vs Absent	0.84 (0.66-1.08)	0.0351	0.83 (0.66-1.03)	0.217
	Not known vs Absent	1.11 (0.98-1.25)		0.95 (0.85-1.06)	
Melanoma subtype	NM vs SSM	1.16 (1.03-1.31)	0.0027	1.24 (1.10-1.39)	<0.0001
	LMM vs SSM	0.66 (0.34-1.28)		0.68 (0.35-1.33)	
	ALM vs SSM	1.45 (1.04-2.03)		1.96 (1.48-2.61)	
	DM vs SSM	1.30 (0.69-2.44)		1.40 (0.75-2.64)	
	Not known vs SSM	0.86 (0.71-1.04)		1.07 (0.90-1.27)	

ALM, acral lentiginous melanoma; CI, confidence interval; DM, desmoplastic melanoma; HR, hazard ratio; LMM, lentigo maligna melanoma; NM, nodular melanoma; SN, sentinel node; SSM, superficial spreading melanoma.

[a] The Cox regression results were based on 7687 patients. Patients with missing ulceration status (6%) or anatomical site (6%) were excluded. A 'not known' category was created for missing status for regression, mitoses and melanoma subtype as recommended by Johansson and Karlsson.[10] Multiple imputation was not considered, given the pathologists involved in this study believe these histopathological parameters are not missing at random, but rather because they were not seen during pathological assessment. The missing at random assumption (a condition for multiple imputation) would therefore be too strong.[37]

C-statistics

Breslow thickness had the highest C-statistic in both cohorts and for all three clinical outcomes (supplementary Figures S1 and S2, available at https://doi.org/10.1016/j.annonc.2020.11.015). Figure 1 depicts the C-statistics for the multivariable risk models for RFS and OS in the development and validation cohorts. For both cohorts, adding SN status to each of the three multivariable models (Breslow thickness only, Breslow thickness and ulceration, and all clinicopathological parameters) showed an increase in terms of discriminative performance for RFS and OS. Similar results were seen for MSS using MIA data (supplementary Figure S2, available at https://doi.org/10.1016/j.

annonc.2020.11.015). When combining all clinicopathological variables for OS using the development cohort, the C-statistic was 0.74 (95% CI 0.72-0.76) (supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2020.11.015). When SN status was added, the C-statistic improved to 0.78 (95% CI 0.76-0.79) (also depicted in Figure 1). For RFS, the C-statistic improved from 0.74 (95% CI 0.72-0.76) to 0.76 (95% CI 0.74-0.77) when SN status was included. Similar incremental improvements were found in the external validation: the C-statistic increased from 0.70 (95% CI 0.69-0.72) to 0.73 (95% CI 0.72-0.75) for OS and from 0.70 (95% CI 0.68-0.72) to 0.74 (95% CI 0.72-0.75) for RFS when SN status was included. As expected, the C-statistics from the validation cohort were all lower than the C-statistics of the development cohort because they were calculated using coefficients from the development cohort.16 For MSS, using only MIA data, the C-statistic increased from 0.72 (95% CI 0.69-0.74) to 0.76 (95% CI 0.74-0.78).

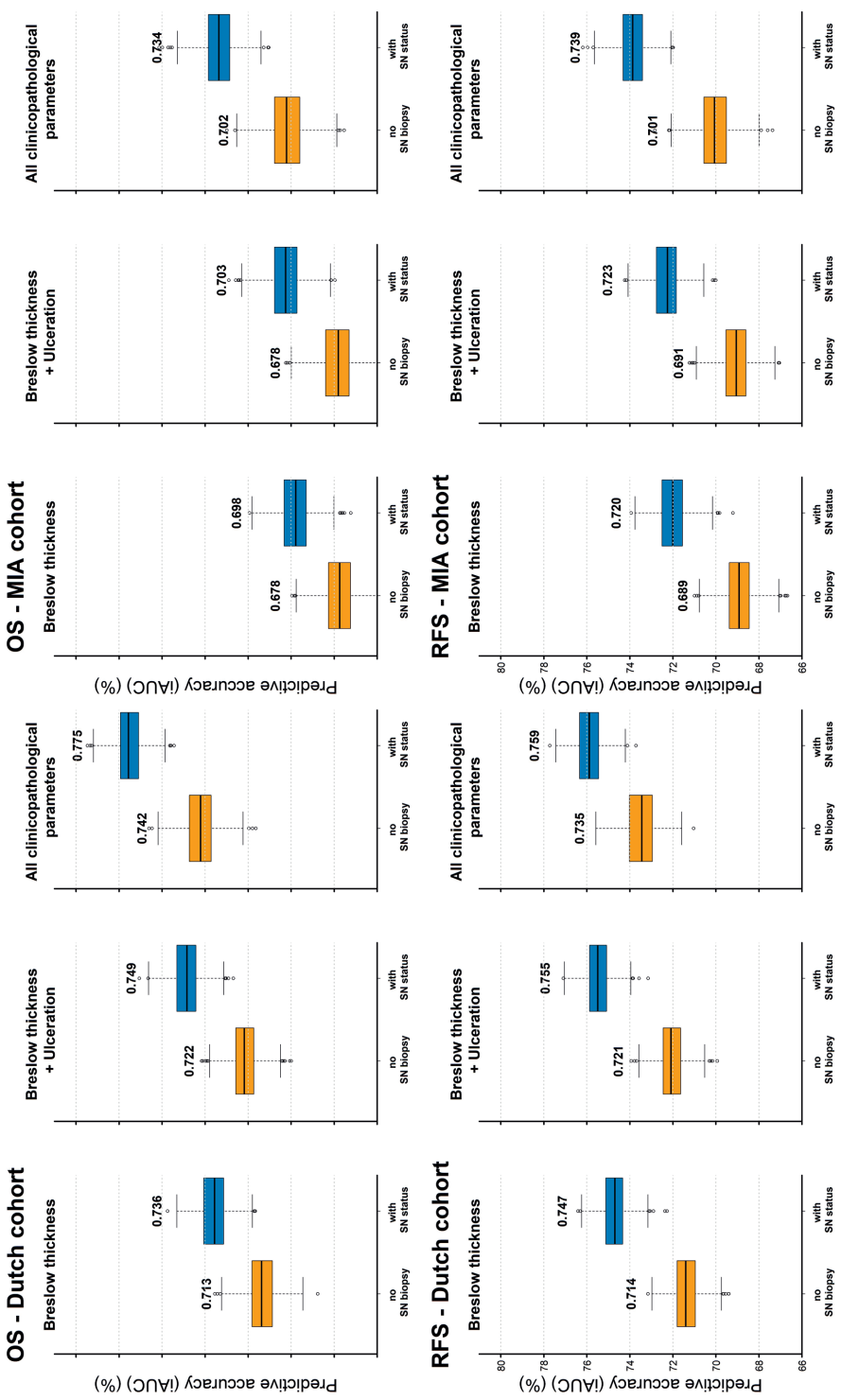
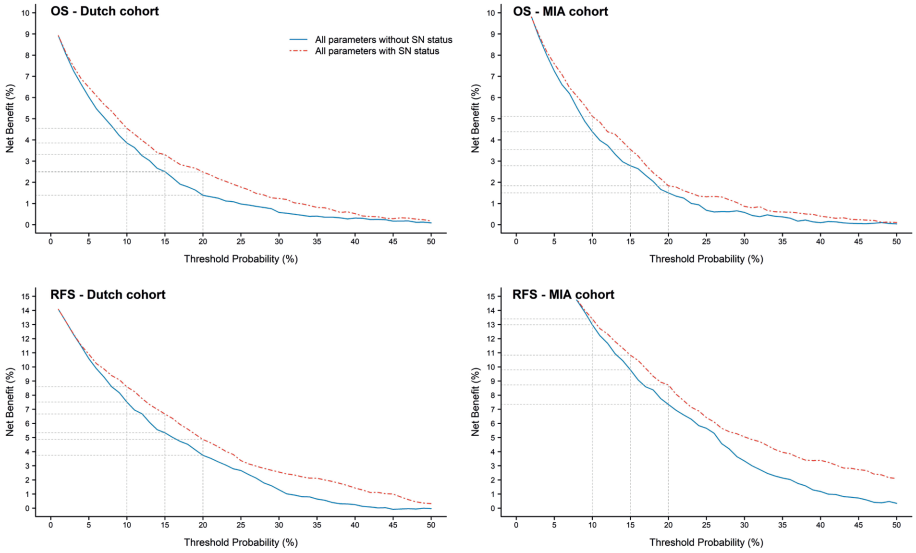


Figure 1. C-statistics for all six multivariable models for overall survival and recurrence free survival in the Dutch (development) and MIA dataset (validation). The C-statistic is equivalent to the area under the curve (AUC) and indicates the discriminative performance of the model, such that, the higher the C-statistic, the better the model separates patients who will have a recurrence (or will die) from those who will have no recurrence (or will not die).[12]

Decision analysis curves

Figure 2 depicts the decision analysis curves for 3-year survival outcomes. For all three clinical outcomes (RFS, OS and MSS), the multivariable model that included SN status outperformed the multivariable model without SN status across the full range of cut-off risks. Five-year decision curve analysis results, provided in supplementary Figure S3, available at https://doi.org/10.1016/j.annonc.2020.11.015, confirmed the 3-year results. Three-year and 5-year decision curve analyses of MSS showed similar results (supplementary Figure S4, available at https://doi.org/10.1016/j.annonc.2020.11.015).

Figure 2. Decision analysis curves for different 3-year survival outcomes for the Dutch cohort (development set) and MIA cohort (validation set).



The solid blue lines represent the estimated risk of experiencing any of the corresponding outcomes using a predictive model that included all clinicopathological factors derived from the primary tumor, excluding SN-status. The dashed red lines represent the estimated risk of experiencing any of the corresponding outcomes combining all clinicopathological variables with SN-status. The X-axes represent the threshold probabilities to define the rates at which a patient is considered as high risk of the corresponding outcome in the figure. The net benefits are represented on the Y-axes. The decision curve analyses provide no specific optimal threshold, but instead displays a wide range of thresholds. This allows clinicians to define a meaningful threshold for each individual patient as there is no threshold that is universally acceptable.[17] For example, if the threshold risk is set at 15% to define low and high risk categories for patients to experience recurrence within the first 3-years, the Dutch RFS data analysis (bottom left) provides a net benefit of 5.3% for the model without SN-status and 6.7% for the model with SN-status. The net benefit difference between the two models is therefore 1.4%, meaning that the model including SN-status detects 1.4 more net high-risk cases for every 100 patients who experienced recurrence within 3 years.

NRI

Table 3 presents an overview of NRI results using <5%, 5%-10% and >10% death or recurrence cut-off risks at 3 years and 5 years for RFS, MSS and OS for the Dutch and the MIA cohorts. Along with the overall NRI figures, the improvements in sensitivity and specificity are shown, expressed as the net percentages of individuals correctly reclassified as having worse or better survival outcomes. For all three survival outcomes, at 3 years and 5 years, the overall NRI improved. The overall NRI was uniformly driven by a higher sensitivity, i.e. patients who developed recurrence or who died were more accurately identified by the model as having a high-risk melanoma (lower predicted survival) when SN biopsy was included. Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2020.11.015, provides the raw numbers that were used to calculate the NRI values.

Table 3. Net reclassification index for all Dutch and MIA melanoma patients for 3-year and 5-year overall survival, recurrence-free survival and melanoma-specific survival for the model with all clinicopathological variables without sentinel node (SN) status, and for the model with all clinicopathological variables with SN status.

	Dutch cohort (development)		MIA cohort (validation)	
	Overall survival			
	3-year	**5-year**	**3- year**	**5-year**
Overall NRI	0.11	0.09	0.10	0.09
Sensitivity gain (%)	0.12	0.09	0.10	0.08
Specificity gain (%)	-0.01	0.00	0.00	0.00
	Recurrence-free survival			
	3-year	**5-year**	**3- year**	**5-year**
Overall NRI	0.15	0.11	0.15	0.12
Sensitivity gain (%)	0.12	0.09	0.06	0.03
Specificity gain (%)	0.03	0.03	0.09	0.09
	Melanoma-specific survival			
	3-year	**5-year**	**3- year**	**5-year**
Overall NRI	-	**-**	0.05	0.04
Sensitivity gain (%)	**-**	**-**	0.05	0.04
Specificity gain (%)	**-**	**-**	0.00	0.00

The sensitivity gain (also called NRI-event) presents the net percentage of persons with the event of interest (i.e. recurrence or death) correctly reclassified upward when SN status is added to the model; if positive this percentage reflects an improvement in sensitivity of the model including SN status. On the other hand, the specificity gain (also called NRI-nonevent) represents the net percentage of persons without the event of interest (i.e. no death or recurrence) correctly reclassified downward when SN status is added to the model; if positive, it reflects an improvement in specificity of the model with SN status over the model without SN status.[15] Overall NRI combines sensitivity and specificity; it is computed as $(P_{up,events} - P_{down,events}) - (P_{up,nonevents} - P_{down,nonevents})$, where an event refers to death (for survival outcomes) or recurrence (for recurrence-free survival). Pre-specified risk thresholds for the NRI assessment of recurrence or death events were <5%, 5%-10% and >10%.
NRI, net classification index.

DISCUSSION

It has been suggested, without supporting evidence, that knowledge of SN status in patients with cutaneous melanoma does not improve the accuracy of the prognostic estimate that can be obtained from standard clinicopathological assessment of the primary tumor, including its Breslow thickness and ulceration status.[5, 6, 7, 8] The results of this study involving 9272 Dutch and 5644 MIA patients, of whom 2106 and 997, respectively, had positive SN biopsies, clearly show that SN status did provide significant additional prognostic information, even when eight other important clinicopathological predictors were included. This finding was consistent across several different measures of discrimination and, importantly, was closely replicated in an independent dataset from another country in which patient characteristics differed.

The current results are in line with those of Mitra et al., the only other study that has calculated a C-statistic for the same research question.[6] They analyzed 561 patients with melanomas ≥0.75 mm in Breslow thickness (286 with a positive SN) in the UK. Their model included Breslow thickness, mitotic rate, ulceration, vascular invasion, site, age and sex. The AUC increased from 0.70 to 0.74 for OS when SN status was added.

In the current study, the C-statistic in the two independent patient cohorts increased by 3% for OS when SN status was included in the model. For RFS, there was an increase of 2% in the Dutch cohort and a 4% increase in the MIA cohort when SN status was included. A 4% increase for MSS was seen in the MIA cohort. Whether the improvements in prognostic accuracy provided by SN biopsy justify the cost and potential morbidity associated with this procedure requires further evaluation. Nevertheless, an absolute gain in the C-statistic of 3%-4% is considered by statisticians to be a substantial gain in predictive ability, especially when other important predictors are already incorporated in the model.[16] Improvement in the AUC depends strongly on the strength of the baseline model; for example, a binary predictor can increase a poor model (AUC 0.60) by 13%, whereas the same predictor will increase an excellent model (AUC 0.85) by only 3%.[16] Another striking feature of the AUC analyses in the present study was that the model combining only Breslow thickness and SN status outperformed the model including all other clinicopathological factors (without SN status) for predicting RFS and MSS. The contribution of age was most striking in the OS model (HR in both cohorts 0.64) and less in the RFS and MSS models (HRs in both cohorts and for both outcomes 0.58). This may be related to other competing risks of (non-melanoma) death. Although we found consistent results regarding the additional prognostic information of SN status, the SN-positivity rate was 22.7% in the Dutch cohort and 17.6% in the MIA cohort. The median Breslow thickness was 1.7 mm in the Dutch cohort and 2.0 mm in the MIA cohort. A possible explanation for these differences could be the higher percentage of desmoplastic melanomas in the MIA

cohort (10.8% versus 0.5% in the Dutch cohort). Desmoplastic melanomas are known to have a greater Breslow thickness at diagnosis compared with other melanoma subtypes, and are less frequently SN-positive.[17,18]

To our knowledge, others have not assessed the NRI and carried out decision curve analysis for the use of SN biopsy as a predictor of outcome in melanoma patients. These are complementary measures of predictive performance and clinical utility. For all three survival outcomes, the overall NRI improved. It was apparent that the NRI gain was driven mainly by the correct reclassification of patients who developed a recurrence or died as having a high-risk melanoma (3-year improvement in sensitivity of 12% for OS and 12% for RFS in the Dutch cohort, and 10% for OS and 6% for RFS in the MIA cohort). This finding indicates that patients at higher risk of recurrence or death were more accurately identified by the model when SN biopsy was included. This improvement in the net classification of people at higher risk of recurrence or death could contribute to decreasing the burden of advanced disease by enabling clinicians to consider more intensive follow-up and adjuvant systemic therapy to potentially prevent death from melanoma.[15]

Decision curve analyses were carried out to see if a model improved clinical decision making. When comparing two risk models, the one with the highest net benefit for any specified threshold should lead to better clinical decisions. A net benefit increase was observed across all threshold probabilities for all three survival outcomes and in the two independent cohorts.[15] This indicated that patients were more accurately identified as having a high-risk melanoma when SN status was included in the model compared to a model without SN status.[19] From a health economic point of view, the net benefit of 1.4% difference at a 15% threshold for RFS in the Dutch cohort is particularly important at the population level, where the net benefit is 140 per 10 000 patients and where the cost of treating advanced disease represents a large economic burden.[20]

Clinical implications

That knowledge of SN status provides more accurate prognostic and staging information than clinicopathological features of a primary melanoma alone is clearly demonstrated in the present study. However, although it is clear-cut, clinicians and patients will have to decide whether its value outweighs the cost and potential morbidity of the SN biopsy. The cost-effectiveness of SN biopsy has been evaluated in two studies, with contradictory results reported.[21,22] Side-effects of SN biopsy such as infection, seroma and lymphedema have been evaluated in a recent meta-analysis, showing an overall complication rate of 11.3%.[23] However, the majority of complications are minor and self-limiting.[23] To an individual newly diagnosed with melanoma, knowledge of SN status has been shown to have a positive psychological benefit, regardless of its result.[24]

Regulatory approval and funding of adjuvant systemic therapy for patients with stage IIIA patients currently differs from country to country. In most, adjuvant drug therapies are only approved or reimbursed for stage IIIA patients with SN metastases >1 mm in maximum dimension, while patients with resected stage IIIB, IIIC and IIID disease, many staged as such by SN biopsy, are generally eligible for adjuvant targeted or immune therapy.[25] It has been claimed that the use of SN status alone to accept and stratify patients into clinical trials or to receive adjuvant systemic treatment is "not rational".[5] However, other methods of selecting high-risk patients are limited. At present, only approximately 20% of patients who have an SN biopsy are found to be SN-positive. Most current melanoma management guidelines recommend that SN biopsy should be considered in all patients with cutaneous melanomas ≥1 mm in thickness and in patients with thinner tumors that have high-risk features.[26,27] Given the small improvement in the accuracy of prognosis with the addition of SN biopsy to standard clinicopathological parameters, our data challenge the appropriateness of these recommendations. Tools that predict SN-positivity are now available and can improve patient selection, particularly by indicating those who are very unlikely to be SN-positive and in whom SN biopsy can reasonably be omitted.[28] Biomarkers based on gene expression profiling that predict outcome are also available, but their accuracy has not been shown to be better than that provided by SN biopsy and reliable validation of their predictive ability is in any case still required.[29, 30, 31] Until these methods of predicting or detecting nodal metastasis are validated, SN biopsy remains the best method of identifying actual lymph node metastases, predicting those patients who may benefit from adjuvant systemic therapy and those higher-risk patients who may be eligible for enrollment in clinical trials of systemic therapy.

Melanomas with a high Breslow thickness may be considered for adjuvant therapy irrespective of SN status, as these patients have a poor prognosis comparable to that of patients with thinner melanomas who are SN-positive.[1,32] As well, trials of adjuvant systemic therapies for patients with stage II melanomas are currently in progress, based on Breslow thickness and ulceration, and will demonstrate whether these patients derive as much benefit from adjuvant therapy as patients with a positive SN biopsy.[33,34] Selecting only the most appropriate patients for early adjuvant systemic therapy according to their SN status is of great importance, considering the relatively frequent and occasionally fatal side-effects and the costs of adjuvant systemic treatment.[35] Nor can the therapeutic value of SN biopsy in achieving regional node control be overlooked, with node field recurrence common if an SN biopsy procedure is not carried out.[4] This benefit will not be achieved by predicting the likelihood of SN-positivity using a nomogram or gene expression profiling, no matter how accurately. If positive SNs are not removed, therapeutic node dissection at a later date will usually be required, at a cost of potentially increased morbidity.

Strengths of our study are the large sample sizes from two independent datasets across two countries, which greatly increases the generalizability of the results. Also, in both cohorts, we had access to long-term and near-complete follow-up. Another strength is the external validation of our model and the assessment of multiple measures of predictive performance to indicate the true additional value of SN status. A limitation of the study is that criteria for SN biopsy may have changed over time, with the possibility of selection bias, although the consistency of the results in the two cohorts suggests that minimal bias was introduced. A limitation is that because the information was not available in the dataset, we were not able to account for the size and localization of SN metastases, which has been shown to impact prognosis.[36] The mitotic rate as a count was not recorded in the Dutch dataset, a limitation preventing exploration of its effect, however, separate analysis of the MIA dataset including mitosis as a count showed that the incremental value of SN status remained a prognostic parameter (data not shown). Another limitation is that pre-specified risk thresholds were used for the NRI calculations. This is an inherent limitation of the NRI, and the choice of these pre-specified risk thresholds influences the NRI result.[15] However, given the lack of consensus in defining appropriate risk thresholds in melanoma management, we chose what we believed were reasonable thresholds. Future studies should focus on formal cost-effectiveness analyses using health economic methodology, accounting for both long-term savings and local health system costs of the SN biopsy procedure.[11,16]

CONCLUSIONS

Knowledge of SN status provides additional staging and prognostic accuracy over and above traditional clinicopathological characteristics of a primary melanoma. A particular benefit is more reliable identification of patients at higher risk of recurrence and death, which has obvious implications for selection of patients likely to benefit from adjuvant systemic therapy. However, bearing in mind the magnitude of the improvements in accuracy, clinicians and patients must weigh the advantages of determining actual SN status against the cost and potential morbidity of SN biopsy.

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Supplementary Table 1. Clinicopathological factors comparison between positive SN versus negative SN patients for Dutch and MIA patients.

	Dutch cohort (development)			MIA cohort (validation)		
Characteristic	**Negative SN n = 7166**	**Positive SN n = 2106**	***p*-value**	**Negative SN n = 4647**	**Positive SN n = 997**	***p*-value**
Gender (n (%))						0.12
Female	3796 (53.0)	939 (44.6)	<0.0001	1918 (41.3)	385 (38.6)	
Male	3370 (47.0)	1167 (55.4)		2729 (58.7)	612 (61.4)	
Age at diagnosis in years						
Median (range)	55.0 (18-94)	55.0 (18-100)	0.10	59 (18-102)	55 (18-93)	<0.0001
Breslow thickness (mm)			<0.0001			
Median (range)	1.6 (0.2-62)	2.4 (0.4-38)		1.8 (0.3-47)	2.6 (0.5-28)	<0.0001
Mitoses (n (%))			<0.0001			<0.0001
No	679 (9.5)	83 (3.9)		436 (9.4)	30 (3.0)	
Yes	4261 (59.5)	1406 (66.8)		3993 (85.9)	939 (94.2)	
Not known	2226 (31.1)	617 (20.3)		218 (4.7)	28 (2.8)	
Ulceration (n (%))			<0.0001			<0.0001
No	5217 (77.8)	1243 (62.6)		3231 (69.5)	567 (56.9)	
Yes	1485 (22.2)	744 (37.4)		1112 (23.9)	391 (30.2)	
Regression (n (%))			<0.0001			<0.0001
Absent	2346 (32.7)	740 (35.1)		1355 (29.2)	388 (38.9)	
Present	625 (8.7)	94 (4.5)		2585 (55.6)	509 (51.1)	
Not known	4195 (58.5)	1272 (60.4)		707 (15.2)	100 (10.0)	
Primary tumor site (n (%))			<0.0001			<0.0001
Arm	1175 (16.8)	218 (10.7)		941 (20.3)	107 (10.7)	
Head and Neck	447 (6.4)	114 (5.6)		794 (17.1)	158 (15.9)	
Leg	2297 (32.9)	703 (34.4)		1161 (25.0)	314 (31.5)	
Trunk	3056 (43.8)	1008 (49.3)		1751 (37.7)	418 (41.9)	

Supplementary Table 1. (continued)

	Dutch cohort (development)			MIA cohort (validation)		
Characteristic	**Negative SN n = 7166**	**Positive SN n = 2106**	***p*-value**	**Negative SN n = 4647**	**Positive SN n = 997**	***p*-value**
Melanoma subtype (n (%))			<0.0001			<0.0001
Superficial spreading	4671 (65.2)	1199 (56.9)		1871 (40.3)	433 (43.3)	
Nodular	1524 (21.3)	630 (29.9)		1439 (31.0)	366 (36.7)	
Lentigo maligna	80 (1.1)	5 (0.2)		95 (2.0)	9 (0.9)	
Acral lentiginous	99 (1.4)	54 (2.2)		66 (1.4)	44 (4.4)	
Desmoplastic	43 (0.6)	5 (0.2)		486 (10.5)	37 (3.7)	
Not known	749 (10.5)	213 (10.1)		690 (14.9)	108 (10.8)	
Median follow-up in years (IQR)	5.3 (3.5-7.9)	4.4 (2.6-6.9)	<0.0001	3.9 (1.7-7.2)	3.9 (1.9-6.9)	0.55

**There is 6% missing for ulceration in both Dutch and MIA data.
**There is 3% missing for anatomical site in Dutch data.
** There is 0.4% missing for Breslow thickness in MIA data.

Supplementary Table 2. Univariable HRs for OS and RFS for Dutch patients (development cohort). SSM = Superficial spreading melanoma, NM = Nodular melanoma, LMM = Lentigo maligna melanoma, ALM = Acral lentiginous melanoma, DM = Desmoplastic melanoma

		OS		RFS	
		Univariable		Univariable	
Variable	Class	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
SN-status	Positive vs Negative	3.13 (2.84-3.46)	<0.0001	3.56 (3.24-3.91)	<0.0001
Breslow thickness	Per mm	1.08 (1.07-1.09)	<0.0001	1.08 (1.07-1.09)	<0.0001
Gender	Male vs Female	1.79 (1.62-1.98)	<0.0001	1.47 (1.34-1.61)	<0.0001
Age at diagnosis	Per year	1.04 (1.04-1.04)	<0.0001	1.02 (1.02-1.02)	<0.0001
Primary tumor site	Trunk vs Head and Neck	0.70 (0.58-0.84)	<0.0001	0.69 (0.58-0.83)	<0.0001
	Arm vs Head and Neck	0.56 (0.45-0.69)		0.45 (0.36-0.56)	
	Leg vs Head and Neck	0.52 (0.43-0.63)		0.72 (0.60-0.86)	
Mitosis	Yes vs No	2.12 (1.65-2.74)	<0.0001	2.69 (2.09-3.46)	<0.0001
	Not known vs No	1.81 (1.40-2.35)		1.95 (1.50-2.54)	
Ulceration	Yes vs No	3.09 (2.79-3.42)	<0.0001	2.98 (2.70-3.28)	<0.0001
Regression	Present vs Absent	0.67 (0.53-0.85)	<0.0001	0.58 (0.47-0.73)	<0.0001
	Not known vs Absent	1.13 (1.01-1.27)		0.95 (0.86-1.05)	
Melanoma subtype	NM vs SSM	1.88 (1.69-2.10)	<0.0001	1.88 (1.69-2.08)	<0.0001
	LMM vs SSM	1.03 (0.54-2.00)		0.84 (0.43-1.61)	
	ALM vs SSM	2.20 (1.61-3.01)		2.88 (2.20-3.77)	
	DM vs SSM	1.93 (1.06-3.50)		1.68 (0.93-3.05)	
	Not known vs SSM	1.19 (1.01-1.40)		1.32 (1.13-1.53)	

The Cox regression results were based on 7687 patients. Patients with missing ulceration status (6%) or anatomical site (6%) were excluded. A "not known" category was created for missing status for regression, mitoses and melanoma subtype as recommended by Johansson and Karlsson.[10] Multiple imputation was not considered, given the pathologists involved in this study believe these histopathological parameters are not missing at random, but rather because they were not seen during pathological assessment. The missing at random assumption (a condition for multiple imputation) would therefore be too strong.[37]

Supplementary Table 3. Overview of C-statistics for OS, RFS and MSS for the Dutch and MIA cohort for the model with all clinicopathological variables without SN-status, and for the model with all clinicopathological variables with SN-status. The C-statistic is equivalent to the area under the curve (AUC) and indicates the discriminative performance of the model, such that, the higher the C-statistic, the better the model separates patients who will have a recurrence (or will die) from those who will have no recurrence (or will not die).[14]

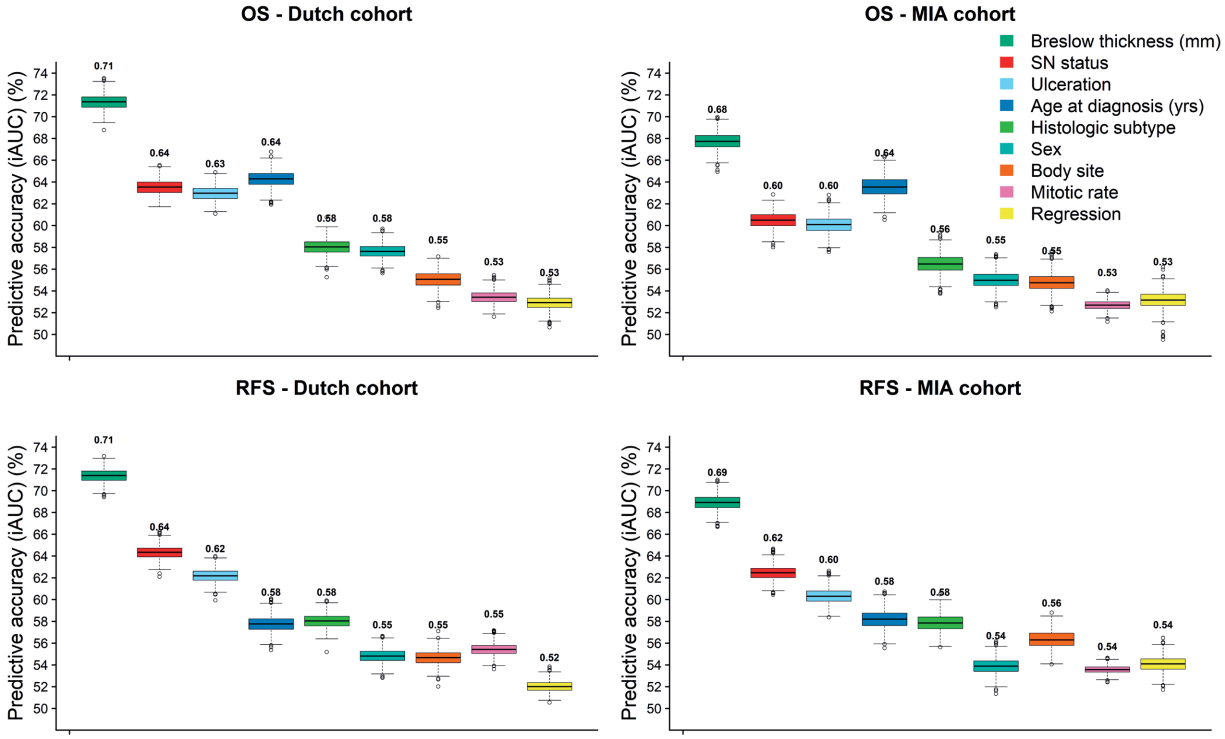
	Dutch cohort (development)	MIA cohort (validation)
	Overall survival (95% CI)	
Without SN-status	0.74 (0.72-0.76)	0.70 (0.69-0.72)
With SN-status	0.78 (0.76-0.79)	0.73 (0.72-0.75)
	Recurrence-free survival (95% CI)	
Without SN-status	0.74 (0.72-0.76)	0.70 (0.68-0.72)
With SN-status	0.76 (0.74-0.77)	0.74 (0.72-0.75)
	Melanoma-specific survival (95% CI)	
Without SN-status	-	0.72 (0.69-0.74)
With SN-status	-	0.76 (0.74-0.78)

Supplementary Table 4. Reclassification of all melanoma patients using the model with SN-status over the model without. Numbers in orange represent patients with a decreased risk of recurrence or death (i.e. higher survival) when the model with SN-status was used, numbers in blue represent patients with an increased risk of recurrence or death (i.e. lower survival) predicted by the model including SN-status.

		Model with all covariates excluding SN-status											
		Dutch cohort (development)						MIA cohort (validation)					
		Event			No event			Event			No event		
Model with all covariates including SN-status	Outcomes by event rates	<5%	5% to 10%	≥10%	<5%	5% to 10%	≥10%	<5%	5% to 10%	≥10%	<5%	5% to 10%	≥10%
	3-year OS												
	<5%	529	48	0	7175	170	0	354	26	0	4725	125	0
	5% to 10%	105	44	13	177	65	6	58	29	6	123	75	27
	≥10%	0	50	8	1	37	8	0	24	11	0	32	5
	5-year OS												
	<5%	865	78	0	6839	140	0	620	35	0	4459	116	0
	5% to 10%	143	51	14	139	58	5	84	41	14	97	63	19
	≥10%	1	63	9	0	24	7	0	35	12	0	21	4
	3-year RFS												
	<5%	238	124	0	5015	833	0	194	107	0	2752	553	0
	5% to 10%	193	96	120	662	478	393	110	113	86	248	408	280
	≥10%	6	190	239	7	342	336	8	129	221	16	139	256
	5-year RFS												
	<5%	358	174	0	4895	783	0	284	145	0	2662	515	0
	5% to 10%	231	133	154	624	441	359	133	141	105	225	380	261
	≥10%	7	223	269	6	309	306	8	147	237	16	121	240
	3-year MSS												
	<5%	-	-	-	-	-	-	361	2	0	5206	2	0
	5% to 10%	-	-	-	-	-	-	22	1	0	20	3	1
	≥10%	-	-	-	-	-	-	0	1	0	0	0	1
	5-year MSS												
	<5%	-	-	-	-	-	-	595	3	0	4972	1	0
	5% to 10%	-	-	-	-	-	-	29	3	0	13	1	1
	≥10%	-	-	-	-	-	-	0	1	0	0	0	1

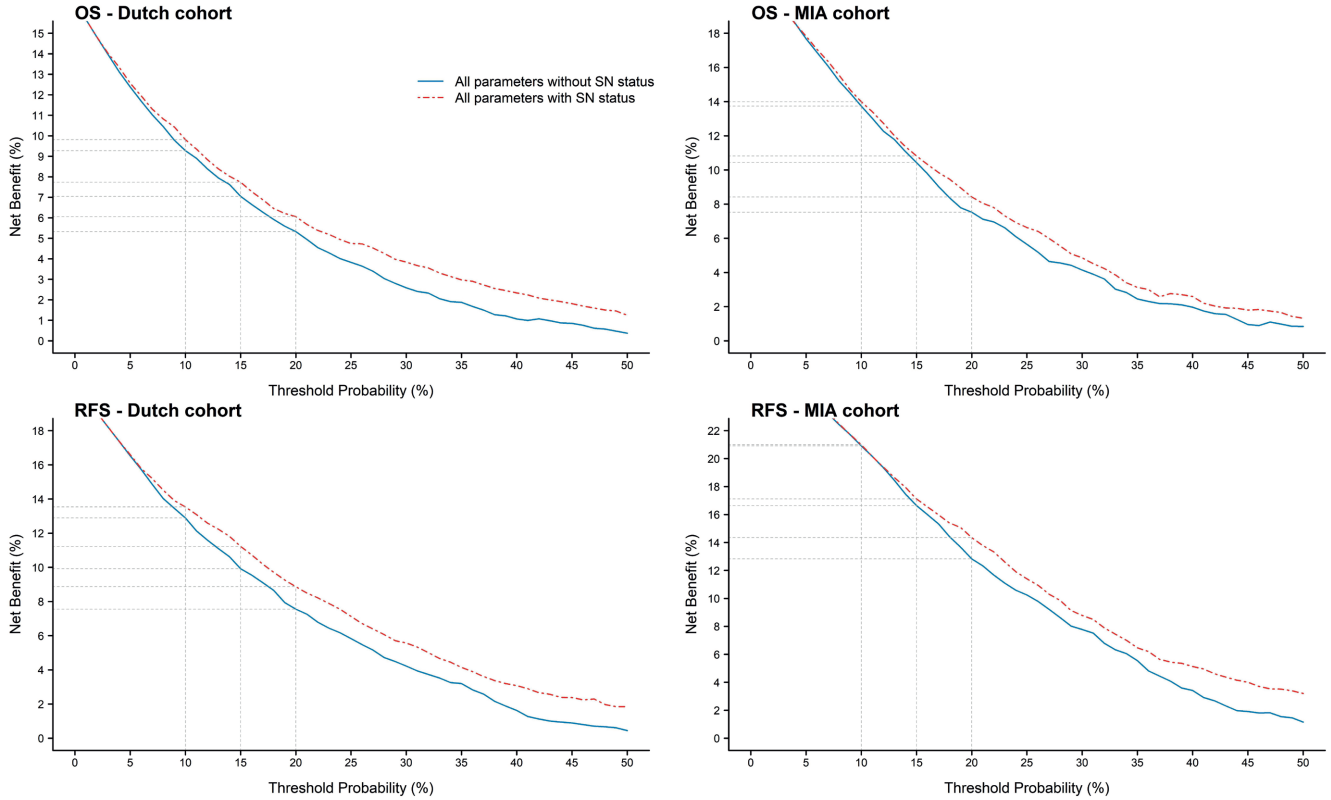
The sum of the net percentages of correctly reclassified persons with and without the event of interest (death or recurrence) represent the overall indicator of the model performance. As an example, among patients who experienced recurrence within the first 3-year after diagnosis in the Dutch cohort, 389 (193 + 6 + 190) had higher event risk estimates (improved classification of a high-risk melanoma) using the model with SN-status and 244 (124 + 0 + 120) had lower event risk estimates (worse classification of a high-risk melanoma), therefore the gain in reclassification of high risk patients of recurrence is 145 (389 - 244) patients, out of 1206 patients. Similarly, 1226 (833 + 0 + 393) patients were reclassified down (lower risk estimates) and 1011 (662 + 7 + 342) were reclassified up (higher risk). This translated into a reclassification gain of 215 (1226 - 1011) patients, out of 8066 patients. The net gain of reclassification was then 360 (145 + 215) or 4% of the 9272 Dutch patients. In our analysis, improvement in sensitivity (upward reclassification of people who had a lower predicted survival) indicates that SN-biopsy aids in identifying people with a higher risk of recurrence or death, over and above the other factors in the model. This enables clinicians to offer adjuvant systemic treatment and potentially prevent death from melanoma. On the other hand, improvement in specificity (downward reclassification of people who had a higher predicted survival) indicates that SN-biopsy aids in identifying people with a lower risk of recurrence or death. This could reduce overtreatment and anxiety, but will have a limited contribution to decreasing the burden of disease.[15] As an example, for a specific patient, the model without SN status can indicate a risk of experiencing a recurrence with the first 3 years after diagnosis of 11%. However, if that patients had a known SN status his or her risk of experiencing a recurrence would be much clearer: if the patient would have a positive SN biopsy, his or her risk would increase to 25%, whilst if he or she had a negative SN biopsy, the risk would decrease to 6%.

Supplementary Figure 1. C-statistics for all univariable models for overall survival and recurrence free survival in the Dutch (development) and MIA dataset (validation). The C-statistic is equivalent to the area under the curve (AUC) and indicates the discriminative performance of the model, such that, the higher the C-statistic, the better the model separates patients who will have a recurrence (or will die) from those who will have no recurrence (or will not die).[14]

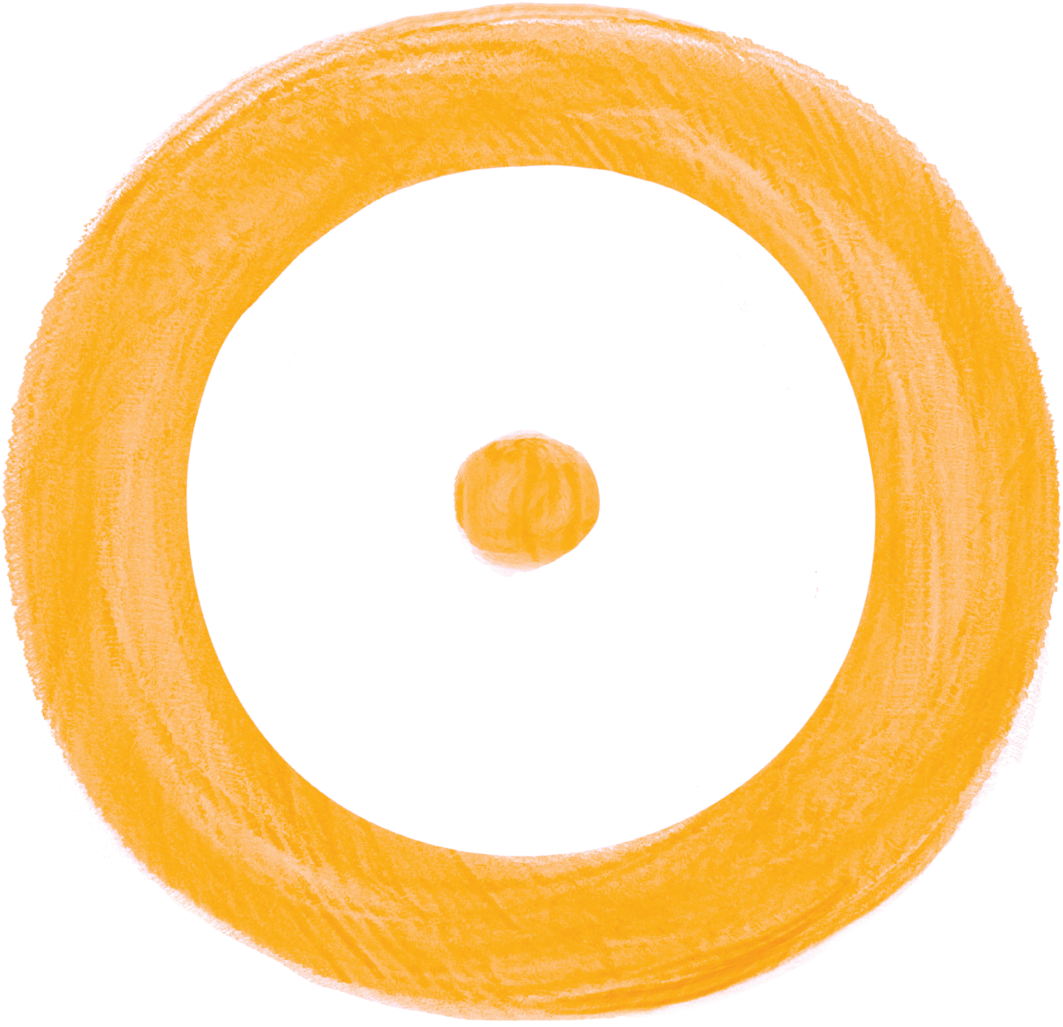


Supplementary Figure 2. Multivariable and univariable C-statistics for melanoma specific survival for the MIA dataset. The C-statistic is equivalent to the area under the curve (AUC) and indicates the discriminative performance of the model, such that, the higher the C-statistic, the better the model separates patients who will have a recurrence (or will die) from those who will have no recurrence (or will not die).[14]

Supplementary Figure 3. Decision analysis curves for different 5-year survival outcomes for the Dutch cohort (development set) and MIA cohort (validation set). The solid blue lines represent the estimated risk of experiencing any of the corresponding outcomes using a predictive model that included all clinicopathological factors derived from the primary tumor, excluding SN-status. The dashed red lines represent the estimated risk of experiencing any of the corresponding outcomes combining all clinicopathological variables with SN-status. The X-axes represent the threshold probabilities to define the rates at which a patient is considered as high risk of the corresponding outcome in the figure. The net benefits are represented on the Y-axes. The decision curve analyses provide no specific optimal threshold, but instead displays a wide range of thresholds. This allows clinicians to define a meaningful threshold for each individual patient as there is no threshold that is universally acceptable.[19]



Supplementary Figure 4. Decision analyses curves for 3-year and 5-year melanoma specific survival for the MIA dataset. The solid blue lines represent the estimated risk of experiencing any of the corresponding outcomes using a predictive model that included all clinicopathological factors derived from the primary tumor, excluding SN-status. The dashed red lines represent the estimated risk of experiencing any of the corresponding outcomes combining all clinicopathological variables with SN-status. The X-axes represent the threshold probabilities to define the rates at which a patient is considered as high risk of the corresponding outcome in the figure. The net benefits are represented on the Y-axes. The decision curve analyses provide no specific optimal threshold, but instead displays a wide range of thresholds. This allows clinicians to define a meaningful threshold for each individual patient as there is no threshold that is universally acceptable.[19]



CHAPTER 5

High discordance rate in assessing sentinel node positivity in cutaneous melanoma: expert review may reduce unjustified adjuvant treatment

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ABSTRACT

Introduction: Identification of sentinel node (SN) metastases can set the adjuvant systemic therapy indication for patients with stage III melanoma. Studies re-evaluating the diagnosis of initially positive SN biopsies are scarce.

Methods: Dutch patients with melanoma who underwent SN biopsy between 2003 and 2014 were selected from PALGA, the Dutch Pathology Registry. Histopathological slides of SN-positive patients were retrieved for review. A random sample was reassessed by an expert melanoma pathologist. Recurrence-free survival (RFS) of patients who were misclassified (false-positive) was compared with those with a true positive SN status. For comparison, a group of SN-negative patients was included. Multivariable logistic analysis was performed to assess clinicopathological characteristics associated with misclassification of SN status.

Results: Diagnosis was downgraded from melanoma metastasis to nodal nevus in 38 of the 322 reviewed patients (11.8%). Considering the inclusion criteria of phase III adjuvant trials, at least 4.3% of patients would have falsely qualified for adjuvant therapy. In multivariable analysis, patients with a low SN tumour burden and subcapsular SN tumour location had a significantly higher chance of being misclassified. The five-year RFS of the 38 downgraded patients was 86.7% (95% confidence interval [CI] = 72.6–96.6), similar to the 85.9% (95% CI = 84.9–86.8, p = 0.18) for 6413 SN-negative patients and better than the 53.2% (95% CI = 47.2–59.9, p = 0.009) of 284 patients who were truly SN positive upon review.

Conclusions: More than 10% of originally positive SN biopsies of patients with melanoma concern misclassified nodal nevi. We advocate that when adjuvant treatment is considered in patients with stage III melanoma, SN biopsies should be reassessed by an expert melanoma pathologist.

INTRODUCTION

Sentinel node (SN) biopsy is an important part of routine staging for patients with clinically localised melanoma[1]. The identification of SN metastases significantly impacts clinical practice as it implicates worse survival[2] and nowadays sets the indication for adjuvant systemic therapies for patients with stage III disease[3]. In line with the inclusion criteria of the phase III adjuvant trials[4-6], for a subset of these patients (stage IIIA as per the 7th edition of the American Joint Committee on Cancer [AJCC] melanoma staging system), a threshold of >1.0 mm SN tumour burden is applicable for the indication for adjuvant systemic therapy[7]. Multiple studies have shown that pathological re-evaluation of initially negative SN biopsies by immunohistochemistry and serial sectioning may detect deposits of occult melanoma cells[8-15]. However, only one study seems to have systematically re-evaluated 'initially positive' SNs[16], while these are the patients who nowadays could be at risk of unjustified exposure to the severe and potentially fatal side-effects of adjuvant systemic treatment in case of a false-positive SN diagnosis[17]. Moreover, by identifying patients with a false-positive SN diagnosis, the high costs of these systemic therapies can be avoided[18]. Therefore, adequate assessment of SN positivity is crucial. The goal of the present study was to review SN biopsies of patients with melanoma that were initially diagnosed positive for melanoma metastases and determine concordance when reassessed by a dedicated melanoma pathologist. In addition, clinicopathological characteristics associated with misdiagnosis were assessed, and survival of patients who were initially misdiagnosed as having stage III disease upon review was compared with that of SN-negative patients and truly SN-positive patients.

METHODS

Collection of data

Data for this retrospective nationwide study were obtained from PALGA, the Dutch Nationwide Network and Registry of Histopathology and Cytopathology. Since 1991, PALGA has prospectively been collecting data from all pathology laboratories in the Netherlands[19]. All data were encoded and used anonymously. Ethical approval was granted by the board of PALGA.

Study population

Pathology reports of all newly diagnosed patients with invasive melanoma in the Netherlands between January 2003 and December 2014 for whom SN biopsy was performed were analysed. Patients presenting with stage III locoregional metastases (defined as in-transit, satellite or lymph node metastases other than in the SN biopsy) or stage IV disease (distant metastases) within 12 weeks of initial diagnosis were excluded. Patients with multiple primary melanoma, non-cutaneous melanoma, desmoplastic melanoma, microsatellites and melanomas occurring in children (age <18 years), were also excluded. For each patient, clinical and pathological variables were extracted from the pathology files, including the date of diagnosis, age, gender, Breslow thickness (in millimetre), presence of ulceration, melanoma subtype, anatomical localisation, recurrence (date, site and type, skin—local or in-transit, in regional nodes or at a distant site), SN status and the number of positive SN biopsies. Patients were classified as per the 7th AJCC melanoma staging system because this version was used in the randomised controlled trials studying the efficacy of adjuvant systemic therapy in patients with stage III melanoma[4-6].

For all positive SN biopsies, all anonymously coded histopathological slides (haematoxylin and eosin [H&E] and all available immunochemical staining) were requested at each individual hospital and/or pathology laboratory in the Netherlands for review (n = 26 pathology laboratories, n = 1279 cases). Not all patients were reassessed because of efficiency and time reasons. Only 8 pathology laboratories did not have their slides available for review, for reasons that they did not have time or personnel to retrieve their slides: they did not respond to our invite on multiple occasions, or there were additional costs that we could not account for. For the present study, of the 26 packages with slides that we received, we randomly selected all cases from one pathology laboratory and all cases from the consecutive four pathology laboratories that we received thereafter (n = 322 from 5 pathology laboratories; two academic and three non-academic). These 322 cases were reviewed by a dedicated European Organisation for Research and Treatment of Cancer (EORTC) melanoma pathologist (W.A.M.B.), and the first two authors (M.-A.E.S. and A.E.L.), to review the diagnosis and to assess tumour burden (in mm) and localisation (subcapsular and non-subcapsular). Tumour burden was defined as the single measurement of the

maximum diameter of the largest lesion in any direction, as per the EORTC protocol[20]. SN-negative patients were included to compare survival, and their pathology reports were analysed to see if nodal nevi were present. A nodal nevus was defined as a collection of non-atypical nevoid melanocytes located within the capsule, sometimes extending within the septa or trabecula of the capsule deeper within the lymph node. Melanoma metastasis was defined by the presence of morphological atypical melanocytes present within the subcapsular region or parenchyma of a lymph node. Patients with both subcapsular and non-subcapsular SN tumour deposits were classified as non-subcapsular. The primary melanoma was not available for comparison. We had access to the slides analysed in the initial evaluation of the SN biopsy (mostly H&E, S100 and Melan-A or MART staining). No PRAME, p16, HMB45 or Ki-67 staining was available.

To check if the downstaging to nodal nevus was justified, recurrence-free survival (RFS) was compared between patients with an initially positive SN status who were downgraded to a nodal nevus, those with a persistent positive SN status upon review, SN-negative patients and SN-negative patients with a nodal nevus. In patients with multisite first recurrences, the site associated with the worst prognosis was scored as the first site. RFS was calculated from the date of initial melanoma diagnosis to the date of diagnosis of recurrence. Patients without recurrence were censored at either their date of death or the last date known alive or 1st January 2018 (the data collection cut-off date), whichever occurred earlier.

Statistical analysis

Categorical variables were summarised as numbers and percentages. Continuous variables were summarised as median with interquartile range for non-normally distributed data or mean with standard deviation for normally distributed data. Differences in proportions and medians were analysed using chi-square tests or the Mann-Whitney U test, respectively. Differences in means were assessed using Student's t-test. Kaplan-Meier curves were generated to compare RFS using paired log-rank tests among SN-negative patients, SN-positive patients, patients with a nodal nevus upon review and all SN-negative patients with a nodal nevus reported in their histology report. A logistic regression analysis was performed for all reviewed patients to assess which variables predicted a downgraded diagnosis. The model included Breslow thickness, tumour burden, gender, age, Dewar localisation of tumour burden, ulceration status, anatomical location and melanoma subtype. A 'not known' category was created for missing status for ulceration and anatomical location.

Data were analysed using R, version 3.6.1, and SPSS, version 26. A two-sided p-value of <0.05 was considered statistically significant.

RESULTS

Recruitment and review

A random sample of 322 slides of sufficient quality was reviewed (Supplementary Fig. 1). There were no statistically significant differences in clinicopathological variables between the reviewed and non-reviewed cases, except for localisation of the melanoma (Table 1). In 287 of the 322 (89.1%) reviewed cases, an additional S100 and/or Melan-A staining was available besides H&E staining.

Table 1. Baseline clinicopathological data of pathologically reviewed and non-reviewed patients with cutaneous melanoma with a positive sentinel node status.

Characteristic	Reviewed cases (n=322)	Non-reviewed cases (n=957)	p-value
Gender (n (%))			0.74
Female	147 (45.7)	447 (46.7)	
Male	175 (54.3)	510 (53.3)	
Mean age in years (SD)	54.1 (15.4)	54.3 (14.7)	0.88
Median Breslow thickness in mm (IQR)	2.5 (1.7-4.0)	2.4 (1.6-3.7)	0.27
Breslow thickness in mm (n (%))			0.17
0.1-0.7	1 (0.3)	10 (0.1)	
0.8-1.0	7 (2.2)	42 (4.4)	
1.1-2.0	106 (32.9)	336 (35.1)	
2.1-4.0	140 (43.5)	396 (41.4)	
>4.1	68 (21.1)	173 (18.1)	
Ulceration (n (%))			0.38
No	179 (55.6)	548 (57.3)	
Yes	119 (37.0)	320 (33.4)	
Unknown	24 (7.5)	89 (9.3)	
Localization (n (%))			0.01
Head and neck	8 (2.5)	47 (4.9)	
Trunk	145 (45.0)	474 (49.5)	
Arms	27 (8.4)	88 (9.2)	
Legs	124 (38.5)	325 (34.0)	
Unknown	18 (5.6)	23 (2.4)	
Subtype (n (%))			0.20
Superficial spreading	191 (59.5)	545 (56.9)	
Nodular	84 (26.2)	270 (28.2)	
Lentigo maligna	1 (0.3)	0 (0)	
Acral lentiginous	11 (3.4)	21 (2.2)	
Unknown	34 (10.6)	122 (12.7)	
Stage as per the 7th AJCC (n (%))			0.29
IIIA	175 (54.3)	544 (56.8)	
IIIB/C	123 (38.2)	324 (33.9)	
Unknown	24 (7.5)	89 (9.3)	
Stage as per the 8th AJCC (n (%))			0.40
IIIA	87 (27.0)	287 (30.0)	
IIIB	85 (26.4)	270 (28.2)	
IIIC	130 (40.4)	330 (34.5)	
IIID	0 (0.0)	1 (0.1)	
Unknown	20 (6.2)	69 (7.2)	

AJCC = American Joint Committee on Cancer, SD = standard deviation, IQR = interquartile range.

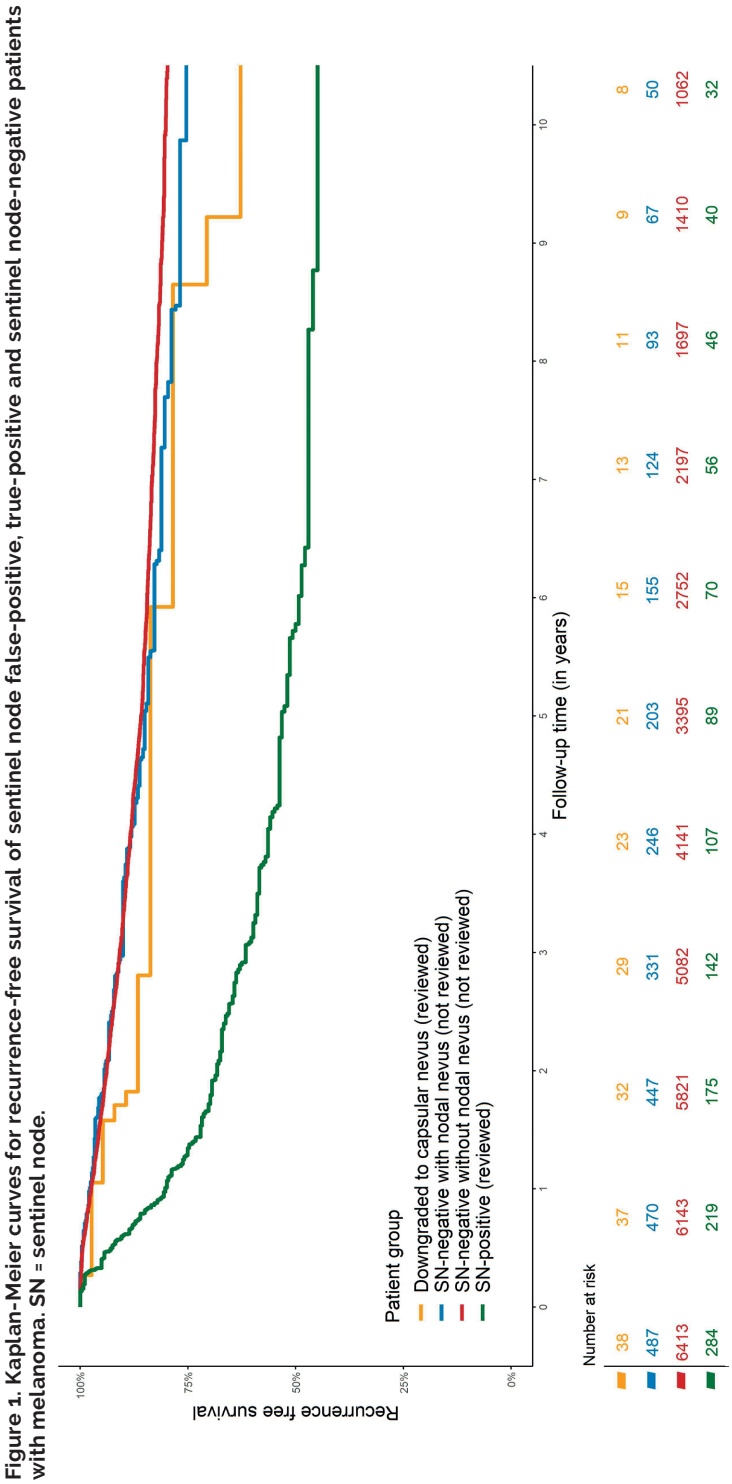

Figure 1. Kaplan-Meier curves for recurrence-free survival of sentinel node false-positive, true-positive and sentinel node-negative patients with melanoma. SN = sentinel node.

Downgraded diagnoses in relation to indication of adjuvant therapy

The diagnosis was downgraded from melanoma metastasis to nodal nevi in 38 patients (11.8%) (Table 2). S100 and/or Melan-A staining was available for all of these patients. The percentage of downgraded cases was comparable in each of the five pathology laboratories: 10.3%, 11.5%, 12.4% and 14.7%, except for one academic pathology laboratory that had 5.0% of their cases downgraded. Of the 322 reviewed patients, 175 patients were initially staged IIIA, 123 were staged IIIB/C and in 24, further determination was not possible because of missing ulceration status (Table 1)[7]. The size of the nodal nevus of the 38 downgraded patients ranged from 0.005 mm to 1.5 mm. Sixty-four of the 175 patients staged as IIIA (36.6%) had a SN tumour burden of >1.0 mm. Of the 38 misdiagnosed patients, 25 patients would have been incorrectly staged as IIIA if they would not have been reviewed. Of these 25 patients, 4 had an SN tumour burden >1.0 mm. Eight patients would have been incorrectly staged as stage IIIB/C, and in 5 patients, further determination was not possible because of missing ulceration status, but regardless, 2 had a SN tumour burden >1.0 mm. Thus, considering the inclusion criteria of the phase III adjuvant trials, at least 14 patients (4.3%) would have been falsely qualified for adjuvant therapy: 4 patients with stage IIIA disease with a nodal nevus of >1.0 mm, 8 patients with stage IIIB/IIIC disease and 2 patients with unknown stage III, but with a SN tumour burden >1.0 mm.

Table 2. Baseline data of 322 patients with melanoma who were originally diagnosed as sentinel node positive, stratified by pathology review status.

Characteristic	SN-negative upon review (n=38)	SN-positive upon review (n=284)	p-value
Gender (n (%))			0.42
Female	15 (10.2)	132 (89.8)	
Male	23 (13.1)	152 (86.9)	
Median tumour burden in mm (IQR)	0.3 (0.2-0.6)	0.9 (0.3-2.3)	<0.001
Tumor burden in mm (n (%))			0.007
<0.1	4 (18.2)	18 (81.8)	
0.1-1.0	27 (16.5)	137 (83.5)	
>1.0	7 (5.1)	129 (94.9)	
Tumor burden location (n (%))			<0.001
Subcapsular	12 (37.5)	20 (62.5)	
Non-subcapsular*	26 (9.0)	264 (91.0)	
Mean age at diagnosis in years (SD)	51.6 (13.9)	54.5 (15.6)	0.29
Median age at diagnosis in years (IQR)	52 (41-64)	55 (43-67)	0.23
Median Breslow thickness in mm (IQR)	1.8 (1.2-2.5)	2.6 (1.8-4.0)	<0.001
Breslow thickness in mm (n (%))			0.01
0.1-0.7	0 (0.0)	1 (100)	
0.8-1.0	2 (28.6)	5 (71.4)	
1.1-2.0	21 (20.0)	85 (80.2)	
2.1-4.0	11 (7.9)	129 (92.1)	
>4.1	4 (5.9)	64 (94.1)	

Table 2. (continued).

Characteristic	SN-negative upon review (n=38)	SN-positive upon review (n=284)	p-value
Localization (n (%))			0.84
Head and neck	1 (12.5)	7 (87.5)	
Trunk	19 (13.1)	126 (86.9)	
Arms	2 (7.4)	25 (92.6)	
Legs	15 (12.1)	109 (87.9)	
Unknown	1 (5.6)	17 (94.4)	
Subtype (n (%))			0.64
Superficial spreading	26 (13.5)	166 (86.5)	
Nodular	9 (10.7)	75 (89.3)	
Lentigo maligna	0 (0.0	1 (100)	
Acral lentiginous	0 (0.0)	11 (100)	
Unknown	3 (8.8)	31 (91.2)	
Ulceration (n (%))			0.06
No	25 (14.0)	154 (86.0)	
Yes	8 (6.7)	111 (93.3)	
Unknown	5 (20.8)	19 (79.2)	
Stage as per the 7th AJCC (n (%))			0.04
IIIA	25 (14.3)	150 (85.7)	
IIIB/C	8 (6.5)	115 (93.5)	
Unknown	5 (20.8)	19 (79.2)	

SN = sentinel node, AJCC = American Joint Committee on Cancer, SD = standard deviation, IQR = interquartile range.
[a] Patients with both subcapsular and non-subcapsular SN tumour deposits were classified as non-subcapsular.

Logistic regression

When assessing the association between clinicopathological characteristics and the chance of downgrading an initial SN-positive biopsy to a nodal nevus, on multivariable analysis, two predictors remained statistically significant: SN tumour burden (odds ratio [OR] = 0.39 [95% confidence interval [CI] = 0.19–0.78], p = 0.008) and non-subcapsular location of the nodal nevus (OR = 0.31 (95% CI = 0.13–0.72, p = 0.006) (Table 3). Examples of cases for which the diagnosis was downgraded from melanoma metastases to nodal nevi are displayed in Fig. 2. Some display unusual large nevi some with paraseptal and/or focal parenchymal extension.

Table 3. Multivariable logistic regression for misdiagnosis of nodal nevus as melanoma metastasis in 322 patients.

		Univariable		Multivariable[a]	
Variable	Definition	OR (95% CI)	p-value	OR (95% CI)	p-value
Breslow thickness	Per mm	0.64 (0.47-0.87)	0.004	-	-
Tumor burden[b]	Per mm	0.32 (0.16-0.65)	0.002	0.39 (0.19-0.78)	0.008
Dewar localisation	Subcaps	1		1	
	Non-subcaps	0.16 (0.07-0.37)	<0.001	0.31 (0.13-0.72)	0.006

Table 3. (continued).

		Univariable		Multivariable*	
Variable	**Definition**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Age	Per year	0.99 (0.97-1.01)	0.29	-	-
Gender	Male	1		-	-
	Female	0.75 (0.38-1.50)	0.42	-	
Ulceration	No	1		-	-
	Yes	0.44 (0.19-1.02)	0.06	-	
	Missing	1.62 (0.56-4.74)	0.38	-	
Anatomic location	Head and neck	1		-	-
	Trunk	1.06 (0.12-9.06)	0.96	-	
	Arm	0.56 (0.04-7.12)	0.66	-	
	Legs	0.96 (0.11-8.38)	0.98	-	
	Missing	0.41 (0.02-7.55)	0.55	-	
Melanoma subtype	SSM	1		-	-
	NM	0.77 (0.34-1.72)	0.52	-	
	Other	0.45 (0.13-1.54)	0.20	-	

OR = odds ratio, CI = confidence interval, SSM = superficial spreading melanoma, NM = nodular melanoma.
[a] Only variables that were statically significant are shown. All variables that are shown in the univariable analysis were included in the multivariable analysis.
[b] Defined as the single measurement of the maximum diameter of the largest lesion in any direction.

Survival comparison

A total of 6900 SN-negative patients were included for survival comparison, of which 487 had a nodal nevus. The five-year RFS was 85.1% (95% CI = 81.5–88.8) for the 487 SN-negative patients with a nodal nevus and 85.9 (95% CI = 84.9–86.8) for the remaining SN-negative patients (Fig. 1). The five-year RFS of the 38 patients with a downgraded diagnosis upon review was 86.7 (95% CI = 72.6–96.6), which was not statistically significantly different from that of the 487 SN-negative patients with a nodal nevus (p = 0.41) and from that of the remaining 6413 SN-negative patients (p = 0.18). There was a statistically significant difference in RFS between the 38 downgraded patients (86.7 [95% CI = 72.6–96.6]) and the 5-year RFS of the 284 patients that remained SN positive after review (53.2% [95% CI = 47.2–59.9], p = 0.009).

Figure 2 (A–D). Illustrating examples of four cases in which diagnosis was downgraded from melanoma metastases to nodal nevus. (A–C) Nevus typically located in the capsule. The melanocytic cells lack atypia or mitoses precluding a diagnosis of melanoma metastasis. (C) Extensive and large nodal nevus with however typical capsular location. (B–D) Paratrabecular or septal deeper extension of nevus cells along fibrous bands originating from the capsule. These nevus cells can easily be misdiagnosed as melanoma metastases if pathologists misinterpret septal extension as parenchymal location of melanocytes. Immunostaining (S100 and/or Melan-A) confirms the melanocytic nature of the nevus cells and highlights the nodal/septal location of melanocytes. H&E = haematoxylin and eosin.

DISCUSSION

This study was undertaken to reassess SN biopsies of patients with melanoma who were initially diagnosed positive for melanoma metastases and determine concordance when reassessed by an expert melanoma pathologist. Our results show that more than 10% of originally positive SN biopsies in patients with melanoma concerned capsular nevi that were misclassified as melanoma metastasis and that potentially at least 4.3% of patients with stage III disease nowadays would receive unjustified adjuvant treatment based on an overdiagnosed SN biopsy[7].

Recently, adjuvant therapy for patients with stage III melanoma has been proven to increase relapse-free survival for patients with melanoma[3] and is currently being implemented worldwide. Because a positive SN status is generally considered to be an indication for adjuvant therapy, the number of SN biopsies performed in patients with melanoma, which currently ranges from 40% to only 60% in large nationwide data, is likely to increase[21,22]. Therefore, adequate assessment of SN positivity (and its tumour burden) is more important than ever.

For a subset of these patients (stage IIIA), most adjuvant therapy guidelines apply a threshold of >1.0 mm SN tumour burden, e.g. as approved by the Food and Drug Administration[7]. This is because in the adjuvant setting, all studies with regard to patients with stage IIIA disease have been performed on patients with an SN tumour burden >1.0 mm[4-6]. In line with this, the European Society for Medical Oncology also advocates that treatment decisions for patients with stage IIIA disease and SN ≤1.0 mm should be made on an individual basis, and the European Association of Dermato Oncology and EORTC state it should be carefully discussed with these patients[23,24]. If we would not account for a >1.0 mm SN tumour burden threshold for patients with stage IIIA disease, all 38 misdiagnosed cases (11.8%) would have falsely qualified for adjuvant treatment.

Most studies that have shown that pathological review of 'initially negative' SN biopsies could lead to the detection of melanoma metastases reviewed only SN biopsies of patients who developed metastatic disease during follow-up[8,10-13]. This led to percentages of upgraded diagnoses from 20%[11] to 43%[12]. We could identify only one study that reviewed an unbiased population of negative SN biopsies, which found a 5% upgrade rate[9]. However, to the best of our knowledge, only one study has previously re-evaluated 'initially positive' SNs and found a downgrade rate of 10.1% (16 out of 159 patients)[16]. Identifying falsely positive SNs is of importance because they put patients at risk of unjustified exposure to the potentially fatal side-effects of adjuvant systemic treatment[17]. Moreover, by identifying patients with a false-positive SN, the high costs of these systemic therapies can be avoided[18]. As the present results show a high downgrade rate (11.8%) of initially positive SN biopsies and that 44 of

1000 patients might receive unjustified adjuvant therapy, we advocate that in case adjuvant treatment is considered in patients with stage III melanoma, SN biopsies should be reviewed by an expert melanoma pathologist.

In most cases, differentiation between a nodal nevus and melanoma metastasis is straightforward, based on location and cytomorphological features of the melanocytic cells in the lymph node. However, in a subset of cases, nodal nevi may be difficult to discriminate from melanoma metastasis. Small melanoma metastasis or metastasis from a primary nevoid melanoma can be difficult to discriminate from a nodal nevus[25]. In typical cases, nodal nevi are located within the capsule; they are small, are often triangular shaped and lack the cytonuclear atypia and mitotic activity of melanoma cells. However, capsular nevi may be quite extensive and may show some parenchymal and paratrabecular extension mimicking localisation within the lymph node parenchyma and therefore melanoma metastasis. Indeed, subcapsular location of the nodal nevus was, besides low SN tumour burden, one of the predictors of misdiagnosis as metastases. In cases of subcapsular location, differential diagnosis may therefore be difficult and mostly relies on cytomorphology. Melanomas can have nevoid cytomorphology and bland appearance. In such cases, the discrimination of a metastasis from nevus is difficult especially when metastasis is small, e.g. as isolated cells instead of nests[26]. S100, Sox-10 and Melan-A/MART1 immunohistochemistry help to identify nevoid cells, but do not differentiate between nodal nevi and metastatic melanoma cells[26-29]. Weak or absent immunohistochemical staining for HMB-45, low Ki-67 proliferation, expression of p16 or absence of PRAME staining all favour a diagnosis of nevus[27-29].

One of the strengths of the present study is the generalisability of the results because we randomly selected cases from five different pathology laboratories all over the Netherlands. The rate of downgraded cases was comparable for four of the laboratories, except for one that had only 5.0% of their cases downgraded. One could argue that pathologists working in laboratories with less experience or lower volume of cases are more likely to have a high downgraded rate in the present study. However, we reviewed cases of two academic laboratories and three non-academic laboratories. Although the laboratory with the 5.0% downgraded rate was academic, the other academic laboratory was in between the 10.3–14.7% downgraded rate of the non-academic laboratories. Therefore, in addition, in academic laboratories, there is still a significant number of misdiagnosis of SN biopsies, which might be related to the fact that also in these pathology departments, SN biopsies are not always seen by a dedicated melanoma pathologist.

Another strength is the review by an expert EORTC melanoma pathologist and the comparison of RFS of the downgraded cases with that of other patients with melanoma. No statistically significant difference in RFS was found between the 38

patients who were downgraded upon review and SN-negative patients, implying that the downgrading of these patients is justified. Moreover, there was a statistically significant difference in RFS between the 38 downgraded patients and the remaining 284 SN-positive patients (p = 0.009). A limitation is that all cases were reviewed by a single expert pathologist, which may be related to differences in interpretation, even at the expert level. Another limitation is that not all 1279 cases were reviewed, but a random sample. To minimise bias and to optimise efficiency, all cases from one randomly selected pathology laboratory and all cases from the consecutive four pathology laboratories that were received thereafter were reviewed. However, we cannot completely exclude any bias in this approach, although comparison of clinicopathological characteristics between the 322 reviewed cases and the 957 non-reviewed cases showed no statistically significant differences, except for localisation of the melanoma (Table 1). A final limitation is that we were not able to explore the mitotic rate as it was not systematically recorded in the database.

CONCLUSIONS

A large number of originally positive SN biopsies in patients with melanoma are misclassified, indicating that some patients with melanoma might receive unjustified adjuvant treatment. We therefore advocate that when adjuvant treatment is considered in patients with stage III melanoma, SN biopsies should be reassessed by an expert melanoma pathologist.

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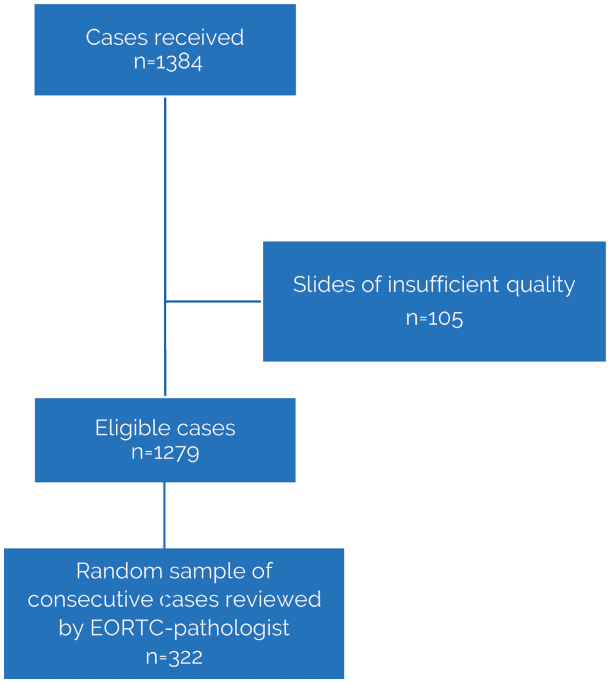
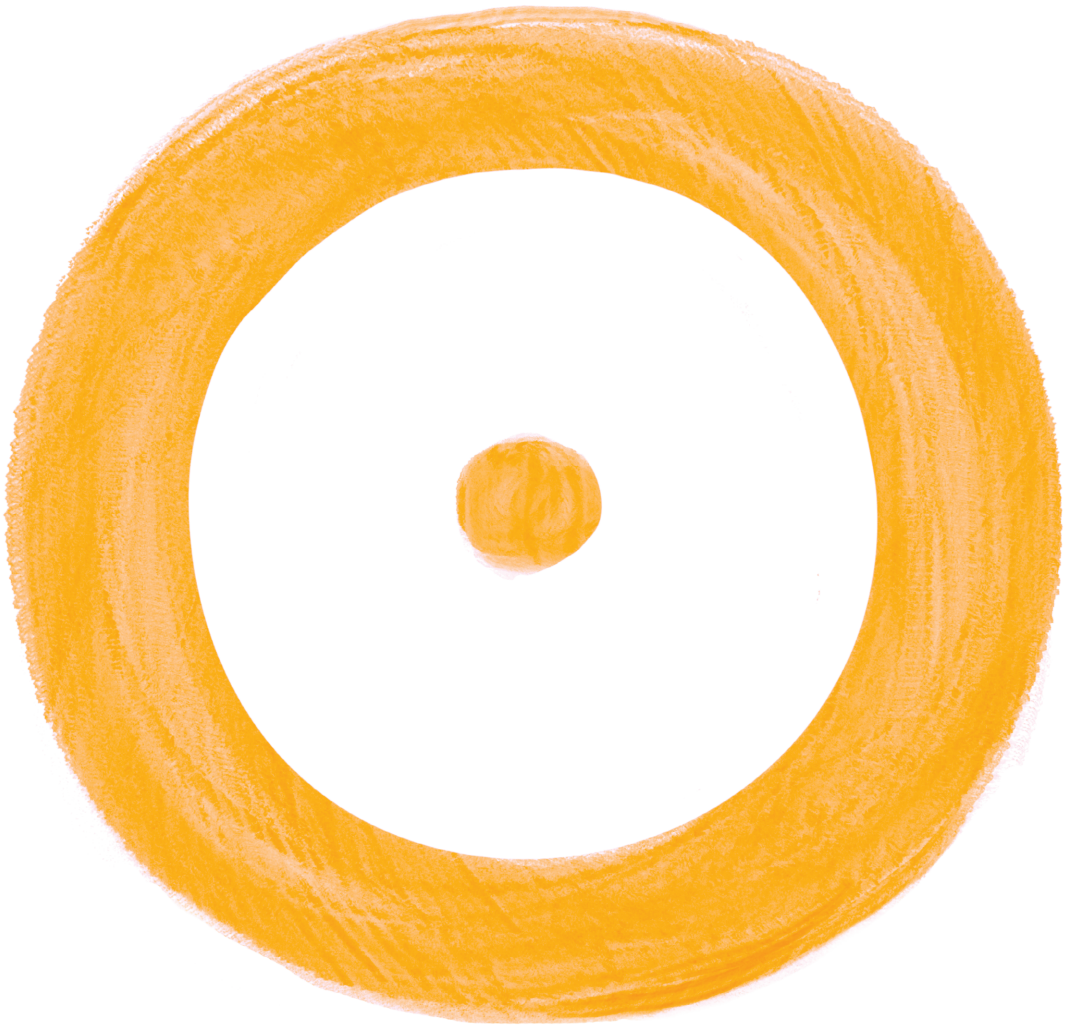
Supplementary Figure 1. Flowchart of included patients.





CHAPTER 6

Time interval between diagnostic excision-biopsy of a primary melanoma and sentinel node biopsy: effects on the sentinel node positivity rate and survival outcomes

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ABSTRACT

Introduction: The optimal time interval between diagnostic excision of a primary cutaneous melanoma and sentinel node (SN) biopsy is unknown. The current study sought to determine whether this interval influenced the SN-positivity rate, recurrence or survival.

Methods: Data collected from 2004 to 2014 for a Dutch population-based cohort of melanoma patients who underwent SN biopsy (SNB) within 100 days of initial diagnosis (n=7660) and for a similarly specified cohort from a large Australian melanoma treatment centre (n=3478) were analysed. Time to SNB was analysed continuously (in weeks) and categorically (per month). The effects of SN timing on SN-positivity were based on multivariable logistic regression, and its effects on recurrence-free survival (RFS) and overall survival (OS) were based on standard Cox proportional hazard regression analyses. Advanced modelling using a multivariable Cox model with penalised splines for modelling the continuous effects of time to SNB on RFS and OS was also performed.

Results: The median times to SNB were 36 and 27 days in the Dutch and Australian cohorts, respectively. There was no significant association between time to SNB and SN-positivity in either cohort, nor did either cohort show an impact of time to SNB on RFS or OS. The spline-based HR curves for RFS and OS confirmed these findings.

Conclusions: The time interval between diagnostic excision of a primary melanoma and SNB did not influence the SN-positivity rate or survival outcomes. This provides reassurance that neither early nor delayed definitive wide excision and SNB will adversely affect prognosis.

INTRODUCTION

Sentinel node biopsy (SNB) is well established as an important staging investigation for patients with melanoma, and adjuvant systemic drug therapy has been shown to reduce the risk of recurrence in some groups of SN-positive patients[1, 2]. Current melanoma management guidelines make no recommendations about the optimal time interval between melanoma diagnosis (i.e. the date of complete diagnostic excision of a primary melanoma) and SNB (usually performed at the time of definitive wide excision of the melanoma)[3-5]. A delay in carrying out a SNB could theoretically affect the likelihood of a positive result or survival because cells shed from the primary melanoma have had more time to reach spread regional lymph nodes or spread to distant sites. The results of two previous studies that examined the effect of time to SNB suggested that there is no significant association between the time interval and SN-positivity[6, 7]. Literature on the effect of time interval to SNB on survival shows mixed results, with three studies finding no association[6-8], two finding worse survival with early SNB[9, 10], and one finding improved survival with early SNB[6, 11]. The aim of the present study was to re-assess the influence of the time interval between diagnostic excision biopsy of a melanoma and the SNB on the SN-positivity rate and survival using two large patient cohorts. Data from a nation-wide Dutch cohort, and from a large, specialised melanoma treatment centre in Australia were obtained and analysed independently.

METHODS

Collection of data

For the Dutch cohort, data for all patients with invasive cutaneous melanoma newly diagnosed in the Netherlands between January 2004 and December 2014 were obtained from PALGA, the Dutch Pathology Registry[12]. All data were encoded and used anonymously and ethical approval was granted by the board of PALGA. Follow-up data were obtained from the Netherlands Cancer Registry.

For the Australian cohort, a search was performed of the prospectively maintained database of Melanoma Institute Australia (MIA) in Sydney for patients who were diagnosed and treated over the same time period. To eliminate referral bias, patients who were initially treated by wide excision elsewhere but later referred to MIA for further management or follow-up were excluded. All patients gave consent prospectively for use of their database information for research purposes.

Study population

The study population consisted of patients ≥18 years who underwent SNB within 100 days of initial diagnosis of their primary cutaneous melanoma. Excluded were those who underwent SNB on the same day as diagnostic excision biopsy of their melanoma, those with synchronous, clinically-detected in-transit, nodal or distant metastases, those who had an initial partial biopsy but not a complete excision of the melanoma prior to the wide excision/SN biopsy procedure, those with multiple primary melanomas, and those with incomplete follow-up. For each patient demographic characteristics were collected, including date of diagnosis, age, sex, primary tumour anatomical site and recurrence. Recurrence was defined as either cutaneous or subcutaneous (local or in-transit), nodal (regional only) or distant metastasis. Pathological data collected included Breslow thickness, melanoma subtype, SN status, presence or absence of ulceration, and mitoses per mm2. Only patients with all predefined clinicopathological characteristics available were included. Time interval to SNB was calculated in weeks from the date of complete excision of the primary melanoma to the date of SNB and was analysed both continuously and categorically (per month). For the categorical assessment, patients were divided into three groups: (i) those who had their SNB performed within the first month after complete diagnostic excision of their primary melanoma, (ii) those who had it performed in the second month, and (iii) those who had it performed in the third month. Clinical outcomes that were assessed were SN-positivity, recurrence-free survival (RFS) and overall survival (OS). RFS was calculated from the date of diagnosis to the date of recurrence or death. OS was calculated from the date of diagnosis to the date of death. Patients without the corresponding event were censored at the last date known alive or January 1st 2018 (the data collection cut-off date), whichever

occurred earlier. The size of each SN tumour deposit was also measured as an outcome, but this information will be reported and discussed in a separate article.

Statistical analysis

Data for the Dutch and MIA patients were analysed independently. Categorical variables were summarised as numbers and percentages. Continuous variables were summarised as medians with interquartile ranges (IQRs). Differences in proportions and medians were analysed using Chi-square and Mann-Whitney U tests, respectively. Kaplan-Meier curves were generated for RFS and OS. SN-positivity was assessed using univariable and multivariable logistic regression. The variables analysed included sex, Breslow thickness, age, primary site, ulceration, mitoses and melanoma subtype. The associations between time interval to SNB and RFS and OS were assessed using univariable and multivariable Cox proportional hazard regression; hazard ratios (HRs) and 95% confidence intervals (CIs) were reported. In addition to the aforementioned variables, SN status was also included as a predictor. The proportional hazards assumption was evaluated using the Schoenfeld residuals test for each variable candidate for the multivariable analysis. When the proportional hazard assumption did not hold for a specific variable, that variable was included as a stratification factor in the multivariable model. Linearity of the association between age and Breslow thickness with respect to each clinical outcome (SN-positivity, RFS and OS) was assessed using the test of deviance[13]. Advanced modelling using a multivariable Cox model with penalised splines was also performed to model the continuous effects of time to SNB on RFS and OS[14]. Flexible hazard ratio curves were produced to depict these effects. Early SN biopsies, conducted within the first week after diagnosis, were taken as the reference.

All statistical analyses were performed using R version 3.6.1 (R Core Team, Vienna, Austria). A two-sided p-value of <0.05 was considered statistically significant.

This study adhered to the guideline for the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) and the checklist was completed (Supplementary Materials Table 1)[15].

RESULTS

Clinicopathological features of the 7660 Dutch and 3478 MIA patients are presented in Table 1, and a flowchart of the patient selection process is shown in Supplementary Figure 1. The median time to SNB was 36 days (IQR 28-49, range 1-100) for the Dutch cohort and 27 days (IQR 20-37, range 1-100) for the MIA cohort (Figure 1). Figures 2 and 3 show the RFS and OS of the Dutch and MIA patients when SNB was performed during the first, second and third months after complete diagnostic excision of their primary melanoma, respectively. The median follow-up duration was 5.0 years

(IQR 3.3-7.5) for Dutch patients and 3.1 years (IQR 1.4-5.7) for MIA patients. SN tumor diameter was available for 13.4% and 15.6% of patients in the Dutch and MIA cohorts, respectively. These data will be reported in a separate article.

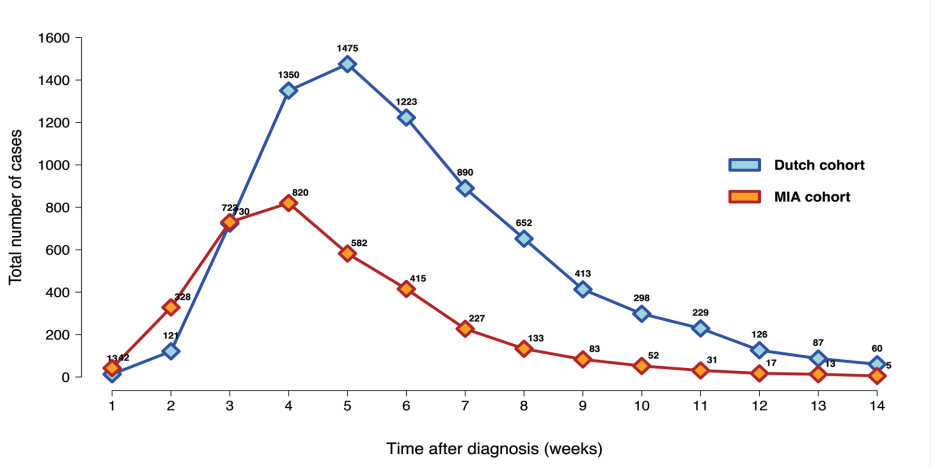
Table 1. Clinicopathological characteristics of patients who underwent SN biopsy, stratified for the Dutch and MIA cohorts.

Characteristic	Dutch (n = 7660)	MIA (n = 3478)	p-value
Sex (n (%))			<0.001
Female	3925 (51.2)	1426 (41.0)	
Male	3735 (48.8)	2052 (59.0)	
Median age in years (IQR)	55 (44-66)	59 (47-69)	<0.001
Median Breslow thickness in mm (IQR)	1.8 (1.2-2.8)	2.0 (1.2-3.2)	<0.001
Breslow thickness in mm (n (%))			<0.001
≤1.0	930 (12.1)	417 (12.0)	
1.1-2.0	3671 (47.9)	1401 (40.3)	
2.1-4.0	2256 (29.5)	1095 (31.5)	
>4.0	803 (10.5)	565 (16.2)	
Ulceration (n (%))			0.01
No	5683 (74.2)	2502 (71.9)	
Yes	1977 (25.8)	976 (28.1)	
Mitoses (n (%))			<0.001
No	638 (8.3)	260 (7.5)	
Yes	4894 (63.9)	3152 (90.5)	
Missing	2128 (27.8)	72 (2.1)	
Primary site (n (%))			<0.001
Head & Neck	478 (6.2)	634 (18.2)	
Trunk	3458 (45.1)	1336 (38.4)	
Upper limb	1165 (15.2)	672 (19.3)	
Lower limb	2559 (33.4)	836 (24.0)	
Melanoma subtype (n (%))			<0.001
Superficial spreading	4919 (64.2)	1534 (44.1)	
Nodular	1793 (23.4)	1121 (32.2)	
Lentigo maligna	67 (0.9)	75 (2.2)	
Acral lentignious	129 (1.7)	63 (1.8)	
Desmoplastic	42 (0.5)	328 (9.4)	
Missing	710 (9.3)	357 (10.3)	
SN result (n (%))			<0.001
Negative	5892 (76.9)	2818 (81.0)	
Positive	1768 (23.1)	660 (19.0)	

Table 1. (continued).

Characteristic	Dutch (n = 7660)	MIA (n = 3478)	p-value
Number of positive SN			<0.001
1	1364 (77.1)	434 (65.8)	
2	282 (16.0)	91 (13.8)	
3	70 (4.0)	11 (1.7)	
4	13 (0.7)	4 (0.6)	
5	1 (0.1)	1 (0.2)	
6	1 (0.1)	0 (0.0)	
Missing	37 (2.1)	119 (18.0)	
Median follow-up time in years (IQR)	5.0 (3.3-7.5)	3.1 (1.4-5.7)	<0.001
Median time to SNB in days (IQR)	36 (28-49)	27 (20-37)	<0.001

Figure 1. Number of sentinel node biopsy procedures according to time since melanoma diagnosis.



SN-positivity

Table 2 shows the logistic regression for time to SNB associated with SN-positivity. In both the Dutch and MIA cohorts, patients in whom SNB was performed during the second month after complete diagnostic excision of their primary melanoma had no greater chance of SN-positivity than patients who had SNB during the first month (Dutch OR 1.00 (95%CI 0.88-1.14), p=0.99; MIA OR 0.96 (95%CI 0.79-1.16), p=0.65). Likewise, patients who had SNB during the third month after diagnostic excision of their primary melanoma had no greater chance of SN positivity (Dutch OR 1.11 (95%CI 0.94-1.32), p=0.23; MIA OR 0.97 (95%CI 0.64-1.45), p=0.90). Nor was there any significant effect on SN-positivity when time to SNB was assessed as a continuous variable (in weeks) for the Dutch cohort (OR 1.01 (95%CI 0.98-1.03), p=0.56) or for the MIA cohort (OR 0.99 (95%CI 0.94-1.03), p=0.53).

Figure 2. Recurrence-free survival of Dutch and MIA patients, stratified by the number of months between diagnostic excision and sentinel node biopsy

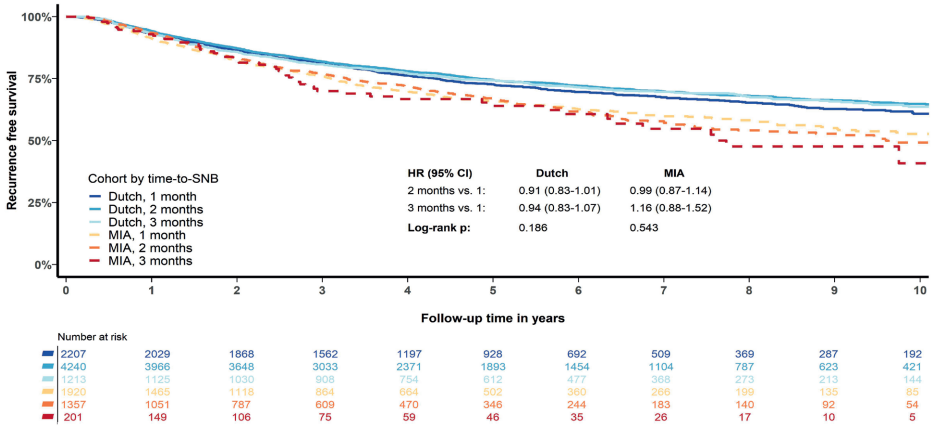


Figure 3. Overall survival of Dutch and MIA patients, stratified by the number of months between diagnostic excision and sentinel node biopsy

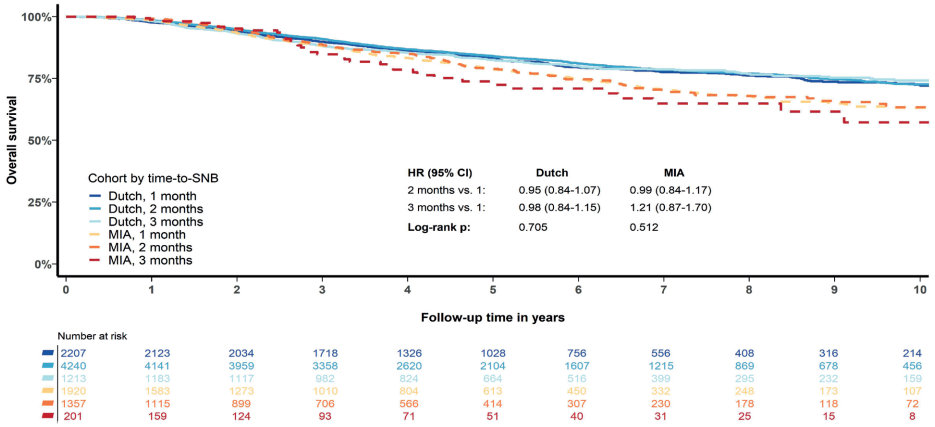


Table 2. Multivariable logistic regression for SN status, stratified for the total Dutch (n=7660) and MIA cohorts (n=3478).

		Dutch cohort				MIA cohort			
		Multivariable 1***		Multivariable 2***		Multivariable 1***		Multivariable 2***	
Variable	Class	OR (95% CI)*	p-value	OR (95% CI)*	p-value	OR (95% CI)**	p-value	OR (95% CI)**	p-value
Time interval to SN	Per week	1.01 (0.98-1.03)	0.56	-	-	0.99 (0.94-1.03)	0.53	-	-
Time interval to SN****	1st month	-	-	1		-	-	1	
	2nd month	-	-	1.00 (0.88-1.14)	0.99	-	-	0.96 (0.79-1.16)	0.65
	3rd month	-	-	1.11 (0.94-1.32)	0.23	-	-	0.97 (0.64-1.45)	0.90

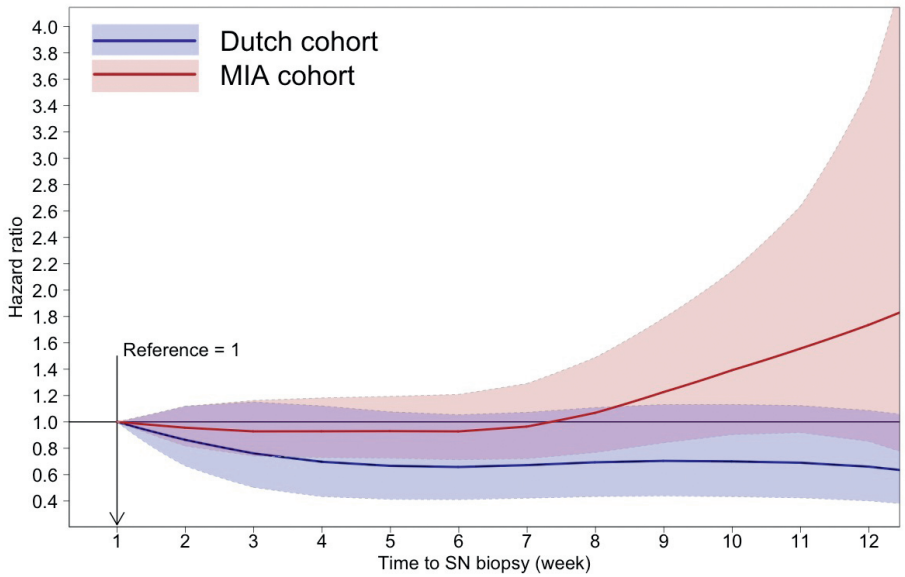
* The β-coefficients of the intercept for the Dutch multivariable model were -2.74 (standard error 0.23, p-value <0.0001) and -2.72 (standard error 0.22, p-value <0.0001) for the continuous and categorical time interval.
** The β-coefficients of the intercept for the MIA multivariable model were -2.40 (standard error 0.35, p-value <0.0001) and -2.46 (standard error 0.34, p-value <0.0001) for the continuous and categorical time interval.
*** Multivariable analyses adjusted for: sex, Breslow thickness, age at diagnosis, primary site, ulceration status, melanoma subtype, presence of mitoses.
**** Number of patients with a positive SN biopsy in the 1st, 2nd and 3rd month: 520 (23.6%), 953 (22.5%), 295 (24.3%) in the Dutch cohort, and 377 (19.6%), 249 (18.3%), 34 (16.9%) in the MIA cohort.

Survival outcomes

There was no significant association between time to SNB and RFS or OS in multivariable analyses (analysed either continuously and categorically) in either of the two cohorts (Tables 3 and 4), when adjusting for SN status, sex, Breslow thickness, age, primary site, ulceration, mitoses and SN status. No significant associations between time to SNB (continuously, in weeks) and RFS or OS were found in either the Dutch cohort or the MIA cohort. The proportional hazards assumption was not violated.

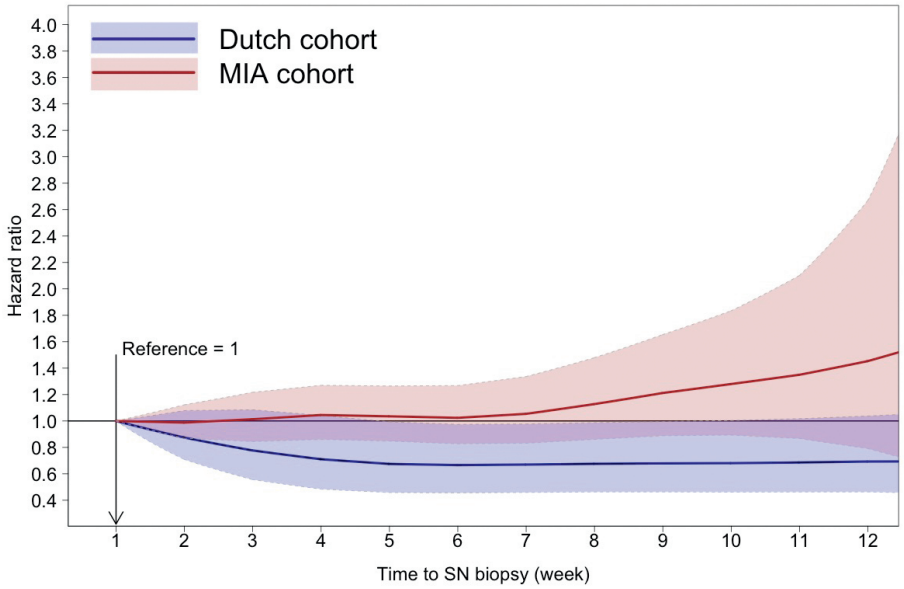
When time to SNB was analysed categorically, no significant associations between time to SNB and RFS or OS were found in either the Dutch or the MIA cohorts, except for RFS for Dutch patients who underwent SNB in the second month compared to those who underwent SNB in the first month. An additional analysis that included only SNB-positive patients showed similar results (data not shown). The spline-based HR curves for RFS and OS, examining the two cohorts separately, are presented in Figures 4 and 5. In both cohorts, the HR curves for RFS and OS did not show any survival disadvantage or advantage for a delayed time to SNB.

Figure 5. Penalised spline model of the continuous effect of time to sentinel node biopsy on overall survival for the Dutch and the MIA cohorts, reflecting hazard ratios with their 95% confidence intervals. Early biopsies (conducted within the first week after diagnosis) were taken as the reference.



Smooth HRs in both cohorts were adjusted for SN status, sex, Breslow thickness, age at diagnosis, primary site, ulceration status, presence of mitoses. The 95% confidence interval of the HR includes "1" across the entire range of time interval to SNB. This means that there is no statistically significant difference in terms of overall survival between patients who underwent SNB within the first week after initial diagnosis compared to those who underwent SNB later (up to 100 days).

Figure 4. Penalised spline model of the continuous effect of time to sentinel node biopsy on recurrence-free survival for the Dutch and the MIA cohorts, reflecting hazard ratios with their 95% confidence intervals. Early biopsies (conducted within the first week after diagnosis) were taken as the reference.



Smooth HRs in both cohorts were adjusted for SN status, sex, Breslow thickness, age at diagnosis, primary site, ulceration status, presence of mitoses. The 95% confidence interval of the HR includes "1" across the entire range of time interval to SNB. This means that there is no statistically-significant difference in terms of recurrence-free survival between patients who underwent SNB within the first week after initial diagnosis compared to those who underwent SNB later (up to 100 days).

Table 3. Multivariable Cox regression for recurrence-free survival for the Dutch and MIA cohorts.

		Dutch cohort (n events = 2143)				MIA cohort (n events = 945)			
		Multivariable 1		Multivariable 2		Multivariable 1		Multivariable 2	
Variable	Class	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Time interval to SN	Per week	0.99 (0.97-1.01)	0.19	-	-	1.02 (0.98-1.05)	0.20	-	-
Time interval to SN	1st month	-	-	1		-	-	1	
	2nd month	-	-	0.90 (0.81-0.99)	0.03	-	-	1.04 (0.92-1.19)	0.54
	3rd month	-	-	0.90 (0.79-1.03)	0.13	-	-	1.30 (0.99-1.71)	0.06

P-value for test of deviance for time to SN biopsy for Dutch cohort: 0.662, for MIA cohort: 0.930
Multivariable analyses were adjusted for: presence of mitoses, SN status, sex, Breslow thickness, age at diagnosis, primary site, ulceration status. The latter 5 variables were included as a stratification factor.

Table 4. Multivariable Cox regression for overall survival for the Dutch and MIA cohorts.

		Dutch cohort (n events = 1430)				MIA cohort (n events = 607)			
		Multivariable 1		Multivariable 2		Multivariable 1		Multivariable 2	
Variable	Class	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Time interval to SN	Per week	0.99 (0.97-1.01)	0.28	-	-	1.02 (0.97-1.06)	0.37	-	-
Time interval to SN	1st month	-	-	1		-	-	1	
	2nd month	-	-	0.93 (0.83-1.05)	0.24	-	-	1.00 (0.85-1.18)	0.99
	3rd month	-	-	0.93 (0.79-1.10)	0.39	-	-	1.38 (0.98-1.93)	0.06

P-value for test of deviance for time to SN biopsy for Dutch cohort: 0.504, for MIA cohort: 0.879.
Multivariable analyses were adjusted for: presence of mitoses, SN status, sex, Breslow thickness, age at diagnosis, primary site, ulceration status. The latter 5 variables were included as a stratification factor.

DISCUSSION

In this study, using two large, independent, cohorts of patients with primary cutaneous melanoma, no statistically significant effects were found on the rate of SN-positivity, RFS or OS of the time interval between diagnostic excision and SNB, when SNB was performed within 100 days of diagnostic excision of the primary tumour. Although early diagnosis of melanoma and other cancers is known to favourably influence prognosis, a hitherto unanswered question is whether the time between complete diagnostic excision of a primary melanoma and SNB has any influence on prognosis. It could be argued that the most important time interval is that between melanoma development and when the patient consults a physician, or a physician diagnoses the melanoma. A previous study analysing delays in diagnostic excision of a primary melanoma found that the delay was mainly caused by patients not seeking medical attention in the phase preceding diagnostic excision[16].

To date, only two studies have attempted to investigate the effect on SN-positivity of variation in time interval between diagnosis and SNB[6, 7]. Parrett et al. reported no statistically significant difference between the median delay time for 78 patients with a positive SNB and that of 414 patients with a negative SNB (41 days versus 35 days, respectively, p=0.50)[7]. The other study, by Oude Ophuis et al., analysed time to SNB (per day) in relation to SN-positivity in 3546 patients from four EORTC centres[6]. An odds ratio (OR) of 1.00 (95%CI 0.99-1.00, p=0.92) was observed after adjustment for other clinicopathological factors. The authors also assessed the effect of the interval (earlier and later than the median of 43 days) on SN metastasis diameter, and no statistically-significant difference was found.

Multiple previous studies have investigated the effect of variation in time to SNB on survival (including the two studies mentioned previously)[6-11]. Parrett et al. found no statistically significant effect of time to SNB on RFS or OS, after adjusting for confounders[7]. Similarly, Nelson et al. found no difference in the effect of time to SNB on RFS and melanoma-specific survival (MSS) when analysing 2483 patients with early and delayed SNB (defined as less than 30 days and 30 or more days from initial diagnosis, respectively)[8]. Oude Ophuis et al. analysed MSS as an outcome and found no statistically significant difference either (HR 1.00 (95%CI 0.99-1.00), p=0.92) per day)[6]. However, Tejera-Vaquerizo et al. reported the surprising finding that a SNB conducted in the first 40 days (versus 41-120 days) was associated with worse MSS when analysing outcomes for 1963 patients (HR 1.7 (95%CI 1.2–2.5), p=0.007)[10], and Mandala et al. also reported a reduction in survival with earlier SNB and an increased survival with delayed SNB, based on a study of 8953 patients from 6 Italian centres. Mandala et al. analysed time to SNB both continuously and categorically, as we did[9]. When they analysed time to SNB continuously (per week), the HR for both RFS and OS was 0.98 (95%CI 0.97-0.99) after adjusting for age, sex, Breslow thickness, primary

site, ulceration and SN status. Regarding categorical time to SNB, the HR for OS was 0.76 (95%CI 0.69-0.85, p<0.0001) comparing the second to the first month and for the third versus the first month this was 0.85 (95%CI 0.75-0.95, p=0.0062). For RFS the HR for the second versus the first month was 0.83 (95%CI 0.75-0.91, p=0.0001) and for the third versus the first month 0.82 (95%CI, 0.74-0.92, p=0.0004)[9]. In contrast, Fortes et al. analysed data from a much smaller series of 748 patients and found an improved MSS when SNB was performed within 30 days compared to >30 days (HR for MSS 0.29 (95% CI 0.11-0.77))[11].

In the present study, no significant association between time to SNB (either continuous or categorical) and survival was found. This is consistent with the results of studies in the pre-SNB era that deliberately delayed elective regional lymph node dissection until 3 months, which also showed no significant benefit or detriment[17, 18]. The hypothesis on which these studies were based was that delayed nodal surgery would allow more time for any tumour cells that might have remained for a time in the region of the primary melanoma or in afferent lymphatics to reach the regional nodes, and that their removal would improve survival.

Strengths of our study include the large size of the patient cohorts and the use of nationwide data as well as data from a large, well-maintained institutional database. Another strength is that we assessed relevant outcomes (SN-positivity, recurrence and overall survival) using comprehensive statistical methodology. A limitation is that the study did not have any data reported on the psychological stress and anxiety that can be associated with waiting for a SNB procedure. Psychological stress following a melanoma diagnosis is well known to have a negative impact on quality of life for melanoma patients, irrespective of pathological stage[19, 20] and longer waiting times for additional diagnostic tests or treatment may play a role in increasing patients' stress levels. Another limitation is that although timely diagnosis of melanoma and other cancers is a known favorable prognostic factor, the question remains whether the time interval between the diagnostic biopsy and SNB is the most important time variable that influences prognosis. We suspect that the most important delay is the time interval between development of a melanoma until histopathological confirmation of the diagnosis. Unfortunately, this remains merely a hypothesis, as it would be unethical to perform a study to prospectively investigate this. A final limitation is the relatively short follow-up of 3.1 years in the MIA cohort, compared to 5.0 years in the Dutch cohort; however, the two cohorts showed similar results in terms of SN-positivity rates and survival outcomes.

CONCLUSIONS

The time interval between diagnostic excision of a melanoma and SNB, ranging between 0 and 100 days, did not influence the SN-positivity rate or survival outcome.

The practical implication of our findings is that patients can be reassured that if logistic considerations result in definitive wide local excision and SNB being undertaken early or with a delay of up to 100 days, their survival outcome is not likely to be adversely affected.

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Supplementary Table 1. STROBE Statement—Checklist of items that should be included in reports of cohort studies.

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6,7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-8
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6,7,33
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	7,8
		(*c*) Explain how missing data were addressed	7,8,33
		(*d*) If applicable, explain how loss to follow-up was addressed	7,8
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9,10,33
		(b) Give reasons for non-participation at each stage	6,7
		(c) Consider use of a flow diagram	33

Supplementary Table 1. (continued).

	Item No	Recommendation	Page No
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9,Table1, Suppl T1
		(b) Indicate number of participants with missing data for each variable of interest	7,33
		(c) Summarise follow-up time (eg, average and total amount)	9,Table1
Outcome data	15*	Report numbers of outcome events or summary measures over time	21-26
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9,Table2, Table 3, Table 4
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for exposed and unexposed groups.

Supplementary Table 2. Clinicopathological characteristics of patients who underwent SN biopsy, stratified for the Dutch and MIA cohorts, stratified for the 1st, 2nd and 3rd month after excisional biopsy.

	Dutch cohort (n=7660)				MIA cohort (n=3478)			
Characteristic	**1st month (n=2207)**	**2nd month (n=4240)**	**3rd month (n=1213)**	**p-value**	**1st month (n=1920)**	**2nd month (n=1357)**	**3rd month (n=201)**	**p-value**
Sex (n (%))				0.46				0.62
Female	1112 (50.4)	2175 (51.3)	638 (52.6)		788 (41.0)	562 (41.4)	76 (37.8)	
Male	1095 (49.6)	2065 (48.7)	575 (47.4)		1132 (59.0)	795 (58.6)	125 (62.2)	
Median age in years (IQR)	54 (43-65)	55 (44-66)	56 (44-66)	0.003	59 (48-68)	59 (47-69)	61 (47-71)	0.30
Median Breslow thickness in mm (IQR)	1.8 (1.2-2.8)	1.7 (1.2-2.7)	1.7 (1.2-2.9)	0.12	2.0 (1.3-3.3)	1.9 (1.3-3.1)	1.8 (1.2-3.3)	0.26
Breslow thickness in mm (n (%))				0.12				0.13
≤1.0	245 (11.1)	517 (12.2)	168 (13.8)		226 (11.8)	156 (11.5)	35 (17.4)	
1.1-2.0	1050 (47.6)	2055 (48.5)	566 (46.7)		755 (39.3)	573 (42.2)	73 (36.3)	
2.1-4.0	677 (30.7)	1241 (29.3)	338 (27.9)		612 (31.9)	423 (31.2)	60 (29.9)	
>4.0	235 (10.6)	427 (10.1)	141 (11.6)		327 (17.0)	205 (15.1)	33 (16.4)	
Ulceration (n (%))				0.03				0.03
No	1592 (72.1)	3178 (75.0)	913 (75.3)		1346 (70.1)	1008 (74.3)	148 (73.8)	
Yes	615 (27.9)	1062 (25.0)	300 (24.7)		574 (29.9)	349 (25.7)	53 (26.4)	
Mitoses (n (%))				0.03				0.48
No	152 (6.9)	373 (8.8)	113 (9.3)		149 (7.8)	93 (6.9)	18 (9.0)	
Yes	1415 (64.1)	2720 (64.2)	759 (62.6)		1728 (90.0)	1242 (91.5)	180 (89.6)	
Missing	640 (29.0)	1147 (27.1)	341 (28.1)		43 (2.2)	22 (1.6)	3 (1.5)	
Primary site (n (%))				<0.001				0.73
Head & Neck	84 (3.8)	285 (6.7)	109 (9.0)		339 (17.7)	249 (18.3)	46 (22.9)	
Trunk	1019 (46.2)	1899 (44.8)	540 (44.5)		745 (38.8)	519 (38.2)	72 (35.8)	

Supplementary Table 2. (continued).

Characteristic	Dutch cohort (n=7660)				MIA cohort (n=3478)			
	1st month (n=2207)	2nd month (n=4240)	3rd month (n=1213)	p-value	1st month (n=1920)	2nd month (n=1357)	3rd month (n=201)	p-value
Upper limb	334 (15.1)	663 (15.6)	168 (13.8)		371 (19.3)	262 (19.3)	39 (19.4)	
Lower limb	770 (34.9)	1393 (32.9)	396 (32.6)		465 (24.2)	327 (24.1)	44 (21.9)	
Melanoma subtype (n (%))				<0.001				0.03
Superficial spreading	1387 (62.8)	2763 (65.2)	769 (63.4)		819 (42.7)	628 (46.3)	87 (43.3)	
Nodular	538 (24.4)	970 (22.9)	285 (23.5)		660 (34.4)	405 (29.8)	56 (27.9)	
Lentigo maligna	13 (0.6)	34 (0.8)	20 (1.6)		49 (2.6)	23 (1.7)	3 (1.5)	
Acral lentignious	35 (1.6)	55 (1.3)	39 (3.2)		34 (1.8)	27 (2.0)	2 (1.0)	
Desmoplastic	7 (0.3)	27 (0.6)	8 (0.7)		171 (8.9)	135 (9.9)	22 (10.9)	
Missing	227 (10.3)	391 (9.2)	92 (7.6)		187 (9.7)	139 (10.2)	31 (15.4)	
SN result (n (%))				0.33				0.49
Negative	1687 (76.4)	3287 (77.5)	918 (75.7)		1543 (80.4)	1108 (81.7)	167 (83.1)	
Positive	520 (23.6)	953 (22.5)	295 (24.3)		377 (19.6)	249 (18.3)	34 (16.9)	
Median follow-up time in years (IQR)	4.7 (3.2-7.0)	5.0 (3.3-7.5)	5.3 (3.6-7.9)	<0.001	3.2 (1.4-5.8)	3.1 (1.4-5.5)	2.7 (1.1-5.1)	0.27

Supplementary Table 3. Multivariable logistic regression for SN status, stratified for the total Dutch (n=7660) and MIA cohorts (n=3478).

		Dutch cohort				MIA cohort			
		Multivariable 1		Multivariable 2		Multivariable 1		Multivariable 2	
Variable	Class	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Time interval to SN	Per week	1.01 (0.98-1.03)	0.56	-	-	0.99 (0.94-1.03)	0.53	-	-
Time interval to SN	1st month	-	-	1		-	-	1	
	2nd month	-	-	1.00 (0.88-1.14)	0.99	-	-	0.96 (0.79-1.16)	0.65
	3rd month	-	-	1.11 (0.94-1.32)	0.23	-	-	0.97 (0.64-1.45)	0.90
Sex	Male	1		1		1		1	
	Female	0.71 (0.64-0.79)	<0.0001	0.79 (0.70-0.88)	<0.0001	0.93 (0.78-1.10)	0.37	0.97 (0.79-1.17)	0.73
Breslow thickness	T1	1		1		1		1	
	T2	1.88 (1.49-2.39)	<0.0001	1.78 (1.41-2.27)	<0.0001	1.75 (1.22-2.60)	0.004	1.93 (1.32-2.90)	0.0001
	T3	4.46 (3.54-5.67)	<0.0001	3.94 (3.09-5.07)	<0.0001	3.49 (2.43-5.14)	<0.0001	4.28 (2.90-6.49)	<0.0001
	T4	7.19 (5.57-9.36)	<0.0001	6.02 (4.55-8.00)	<0.0001	4.54 (3.11-6.80)	<0.0001	6.19 (4.04-9.70)	<0.0001
Age at diagnosis	18-35	1		1		1		1	
	36-55	0.94 (0.27-0.37)	0.47	0.88 (0.73-1.07)	0.19	0.74 (0.28-0.45)	0.04	0.64 (0.47-0.86)	0.004
	56-75	0.94 (0.80-1.14)	0.57	0.74 (0.62-0.90)	0.002	0.59 (0.45-0.78)	0.0002	0.41 (0.31-0.56)	<0.0001
	>75	1.02 (0.79-1.32)	0.87	0.68 (0.52-0.89)	0.006	0.41 (0.28-0.60)	<0.0001	0.25 (0.17-0.38)	<0.0001
Primary site	H&N	1		1		1		1	
	Trunk	1.27 (1.02-1.62)	0.04	1.48 (1.16-1.90)	0.002	1.18 (0.93-1.51)	0.18	1.14 (0.89-1.48)	0.31
	Upper limb	0.72 (0.55-0.94)	0.02	0.90 (0.68-1.20)	0.45	0.54 (0.39-0.74)	0.0001	0.55 (0.39-0.77)	0.0006
	Lower limb	1.22 (0.97-1.56)	0.10	1.49 (1.16-1.93)	0.002	1.42 (1.10-1.83)	0.008	1.33 (1.00-1.77)	0.048
Ulceration	No	1		1		1		1	
	Yes	2.10 (1.88-2.36)	<0.0001	1.37 (1.21-1.56)	<0.0001	1.97 (1.65-2.35)	<0.0001	1.49 (1.22-1.82)	<0.0001

Supplementary Table 3. (continued).

		Dutch cohort				MIA cohort			
		Multivariable 1		Multivariable 2		Multivariable 1		Multivariable 2	
Variable	Class	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Melanoma subtype	SSM	1		1		1		1	
	Nodular	1.60 (1.42-1.81)	<0.0001	0.95 (0.83-1.09)	0.45	1.03 (0.86-1.25)	0.73	0.64 (0.52-0.80)	<0.0001
	ALM	2.20 (1.52-3.16)	<0.0001	1.52 (1.02-2.24)	0.04	2.67 (1.58-4.45)	0.0002	1.77 (1.00-3.11)	0.048
	Other	1.06 (0.88-1.27)	0.53	0.85 (0.70-1.02)	0.08	0.41 (0.31-0.54)	<0.0001	0.36 (0.27-0.48)	<0.0001
Mitoses	No	1		1		1		1	
	Yes	2.51 (1.97-3.24)	<0.0001	1.33 (1.55-2.23)	<0.0001	3.86 (2.39-6.72)	<0.0001	2.27 (1.37-4.01)	0.002
	Not known	2.11 (1.63-2.76)	<0.0001	1.28 (1.38-2.20)	0.0002	1.91 (0.74-4.54)	0.16	1.09 (0.39-2.77)	0.87

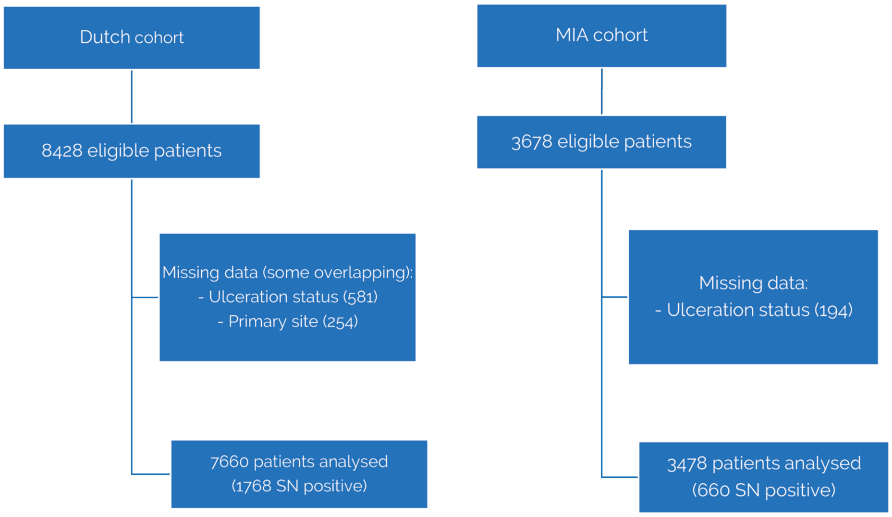
Supplementary Table 4. Uni- and multivariable Cox regression for recurrence-free survival for the Dutch and MIA cohorts.

		Dutch cohort (n events = 2143)						MIA cohort (n events = 945)					
		Univariable		Multivariable 1		Multivariable 2		Univariable		Multivariable 1		Multivariable 2	
Variable	**Class**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Time interval to SN	Per week	0.99 (0.97-1.01)	0.27	0.99 (0.97-1.01)	0.19	-	-	1.01 (0.98-1.04)	0.75	1.02 (0.98-1.05)	0.20	-	-
Time interval to SN	1st month	1		-	-	1		1		-	-	1	
	2nd month	0.91 (0.83-1.01)	0.07	-	-	0.90 (0.81-0.99)	0.03	0.99 (0.87-1.14)	0.92	-	-	1.04 (0.92-1.19)	0.54
	3rd month	0.94 (0.83-1.07)	0.37	-	-	0.90 (0.79-1.03)	0.13	1.16 (0.88-1.52)	0.29	-	-	1.30 (0.99-1.71)	0.06
SN status	Negative	1		1		1		1		1		1	
	Positive	3.24 (2.97-3.53)	<0.0001	2.51 (2.30-2.75)	<0.0001	2.51 (2.30-2.75)	<0.0001	2.54 (2.23-2.90)	<0.0001	2.48 (2.16-2.85)	<0.0001	2.48 (2.16-2.86)	<0.0001
Sex	Male	1		1		1		1		1		1	
	Female	0.66 (0.60-0.71)	<0.0001	0.81 (0.74-0.89)	<0.0001	0.81 (0.74-0.89)	<0.0001	0.68 (0.59-0.78)	<0.0001	0.78 (0.68-0.90)	0.0006	0.78 (0.68-0.90)	0.0005
Breslow thickness	T1	1		1		1		1		1		1	
	T2	2.21 (1.74-2.80)	<0.0001	1.79 (1.41-2.28)	<0.0001	1.79 (1.41-2.28)	<0.0001	2.37 (1.66-3.36)	<0.0001	1.78 (1.25-2.53)	0.001	1.78 (1.25-2.56)	0.001
	T3	5.59 (4.42-7.06)	<0.0001	3.12 (2.45-3.97)	<0.0001	3.12 (2.45-3.97)	<0.0001	4.42 (3.12-6.25)	<0.0001	2.38 (1.66-3.40)	<0.0001	2.38 (1.67-3.40)	<0.0001
	T4	10.70 (8.40-13.62)	<0.0001	4.59 (3.56-5.91)	<0.0001	4.59 (3.56-5.91)	<0.0001	7.25 (5.09-10.31)	<0.0001	3.47 (2.40-5.01)	<0.0001	3.49 (2.42-5.03)	<0.0001
Age at diagnosis	18-35	1		1		1		1		1		1	
	36-55	1.49 (1.24-1.80)	<0.0001	1.55 (1.28-1.87)	<0.0001	1.55 (1.28-1.87)	<0.0001	1.32 (0.99-1.78)	0.06	1.41 (1.05-1.90)	0.02	1.41 (1.05-1.90)	0.02
	56-75	2.56 (2.13-3.07)	<0.0001	2.35 (1.95-2.82)	<0.0001	2.35 (1.96-2.83)	<0.0001	2.15 (1.62-2.84)	<0.0001	2.11 (1.59-2.81)	<0.0001	2.12 (1.59-2.82)	<0.0001
	>75	4.57 (3.70-5.64)	<0.0001	3.64 (2.94-4.51)	<0.0001	3.66 (2.96-4.54)	<0.0001	3.74 (2.75-5.09)	<0.0001	3.50 (2.54-4.81)	<0.0001	3.51 (2.55-4.83)	<0.0001
Primary site	H&N	1		1		1		1		1		1	
	Trunk	0.67 (0.58-0.79)	<0.0001	0.68 (0.58-0.80)	<0.0001	0.68 (0.58-0.80)	<0.0001	0.66 (0.56-0.78)	<0.0001	0.68 (0.57-0.80)	<0.0001	0.68 (0.58-0.81)	<0.0001
	Upper limb	0.50 (0.42-0.61)	<0.0001	0.56 (0.47-0.68)	<0.0001	0.56 (0.47-0.68)	<0.0001	0.53 (0.42-0.65)	<0.0001	0.63 (0.50-0.78)	<0.0001	0.63 (0.50-0.78)	<0.0001
	Lower limb	0.63 (0.54-0.75)	<0.0001	0.72 (0.61-0.85)	<0.0001	0.71 (0.60-0.84)	<0.0001	0.80 (0.67-0.95)	0.01	0.86 (0.71-1.03)	0.11	0.86 (0.72-1.04)	0.12
Ulceration	No	1		1		1		1		1		1	
	Yes	2.89 (2.67-3.15)	<0.0001	1.76 (1.60-1.93)	<0.0001	1.76 (1.60-1.93)	<0.0001	2.25 (1.97-2.56)	<0.0001	1.51 (1.31-1.73)	<0.0001	1.51 (1.32-1.73)	<0.0001
Mitoses	No	1		1		1		1		1		1	
	Yes	2.31 (1.87-2.87)	<0.0001	1.25 (1.01-1.57)	0.045	1.25 (1.01-1.57)	0.045	3.80 (2.46-5.85)	<0.0001	2.20 (1.42-3.42)	0.0004	2.20 (1.42-3.41)	0.0004
	Not known	1.77 (1.41-2.21)	<0.0001	1.16 (0.93-1.46)	0.19	1.16 (0.93-1.46)	0.20	3.23 (1.67-6.27)	0.0005	2.09 (1.07-4.05)	0.03	2.10 (1.07-4.09)	0.03

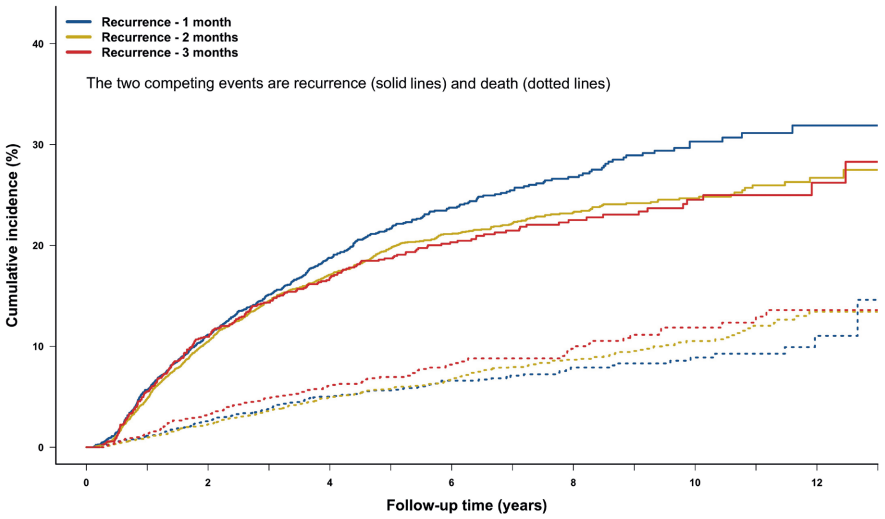
Supplementary Table 5. Uni- and multivariable Cox regression for overall survival for the Dutch and MIA cohorts.

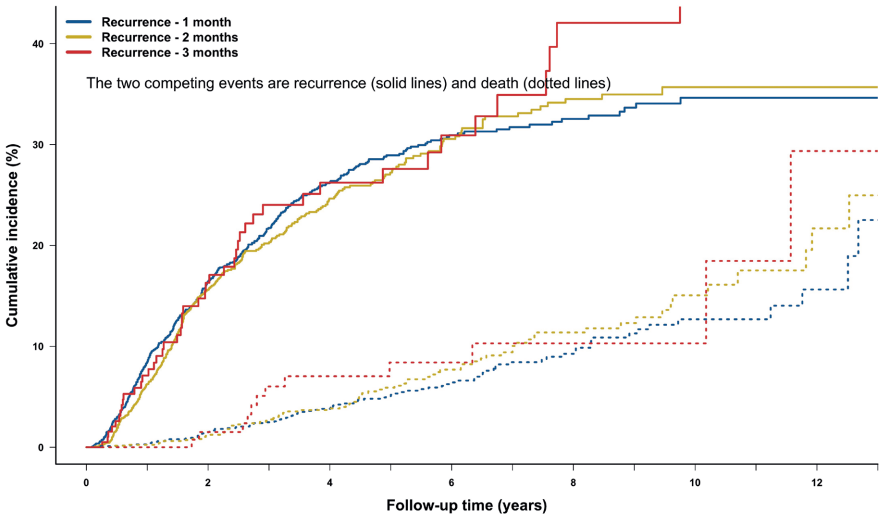
		Dutch cohort (n events = 1430)						MIA cohort (n events = 607)					
		Univariable		Multivariable 1		Multivariable 2		Univariable		Multivariable 1		Multivariable 2	
Variable	Class	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Time interval to SN	Per week	0.99 (0.97-1.02)	0.59	0.99 (0.97-1.01)	0.28	-	-	1.01 (0.97-1.05)	0.59	1.02 (0.97-1.06)	0.37	-	-
Time interval to SN	1st month	1		-	-	1		1		-	-	1	
	2nd month	0.95 (0.84-1.07)	0.41	-	-	0.93 (0.83-1.05)	0.24	0.99 (0.84-1.17)	0.88	-	-	1.00 (0.85-1.18)	0.99
	3rd month	0.98 (0.84-1.15)	0.80	-	-	0.93 (0.79-1.10)	0.39	1.21 (0.87-1.70)	0.26	-	-	1.38 (0.98-1.93)	0.06
SN status	Negative	1		1		1		1		1		1	
	Positive	3.14 (2.83-3.49)	<0.0001	2.41 (2.16-2.69)	<0.0001	2.41 (2.16-2.69)	<0.0001	2.35 (1.99-2.76)	<0.0001	2.31 (1.95-2.75)	<0.0001	2.32 (1.95-2.75)	<0.0001
Sex	Male	1		1		1		1		1		1	
	Female	0.56 (0.50-0.62)	<0.0001	0.71 (0.64-0.80)	<0.0001	0.71 (0.64-0.80)	<0.0001	0.63 (0.53-0.75)	<0.0001	0.77 (0.64-0.92)	0.004	0.77 (0.64-0.92)	0.003
Breslow thickness	T1	1		1		1		1		1		1	
	T2	2.01 (1.49-2.70)	<0.0001	1.63 (1.21-2.20)	0.001	1.64 (1.21-2.20)	0.001	2.43 (1.53-3.86)	0.0002	1.74 (1.09-2.78)	0.02	1.75 (1.10-2.80)	0.02
	T3	5.28 (3.95-7.07)	<0.0001	2.89 (2.14-3.89)	<0.0001	2.89 (2.14-3.90)	<0.0001	4.47 (2.83-7.05)	<0.0001	2.22 (1.38-3.55)	0.0009	2.22 (1.38-3.55)	0.0009
	T4	10.75 (7.97-14.48)	<0.0001	4.41 (3.23-6.03)	<0.0001	4.42 (3.24-6.03)	<0.0001	7.69 (4.84-12.20)	<0.0001	3.32 (2.05-5.36)	<0.0001	3.34 (2.06-5.39)	<0.0001
Age at diagnosis	18-35	1		1		1		1		1		1	
	36-55	1.43 (1.12-1.82)	0.004	1.40 (1.10-1.79)	0.006	1.40 (1.10-1.79)	0.006	1.38 (0.93-2.06)	0.11	1.39 (0.93-2.07)	0.11	1.38 (0.92-2.07)	0.11
	56-75	2.91 (2.31-3.67)	<0.0001	2.47 (1.95-3.12)	<0.0001	2.47 (1.95-3.12)	<0.0001	2.41 (1.65-3.52)	<0.0001	2.18 (1.48-3.22)	<0.0001	2.19 (1.49-3.22)	<0.0001
	>75	6.30 (4.86-8.15)	<0.0001	4.74 (3.64-6.15)	<0.0001	4.74 (3.65-6.16)	<0.0001	5.65 (3.77-8.47)	<0.0001	5.07 (3.34-7.70)	<0.0001	5.10 (3.36-7.74)	<0.0001
Primary site	H&N	1		1		1		1		1		1	
	Trunk	0.69 (0.57-0.83)	<0.0001	0.74 (0.61-0.89)	0.002	0.74 (0.61-0.90)	0.002	0.78 (0.63-0.96)	0.02	0.80 (0.65-0.99)	0.04	0.81 (0.66-1.00)	0.05
	Upper limb	0.54 (0.44-0.68)	<0.0001	0.66 (0.53-0.83)	0.0004	0.67 (0.53-0.84)	0.0004	0.57 (0.44-0.75)	<0.0001	0.70 (0.53-0.92)	0.01	0.70 (0.53-0.93)	0.01
	Lower limb	0.51 (0.42-0.63)	<0.0001	0.61 (0.50-0.74)	<0.0001	0.61 (0.50-0.75)	<0.0001	0.73 (0.58-0.92)	0.008	0.77 (0.60-0.97)	0.03	0.77 (0.61-0.98)	0.03
Ulceration	No	1		1		1		1		1		1	
	Yes	3.15 (2.84-3.49)	<0.0001	1.88 (1.68-2.10)	<0.0001	1.88 (1.68-2.10)	<0.0001	2.31 (1.97-2.71)	<0.0001	1.52 (1.29-1.81)	<0.0001	1.53 (1.29-1.81)	<0.0001
Mitoses	No	1		1		1		1		1		1	
	Yes	2.07 (1.59 (2.69)	<0.0001	1.04 (0.79-1.36)	0.79	1.04 (0.79-1.36)	0.79	3.50 (2.02-6.07)	<0.0001	2.03 (1.16-3.55)	0.01	2.03 (1.16-3.55)	0.01
	Not known	1.76 (1.34-2.31)	<0.0001	1.10 (0.84-1.45)	0.49	1.10 (0.84-1.45)	0.48	2.11 (0.80-5.54)	0.13	1.37 (0.52-3.63)	0.53	1.38 (0.52-3.66)	0.52

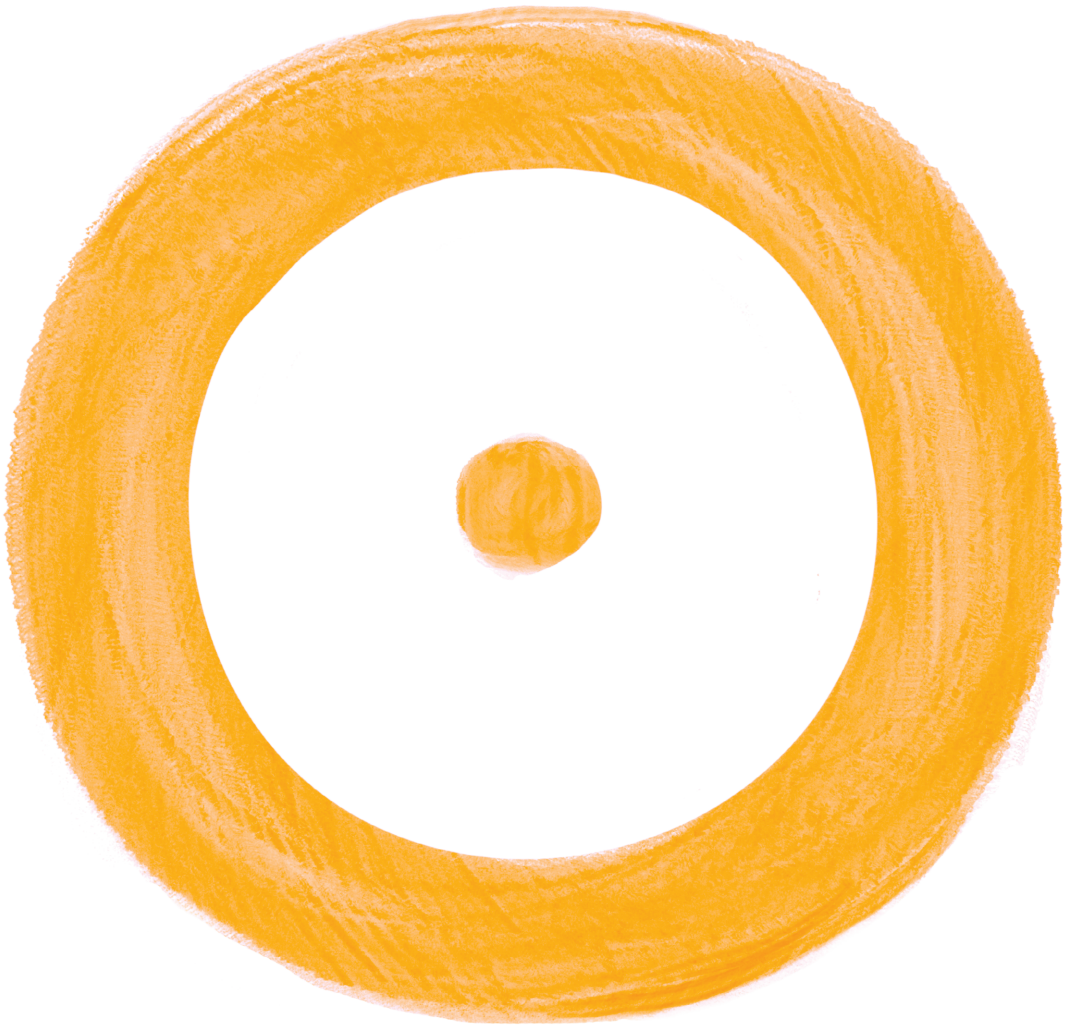
Supplementary Figure 1. Flowchart of patient selection for the Dutch and MIA cohorts.



Supplementary Figure 2. Cumulative incidence for patients with melanoma in the Dutch (upper) and MIA (lower) cohorts for recurrence or death in the 1st, 2nd and 3rd month after excisional biopsy.







CHAPTER 7

Effect of the time interval between melanoma diagnosis and sentinel node biopsy on the size of metastatic tumour deposits in node-positive patients

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ABSTRACT

Introduction: This study sought to assess whether the interval between diagnostic excision-biopsy of a primary melanoma and definitive wide excision with sentinel node biopsy (SNB) influenced the size of SN metastatic deposits, which might have implications for prognosis and management.

Methods: Data were collected for (i) a Dutch population-based cohort of patients treated between 2004 and 2014 who underwent SNB within 100 days of complete excision of their primary melanoma and who were SN-positive with known SN metastasis diameter (n=1027) and (ii) a cohort from a large Australian melanoma treatment centre (n=541) who presented in the same time period. The effects of SNB timing on the size of SN metastatic deposits were analysed.

Results: Dutch patients whose SNB was performed in the second or third months after diagnosis had significantly larger SN metastasis diameters than patients who had their SNB in the first month (median increases of 17% (95%CI -14, 60%, p=0.211) and 71% (95%CI 15, 119%, p=0.004), respectively). No significant difference in tumour diameter for early and late SNB was found in the Australian cohort.

Conclusions: SN metastasis diameter became progressively greater with SN biopsy in the second and third months after primary melanoma diagnosis in the larger, population-based patient cohort. An increase in metastasis diameter was not observed in the smaller, institutional cohort, possibly due to detection of larger SN metastases by routine pre-operative ultrasound, with fine-needle biopsy confirmation. These patients did not proceed to SNB and were therefore not able to be included in the study.

INTRODUCTION

The importance of sentinel node biopsy (SNB) for staging patients with newly-diagnosed melanomas is widely-recognised, and has become even more important since it has been shown that adjuvant systemic therapy reduces the risk of recurrence in SN-positive patients[1, 2]. However, the optimal time interval between melanoma diagnosis (i.e. the date of excisional biopsy of a primary melanoma) and SNB (at the time of definitive wide excision) is not known[3-5]. An early SNB might remove very small metastatic tumour deposits before they have had the opportunity to disseminate further, while a delayed SNB could reveal tumour cells that may have lingered at the primary lesion site or in afferent lymphatics and that would have been missed with an early SNB. On the other hand, a delayed SNB is likely to increase the likelihood of a larger metastatic deposit being present, as cells from the primary melanoma that have spread to regional lymph nodes have had more time to proliferate, potentially affecting the patient's outcome adversely[6, 7]. Only one previous study has assessed the effect of time to SNB on the size of SN metastases, and no significant difference was found[8]. However, that study involved a sample of only 568 patients, indicating that more data are needed to address this matter and consequently guide the timing of SNB.

The aim of the present study was therefore to re-assess the influence of the time interval between diagnostic biopsy of a melanoma and SNB on the SN metastasis diameter. To do this, population-based data from the Netherlands and data from a large specialised melanoma treatment centre in Australia were analysed.

METHODS

Collection of data

Data were collected for patients with newly diagnosed primary invasive cutaneous melanomas treated between January 2004 and December 2014, whose SNB was performed within 100 days of complete diagnostic excision of their primary melanoma and with a known SN metastasis diameter. Information for a Dutch cohort was obtained from PALGA, the Dutch Pathology Registry[9], and information for an Australian cohort was obtained from the prospectively-maintained database of Melanoma Institute Australia (MIA) in Sydney. Patients whose initial wide excision was performed elsewhere but who were later referred to MIA for further management or follow-up were excluded, to eliminate referral bias. De-identified data from both sources were analysed. Ethical approval was obtained from the board of PALGA for the Dutch cohort and from the Research Committee of MIA for the Australian cohort. All MIA patients had given consent for use of their database information for research purposes.

Study population

Patients were excluded if they underwent their SNB on the same day as the diagnostic excision-biopsy of their melanoma (n=34 and n=69 in the Dutch and MIA cohorts, respectively). Patients who had an initial partial biopsy revealing invasive melanoma but who did not have a complete excision of the melanoma prior to definitive wide excision and SNB were also excluded, as were those with synchronous, clinically-detected in-transit, nodal or distant metastases, patients aged <18 years and those with multiple primary melanomas. For each patient, the following demographic and pathological data were collected: date of diagnosis, age, sex, primary tumour anatomical site, Breslow thickness, melanoma subtype, SN status, presence or absence of ulceration and mitoses. Patients with missing pre-specified clinicopathological characteristics were excluded. Time interval was calculated in weeks from the date of complete diagnostic excision of the melanoma to the date of SNB and was analysed both continuously and categorically. Patients were categorised into three groups: (i) those who had their SNB performed within the first month after complete diagnostic excision of their primary melanoma, (ii) those who had it performed in the second month, and (iii) those who had it performed in the third month. The clinical outcome assessed in this study was the maximum diameter (in mm) of the largest nodal tumour deposit, measured as recommended in the guidelines published by the EORTC[10]. Other clinical outcomes (SN-positivity, recurrence-free survival and overall survival) were analysed, and will be reported elsewhere.

Statistical analysis

Analyses were performed independently for the Dutch and MIA cohorts. Patient and tumour characteristics were summarised using descriptive statistics and stratified by cohort. Differences in terms of clinicopathological features between the two cohorts were tested using chi-square and Mann-Whitney U tests for categorical and continuous variables, respectively. The effects of the interval between complete diagnostic excision of a primary melanoma and SNB on mean SN metastasis diameter were assessed using univariable and multivariable linear regression models. The multivariable models were adjusted for sex, Breslow thickness, age, primary site, ulceration, mitoses and melanoma subtype. The distribution of SN metastasis diameter was examined to check whether there was a normal distribution. The effects of the interval between complete diagnostic excision of a primary melanoma and SNB on median SN metastasis diameter was also evaluated using relative change in medians and Wilcoxon signed-rank test.

The study was conducted according to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) recommendations and a flowchart of patient selection procedures is provided (Supplementary Materials Table 1 and Supplementary Figure 1, respectively)[11]. All statistical analyses were performed using R version 3.6.1 (R Core Team, Vienna, Austria). A two-sided p-value of <0.05 was considered statistically significant.

RESULTS

Clinicopathological features of the 1027 Dutch and 541 MIA patients who were found to be SN-positive are presented in Table 1. A flowchart of the patient selection process is shown in Supplementary Figure 1. The median time to SNB was 34 days (IQR 27-46 days, range 1-100 days) for the Dutch cohort and 27 days (IQR 20-37 days, range 1-100 days) for the MIA cohort.

Table 1. Clinicopathological characteristics of sentinel node-positive patients for whom SN metastasis diameter measurements were available, stratified for the Dutch and the MIA cohorts.

Characteristics	Dutch (n = 1027)	MIA (n = 541)	p-value
Sex (n (%))			0.02
Female	470 (45.8)	212 (39.2)	
Male	557 (54.2)	329 (60.8)	
Median age in years (IQR)	56 (45-66)	56 (44-66)	0.85
Median Breslow thickness in mm (IQR)	2.4 (1.6-3.8)	2.5 (1.8-4.1)	0.03
Breslow thickness in mm (n (%))			0.02
≤1.0	46 (4.5)	28 (5.2)	

Table 1. (continued).

Characteristics	Dutch (n = 1027)	MIA (n = 541)	p-value
1.1-2.0	354 (34.5)	157 (29.0)	
2.1-4.0	424 (41.3)	217 (40.1)	
>4.0	203 (19.8)	139 (25.7)	
Ulceration (n (%))			0.25
No	643 (62.6)	322 (59.5)	
Yes	384 (37.4)	219 (40.5)	
Mitoses (n (%))			<0.001
No	40 (3.9)	11 (2.0)	
Yes	727 (70.8)	530 (98.0)	
Missing	260 (25.3)	0 (0.0)	
Primary site (n (%))			<0.001
Head & Neck	46 (4.5)	91 (16.8)	
Trunk	517 (50.3)	223 (41.2)	
Upper limb	103 (10.0)	57 (10.5)	
Lower limb	361 (35.2)	170 (31.4)	
Melanoma subtype (n (%))			<0.001
Superficial spreading	604 (58.8)	287 (53.0)	
Nodular	284 (27.7)	200 (37.0)	
Lentigo maligna	1 (0.1)	24 (4.4)	
Acral lentignious	27 (2.6)	20 (3.7)	
Desmoplastic	0 (0.0)	8 (1.5)	
Missing	111 (10.8)	2 (0.4)	
Median time to SNB in days (IQR)	34 (27-46)	27 (20-36)	<0.001
Median tumour diameter in mm (IQR)	0.7 (0.3-2.0)	1.1 (0.5-3.0)	<0.001

The median SN metastasis diameter was 0.7mm (IQR 0.3-2.0mm) in the Dutch cohort and 1.1mm (IQR 0.5-3.0mm) in the MIA cohort. The median diameter increased per month in the Dutch cohort but decreased in the MIA cohort. In Dutch patients, the median SN metastasis diameter was 0.6mm (IQR 0.2-1.6mm) when SNB was performed during the first month after complete diagnostic excision of their primary melanoma, 0.7mm (IQR 0.3-2.0mm) during the second month, and 1.2mm (IQR 0.4-3.8mm) when performed during the third month (Table 2). When SNB was performed during the first month after complete diagnostic excision of a primary melanoma, 37.6% of Dutch patients had a SN metastasis diameter >1.0mm, compared to 41.0% and 52.7% during the second and third months, respectively. In MIA patients, the median metastasis diameter was 1.3mm (IQR 0.5-3.0mm) when SNB was performed during the first month, 1.0mm (IQR 0.4-3.0mm) during the second month and 1.0mm (IQR 0.3-2.5mm) during the third month. For MIA patients, 52.3% had a SN metastasis diameter >1.0mm during the first month, compared to 49.1% and 34.8% during the second and third months, respectively. Supplementary Figure 2 shows that the distribution of SN metastasis diameter was normally distributed on a logarithmic scale but not on a linear scale. Therefore, SN metastasis diameter was log-transformed for the univariable and multivariable linear regressions shown in Table 3. Figure 1 shows the distribution of SN metastasis diameter in both cohorts when SNB was performed during the first, second and third month after complete diagnostic excision. In multivariable analysis, Dutch patients in whom SNB was performed in the second or third month after diagnosis had significantly greater metastasis diameters than those in whom it was performed during the first month after complete diagnostic excision of their primary melanoma (effect 26% (95%CI 0 to 59%, p=0.049) and 78% (95%CI 25 to152%, p=0.001), respectively. These results were consistent with the median analysis. In the Dutch cohort, the relative change in medians between the second or third month after diagnosis with respect to the first month were 17% (95%CI -14 to 60%, p=0.211) and 71% (95%CI 15 to 119%, p=0.003) respectively. No statistically significant association between time to SNB and metastasis diameter was found in the MIA cohort.

Table 2. Median tumour diameter per month after complete diagnostic excision of a primary melanoma.

	Dutch cohort				MIA cohort			
Month(s) after diagnosis	N total =1027	Median diameter (IQR)	N (%) diameter ≤1.0mm	N (%) diameter >1.0mm	N total =541	Median diameter (IQR)	N (%) diameter ≤1.0mm	N (%) diameter >1.0mm
1st	330	0.6 (0.2-1.6)	206 (62.4)	124 (37.6)	306	1.3 (0.5-3.0)	146 (47.7)	160 (52.3)
2nd	568	0.7 (0.3-2.0)	335 (59.0)	233 (41.0)	212	1.0 (0.4-3.0)	108 (50.9)	104 (49.1)
3rd	129	1.2 (0.4-3.8)	61 (47.3)	68 (52.7)	23	1.0 (0.3-2.5)	15 (65.2)	8 (34.8)

Table 3. Multivariable multiple linear regression for tumour diameter (log-transformed) in SN-positive patients, stratified for the Dutch (n=1027) and MIA cohorts (n=541).

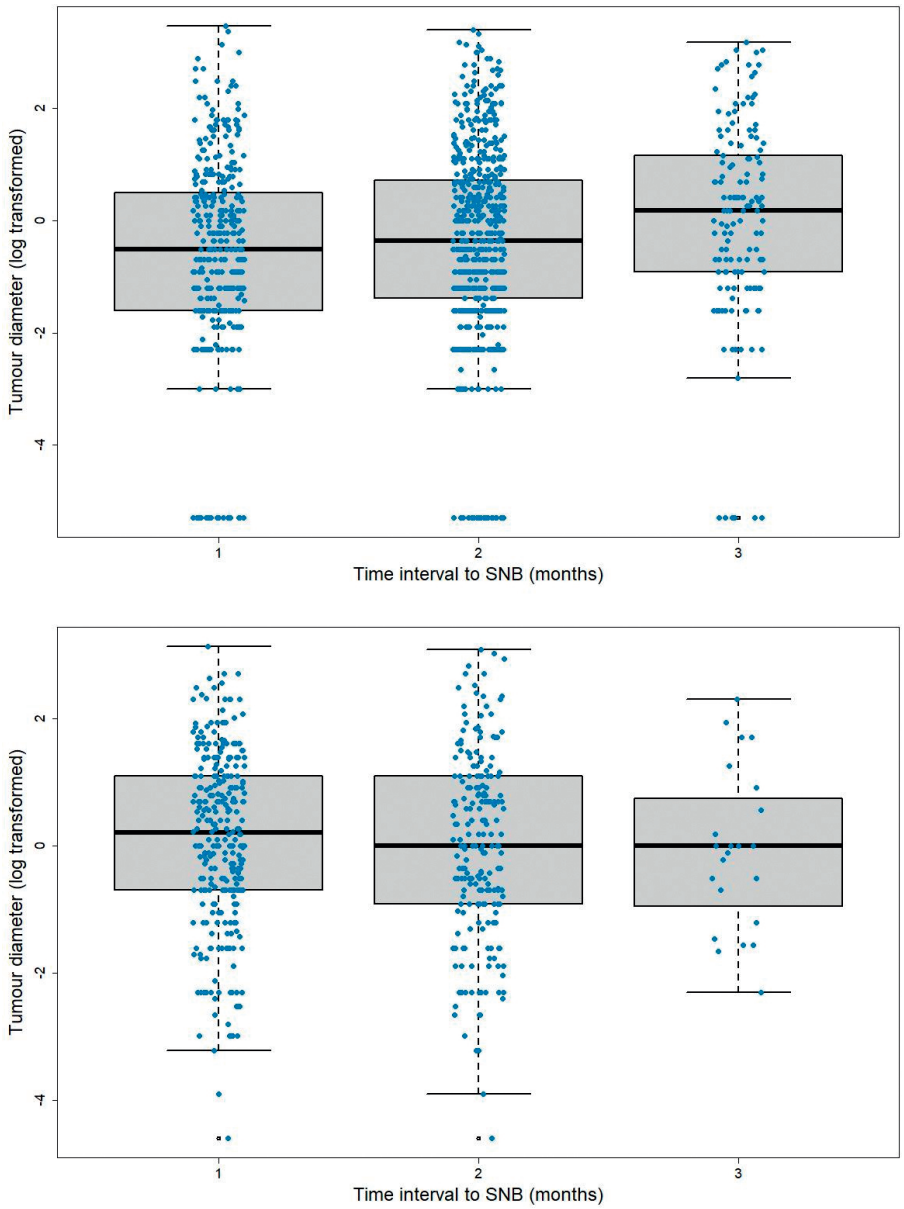
		Dutch cohort				MIA cohort			
		Multivariable 1		Multivariable 2		Multivariable 1		Multivariable 2	
Variable	Class	Effect§ (95% CI)*	p-value	Effect§ (95% CI)*	p-value	Effect§ (95% CI)**	p-value	Effect§ (95% CI)**	p-value
Time interval to SN	Per week	9% (4,14)	0.0003	-	-	-2% (-7,44)	0.55	-	-
Time interval to SN	1st month	-	-	1		-	-	1	
	2nd month	-	-	26% (0,59)	0.049	-	-	2% (-19,29)	0.87
	3rd month	-	-	78% (25,152)	0.001	-	-	0% (-44,76)	0.98
Sex	Male	1		1		1		1	
	Female	-30% (-43,-12)	0.002	-18% (-35,2)	0.07	-20% (-37,2)	0.07	-9% (-29,15)	0.42
Breslow thickness	T1	1		1		1		1	
	T2	60% (-6,171)	0.08	59% (-6,170)	0.08	35% (-21,129)	0.27	16% (-33,104)	0.559

Table 3. (continued).

		Dutch cohort				MIA cohort			
		Multivariable 1		Multivariable 2		Multivariable 1		Multivariable 2	
Variable	Class	Effect[§] (95% CI)*	p-value	Effect[§] (95% CI)*	p-value	Effect[§] (95% CI)**	p-value	Effect[§] (95% CI)**	p-value
	T3	166% (58,349)	0.0003	141% (41,310)	0.001	133% (39,292)	0.001	90% (9,234)	0.03
	T4	499% (245,937)	<0.0001	382% (173,752)	<0.0001	348% (162,665)	<0.0001	229% (81,497)	0.0001
Age at diagnosis	18-35	1		1		1		1	
	36-55	29% (-12,89)	0.19	16% (-19,68)	0.42	8% (-27,59)	0.71	-8% (-36,34)	0.67
	56-75	60% (10,134)	0.01	27% (-12,83)	0.20	7% (-27,56)	0.74	-21% (-45,14)	0.21
	>75	155% (51,332)	0.0005	88% (13,212)	0.02	61% (-5,172)	0.08	2% (-39,71)	0.95
Primary site	H&N	1		1		1		1	
	Trunk	-32% (-60,17)	0.16	-19% (-52,36)	0.42	9% (-22,53)	0.62	10% (-20,53)	0.56
	Upper limb	-33% (-64,24)	0.20	-9% (-50,67)	0.76	10% (-31,74)	0.69	-4% (-38,49)	0.90
	Lower limb	-51% (-71,-15)	0.01	-41% (-65,1)	0.06	-25% (-47,8)	0.12	-20% (-44,14)	0.23
Ulceration	No	1		1		1		1	
	Yes	69% (35,112)	<0.0001	24% (-2,56)	0.07	88% (49,138)	<0.0001	39% (8,78)	0.01
Melanoma subtype	SSM	1		1		1		1	
	Nodular	82% (42,134)	<0.0001	23% (-4,59)	0.11	43% (11,84)	0.005	0% (-23,29)	0.99
	ALM	16% (-41,129)	0.68	-4% (-51,88)	0.90	4% (-42,85)	0.90	-2% (-45,75)	0.95
	Other	55% (8,121)	0.02	34% (-5,90)	0.10	51% (-11,155)	0.12	23% (-26,105)	0.43
Mitoses	No	1		1		1		1	
	Yes	31% (-26,131)	0.36	-14% (-50,50)	0.60	158% (12,494)	0.03	49% (-35,244)	0.35
	Not known	28% (-29,133)	0.41	-11% (-50,57)	0.68	NA	NA	NA	NA

[§]Effect is calculated as (exp(coefficient)-1) * 100 and represent the relative change in tumour diameter for every unit increase in in each variable in the model.
* The β-coefficients of the intercept for the Dutch multivariable model were -1.67 (standard error 0.47, p-value 0.0005) and -1.32 (standard error 0.46, p-value=0.004) for the continuous and categorical time interval.
** The β-coefficients of the intercept for the MIA multivariable model were -0.80 (standard error 0.47, p-value 0.09) and -0.89 (standard error 0.45, p-value=0.05) for the continuous and categorical time interval.

Figure 1. Distribution of SN metastasis diameter in in Dutch cohort (upper) and the MIA cohort (lower) when SNB was performed during the first, second and third month after complete diagnostic excision.



DISCUSSION

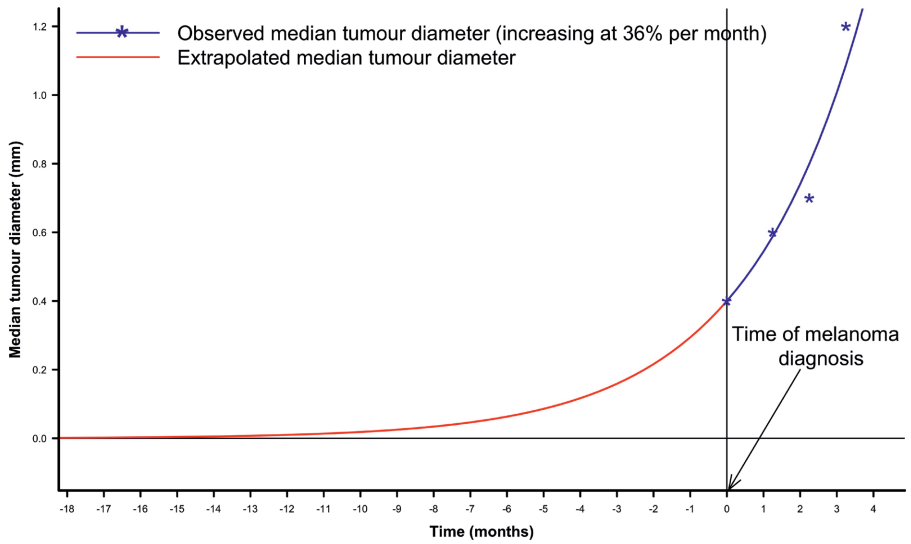
In an attempt to clarify whether the time interval between complete diagnostic excision of a primary melanoma and SNB influenced the size of metastatic tumour deposits found in SNs, we undertook this study using two large, independent cohorts of SN positive melanoma patients, one from the Netherlands and the other from Australia. Dutch patients whose SNB was performed during the second month or the third month after diagnosis had significantly greater metastasis diameters than those whose SNB was performed in the first month. This is what might have been anticipated. However, no significant difference between time to SNB and diameter of SN metastases was observed in the Australian cohort.

Although the presence of larger tumour deposits might be expected if SNB is delayed, clear-cut evidence of this has not been provided in any previous studies. Only one prior investigation has assessed the effect of the time to SNB[8]. This was by Oude Ophuis et al., who compared the SN metastasis diameter of patients with a SNB performed earlier and later than the median of 43 days. They used a chi-square test, and no statistically significant difference was found (p=0.12). As far as we are aware, no other studies assessing the effect of later SNB on SN metastasis size have been published to date. In the current study, discrepant results were found in the two cohorts that were analysed to assess the effect of the time interval on SN metastasis diameter. As previously reported, the latter is at least partly subjective, as it often requires pathologists to make a judgement as to whether tumour cells represent part of a single deposit or multiple separate deposits[12]. The increase in metastasis diameter from 0.6mm (IQR 0.2-1.6) in the first month in the Dutch cohort to 1.2mm (IQR 0.4-3.8) in the third month could be of great clinical significance, because in some countries and in a number of clinical trials a 1.0mm threshold of metastasis diameter determines eligibility for adjuvant systemic therapy for patients with stage IIIA disease and this was exceeded in many cases in the third month. For instance, in the Dutch cohort the diameter of the tumour deposits exceeded 1.0mm in 53% of cases in the third month, whereas only 38% had a metastasis exceeding 1.0mm in the first month. Thus delayed SNB meant that many more patients would have been considered eligible to receive adjuvant systemic therapy.

The finding of an increase in SN metastasis size in patients with a longer time to SNB in the much larger Dutch patient cohort (17% median increase at 2 months, 71% median increase at 3 months) is consistent with what might be expected. There is evidence from the literature that the tumour doubling time for metastatic melanoma at distant sites ranges widely from 1.43 to 4.80 months (although tumour doubling time for metastatic disease in regional lymph nodes has not been reported, nor would it be easily possible to study this)[13]. It should be noted that most estimates of tumour doubling time in both preclinical and clinical situations have been based on

measured tumour diameter, rather than tumour volume. Our results for the Dutch cohort suggest that a period of up to 3 months represents only a small component of the time that the metastatic melanoma has been present in a sentinel node. This would be consistent with the concept that metastasis to regional nodes can occur some time before a melanoma becomes clinically obvious and is diagnosed by excision and histological examination[14]. To demonstrate this, we estimated the time at which the original metastatic melanoma cell reached the SN to initiate what later became a measurable melanoma metastasis in that SN. This was done by estimating the median growth rate using the Dutch data and extrapolating the diameter size over the months before the diagnosis time (see Figure 2). As can be seen in the figure, the estimated time at which the metastasis originated was approximately 18 months before the time at which the primary melanoma was diagnosed by a pathologist (time 0 in Figure 2). According to this growth rate, the tumour-doubling time was 10 weeks, this is the time period at which the tumour diameter would double in size. That no significant increase in size in a 3-month period was observed in the much smaller MIA patient cohort could perhaps be related to inadequate sample size, with this limitation exaggerated by the fact that all patients had high-resolution ultrasound examination of their SNs at the time of their preoperative lymphoscintigram. Those with an ultrasound-detected metastasis in a SN had fine needle biopsy confirmation of the diagnosis, then proceeded directly to a full regional lymph node dissection. Thus, these patients were excluded from the MIA series of SN-positive patients selected for the present study who were included only if they had a SNB procedure.

Figure 2. Extrapolation of SN tumour diameter over time in the Dutch cohort.



Strengths of our study include the large numbers of patients in the two cohorts and the use of nationwide data as well as data from a large, well-maintained institutional database containing prospectively-collected information. While the inclusion of a large number of patients with SN metastasis diameter as an outcome was a strength, SN metastasis volume (rather than diameter) might have been an even more accurate measure of SN metastasis size[15, 16]. However, measuring the volume of SN metastases is difficult and not routinely performed in day-to-day clinical practice. When using diameter as an indicator of tumour deposit size, the assumption is made that the deposit is spherical, which in reality is often not the case. Indeed, they are sometimes thin and elongated, in which circumstance diameter is not a good measure of the number of tumour cells in the metastasis[17]. A major limitation that might have been introduced by the retrospective nature of the current study is selection bias. Another limitation is that although timely diagnosis of melanoma and other cancers is a known favorable prognostic factor, the question remains whether the time interval between the diagnostic biopsy and SNB is the most important time period that influences prognosis. It seems likely that the most important interval is actually from the time that a melanoma first develops until treatment by a physician is sought, a biopsy is performed and a histopathological diagnosis is obtained. Although it is not possible to substantiate this hypothesis, delaying a SNB by a few weeks may be largely inconsequential compared with the latency between malignant transformation of a pre-malignant lesion into a melanoma and its diagnosis.

CONCLUSIONS

The time interval between complete diagnostic excision of a primary melanoma and SNB was associated with SN metastasis diameter in the larger, population-based study cohort, with larger tumour deposits if SNB was delayed, but this association was not observed in the smaller, institution-based cohort. The reasons for this discrepancy were not apparent, and the inconclusive results indicate that further studies are required to clarify this issue. This matter has important clinical implications if the size of SN tumour deposits is to be used as a criterion for recommending adjuvant systemic therapy.

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Supplementary Table 1. STROBE Statement—Checklist of items that should be included in reports of cohort studies.

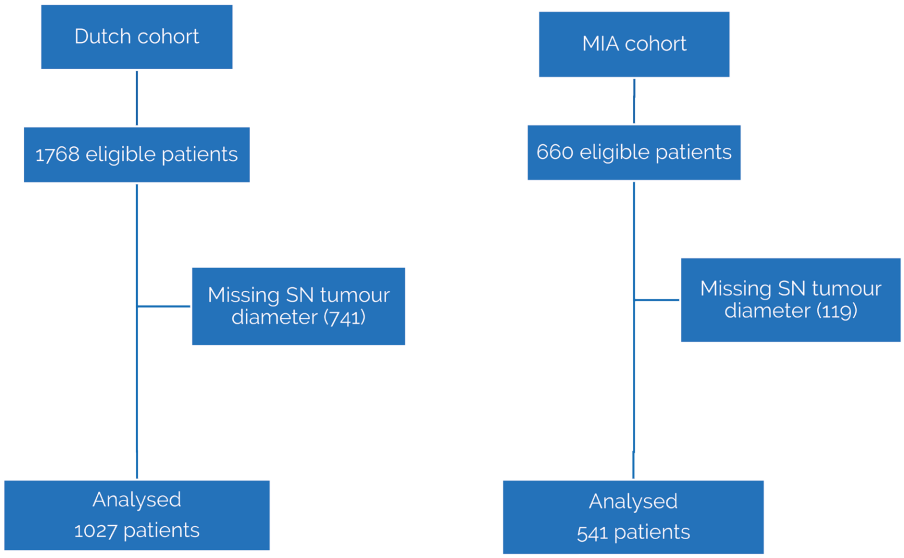
	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6,7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-8
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6,7,22
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	7,8
		(*c*) Explain how missing data were addressed	7,8,22
		(*d*) If applicable, explain how loss to follow-up was addressed	7,8
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9,10,22
		(b) Give reasons for non-participation at each stage	6,7
		(c) Consider use of a flow diagram	22

Supplementary Table 1. (continued).

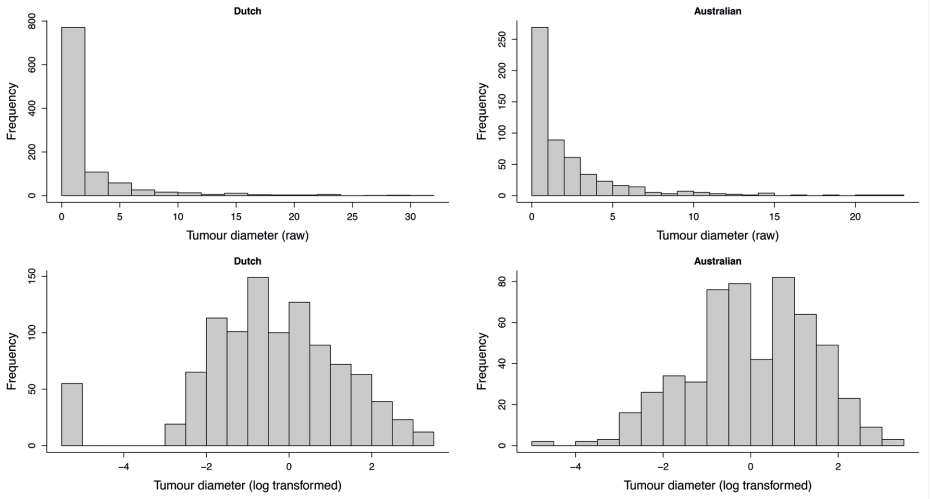
	Item No	Recommendation	Page No
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9,Table1
		(b) Indicate number of participants with missing data for each variable of interest	7,22
		(c) Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Report numbers of outcome events or summary measures over time	Table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9,Table 3
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Supplementary Figure 1. Flowchart of patient selection for the Dutch and MIA cohorts.



Supplementary Figure 2. Histograms of tumour diameter frequencies without and with log transformation. The raw numbers display clearly right-skewed distributions, whereas the log-transformed scales show normal distributions.



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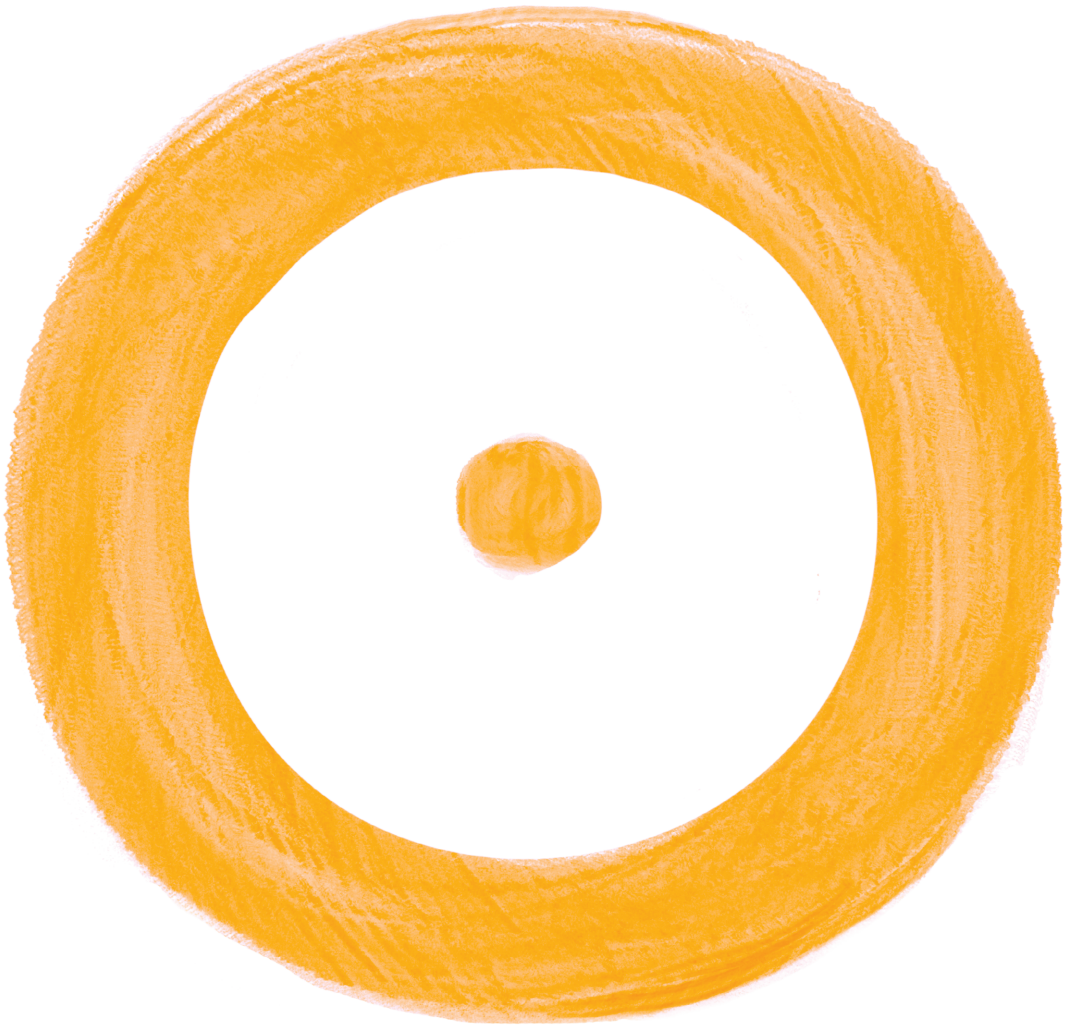
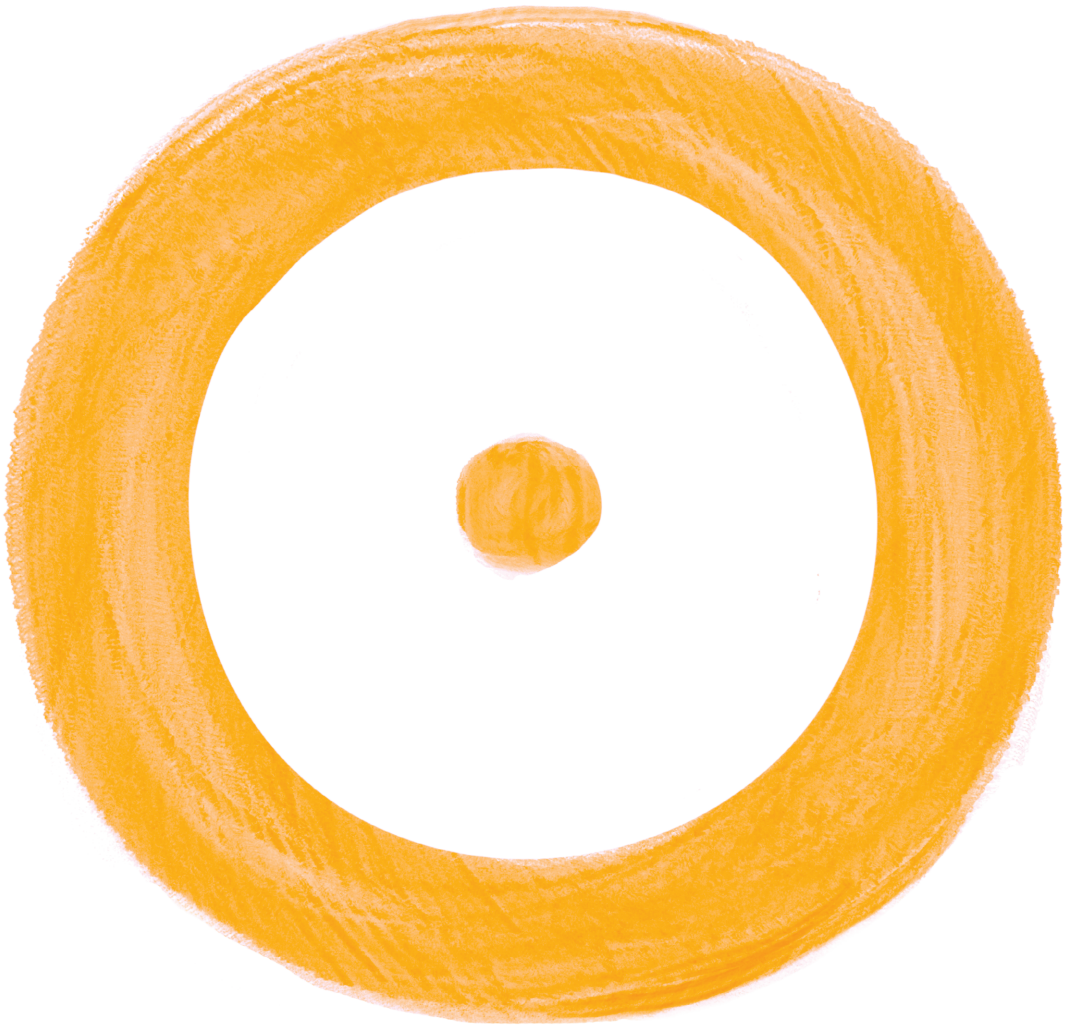


PART II

INDIVIDUAL CLINICOPATHOLOGICAL VARIABLES FOR SURVIVAL



CHAPTER 8

Association of histologic regression with a favorable outcome in patients with stage 1 and stage 2 cutaneous melanoma

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ABSTRACT

Importance: Although regression is commonly observed in cutaneous melanoma, it is uncertain whether it is associated with patient prognosis.

Objective: To determine whether histologically confirmed regression was associated with better or worse survival in patients with primary cutaneous melanoma.

Design, Setting, and Participants: This cohort study analyzed data from 2 large cohorts of adults (one in the Netherlands and the other in Australia) with histologically proven, stage 1 and 2 primary, invasive cutaneous melanoma with known regression status treated between 2000 and 2014, with median follow-up times of 4.5 and 11.1 years for the Dutch and Australian cohorts, respectively. For the Dutch patients, population-based data from PALGA, the Dutch Pathology Registry, were used, and follow-up data were retrieved from the Netherlands Cancer Registry. For the Australian patients, data from the database of a large, specialized melanoma treatment center were used.

Main Outcomes and Measures: Multivariable Cox proportional hazards analyses were performed per cohort to assess recurrence-free survival (RFS) and overall survival (OS), and subgroup analyses according to Breslow thickness category and melanoma subtype were performed.

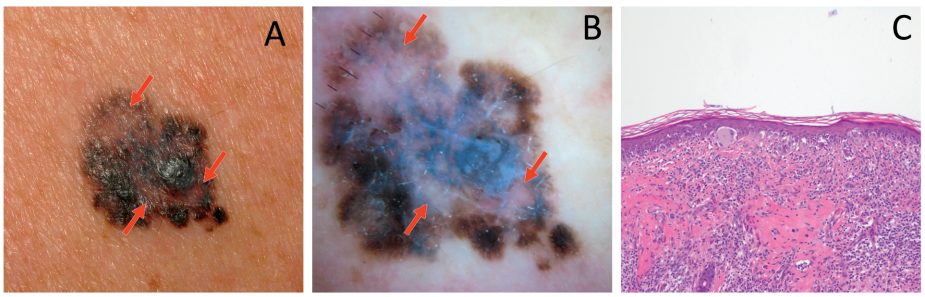
Results: A total of 17 271 Dutch patients and 4980 Australian patients were included. In both cohorts, survival outcomes were better for patients with disease regression. For Dutch patients, the hazard ratio (HR) for those with disease regression was 0.55 (95% CI, 0.48-0.63; P < .001) for RFS and 0.87 (95% CI, 0.79-0.96; P = .004) for OS; for the Australian patients, the HR was 0.61 (95% CI, 0.52-0.72; P < .001) for RFS and 0.73 (95% CI, 0.64-0.84; P < .001) for OS. Subgroup analyses showed that the presence of regression improved RFS within thin and intermediate Breslow thickness melanomas in both cohorts. For patients with superficial spreading melanoma (SSM) subtype, regression improved RFS and OS in both cohorts. For Dutch patients with SSM, the HR for those with disease regression was 0.54 (95% CI, 0.46-0.63; P < .001) for RFS and 0.86 (95% CI, 0.76-0.96; P = .009) for OS; for the Australian patients with SSM, the HR was 0.67 (95% CI, 0.52-0.85; P = .001) for RFS and 0.72 (95% CI, 0.59-0.88; P = .001) for OS.

Conclusions and Relevance: In 2 large patient cohorts from 2 different continents, regression was a favorable prognostic factor for patients with stage 1 and 2 melanomas, especially in those with thin and intermediate thickness tumors and those with SSM subtype.

INTRODUCTION

The phenomenon of regression in a melanoma is commonly observed. It refers to disappearance or loss of part or all of a melanoma that is thought to occur as a consequence of a host immunological response directed against the tumor cells. It can be identified both clinically (macroscopically) and histologically (microscopically). Sometimes apparent to the naked eye (Figure 1A), macroscopic regression can best be appreciated using a dermatoscope to examine a pigmented lesion, revealing the presence of bluish-gray or white scarlike depigmentation (of lighter color than the surrounding skin, and corresponding histopathologically to fibrosis, Figure 1B), or as "peppering" (very fine gray dots seen with a dermatoscope and histologically corresponding to the presence of pigment-laden macrophages).[1] The presence of regression is observed not only in melanoma, but also in benign nevi,[1] and it has been suggested that the decline in the number of nevi after the fifth decade of life may be partially caused by progressive regression of these nevi.[2] Despite the lack of standardized criteria for reporting histopathological regression in melanomas, it is generally characterized by a variable decrease in the number of dermal invasive melanoma cells in a tumor, accompanied by the presence of a host response consisting of dermal fibrosis, an inflammatory infiltrate, melanophages, increased vascularity, and epidermal attenuation (Figure 1C).[3]

Figure 1. Macroscopic regression (A, red arrows), dermatoscopic scar-like depigmentation (B, red arrows), and histopathological regression characterised by immature scar-like fibrosis and a mixed inflammatory cell infiltrate including numerous lymphocytes and pigment-laden macrophages (C). A-C represent the same lesion.



The presence of some histopathologic regression is estimated to occur in between 10% and 58%[4,5] of cutaneous melanomas. Although it is common, divergent conclusions have been drawn about the prognostic significance of regression in cutaneous melanoma. Some have suggested that its presence is associated with a worse prognosis, because it can reduce the measured Breslow thickness of the primary tumor (when the deepest melanoma cells are no longer present). Others have argued that the presence of regression implies better survival, because effective activation of the host immune system against the tumor is presumed to be the basis

of regression.[6,7] When attempts have been made to determine which supposition is correct, several studies found regression to be a favorable prognostic factor,[8,9] whereas multiple others found that regression was not significantly associated with either recurrence-free survival (RFS) or overall survival (OS),[4,10-13] and 2 found that histologic regression was associated with worse OS.[14,15] These divergent results are possibly related to small study group numbers and relatively short follow-up. Hence the aim of the present study was to clarify whether histologic regression was associated with better or worse survival in patients with primary cutaneous melanomas by analyzing data from a nationwide European cohort, as well as data from the well-maintained database of a large, specialized melanoma treatment center in Australia.

METHODS

Collection of data

For the Dutch nationwide cohort, encoded and anonymous data for all patients with newly diagnosed stage 1 and 2 melanomas treated between January 2000 and December 2014 were obtained from PALGA, the Dutch Pathology Registry. PALGA has been collecting data prospectively from all pathology laboratories in the Netherlands since 1991.[16] Follow-up data were obtained from the Netherlands Cancer Registry, which gathers information on every cancer patient treated in the Netherlands. Follow-up was calculated from date of diagnosis until date of death, the date last known to be alive, or January 1, 2018, whichever occurred earlier. Ethical approval was granted by the board of PALGA, and all data were deidentified.

For the Australian institutional cohort, a search was performed of the prospectively maintained database at Melanoma Institute Australia (MIA) for all patients with stage 1 and 2 melanomas treated over the same time period. All patients had given permission for their deidentified data to be used for research purposes. Approval for use of the data was obtained from the Sydney Local Health District Ethics Committee.

Study population

Patients with noncutaneous melanomas were excluded, as well as those with more than 1 primary melanoma. For each patient, demographics collected included date of diagnosis, age, sex, location of the melanoma, and recurrence details. Pathologic data included Breslow thickness (mm), melanoma subtype, sentinel node (SN) biopsy (performed or not performed), ulceration (present or absent), and regression (present or absent). Breslow thickness was measured to the deepest invasive tumor cell (not the base of any regression). Mitotic rate (per mm2) was able to be included for the MIA cohort only because it was not available in the Dutch cohort. The pathology of the cases was reported by a large number of pathologists in the Netherlands (n = 750) and by MIA-affiliated pathologists (n = 17). As such, definitions of regression

used by the pathologists reflected those provided in contemporary literature and textbooks.[17-22] Regression was defined as loss of part or all of a melanoma as a consequence of an immunologic response directed against the tumor and was coded as present or absent. It was broadly recognized by the presence of dermal fibrosis that was unrelated to prior trauma and usually accompanied by increased vascularity, pigment-laden macrophages, and some lymphocytes with or without epidermal thinning and loss of rete ridges (Figure 1C). In cases with residual in situ or invasive melanoma overlying the area of fibrosis, regression was regarded as present as long as the above criteria were fulfilled. Tumor-infiltrating lymphocytes were not considered sufficient for regression in the absence of identifiable dermal fibrosis. For both data sets, primary and secondary outcome measures were RFS and OS. Recurrence was defined as either cutaneous (local or in transit), nodal (regional), or distant metastasis. In patients with synchronous first recurrences at multiple sites, the site with the worst prognosis was recorded as first site. Patient RFS and OS were calculated from the date of diagnosis to the date of recurrence or death from any cause, respectively. Patients without recurrence were censored at either their date of death or the last date known alive or January 1, 2018 (the data collection cutoff date), whichever occurred earlier. Patients were categorized as stage 1 or stage 2 according to the American Joint Committee on Cancer (AJCC) Staging Manual, 8th edition.[23] When no SN biopsy was performed, it was assumed that patients had stage 1 or 2 disease.

Statistical analysis

Data for the Dutch and MIA patients were analyzed separately. Categorical variables were summarized as numbers and percentages. Continuous variables were summarized as medians with interquartile ranges (IQR). Differences in proportions and medians were analyzed using $\chi 2$ or Mann-Whitney U tests, respectively. Kaplan-Meier curves were generated for OS and RFS. Statistical analysis was performed using multivariable Cox proportional hazard models for both cohorts. The variables analyzed included Breslow thickness, sex, age, ulceration, regression, and SN biopsy.[9] Only patients with all these predefined variables available were selected. Age and Breslow thickness were included as continuous variables. The proportional hazards assumption was evaluated using the Schoenfeld residuals test. An additional multivariable Cox analysis was performed including mitotic rate (/mm2) for patients in the MIA cohort. In addition, subgroup analyses were performed considering 2 stratification factors: melanoma subtype (superficial spreading and nodular, other subtypes were not analyzed owing to the small number of events observed), and Breslow thickness category (thin [≤1.0 mm], intermediate [1.1-4.0 mm], and thick [>4.0 mm]). This study adhered to the guideline for the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines, and the checklist was completed as well as a flowchart of patient selection (eTable 1 and eFigure in the Supplement, respectively).[24]

All statistical analyses were performed using R (version 3.6.1, R Core Team). A 2-sided P<.05 was considered statistically significant.

RESULTS

Clinicopathological features of the patients in the Dutch and MIA cohorts with and without histologic regression are presented in Table 1. Of the entire cohort of 17 271 Dutch patients, 6121 (35.4%) showed regression of the primary melanoma. Of the total 4980 patients in the MIA cohort, 2198 (44.1%) showed regression. In both cohorts, similar associations were observed: presence of regression was significantly associated with male sex, lower Breslow thickness, absence of ulceration, superficial spreading melanoma subtype, and location on the trunk. There was no significant association between age and presence of regression in either of the cohorts. The median (IQR) follow-up time was 4.5 (3.1-6.5) years for the Dutch cohort and 11.1 (4.0-17.9) years for the MIA cohort.

Survival analyses

Figure 2 shows the Kaplan-Meier OS and RFS curves for the Dutch and MIA cohorts. For both survival outcome measures and in both cohorts, patients with regression had better survival. All 17 271 Dutch patients and 4980 Australian patients were included in the Cox regression model. Multivariable analyses showed a hazard ratio (HR) of 0.55 (95% CI, 0.48-0.63; P<.001) for RFS and 0.87 (95% CI, 0.79-0.96; P = .004) for OS associated with regression in the Dutch cohort (Table 2). Similarly, an HR of 0.61 (95% CI, 0.52-0.72; P<.001) for RFS and 0.73 (95% CI, 0.64-0.84; P<.001) for OS associated with regression in the MIA cohort was observed. When mitotic rate was included in the model, the HR associated with regression was 0.74 (95% CI, 0.63-0.86; P = .002) for RFS and 0.80 (95% CI, 0.70-0.92; P = .002) for OS in the MIA cohort (eTable 2 in the Supplement).

Subgroup analysis by Breslow thickness

eTable 3 in the Supplement shows the number of included patients for each Breslow thickness category and according to melanoma subtype, together with the number of events (recurrence for RFS and death for OS) for the Dutch and MIA cohorts. When stratifying the Cox analysis according to Breslow thickness, patients with thin and intermediate-thickness melanomas in both cohorts had better RFS if regression was present (eTable 4 in the Supplement). For patients with thin melanomas, the presence of regression was associated with better OS for the MIA cohort only (HR, 0.66; 95% CI, 0.50-0.88; P = .004). In contrast, there was no statistically significant association between regression and RFS or OS in patients with thick melanomas in either cohort; in the Dutch cohort the HRs were 0.74 (95% CI, 0.53-1.02; P = .06) and 1.07 (95% CI, 0.83-1.38; P = .62) for RFS and OS, respectively. In the MIA cohort the HRs were 0.91 (95% CI, 0.65-1.29; P = .60) and 0.78 (95% CI, 0.57-1.08; P = .14), respectively.

Table 1. Clinicopathological factors of all Dutch and MIA patients with stage 1 and 2 primary cutaneous melanoma stratified for regression.

	Dutch cohort			MIA cohort		
Characteristic	**Regression absent (n = 11150)**	**Regression present (n = 6121)**	**p-value**	**Regression absent (n = 2782)**	**Regression present (n = 2198)**	**p-value**
Sex (n (%))						
Female	6444 (57.8)	2872 (46.9)	<0.0001	1280 (46.0)	791 (36.0)	<0.0001
Male	4706 (42.2)	3249 (53.1)		1502 (54.0)	1407 (64.0)	
Median age at diagnosis in years (IQR)	58.0 (46.0-69.0)	58.0 (47.0-68.0)	0.576	59.0 (45.0-71.0)	59.0 (48.0-70.0)	0.236
Median Breslow thickness in mm (IQR)	0.9 (0.5-1.7)	0.6 (0.5-1.0)	<0.0001	1.5 (0.8-3.0)	0.8 (0.5-1.4)	<0.0001
Breslow thickness (n (%))			<0.0001			<0.0001
≤1.0mm	6467 (58.0)	4857 (79.3)		991 (35.6)	1395 (63.5)	
1.1-2.0mm	2509 (22.5)	863 (14.1)		701 (25.2)	465 (21.2)	
2.1-4.0mm	1433 (12.9)	289 (4.7)		651 (23.4)	227 (10.3)	
>4.0mm	741 (6.6)	112 (1.8)		439 (15.8)	111 (5.1)	
Ulceration (n (%))						
No	9696 (87.0)	5748 (93.9)	<0.0001	2171 (78.0)	1972 (89.7)	<0.0001
Yes	1454 (13.0)	373 (6.1)		661 (22.0)	116 (10.3)	
Median mitotic rate / mm2 (IQR)	NA	NA	-	3.0 (1.0-6.0)	1.0 (0.0-3.0)	<0.0001
Primary site (n (%))						
Head & Neck	1484 (13.3)	371 (6.1)	<0.0001	586 (21.1)	189 (8.6)	<0.0001
Trunk	4582 (41.1)	3711 (60.6)		827 (29.7)	1189 (54.1)	
Upper limb	1835 (16.5)	811 (13.2)		550 (19.8)	372 (16.9)	
Lower limb	3249 (29.1)	1228 (20.1)		819 (29.4)	448 (20.4)	

Table 1. Continued.

Characteristic	Dutch cohort			MIA cohort		
	Regression absent (n = 11150)	Regression present (n = 6121)	p-value	Regression absent (n = 2782)	Regression present (n = 2198)	p-value
Melanoma subtype (n (%))			<0.0001			<0.0001
Superficial spreading	8518 (76.4)	5434 (88.8)		1191 (42.8)	1570 (71.4)	
Nodular	1394 (12.5)	249 (4.1)		754 (27.1)	216 (9.8)	
Lentigo maligna	497 (4.5)	160 (2.6)		106 (3.8)	90 (4.1)	
Acral lentignious	74 (0.7)	21 (0.3)		62 (2.2)	16 (0.7)	
Other	667 (6.0)	257 (4.2)		669 (24.0)	306 (13.9)	
SN biopsy (n (%))						
No	8839 (79.3)	5501 (89.9)	<0.0001	1830 (65.8)	1637 (74.5)	<0.0001
Yes	2311 (20.7)	620 (10.1)		952 (34.2)	561 (25.5)	
Median follow-up in years (IQR)	4.2 (2.9-5.9)	5.1 (3.4-7.6)	<0.0001	11.6 (4.2-19.2)	10.7 (3.8-16.7)	<0.0001

Abbreviations: IQR, interquartile range; MIA, Melanoma Institute Australia; NA, not applicable; SN, sentinel node.

Figure 2. Kaplan-Meier curves for RFS and OS for regression status for Dutch and MIA stage I and II melanoma patients.

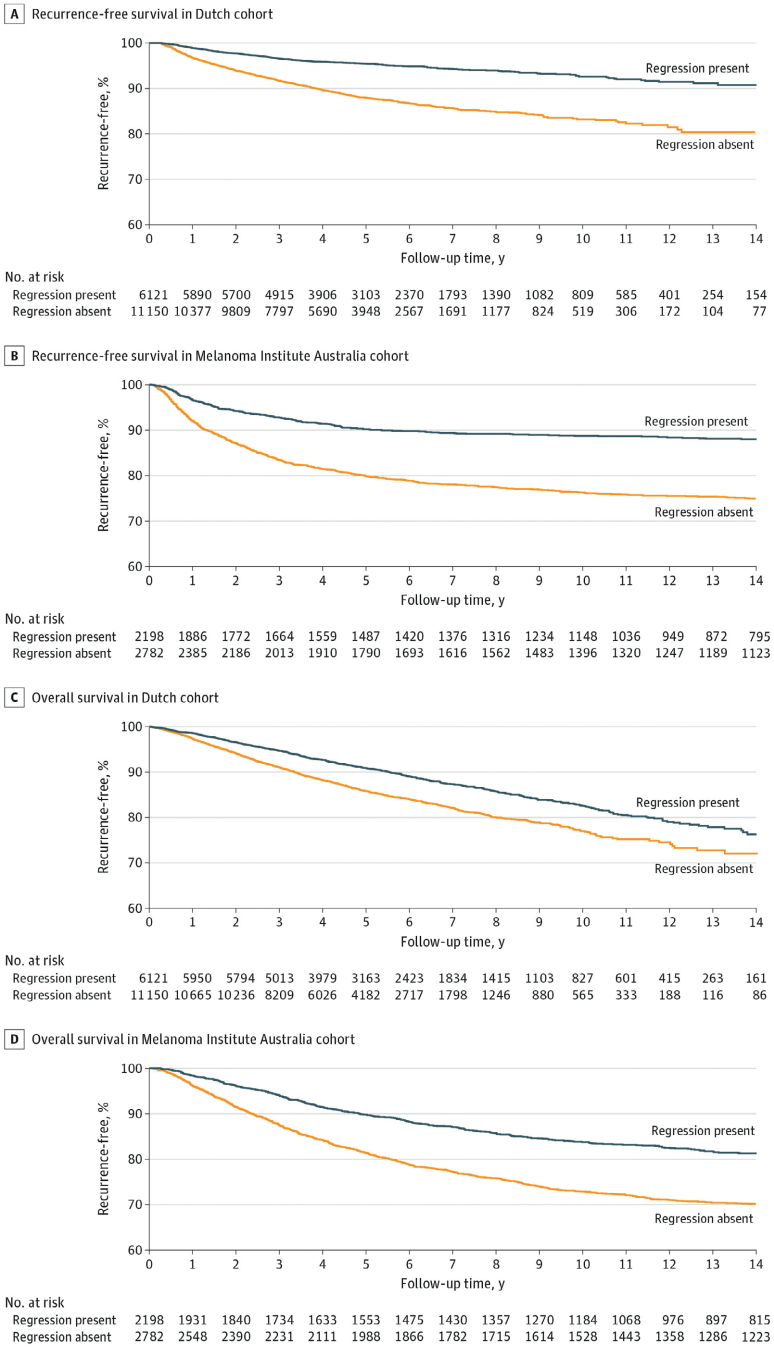


Table 2. Multivariable Cox regression for regression-free and overall survival for all Dutch and MIA patients with stage 1 and 2 melanoma not adjusted for mitotic index.

		Recurrence free survival				Overall survival			
		Dutch (1522 events)		MIA (836 events)		Dutch (2231 events)		MIA (1078 events)	
Variable	Class	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Regression	Present vs Absent	0.55 (0.48-0.63)	<0.0001	0.61 (0.52-0.72)	<0.0001	0.87 (0.79-0.96)	0.004	0.73 (0.64-0.84)	<0.0001
Ulceration	Yes vs No	2.30 (2.03-2.61)	<0.0001	2.00 (1.70-2.34)	<0.0001	1.82 (1.64-2.02)	<0.0001	1.84 (1.61-2.12)	<0.0001
SN biopsy	Yes vs No	1.09 (0.97-1.22)	0.148	1.00 (0.87-1.16)	0.97	0.72 (0.63-0.82)	<0.0001	0.83 (0.73-0.95)	0.006
Breslow thickness	Per mm	1.41 (1.38-1.44)	<0.0001	1.25 (1.22-1.29)	<0.0001	1.28 (1.26-1.31)	<0.0001	1.21 (1.18-1.24)	<0.0001
Sex	Male vs Female	1.41 (1.27-1.56)	<0.0001	1.26 (1.09-1.45)	0.002	1.49 (1.37-1.62)	<0.0001	1.46 (1.28-1.66)	<0.0001
Age	Per year	1.01 (1.00-1.01)	<0.0001	1.01 (1.01-1.01)	<0.0001	1.06 (1.06-1.07)	<0.0001	1.04 (1.04-1.05)	<0.0001

Abbreviations: HR, hazard ratio; MIA, Melanoma Institute Australia; SN, sentinel node.

Subgroup analysis by melanoma subtype

Analysis was conducted only for patients with superficial spreading melanoma (SSM) and nodular melanoma (NM), given the small number of events in the other melanoma subtype categories (eTable 3 in the Supplement). eTable 5 in the Supplement shows the multivariable Cox analyses for RFS and OS stratified by SSM and NM subtypes, for the Dutch and MIA cohorts. In the Dutch cohort, regression remained a significant predictor of RFS (HR, 0.54; 95% CI, 0.47-0.63; P < .001) and OS (HR, 0.86; 95% CI, 0.76-0.96; P = .009) for SSM only. In the MIA cohort, the presence of regression was a significant predictor of better RFS and OS for both these melanoma subtypes: for SSM, the HR was 0.67 (95% CI, 0.52-0.85; P = .001) for RFS and 0.72 (95% CI, 0.59-0.88; P = .001) for OS. For NM, the HR was 0.71 (95% CI, 0.53-0.95; P = .02) for RFS and 0.73 (95% CI, 0.55-0.97; P = .03) for OS. There was no statistically significant difference in the percentage of melanomas that were of nodular subtype in each Breslow thickness category in the 2 cohorts (P = .30, eTable 6 in the Supplement).

DISCUSSION

This study, to our knowledge the largest examination of regression in melanoma patients performed to date, showed in cohorts from 2 continents that the presence of regression was a favorable prognostic factor for patients with stage 1 and 2 melanomas, especially those with thin and intermediate-thickness tumors (Breslow thickness ≤4.0 mm) and those with SSM subtype.

Two previous studies have also found regression to be a favorable prognostic factor,[8,9] but others have reported that regression was not significantly associated with either RFS or OS[4,10-13] (eTable 7 in the Supplement), and 2 found that histologic regression was associated with worse OS.[14,15] A systematic review and meta-analysis published by Gualano et al25 in 2018 included 10 studies comprising 8557 patients, and indicated that histological regression is associated with improved survival. However, the studies that were included were very heterogeneous in melanoma subtype, used differing definitions of regression, and most had limited samples sizes, so that HRs for RFS ranged from 0.62 (95% CI, 0.43-0.90) in 1 study,[9] to 1.62 (95% CI, 0.58-4.54) in another study[12] that included only acral lentiginous melanomas. Three additional studies have been published since that review, with differing conclusions: Maurichi et al[14] developed a nomogram to predict 12-year OS in 2243 patients with thin (≤1.0 mm) melanomas and reported that regression was an independent predictor for worse survival (in addition to age, mitotic rate, ulceration, lymphovascular invasion, and SN status). Zugna et al[8] reported better survival associated with regression when analyzing 264 patients with stage 3 SN-positive disease, and Ribero et al[10] assessed 954 patients with melanomas smaller than 1 mm in thickness and determined its predictive value for SN status, RFS and melanoma-specific survival. In the latter study,[10] regression was not found to be an independent prognostic factor for survival,

but was associated with a lower incidence of SN-positivity. The lack of agreement in the literature may be partially explained by an absence of standardized criteria for defining disease regression. In the earliest study examining its prognostic utility, Clark et al[15] required a complete absence of tumor overlying or deep to the area of regression. In contrast, other studies only required an area of dermal regressive fibrosis to be present.[9,10] This less restrictive definition may have resulted in significantly more tumors being classified as having regression, potentially altering the calculated associations with outcomes. In our experience, histologically unambiguous regression is often associated with residual in situ or invasive melanoma. It is possible that this difference in definition is, at least in part, the reason for discordant findings between our study and that of Clark et al.[15]

Only 2 previous studies have focused exclusively on patients with stage 1 and 2 disease; 1 showed no survival benefit when regression was present,[11] whereas the other did show a benefit.[9] Nagore et al[11] studied the histology of 823 stage 1 and 2 patients, with 10.3% showing regression. On univariable analysis for RFS and OS they found no significant benefit for regression (assessed by calculating HRs), and therefore did not include it in their final prognostic model that comprised Breslow thickness, primary tumor site, sex, vascular invasion, mitoses, and ulceration. Ribero et al[9] studied 1693 patients with stage 1 and 2 cancer from a single center in Italy; 20.6% showed regression, and they reported an HR of 0.62 (95% CI, 0.43-0.90) for RFS and an HR of 0.43 (95% CI, 0.23-0.80) for OS in the overall group. These results are similar to ours, even though the percentages of patients with regression in the current cohorts were substantially higher (6121 Dutch patients [35.4%] showed regression and 2198 MIA patients [44.1%]). In addition, we found that regression was only a statistically significant prognostic indicator in patients with thin or intermediate thickness melanomas. In those with thick melanomas, the presence of regression was less common (112 [13.1%] and 111 [20.2%] in the Dutch and MIA cohorts, respectively). A statistical consequence of this may be that regression lost its relative prognostic significance compared with other prognostic predictors in thick melanomas.

For patients with the SSM subtype, regression was associated with improved RFS and OS in both cohorts. However, for NM, the 2 cohorts showed mixed results. For RFS, the HRs in both cohorts were less than 1, indicating consistent results between the 2 cohorts, even though this was only statistically significant in the MIA cohort. For OS, the HR was 1.11 (95% CI, 0.88-1.40; P = .37) in the Dutch cohort, and 0.73 (95% CI, 0.55-0.97; P = .03) in the MIA cohort. This likely reflects the known stronger influence of other prognosis factors in patients with nodular melanomas.[26]

Strengths and limitations

Strengths of our study include the large size of the patient cohorts from 2 continents who were studied, the relatively long follow-up in both cohorts, and the use of

nationwide data as well as data from a large, well-maintained institutional database. Another strength is that patients with stage 1 and 2 cancer were stratified according to Breslow thickness category and melanoma subtype. A limitation is that there are no established guidelines for histologic assessment and reporting of regression; it was interpreted subjectively by pathologists on the basis of the presence of a widely accepted pattern of characteristics.20 Even though a recent study[27] showed a high concordance between pathologists (95.0%) for the reporting of regression, others have reported lower concordance rates (74.2%).[28] It is possible that variation in the reporting of regression could account, at least in part, for the inconsistent results of some previous, smaller studies. However, given the large numbers that were included in the present study, and the fact that the data were derived from 2 independent cohorts, the assessment of histopathologic regression was not limited to the interpretation of a few pathologists, but reflects how regression is interpreted in current clinical practice by a large number of pathologists, increasing the generalizability of our results. Another limitation is that when no SN biopsy was performed, it was assumed that patients had stage 1 or 2 disease. Although this is a plausible assumption because most patients had a Breslow thickness of 1.0 mm or smaller, we cannot exclude the possibility that some patients might have been upstaged to stage 3 if SN biopsy had been performed. Another limitation is that we had no information regarding treatment with immunotherapy. The time frame of enrollment overlapped with the advent of effective immunotherapy for patients with stage 4 disease (from 2012), which might have had a different efficacy in the presence or absence of regression. However, the stronger association with improved RFS than with OS suggests that this was not a dominant factor. A final limitation is that mitotic rate data were not available for the Dutch cohort; however, when mitotic rate was included in the multivariable analysis using MIA data our findings remained unchanged.

CONCLUSIONS

Consistent with several previous reports, the results of this study, by far the largest reported to date, indicate that regression can be considered a favorable prognostic factor for patients with stage 1 and stage 2 melanomas. Those with thin and intermediate-thickness tumors (Breslow thickness ≤4.0 mm) and those with SSM subtype are most likely to have an improved prognosis when regression is present.

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Supplementary Table 1. STROBE Statement—Checklist of items that should be included in reports of cohort studies.

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4-5
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	6-7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8-9
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-9
Bias	9	Describe any efforts to address potential sources of bias	7-8
Study size	10	Explain how the study size was arrived at	8-10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9-10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	9-10
		(*d*) If applicable, explain how loss to follow-up was addressed	10
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	32
		(b) Give reasons for non-participation at each stage	32
		(c) Consider use of a flow diagram	32

Supplementary Table 1. (continued).

	Item No	Recommendation	Page No
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	32
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	21
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	19, 24
		(*b*) Report category boundaries when continuous variables were categorized	19, 24
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for exposed and unexposed groups.

Supplementary Table 2. Multivariable Cox regression for RFS and OS for all stage I and II MIA patients, including mitotic rate.

		Recurrence free survival		Overall survival	
Variable	**Class**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Regression	Present vs Absent	0.74 (0.63-0.86)	0.0002	0.80 (0.70-0.92)	0.002
Ulceration	Yes vs No	1.46 (1.25-1.72)	<0.0001	1.56 (1.35-1.81)	<0.0001
SN biopsy	Yes vs No	0.77 (0.66-0.89)	0.004	0.72 (0.63-0.82)	<0.0001
Breslow thickness	Per mm	1.17 (1.13-1.20)	<0.0001	1.16 (1.13-1.20)	<0.0001
Sex	Male vs Female	1.22 (1.06-1.41)	0.007	1.43 (1.26-1.64)	<0.0001
Age	Per year	1.01 (1.00-1.01)	0.006	1.04 (1.03-1.04)	<0.0001
Mitotic rate / mm^2	0	1		1	
	1	2.65 (1.89-3.71)	<0.0001	1.58 (1.24-2.03)	0.0003
	2	3.92 (2.82-5.46)	<0.0001	1.67 (1.24-2.16)	0.0001
	3	4.73 (3.36-6.66)	<0.0001	2.28 (1.75-2.96)	<0.0001
	4	4.76 (3.28-6.91)	<0.0001	1.75 (1.29-2.38)	0.0004
	5	4.35 (2.96-6.39)	<0.0001	1.87 (1.37-2.56)	<0.0001
	6+	6.17 (4.51-8.46)	<0.0001	2.28 (1.80-2.89)	<0.0001

Supplementary Table 3. Presence of regression per Breslow thickness category and melanoma subtype for the Dutch and MIA cohort.

		Dutch cohort						MIA cohort					
		Regression		Death		Recurrence		Regression		Death		Recurrence	
Variable	Class	Present (%)	Absent (%)	Regression present (%)	Regression absent (%)	Regression present (%)	Regression absent (%)	Present (%)	Absent (%)	Regression present (%)	Regression absent (%)	Regression present (%)	Regression absent (%)
Breslow (mm)	≤1.0	4857 (42.9)	6467 (57.1)	388 (8.0)	369 (5.7)	83 (1.7)	166 (2.6)	1395 (58.5)	991 (41.5)	103 (7.4)	99 (10.0)	52 (3.7)	64 (6.5)
	1.1-2.0	863 (25.6)	2509 (74.4)	125 (14.5)	327 (13.0)	99 (11.5)	352 (14.0)	465 (39.9)	701 (60.1)	97 (20.9)	158 (22.5)	62 (13.3)	140 (20.0)
	2.1-4.0	289 (168)	1433 (83.2)	110 (38.1)	439 (30.6)	91 (28.0)	395 (27.6)	227 (25.9)	651 (74.1)	78 (34.4)	254 (39.0)	62 (27.3)	224 (34.4)
	>4.0	112 (13.1)	741 (86.9)	71 (63.4)	402 (54.3)	43 (38.4)	303 (40.9)	111 (20.2)	439 (79.8)	46 (41.4)	243 (55.4)	42 (37.8)	190 (43.3)
Subtype	SSM	5434 (38.9)	8518 (61.1)	525 (9.7)	859 (10.1)	222 (4.1)	702 (8.2)	1570 (56.9)	1191 (43.1)	188 (12.0)	219 (18.4)	120 (7.6)	175 (14.7)
	NM	249 (15.2)	1394 (84.8)	90 (36.1)	473 (33.9)	62 (24.9)	390 (28.0)	216 (22.3)	754 (77.7)	61 (28.2)	316 (41.9)	56 (25.9)	279 (37.0)
	LMM	160 (24.4)	497 (75.6)	35 (21.9)	74 (14.9)	7 (4.4)	24 (4.8)	90 (45.9)	106 (54.1)	17 (18.9)	29 (27.4)	4 (4.4)	11 (10.4)
	ALM	21 (22.1)	74 (77.9)	5 (23.8)	20 (27.0)	2 (9.5)	21 (28.4)	16 (20.5)	62 (79.5)	3 (18.8)	23 (37.1)	1 (6.3)	20 (32.3)

Supplementary Table 4. Multivariable Cox regression for RFS and OS for stage I and II Dutch and MIA melanoma patients, stratified for Breslow thickness.

		DUTCH COHORT											
		Thin (≤1.0mm) (n=11324)				Intermediate (1.1-4.0mm) (n=5094)				Thick (>4.0mm) (n=853)			
		RFS		OS		RFS		OS		RFS		OS	
Variable	Class	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Regression	Present vs Absent	0.57 (0.44-0.75)	<0.0001	0.91 (0.79-1.06)	0.22	0.76 (0.65-0.90)	0.001	0.92 (0.80-1.07)	0.30	0.74 (0.53-1.02)	0.06	1.07 (0.83-1.38)	0.62
Ulceration	Yes vs No	2.34 (1.38-3.98)	0.002	1.18 (0.78-1.78)	0.44	1.45 (1.26-1.68)	<0.0001	1.37 (1.20-1.58)	<0.0001	1.68 (1.35-2.11)	<0.0001	1.94 (1.59-2.36)	<0.0001
SN biopsy	Yes vs No	0.86 (0.52-1.43)	0.55	0.64 (0.38-1.10)	0.11	0.62 (0.54-0.71)	<0.0001	0.49 (0.42-0.57)	<0.0001	0.62 (0.47-0.81)	0.0005	0.55 (0.42-0.73)	<0.0001
Breslow thickness*	Per mm	1.35 (1.27-1.44)	<0.0001	1.07 (1.03-1.10)	0.0003	1.68 (1.56-1.82)	<0.0001	1.52 (1.41-1.64)	<0.0001	1.12 (0.96-1.48)	0.0001	1.17 (1.12-1.23)	<0.0001
Sex	Male vs Female	1.25 (0.97-1.62)	0.08	1.38 (1.19-1.60)	<0.0001	1.37 (1.20-1.56)	<0.0001	1.47 (1.30-1.67)	<0.0001	1.19 (0.96-1.48)	0.11	1.30 (1.08-1.57)	0.005
Age	Per year	1.01 (1.00-1.02)	0.01	1.10 (1.09-1.10)	<0.0001	1.00 (1.00-1.01)	0.04	1.05 (1.04-1.05)	<0.0001	0.99 (0.98-0.99)	0.004	1.03 (1.02-1.03)	<0.0001
		Thin (≤1.0mm) (n=2386)				Intermediate (1.1-4.0mm) (n=2044)				Thick (>4.0mm) (n=550)			
		RFS		OS		RFS		OS		RFS		OS	
Variable	Class	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Regression	Present vs Absent	0.64 (0.44-0.93)	0.02	0.66 (0.50-0.88)	0.004	0.78 (0.63-0.96)	0.02	0.99 (0.83-1.19)	0.90	0.91 (0.65-1.29)	0.60	0.78 (0.57-1.08)	0.14
Ulceration	Yes vs No	2.80 (1.40-5.61)	0.004	4.17 (2.52-6.89)	<0.0001	1.48 (1.34-1.65)	<0.0001	1.33 (1.11-1.59)	0.002	1.56 (1.19-2.04)	0.001	1.74 (1.36-2.23)	<0.0001
SN biopsy	Yes vs No	0.68 (0.36-1.29)	0.24	0.64 (0.35-1.17)	0.15	0.73 (0.61-0.87)	0.0007	0.66 (0.56-0.79)	<0.0001	0.58 (0.43-0.77)	0.0002	0.53 (0.40-0.70)	<0.0001
Breslow thickness*	Per mm	1.29 (1.18-1.40)	<0.0001	1.12 (1.06-1.19)	0.0002	1.49 (1.34-1.65)	<0.0001	1.34 (1.22-1.48)	<0.0001	1.05 (0.99-1.12)	0.12	1.09 (1.03-1.15)	0.004
Sex	Male vs Female	1.42 (0.96-2.08)	0.08	1.74 (1.28-2.35)	0.0004	1.20 (0.99-1.45)	0.05	1.43 (1.20-1.70)	<0.0001	1.01 (0.77-1.33)	0.94	1.25 (0.97-1.61)	0.09
Age	Per year	1.01 (1.00-1.03)	0.04	1.07 (1.28-2.35)	<0.0001	1.00 (0.99-1.01)	0.61	1.03 (1.03-1.04)	<0.0001	1.00 (0.99-1.01)	0.46	1.02 (1.01-1.03)	<0.0001

*For thin melanomas per 0.1mm

Supplementary Table 5. Multivariable Cox regression for RFS and OS for stage I and II Dutch and MIA melanoma patients, stratified for superficial spreading and nodular melanoma subtypes.

		Dutch cohort								MIA cohort							
		SSM (n=13952)				NM (n=1643)				SSM (n=2761)				NM (n=970)			
		RFS		OS		RFS		OS		RFS		OS		RFS		OS	
Variable	Class	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Regression	Present vs Absent	0.54 (0.46-0.63)	<0.0001	0.86 (0.76-0.96)	0.009	0.82 (0.62-1.07)	0.14	1.11 (0.88-1.40)	0.37	0.67 (0.52-0.85)	0.001	0.72 (0.59-0.88)	0.001	0.71 (0.53-0.95)	0.02	0.73 (0.55-0.97)	0.03
Ulceration	Yes vs No	2.50 (2.13-2.95)	<0.0001	1.82 (1.58-2.10)	<0.0001	1.36 (1.12-1.66)	0.002	1.52 (1.28-1.82)	<0.0001	1.83 (1.33-2.52)	0.0002	1.77 (1.36-2.30)	<0.0001	1.30 (1.04-1.64)	0.02	1.45 (1.17-1.81)	0.0007
SN biopsy	Yes vs No	1.13 (0.97-1.32)	0.12	0.83 (0.71-0.98)	0.03	0.70 (0.57-0.87)	0.001	0.43 (0.34-0.55)	<0.0001	0.85 (0.66-1.10)	0.22	0.84 (0.67-1.05)	0.12	0.78 (0.62-0.98)	0.03	0.67 (0.54-0.84)	0.0004
Breslow thickness	Per mm	1.54 (1.49-1.58)	<0.0001	1.31 (1.27-1.34)	<0.0001	1.20 (1.16-1.25)	<0.0001	1.22 (1.18-1.26)	<0.0001	1.48 (1.39-1.57)	<0.0001	1.34 (1.27-1.41)	<0.0001	1.14 (1.09-1.20)	<0.0001	1.13 (1.08-1.18)	<0.0001
Sex	Male vs Female	1.41 (1.23-1.60)	<0.0001	1.48 (1.33-1.65)	<0.0001	1.43 (1.19-1.73)	0.0002	1.40 (1.19-1.66)	<0.0001	1.41 (1.11-1.79)	0.005	1.70 (1.37-2.11)	<0.0001	1.16 (0.93-1.45)	0.19	1.57 (1.26-1.96)	<0.0001
Age	Per year	1.01 (1.00-1.01)	0.0008	1.07 (1.06-1.07)	<0.0001	1.00 (0.99-1.01)	0.39	1.03 (1.03-1.04)	<0.0001	1.01 (1.00-1.02)	0.003	1.05 (1.04-1.06)	<0.0001	1.00 (0.99-1.01)	0.31	1.03 (1.02-1.04)	<0.0001

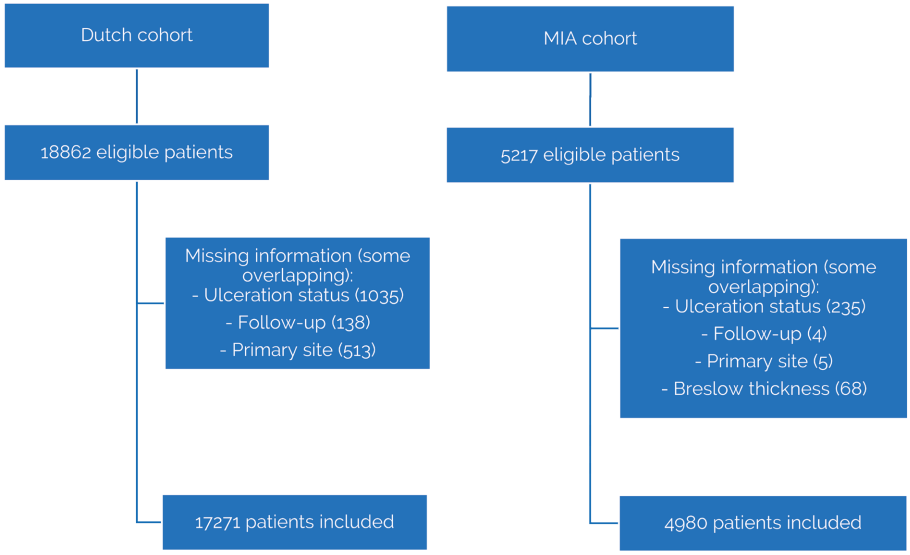
Supplementary Table 6. Distribution of Breslow thickness categories for superficial spreading melanoma and nodular melanoma for the Dutch and MIA cohorts.

Breslow thickness category in mm (n (%))	SSM		p-value	NM		p-value
	Dutch	MIA		Dutch	MIA	
			<0.0001			0.30
0.1-1.0	10119 (72.5)	1796 (65.0)		119 (7.2)	55 (5.7)	
1.1-4.0	3534 (25.3)	867 (31.4)		1064 (64.8)	639 (65.9)	
>4.0	299 (2.1)	98 (3.5)		460 (28.0)	276 (28.5)	

Supplementary Table 7. Overview of hazard ratio's (HRs) of presence of regression in large, previously published studies after 2000 and that performed a multivariable Cox analysis.

Author	Included patients	Median follow-up	N (N in Cox)	N (%) with regression	HR (95% CI) for regression per survival outcome	Other variables in Cox model
Callender et al. [4]	>1.0mm Breslow thickness who underwent SN biopsy (Sunbelt trial)	5.7 years	2500 (2500)	261 (10.4%)	OS (uni): 0.97 (0.73-1.27) RFS (uni): 0.94 (0.68-1.27) OS and RFS (multi): not analyzed	Age, Breslow thickness, ulceration status, melanoma subtype, sex, SN status, Clark level, lymphovascular invasion, anatomical site
Zugna et al. [8]	SN-positive patients from 2 centers in Italy	2.7 years	264 (264)	43 (16.3)	MSS (uni): 0.26 (0.09-0.71) MSS (multi): 0.34 (0.12–0.92)	Age, Breslow thickness, ulceration status, melanoma subtype, number of positive SN biopsies, SN tumor burden
Ribero et al. [9]	Stage I and II from a single center in Italy	4.8 years	1693 (1693)	349 (20.6)	OS (uni): not reported OS (multi): 0.43 (0.23-0.80) RFS (uni): 0.34 (95% CI not reported), p<0.001 RFS (multi): 0.62 (0.43-0.90)	Age, Breslow thickness, ulceration status, sex, SN status
Ribero et al. [10]	>1.0mm Breslow thickness from 4 centers in Italy	6.4 years	1182 (954)	304 (31.9)	MSS (uni): 0.99 (0.77-1.27) MSS (multi): 1.05 (0.77-1.44) RFS (uni): 0.96 (0.77-1.20) RFS (multi): 1.11 (0.85-1.46)	Age, Breslow thickness, ulceration status, sex, SN status, tumor-infiltrating lymphocytes, mitotic rate
Nagore et al. [11]	Primary localized invasive cutaneous melanoma (no comment on SN status, or if SN biopsy was performed or not) from a single center in Spain	5.2 years	823 (823)	85 (10.3%)	OS and DFS (uni): univariable not significant, but no HRs provided OS and DFS (multi): not analyzed	Age, Breslow thickness, ulceration status, sex, SN status, anatomical site, mitotic rate, vascular invasion
Ito et al. [12]	Stage I-III acral lentiginous melanoma from single center in Japan	2.6 years	84 (84)	13 (11.8)	MSS (uni): 2.04 (0.69-6.03) MSS (multi): not analyzed RFS (uni): 2.36 (0.88-6.34) RFS (multi): 1.62 (0.58-4.54)	Age, Breslow thickness, ulceration status, sex, SN status, anatomical site
Testori et al. [13]	Stage I-III from 23 Italian centers	4.5 years	1313 (1230)	386 (29.4)	OS (uni): univariable not significant, but no HRs provided OS (multi): not analyzed	Age, Breslow thickness, ulceration status, sex, SN status, anatomical site, Clark level, number of positive SN biopsies
Maurichi et al. [14]	≤1.0mm Breslow thickness from six European centers	10.3 years	2234 (2234)	27.7%	OS (uni): not reported OS (multi): 3.32 (2.31-4.77)	Age, Breslow thickness, ulceration status, mitotic rate, SN status, lymphovascular invasion

Supplementary Figure 1. Flowchart of patient selection for Dutch and the MIA cohorts.



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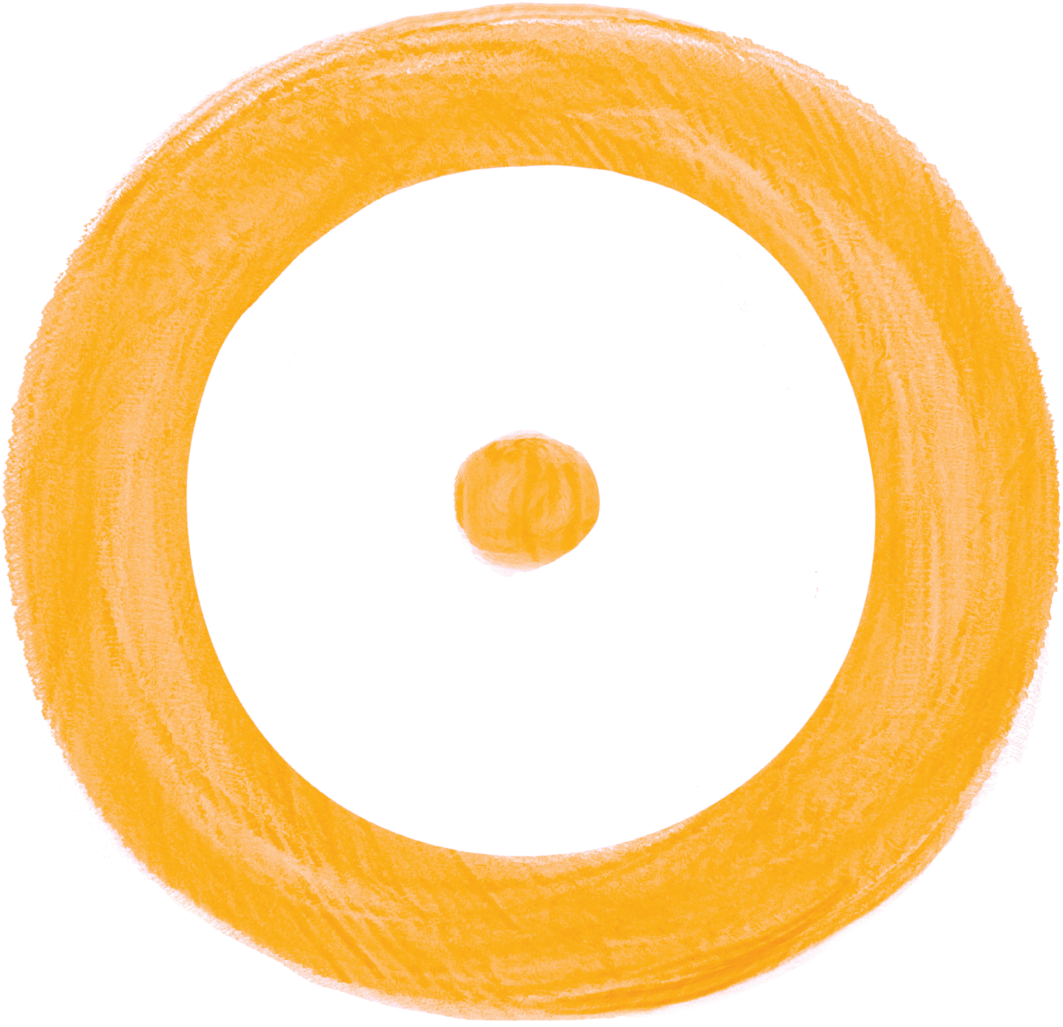

CHAPTER 9

Subtyping cutaneous melanoma matters

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ABSTRACT

Background: Our aim was to investigate the role of melanoma subtype on survival and focus on the effects stratified by Breslow thickness and ulceration status.

Methods: Patients with cutaneous melanoma stage I, II, or III diagnosed between 2000 and 2014 were derived from the Dutch Nationwide Pathology Registry and overall survival data from the Netherlands Cancer Registry. Patients were followed until 2018. Using multivariable Cox proportional hazards models, hazard ratios were calculated for each melanoma subtype, per Breslow thickness category and ulceration status, and adjusted for age, sex, stage, and localization.

Results: A total of 48 361 patients were included: 79.3% had superficial spreading melanoma (SSM), 14.6% nodular melanoma (NM), 5.2% lentigo maligna melanoma, and 0.9% acral lentiginous melanoma (ALM). In the total patient group, using SSM as the reference category, adjusted hazard ratios were 1.06 (95% confidence interval [CI] = 1.01 to 1.12) for NM, 1.02 (95% CI = 0.93 to 1.13) for lentigo maligna melanoma, and 1.26 (95% = CI 1.06 to 1.50) for ALM. Among patients with 1.0 mm or less Breslow thickness and no ulceration, NM showed a twofold increased risk (hazard ratio = 1.96, 95% CI = 1.58 to 2.45) compared with SSM. Compared with 1.0 mm or less SSM without ulceration, the hazard ratio for 1.0 mm or less SSM with ulceration was 1.94 (95% CI = 1.55 to 2.44), and the hazard ratio for 1.0 mm or less NM with ulceration was 3.46 (95% CI = 2.17 to 5.50). NM patients with tumors greater than 1.0 mm did not show worse survival than SSM patients with tumors greater than 1.0 mm.

Conclusions: In this large nationwide study, ALM patients showed worse survival than SSM patients. Among patients with melanomas that were thin (1.0 mm or less), NM subtype patients also showed worse survival than SSM patients.

INTRODUCTION

Melanoma can be classified into 4 major histologic subtypes: superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo maligna melanoma (LMM), and acral lentiginous melanoma (ALM)[1]. SSM is the most common subtype (70%) and usually presents as a flat, slowly growing lesion[2]. NM accounts for 20% of all melanomas. As the name suggests, it grows as a nodule, which may be pigmented or amelanotic. NM tends to have a faster growth rate than SSM[3]. LMM represent 5%-10% of all melanomas[4] and are mostly diagnosed as large, flat macules on the face in older patients. ALM, by definition, involves the acral sites (palms and soles). It is the most common type of melanoma in the Asian population[5] but is rare (1%-2%) in Western populations[4-6].

Apart from clinical and histological differences, recent studies have shown that there are genetic differences between melanoma subtypes as well. As an example, only a small proportion (16%) of ALM carries a BRAF-mutation compared with up to 66% of SSM[7].

Although current melanoma staging for stage I-III melanoma patients is based on Breslow thickness, ulceration status, and presence of sentinel lymph node metastases[8], it is known that prognosis of patients is also driven by other features, such as age, sex, and anatomic localization[9,10]. Regarding histological subtype, there is controversy as to what extent survival differences between melanoma subtypes are driven by the tumor subtype itself or by other well-known correlated prognostic factors, such as a thicker Breslow thickness and more frequent presence of ulceration in some subtypes. The few studies that included a sufficient number of patients to address the prognostic importance of subtype show conflicting results[11-13]. However, none of these studies have disentangled the effects of subtype, Breslow thickness, and ulceration status. Therefore, our aim was to investigate the role of melanoma subtypes on survival using nationwide data from the Netherlands. We focused on the 4 major melanoma subtypes in combination with Breslow thickness and ulceration status.

METHODS

Collection of data

Data for this retrospective nationwide study were obtained from "PALGA," the Dutch Nationwide Network and Registry of Histopathology and Cytopathology[14]. Since 1991, PALGA has prospectively been collecting data from all pathology laboratories in the Netherlands. All data were encoded and used anonymously. Ethical approval was granted by the board of PALGA.

Study population

For this cohort study, pathologic reports of all newly diagnosed invasive melanoma patients in the Netherlands between January 1, 2000, and December 31, 2014, were analyzed. Patients presenting with locoregional (defined as in transit, satellite, or lymph node metastases other than sentinel node biopsy [SLNB]) or distant metastases (stage IV) within 100 days of initial diagnosis were excluded. Patients with noncutaneous melanoma, desmoplastic melanoma, melanoma of unknown primary, and patients without a defined melanoma subtype were excluded. We also excluded patients with multiple primary melanoma, because we previously showed that these patients have worse prognosis[15]. Melanoma occurring in children (age <18 years) were excluded as well. For this study, this yielded a dataset of adults with histologically proven invasive, primary, single, cutaneous melanoma diagnosed between 2000 and 2014 in the Netherlands. For each patient, clinical and pathological variables were extracted from the pathology files, including date of diagnosis, age, sex, Breslow thickness in millimeters, T stage, ulceration (present or absent), body site (head and neck, trunk, arms, or legs), melanoma subtype (SSM, NM, LMM, or ALM), and SLNB result (positive, negative, or not performed). Because guidelines do not address the maximum time between primary excision and SLNB, we decided in a multidisciplinary setting to include as SLNB all SLNB performed within 100 days after initial diagnosis, as previously described[16]. Patients were categorized as stage I, II, and III according to the 8th edition of the American Joint Committee on Cancer[8]. When no SLNB was performed, it was assumed patients were stage I or II. Overall survival data and vital status (dead or alive) were obtained from the Netherlands Cancer Registry hosted by the Comprehensive Cancer Organization of the Netherlands. The Netherlands Cancer Registry is a nationwide, population-based cancer registry with information on vital status and date of death retrieved from the database of deceased persons of the Central Bureau of Genealogy and the municipal demography registries. Follow-up was calculated from date of diagnosis until date of death, the date last known alive, or January 1, 2018, whichever occurred earlier.

Statistical analysis

Categorical variables were summarized as numbers and percentages. Continuous variables were summarized as median with interquartile range (IQR) for nonnormally distributed data or mean with SD for normally distributed data. Differences in proportions and medians were analyzed using $\chi 2$ tests or Mann-Whitney U test, respectively. Differences in means were assessed with Student t test. Patients were stratified in 4 Breslow thickness strata; 1.0 or less, 1.1-2.0, 2.1-4.0, and greater than 4.0 mm, as well as per ulceration status and stage: I, II, and III. Complete case Cox proportional hazards regression analyses were performed to calculate the main effects of melanoma subtype to estimate hazard ratios (HRs) and 95% confidence intervals (CIs), and time to all-cause death (overall survival) was selected as outcome. Variables selected for multivariable analyses were subtype, Breslow thickness, age, sex, ulceration, localization, and stage. In case of missing ulceration status, ulceration was assumed to be absent. To test if this assumption was valid, we compared the outcomes of a Cox regression model with missing ulceration status as a separate category in a categorical variable with that of a model with missing ulceration status included in the "negative" category. Multiple imputation was not considered, given the pathologist involved in this study (P. J. van Diest) believes from clinical experience that it is plausible that this histopathological parameter is not missing at random but rather because it was not seen during pathological assessment. The missing at random assumption (a condition for multiple imputation) would therefore be too strong. The proportional hazards assumption was examined by plotting a log-minus-log graph for categorical variables. If the lines were parallel, it was assumed that the proportional hazards assumption was not violated. For continuous variables (Breslow thickness and age), Schoenfeld residuals were plotted as a function of time, and a loess curve was fitted. If the curve was horizontal, it was assumed that the proportional hazards assumption was not violated. To assess linearity of continuous variables, Martingale residuals were plotted against time. In case of nonlinearity, continuous variables were categorized. We hypothesized that the effect of melanoma subtype was different for tumors with different Breslow thickness. Hence, we constructed an interaction term of Breslow thickness (categorized as ≤1.0 mm, 1.1-2.0 mm, 2.1-4.0 mm, and >4.0 mm) and ulceration with the 4 subtypes of melanoma and added this to the aforementioned multivariable Cox model. We tested for the presence of statistical interaction by subtracting the deviance (−2*[log likelihood]) from the model with the interaction term from the deviance of the model without the interaction term, evaluating the difference in degrees of freedom and using a $\chi 2$ distribution to determine the corresponding P value. A statistically significant P value would indicate that the effect of melanoma subtype is different at different values of Breslow thickness. Finally, we graphically represented the hazard ratios for each melanoma subtype per Breslow thickness category and ulceration. All data were analyzed using SPSS version 26. A 2-sided P value of less than .05 was considered statistically significant.

RESULTS

Paitent characteristics

A total of 48 361 melanoma patients were included with a female predominance of 56.4% (Table 1). Patients had a mean age at diagnosis of 56.39 years (SD =16.07). The median Breslow thickness was 0.86 mm (IQR = 0.50-1.60 mm). Ulceration was present in 12.5% of patients, and most melanomas were located on the trunk (42.3%). Follow-up data were available in 93.7% of patients, and the median follow-up time was 73.8 months (IQR = 43.5-120.7 months). The median follow-up time among survivors was 82.9 months (IQR = 51.1-129.7 months). The majority of patients were diagnosed with SSM (79.3%), followed by NM (14.6%), LMM (5.2%), and ALM (0.9%). Patients with LMM had a mean age of 71.09 years (SD = 12.37) at the time of diagnosis compared with 54.49 years (SD = 15.44) for SSM patients. The median Breslow thickness varied between 0.60 mm (IQR = 0.38-1.00 mm) for LMM and 2.80 mm (IQR = 1.75-4.50 mm) for NM. Most SSM and NM were located on the trunk, most LMM on the face, and most ALM on the feet. Ulceration was present in 38.7% of NM, 34.4% of ALM, 7.9% of SSM, and 5.1% of LMM.

Survival analyses

Before multivariable analysis, we found no linear association between age and survival when assessing linearity for continuous variables. Therefore, age was categorized into 10 equal groups based on the number of events (death). No other violations in proportionality or linearity were found. The hazard ratios and 95% confidence intervals related to subtype were identical when missing ulceration status (16.2%) was regarded as a separate "missing" category or when missing ulceration status was included in the "negative" category (data not shown). In all of the following analyses, we therefore regarded missing ulceration status as negative. A total of 43 872 (90.7%) patients were included in the multivariable analysis. To calculate the main effect of each melanoma subtype, using SSM as a reference, statistically significant hazard ratios for NM (HR = 1.06, 95% = CI 1.01 to 1.12, P = .04) and ALM (HR = 1.26, 95% CI = 1.06 to 1.50, P = .008) were found. For LMM, no statistically significant difference was found (P = .65).

Table 1. Baseline table of all patients with a single primary cutaneous melanoma in the Netherlands from 2000 to 2014[a]

Characteristic	Total (N=48,361)	SSM (n = 38,373)	NM (n = 7059)	LMM (n= 2500)	ALM (n=429)
Subtype, No. (%)		–	–	–	–
SSM	38,373 (79.3)				
NM	7095 (14.6)				
LMM	2500 (5.2)				
ALM	429 (0.9)				
Sex, No. (%)					
Female	27,270 (56.4)	21,978 (57.3)	3605 (51.1)	1415 (56.6)	272 (63.4)
Male	21,091 (43.6)	16,395 (42.7)	3454 (48.9)	1085 (43.4)	157 (36.6)
Age at diagnosis in years, mean (SD)	56.39 (16.07)	54.49 (15.44)	61.07 (16.88)	71.09 (12.37)	63.70 (14.82)
18-35	5118 (10.6)	4507 (11.7)	547 (8.1)	22 (0.9)	15 (3.5)
36-55	17,974 (37.2)	15,629 (40.7)	1975 (28.0)	261 (10.4)	109 (25.4)
55-75	18,899 (39.1)	14,583 (38.0)	2919 (41.4)	1193 (47.7)	204 (47.6)
>75	6370 (13.2)	3554 (9.5)	1591 (22.5)	1024 (41.0)	101 (23.5)
Breslow in mm, median (IQR)	0.86 (0.50-1.60)	0.76 (0.50-1.20)	2.80 (1.75-4.50)	0.60 (0.38-1.00)	2.02 (1.20-4.00)
0.1-0.7					
0.8-1.0	20,545 (42.5)	18,716 (48.8)	173 (2.5)	1601 (64.0)	55 (12.8)
1.1-2.0	7987 (16.5)	7269 (18.9)	378 (5.4)	309 (12.4)	31 (7.2)
2.1-4.0	10,576 (21.9)	8263 (21.5)	1815 (25.7)	368 (14.7)	130 (30.3)
>4.0	6148 (12.7)	3161 (8.2)	2717 (38.5)	159 (6.4)	111 (25.9)
	3105 (6.4)	964 (2.5)	1976 (28.0)	63 (2.5)	102 (23.8)
Localization, No. (%)					
Head & neck	5983 (12.4)	3186 (8.3)	1137 (16.1)	1660 (66.4)	0 (0)
Trunk	20,438 (42.3)	17527 (45.7)	2651 (37.6)	260 (10.4)	0 (0)
Arms	7035 (14.5)	5442 (14.2)	1231 (17.4)	280 (11.2)	82 (19.1)
Legs	13,358 (27.6)	10969 (28.6)	1805 (25.6)	240 (9.6)	334 (77.9)
Missing	1547 (3.2)	1249 (3.3)	235 (3.3)	60 (2.4)	3 (0.7)
Ulceration, No. (%)					
No	34,480 (71.3)	29,233 (76.2)	3243 (45.9)	1176 (47.0)	228 (53.2)
Yes	6042 (12.5)	3035 (7.9)	2732 (38.7)	128 (5.1)	147 (34.3)
Missing	7839 (16.2)	6105 (15.9)	1084 (15.4)	1196 (47.8)	54 (12.6)
AJCC Stage at time of diagnosis, No. (%)					
I	35,442 (73.3)	31,586 (82.3)	1535 (21.7)	2160 (86.4)	161 (37.5)
II	10,618 (22.0)	5347 (13.9)	4727 (67.0)	335 (13.4)	209 (48.7)
III	2301 (4.8)	1140 (3.8)	797 (11.3)	5 (0.2)	59 (13.8)
Number of deaths, No. (%)	8619 (17.8)	5230 (13.6)	2665 (37.8)	583 (23.3)	141 (32.9)
Follow-up in months, median (IQR)	73.8 (43.5-120.7)	76.9 (45.8-123.9)	62.6 (33.6-114.0)	59.4 (34.4-97.2)	56.6 (34.9-93.2)

[a] AJCC = American Joint Committee on Cancer; ALM = acral lentiginous melanoma; IQR = interquartile range; LMM = lentiginous malignant melanoma; NM = nodular melanoma; SSM = superficial spreading melanoma.

Table 2. Overview of hazard ratios of NM and ALM vs SMM in large, previously published studies[a]

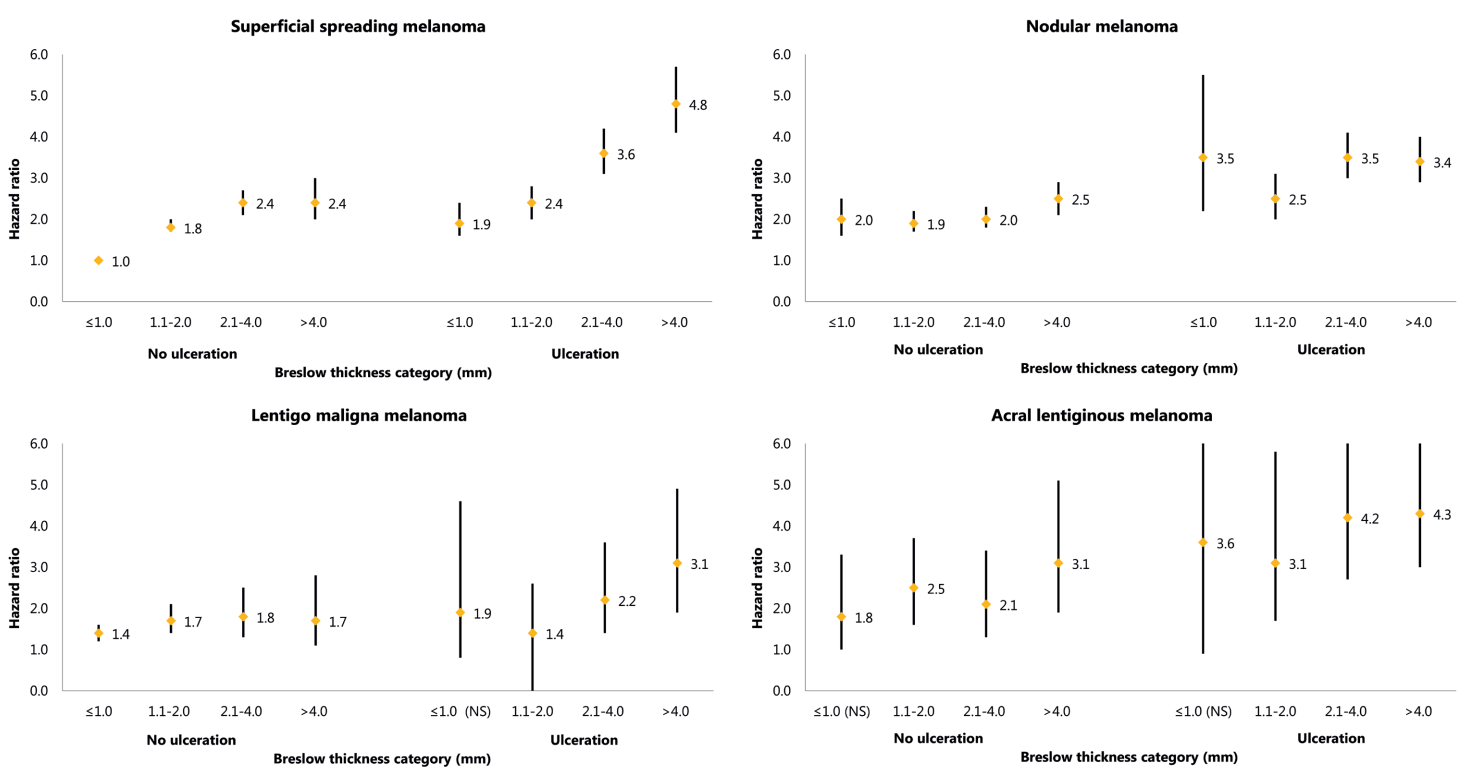
Study	No. total (No. of NM, and No. of ALM)	HR subtype (95% CI)	No. (%) ≤1.0mm Breslow thickness	Other variables in Cox analysis	Outcome
Lattanzi et al. 2019 [12]	118,508 (21,399 NM, ALM excluded) No. in Cox not mentioned	NM: 1.55 (1.41-1.70) ALM: -	37596 (31.7)	Breslow thickness, ulceration, age, gender, stage, year of diagnosis	OS
Lindholm et al., 2004 [11]	9515 (1821 NM, 156 ALM) 6191 in Cox	NM: 1.35 (1.08-1.70) ALM: 0.91 (0.49-1.70)	2933 (47.4)	Breslow thickness, ulceration, age, gender, localization, tumour dimension, Clark level, domicil	MSS
Robsahm et al., 2018 [13]	8087 (1527 NM, 32 ALM) 5010 in Cox	NM: 1.01 (0.79-1.29) ALM (merged with 106 'other'): 0.67 (0.40-1.14)	3745 (46.3)	Breslow thickness, ulceration, age, gender, localization, stage, second primary melanoma	MSS
Dessinioti et al., 2019 [17]	20,132 (5062 NM, ALM excluded) 8370 in Cox (T1)	NM: T1: 2.20 (1.28-3.78) T2: 1.23 (0.95-1.60) T3: 0.84 (0.69-1.03) T4: 0.96 (0.79-1.17) ALM: -	9681 (48.1)	Breslow thickness, ulceration, age, gender, center	MSS

a ALM = acral lentiginous melanoma; CI = confidence interval; HR = hazard ratio; MSS = melanoma-specific survival; NM = nodular melanoma; OS = overall survival; — = Not applicable.

Effect of subtype per Breslow thickness and ulceration status

Because we hypothesized that the effect of melanoma subtype was different for tumors with different Breslow thickness, an interaction term of Breslow thickness with melanoma subtype was included in the model. A statistically significant interaction effect between melanoma subtype and Breslow thickness was observed (P = .001). The effect of melanoma subtype at different values of Breslow thickness and stratified for ulceration is shown in Figure 1. SSM of 1.0 mm or less without ulceration was used as a reference category for all analyses presented in the different figure panels. Among patients with Breslow thickness 1.0 mm or less and no ulceration, NM showed a 2-fold increased risk (HR = 1.96, 95% CI = 1.58 to 2.45) compared with SSM. Compared with 1.0 mm or less SSM without ulceration, the hazard ratio for 1.0 mm or less SSM with ulceration was 1.94 (95% CI = 1.55 to 2.44), and that for 1.0 mm or less NM with ulceration was 3.46 (95% CI = 2.17 to 5.50). NM patients with tumors greater than 1.0 mm did not show worse survival than SSM patients.

Figure 1. Graphical representation of hazard ratios with 95% confidence interval for each Breslow thickness category, per melanoma subtype and per ulceration status for death from all causes. Superficial spreading melanoma 1.0mm or less without ulceration is used as a refference caregory. NS - not statistically significant.



DISCUSSION

In this study, we showed that NM and ALM melanoma subtypes had worse survival than SSM and LMM subtypes. NM subtype especially affected survival among melanomas that were thin (≤1.0 mm).

Interestingly, there is little literature with sufficient number of patients evaluating the role of melanoma subtype on survival (Table 2 provides an overview, including all variables included in the models). The most recent and largest study was performed by Lattanzi et al.[12], who included 118 508 patients using Surveillance, Epidemiology, and End Results data from 1973 to 2012. They showed that compared with SSM, NM was a statistically significant risk factor for all-cause mortality (HR = 1.55, 95% CI = 1.41 to 1.70). As in our study, stage IV patients were excluded. Other melanoma subtypes besides SSM and NM were not analyzed. Lindholm et al. (11) included 6191 Swedish stage I and II melanoma patients diagnosed with SSM, NM, LMM, or ALM between 1990 and 1999. They observed a hazard ratio for disease-specific-survival of 1.35 (95% CI = 1.08 to 1.70) for NM compared with SSM. LMM and ALM were not found to be independent predictors for mortality. On the contrary, Robsahm et al. (13) did not find melanoma subtype to be an independent predictor for melanoma-specific survival when they analyzed 5010 Norwegian melanoma patients diagnosed between 2008 and 2012. They found a hazard ratio of 1.01 (95% CI = 0.79 to 1.29) for NM and a hazard ratio of 0.93 (95% CI = 0.45 to 1.86) for LMM. Although we found that NM was statistically significantly associated with worse survival, the hazard ratio was only 1.06 (95% CI = 1.01 to 1.12), and its statistical significance might also be affected by the large numbers that this study was based on.

Our most interesting finding is that we found higher hazard ratios for death for 1.0 mm or less NM compared with 1.0 mm or less SSM in both ulcerated and nonulcerated melanomas. This might reflect the biological aggressiveness of NM. So in case of timely diagnosis of this melanoma subtype, its Breslow thickness can be misleading, because the tumor seems to behave in a more aggressive way than would be expected on the basis of its Breslow thickness. Our finding is supported by Dessinioti et al.[17], who recently compared melanoma-specific survival of 297 thin (defined as ≤1.0 mm Breslow thickness) NM with 9384 thin SSM. They concluded that thin NM is a high-risk melanoma subtype when adjusted for age, sex, Breslow thickness, ulceration, and center heterogeneity (HR = 2.20, 95% CI = 1.28 to 3.78) (Table 2). The biological aggressiveness of relatively thin NM might also be an explanation for the fact that mortality from NM has not decreased with the years[18], even though the median thickness of NM has decreased[19]. Also on a molecular level, NM seems to be a distinct melanoma subtype, because it is more frequently associated with NRAS mutations than SSM[20-22], and it has been shown that this mutation is associated with progressive disease[20].

Our data also show worse survival of ALM than of SSM. Although ALM is a relatively rare melanoma subtype, studies have shown that it is an independent predictor for survival[23,24]. Gumaste et al.[23] compared 61 ALMs with 183 non-ALMs and found a hazard ratio of 2.64 (P = .001) for melanoma-specific survival for ALMs vs non-ALMs. A potential reason that Lindholm et al.[11] and Robsahm et al.[13] found no statistically significantly worse survival for ALM patients could be due to the relatively small number of patients with ALM subtype in these studies (156 and 32 patients, respectively). A delay in diagnosis, and therefore a worse prognosis, might also be caused by the atypical presentation of this melanoma subtype.

Because melanoma subtyping is of prognostic relevance, accuracy of subtyping in daily practice is important and needs to be reproducible between pathologists. We could find only 1 study on reproducibility of melanoma subtyping, describing a substantial to almost perfect agreement for SSM, NM, LMM, and ALM subtypes as kappa values of 0.73, 0.70, 0.70, and 0.83, respectively, were found[25]. Furthermore, in the evolving landscape of adjuvant therapies for melanoma patients[26], the role of NM and ALM subtypes may need to be evaluated for the indication of SLNB and adjuvant therapy.

Our main strength is that we thoroughly assessed the effect of melanoma subtype in different strata of Breslow thickness and ulceration status. Our large sample size allowed us to do this not only for SSM and NM but also for the less prevalent LMM and ALM subtypes. The use of nationwide data resulted in an unselected study population and increased the generalizability of our results. Limitations that go hand in hand with the retrospective nature of our study are missing data. In our study, the missing data were relatively few (9.3%). For our analyses, we regarded missing ulceration status as absent. Although this is an assumption, it is likely to be true for the majority of patients[27]. Eigentler et al.[27] used a predictive model for missing ulceration status (n = 7107) in their nationwide study in stage I-III patients (n = 15158) and estimated 4.9% to be ulcerated. In addition, we have performed a sensitivity analysis including missing ulceration status as a separate "missing" category, which showed no changes in hazard ratios and 95% confidence intervals. Another limitation is that we assumed an SLNB negative outcome in cases where no SLNB was performed. Because SLNB was performed in 44% of patients with a melanoma greater than 1.0 mm Breslow thickness, we might have missed patients who should have been categorized as stage III when SLNB would have been performed and are now categorized as stage II. Because NM and ALM have a higher chance of SLNB positivity, the staging category of these patients might have been underestimated. Although we correct for stage in multivariable analysis, there may thus be some residual confounding effect in NM and ALM patients. A final limitation regarding the analyses is that one could argue that multiple comparisons have been made and that a multiple hypothesis testing

correction should have been performed. In that case, our findings would be no longer statistically significant and therefore should be interpreted with care.

CONCLUSIONS

All in all, we have shown that melanoma subtype is an independent predictor for survival for melanoma patients, NM and ALM being prognostically worse. NM subtypes especially showed worse survival among melanomas that were thin (≤1.0 mm). Incorporation of histologic subtype into prediction models may lead to better prognostication of melanoma patients.

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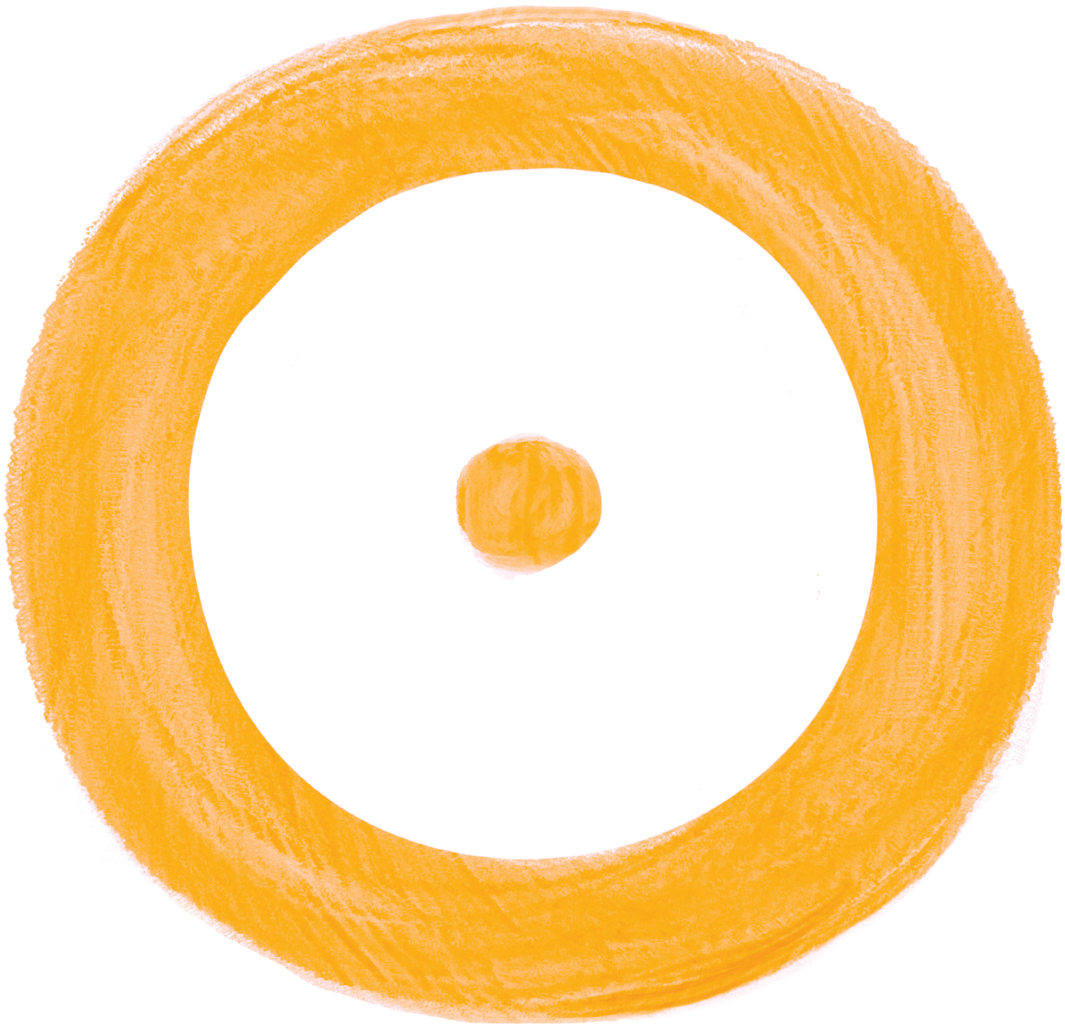

CHAPTER 10

Comparison of survival between patients with single vs multiple primary cutaneous melanomas

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ABSTRACT

Importance: Melanoma is one of the most rapidly increasing forms of cancer worldwide. Most studies about survival among patients with melanoma consider only the primary tumor and disregard the potential effect of multiple primary tumors. A better understanding of the prognosis of patients with multiple primary melanoma is important for patient counselling and follow-up strategies.

Objective: To describe the epidemiologic features of multiple primary melanoma in patients from the Netherlands.

Design, Setting, and Participants: This retrospective, population-based cohort study included adults with histologically proven, primary, invasive cutaneous melanoma in the Netherlands between January 1, 2000, and December 31, 2014, with a median follow-up of 75.1 months, using data from PALGA, the Dutch Nationwide Network and Registry of Histopathology and Cytopathology. Follow-up data were retrieved from the Netherlands Cancer Registry. Statistical analysis was performed from August 1, 2018, to September 3, 2018.

Main Outcomes and Measures: A multivariable Cox model with a time-varying covariate was performed to assess overall survival between patients with a single primary melanoma vs those with multiple primary melanomas. Secondary outcomes included incidence of multiple primary melanoma, differences in Breslow thickness, and time between first and second multiple primary melanoma.

Results: Of the 56 929 study patients, 31 916 (56.1%) were female, with a mean (SD) age of 56.4 (16.2) years. A total of 54 645 single primary melanomas and 4967 multiple primary melanomas in 2284 patients were included. The median Breslow thickness decreased from 0.90 mm (interquartile range, 0.55-1.70 mm) for the first melanoma to 0.65 mm (interquartile range, 0.45-1.10 mm) for the second melanoma (P<.001). For their second melanoma, 370 patients (16.2%) had a higher T stage, 1112 (48.7%) had the same T stage, and 802 (35.1%) had a lower T stage. In addition, 841 of 2284 second melanomas (36.8%) in patients with multiple primary melanomas were found during the first year of follow-up, whereas 624 of 2284 (27.3%) were found after 5 years of follow-up. These proportions did not vary when stratified for melanoma stage. Worse overall survival was seen among patients with multiple primary melanomas compared with patients with a single primary melanoma (hazard ratio, 1.31; 95% CI, 1.20-1.42; P<.001).

Conclusions and Relevance: A significant decrease in Breslow thickness between the first and second multiple primary melanoma was found, and overall survival among patients with multiple primary melanomas was significantly worse than that among patients with a single primary melanoma. These findings suggest that more strict follow-up strategies may be warranted for patients with multiple primary melanomas.

INTRODUCTION

Melanoma is one of the most rapidly increasing forms of cancer worldwide and is accountable for most skin cancer–related deaths.[1-4] Familial atypical mole and melanoma syndrome leads to multiple primary melanomas, but primary melanoma can manifest multiple times outside the framework of familial syndromes. Follow-up guidelines and survival analyses usually consider only the initial melanoma and disregard the potential effects of multiple primary melanomas, which occur in 0.2% to 12.7% of patients with melanoma.[5-16] Few epidemiologic data about tumor and patient characteristics of patients with multiple primary melanomas are available, necessitating better characterization of and prognostication for this group of patients.

Melanoma staging and prognosis relies on Breslow thickness. A rationale for close follow-up after a first melanoma is diagnosed is to detect metastases and a subsequent melanoma as early as possible, ideally at a decreased Breslow thickness. However, no strong evidence exists to support a specific follow-up interval.[17-20] Current Dutch melanoma guidelines recommend 1 follow-up visit within 1 month after initial diagnosis for stage pathologic (p)T1a melanoma. For pT1b melanoma and higher, a follow-up visit every 3 months is recommended for the first year after diagnosis, twice a year for year 2, and annually for years 3 to 5. Further follow-up is recommended for patients with a family history of melanoma and is debated for patients with a higher risk for developing melanoma, such as those with more than 100 nevi or 5 or more atypical nevi. No recommendation is made regarding follow-up for patients with multiple primary melanomas in Dutch guidelines, similar to most international guidelines.[20]

Conflicting results have been reported regarding survival among patients with a single primary melanoma vs multiple primary melanomas; increased, equal, and decreased survival has been documented.[16,21-27] Some authors argue that these conflicting results have been found because of methods that disregard survival bias in patients with multiple primary melanomas; despite different methodologic approaches to prevent survival bias, conflicting results remain.[21,24,26]

Because knowledge about the epidemiologic characteristics of multiple primary melanomas is important for guidelines, patient guidance, and follow-up, the aim of this study was to gain insight into these epidemiologic characteristics of multiple primary melanomas in patients in the Netherlands. This study focused on incidence, differences in Breslow thickness, time between subsequent melanomas, and overall survival among patients with a single primary melanoma vs multiple primary melanomas.

METHODS

Collection of data

Data for this retrospective nationwide cohort study were obtained from PALGA, the Dutch Nationwide Network and Registry of Histopathology and Cytopathology.[28] Since 1987, PALGA has prospectively collected data from all pathology laboratories in the Netherlands. All data were encoded and used anonymously. Ethical approval was granted by the board of PALGA, Houten, the Netherlands. The ethical board of PALGA approves or disapproves all applications based on internal procedures. Anonymous data were used; therefore, patients could not be asked directly for informed consent. Statistical analysis was performed from August 1, 2018, to September 3, 2018.

Study population

The pathology reports of all patients with newly diagnosed melanoma in the Netherlands between January 1, 2000, and December 31, 2014, were analyzed. Melanoma in situ, spitzoid tumors of unknown malignant potential, melanocytic tumors of unknown malignant potential, and superficial atypical melanocytic proliferation of uncertain significance were excluded, as were melanomas lacking or having unclear Breslow thickness. We excluded patients with positive sentinel lymph node biopsy findings, lymph node dissection, fine needle aspiration, or otherwise diagnosed positive lymph nodes within 14 days of the diagnosis of the melanoma to ensure that patients were free of clinically detectable nodal disease in the study. This evaluation led to the exclusion of 188 patients with a single primary melanoma (0.34%) and 15 patients with multiple primary melanomas (0.65%). Furthermore, noncutaneous, desmoplastic melanoma, melanoma of unknown primary, recurrences, intransit melanoma, and melanomas occurring among children (<18 years of age) were excluded. This yielded a data set of adults with histologically proven, invasive, primary cutaneous melanoma diagnosed between January 1, 2000, and December 31, 2014, in the Netherlands.

For each patient, clinical and pathologic variables were extracted from the findings on the pathology files, including date of diagnosis, age, sex, Breslow thickness, T stage, ulceration presence or absence, type of melanoma (superficial spreading, nodular, lentigo maligna, or acral lentiginous), and body site (head and neck, trunk, arms, or legs). The TNM staging was in accordance with the American Joint Committee on Cancer staging at the time of diagnosis. Mitoses were included for melanoma for the period that the seventh edition of the American Joint Committee on Cancer Cancer Staging Manual was valid because mitotic rate (≥1 mitoses/mm2) indicated sentinel lymph node biopsy.

Patients with multiple primary melanomas were defined as those with a new primary melanoma diagnosed on or after the date of first melanoma diagnosis irrespective

of topography. Thus, patients diagnosed with 2 simultaneous melanomas were registered with the diagnosis of multiple primary melanomas. Multiple melanomas were counted separately in the analysis, resulting in total number of melanomas instead of patients.

Follow-up data, including vital status (dead or alive), were obtained from the Netherlands Cancer Registry, which gathers information about every patient with cancer in the Netherlands, through January 1, 2018. Some patients classified with a single primary melanoma between January 1, 2000, and December 31, 2014, were reclassified as having multiple primary melanomas if the second melanoma occurred between January 1, 2015, and January 1, 2018.

Statistical analysis

Categorical variables are presented as numbers and percentages. Continuous variables are presented as medians with interquartile ranges (IQRs) for nonnormally distributed data or means (SDs) for normally distributed data. Univariate variables were analyzed using $\chi 2$ tests or Mann-Whitney test, as appropriate. For multiple primary melanomas, analyses were performed until the sixth multiple primary melanoma. Patients with multiple primary melanomas diagnosed simultaneously were registered with the diagnosis of multiple primary melanomas, and a random order for difference in Breslow thickness calculations was used. Absolute difference in Breslow thickness was calculated between subsequent primary melanoma and tested for significance on group level using the Kruskal-Wallis test and a post hoc pairwise Mann-Whitney test for significance between groups. The difference in time between subsequent melanoma diagnosis was calculated per day.

To prevent survival (also known as immortal time) bias for multiple primary melanomas, we performed Cox regression analysis with a time-varying covariate to assess differences in survival between patients with a single primary melanoma and those with multiple primary melanomas, yielding a hazard ratio (HR). Immortal time refers to a period of follow-up during which, by design, the study outcome (death) cannot occur. By definition, multiple primary melanomas can only be multiple if patients have survived to develop a second melanoma.[29-31] Death, using overall survival data, was selected as the primary outcome and variables were age, Breslow thickness, ulceration, type of melanoma, localization, and sex. The proportional hazards assumption was checked by plotting a log-minus-log graph for all variables. For multiple primary melanomas, all variables of the first melanoma were considered. We performed an additional worst case analysis, in which we included the pathologic characteristics most likely to be associated with death (defined as highest Breslow thickness and its corresponding ulceration status) for each patient with multiple primary melanomas. For example, the initial melanoma is 0.6 mm thick without ulceration; 1 year later, a 2.5-mm-thick melanoma with ulceration occurs. For the

regular analysis, we evaluated the tumor characteristics of the first melanoma (0.6 mm without ulceration), whereas in the additional worst case analysis, the characteristics of the 2.5-mm-thick melanoma were used. Cox regression was performed using SAS, version 9.4 (SAS Institute Inc); all other data were analyzed using SPSS, version 21 (IBM Corporation). Two-sided P<.05 was corrected for multiple hypothesis testing according to Bonferroni before it was considered to be statistically significant.

RESULTS

Recruitment

Of the 56 929 patients included in the analysis, 31 916 (56.1%) were female, with a mean (SD) age of 56.4 (16.2) years. Median follow-up time was 75.1 months (range, 43.5-123.5 months). A total of 56 929 patients with 59 612 primary cutaneous melanomas (54 645 single primary melanomas and 4967 multiple primary melanomas in 2284 patients [4.0%]) met our inclusion criteria. In total, 2008 patients had 2 primary melanomas, 206 patients had 3 melanomas, and 70 patients had 4 or more (up to 10) primary cutaneous melanomas. A total of 339 patients had simultaneous multiple primary melanomas.

Differences between patients with single and multiple melanomas

When comparing single primary melanomas (n = 54 645) with multiple primary melanomas (n = 2284), more males had multiple primary melanomas (1134 [49.6%] vs 23 879 [43.7%]; P<.001). Melanomas on the trunk (1074 [47.0%] vs 22 818 [41.8%]) were more frequent and melanomas on the leg (529 [23.2%] vs 15 196 [27.8%]) were less frequent for multiple primary melanomas than for single primary melanomas (P<.001). Mean (SD) age (56.4 [16.2] years vs 58.8 [15.2] years; P<.11) and median Breslow thickness (0.90 mm [IQR, 0.52-1.80 mm] vs 0.88 mm [IQR, 0.60-1.45 mm]; P<.64) did not differ significantly between the 2 groups, neither did ulceration (71.3% vs 68.0%) or subtype of melanoma (superficial spreading: 2.4% vs 70.6%; nodular: 14.6% vs 13.4%; lentigo maligna: 4.4% vs 4.5%; and acral lentiginous: 0.4% vs 0.8%) (Table 1). Follow-up of 5 years or more was available for 1542 multiple primary melanomas (67.8%) compared with 31 204 single primary melanomas (60.8%).

Table 1. All patients with cutaneous melanoma in the Netherlands from 2000-2014, stratified by SPM vs MPM.

	SPM (n=54,645)	MPM[a] (n=2,284)	p-value
Sex (N (%))	30,766 (56.3)	1,150 (50.4)	<0.001[b]
Female	23,879 (43.7)	1,134 (49.6)	
Male			
Age (mean (SD))	56.42 (16.16)	58.80 (15.19)	0.110
18-35	5,849 (10.7)	232 (10.2)	<0.001[b]
36-55	20,237 (37.0)	770 (33.7)	
56-75	21,244 (38.9)	1,029 (45.1)	
>75	7,315 (13.7)	253 (11.1)	
Breslow thickness (median (IQR))	0.90 (0.52-1.80)	0.88 (0.60-1.45)	0.638
0.01-1.00	31,021 (56.8)	1,305 (57.1)	0.023[b]
1.01-2.00	12,301 (22.5)	524 (22.9)	
2.01-3.00	4,881 (8.9)	222 (9.7)	
3.01-4.00	2,490 (4.6)	108 (4.7)	
>4.00	3,952 (7.2)	125 (5.5)	
Body site (N (%))			
HN	6,860 (12.6)	267 (11.7)	<0.001[b]
Trunk	22,818 (41.8)	1,074 (47.0)	
Arms	7,958 (14.5)	369 (16.1)	
Legs	15,196 (27.8)	529 (23.2)	
Missing	1,813 (3.3)	45 (2.0)	
Ulceration (N (%))			
Yes	37,401 (68.4)	1,629 (71.3)	0.505
No	7,283 (13.3)	304 (13.3)	
Missing	9,961 (18.2)	351 (15.3)	
Subtype (N (%))			
SSM	38,562 (70.6)	1,654 (72.4)	0.058
NM	7,345 (13.4)	334 (14.6)	
LMM	2,506 (45.9)	100 (4.4)	
ALM	433 (7.9)	8 (0.4)	
Missing	5,799 (10.6)	188 (8.2)	
Deaths per stage (N (%))			
T1	2,851 (26.6)	148 (33.7)	<0.001[b]
T2	2,441 (22.8)	106 (24.1)	
T3	2,933 (27.4)	117 (26.7)	
T4	2,498 (23.3)	68 (15.5)	
Deaths per stage (proportion*100 (95% CI))			
T1	9.1 (8.8-9.5)	11.3 (9.7-13.1)	<0.001[b]
T2	20.2 (19.5-20.9)	20.5 (17.3-24.2)	
T3	40.0 (38.8-41.1)	35.6 (30.6-40.9)	
T4	63.4 (61.9-64.9)	54.0 (45.3-62.4)	

Abbreviations: IQR, interquartile range; MPMs, multiple primary melanomas; SPM, single primary melanoma.
[a] For MPMs, all variables are given for the first melanoma.
[b] Statistically significant, corrected for multiple testing.

Table 2. Time from first to second melanoma in patients with MPMs in the Netherlands, 2000-2014, stratified by stage of the first melanoma[a]

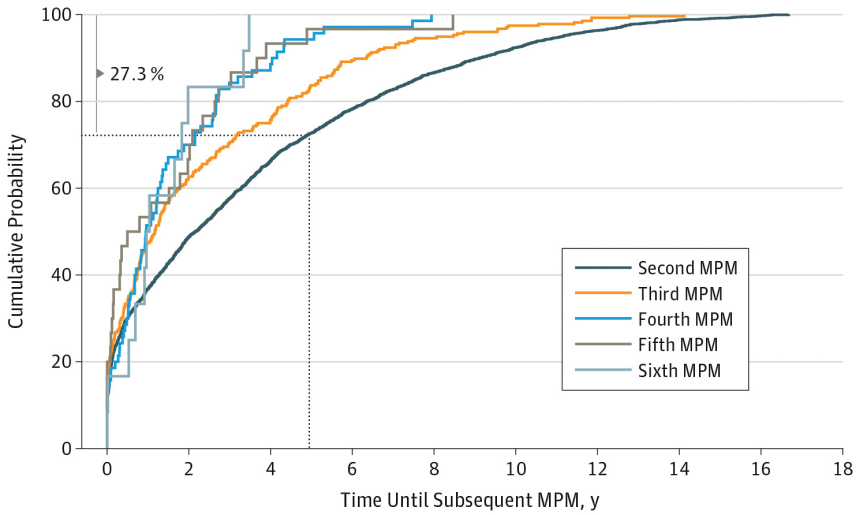
Stage first melanoma	T1a	T1b	T1nos	T2a	T2b	T2nos	T3a	T3b	T3nos	T4a	T4b	T4nos	Total
Time in years	282	139	38	142	27	16	73	54	9	25	36	0	841
≤1	85	46	10	51	10	6	25	24	2	6	7	0	272
1-2	68	33	13	30	8	8	18	12	1	6	5	1	203
2-3	70	29	8	44	3	5	18	11	1	5	4	0	198
3-4	52	23	11	22	4	10	10	4	1	3	6	0	146
4-5	45	19	11	23	5	4	8	7	0	4	2	0	128
5-6	45	24	7	13	1	2	6	3	1	0	2	0	104
6-7	23	18	12	11	5	8	1	4	2	1	1	0	86
7-8	24	16	13	7	0	3	5	2	1	0	1	0	72
8-9	13	14	10	8	3	4	2	2	2	1	1	0	60
9-10	33	37	39	17	3	16	10	2	8	1	5	3	174
>10													
Total	740	398	172	368	69	82	176	125	28	52	70	4	2284
% after 5 years	24.7	32.2	53.5	21.5	24.6	45.1	18.2	16.0	50.0	13.5	17.1	75.0	27.3

Abbreviation: MPMs, multiple primary melanomas.
[a] According to the eighth edition of the American Joint Committee on Cancer Cancer Staging Manual.

Time between first and subsequent melanomas

In 841 of 2284 (36.8%), the second multiple primary melanoma was found in the first year of follow-up after the first melanoma. However, 624 of 2284 second melanomas (27.3%) were found more than 5 years after diagnosis of the first melanoma (Figure 1 and eTable in the Supplement), with a median difference of 2.2 years (IQR, 0.3 years to 5.4 years) and a maximum time of 16.7 years between the first and second melanoma. When stratifying for stage of the first melanoma, second melanomas later than 5 years after diagnosis of the first melanoma were found in 183 first-stage T1a melanomas (24.7%), 128 T1b melanomas (32.2%), 79 T2a melanomas (21.5%), and 17 T2b melanomas (24.6%) (Table 2).

Figure 1. Cumulative probability curves of multiple primary melanoma (MPM) in the Netherlands 2000-2014.



Grey vertical lines represents percentage of second melanomas found more than 5 years after the diagnosis of the first melanoma.
Dotted lines indicate the 5-year point for patients with their second melanoma.

Difference in Breslow thickness between subsequent melanomas

The median Breslow thickness in multiple primary melanomas was 0.90 mm (IQR, 0.55-1.70 mm) for the first melanoma and 0.65 mm (IQR, 0.45-1.10 mm) for the second melanoma (P < .001). No significant differences were found in Breslow thickness between the second and subsequent melanomas (Figure and eTable in the Supplement). When selecting all first and second multiple primary melanomas, 1379 (60.4%) had a decreased Breslow thickness, 82 (3.6%) had the same Breslow thickness, and 823 (36.0%) had an increased Breslow thickness of the second melanoma.

Translated to stages, 370 (16.2%) of second melanomas had a higher T stage, 1112 (48.7%) had the same T stage, and 802 (35.1%) had a lower T stage (P < .001) (Table 3).

Table 3. T-stage of second melanomas in patients with multiple primary melanoma in the Netherlands, 2000-2014[a]

T-stage second melanoma	T1	T2	T3	T4	Total
T-stage first melanoma					
T1	1001[b]	203[c]	74[c]	35[c]	1,313
T2	397[d]	75[b]	28[c]	16[c]	516
T3	218[d]	72[d]	25[b]	14[c]	329
T4	79[d]	23[d]	13[d]	11[b]	126
Total	1695	373	140	76	2,284

[a] All converted to the eighth edition of the American Joint Committee on Cancer Cancer Staging Manual. Overall P < .001.
[b] Same T stage as first melanoma.
[c] Higher T stage than first melanoma.
[d] Lower T stage than first melanoma.

Overall survival among patients with single vs multiple primary melanoma

Because of missing data, a total of 38 816 cases were analyzed: 37 049 single primary melanomas and 1767 multiple primary melanomas. Corrected for all variables, an HR of 1.31 (95% CI, 1.20-1.42) (P < .001) for multiple primary melanomas vs a single primary melanoma was found (Table 4). In multivariable analysis, age per year (HR, 1.06; 95% CI, 1.05-1.06), Breslow thickness per mm (HR, 1.11; 95% CI, 1.10-1.12), and presence of ulceration (HR, 2.20; 95% CI, 2.14-2.25) increased. Females were less likely than males to die at any given time (overall HR, 0.71; 95% CI, 0.67-0.76). Compared with a single primary melanoma, all other types of melanoma had a lower HR, although the HR for lentigo maligna melanoma was not significant. Analysis including pathologic characteristics most likely to be associated with death yielded an HR of 1.12 (95% CI, 1.01-1.24; P = .04).

Table 4. Cox multivariable regression with time-varying covariate for death due to SPM vs MPM in the Netherlands, 2000-2014.

	HR (95% CI)	p-value
Age (per year)	1.06 (1.05-1.06)	<0.001[a]
Breslow thickness (per mm)	1.11 (1.10-1.12)	<0.001[a]
Sex		
Male	Reference	
Female	0.71 (0.67-0.76)	<0.001[a]

Table 4. (continued).

	HR (95% CI)	p-value
Body site		
H&N	Reference	
Trunk	0.92 (0.86-0.99)	0.023[a]
Arms	0.72 (0.63-0.80)	<0.001[a]
Legs	0.80 (0.73-0.88)	<0.001[a]
Ulceration		
No	Reference	
Yes	2.20 (2.14-2.25)	<0.001[a]
Type melanoma		
SSM	Reference	
Nodular	1.33 (1.27-1.39)	<0.001[a]
LMM	0.99 (0.88-1.10)	0.87
ALM	1.52 (1.39-1.70)	<0.001[a]
Multiple melanoma		
No (SPM)	Reference	
Yes (MPM)	1.31 (1.20-1.42)	<0.001[a]

Abbreviations: MPM, multiple primary melanoma; SPM, single primary melanoma.
[a] Statistically significant.

DISCUSSION

We found a 4.0% prevalence of multiple primary melanomas among all patients with melanoma in the Netherlands from 2000 through 2014. A total of 36.8% of second multiple primary melanomas were found during the first year of follow-up, and 27.3% of these melanomas were found after 5 years. We observed a decrease in Breslow thickness between subsequent melanomas and that overall survival among patients with multiple primary melanomas was significantly worse compared with that among patients with a single primary melanomas.

The 4.0% observed multiple primary melanoma prevalence that we found is in line with the literature, in which a range of 0.2% to 12.7% has been described.[5-16] Some studies incorporated melanoma in situ in this percentage.[6-8,13] We observed a significant decrease in Breslow thickness between the first and second multiple primary melanomas. Whereas many other studies confirmed this finding,[5,8,9,12,14,16,32] few assessed the question of why subsequent melanomas tend to have decreased Breslow thickness. It has been argued that patients themselves were likely to detect their first primary melanoma.[17,33] However, little is known about the detection of subsequent primary melanomas. Several studies[18,33,34] reported that subsequent melanomas were most often detected by physicians (up to 94%-95%). Francken et al[35] showed that a history of melanoma was not associated with an increase in the ability

of patients to detect new primary melanoma themselves. De Giorgi et al36 showed that patients with melanoma who did not attend follow-up visits had significantly increased Breslow thickness for the second melanoma, suggesting that decreased Breslow thickness is associated with dermatologic surveillance.

A total of 36.8% of second multiple primary melanomas were found in the first year of follow-up, but no less than 27.3% of second melanomas were found after 5 years of follow-up, comparable with another study.[6] Ferrone et al[8] observed a 59% rate within the first year and Murali et al[9] found a 58% rate in the first 3 years, but neither reported on the incidence of second melanoma after 5 years. Menzies et al[13] and Moore et al[32] found a median time of 2.8 years and 3.8 years between the first and second multiple primary melanomas, comparable with the 2.2 years that we observed. We stratified the 36.8% of multiple primary melanomas by stage; when selecting only patients with initial T1a melanoma, this percentage stayed almost stable because 24.8% of the second melanomas in this selected group of patients were found after 5 years. Current Dutch and international guidelines recommend discontinuation of follow-up in all patients with melanoma 5 years after the initial diagnosis.[37] International follow-up guidelines differ considerably, ranging from no follow-up for pT1a melanoma to lifelong follow-up for all patients.20 Few guidelines comment about patients with multiple primary melanomas. The American Association of Dermatology states that multiple primary melanoma is one of the factors that "may influence follow-up interval."[38(p22)] Other guidelines do not comment on follow-up but instead focus on the hereditary component in patients with multiple primary melanomas.[39,40] The review by Francken et al[17] suggests that no strong evidence exists to support a specific follow-up interval for regular melanoma. Even though the article is from 2005, it reported that most investigators advocated long-term or even lifelong follow-up for patients with multiple melanoma. Follow-up surveillance in patients with melanoma can serve several goals: detect metastases, find a subsequent melanoma, or reassure and educate patients. One could argue that finding a subsequent melanoma is not a main goal, as in the Netherlands, because pT1a melanomas are not incorporated in the follow-up surveillance. Because the risk of metastasis in this group of patients is low, it is not useful to screen these patients for a subsequent melanoma. However, because the prevalence of melanoma has increased and the risk of metastasis and thus death is low, paradoxically, this group of patients may have a high risk of developing a subsequent melanoma.

Several studies have analyzed survival among patients with a single primary melanoma vs that among patients with multiple primary melanomas; 3 found better survival,[16,22,23] 1 found similar survival,[21] and 4 found worse survival among patients with multiple primary melanomas vs patients with a single primary melanoma,[24-27] as we did in the present study. Only 3 studies corrected for immortal time bias of multiple primary melanomas.21,24,26 Pardo et al[24] used Cox time-varying analysis

and found an HR of 1.32 (95% CI, 1.17-1.50) among 1210 patients with multiple primary melanomas. Youlden et al[26] applied delayed-entry methods and found worse survival among 2330 patients with multiple primary melanomas (HR, 2.01; 95% CI, 1.57-2.59). On the contrary, Grossmann et al[21] recently analyzed survival data obtained from the Surveillance, Epidemiology, and End Results registry using a 1:1 matching technique to prevent bias and found no survival difference in 887 cases (HR of 1.07 [95% CI, 0.87-1.31] for all melanomas and HR of 0.99 [95% CI, 0.76-1.29] for only invasive melanomas). Our results are in line with Pardo et al[24] and Youlden et al.[26] We found an HR of 1.31 (95% CI, 1.20-1.42; P < .001) for multiple primary melanomas vs a single primary melanoma, indicating that the hazard of dying of multiple primary melanomas at any given time was 1.31 times higher than the hazard of dying of a single primary melanoma, corrected for all potential confounders included in the analysis.

Because patients with multiple primary melanomas had worse survival than patients with a single primary melanoma in our multivariable analysis, we believe that a patient, once proven to have developed a second melanoma, may benefit from more thorough surveillance. Adjuvant therapies are being developed and studied, and in the near future, their use may extend beyond patients with stage IV disease. We argue that patients with multiple primary melanomas may benefit from being monitored more closely, not only for subsequent melanoma, but especially for metastases.

Strengths and limitations

One of the strengths of this study is that, to our knowledge, we used the largest epidemiologic cohort thus far for answering this research question. We also used the appropriate statistical techniques to assess survival between patients with a single primary melanoma and those with multiple primary melanomas (time-dependent exposures), thus best preventing immortal time bias.[29-31]

A limitation is the lack of information about family history because patients with familial atypical mole and melanoma syndrome are known to have multiple primary melanomas and to be younger at the time of diagnosis of their first melanoma. Another possible limitation was that we chose not to include melanoma in situ. Previous studies were not always clear if they included melanoma in situ. We argue that we would have found even more multiple primary melanomas if we had included melanoma in situ, since Leiter et al[6] showed 33.6% of secondary melanoma to be melanoma in situ. However, since we aimed to analyze differences in Breslow thickness and association with overall survival, 2 features not related to melanoma in situ, we chose not to include noninvasive melanoma. All multiple primary melanoma studies are inherently hampered by the lack of an adequate definition of multiple primary melanoma. Some studies excluded[8,15,32] and 1 study[9] separately analyzed simultaneous multiple primary melanomas, defined as a second primary melanoma diagnosed within 1 month after the first melanoma. Others defined multiple primary

melanoma as 2 or more primary melanomas diagnosed 1 year or further from each other,[21] but most have not reported their definition. We defined patients with multiple primary melanomas as having another melanoma on or after the date of the first diagnosis of melanoma irrespective of topography. A final limitation was that we did not have data available about immunosuppressive therapy, which is a known risk factor for survival in general and for developing melanoma.[41]

CONCLUSIONS

In this study, Breslow thickness decreased with subsequent melanomas. A high percentage of second melanomas occurred later than 5 years after the first melanoma, and overall survival among patients with multiple primary melanomas was significantly worse compared with that among patients with a single primary melanoma. The findings suggest that current melanoma follow-up strategies need to be reconsidered for patients with multiple primary melanomas and guidelines should comment on this.

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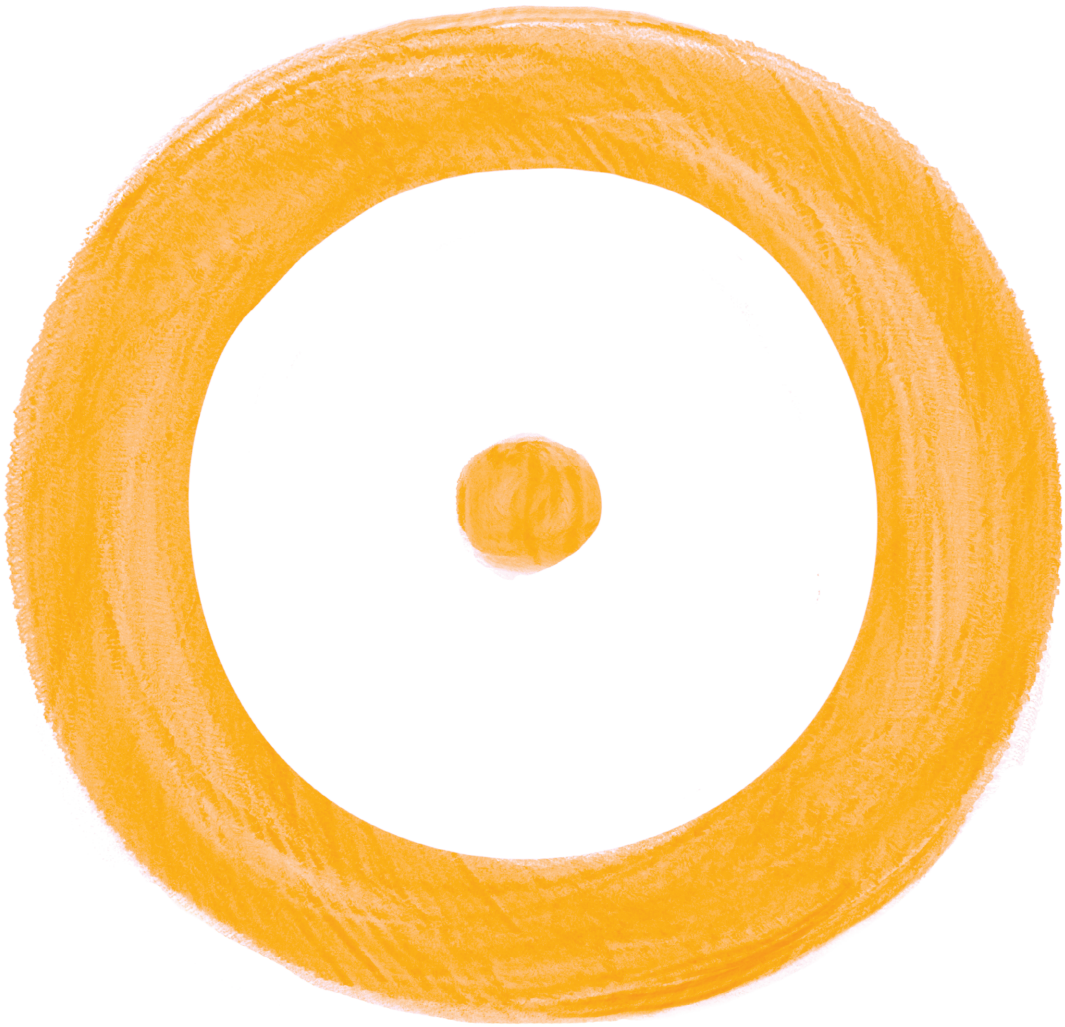

CHAPTER 11

The progressive relationship between increasing Breslow thickness and decreasing survival is lost in patients with ultra-thick melanomas (≥15mm in thickness)

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ABSTRACT

Background: Survival tends to decrease as the Breslow thickness of a primary melanoma increases. However, little is known about the prognostic value of Breslow thickness in patients with very thick melanomas. We sought to assess survival in patients with melanomas ≥4.0mm in Breslow thickness.

Methods: A pooled cohort of 5595 patients (4107 Dutch and 1488 Australian) with melanomas ≥4.0mm in thickness diagnosed from 2000-2014 was analyzed. Standard and spline Cox regressions were generated for overall survival (OS) and recurrence-free survival (RFS).

Results: Median follow-up was 3.4 years. Beyond 4mm, Breslow thickness was not linearly associated with survival. The continuous hazard ratio (HR) for OS and RFS increased steadily as Breslow thickness increased to 15mm, stabilized up to 20mm, and decreased thereafter. Using patients with melanomas 4-<10mm thick as a reference group, the categorical HR for OS increased up to the 15-<20mm thickness category and then decreased (HR 1.46 (95%CI 1.29-1.66), p<0.0001 for 10-<15mm, HR 1.97 (95%CI 1.55-2.51), p<0.0001 for 15-<20mm, and HR 1.36 (95%CI 1.07-1.84), p=0.045 for ≥20mm). For RFS, similar trends were observed.

Conclusions: The progressive relationship between increasing Breslow thickness and decreasing survival is lost in patients with melanomas ≥15mm in thickness.

INTRODUCTION

In 1970, Alexander Breslow observed that the deepest extent of invasion of a primary melanoma, measured in millimeters from the top of the granular layer of the epidermis to the deepest invasive tumor cell, was related to lymph node involvement and prognosis[1]. Today, this Breslow thickness measurement remains one of the most important predictors of melanoma recurrence and death[2], and it forms the basis for staging primary melanoma in the American Joint Committee on Cancer (AJCC) staging system and in the AJCC 8th Edition Staging Manual[2,3]. Survival tends to decrease as Breslow thickness increases. The AJCC staging system regards melanomas with a Breslow thickness of >4.0mm as 'thick', with a generally poor prognosis, and these represent approximately 7-8% of new melanoma diagnoses[2,4]. Most thick melanomas exceed 4mm in thickness by just a few mm (Supplementary Figure 1). However, little is known about the prognostic value of Breslow thickness in patients with melanomas >8mm. Our clinical impression has been that long-term survival occurs more often in patients with "ultra-thick" melanomas (which we defined as ≥15mm) than in some patients with melanomas in the 4-8mm range. The aims of the present study were to assess overall survival and recurrence-free survival in patients with melanomas ≥4.0mm in Breslow thickness, to determine whether increasing Breslow thickness beyond 4mm is still a significant progressive predictor of survival in these patients and to evaluate other predictors of outcome in them.

METHODS

Collection of data

For a Dutch melanoma cohort, data for all patients in the Netherlands with newly-diagnosed invasive melanoma diagnosed between January 2000 and December 2014 were obtained from PALGA, the Dutch Pathology Registry, which collects data from all pathology laboratories in the Netherlands[5]. Follow-up data were obtained from the Netherlands Cancer Registry, which records information on every cancer patient treated in the Netherlands. Follow-up was calculated from date of melanoma diagnosis until date of death, the date last known to be alive or January 1st 2018, whichever occurred earlier. All data were encoded and used anonymously. Ethical approval for the use of the data was granted by the PALGA board. For Australian melanoma patients, a cohort was obtained from the prospectively-maintained research database of Melanoma Institute Australia (MIA), a tertiary referral center that manages around a third of melanoma patients in the state of New South Wales, Australia. A search of the MIA database was performed for all patients with melanoma diagnosed in the same time period as the Dutch cohort. Prospective approval for use of the de-identified data was obtained from the Human Research Ethics Committee of the Royal Prince Alfred Hospital (Protocol X15-0454 & HREC/11/RPAH/444).

Study population

The study population consisted of 5595 patients who had clinically-localized primary cutaneous melanomas with a Breslow thickness ≥4.0mm. Patients in whom distant metastasis (other than in a sentinel node) was diagnosed within 12 weeks of diagnosis were excluded (n=406), as were those with more than one primary melanoma (n=79) and those with missing follow-up information (n=259); numbers for patients within 4-<10mm, 10-<15mm, 15-<20mm and ≥20mm Breslow thickness, 219/5182, 27/476, 9/116 and 4/80, respectively. As well, patients who were initially treated elsewhere but later referred to MIA for follow-up or further management (n=209 were excluded, to eliminate referral bias).

For each patient, the demographic data collected included date of primary diagnosis, age, gender, location of the melanoma and recurrence. Pathological data included Breslow thickness, melanoma subtype, SN status, presence or absence of ulceration and presence or absence of mitoses. For both datasets, the clinical outcomes recorded were recurrence-free survival (RFS) and overall survival (OS). RFS was defined as the time from primary melanoma diagnosis until first recurrence (either cutaneous or subcutaneous (local or in-transit), nodal (regional) or distant metastasis) or death from any cause, whichever occurred first. Patients without recurrence were censored at either their date of death or the date they were last known to be alive or January 1st 2018 (the data collection cut-off date), whichever occurred earlier.

Statistical analysis

Clinicopathological characteristics were summarized using descriptive statistics. Patients were stratified into four different Breslow thickness categories: 4-<10mm, 10-<15mm, 15-<20mm and ≥20mm. To assess whether there were any differences between the Dutch patients and Australian patients, the two cohorts were initially analyzed separately. Survival distributions for RFS and OS were estimated using Kaplan-Meier curves and compared using the log-rank two-tailed test. Five-year survival rates were computed for the whole cohort, and for the cohort excluding patients with desmoplastic melanoma. To have sufficient numbers of events to allow meaningful analyses, the two cohorts were combined for the modelling analysis. The association between survival outcomes and Breslow thickness as a continuous variable was investigated using spline-based hazard ratio curves, taking patients with melanomas of 4.0mm in thickness as the reference group[6]. Furthermore, multivariable survival analyses using stratified Cox regression were performed[7]. Further specifications are provided in the Supplementary materials. This study adhered to the guideline for the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) and the checklist was completed (Supplementary Table 1)[8]. All statistical analyses were performed using R version 3.6.1 (R Core Team, Vienna, Austria). A two-sided p-value of <0.05 was considered statistically significant.

RESULTS

Clinicopathological features of all 5595 included patients with thick melanomas are summarized in Table 1. The majority of them were male (57.87%), with the most common primary melanoma site being on the trunk (36.4%). Nodular melanoma was the most common subtype (59.8%), and 61.8% of the thick melanomas were ulcerated. The SN was positive in 37.2%. Supplementary Figure 1 shows the distribution of Breslow thickness for all patients. Overall, patients with Breslow thickness ≥4.0mm represented 8.1% of the pooled cohorts; 8.2% in the Dutch registry and 7.8% from the MIA research database. The numbers of patients categorized with melanomas 4-<10mm, 10-<15mm, 15-<20mm and ≥20mm in thickness were 4963, 449, 107 and 76, respectively.

Clinicopathological features of the Dutch and MIA melanoma patients with thick melanomas are shown separately in Supplementary Table 2. There was no statistically significant difference in Breslow thickness for the Dutch and the MIA cohorts (median Breslow thickness 5.4mm and 5.5mm, respectively, p=0.14). In total, 45.7% of Dutch patients were female, compared to 32.8% of MIA patients (p<0.001). The melanoma subtype was desmoplastic in 20.3% of MIA patients compared to 2.3% of Dutch patients (p<0.0001).

Table 1. Summary* of clinicopathological factors for pooled cohorts of patients with melanomas ≥4.0mm thick.

Characteristic	All patients (n=5595)	4-<10mm (n=4963)	10-<15mm (n=449)	15-<20mm (n=107)	≥20mm (n=76)	p-value
Sex (n (%))						0.39
Female	2365 (42.3)	2112 (42.6)	173 (38.5)	48 (44.9)	32 (42.1)	
Male	3230 (57.7)	2851 (57.4)	276 (61.5)	59 (55.1)	44 (57.9)	
Median age at diagnosis in years (IQR)	69 (57-79)	69 (57-79)	71 (57-82)	73 (56-83)	67 (56-76)	0.04
Primary site (n (%))						0.17
Head & Neck	1276 (22.8)	1128 (22.7)	111 (24.7)	21 (19.6)	16 (21.1)	
Trunk	2034 (36.4)	1778 (35.8)	170 (37.9)	51 (47.7)	35 (46.1)	
Upper limb	844 (15.1)	756 (15.2)	64 (14.3)	15 (14.0)	9 (11.8)	
Lower limb	1441 (25.8)	1301 (26.2)	104 (23.2)	20 (18.7)	16 (21.1)	
Median Breslow thickness in mm (IQR)	5.5 (4.5-7.0)	5.0 (4.4-6.5)	11.0 (10.0-12.0)	16.0 (15.0-17.0)	25.0 (22.0-35.5)	<0.0001
Subtype (n (%))						<0.0001
Superficial spreading	1295 (26.8)	1211 (28.2)	60 (15.6)	11 (11.5)	13 (23.2)	
Nodular	2889 (59.8)	2536 (59.0)	250 (65.1)	66 (68.8)	37 (66.1)	
Lentigo maligna	101 (2.1)	94 (2.2)	6 (1.6)	1 (1.0)	0 (0.0)	
Acral lentiginous	148 (3.1)	136 (3.2)	11 (2.9)	1 (1.0)	0 (0.0)	
Desmoplastic	398 (8.1)	318 (7.4)	57 (14.8)	17 (17.7)	6 (10.7)	
Not known	764	668	65	11	20	
Ulceration (n (%))						<0.0001
No	1892 (38.2)	1751 (39.9)	103 (25.5)	22 (23.2)	16 (23.5)	
Yes	3059 (61.8)	2633 (60.1)	301 (74.5)	73 (76.8)	52 (76.5)	
Not known	644	579	45	12	8 ()	
Mitoses						0.11

Table 1. (continued).

Characteristic	All patients (n=5595)	4-<10mm (n=4963)	10-<15mm (n=449)	15-<20mm (n=107)	≥20mm (n=76)	p-value
No	95 (3.6)	82 (3.4)	12 (6.5)	1 (2.0)	0 (0.0)	
Yes	2568 (96.4)	2320 (96.6)	173 (93.5)	48 (98.0)	27 (100.0)	
Not known	2932	2561	264	58	49	
SN status (n (%))						0.99
Negative	1395 (62.8)	1275 (62.9)	80 (61.5)	26 (61.9)	14 (63.6)	
Positive	825 (37.2)	751 (37.1)	50 (38.5)	16 (38.1)	8 (36.4)	
Not performed	1230	2937	319	65	54	
Median follow-up in years (IQR)	3.4 (1.6-6.5)	3.6 (1.7-6.8)	2.3 (1.0-4.2)	2.0 (0.7-4.5)	2.2 (0.8-5.8)	<0.0001

* Categorical variables were summarized as numbers and percentages. Continuous variables were summarized as medians with interquartile ranges (IQR). Differences in proportions and medians were analyzed using the chi-square test and the Mann-Whitney U test, respectively.

Kaplan-Meier analyses

Figures 1 and 2 show the Kaplan-Meier curves for OS and RFS for the combined cohorts, stratified according to Breslow thickness. Five-year OS for patients with melanomas of 4-<10mm, 10-<15mm, 15-<20mm and ≥20mm was 52.9% (95%CI 51.4-54.4), 35.9% (95%CI 31.2-41.3), 34.5% (95%CI 25.9-46.0) and 47.9% (95%CI 37.3-61.6), respectively. Five-year RFS was 38.2% (95%CI 36.7-39.7), 25.0% (95%CI 21.0-29.9), 19.3% (95%CI 12.7-29.4) and 32.0% (95%CI 22.8-45.1), respectively. Five-year survival rates excluding patients with desmoplastic melanomas (n=398) are shown in Supplementary Table 3.

Figure 1. Kaplan-Meier curves for overall survival in patients with thick melanomas.

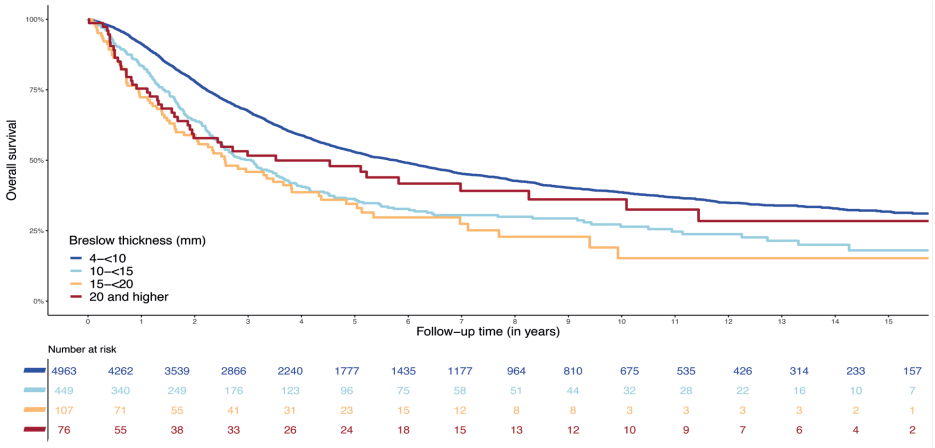
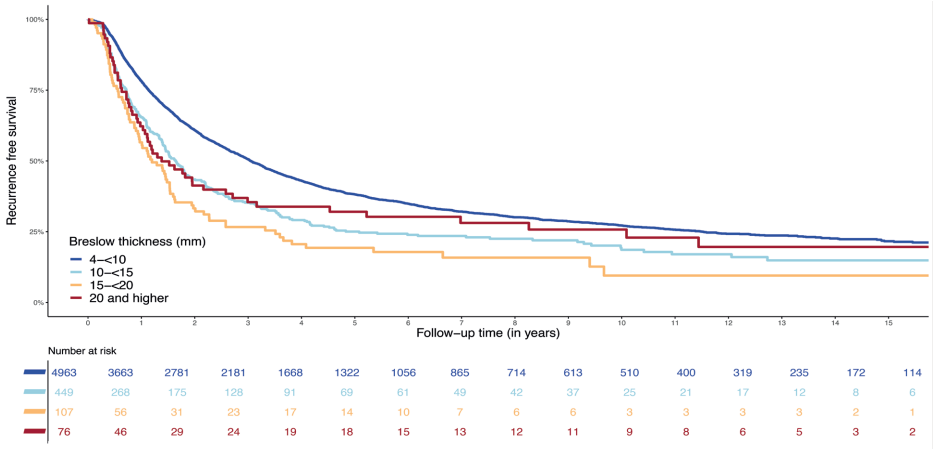


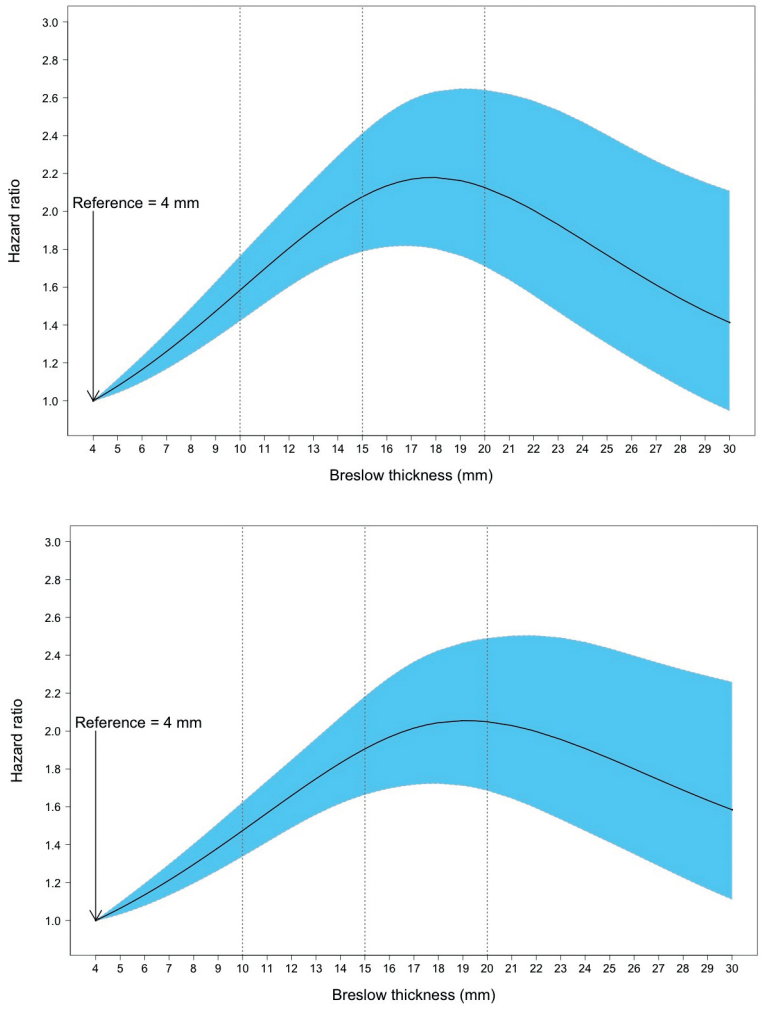
Figure 2. Kaplan-Meier curves for recurrence-free survival in patients with thick melanomas.



Smooth HR curves

The derived spline-based hazard ratio curves and the associated 95% CIs for OS and RFS are depicted in Figure 3. The curves showed a nonlinear association between Breslow thickness beyond 4mm and either OS or RFS. For both survival outcomes, the HR increased in patients with a Breslow thickness up to 15mm, stabilized up to 20mm, and steadily decreased thereafter.

Figure 3. Multivariable penalized spline model of Breslow thickness as a continuous variable on overall survival (top panel) and recurrence-free survival (bottom panel), reflecting hazard ratios with their 95% confidence intervals. A Breslow thickness of 4mm was taken as the reference (HR=1.0).



406 patients with synchronous metastasis were excluded, however the patterns remained the same when the 406 patients were included in a sensitivity analysis (data not shown).

Cox analyses in the total cohort

Univariable and multivariable Cox analyses are shown in Table 2. When correcting for sex, age, primary tumor site, ulceration, mitoses, melanoma subtype and SN status, OS and RFS were significantly lower for all patients compared to those with melanomas 4-<10mm in thickness.

Cox analysis by Breslow thickness subgroups

Univariable and multivariable Cox analyses for OS and RFS stratified by the four Breslow thickness groups are shown in Supplementary Tables 4-7. Because there were too few events, no multivariable analysis was performed in patients with melanomas ≥20mm thick. In patients with melanomas 4-<10mm in thickness, significant predictors of OS and RFS in multivariable analysis were sex, age, Breslow thickness, primary site, ulceration status and SN status. In patients with melanomas 10-<15mm thick, significant predictors for OS were age and ulceration status, and for RFS were age, ulceration status and SN status. In patients with melanomas 15-<20mm thick the number of deaths was 70, and only age and sex remained significant predictors for OS in multivariable analysis, whereas none of the variables reached statistical significance for RFS. In patients with melanomas ≥20mm thick 43 patients died, and the only significant predictor of OS was ulceration status, and for RFS significant predictors were ulceration status, melanoma subtype and SN status.

Table 2. Univariable and multivariable Cox regression hazard ratio estimates of overall survival and recurrence-free survival in all patients with thick melanomas (n=5595).

			OS (n=5595, 2895 events)				RFS (n=5595, 3616 events)			
			Univariable		Multivariable		Univariable		Multivariable	
Breslow thickness (mm)	N	N deaths	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
4-<10	4963	2512	1		1		1		1	
10-<15	449	270	1.61 (1.42-1.82)	<0.0001	1.46 (1.29-1.66)	<0.0001	1.48 (1.32-1.66)	<0.0001	1.34 (1.19-1.50)	<0.0001
15-20	107	70	2.03 (1.60-2.58)	<0.0001	1.97 (1.55-2.51)	<0.0001	1.99 (1.60-2.48)	<0.0001	1.94 (1.56-2.41)	<0.0001
≥20	76	43	1.38 (1.02-1.87)	0.04	1.36 (1.07-1.84)	0.045	1.36 (1.04-1.78)	0.03	1.39 (1.06-1.81)	0.02

Multivariable analyses corrected for: sex, age (18-35, 36-55, 56-75, >75), primary tumor site (head and neck, trunk, upper limb, lower limb), ulceration status (present, absent, not known), presence of mitoses (present, absent, not known), melanoma subtype (non-nodular* vs nodular), SN status (negative, positive, not performed). * Given a low number of events for some melanoma subtypes, patients were categorized as non-nodular (including missings) or nodular.

DISCUSSION

In previously published reports, 7-8% of patients presenting with primary cutaneous melanomas had tumors ≥4.0mm in Breslow thickness, categorized as "thick" melanomas[2-4]. In the present study, 8.1% of the pooled cohorts had tumors ≥4.0mm. These 5595 patients constitute the largest study of thick melanomas reported to date, as far as we are aware. Our thick melanomas were more commonly of nodular, acral or desmoplastic subtype, more frequently involved the head and neck region and were more often ulcerated than is reported to occur in thinner melanomas[9]. While increasing Breslow thickness is strongly associated with progressively worsening survival outcomes in patients with thin melanomas (≤1.0mm) and intermediate thickness melanomas (1.1-4.0mm)[2,10], its progressive association with survival outcomes was lost in patients with "ultra-thick" melanomas (which we defined arbitrarily as ≥15mm).

We were able to identify only three previously published studies in which survival in patients with very thick melanomas was analyzed. Blakely et al. reported 37 patients with melanomas ≥8mm thick, and compared OS and RFS to that of 58 patients with melanomas 4.0-<8.0mm[11]. RFS for patients with melanomas ≥8mm was significantly shorter than for those with melanomas ≥4.0mm (p<0.0006), whereas OS for the two groups was similar (p=0.40). On multivariable Cox analysis (included variables age, sex, primary tumor site, Breslow thickness (4-8mm vs ≥8mm), margin of excision, ulceration, lymphovascular invasion, mitotic rate, complex wound coverage, SN status and neutrophil to lymphocyte ratio), the authors found a HR of 2.9 (95%CI 1.46-5.93), p=0.003) for patients with melanomas ≥8mm for RFS. HRs for OS were not provided. We note that their dataset was small and there were only 21 events. In the current study, ulceration status was the only significant predictor for OS in patients with melanomas ≥20mm in thickness, as it was for RFS in addition to SN status and melanoma subtype. When assessing the progressive impact of Breslow thickness continuously, its progressive value was lost in patients with melanomas ≥15mm in thickness. Han et al. analyzed 1235 patients, reported in two separate articles, with a median follow-up of 2.3 years; 279 patients had a Breslow thickness of ≥8mm[12,13]. Their results showed that for melanoma-specific survival (MSS), the correlation between thickness and MSS was lost once melanomas became thicker than 8mm[11]. When they analyzed OS, they found that patients with melanomas >10mm thick had a HR of 1.41 (95%CI 1.07-1.87, p=0.02) compared to those with melanomas 4-6mm thick (consistent with our results)[13]. Patients with melanomas >6-10mm had a HR of 1.11 (95%CI 0.89-1.38), p=0.35) compared to those with melanomas 4-6mm thick. For RFS, compared to patients with melanomas 4-6mm thick, the HR was 1.39 (95%CI 1.14-1.71, p=0.001) for patients with melanomas 6-10mm thick, and 1.25 (95%CI 0.95-1.66, p=0.11) for those with melanomas >10mm thick. HRs for patients with melanomas 6-10mm thick compared to those with >10mm thick were not provided.

A biological explanation for the more favorable than expected outcomes for patients with ultrathick melanomas remains uncertain. Anecdotally, we have recognized that a number of these ultrathick primary tumors had a polypoid architecture, raising the possibility that perhaps they had less capacity to access lymphatics or blood vessels as a consequence of their predominantly exophytic growth pattern. Indeed, when one of us (RVR) reviewed the available pathology reports, clinical images and / or slides of 16 MIA patients with melanomas ≥20.0mm thick included in the present study, 12 of the 16 (75%) had a polypoid, exophytic architecture. It is possible that Breslow thickness in polypoid tumors may not have the same prognostic implications as it does in conventional, non-polypoid tumors. Alternative hypotheses to explain the finding include biologically intrinsic factors unique to these tumors. For example, that these melanomas are able to reach such a large size without the development of detected metastatic disease probably indicates that they are less biologically aggressive. Our data suggest that desmoplastic melanomas are overrepresented in ultrathick primary melanomas. This melanoma subtype, particularly pure desmoplastic melanoma, is known to have a lower risk of SN metastasis and more a favorable outcome than melanomas without desmoplasia[14,15]. Nevertheless, the underlying molecular mechanisms and features of the tumor microenvironment that underpin the uncommon ultrathick melanoma subgroup require further study. Such studies may not only provide important insights into mechanisms of melanoma metastasis but may also identify novel drivers of prognosis in melanoma patients.

We excluded from the current study 406 patients with metastases detected clinically or by imaging within 12 weeks of diagnosis. Of these, 346 (85.2%) had locoregional disease and 60 (14.8%) had distant metastases. Blakely et al. excluded 9 of 61 patients (14.8%) with melanomas ≥4.0mm in thickness from their study because of clinically apparent loco-regional metastasis, compared to 406 of 5595 patients (7.3%) who we excluded. The two studies performed by Han et al. did not comment on the number of patients with synchronous locoregional or distant metastases. If we had included patients with synchronous metastases, the study population would have been very heterogeneous, since these patients are considered to have advanced disease and likely to have a poor prognosis. We therefore chose to exclude patients with metastases detected within 12 weeks from the initial melanoma diagnosis.

Strengths of our study include the large sample size and the use of nationwide data as well as data from a well-maintained database at a specialized tertiary institution. This increases the generalizability of our results and enabled us to include a sufficient number of events to distinguish different thickness subgroups among patients with melanomas ≥4.0mm. A limitation is the relatively small sample size of the subgroups of patient with melanomas 15mm-<20mm thickness (n=107) and ≥20mm (n=76). Another limitation is the large number of missing values for mitoses in the Dutch dataset. However, we suspect that it would not have been significantly associated with

RFS, as mitoses were nearly uniformly present in the MIA patients (94.2%), negating its discriminatory utility. Evaluation of the prognostic significance of mitotic rate (qualified as a number/mm2) was not addressed in our study as data on tumor mitotic rate was not available in the Dutch dataset. A final limitation was the median follow-up of only 3.4 years (ranging from 2.0 years in patients with melanomas 15-<20mm in thickness to 3.6 years in those with 4-<10mm thick melanomas). However, in this selected group of patients, 3.4 years could be considered relatively long as approximately 50% of those with melanomas >4mm in thickness who ultimately die of melanoma will have done so by 3.5 years[2].

CONCLUSIONS

While increasing Breslow thickness is strongly associated with worse survival outcomes in patients with cutaneous melanoma, its progressive prognostic value is lost in patients with "ultra-thick" melanomas (≥15mm in thickness).

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Additional Statistical Methods

The Cox stratification procedure is used to account for potential baseline risk heterogeneity when data from different populations are pooled. The stratification procedure is different from that of splitting the data and running 2 parallel regressions. Instead, a variable "cohort" is included in the model, assuming the baseline risk of experiencing an event is different for both populations. No variable selection was conducted; instead, all basic and readily available clinicopathological variables were included. In both the standard Cox and the spline regressions, the effect of Breslow thickness was adjusted for gender, age, primary tumor site, ulceration, mitoses, melanoma subtype and SN status. The proportional hazards assumption was evaluated using the Schoenfeld residuals test. An additional Cox analysis for each of the four Breslow thickness subgroups was performed, to evaluate which of the aforementioned variables were associated with survival. In these subgroup analyses, Breslow thickness itself was included based on the median thickness within each subgroup.

Supplementary Table 1. STROBE Statement—Checklist of items that should be included in reports of cohort studies.

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6,7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-8

Supplementary Table 1. (continued).

	Item No	Recommendation	Page No
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6,7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	7,8
		(*c*) Explain how missing data were addressed	7,8
		(*d*) If applicable, explain how loss to follow-up was addressed	7,8
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	9
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders	9, 19, 20, 26, 27
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) Summarize follow-up time (e.g., average and total amount)	18,27
Outcome data	15*	Report numbers of outcome events or summary measures over time	21
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9,21
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	7,8
Discussion			
Key results	18	Summarize key results with reference to study objectives	13

Supplementary Table 1. (continued).

	Item No	Recommendation	Page No
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-17
Generalizability	21	Discuss the generalizability (external validity) of the study results	16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for exposed and unexposed groups.

Supplementary Table 2. Clinicopathological features of Dutch and Australian patient cohorts with thick melanomas.

Characteristic	Dutch (n= 4107)	MIA (n=1488)	p-value
Sex (n (%))			<0.001
Female	1877 (45.7)	488 (32.8)	
Male	2230 (54.3)	1000 (67.2)	
Median age at diagnosis in years (IQR)	69 (56-80)	70 (58-79)	0.26
Primary site (n (%))			<0.001
Head & Neck	840 (20.5)	436 (29.3)	
Trunk	1506 (36.7)	528 (35.5)	
Upper limb	612 (14.9)	232 (15.6)	
Lower limb	1149 (28.0)	292 (19.6)	
Median Breslow thickness in mm (IQR)	5.4 (4.5-7.0)	5.5 (4.5-7.2)	0.14
Breslow thickness in mm (n (%))			0.65
4-<10	3645 (88.8)	1318 (88.6)	
10-<15	332 (8.1)	117 (7.9)	
15-<20	73 (1.8)	34 (2.3)	
≥20	57 (1.4)	19 (1.3)	
Melanoma subtype (n (%))			<0.001
Superficial spreading	1069 (30.7)	226 (16.7)	
Nodular	2129 (61.2)	760 (56.1)	
Lentigo maligna	76 (2.2)	25 (1.9)	
Acral lentiginous	107 (3.1)	41 (3.0)	
Desmoplastic	96 (2.8)	302 (22.3)	

Supplementary Table 2. Continued.

Characteristic	Dutch (n= 4107)	MIA (n=1488)	p-value
Not known	630	134	
Ulceration (n (%))			<0.001
No	1231 (34.8)	661 (46.9)	
Yes	2310 (65.2)	749 (53.1)	
Not known	566	78	
Mitoses			0.003
No	58 (4.7)	37 (2.56)	
Yes	1166 (95.3)	1402 (97.4)	
Not known	2883	49	
SN status (n (%))			<0.001
Negative	712 (55.6)	683 (72.7)	
Positive	568 (44.4)	257 (27.3)	
Not performed	2827	548	
Median follow-up in years (IQR)	3.6 (1.8-7.0)	3.0 (1.2-5.5)	<0.001

Supplementary Table 3. Overview of five-year overall survival and recurrence-free survival rates for patients with thick melanomas, stratified for all patients, and for all patients excluding those with desmoplastic melanomas (n=398).

	5-year overall survival (95% CI)		5-year recurrence-free survival (95% CI)	
Breslow thickness	All patients	Excluding patients with desmoplastic melanomas	All patients	Excluding patients with desmoplastic melanomas
4-<10	52.9 (51.4-54.4)	52.0 (50.5-53.6)	38.2 (36.7-39.7)	37.4 (35.9-38.9)
10-<15	35.9 (31.2-41.3)	32.5 (27.7-38.2)	25.0 (21.0-29.9)	21.4 (17.4-26.4)
15-<20	34.5 (25.9-46.0)	27.1 (18.6-39.5)	19.3 (12.7-29.4)	14.4 (8.3-25.0)
≥20	47.0 (37.3-61.6)	43.8 (33.1-58.2)	32.0 (22.8-45.1)	31.1 (21.7-44.7)

Supplementary Table 4. Univariable and multivariable Cox regression hazard ratio estimates of overall survival and recurrence-free survival in patients with melanomas 4-<10mm in thickness (n=4963).

			Overall survival (2512 events)				Recurrence-free survival (3162 events)			
			Univariable		Multivariable		Univariable		Multivariable	
Characteristic	N patients	N deaths	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Sex										
Male	2851	1469	1		1		1		1	
Female	2112	1043	0.87 (0.80-0.94)	0.0005	0.83 (0.76-0.91)	<0.0001	0.90 (0.84-0.97)	0.005	0.88 (0.82-0.95)	0.002
Age at diagnosis (years)										
18-35	217	53	1		1		1		1	
36-55	935	359	1.78 (1.33-2.37)	<0.0001	1.66 (1.24-2.21)	0.0006	1.76 (1.40-2.22)	<0.0001	1.67 (1.32-2.11)	<0.0001
56-75	2097	972	2.58 (1.96-3.41)	<0.0001	2.39 (1.81-3.15)	<0.0001	2.23 (1.78-2.79)	<0.0001	2.13 (1.70-2.66)	<0.0001
>75	1714	1128	5.48 (4.16-7.23)	<0.0001	5.02 (3.79-6.64)	<0.0001	3.48 (2.78-4.36)	<0.0001	3.12 (2.48-3.93)	<0.0001
Breslow thickness (mm)										
≤5	3156	1505	1		1		1		1	
>5	1807	1007	1.27 (1.18-1.38)	<0.0001	1.17 (1.08-1.26)	0.0001	1.23 (1.15-1.32)	<0.0001	1.15 (1.07-1.23)	0.0001
Primary site										
H&N	1128	615	1		1		1		1	
Trunk	1778	913	0.81 (0.73-0.90)	<0.0001	1.07 (0.96-1.19)	0.21	0.82 (0.75-0.90)	<0.0001	0.99 (0.90-1.10)	0.91
Arm	756	346	0.74 (0.65-0.84)	<0.0001	0.83 (0.72-0.95)	0.006	0.70 (0.62-0.79)	<0.0001	0.77 (0.68-0.87)	<0.0001
Leg	1301	638	0.72 (0.64-0.80)	<0.0001	0.85 (0.76-0.96)	0.008	0.87 (0.79-0.96)	0.005	1.00 (0.90-1.11)	0.93
Melanoma subtype										
SSM and other	2427	1197	1		1		1		1	
NM	2536	1315	1.07 (0.99-1.15)	0.11	0.98 (0.91-1.06)	0.65	1.03 (0.96-1.11)	0.36	0.99 (0.92-1.06)	0.68
Ulceration										
No	1751	692	1		1		1		1	

Supplementary Table 4. (continued).

			Overall survival (2512 events)				Recurrence-free survival (3162 events)			
			Univariable		Multivariable		Univariable		Multivariable	
Characteristic	N patients	N deaths	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Yes	2633	1507	1.68 (1.54-1.84)	<0.0001	1.55 (1.41-1.70)	<0.0001	1.58 (1.46-1.71)	<0.0001	1.46 (1.35-1.58)	<0.0001
Not known	579	313	1.11 (0.97-1.27)	0.13	1.08 (0.94-1.24)	0.27	1.02 (0.90-1.15)	0.74	0.99 (0.88-1.12)	0.90
Mitosis										
No	82	29	1				1			
Yes	2320	885	1.27 (0.88-1.84)	0.21	1.23 (0.85-1.79)	0.27	1.26 (0.93-1.72)	0.14	1.15 (0.84-1.57)	0.39
Not known	2561	1598	1.72 (1.18-2.48)	0.004	1.32 (0.91-1.93)	0.14	1.60 (1.17-2.18)	0.003	1.22 (0.89-1.67)	0.22
SN status										
Negative	1275	381	1		1		1		1	
Positive	751	424	2.05 (1.78-2.35)	<0.0001	2.09 (1.82-2.41)	<0.0001	1.97 (1.75-2.22)	<0.0001	1.98 (1.75-2.23)	<0.0001
Not performed	2937	1707	2.26 (2.02-2.54)	<0.0001	1.62 (1.42-1.84)	<0.0001	2.04 (1.85-2.25)	<0.0001	1.65 (1.47-1.84)	<0.0001

Supplementary Table 5. Univariable and multivariable Cox regression hazard ratio estimates of overall survival and recurrence-free survival in patients with melanomas 10-<15mm in thickness (n=449).

			Overall survival (270 events)				Recurrence-free survival (317 events)			
			Univariable		Multivariable		Univariable		Multivariable	
Characteristic	**N patients**	**N deaths**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Sex										
Male	276	157	1		1		1		1	
Female	173	113	1.00 (0.78-1.27)	0.97	0.93 (0.71-1.20)	0.56	0.95 (0.76-1.19)	0.67	0.91 (0.72-1.17)	0.46
Age at diagnosis (years)										
18-35	13	4	1		1		1		1	
36-55	83	34	1.28 (0.45-3.61)	0.64	1.46 (0.51-4.15)	0.48	1.68 (0.67-4.23)	0.27	2.04 (0.80-5.19)	0.14
56-75	176	103	2.50 (0.92-6.80)	0.07	2.36 (0.86-6.46)	0.09	2.37 (0.97-5.82)	0.06	2.32 (0.94-5.73)	0.07
>75	177	129	4.23 (1.56-11.48)	0.005	4.49 (1.65-12.24)	0.003	3.19 (1.30-7.83)	0.01	3.29 (1.34-8.11)	0.01
Breslow thickness (mm)										
≤11	255	152	1		1		1		1	
>11	194	118	0.94 (0.74-1.19)	0.60	0.93 (0.73-1.19)	0.57	0.93 (0.75-1.17)	0.54	0.90 (0.72-1.13)	0.36
Primary site										
H&N	111	66	1		1		1			
Trunk	170	110	0.87 (0.64-1.18)	0.37	0.95 (0.69-1.31)	0.76	0.97 (0.72-1.29)	0.81	0.99 (0.74-1.34)	0.96
Arm	64	36	0.79 (0.52-1.18)	0.25	0.79 (0.51-1.20)	0.27	0.76 (0.52-1.12)	0.17	0.76 (0.51-1.13)	0.17
Leg	104	58	0.69 (0.48-0.98)	0.04	0.67 (0.46-0.98)	0.04	0.93 (0.67-1.28)	0.65	0.91 (0.65-1.28)	0.60
Melanoma subtype										
SSM and other	199	99	1				1		1	
NM	250	171	1.33 (1.04-1.71)	0.003	1.24 (0.95-1.61)	0.12	1.30 (1.03-1.64)	0.003	1.22 (0.96-1.56)	0.11
Ulceration										
No	103	48	1		1		1		1	

Supplementary Table 5. Continued.

			Overall survival (270 events)				Recurrence-free survival (317 events)			
			Univariable		Multivariable		Univariable		Multivariable	
Characteristic	N patients	N deaths	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Yes	301	197	1.72 (1.25-2.37)	0.0009	1.81 (1.29-2.53)	0.0005	1.57 (1.18-2.08)	0.002	1.54 (1.14-2.08)	0.005
Not known	45	25	0.85 (0.52-1.39)	0.52	1.00 (0.61-1.65)	0.99	0.83 (0.53-1.30)	0.41	0.85 (0.54-1.36)	0.50
Mitosis										
No	12	4	1				1			
Yes	173	65	1.66 (0.60-4.58)	0.33	1.13 (0.40-3.16)	0.82	2.07 (0.84-5.11)	0.11	1.52 (0.61-3.78)	0.38
Not known	264	201	1.82 (0.67-4.92)	0.24	1.21 (0.43-3.40)	0.72	2.01 (0.82-4.93)	0.13	1.27 (0.50-3.23)	0.62
SN status										
Negative	80	31	1		1		1		1	
Positive	50	30	1.34 (0.81-2.23)	0.26	1.38 (0.83-2.32)	0.22	1.43 (0.91-2.25)	0.13	1.45 (0.91-2.30)	0.12
Not performed	319	209	1.53 (1.04-2.27)	0.03	1.18 (0.77-1.80)	0.46	1.58 (1.11-2.24)	0.01	1.48 (1.01-2.15)	0.04

Supplementary Table 6. Univariable and multivariable Cox regression hazard ratio estimates of overall survival and recurrence-free survival in patients with melanomas 15-<20mm in thickness (n=107).

			Overall survival (70 events)				Recurrence-free survival (83 events)			
			Univariable		Multivariable		Univariable		Multivariable	
Characteristic	**N patients**	**N deaths**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Sex										
Male	59	37	1		1		1		1	
Female	48	33	1.01 (0.62-1.63)	0.98	0.50 (0.26-0.95)	0.03	1.03 (0.66-1.60)	0.90	0.63 (0.35-1.12)	0.11
Age at diagnosis (years)										
36-55	24	11	1		1		1		1	
56-75	38	22	1.19 (0.57-2.49)	0.64	1.11 (0.50-2.49)	0.79	1.65 (0.85-3.17)	0.14	1.30 (0.62-2.73)	0.49
>75	45	37	4.03 (1.92-8.48)	0.0002	4.46 (1.93-10.34)	0.0005	2.93 (1.50-5.74)	0.002	2.08 (0.97-4.46)	0.06
Breslow thickness (mm)										
≤16	62	42	1		1		1		1	
>16	45	28	1.38 (0.83-2.29)	0.22	1.70 (0.94-3.10)	0.08	1.12 (0.71-1.77)	0.62	1.28 (0.76-2.18)	0.36
Primary site										
H&N	21	11	1		1		1		1	
Trunk	51	31	0.88 (0.43-1.77)	0.71	1.16 (0.52-2.58)	0.71	0.71 (0.38-1.31)	0.27	0.99 (0.49-2.02)	0.99
Arm	15	11	1.74 (0.74-4.10)	0.21	1.60 (0.58-4.44)	0.37	1.19 (0.56-2.53)	0.66	1.23 (0.51-2.96)	0.65
Leg	20	17	0.86 (0.39-1.89)	0.70	1.18 (0.49-2.87)	0.71	0.81 (0.40-1.65)	0.56	1.11 (0.50-2.44)	0.80
Melanoma subtype										
SSM and other	41	25	1		1		1		1	
NM	66	45	1.34 (0.81-2.17)	0.26	1.47 (0.84-2.58)	0.18	1.06 (0.68-1.67)	0.79	0.98 (0.59-1.61)	0.92
Ulceration										
No	22	10	1		1		1		1	
Yes	73	52	1.68 (0.85-3.33)	0.14	1.51 (0.69-3.31)	0.31	1.36 (0.76-2.41)	0.30	1.25 (0.60-2.63)	0.55
Not known	12	8	0.90 (0.33-2.44)	0.83	0.73 (0.25-2.16)	0.57	0.58 (0.23-1.47)	0.25	0.59 (0.19-1.81)	0.36
Mitosis										
Yes	24	24	1		1		1		1	

Supplementary Table 6. (continued).

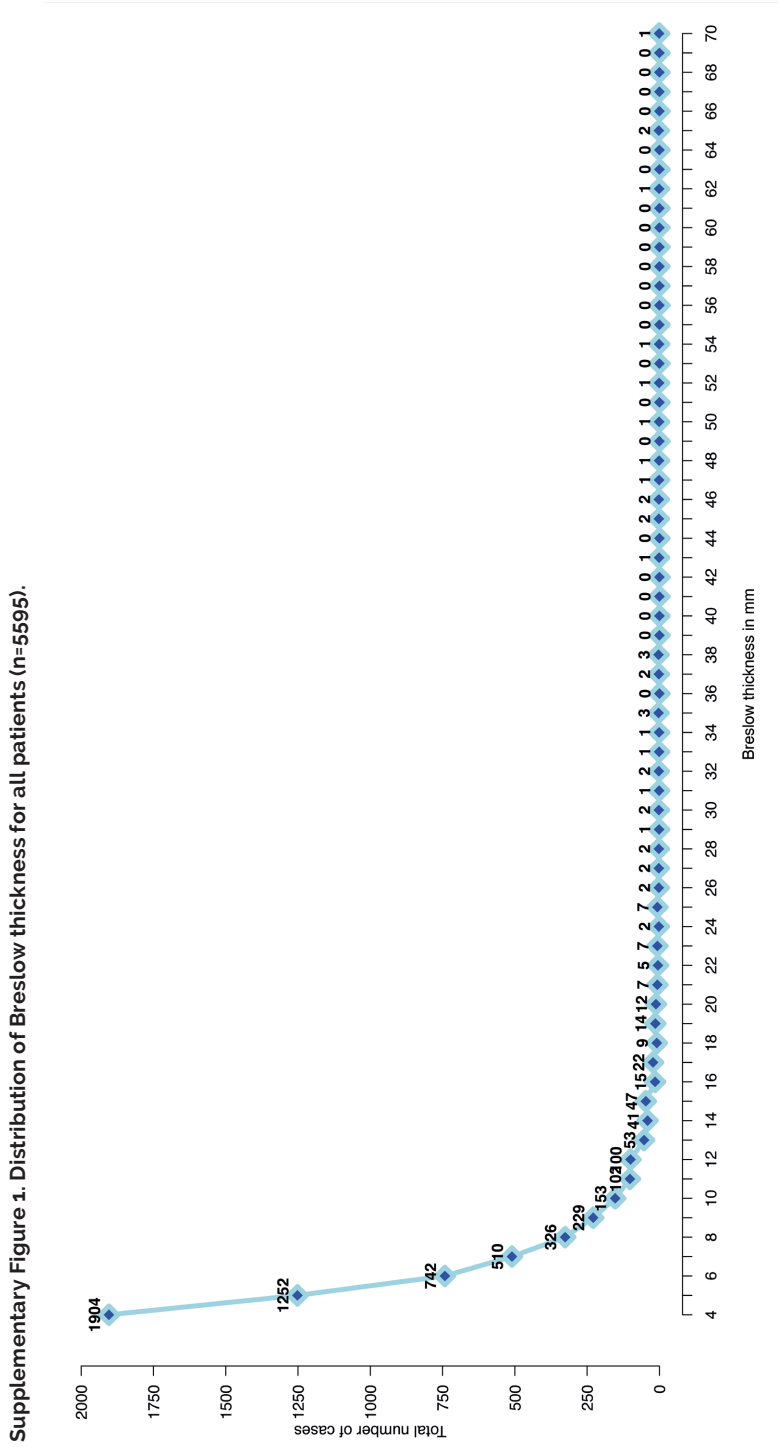
			Overall survival (70 events)				Recurrence-free survival (83 events)			
			Univariable		Multivariable		Univariable		Multivariable	
Characteristic	**N patients**	**N deaths**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Not known	49	46	0.93 (0.51-1.69)	0.81	0.76 (0.32-1.78)	0.52	0.82 (0.48-1.40)	0.47	0.59 (0.27-1.26)	0.17
SN status										
Negative	26	12	1		1		1		1	
Positive	16	13	1.47 (0.65-3.32)	0.36	1.13 (0.41-3.16)	0.81	1.76 (0.83-3.72)	0.14	1.44 (0.55-3.78)	0.45
Not performed	65	45	1.64 (0.84-3.20)	0.15	1.39 (0.57-3.35)	0.47	2.11 (1.15-3.89)	0.02	2.07 (0.95-4.54)	0.07

Supplementary Table 7. Univariable Cox regression hazard ratio estimates of overall survival and recurrence-free survival in patients with melanomas ≥20mm in thickness (n=76).

			Overall survival (43 events)		Recurrence-free survival (54 events)	
Characteristic	**N patients**	**N deaths**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Sex						
Male	44	26	1		1	
Female	32	17	0.83 (0.49-1.55)	0.56	0.79 (0.45-1.37)	0.39
Age at diagnosis (years)						
18-35	4	3	1		1	
36-55	15	3	0.27 (0.05-1.35)	0.11	0.65 (0.16-2.63)	0.55
56-75	35	19	1.19 (0.35-4.09)	0.78	2.11 (0.62-7.12)	0.23
>75	22	18	1.51 (0.44-5.16)	0.51	1.66 (0.49-5.65)	0.42
Breslow thickness (mm)						
≤25	40	23	1		1	
>25	36	20	0.65 (0.35-1.19)	0.16	0.62 (0.36-1.08)	0.09
Primary site						
H&N	16	8	1		1	
Trunk	35	18	1.18 (0.51-2.74)	0.71	0.91 (0.45-1.84)	0.79
Arm	9	7	1.92 (0.69-5.31)	0.21	1.08 (0.42-2.75)	0.87
Leg	16	10	1.32 (0.52-3.34)	0.56	0.92 (0.40-2.09)	0.84
Melanoma subtype						
SSM and other	39	21	1		1	
NM	37	22	1.57 (0.85-2.89)	0.15	1.76 (1.02-3.05)	0.04
Ulceration						
No	16	4	1		1	
Yes	52	34	5.31 (1.62-17.39)	0.006	2.48 (1.15-5.35)	0.02
Not known	8	5	3.18 (0.75-13.46)	0.11	1.15 (0.37-3.57)	0.81
Mitosis						

Supplementary Table 7. (continued).

			Overall survival (43 events)		Recurrence-free survival (54 events)	
Characteristic	**N patients**	**N deaths**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Not known	49	34	1		1	
Yes	27	9	1.15 (0.44-2.95)	0.78	0.94 (0.39-2.23)	0.88
SN status						
Negative	14	5	1		1	
Positive	8	3	1.07 (0.24-4.87)	0.93	2.37 (0.68-8.30)	0.18
Not performed	54	35	2.74 (0.94-8.02)	0.07	3.61 (1.38-9.44)	0.009



Supplementary Figure 1. Distribution of Breslow thickness for all patients (n=5595).

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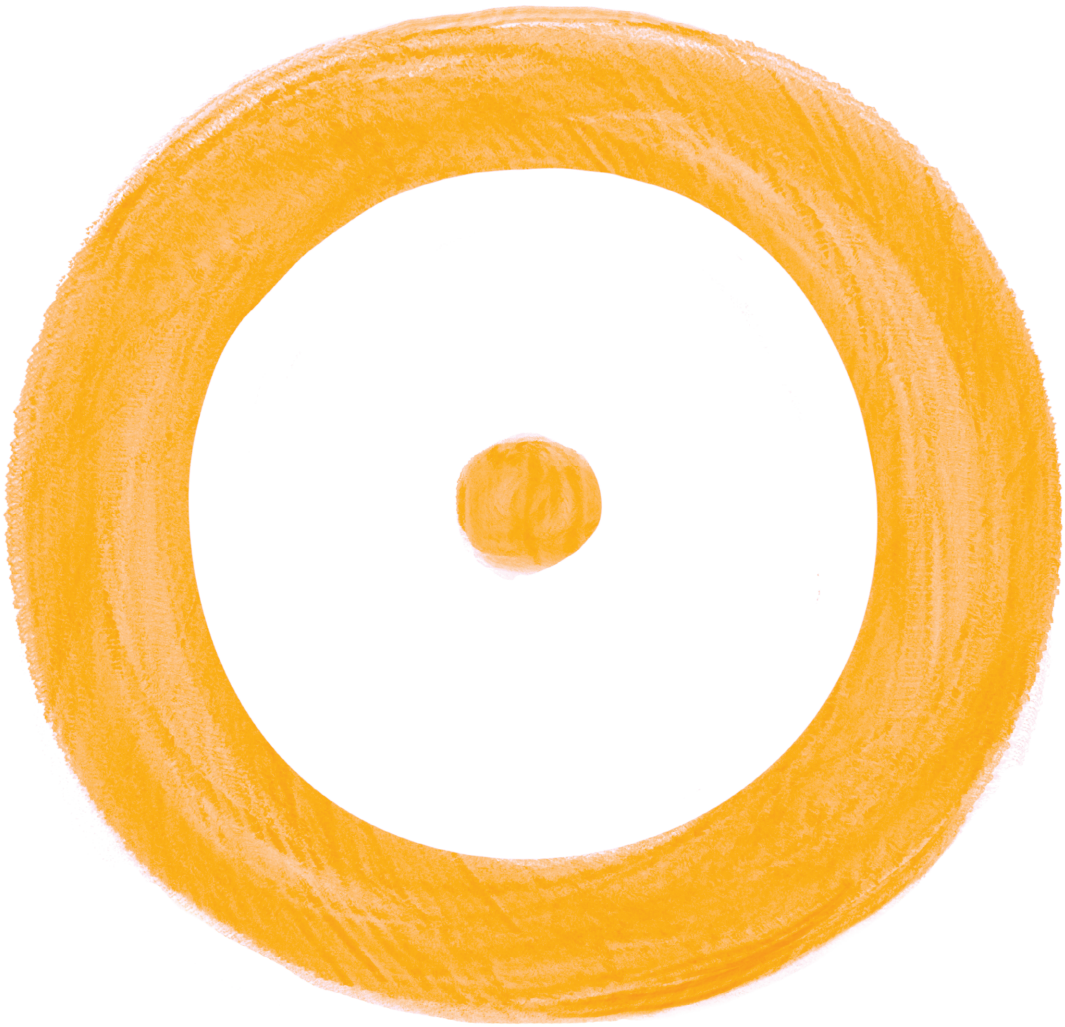

CHAPTER 12

Thick melanomas without lymph node metastases: a forgotten group with poor prognosis

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ABSTRACT

Introduction: Although adjuvant therapy is available for melanoma patients with sentinel lymph node (SLN) metastases (pN+), this is not the case for thick melanomas without SLN involvement (pN-).

Objectives: We assessed overall and relative survival (OS, RS) in patients with >4.0 mm Breslow thickness (BT) pN- and pN + melanomas and ≤4.0 mm pN+ patients.

Methods: Clinicopathological data were retrieved from a cohort of >4.0 mm thick and/or pN + melanoma patients in the Netherlands from 2000 to 2014. OS and RS was compared using Kaplan-Meier-curves. A Cox-regression-model was developed to assess determinants of OS in >4.0 mm pN- patients.

Results: In 54 645 patients, 3940 (7.2%) had >4.0 mm thick melanomas. SLN biopsy was performed in 1150 (29.2%) patients. Five-year OS was 70.5% for >4.0 mm pN- and 48.1% for >4.0 mm pN+ patients ($p < 0.001$), with a decreasing trend in OS for every mm BT. Five-year OS in 1877 ≤ 4.0 mm pN+ patients was 71.5%, which was not different from >4.0 mm pN- ($p = 0.24$). Higher age, higher BT category, ulceration and male gender were significantly associated with poor survival in >4.0 mm pN- patients.

Conclusions: Thick pN- melanomas have a poor prognosis, comparable to that of less thick pN + melanomas, which is not accounted for in current guidelines. We encourage including these high-risk patients in adjuvant trials.

INTRODUCTION

Building upon the legacy of Wallace Clark, in 1970, Alexander Breslow observed in his pioneering work on 98 patients that deepest level of invasion, measured in millimeters starting from the granular layer to the deepest tumor cell into the skin, was related with lymph node involvement and prognosis[1]. Still, this Breslow thickness (BT) is one of the most important predictors of dying from melanoma[2]. Together with ulceration status, BT determines the indication for sentinel lymph node biopsy (SLNB). Although there is no international consensus in follow-up strategies for melanoma patients[3], most (inter)national guidelines do not specifically comment on thick (>4.0 mm) melanomas and focus on thin and lymph node positive melanomas. For example, guidelines of The British Association of Dermatologists (BAD) as well as Dutch guidelines recommend to perform the same follow-up in patients with melanoma of BT > 4.0 mm without further lymph node involvement as in patients with a melanoma of 1.1 mm[3,4]. Both have a follow-up regime of 5 years with more regular intervals in the first two years, even though patients with higher BT are well known to have a much higher risk of distant metastases[2]. Current guidelines do not recommend adjuvant therapy in stage IIB/C patients[3,4].

Recent studies have shown significantly improved relapse free (RFS) and overall survival for adjuvant targeted- and immunotherapy in stage III melanoma patients (e.g. with lymph node involvement regardless of BT)[5-7]. Unfortunately, patients with thick melanomas without lymph node involvement (stage IIB and IIC) were not included in these studies. BRIM8, the only adjuvant study that included stage IIC patients, did not meet its primary endpoint[8]. Currently, adjuvant targeted- and immunotherapy is not available for thick melanoma without lymph node involvement, even though these patients have worse prognosis than IIIA[2]. Furthermore, despite significant changes in the classification of T1 and stage III and IV melanoma in the recent 8th American Joint Committee on Cancer (AJCC) classification, the definition of stage IIB and IIC has remained unchanged[2]. Here, we aimed to assess the survival of melanoma patients >4.0 mm melanoma with (pN+) and without lymph node involvement (pN-) in comparison with ≤4.0 mm pN+ patients in the Netherlands.

METHODS

Collection of data

Data for this retrospective nationwide study were derived from "PALGA", the Dutch Nationwide Network and Registry of Histopathology and Cytopathology, that prospectively collects all pathology data from all pathology labs in the Netherlands since 1987 (http://www.palga.nl/). All data was encoded and used anonymously. Ethical approval was granted by the scientific review board of PALGA.

Study population

For this cohort study, we included the pathologic reports of all histologically newly diagnosed primary, cutaneous melanomas in adult patients without clinical signs of lymph node macro metastases in the Netherlands between 2000 and 2014. Melanoma in situ, Spitzoid tumours of unknown malignant potential, melanocytic tumours of unknown malignant potential, superficial atypical melanocytic proliferation of uncertain significance, melanoma of unknown primary and patients with multiple melanoma were excluded[9,10], as well as melanoma lacking or having unclear BT. Due to their distinct biologic behaviour, desmoplastic melanoma were excluded as well.

For each patient, clinical and pathological variables were extracted from the pathology text files, including date of diagnosis, age (18–35, 36–54, 55–74, >75), sex, BT in mm (≤4.0 mm, 4.0–4.9, 5.0–5.9, 6.0–7.9, >8.0), ulceration (present or absent), type of melanoma (superficial spreading, nodular, lentigo maligna melanoma or acral lentiginous) and body site (head and neck, trunk, arms or legs). Lymph node involvement (pN- or pN+) was defined as melanoma metastases in SLNB. As guidelines do not comment on the time between primary excision and SLNB, we decided to include all SLNB performed within 100 days after initial diagnosis as SLNB[11]. A positive SLNB also included patients with isolated tumor cells. To ensure that patients did not present with metastases (other than diagnosed with SLNB), we excluded patients with skin, distant or non-SLNB nodal metastases within 100 days of the initial diagnosis from survival analyses. Next, patients were divided into three groups: >4.0 mm pN-, >4.0 mm pN+ and ≤4.0 mm pN+. If no SLNB was performed, patients were excluded from survival analyses. Overall survival (OS) data and vital status (dead or alive) were obtained through linkage with the Netherlands Cancer Registry (NCR) hosted by the Comprehensive Cancer Organization of the Netherlands (IKNL). The NCR is a nation-wide population-base cancer registry with information on vital status, follow-up and date of death annually retrieved from the database of deceased persons of the Central Bureau of Genealogy and the municipal demography registries (GBA). Cohort-based relative survival (RS) was calculated as a proxy for melanoma specific survival, as it adjusts for gender- and age-specific background mortality[12]. Follow-up was gathered until January 1st, 2018.

Statistical analysis

Univariable analysis was performed using chi-square tests or Mann-Whitney U test, as appropriate. Continuous variables are presented as median with interquartile range (IQR) or mean with standard deviation (SD) for non-normally distributed data and normally distributed data, respectively. Categorical variables are presented as numbers and percentages. Kaplan-Meier survival plots were generated for OS and RS according to staging system and a post-hoc test for OS was performed to assess significance between the three aforementioned groups of patients. A Cox proportional hazard analysis was performed to estimate hazard ratio's (HRs) for >4.0 mm pN- patients with OS as outcome. The proportional hazards assumption was examined by plotting a log-minus-log graph per prognostic variable in the model. If the lines were parallel, it was assumed that the proportional hazards assumption was not violated. Determinants fitted in the model were age per category, sex, BT category, ulceration status, body site and melanoma subtype. All determinants were categorized as described earlier. Five and ten year OS rates for patients >4.0 mm pN-, >4.0 mm pN+ and ≤4.0 mm pN+ were analysed using SPSS version 21 statistical software. Likewise, SAS version 9.4 (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA) was used for RS. Two-sided p-values <0.05 were considered significant.

RESULTS

Between 2000 and 2014 a total of 54 645 primary, single, cutaneous melanoma patients were diagnosed. A total of 3940 (7.2%) patients had BT > 4.0 mm, with a median BT of 5.90 (IQR 4.95–7.40). The mean age was 66.87 (SD 16.12) years and a slight male predominance was observed (55.5%). Ulceration was observed in 2320 (58.9%) of melanomas and the most frequent type of melanoma was the nodular subtype (Table 1). The median follow-up time was 40.5 months (IQR 19.3–79.7).

Lymph node status in thick melanomas

A total of 429 patients had metastases within 100 days of the initial diagnosis. In 79 patients, these metastases were in-transit or satellites, in 385 nodal (other than SLNB, e.g. a positive FNA, direct lymphadenectomy or a positive lymphadenectomy after a previous negative SLNB) and in 45 visceral. Lymph node status was available in 1150 (29.2%) of the remaining patients with >4.0 mm melanoma, with follow-up data available in 1066 (92.7%) patients. One-hundred thirty four SLNBs were regarded positive because of isolated tumor cells. A total of 502 > 4.0 mm pN+ patients and 648 > 4.0 mm pN- patients were identified. Ulceration was present in 58.9% of all >4.0 mm pN- patients. A total of 2018 patients had BT ≤ 4.0 mm pN+, with follow-up data available in 1877 (93.0%) patients (Table 1 and Fig. 1).

Table 1. Baseline table of all >4.0 mm cutaneous melanoma in the Netherlands diagnosed between 2000 and 2014 and of all patients categorized >4.0 mm pN-, >4.0 mm pN+ or ≤4.0 mm pN+.

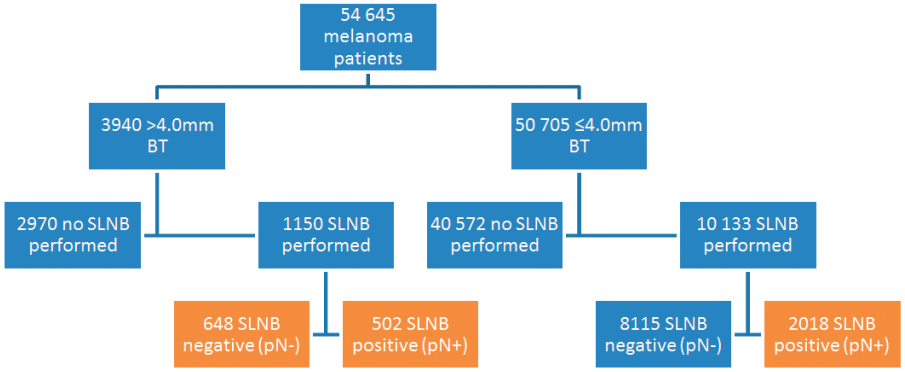
Characteristic	All >4.0mm	>4.0mm pN-	>4.0mm pN+	≤4.0mm pN+
Total	3940	648	502	2018
Gender (N (%))	1753 (44.5)	268 (41.4)	185 (36.9)	964 (47.8)
Female	2187 (55.5)	380 (58.6)	317 (63.1)	1054 (52.2)
Male				
Age in years (mean (SD))	66.87 (16.12)	60.41 (14.25)	57.51 (14.59)	52.78 (14.42)
18-35	169 (4.3)	41 (6.3)	39 (7.8)	254 (12.6)
36-55	761 (19.3)	181 (27.9)	169 (33.7)	877 (43.5)
56-75	1648 (41.8)	333 (51.4)	236 (47.0)	778 (38.6)
>75	1362 (34.6)	93 (14.4)	58 (11.6)	109 (5.4)
Breslow thickness in mm (median (IQR))	5.90 (4.95-7.40)	5.34 (4.70-7.00)	5.50 (4.80-7.00)	2.10 (1.50-2.90)
4.1-4.9				
5.0-5.9	982 (24.9)	200 (30.9)	132 (26.3)	*0-0.7mm* 28 (1.4)
6.0-7.9	1004 (25.5)	182 (28.1)	145 (28.9)	*0.8-1.0mm* 101 (5.0)
≥8.0	627 (15.9)	155 (23.9)	122 (24.3)	*1.1-2.0mm* 859 (42.6)
	1327 (33.7)	111 (17.1)	103 (20.5)	*2.1-4.0mm* 1030 (51.0)
Localization (N (%))	759 (19..3)	63 (9.7)	35 (7.0)	98 (4.9)
Head and neck	1392 (35.3)	269 (41.5)	226 (45.0)	979 (48.5)
Trunk	553 (14.0)	114 (17.6)	49 (9.8)	210 (10.4)
Arms	1101 (27.9)	185 (28.5)	170 (33.9)	671 (33.3)
Legs	135 (3.4)	17 (2.6)	22 (4.4)	60 (3.0)
Missing				
Type of melanoma (N (%))	1036 (26.3)	162 (25.0)	164 (32.7)	1231 (61.0)
Superficial spreading	2145 (54.4)	354 (54.6)	261 (52.0)	518 (25.7)
Nodular	65 (1.6)	4 (0.6)	0 (0.0)	5 (0.2)
Lentigo maligna melanoma	102 (2.6)	29 (4.5)	20 (4.0)	34 (1.7)
Acral lentiginous	592 (15.0)	99 (15.3)	57 (11.4)	230 (11.4)
Missing				
Ulceration (N (%))	1141 (29.0)	234 (36.1)	158 (31.5)	1201 (59.5)
No	2320 (58.9)	351 (54.2)	297 (59.2)	578 (28.6)
Yes	479 (12.2)	63 (9.7)	47 (9.4)	239 (11.8)
Missing				
SLNB assessment and result (N (%))	2790 (70.8)	NA	NA	NA
Not performed	1150 (29.2)	648 (100)	502 (100)	2018 (100)
Performed	648 (56.3)	648 (100)	0 (0)	0 (0)
Negative	502 (43.7)	0 (0)	502 (100)	2018 (100)
Positive				

Table 2. Overview of 5- and 10-year overall and relative survival with 95% CI for >4.0 mm pN-, >4.0 mm pN+ and melanoma ≤4.0 mm pN+ per Breslow thickness category in the Netherlands from 2000 to 2014.

	>4.0mm pN- n=598 (of 648)		>4.0mm pN+ n=468 (of 502)			≤4.0mm pN+ n=1877 (of 2018)	
BT cat in mm	5-yr	10-yr	5-yr	10-yr	BT cat in mm	5-yr	10-yr
Overall survival							
4.1-4.9	77.8 (71.8-84.4)	61.1 (52.9-70.5)	61.7 (53.1-71.6)	47.6 (37.6-60.2)	0-0.7	NA	NA
5.0-5.9	73.5(66.8-80.9)	58.0 (49.6-67.9)	48.5 (40.4-58.2)	40.7 (32.5-51.0)	0.8-1.0	88.2 (81.5-95.4)	81.5 (72.3-92.0)
6.0-7.9	68.3 (60.7-76.8)	53.1 (44.2-63.7)	38.5 (30.4-48.8)	25.7 (18.1-36.4)	1.1-2.0	80.1 (77.2-83.1)	68.8 (64.9-72.9)
≥8.0	55.0 (45.5-66.4)	32.6 (22.0-48.3)	41.7 (32.3-53.8)	32.3 (22.9-45.5)	2.1-4.0	62.7 (59.5-66.0)	47.1 (43.3-51.2)
All	70.5 (66.8-74.5)	53.7 (48.9-58.9)	48.1 (43.5-53.1)	36.8 (32.1-42.3)	All	71.5 (69.4-73.7)	58.3 (55.6-61.1)
Relative survival							
4.1-4.9	84.5 (77.7-91.3)	73.7 (63.3-84.2)	66.4 (56.6-76.2)	54.3 (41.3-67.3)	0-0.7	NA	NA
5.0-5.9	79.8 (72.1-87.5)	68.6 (58.0-79.2)	51.3 (41.8-60.9)	47.5 (36.7-58.3)	0.8-1.0	91.7 (84.5-98.9)	88.8 (78.0-99.6)
6.0-7.9	75.6 (66.7-84.4)	66.4 (54.3-78.4)	42.5 (32.6-52.3)	31.2 (20.4-42.0)	1.1-2.0	83.5 (80.5-86.6)	75.1 (70.8-79.5)
≥8.0	60.6 (49.1-72.3)	39.0 (24.9-53.0)	45.4 (34.2-56.7)	36.6 (24.1-49.0)	2.1-4.0	66.4 (63.0-69.8)	53.2 (48.7-57.7)
All	77.1 (72.9-81.4)	65.1 (59.1-71.1)	51.8 (46.7- 56.9)	42.9 (36.9-48.8)	All	75.1 (72.8-77.3	64.6 (61.6-67.7)

NA = Too small numbers for reliable calculations.
BT = Breslow thickness

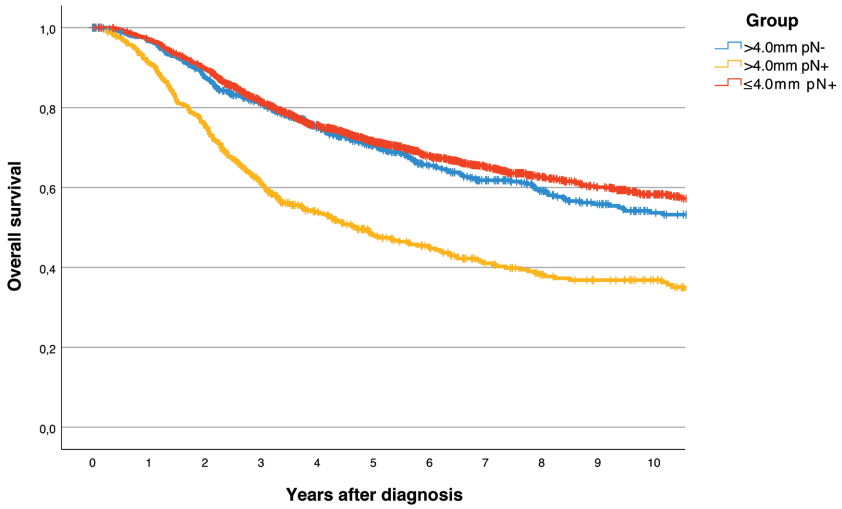
Figure 1. Flowchart of patients included in the current study. Patients in the orange boxes were included.



Overall and relative survival

OS curves of >4.0 mm pN-, >4.0 mm pN+ and ≤4.0 mm pN+ melanoma are depicted in Fig. 2, showing that OS of >4.0 mm pN- and ≤4.0 mm pN+ patients were similar ($p = 0.24$) while >4.0 mm pN+ patients did worse ($p < 0.001$). Table 2 summarizes the 5- and 10-year OS and RS rates with 95% CI for all three groups of patients and per BT category: five-year OS was 70.5% for patients with >4.0 mm pN- melanoma compared to 48.1% for >4.0 mm pN+ melanoma and 71.5% for ≤4.0 mm pN+ melanoma. Likewise, 10-year OS rates were 53.7%, 36.8% and 58.3%. RS rates showed similar numbers (Table 2). In multivariable analysis of >4.0 mm pN- melanoma, patients 56 + years old, patients with a melanoma of 8.0 mm or thicker, presence of ulceration and male gender were significantly associated with worse survival (Table 3). Testing of the proportional hazards assumption showed no violation. Survival curves per BT category for all three groups are shown in Supplementary figures 3, 4 and 5. Survival stratified for ulceration in thick melanomas are shown in Table 4.

Figure 2. Kaplan-Meier overall survival graphs per Breslow-category for >4.0 mm pN-, >4.0 mm pN+ and ≤4.0 mm pN+ melanoma in the Netherlands from 2000 to 2014.



No at risk											
>4.0mm N-	598	570	515	451	362	300	241	207	171	139	115
>4.0mm N+	468	422	349	267	201	154	129	104	88	78	66
≤4.0mm N+	1887	1808	1664	1394	1139	953	765	621	495	432	357

P-value >4.0mm pN- vs >4.0mm pN+: <0.001*
P-value >4.0mm pN- vs ≤4.0mm pN+: 0.24
P-value >4.0mm pN+ vs ≤4.0mm pN+: <0.001*

Table 3. Cox proportional hazard regression for all >4.0 mm pN- melanoma in the Netherlands diagnosed between 2000 and 2014. Localisation and type melanoma not significant.

Variable	HR (95% CI)	p-value
Age (per year)		
18-35	Reference	
36-55	3.13 (0.97-10.12)	0.06
56-75	4.67 (1.47-14.78)	0.009*
>75	7.70 (2.33-25.40)	0.001*
Breslow thickness (mm)		
4.1-4.9	Reference	
5.0-5.9	1.21 (0.79-1.84)	0.358
6.0-7.9	1.39 (0.89-2.16)	0.140
≥8.0	2.36 (1.54-3.63)	<0.001*
Sex		
Male	Reference	
Female	0.60 (0.43-0.83)	0.002*
Ulceration		
No	Reference	
Yes	1.82 (1.31-2.55)	<0.001*

Table 4. Overview of 5- and 10 year overall survival with 95% CI for thick melanomas (>4.0 mm) in the Netherlands from 2000 to 2014, stratified for ulceration status.

	>4.0mm pN- (n=538)		>4.0mm pN+ (n=421)	
	No ulceration (n=214)	Ulceration (n=323)	No ulceration (n=147)	Ulceration (n=274)
5-year OS (95% CI)	81.5 (76.0-87.3)	61.4 (56.1-67.3)	57.5 (49.6-66.5)	42.0 (36.2-48.8)
10-year OS (95% CI)	62.5 (54.2-72.1)	44.4 (38.0-51.8)	42.0 (36.4-56.1)	30.3 (24.5-37.6)

DISCUSSION

We aimed to assess OS in patients with pN- melanomas >4.0 mm BT, compared to >4.0 mm pN+ and ≤4.0 mm pN+. We showed that patients with >4.0 mm pN- melanoma have a poor prognosis, with OS comparable to and in some more advanced BT categories even worse than patients with thinner melanomas of ≤4.0 mm pN+. In addition, we showed that there is an increasing hazard of dying with every mm higher BT.

Survival in IIB/C patients is similar to that of IIIA/B patients, while only the latter groups has access to adjuvant therapy. We found 5-year OS and RS/MSS rates of 70.5% and 77.1% respectively, compared to 5-year OS and RS/MSS rates of 71.5% and 75.1% in ≤4.0 mm pN+ melanoma patients. Gershenwald et al. have found 5-year MSS rates of 82–87% for IIB/C and 83–93% for IIIA/B[2], whereas Haydu et al. showed 64–81% MSS rates for stage IIIA/B[13]. When ulceration is present (IIC), patients have

worse survival compared to IIIA/B patients. We found a 5-year OS of 61.4% for stage IIC patients, compared to 5-year OS of 71.5% ≤ 4.0 mm pN+ melanoma patients. This finding is in line with other studies[2].

For patients with lymph node positive (stage III) melanoma, several adjuvant therapy strategies have proven to increase RFS[5,6]. Unfortunately, no data is available for patients with >4.0 mm melanoma patients without lymph node metastases. This is a worrisome situation, as we have shown here that the latter group of patients has worse OS and RS, warranting studies to evaluate the efficacy of adjuvant therapy. The recently started phase 3 KEYNOTE-716 study, randomizing between pembrolizumab and placebo in high risk stage II melanoma will provide data on the benefits of adjuvant treatment for this group of patients[14].

To the best of our knowledge, this is the largest cohort comparing survival between a group of ≤4.0 mm pN+ and >4.0 mm pN- melanoma patients. Recently, Brancaccio et al. sought attention for this specific group of patients as well[15]. We agree that the poor prognosis of stage IIC must be given more attention in (inter)national guidelines. Future trials should be designed taking these high-risk patients into account as they may derive the same benefit from adjuvant treatment as stage III melanoma patients[16]. Meanwhile, we suggest to intensify the monitoring for patients with thick melanomas., ince response rates to immunotherapy and survival are better in case of lower tumor burden[17], more stringent follow-up could very well result in improved survival for this group of patients. Only some international guidelines comment on this, such as the European Society of Medical Oncology (ESMO), stating "in high-risk patients (e.g. those with thick primary tumours), ultrasound of lymph nodes, CT or whole-body PET/PET–CT scans may lead to an earlier diagnosis of regional or systemic relapses"[18]. The American Association of Dermatology states collaboration with medical oncology is recommended for patients with high-risk cutaneous melanoma (stage IIB and IIC)[19].

Strengths of our study are the fact that we used nationwide data from a very large group of patients. Even though the finding that IIB/C patients have similar and in some cases worse prognosis compared to IIIA/B patients is already known, this has not been verified with nationwide data. This enabled us to obtain reliable data on the relatively rare >4.0 mm BT melanoma patient subgroup and increased the generalizability of our results. A limitation we have to address is the fact that lymph node involvement has only been investigated in 29.2% of all >4.0 mm patients. Possibly, this low rate of SLNB in thick melanomas is explained by the controversy that exists regarding its benefits, reasoning that the a priori risk of distant metastases is too high to validate this procedure[20]. This will probably change in the current era, as now a positive sentinel node sets the indication for adjuvant treatment. Furthermore, we excluded all patients who did not undergo a SLNB. As we expected lymph nodal

basins to be palpated in all melanoma patients, we assumed that these patients had no macrometastases. However, as information on the presence of micro metastases is missing in these patients as they did not undergo a SLNB, regarding them as node negative would have biased our results. A final limitation is that due to the retrospective nature of our study we have no data on systemic treatment. As all patients were included before 2014, adjuvant therapies for melanoma were not prescribed on a regular basis in the Netherlands. However, if patients developed distant disease during follow-up after 2014, they might have been treated with systemic therapies.

CONCLUSIONS

We have shown that patients with thick melanomas without lymph node metastases have a dismal prognosis, comparable with lymph node positive less thick melanomas, with survival worsening with every additional mm BT. Patients with >4.0 mm BT melanoma without nodal involvement qualify for more stringent follow-up and should be included in adjuvant studies.

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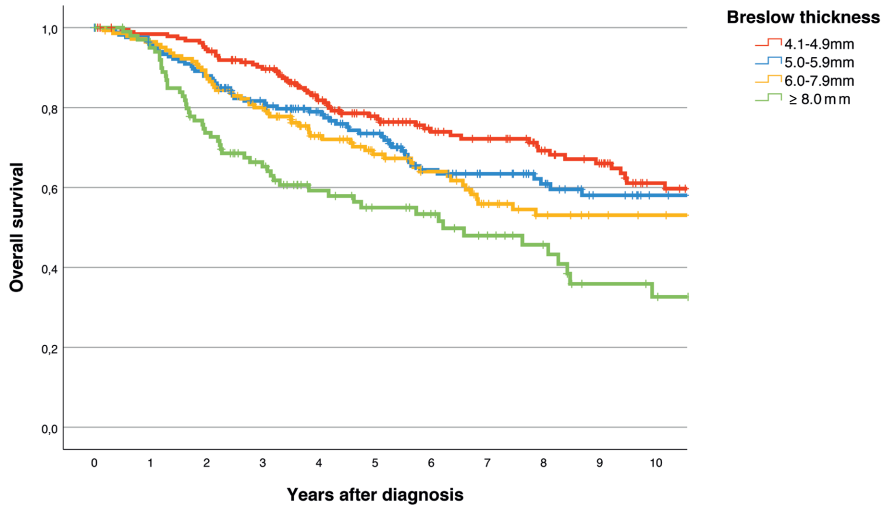
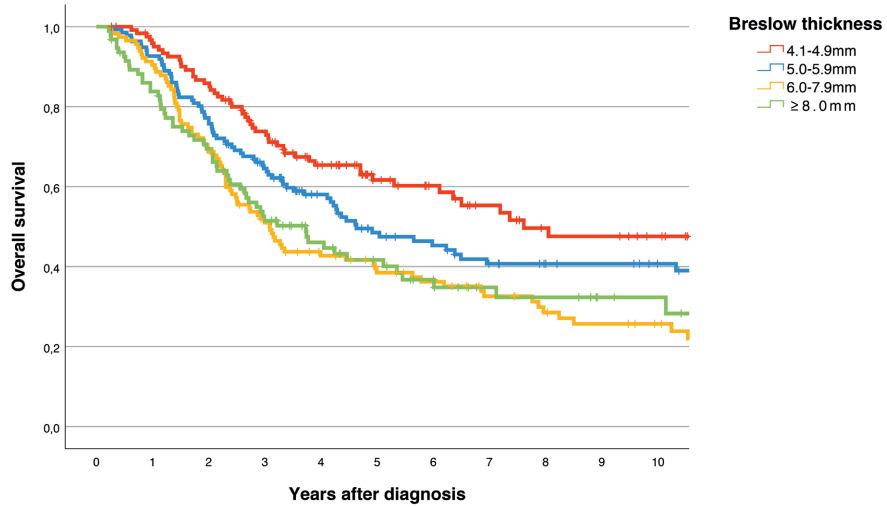
Supplementary Figure 1. Kaplan-Meier overall survival graphs per Breslow-category for 648 (FU in 598) >4.0mm pN- melanoma in the Netherlands diagnosed between 2000 and 2014.



No at risk											
4.1-4.9mm	188	182	175	160	129	107	88	82	68	59	44
5.0-5.9mm	167	159	145	125	105	89	65	57	48	37	31
6.0-7.9mm	142	136	123	108	85	68	57	44	36	32	30
≥8.0mm	101	94	72	58	43	36	31	25	19	12	10

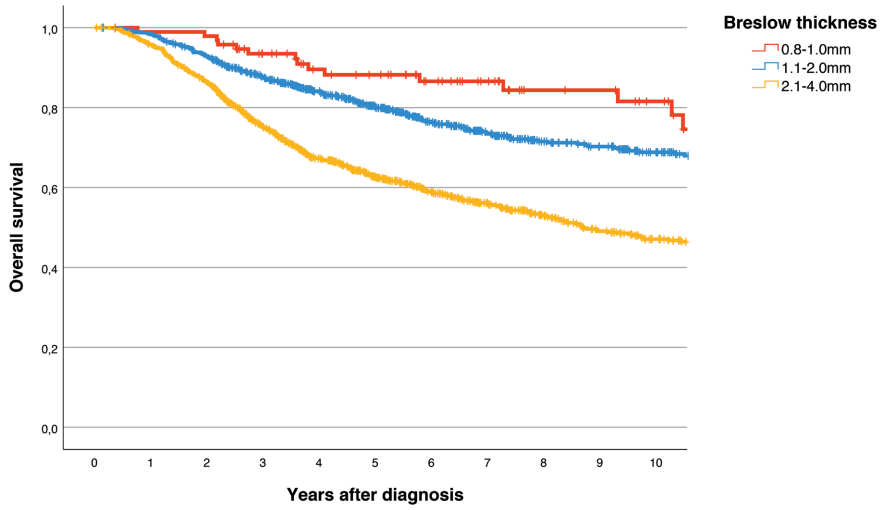
Supplementary Figure 2. Kaplan-Meier overall survival graphs per Breslow-category for 502 (FU in 468) >4.0mm pN+ melanoma in the Netherlands diagnosed between 2000 and 2014.



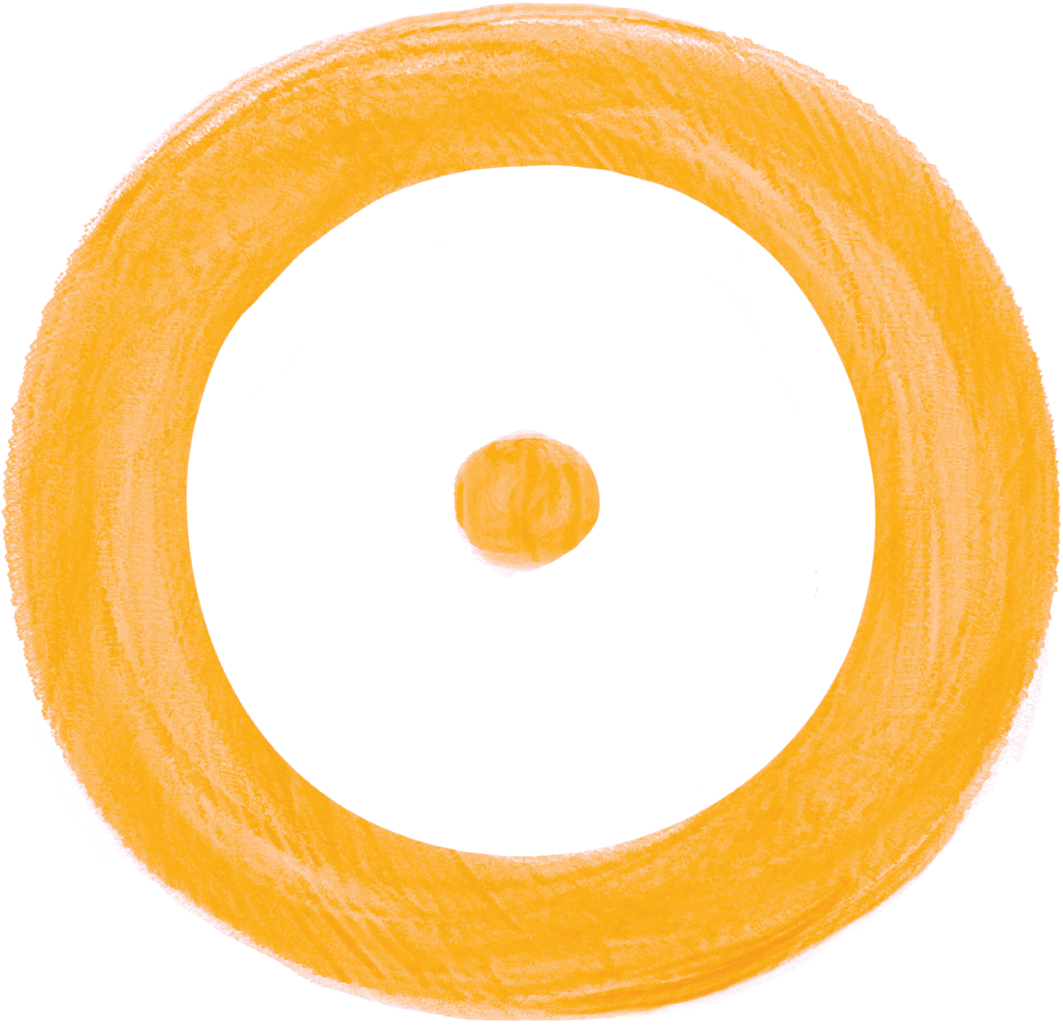
No at risk

4.1-4.9mm	123	115	103	83	63	45	37	30	24	23	19
5.0-5.9mm	136	126	105	83	62	47	41	34	30	28	24
6.0-7.9mm	115	104	79	56	43	36	32	26	21	18	15
≥8.0mm	94	77	63	45	33	26	20	14	13	9	8

Supplementary Figure 3. Kaplan-Meier overall survival graphs per Breslow-category for 2018 (FU in 1877) ≤4.0mm pN+ melanoma in the Netherlands diagnosed between 2000 and 2014.



No at risk											
0.8-1.0mm	95	93	92	76	66	62	51	43	32	31	26
1.1-2.0mm	812	781	732	641	533	448	357	290	237	212	175
2.1-4.0mm	970	910	819	660	527	433	347	281	221	184	151



CHAPTER 13

Sex matters: men with melanoma have a worse prognosis than women

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ABSTRACT

Background: In Europe, one of the highest melanoma incidences is found in the Netherlands. Like in several other European countries, females are more prone to develop melanoma as compared to males, although survival is worse for men.

Objective: To identify clinicopathological gender-related differences that may lead to gender-specific preventive measures.

Methods: Data from the Dutch Nationwide Network and Registry of Histopathology and Cytopathology (PALGA) were retrieved from patients with primary, cutaneous melanoma in the Netherlands between 2000 and 2014. Patients initially presenting as stage I, II and III without clinically detectable nodal disease were included. Follow-up data were retrieved from the Netherlands Cancer Registry. Gender-related differences were assessed, and to compare relative survival between males and females, multivariable relative excess risks (RER) were calculated.

Results: A total of 54.645 patients were included (43.7% men). In 2000, 41.7% of the cohort was male, as compared to 47.3% in 2014 (P < 0.001). Likewise, in 2000, 51.5% of the deceased cohort was male compared to 60.1% in 2014 (P < 0.001). Men had significantly thicker melanomas at the time of diagnosis [median Breslow thickness 1.00 mm (interquartile range (IQR): 0.60–2.00) vs. 0.82 mm (IQR: 0.50–1.50) for females] and were significantly older at the time of diagnosis, more often had ulcerated melanomas and melanomas localized on the trunk or head and neck. Over time, survival for females improved while that of men decreased (P < 0.001). RER for dying was 1.37 (95% CI: 1.31–1.45) for men in multivariable analysis.

Conclusions: There are evident clinicopathological differences between male and female melanoma patients. After multivariable correction for all these differences, relative survival remains worse for men. Clinicians as well as persons at risk for melanoma should be aware of these differences, as awareness and prevention might lead to a lower incidence and mortality of melanoma. This indicates the need of prevention campaigns integrating and targeting specific risk profiles.

INTRODUCTION

The Netherlands has the fifth-highest melanoma incidence rate in the world,[1] and its incidence is expected to rise further.[2] While melanoma is more common in women in the Netherlands, survival is worse for men.[3] The current 8th American Joint Committee on Cancer (AJCC) staging system includes Breslow thickness, ulceration, nodal and visceral metastases as predictors for survival in melanoma patients.[4] The prognostic value of gender in localized melanoma has been studied extensively, with several large studies (>10 000 patients each) showing a survival advantage for females.[5-14]

The cause as to why female patients have better survival remains controversial. Some have argued that biological differences between melanoma in men and women are due to hormonal influences of oestrogen and androgen.[15-17] Enninga et al.[5] showed females in all age groups (18–45, 46–54, and ≥55 years old) with localized and regional disease were less likely to die from melanoma compared to males in the same age group. No difference in survival between males and females with distant disease was found, although conflicting results have been reported.[9, 18] Others argue that there are gender-related differences in behaviour when it comes to sun exposure and self-examination,[19-23] as men are more likely to have thicker melanoma and melanoma located on the trunk, as opposed to the legs in females.[9] However, when adjusting for these confounders, females remain having a survival advantage.[9]

Many studies have used the United States (US) Surveillance, Epidemiology, and End Results (SEER) database or data from Australia, although there are differences in population characteristics and exposure between countries (e.g. in the US and Australia, melanoma is more common in males1). Besides, only few studies present recent data. We assessed time trends in incidence, mortality and survival of Dutch male and female melanoma patients separately. Furthermore, we aimed to identify a specific subgroup of patients who are at higher risk of dying of melanoma by studying gender-related differences in tumour characteristics that may provide starting points for gender-specific preventive measures.

PATIENTS AND METHODS

Collection of data

Data for this retrospective nationwide study were derived from 'PALGA', the Dutch Nationwide Network and Registry of Histopathology and Cytopathology, that prospectively collects all pathology data from all pathology laboratories in the Netherlands (http://www.palga.nl) since 1987. All data were encoded and used anonymously. Ethical approval was granted by the board of PALGA.

Study population

For this cohort study, data were retrieved from the pathologic reports of all newly diagnosed melanoma patients in the Netherlands between 2000 and 2014. Melanoma in situ, spitzoid tumours of unknown malignant potential ('STUMP'), melanocytic tumours of unknown malignant potential ('MELTUMP') and superficial atypical melanocytic proliferation of uncertain significance ('SAMPUS') were excluded, as well as melanoma without or unclear Breslow thickness (BT). We excluded patients with a positive complete lymph node dissection (CLND), fine needle aspiration or otherwise diagnosed positive lymph nodes within 14 days of diagnosis of the melanoma to ensure as optimal as possible that all patients were free of clinically detectable nodal disease when included in the study. Patients presenting with stage IV disease were excluded as well. Furthermore, non-cutaneous or desmoplastic melanoma, melanoma of unknown primary, recurrences, in-transit melanoma, patients <18 years and patients with multiple primary melanoma were excluded. Patients with other primary tumours were not excluded. For the present study, this yielded a data set with histologically proven invasive, primary, cutaneous melanomas diagnosed between 2010 and 2014 in the Netherlands.

For each patient, clinical and pathological variables were extracted from the pathology text files, including date of diagnosis, age, gender, BT in mm, T stage, ulceration (present or absent), subtype of melanoma (superficial spreading, nodular, lentigo maligna melanoma or acral lentiginous) and body site (head and neck, trunk, arms or legs). T stage was defined according to the AJCC at the time of diagnosis: 5th edition from 2000 to 2002, 6th from 2003 to 2009, 7th from 2010 to 2014). Vital status (dead or alive) was obtained through linkage with the Netherlands Cancer Registry (NCR) hosted by the Comprehensive Cancer Organization of the Netherlands (IKNL). The NCR is a nationwide population-based cancer registry which contains information that trained clerks retrieve the patient's medical records and gathers information on patient characteristics, as well as tumour characteristics and treatment. Information on vital status and date of death is annually retrieved from the database of deceased persons of the Central Bureau of Genealogy and the municipal demography registries (GBA). Cohort-based relative survival (RS) was calculated as a proxy for melanoma-specific survival, as it corrects for gender- and age-specific background mortality.[24]

Statistical analysis

Univariate variables were analysed using chi-square tests or Mann–Whitney U-test, as appropriate. Continuous variables are presented as median with interquartile range (IQR) or mean with standard deviation (SD) for non-normal distributed data and normal distributed data, respectively. Categorical variables are presented as numbers and percentages, and chi-square test was used to test for significance. Mann–Whitney test was used to assess significance between non-normal distributed continuous variables, two-sample t-test for normally distributed continuous variables. A linear-by-linear test was used to assess trend in time. RS analyses were stratified by gender and BT. To identify factors associated with excess mortality in male and female melanoma patients, we performed a full-case multivariable regression models using the Poisson generalized linear model framework calculating the relative excess risk of dying (RER) with a 95% confidence interval (CI) due to melanoma.24 Data were analysed using SPSS version 21 and SAS version 9.4 (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). A two-sided p-value < 0.05 was considered significant.

RESULTS

Men vs women

A total of 54.645 primary cutaneous melanoma patients were included, 23.879 (43.7%) men and 30.766 (56.3%) women. Men had thicker tumours at diagnosis; median Breslow thickness was for men 1.00 mm (IQR: 0.60–2.00) and 0.82 mm (IQR: 0.50–1.50) for women (P < 0.001; Table 1). Males also had more frequently ulcerated tumours (15.9% vs. 11.3%, P < 0.001). In males, the most frequent localization of melanoma was the trunk harbouring 55.4% of melanomas, followed by 15.5% on the head and neck. In females, legs were the most prominent localizations with 37.8%, followed by the trunk with 31.1%. Men were older at diagnosis (P < 0.001); mean age was 58.3 (SD: 15.1) for men and 54.9 (SD: 16.7) for women. For both men and women, superficial spreading melanoma was the most frequent subtype of melanoma, (69.2% and 71.7%, respectively), followed by nodular melanoma comprising 15.2% and 12.1% of melanomas, respectively.

Table 1. Baseline characteristics of all 54.645 primary cutaneous melanoma in the Netherlands diagnosed between 2000 and 2014, stratified for gender. *indicates statistical significance

Characteristic	Male (n=23.879, 43.7%)	Female (n=30.766, 56.3%)	p-value
Age in years (mean (SD))	58.33 (15.12)	54.94 (16.72)	<0.001*
18-35	1856 (7.7)	3993 (13.0)	
36-55	7983 (33.4)	12.254 (39.8)	
56-75	10.801 (45.2)	10.443 (33.9)	
>75	3239 (13.6)	4076 (13.2)	
Breslow thickness in mm (median (IQR))	1.00 (0.60-2.00)	0.82 (0.50-1.50)	<0.001*
0.1-0.7	8807 (36.9)	13.534 (44.0)	
0.8-1.0	3752 (15.7)	5175 (16.8)	
1.1-2.0	5389 (22.6)	6709 (55.5)	
2.1-4.0	3744 (15.7)	3595 (21.8)	
>4.1	2187 (9.2)	1753 (5.7)	
Localization (N (%))			<0.001*
Head and neck	3697 (15.5)	3163 (10.3)	
Trunk	13.236 (55.4)	9582 (31.1)	
Arms	2565 (10.7)	5393 (17.5)	
Legs	3573 (15.0)	11.623 (37.8)	
Missing	808 (3.4)	1005 (3.3)	
Subtype of melanoma (N (%))			<0.001*
Superficial spreading	16.512 (69.2)	22.050 (71.7)	
Nodular	3633 (15.2)	3712 (12.1)	
Lentigo maligna	1089 (4.6)	1417 (4.6)	
melanoma	159 (0.7)	274 (0.9)	
Acral lentiginous	2486 (10.4)	3313 (10.8)	
Missing			
Ulceration (N (%))			<0.001*
No	16.064 (67.3)	21.337 (69.4)	
Yes	3796 (15.9)	3487 (11.3)	
Missing	4019 (16.8)	5942 (19.3)	
T-stage (N (%))			<0.001*
T1	12.559 (52.6)	18.709 (60.8)	
T2	5389 (22.6)	6709 (21.8)	
T3	3744 (15.7)	3595 (11.7)	
T4	2187 (9.2)	1753 (5.7)	

Time trends

The total number of melanomas diagnosed per year increased from 2192 in 2000 to 5349 in 2014. During this period, the percentage of male patients increased from 41.7% to 47.3% (Fig. 1). From 2000 to 2014, the number of male patients who died was also higher than the number of females. This trend was especially apparent from 2004 onwards (Fig. 2). Stratifying by age at diagnosis according to 10-year age groups, melanoma in men was more predominant in 60–79 years old for the total cohort. Comparing 2000–2014, we observed >50% male patients in both the age categories 60–69 and 70–79 (Fig. 3). When stratifying for age, in both elderly men and women,

melanomas were found to be of higher BT, more often of nodular subtype, ulcerated and localized in head and neck area (data not shown).

Figure 1. Incidence of male and female melanoma patients according to year of diagnosis in the Netherlands (*P* < 0.001).

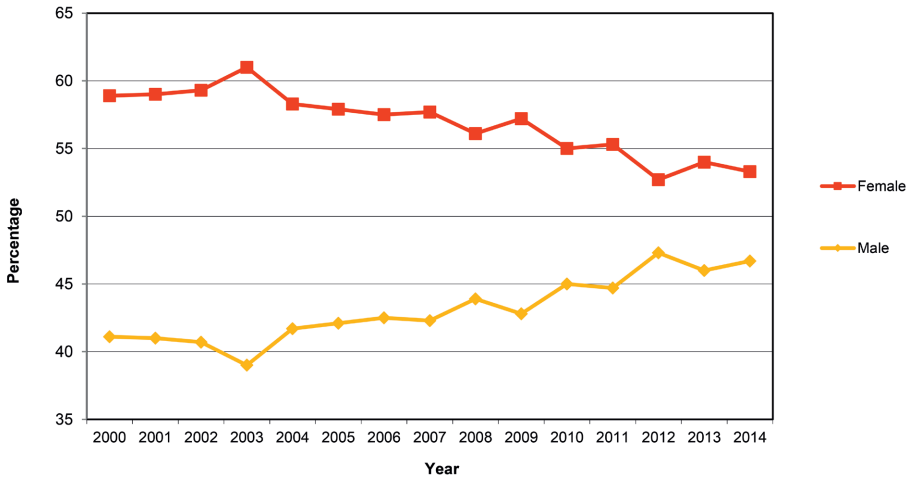


Figure 2. Mortality of male and female melanoma patients according to year of diagnosis in the Netherlands (*P* < 0.001).

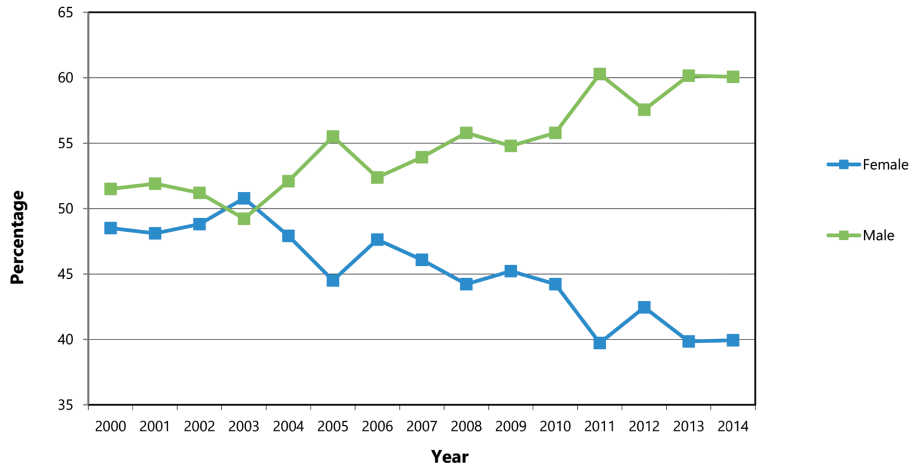


Figure 3. Proporation of male patients according to 10-year age group at diagnosis among patients diagnosed with cutaneous melanoma in the Netherlands in 2000 and 2014

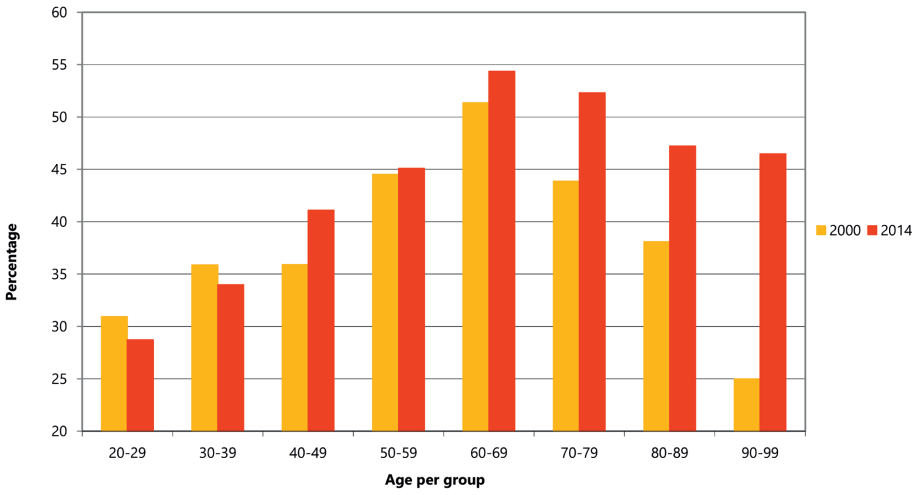


Figure 4. Relative survival curves for male melanoma patients in the Netherlands between 2000 and 2014, stratified for Breslow thickness category with corresponding 5- and 10-year relative survival rates. *N* per Breslow thickness category, respectively, 8253, 3549, 5032, 3529 and 2062.

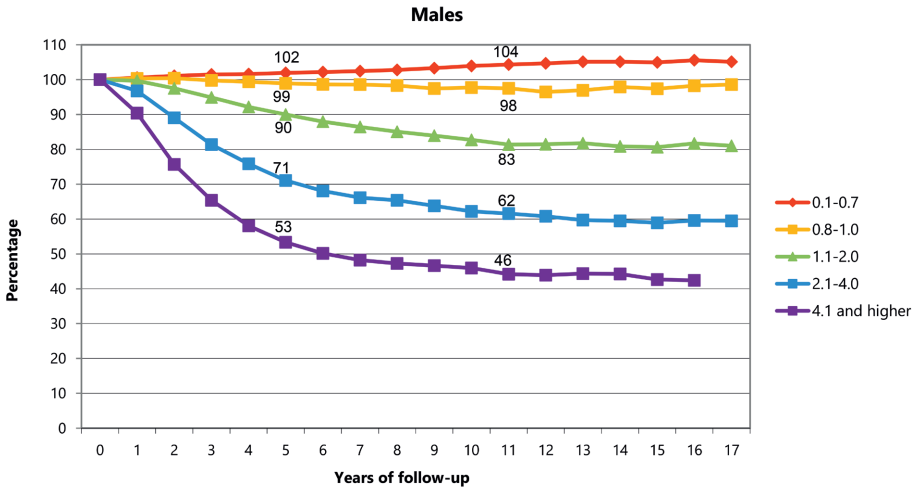
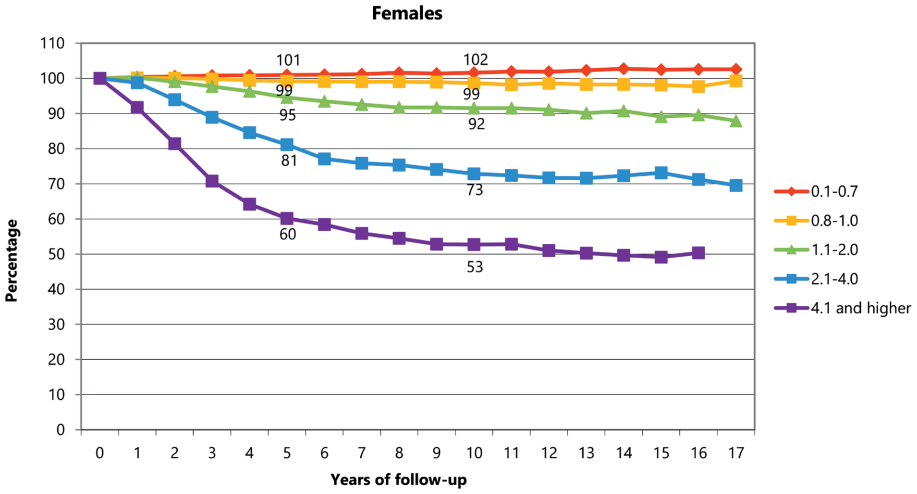


Figure 5. Relative survival curves for female melanoma patients in the Netherlands between 2000 and 2014, stratified for Breslow thickness category with corresponding 5- and 10-year relative survival rates. *N* per Breslow thickness category, respectively, 12 726, 4868, 6262, 3379 and 1651.



Survival analysis

Follow-up data were available in 93.9% of patients. Over time, survival of females improved while that of males decreased (Fig. 2). Five- and ten-year RS curves showed similar survival for males and females in melanoma <1.0 mm BT and worse survival for melanoma >1.1 mm BT (Figs 4 and 5). For melanomas thicker than 1.0 mm BT, RS rates for males were lower compared to females for each Breslow category. The 10-year RS rates for melanomas 1.0–2.0, 2.1–4.0 and ≥4.1 mm were 83% vs. 92%, 62% vs. 73% and 46% vs. 53% for males and females, respectively. After adjustment for age, BT, localization, ulceration and morphological subtype, gender remained significantly associated with a higher risk of dying among males with a RER for males 1.37 (95% CI: 1.31–1.45, Table 2). A total of 37 049 (67.8%) patients were included for this analysis.

Table 2. Relative excess rate (RER) for death among cutaneous melanoma patients diagnosed in the Netherlands between 2000 and 2014.

Variable	N per category	RER (95% CI)
Age in years		
18-35	3797	Reference
36-55	13,666	1.59 (1.38-1.83)
56-75	14,576	3.20 (2.79-3.66)
>75	5010	9.08 (7.91-10.42)
Breslow thickness in mm		
0.1-0.7	15,120	Reference
0.8-1.0	6031	1.36 (1.23-1.50)
1.1-2.0	8119	2.26 (2.09-2.45)
2.1-4.0	5063	4.04 (3.72-4.39)
>4.1	2716	5.79 (5.27-6.36)
Gender		
Female	20,588	Reference
Male	16,461	1.37 (1.31-1.45)
Body site		
Head and neck	4568	Reference
Trunk	16,318	0.97 (0.90-1.04)
Arms	5572	0.77 (0.71-0.84)
Legs	10,591	0.76 (0.71-0.83)
Ulceration		
No	31,292	Reference
Yes	5757	1.67 (1.57-1.76)
Subtype melanoma		
Superficial spreading	29,392	Reference
Nodular	5640	0.98 (0.92-1.04)
Lentigo maligna melanoma	1670	1.19 (1.07-1.33)
Acral lentiginous	347	1.25 (1.04-1.50)

DISCUSSION

Over the past years, the incidence of men diagnosed with melanoma in the Netherlands has increased from 41.7% in 2000 to 47.3% in 2014 as shown in the present study. Men were significantly older at the time of diagnosis and had more often thicker and ulcerated melanomas and melanoma localized on the trunk or head and neck. Correcting for different well-known confounders, survival remained worse for males with a RER of 1.37 (95% CI: 1.31–1.45).

Relative survival rates for men and women were comparable for patients with thin melanomas. RS of those with melanomas 0.1–0.7 mm BT was above 100%, indicating that the patients in this subgroup had better survival than the age- and gender-matched general population. It seems likely that this subgroup of patients with very thin melanomas survives longer because they generally have higher socio-economic

status and less comorbidity, leading to better survival than the general population.[25] It could be speculated that the significant increase in incidence of male melanoma patients over time (from 41.7% in 2000 to 47.3% in 2014) could on the one hand be due to the increase of single households.[26]

The RER of 1.37 we found for melanoma mortality in men is in line with several other large studies [5–14]. It is interesting to note that also in countries with a higher incidence in women (e.g. most European countries), survival adjusted for several known confounders is worse for the opposite sex. As Clark Jr. et al.[27] already noted in 1969, 'the disease is somewhat less malignant in the female when compared with the male'. Until today, gender difference in survival among melanoma patients remains apparent and not fully understood. So for the moment, we should focus on currently available data that might impact the gender-related difference in survival. As our study results show, especially males of older age with a melanoma located on the head and neck could benefit from this. This is supported by data from Lachiewicz et al.,[28] as they compared data from 51 704 adults from the SEER database in the United States from 1992 to 2003 showing a hazard ratio (HR) of 1.84 for scalp/neck melanoma as compared to melanoma with the same clinicopathological characteristics, located on the extremities. There is no consensus whether the subtype of melanoma is an independent prognostic factor of survival. As we have shown in the present study, when correcting for multiple confounders (such as Breslow thickness and ulceration), there was no difference in survival of superficial spreading and nodular melanoma. In contrast, Lattanzi et al.[29] assessed survival difference between superficial spreading and nodular melanoma using SEER data as well and found nodular subtype to be an independent predictor with a HR of 1.55. The recognition that males of older age with a melanoma located on the head and neck have poorer prognosis is important, as campaigns with special focus on this subgroup of (potential) melanoma patients can establish specific prevention campaigns and approaches for early detection of melanoma.[30, 31]

Strengths of our study include the use of nationwide data and therefore its generalizability. Limitations are its retrospective nature. As we have shown, there are more factors predicting survival in melanoma than incorporated in the 8th AJCC (BT and ulceration status). To better inform patient on their prognosis, gender should be taken into account and the development of a prediction model separate for men and women for survival would be useful.

CONCLUSIONS

We have shown that survival for male melanoma patients is worse compared to females, with a worsening trend in time, which cannot be explained by known confounders. Clinicians as well as potential patients should be aware of these

differences, as awareness might lead to a lower incidence of thicker, ulcerated melanomas and thereby reducing mortality of melanoma in males. Primary and secondary prevention campaigns targeting this specific subgroup of melanoma patients are needed.

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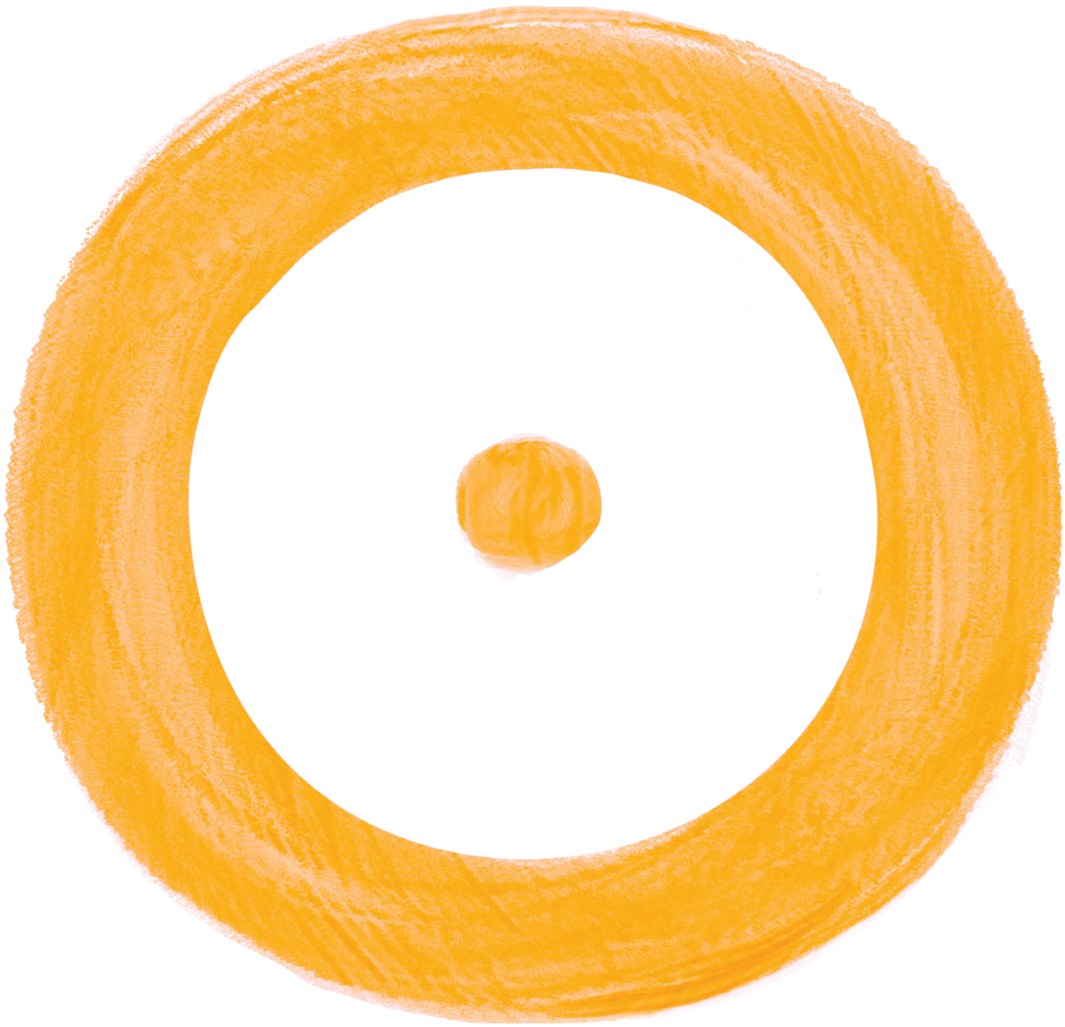
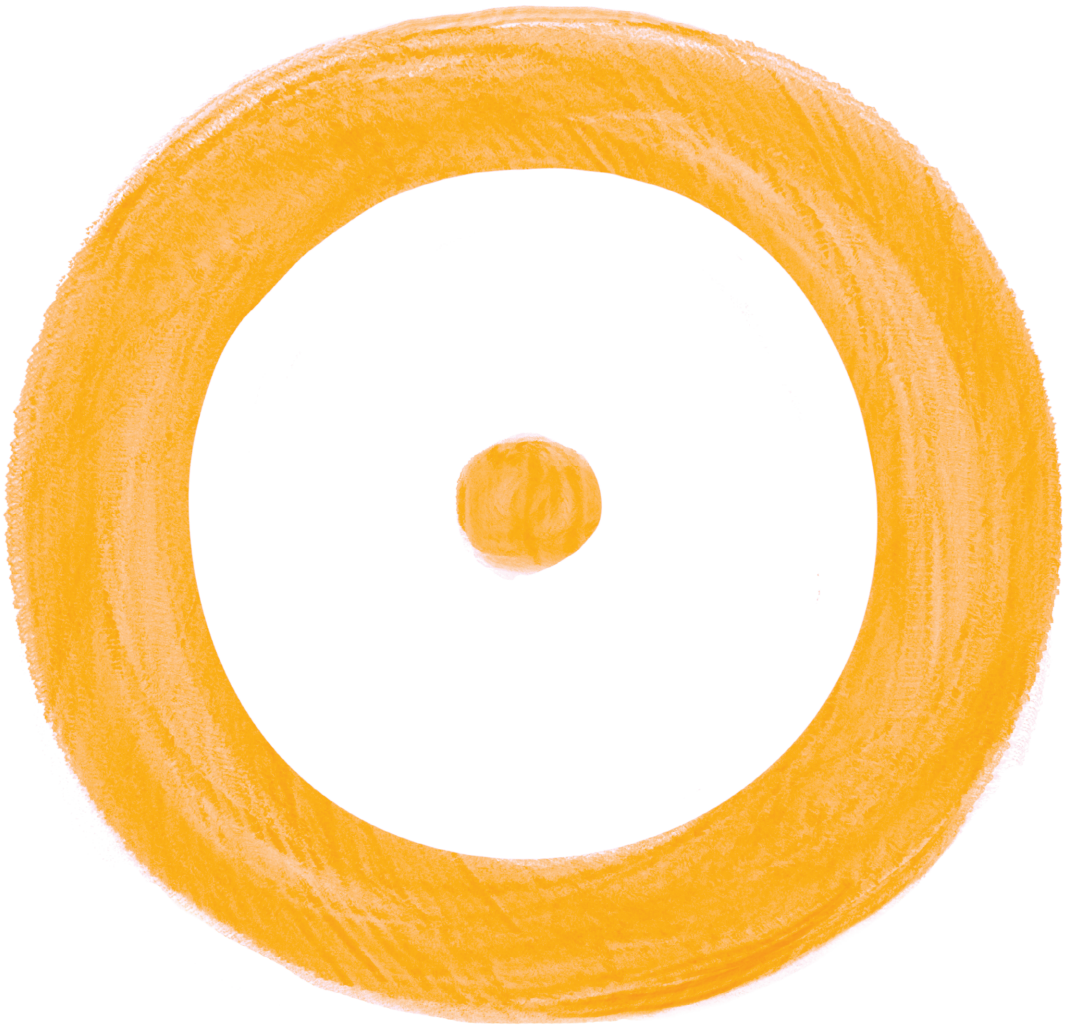


PART III

NOMOGRAM-BASED PREDICTIONS: A MULTICONTINENTAL APPROACH



CHAPTER 14

Development and validation of nomograms to predict local, regional, and distant recurrence in patients with thin (T1) melanomas

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ABSTRACT

Purpose: Although the prognosis of patients with thin primary cutaneous melanomas (T1, ≤ 1.0 mm) is generally excellent, some develop recurrence. We sought to develop and validate a model predicting recurrences in patients with thin melanomas.

Methods: A Dutch population-based cohort (n = 25,930, development set) and a cohort from an Australian melanoma treatment center (n = 2,968, validation set) were analyzed (median follow-up 6.7 and 12.0 years, respectively). Multivariable Cox models were generated for local, regional, and distant recurrence-free survival (RFS). Discrimination was assessed using Harrell's C-statistic for each outcome. Each nomogram performance was evaluated using calibration plots defining low-risk and high-risk groups as the lowest and top 5% of the nomogram risk score, respectively. The nomograms' C-statistics were compared with those of a model including the current American Joint Committee on Cancer staging parameters (T-stage and sentinel node status).

Results: Local, regional, and distant recurrences were found in 209 (0.8%), 503 (1.9%), and 203 (0.8%) Dutch patients, respectively, and 23 (0.8%), 61 (2.1%), and 75 (2.5%) Australian patients, respectively. C-statistics of 0.79 (95% CI, 0.75 to 0.82) for local RFS, 0.77 (95% CI, 0.75 to 0.78) for regional RFS, and 0.80 (95% CI, 0.77 to 0.83) for distant RFS were obtained for the development model. External validation showed C-statistics of 0.80 (95% CI, 0.69 to 0.90), 0.76 (95% CI, 0.70 to 0.82), and 0.74 (95% CI, 0.69 to 0.80), respectively. Calibration plots showed a good match between predicted and observed rates. Using the nomogram, the C-statistic was increased by 9%-12% for the development cohort and by 11%-15% for the validation cohort, compared with a model including only T-stage and sentinel node status.

Conclusions: Most patients with thin melanomas have an excellent prognosis, but some develop recurrence. The presented nomograms can accurately identify a subgroup at high risk. An online calculator is available at www.melanomarisk.org.au.

INTRODUCTION

In population-based cohorts, 58%-81% of patients presenting with primary cutaneous melanomas have thin melanomas (defined as T1, ie, ≤ 1.0 mm in Breslow thickness).[1-5] Attributed to factors such as increased UV-exposure, aging populations, and increasing awareness leading to earlier diagnosis, the number of patients presenting with thin melanomas is steadily increasing worldwide.[6] Overall, their prognosis is very good, with reported 5-year survival rates of 88.6%-98.8%[7,8]; however, a subset develop recurrent disease. Since patients with thin melanomas constitute such a high proportion of all patients diagnosed with melanoma, in absolute numbers, more people ultimately die from T1 melanomas than from T2, T3, or T4 melanomas.[3,9,10] Thus, predicting disease recurrence in patients with T1 melanomas is of great importance. To date, there have been no population-based studies of recurrence in patients with thin melanomas, and the current literature reporting survival of these patients is inconsistent, with uncertainty about which factors are significant predictors of ultimate outcome.[11] In four of the largest published studies, the presence of ulceration was found to be a significant predictor of outcome in two,[7,12] but not in another,[8] while the fourth did not consider it.[1]

The objective of this study was to develop and validate nomograms predicting local, regional, and distant recurrence for patients with thin melanomas, using readily available clinicopathologic information.

METHODS

Collection of data

For the development model, data for all newly diagnosed patients with invasive cutaneous melanomas diagnosed in the Netherlands between January 2000 and December 2014 were obtained from PALGA, the Dutch Pathology Registry,[13] which collects data prospectively from all pathology laboratories in the Netherlands. Data were encoded and used anonymously. Ethical approval was granted by the board of PALGA.

For the validation model, a search was performed of the prospectively maintained database of Melanoma Institute Australia (MIA) for patients with thin melanomas who were initially diagnosed and treated at MIA in the same period. Patients referred to MIA after they had developed melanoma recurrence were excluded, to eliminate referral bias. All patients had given permission for their data to be recorded in the MIA database and used for research purposes. Ethical approval was provided by the Sydney Local Health District Ethics Committee.

Study population

Only patients with melanomas ≤ 1.0 mm in Breslow thickness were included. Patients initially diagnosed with clinically detected stage III disease or stage IV disease, those < 18 years of age, and those with multiple primary melanomas were excluded. For each patient, the information collected included date of diagnosis, age at time of diagnosis, sex, Breslow thickness (in mm, rounded to one decimal place), ulceration (present or absent), T-stage (which combines Breslow thickness and ulceration, as defined by the 8th American Joint Committee on Cancer [AJCC] staging manual),[14] melanoma subtype (superficial spreading, nodular, lentigo maligna, acral lentiginous, or pure desmoplastic), anatomical site of the primary tumor (head and neck, trunk, and upper limb or lower limb), mitoses (present or absent), sentinel node (SN)-biopsy status (positive, negative, or not performed), and recurrence (date, site, and type [local, in-transit or regional nodes, or distant]). Recurrence-free survival (RFS) was calculated from the date of initial melanoma diagnosis to the date of diagnosis of recurrence. The outcomes of interest were local RFS (LRFS), regional (including in-transit) RFS (RRFS), and distant RFS (DRFS). Outcomes were not mutually exclusive, eg, a patient who experienced local recurrence and later regional recurrence contributed to LRFS and RRFS, but at different times, and would have been censored for DRFS. Patients without recurrence were censored at their date of death, the last date known to be alive, or January 1, 2018 (the database cutoff date), whichever occurred earlier.

Statistical analysis

Categorical variables were presented as frequencies (percentages). Continuous variables were summarized as medians (with ranges). To compare the MIA cohort

with the Dutch cohort, variables were analyzed using chi-square or Mann-Whitney U tests, as appropriate. Univariable and multivariable Cox proportional hazards regression analyses were performed to estimate hazard ratios and 95% CIs, using LRFS, RRFS, and DRFS as the outcomes. For each outcome, a backward variable selection procedure was performed on the initial model that included the variables age, sex, site, and readily available parameters in pathology reports (Breslow thickness, ulceration status, mitoses, melanoma subtype, and SN status). Nomograms were derived from the multivariable model to predict each outcome. The model's predictive performance was assessed by calculating Harrell's C-statistic, also known as the area under the curve, reflecting how well the model identified patients with recurrence.[15,16] The 10-year predictive ability of each nomogram was assessed using time-dependent ROC curves for the development and validation cohorts.[16] To correct for overoptimism, internal validation by bootstrapping was performed.[17] External validation was also performed by applying the coefficients and the baseline hazard of the development cohort to the validation cohort. For calibration purposes, the patients in the development data set were divided into three groups based on the nomogram risk scores[18]; the low-risk group included the lowest 5% of the patient distribution of risk scores, the high-risk group included the top 5%, and the intermediate-risk group included the remaining patients. More detailed descriptions of the statistical methods that were used are provided in the Data Supplement (online only).

The study adhered to guidelines for the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD). The TRIPOD checklist and a flowchart outlining the patient selection process are reported in the Data Supplement.[19]

All statistical analyses were performed using R version 3.6.1.[20,21] Two-sided P values < .05 were considered significant.

RESULTS

Baseline characteristics of the 25,930 Dutch patients and the 2,968 MIA patients are detailed in Table 1. SN-biopsy was performed in 1,143 Dutch patients (4.4%) and 284 MIA patients (9.6%). The median follow-up time was 6.7 years (95% CI, 6.6 to 6.7) for the Dutch cohort and 12.0 years (95% CI, 11.7 to 12.3) for the MIA cohort. There was a statistically significant difference in RFS between patients with melanomas < 0.8 mm in thickness and those with melanomas 0.8-1.0 mm in thickness (see the Data Supplement). This finding was consistent between the two cohorts, with the same statistical significance level (P < .0001). RFS curves within each Breslow thickness category were almost identical for the Dutch and MIA patients.

Table 1. Clinicopathologic characteristics of patients with thin melanomas in the Dutch and MIA data sets.

Characteristic	Overall (n = 28,898)	Dutch cohort (n = 25,930)	MIA cohort (n = 2968)	p-value
Gender (n (%))				<0.0001
Female	16,881 (58.4)	15,509 (59.8)	1372 (46.2)	
Male	12,017 (41.6)	10,421 (40.2)	1596 (53.8)	
Median age at diagnosis in years (range)	54.0 (18.0-103.0)	54.0 (18.0-103.0)	55.0 (18.0-95.0)	0.16
Primary site (n (%))				<0.0001
Head & Neck	3387 (11.7)	2983 (11.5)	404 (13.6)	
Trunk	13,215 (45.7)	11,957 (46.1)	1258 (42.4)	
Upper limb	4506 (15.6)	3920 (15.1)	586 (19.7)	
Lower limb	7790 (27.0)	7070 (27.3)	720 (24.3)	
Median Breslow thickness in mm (range)	0.6 (0.1-1.0)	0.6 (0.1-1.0)	0.6 (0.1-1.0)	0.03
Mitoses (n (%))				<0.0001
Not present	8828 (30.5)	7212 (27.8)	1616 (54.4)	
Yes	4777 (16.5)	3701 (14.3)	1076 (36.3)	
Not known	15,293 (52.9)	15,017 (57.9)	276 (9.3)	
Subtype (n (%))				<0.0001
Superficial spreading	26,237 (90.8)	23,663 (91.3)	2574 (86.7)	
Nodular	597 (2.1)	506 (2.0)	91 (3.1)	
Lentigo maligna	1884 (6.5)	1673 (6.5)	211 (7.1)	
Acral lentiginous	97 (0.3)	79 (0.3)	18 (0.6)	
Desmoplastic	83 (0.3)	9 (0.0)	74 (2.5)	
Ulceration (n (%))				<0.0001
Not present	23,454 (81.2)	20,651 (79.6)	2803 (94.4)	
Yes	565 (2.0)	490 (1.9)	75 (2.5)	

Table 1. (continued).

Characteristic	Overall (n = 28,898)	Dutch cohort (n = 25,930)	MIA cohort (n = 2968)	p-value
Not known	4879 (16.9)	4789 (18.5)	90 (3.0)	
8th AJCC T-status (n (%))				<0.0001
T1a	16921 (58.6)	14,886 (57.4)	2035 (68.6)	
T1b	8388 (29.0)	7528 (29.0)	860 (29.0)	
T1nos	3589 (12.4)	3516 (13.6)	73 (2.5)	
SN status (n (%))				<0.0001
Negative	1319 (92.4)	1051 (91.7)	268 (94.4)	
Positive	108 (7.6)	92 (8.3)	16 (5.6)	
Not performed	27.471	24.787	2684	

Abbreviations: AJCC, American Joint Committee on Cancer; MIA, Melanoma Institute Australia; SN, sentinel node.

Table 2. Univariable and multivariable competing risk Cox regression for LRFS, RRFS, and DRFS of patients with thin melanomas for the development (Dutch) cohort (n = 25,930, with 209, 503, and 203 events, respectively).

		Local recurrence free survival				Regional recurrence free survival				Distant recurrence free survival			
		Univariable		Multivariable		Univariable		Multivariable		Univariable		Multivariable	
Variable	Class	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Gender	Male vs Female	1.19 (0.91-1.57)	0.21	-	-	1.59 (1.33-1.89)	<0.0001	1.43 (1.19-1.72)	0.0001	1.99 (1.51-2.62)	<0.0001	1.50 (1.13-2.00)	0.005
SN status	Positive vs Negative	3.00 (1.00-8.98)	0.05	-	-	5.55 (2.90-10.64)	<0.0001	3.59 (1.86-6.90)	0.0001	6.81 (3.01-15.40)	<0.0001	4.80 (2.12-10.87)	0.0002
	Not performed vs Negative	0.49 (0.29-0.82)	0.006	-	-	0.64 (0.44-0.93)	0.018	1.51 (1.03-2.20)	0.03	0.46 (0.28-0.77)	0.003	1.00 (0.59-1.70)	0.99
Breslow thickness	Per mm	1.33 (1.25-1.42)	<0.0001	1.28 (1.20-1.37)	<0.0001	1.44 (1.38-1.51)	<0.0001	1.37 (1.31-1.44)	<0.0001	1.49 (1.39-1.60)	<0.0001	1.43 (1.33-1.54)	<0.0001
Age	Per year	1.04 (1.03-1.05)	<0.0001	1.04 (1.03-1.05)	<0.0001	1.00 (0.99-1.00)	0.24	-	-	1.00 (0.99-1.01)	0.71	-	-
Localisation	Trunk vs Head&Neck	0.39 (0.27-0.56)	<0.0001	0.61 (0.40-0.93)	0.02	1.08 (0.82-1.44)	0.58	0.94 (0.70-1.27)	0.69	0.57 (0.40-0.80)	0.001	0.51 (0.36-0.73)	0.0002
	Arm vs Head&Neck	0.32 (0.19-0.53)	<0.0001	0.40 (0.23-0.69)	0.001	0.52 (0.35-0.77)	0.001	0.45 (0.30-0.68)	0.0001	0.36 (0.22-0.59)	<0.0001	0.34 (0.21-0.57)	<0.0001
	Leg vs Head&Neck	0.50 (0.34-0.73)	0.0003	0.72 (0.46-1.12)	0.14	0.82 (0.60-1.12)	0.21	0.74 (0.53-1.02)	0.069	0.20 (0.12-0.33)	<0.0001	0.20 (0.12-0.33)	<0.0001
Mitosis	Yes vs Not present	2.22 (1.49-3.31)	<0.0001	1.43 (0.94-2.16)	0.091	4.56 (3.34-6.24)	<0.0001	2.66 (1.93-3.66)	<0.0001	3.25 (2.05-5.16)	<0.0001	1.80 (1.12-2.89)	0.015
	Not known vs Not present	0.82 (0.57-1.17)	0.28	0.75 (0.52-1.09)	0.13	1.83 (1.37-2.45)	<0.0001	1.60 (1.19-2.14)	0.002	1.28 (0.84-1.95)	0.26	1.11 (0.73-1.70)	0.63
Ulceration	Yes vs Not present	4.64 (2.81-7.66)	<0.0001	2.72 (1.62-4.55)	0.0001	3.90 (2.73-5.57)	<0.0001	2.15 (1.50-3.09)	<0.0001	4.70 (2.81-7.89)	<0.0001	2.69 (1.59-4.53)	0.0002
	Not known vs Not present	0.77 (0.53-1.10)	0.15	0.83 (0.57-1.21)	0.33	0.91 (0.73-1.13)	0.39	0.94 (0.75-1.17)	0.57	0.93 (0.66-1.31)	0.69	0.97 (0.68-1.37)	0.85
8th AJCC staging	T1b vs T1a	2.97 (2.21-3.98)	<0.0001	-	-	3.46 (2.86-4.19)	<0.0001	-	-	4.43 (3.21-6.10)	<0.0001	-	-
	T1nos vs T1a	0.81 (0.48-1.34)	0.40	-	-	0.76 (0.54-1.08)	0.13	-	-	1.07 (0.64-1.80)	0.80	-	-
Subtype	NM vs SSM	3.98 (2.31-6.88)	<0.0001	1.93 (1.09-3.39)	0.02	4.91 (3.62-6.65)	<0.0001	2.47 (1.80-3.38)	<0.0001	3.63 (2.11-6.26)	<0.0001	-	-
	LMM vs SSM	2.81 (1.87-4.22)	<0.0001	1.64 (0.99-2.69)	0.05	0.50 (0.29-0.87)	0.014	0.62 (0.34-1.10)	0.10	1.10 (0.60-2.02)	0.76	-	-
	ALM vs SSM	7.91 (2.93-21.33)	<0.0001	4.82 (1.75-13.26)	0.002	2.97 (1.11-7.94)	0.03	2.77 (1.02-7.52)	0.046	3.69 (0.92-14.88)	0.07	-	-
	DM vs SSM	20.65 (2.89-147.6)	0.003	9.50 (1.30-69.37)	0.03	NA	NA	NA	NA	NA	NA	-	-

Abbreviations: AJCC, American Joint Committee on Cancer; ALM, acral lentiginous melanoma; DM, pure desmoplastic melanoma; DRFS, distant recurrence-free survival; HR, hazard ratio; LMM, lentigo maligna melanoma; LRFS, local recurrence-free survival; NM, nodular melanoma; RRFS, regional recurrence-free survival; SN, sentinel node; SSM, superficial spreading melanoma.

Cox regression analysis

In the Dutch cohort, local, regional, and distant recurrences occurred in 209 (0.8%), 503 (1.9%), and 203 (0.8%) patients, respectively, ie, in 807 patients altogether (3.1%). For MIA patients, these numbers were 23 (0.8%), 61 (2.1%), and 75 (2.5%), respectively, in 124 patients altogether (4.2%). Results of univariable and multivariable Cox regression analyses for the development cohort for LRFS, RRFS, and DRFS are provided in Table 2. There was no evidence of violation of the proportional hazards assumption. The results of the test of deviance did not support a nonlinear association between each survival outcome and Breslow thickness and age. When categorizing missing ulceration and mitoses status as not present, hazard ratios of other variables were consistent between the multivariable analyses (Table 2 and Data Supplement).

Nomograms and external validation

The C-statistics for the development model for LRFS, RRFS, and DRFS were 0.79 (95% CI, 0.75 to 0.82), 0.77 (95% CI, 0.75 to 0.78), and 0.80 (95% CI, 0.77 to 0.83), respectively (Fig 1). After adjustment for optimism using bootstrapping techniques, these were again 0.79 (95% CI, 0.76 to 0.82), 0.77 (95% CI, 0.75 to 0.79), and 0.80 (95% CI, 0.77 to 0.83), respectively, meaning that there was no overfitting. External validation using the MIA cohort for LRFS, RRFS, and DRFS showed C-statistics of 0.80 (95% CI, 0.69 to 0.90), 0.76 (95% CI, 0.70 to 0.82), and 0.74 (95% CI, 0.69 to 0.80), respectively, confirming the nomogram's high discriminative ability to distinguish patients with low and high risk of local, regional, and distant recurrence. The C-statistics combining T-stage from the AJCC 8th edition14 and SN status (including T1nos patients) for LRFS, RRFS, and DRFS were 0.67 (95% CI, 0.63 to 0.70), 0.68 (95% CI, 0.66 to 0.70), and 0.70 (95% CI, 0.66 to 0.73), respectively, for the development cohort, and 0.65 (95% CI, 0.54 to 0.76), 0.66 (95% CI, 0.59 to 0.73), and 0.65 (95% CI, 0.59 to 0.71), respectively, for the validation cohort. The increase in C-statistics between these models and the proposed nomogram were all statistically significant ($P < .0001$) and ranged between 9% and 12% for the development cohort and between 11% and 15% for the validation cohort. The derived nomograms to define subgroups based on type of recurrence are shown in Figures 2A-2C.

Figure 1. Calibration plots (as Kaplan-Meier curves) and receiver operating characteristics curves at 10 years for local, regional, and distant recurrence-free survival (RFS). The calibration plots are displayed per risk group with corresponding 10-year RFS estimates. The low-risk group includes the bottom 5% of patients (with the lowest total nomogram points), the high-risk group includes patients with the highest 5% of total points, and the remaining patients constitute the intermediate-risk group. AUC, area under the curve; MIA, Melanoma Institute Australia.

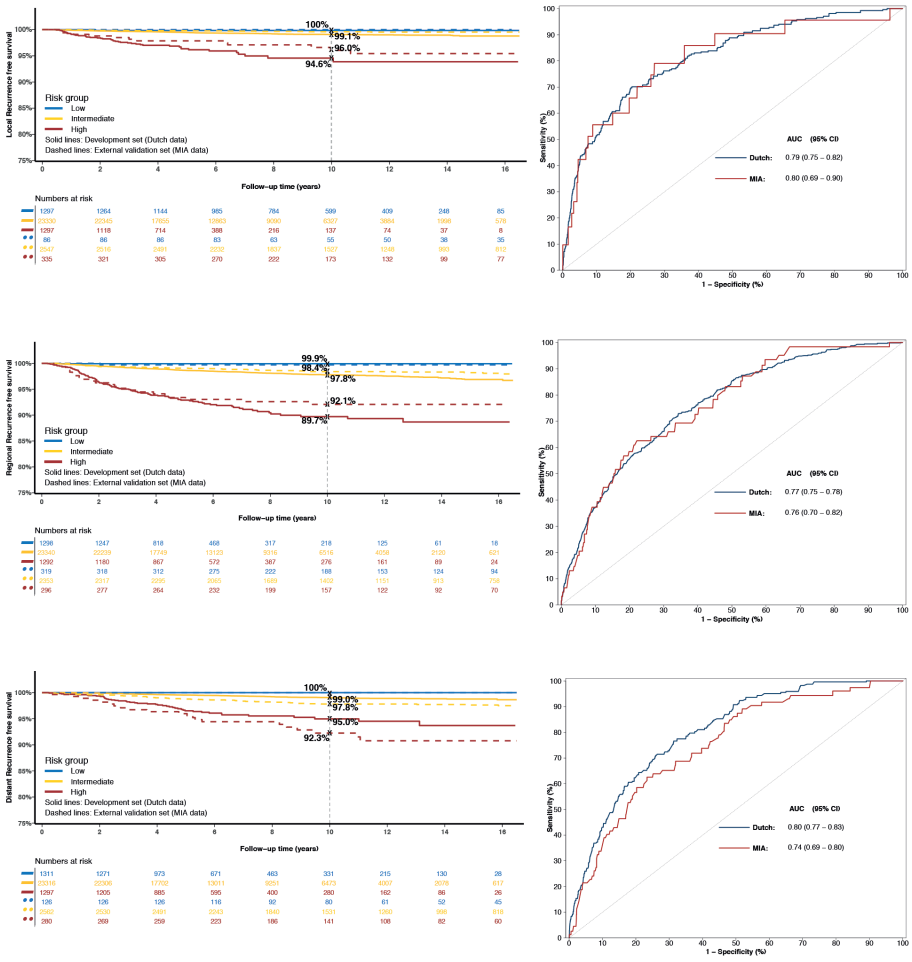
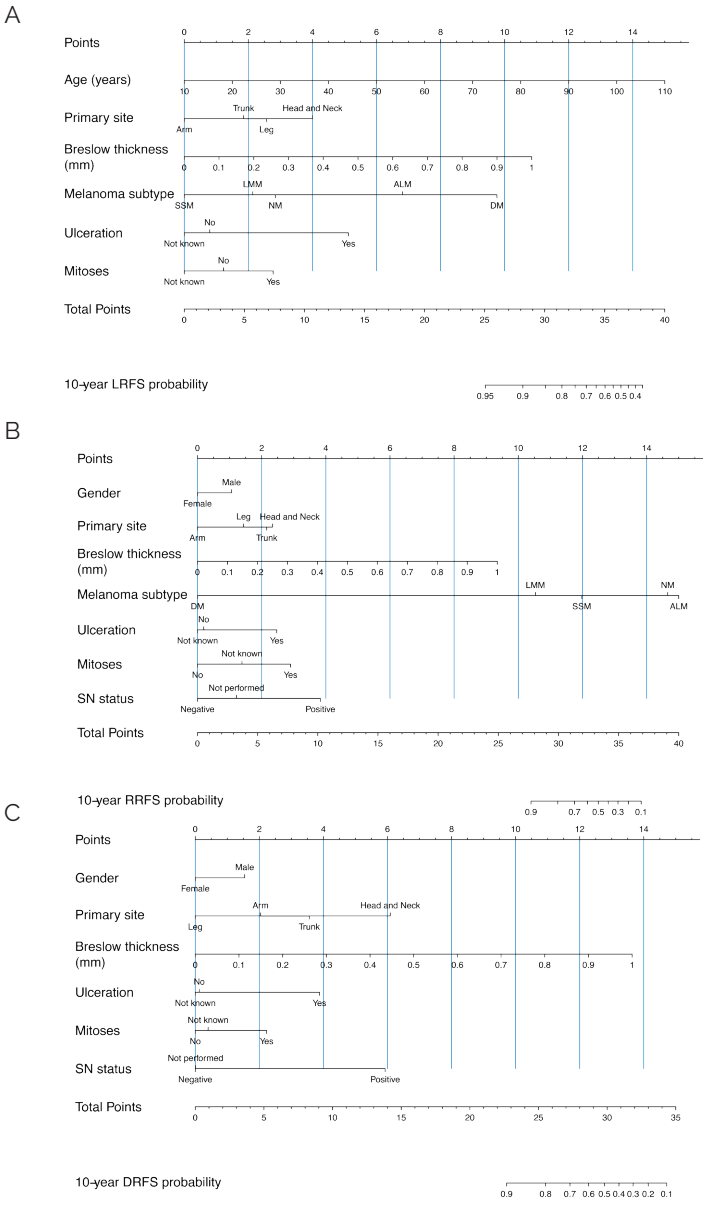


Figure 2. Nomogram to predict (A) local, (B) regional, and (C) distant recurrence-free survival (RFS) in patients with thin melanomas. For each individual clinicopathologic variable, the number of points can be derived from the upper line. When all points are added together the corresponding 10-year RFS probability can be derived by drawing a vertical line from the total number of points downward. A higher number of risk points corresponds to higher risk of recurrence. An online version of the nomogram is available.[34]



ALM, acral lentiginous melanoma; DM, pure desmoplastic melanoma; DRFS, distant recurrence-free survival; LMM, lentigo meligna melanoma; LRFS, local recurrence-free survival; NM, nodular melanoma; RRFS, regional recurrence-free survival; SN, sentinel node; SSM, superficial spreading melanoma.

Risk groups

To display the calibration of the nomograms, Figure 1 shows the Kaplan-Meier curves for the low-risk, intermediate-risk, and high-risk groups, based on the top and lowest 5% patient distribution of risk scores obtained from the nomograms for LRFS, RRFS, and DRFS. The calibration plots indicated that the nomograms were well calibrated, with good agreement of the survival curves for all risk groups between the Dutch and the MIA data. Using the 5% cutoff for the nomogram risk scores, for LRFS the 10-year RFS rate was 99.8% (95% CI, 99.6 to 100) in the low-risk group compared with 94.6% (95% CI, 92.7 to 96.5) in the high-risk group in the development cohort. For the validation cohort, the 10-year RFS rate for LRFS was 100% (95% CI, 100 to 100) in the low-risk group compared with 96.0% (95% CI, 93.7 to 98.4) in the high-risk group. Ten-year RFS rates for RRFS and DRFS are also shown in Figure 1 and the Data Supplement. Similar results were seen using the 10% cutoff for the nomogram risk scores (Data Supplement). Observed and predicted 10-year RFS probabilities for LRFS, RRFS, and DRFS in the development and validation cohorts were very similar in the low-risk and intermediate-risk groups, confirming the good calibration of the nomograms (Data Supplement). The Data Supplement shows histograms of the risk scores in the development and validation sets using the lowest and highest ranking 5% and 10% of patients, respectively, for LRFS, RRFS, and DRFS. An overview of the T1a patients with recurrence who were classified as high-risk by each nomogram using the 5% cutoff is given in the Data Supplement. All had melanomas with a Breslow thickness of ≤ 0.7 mm and no ulceration (hence T1a).

The baseline hazard function curves clearly showed that patients with thin melanomas most often developed regional recurrence, followed by distant recurrence and local recurrence, respectively (Data Supplement). The baseline hazard function for local recurrence was quite stable over time, whereas the curves for distant and regional recurrence were clearly nonlinear, with an increasing risk in the first 3 years after melanoma diagnosis.

DISCUSSION

The counterintuitive fact that patients with T1 primary tumors are responsible for most melanoma deaths is now well documented.[9] However, recurrence of disease in these patients has received little attention, with no population-based studies reporting this published previously. Prior studies have only reported data for patients managed at academic institutions and specialized melanoma treatment centers.

The current AJCC staging system considers only T-stage and SN status to predict survival in patients with T1 melanomas.[14] We found that the predictive performance for LRFS, RRFS, and DRFS (indicated by the C-statistic) increased from 0.67, 0.68, and 0.70, respectively, for the model including T-stage and SN status to 0.79, 0.77,

and 0.80, respectively, by using the current nomogram for the development cohort, and from 0.65, 0.66, and 0.65, respectively, to 0.80, 0.76, and 0.74, respectively, for the validation cohort. An absolute gain in the C-statistic of 9%-15% is considered substantial, especially when the C-statistic of the base model is already quite high.[22] The input needed for each nomogram includes only predictors that are readily available and can be obtained from the primary melanoma itself.

Faries et al[23] studied 2,211 T1 melanomas treated at their institution between 1971 and 2005 and reported a 2.9% recurrence rate in regional nodes, quite similar to the rate in our data set (1.8%). Maurichi et al[24] reviewed 2,243 T1 patients from six European centers. They reported nine local, 168 nodal, and 70 distant recurrences, altogether comprising 11% of patients in their cohort compared with 3.1% and 4.2% in our development and validation data sets, respectively. Of note, Maurichi et al reported that 23.6% of all their T1 melanomas were ulcerated, which is remarkably high, since in our study only 2% were ulcerated, and the ulceration rates in other studies of T1 melanomas have ranged from 1.2% to 4.2%.7[8,12,23,25,26] Calomarde-Rees et al[27] analyzed patients with stage I and II melanomas (of all Breslow thicknesses), 629 of which were ≤ 1.00 mm. Of these, 19 (3.0%) developed lymphatic recurrences (defined as skin and nodal recurrences) and 16 (2.5%) developed distant disease. Kalady et al[28] included 1,158 T1 patients, of whom 1,082 initially presented without recurrences. A total of 101 (9.3%) developed recurrent disease during follow-up; 19 (1.8%) recurred in skin, 38 (3.5%) in regional nodes, and 42 (3.9%) at distant sites. In our study, combining the two cohorts, 232 patients developed skin recurrences, 564 nodal recurrences, and 278 distant recurrences, respectively, ie, 0.8%, 2.0%, and 0.9% of all the T1 patients. Thus, each of the aforementioned studies reported higher recurrence rates than we found. The most likely explanation for this is referral bias in the other studies, and not length of follow-up. Calomarde-Rees et al[27] had a median follow-up comparable with our Dutch cohort (6.3 years v 6.7 years), whereas two of the other three studies had a median follow-up of 10 years[24,28] and one of 13 years,[23] comparable with our MIA cohort (median follow-up 12 years). Our study included nation-wide data and data only for patients initially diagnosed and managed at MIA (patients referred to MIA after they had developed melanoma recurrence were excluded), whereas all other studies were single-institution based, or at tertiary referral hospitals. Thus, referral bias might have occurred, since patients with more advanced disease are more likely to be seen at a tertiary hospital or dedicated institution. However, we cannot exclude the possibility that the shorter follow-up in the Dutch cohort might have led to an underestimation of the number of recurrences in Dutch patients.

Notwithstanding the above, when referral bias was eliminated by analyzing only patients whose initial definitive treatment was at our institution, in a prior study we found that a 0.8 mm cutoff separated patients with thin melanoma at low-risk and high-risk of death because of melanoma.[10] Importantly, in our prior study, we found

that a significant proportion of patients with thin melanomas who died because of melanoma did so relatively late, with only 29% of the deaths occurring within 5 years of diagnosis. The current results showed a small difference between recurrence rates in the two cohorts: 3.1% in the Dutch cohort and 4.2% in the Australian cohort. This difference in recurrence rates might be attributed to several factors, including a longer median follow-up time in the MIA cohort (12.0 years v 6.7 years in Dutch cohort), and a difference in distribution of sex between the two cohorts (53.8% males in the MIA cohort v 40.2% in the Dutch cohort), as there might be different health-seeking behavior by males and females.[29] Recent changes in melanoma staging have led to guideline recommendations that an SN-biopsy procedure be considered for a subset of patients with T1 melanomas that are likely to be associated with a higher risk of nodal involvement (eg, T1b, those with ulceration, or a relatively higher Breslow thickness).[14,30] The current nomograms were able to identify T1a patients, in whom SN-biopsy is not generally recommended, but who were at high-risk of local, regional, or distant recurrence and might therefore benefit from the procedure. Strengths of the current study include the large sample size and the use of population-based data from an entire country for the development model. This resulted in an unselected study population and increases the generalizability of our results. Another strength is the validation of our model in an independent cohort, making it the first validated model for RFS in patients with thin melanomas. The use of pathology data collected in routine clinical practice suggests that the model is useful, applicable, and translatable to patients with melanoma generally. Furthermore, most previous studies included fewer current data elements, with some lacking important prognostic criteria such as ulceration. Limitations that are inevitably associated with the retrospective nature of our study are some missing data and the fact that we could only include patients with melanoma recurrences that were confirmed by histopathologic review. Although in the majority of patients, histopathologic confirmation of suspected disease recurrence would have been obtained, it is likely that some patients were missed in whom recurrent disease was evaluated only by imaging. This might have led to an underestimation of the number of recurrences in these T1 patients. By contrast, there might have been a slight overestimation of the chance of recurrence because we did not account for death as a competing risk. Another limitation is that criteria for SN-biopsy for patients with T1 melanoma in international guidelines have not been specific, simply advising consideration of the procedure.[31-33] This might have led to differences between the two cohorts in deciding whether-or-not to perform SN-biopsy, for clinicians as well as for patients. However, as the majority of T1 melanomas are < 0.8 mm without ulceration or other adverse prognostic factors, most patients will not be eligible for SN-biopsy, regardless of which guideline is followed. The shorter median follow-up in the Dutch cohort (6.7 v 12.0 years) is also a limitation, as some late recurrences may have been missed. A final limitation is that prognostic factors such as regression, lymphovascular invasion, and resection margin status were not incorporated in the models, because they were not adequately documented in the

Dutch data set. Although lymphovascular invasion is uncommon in patients with T1 melanomas, and the literature is inconsistent with regard to the impact of a positive deep margin on prognosis for thin melanomas, the inclusion of these three variables might have improved the performance of the models.

CONCLUSIONS

In conclusion, given the large number of patients who present with thin melanomas, the nomograms that we have developed provide a rational basis for more adequate staging with better selection for SN-biopsy and risk-based management of patients with thin melanomas. They will also assist in the planning of appropriate follow-up schedules and determining clinical trial eligibility and stratification. For ease of implementation in clinical practice, an online calculator able to be accessed readily on a smartphone, tablet, or computer has been developed and is freely available online.[34]

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SUPPLEMENTARY STATISTICAL METHODS

Dealing with missing values

Because of a relatively large number of missing values for ulceration and mitoses, a "not known" category was created for these two variables.[35] Multiple imputation was not considered, given that the pathologists involved in this study believe these histopathological parameters were not missing at random, but rather because they were not seen during pathological assessment. The missing at random assumption (a condition for multiple imputation) would therefore be inappropriate.[36] Since it has been suggested that a missing-indicator variable might lead to bias, a sensitivity analysis was performed, categorizing patients with a "not known" status for mitoses and ulceration as "not present" to assess the impact on HRs of other variables in the multivariable model.[37]

Linearity assumption of continuous variables

The test of deviance was used to assessed the linear relationship between continuous variables (age and Breslow thickness) and each outcome (LRFS, RRFS and DRFS)[38].

Testing the proportional hazards assumption

The proportional hazards assumption was evaluated using the Schoenfeld residuals test.

Additional calibration analysis

A sensitivity analysis was also performed using the bottom 10% and top 10% risk scores for the low-risk and high-risk groups, respectively. These score thresholds (derived from the development cohort) were then used to validate these groupings in the validation dataset and Kaplan-Meier curves were developed accordingly. The 10-year observed versus predicted LRFS, RRFS and DRFS per risk group was calculated. Observed 10-year probabilities were derived from the Kaplan-Meier curves, whilst predicted survival probabilities were calculated by averaging the individual's absolute survival obtained from each respective nomogram across each risk group. In addition, a plot of all the risk scores was generated for the development dataset as well as for the validation dataset to show the spread of the risk scores, as suggested by Royston et al.[18] The differences between models in terms of C-statistics were tested using the Wald test based on standard errors obtained with an estimate of the influence function, as described in detail in the appendix of Blanche et al.[39]

Nomograms predictive performance compared to AJCC 8th edition staging

To assess the incremental discriminative ability of each nomogram, its C-statistic was compared with that of a model including the parameters that are currently used by the AJCC for staging patients with thin melanomas (T-stage and SN-status).[14]

Baseline hazard function

We also described how the risk of recurrence (either local, regional or distant) per year changed during follow-up at baseline levels of covariate by drawing the baseline hazard functions.[40]

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Supplementary Table 1. TRIPOD Checklist: Prediction model development and validation 19.

Section/Topic	Ite		Checklist Item	Page
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	3,4
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	5
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	5
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	6
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	6,7
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centers.	6
	5b	D;V	Describe eligibility criteria for participants.	6,7
	5c	D;V	Give details of treatments received, if relevant.	NA
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	7
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	NA
Predictors	7a	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	6-8
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	NA
Sample size	8	D;V	Explain how the study size was arrived at.	6-8,40
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	32

Supplementary Table 1. (continued).

Section/Topic	Ite		Checklist Item	Page
	10a	D	Describe how predictors were handled in the analyses.	7,8,32,33
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	7,8,32,33
Statistical analysis methods	10c	V	For validation, describe how the predictions were calculated.	7,8,32,33
	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	7,8,32,33
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	NA
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	7,8
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	6,9,24,25
Results				
	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	9,24,25,40
Participants	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	9,24,25,40
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	9,24,25
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	9-11,24,25
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	26,27
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	26,27
	15b	D	Explain how to the use the prediction model.	29-31
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	10,28
Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	NA
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	15

Supplementary Table 1. (continued).

Section/Topic	Ite		Checklist Item	Page
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	12-14
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	12-16
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	12-16
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	4,29
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	17

*Items relevant only to the development of a prediction model are denoted by D, items relating solely to a validation of a prediction model are denoted by V, and items relating to both are denoted D;V.

Supplementary Table 2. Multivariable Cox regression for local, regional and distant RFS in patients with thin melanomas for the development (Dutch) cohort, with missing mitoses and ulceration status categorized as "Not present", (n=25,930).

		LRFS		RRFS		DRFS	
Variable	**Class**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Gender	Female vs male	NA	NA	1.43 (1.19, 1.71)	0.0002	1.50 (1.13, 2.00)	0.0053
SN status	Positive vs Negative	NA	NA	3.64 (1.89, 7.00)	0.0001	4.80 (2.12, 10.89)	0.0002
	Not performed vs Negative	NA	NA	1.50 (1.03, 2.19)	0.0348	1.00 (0.59, 1.70)	0.9900
Breslow thickness	Per mm	1.28 (1.20, 1.37)	<.0001	1.38 (1.31, 1.44)	<.0001	1.44 (1.33, 1.55)	<.0001
Age at diagnosis	Per year	1.04 (1.03, 1.05)	<.0001	NA	NA	NA	NA
Primary site	Trunk vs Head & Neck	0.62 (0.41, 0.95)	0.0288	0.93 (0.69, 1.26)	0.6493	0.51 (0.36, 0.73)	0.0002
	Upper limb vs Head & Neck	0.40 (0.23, 0.70)	0.0012	0.45 (0.30, 0.68)	0.0001	0.34 (0.21, 0.57)	<.0001
	Lower limb vs Head & Neck	0.73 (0.47, 1.13)	0.1557	0.74 (0.53, 1.02)	0.0657	0.20 (0.12, 0.33)	<.0001
Mitoses	Yes vs Not present	1.80 (1.31, 2.48)	0.0003	1.82 (1.49, 2.23)	<.0001	1.66 (1.20, 2.29)	0.0021
Ulceration	Yes vs Not present	2.78 (1.67, 4.62)	<.0001	2.23 (1.56, 3.19)	<.0001	2.72 (1.62, 4.56)	0.0001
Subtype	Nodular vs SSM	1.87 (1.06, 3.29)	0.0299	2.51 (1.83, 3.44)	<.0001	NA	NA
	Lentigo maligna vs SSM	1.62 (0.99, 2.67)	0.0569	0.61 (0.34, 1.09)	0.0936	NA	NA
	Acral lentiginous vs SSM	5.01 (1.82, 13.77)	0.0018	2.68 (0.99, 7.27)	0.0533	NA	NA
	Desmoplastic vs SSM	9.00 (1.23, 65.78)	0.0304	NA	0.9629	NA	NA

Supplementary Table 3. Observed and Predicted 10-year RFS rates for each risk group based on the observed and predicted survival curves using the development and validation cohort for local, regional and distant recurrence-free survival.

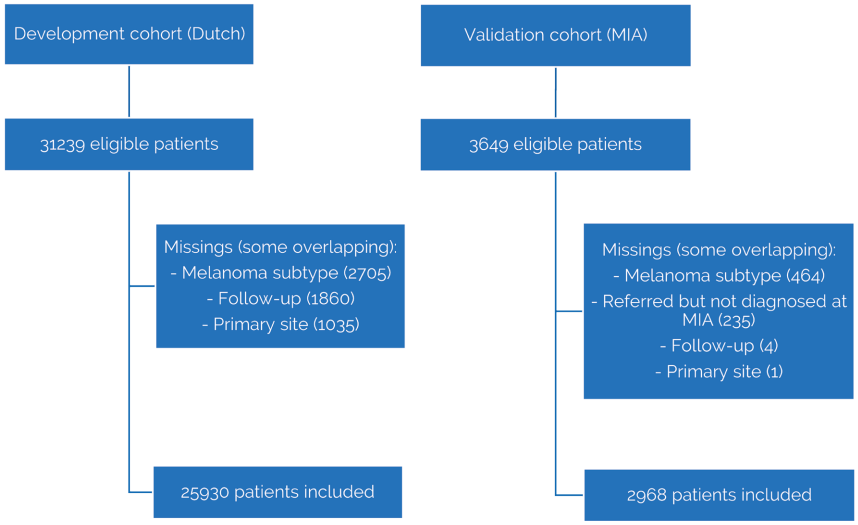
		Development cohort				Validation cohort			
Outcome	Risk group	N	Event	Observed probability (95% CI)	Predicted probability	N	Event	Observed probability (95% CI)	Predicted probability
	Bottom / top 5% of individual risk scores								
Local recurrence free survival	Low	1297	2	99.8 (99.6-100)	99.9	86	0	100 (100-100)	99.9
	Intermediate	23330	163	99.1 (98.9-99.2)	99.0	2547	10	99.6 (99.3-99.9)	98.8
	High	1297	44	94.6 (92.7-96.5)	93.1	335	13	96.0 (93.7-98.4)	91.1
	Bottom / top 10% of individual risk scores								
	Low	2593	3	99.9 (99.7-100)	99.8	181	1	99.4 (98.4-100)	99.8
	Intermediate	20739	118	99.2 (99.1-99.4)	99.1	2226	8	99.6 (99.4-99.9)	99
	High	2592	88	94.8 (93.5-96.0)	95	561	14	97.5 (96.1-98.9)	93.5
	Bottom / top 5% of individual risk scores								
Regional recurrence free survival	Low	1298	1	99.9 (99.8-100)	99.7	319	1	99.7 (99.1-100)	99.8
	Intermediate	23340	402	97.8 (97.5-98.0)	97.9	2353	38	98.4 (97.9-98.9)	97.8
	High	1292	100	89.7 (87.6-91.8)	87.8	296	22	92.1 (88.9-95.3)	87.5
	Bottom / top 10% of individual risk scores								
	Low	2633	2	99.9 (99.8-100)	99.6	487	1	99.8 (99.4-100)	99.7
	Intermediate	20705	324	98.0 (97.8-98.3)	98.1	2009	32	98.4 (97.8-99.0)	98
	High	2592	177	90.9 (89.5-92.3)	90.7	472	28	93.8 (91.6-96.1)	89.7
	Bottom / top 5% of individual risk scores								
Distant recurrence free survival	Low	1311	0	100 (100-100)	100	126	0	100 (100-100)	99.9
	Intermediate	23316	157	99.0 (98.9-99.2)	99.1	2562	54	97.8 (97.2-98.4)	99
	High	1297	46	95.0 (93.4-96.6)	93.3	280	21	92.3 (88.9-95.7)	92.7
	Bottom / top 10% of individual risk scores								
	Low	2688	0	100 (100-100)	99.9	257	0	100 (100-100)	99.9
	Intermediate	20637	124	99.1 (99.0-99.3)	99.2	2261	43	98.1 (97.4-98.7)	99.1
	High	2599	79	95.6 (94.6-96.7)	95.1	450	32	92.5 (89.9-95.2)	94.2

Observed probabilities (and their associated 95% CI) were based on the Kaplan-Meier method while predicted probabilities were calculated by averaging the individual absolute 10-year survival obtained from the relevant nomogram across each risk group. N and Event denote the number of patients at risk and number of recurrences at 10 years in each risk group.

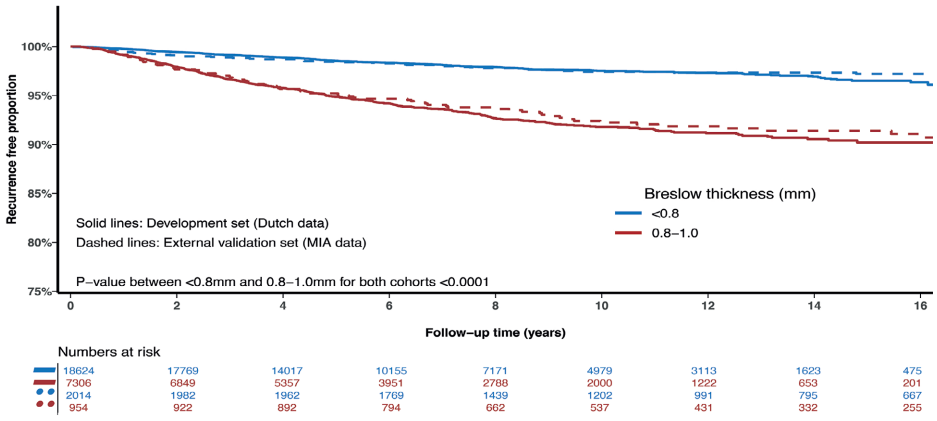
Supplementary Table 4. Clinicopathological characteristics of T1a patients with local, regional and distant recurrence, classified as high-risk by each nomogram using the 5% cut-off. The number of T1a patients with local, regional and distant recurrence who were categorized as high-risk were as follows: 81 (73 Dutch, 8 MIA), 183 (161 Dutch, 22 MIA) and 81 (53 Dutch, 28 MIA), respectively.

Characteristic	T1a with local recurrence (N=81)	T1a with regional recurrence (N=183)	T1a with distant recurrence (N=81)
Risk group (5% cut-off)			
Low	2 (2.5)	2 (1.1)	0 (0.0)
Intermediate	73 (90.1)	175 (95.6)	80 (98.8)
High	6 (7.4)	6 (3.3)	1 (1.2)
Risk group (10% cut-off)			
Low	4 (4.9)	2 (1.1)	0 (0.0)
Intermediate	58 (71.6)	166 (90.7)	78 (96.3)
High	19 (23.5)	15 (8.2)	3 (3.7)
Gender (n (%))			
Female	52 (64.2)	89 (48.6)	28 (34.6)
Male	29 (35.8)	94 (51.4)	53 (65.4)
Median age at diagnosis in years (range)	66.0 (22-87)	52.0 (21-85)	55.0 (23-83)
Primary site (n (%))			
Head & Neck	26 (32.1)	27 (14.8)	17 (21.0)
Trunk	24 (29.6)	110 (60.1)	48 (59.3)
Upper limb	9 (8.8)	16 (8.7)	8 (9.9)
Lower limb	23 (28.4)	30 (16.4)	8 (9.9)
Median Breslow thickness in mm (range)	0.6 (0.2-0.7)	0.6 (0.1-0.7)	0.6 (0.2-0.7)
Mitoses (n (%))			
Not present	30 (37.0)	43 (23.5)	24 (29.6)
Yes	22 (27.2)	49 (26.8)	29 (35.8)
Not known	29 (35.8)	91 (49.7)	28 (34.6)
Subtype (n (%))			
Superficial spreading	60 (74.1)	166 (90.7)	71 (87.7)
Nodular	2 (2.5)	10 (5.5)	3 (3.7)
Lentigo maligna	17 (21.0)	6 (3.3)	6 (7.4)
Acral lentiginous	2 (2.5)	1 (0.5)	0 (0.0)
Desmoplastic	0 (0.0)	0 (0.0)	1 (1.2)
SN status (n (%))			
Negative	4 (4.9)	3 (1.6)	1 (1.2)
Positive	0 (0.0)	6 (3.3)	0 (0.0)
Not performed	77 (95.1)	174 (95.1)	80 (98.8)

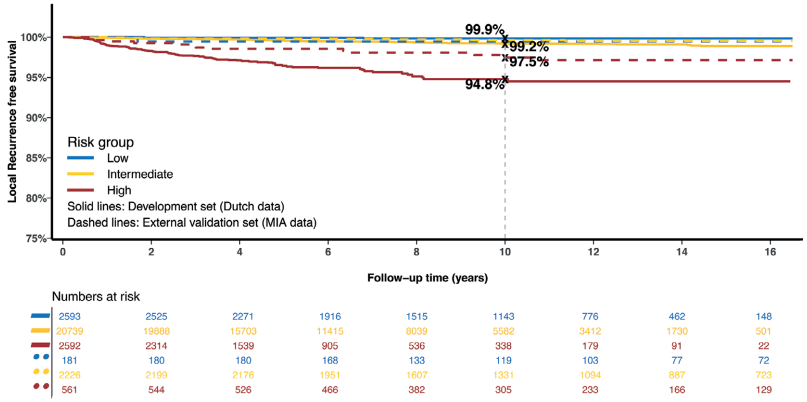
Supplementary Figure 1. Flowchart of patient selection for the development and validation cohorts.

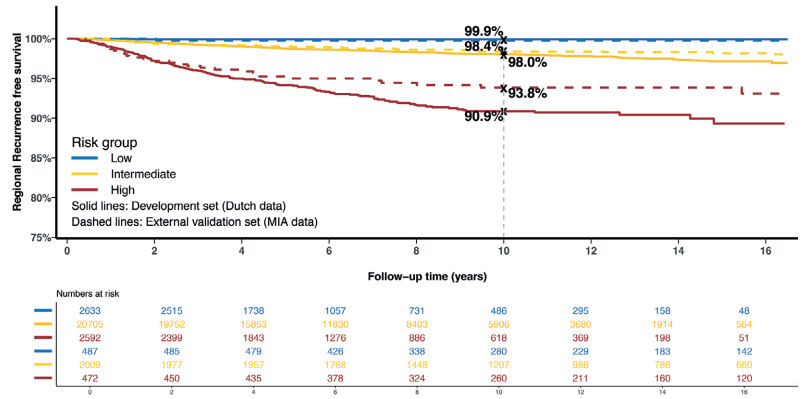


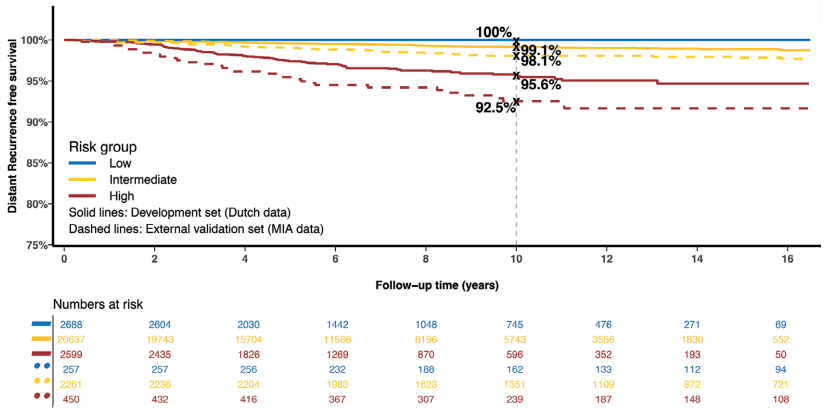
Supplementary Figure 2. Kaplan-Meier curves for RFS according to Breslow thickness in thin melanoma patients in the development set (Dutch data) and the validation set (MIA data).



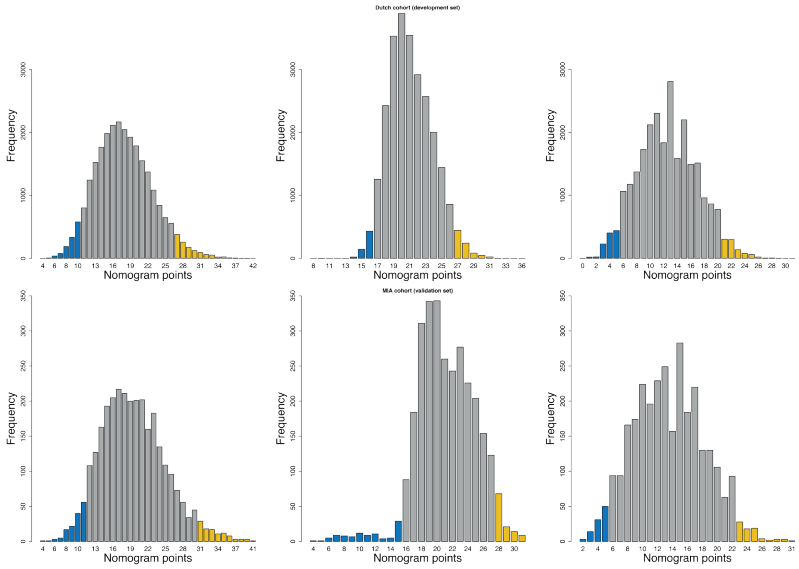
Supplementary Figure 3. Kaplan-Meier curves for local, regional and distant RFS per risk group with corresponding 10-year RFS estimates. The low-risk group includes the bottom 10% of patients (with the lowest total nomogram points), the high-risk group includes patients with the top 10% of total points, and the remaining patients constitute the intermediate group.



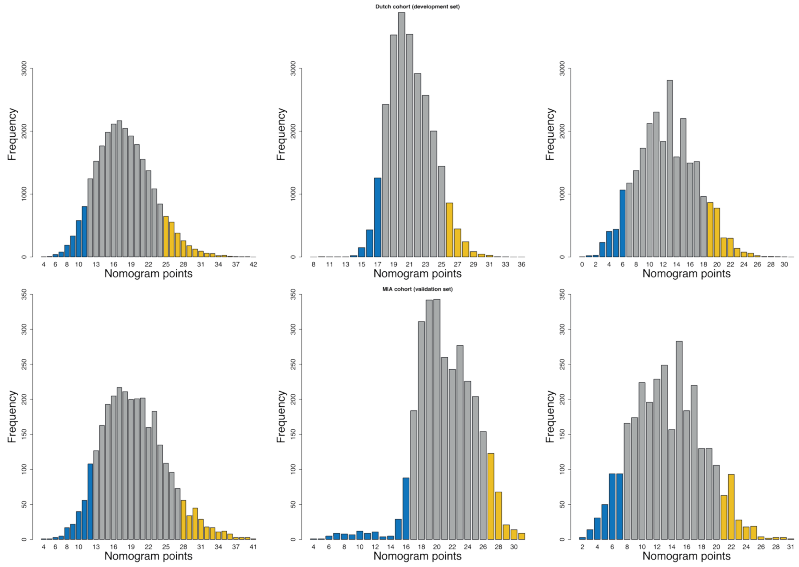




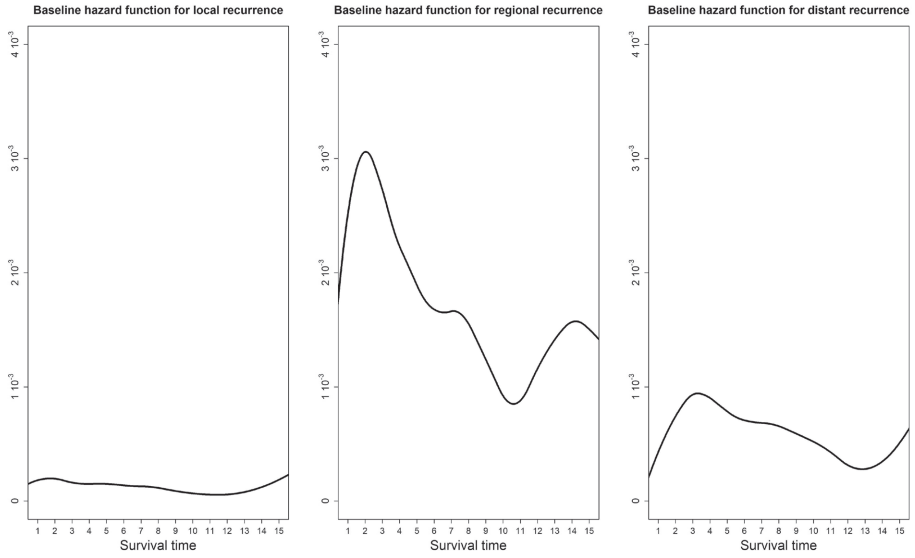
Supplementary Figure 4. Histogram of spread of number of points for the Dutch (development) and MIA (validation) cohorts as computed by each nomogram, using the bottom 5% and top 5% risk scores. The left, middle and right panels show the distribution of scores for the local, regional and distant recurrence nomograms, respectively.

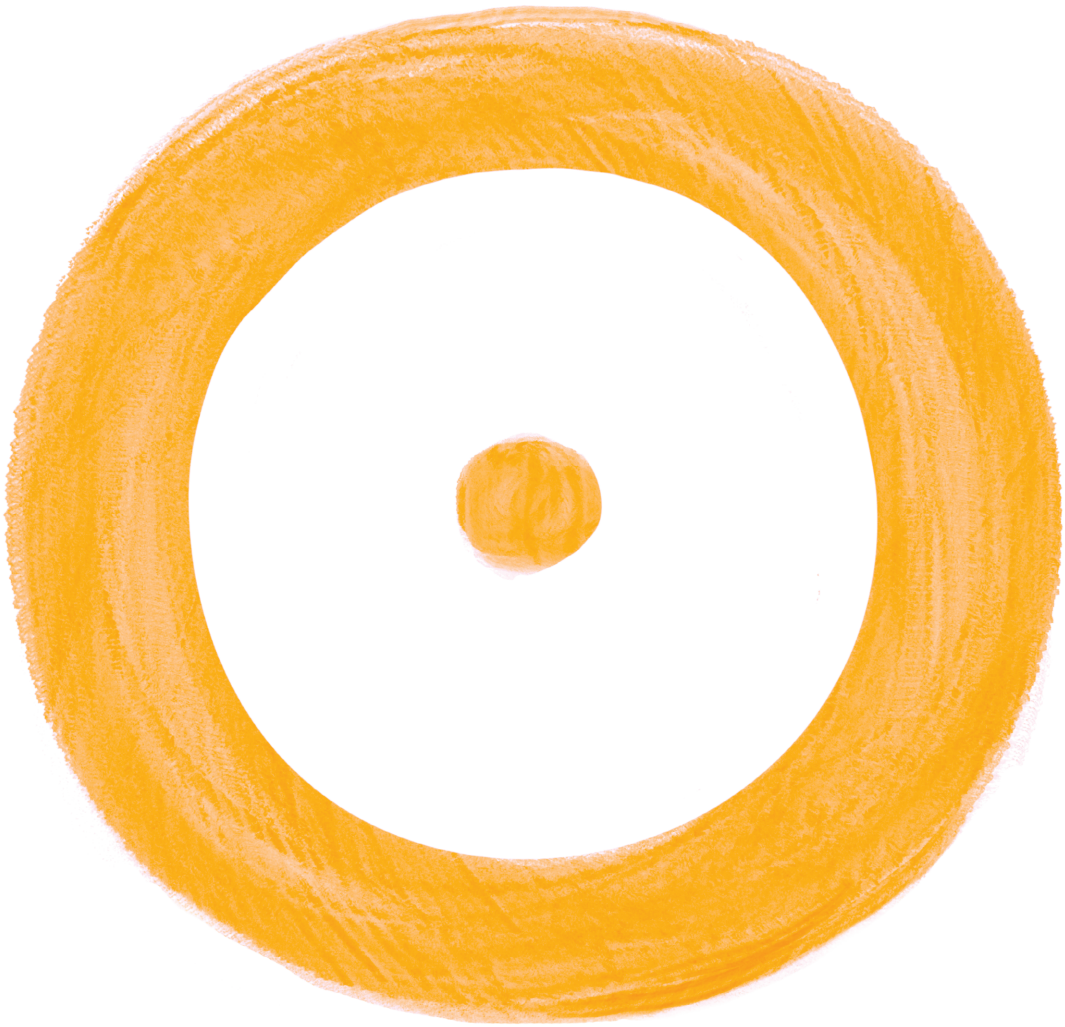


Supplementary Figure 5. Histogram of spread of number of points for the Dutch (development) and MIA (validation) cohorts as computed by each nomogram, using the bottom 10% and top 10% risk scores. The left, middle and right panels show the distribution of scores for the local, regional and distant recurrence nomograms, respectively.



Supplementary Figure 6. Baseline hazard function using maximum penalized likelihood method with penalty function to smooth the estimates for local, regional and distant recurrences in the Dutch cohort.





CHAPTER 15

Predicting recurrence in patients with sentinel node-negative melanoma: validation of the EORTC nomogram using population-based data

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ABSTRACT

Background: Identifying patients with sentinel node (SN)-negative melanoma who are at greatest risk of recurrence is important. The European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group proposed a prognostic model that has not been validated in population-based data. The EORTC nomogram includes Breslow thickness, ulceration status and anatomical location as parameters. The aim of this study was to validate the EORTC model externally using a large national data set.

Methods: Adults with histologically proven, invasive cutaneous melanoma with a negative SN biopsy in the Netherlands between 2000 and 2014 were identified from the Dutch Pathology Registry, and relevant data were extracted. The EORTC nomogram was used to predict recurrence-free survival. The predictive performance of the nomogram was assessed by discrimination (C-statistic) and calibration.

Results: A total of 8795 patients met the eligibility criteria, of whom 14·7 per cent subsequently developed metastatic disease. Of these recurrences, 20·9 per cent occurred after the first 5 years of follow-up. Validation of the EORTC nomogram showed a C-statistic of 0·70 (95 per cent c.i. 0·68 to 0·71) for recurrence-free survival, with excellent calibration (R2 = 0·99; P = 0·999, Hosmer–Lemeshow test).

Conclusions: This population-based validation confirmed the value of the EORTC nomogram in predicting recurrence-free survival in patients with SN-negative melanoma. The EORTC nomogram could be used in clinical practice for personalizing follow-up and selecting high-risk patients for trials of adjuvant systemic therapy.

INTRODUCTION

Sentinel node (SN) status is an important predictor of survival and has become part of the standard staging process for patients with melanoma[1]. Overall, the SN positivity rate ranges from 16 to 27 per cent[2,3], and so the majority of patients are SN-negative with a much better prognosis. A negative SN biopsy does not, however, mean that the disease will not recur. As new adjuvant systemic treatments are now available for patients with AJCC stage III melanoma[4] and being evaluated in those with SN-negative stage II disease, it is important to identify patients with SN-negative melanoma who are at greatest risk of developing metastatic disease.

A recent model and nomogram for predicting SN-negative melanoma recurrence was developed using data from four EORTC Melanoma Group centres[5]. It was successfully validated externally using data from Melanoma Institute Australia (MIA)[6]. A major strength of the EORTC nomogram is its simplicity, as it includes only three prognostic factors related to the primary tumour: presence of ulceration, anatomical location and Breslow thickness. No population-based data have, however, been used to validate the EORTC nomogram. The aim of this study was to determine its generalizability for use in clinical practice by external validation in a Dutch national data set.

METHODS

Data for this retrospective nationwide cohort study were obtained from PALGA, the Dutch Nationwide Network and Registry of Histopathology and Cytopathology. Since 1991, PALGA has been collecting data prospectively from all pathology laboratories in the Netherlands[7]. All data were encoded and used anonymously. Ethical approval was granted by the board of PALGA.

Study population

The pathology reports of all patients newly diagnosed with invasive melanoma in the Netherlands between 1 January 2000 and 31 December 2014 were analysed, and patients with a negative SN biopsy were selected. Patients with locoregional or distant metastases within 6 weeks of diagnosis (stage III and IV), those aged less than18 years and those with multiple primary melanomas were excluded. Patients whose melanoma exceeded 10 mm in Breslow thickness were included in the 10-mm category. SN biopsy was performed according to the Dutch Melanoma Guidelines[8], which from 2005 advised SN biopsy for patients with melanomas thicker than 1·0 mm who wished to be optimally informed about their prognosis, and from 2010 to 2014 also for patients with melanomas of 1·0 mm or less with ulceration and/or a mitotic rate of at least 1/mm2. A SN was defined as any lymph node receiving lymphatic drainage directly from the primary tumour site[9].

Information collected included: date of diagnosis, age at time of diagnosis, sex, Breslow thickness, presence of ulceration (yes or no), presence of mitoses (yes or no), melanoma subtype (superficial spreading, nodular, lentigo maligna, acral or desmoplastic), Clark level (II–V), anatomical site (head and neck, trunk, arms, legs) and recurrence (date, site and type). The outcome of interest was time to first recurrence; in patients with multisite first recurrences, the site with the worst prognosis was scored as the first site. Recurrence-free survival (RFS) was calculated from the date of diagnosis to the date of recurrence or death from any cause. Censoring occurred at the end of follow-up. Follow-up was available until date of death, the date last known alive or 1 January 2018, whichever occurred earlier.

Statistical analysis

Categorical variables are presented as numbers with percentages, and continuous variables as median (i.q.r.). To compare the Dutch cohort with the EORTC cohort, univariable analysis was done using the $\chi 2$ test. The EORTC tool was used to calculate the probability of recurrence for each patient in the Dutch data set. The EORTC model was validated by estimating its discrimination and calibration. Discrimination was assessed by calculating the area under the curve, also known as the C-statistic. It reflects improved classification, that is how well the model identifies patients with a recurrence[10]. For each patient in the cohort, a risk score was calculated using the

EORTC nomogram, which categorized patients as having a low risk of recurrence (score 0–6), an intermediate risk (score 7–9) or a high risk (score 10 or more). Kaplan–Meier curves were produced accordingly. To assess calibration, observed and predicted risks of recurrence were compared and plotted against each other. R2 was calculated as a measure of how closely the data fitted the regression line. The goodness of fit of the model was also tested using the Hosmer–Lemeshow test. Two-sided P<0·050 was considered significant. All statistical analyses were done using R version 3.6.1 (R Core Team, Vienna, Austria).

RESULTS

Baseline characteristics of 8795 Dutch patients with SN-negative melanoma and 3180 patients in the original EORTC cohort[5] are shown in Table 1.

Table 1. Characteristics of Dutch and EORTC patient cohorts.

	Dutch cohort (*n* = 8795)	EORTC cohort[5] (*n* = 3180)	*P*†
Age at diagnosis (years)*	55 (44–65)	55 (44–67)	–
Sex			0.139
F	4719 (53.7)	1668 (52.5)	
M	4076 (46.3)	1510 (47.5)	
Missing	0 (0)	2 (0.1)	
Anatomical site			< 0.001
Head and neck	534 (6.1)	259 (8.1)	
Trunk	3676 (41.8)	1360 (42.8)	
Arm	1420 (16.1)	556 (17.5)	
Leg	2915 (33.1)	996 (31.3)	
Missing	250 (2.8)	9 (0.3)	
Breslow thickness (mm)*	1.6 (1.2–2.4)	1.7 (1.1–3.0)	–
Melanoma subtype			< 0.001
Superficial spreading	5597 (63.6)	1739 (54.7)	
Nodular	1929 (21.9)	885 (27.8)	
Lentigo maligna	99 (1.1)	139 (4.4)	
Acral lentiginous	116 (1.3)	93 (2.9)	
Other	54 (0.6)	46 (1.4)	
Missing	1000 (11.4)	278 (8.7)	
Clark level			< 0.001
II	363 (4.1)	271 (8.5)	
III	2318 (26.4)	1230 (38.7)	
IV	3408 (38.7)	1354 (42.6)	
V	224 (2.5)	140 (4.4)	
Missing	2482 (28.2)	185 (5.8)	

Table 1. (continued).

	Dutch cohort (*n* = 8795)	EORTC cohort[5] (*n* = 3180)	*P*ǂ
Ulceration			< 0.001
No	5756 (65.4)	2264 (71.2)	
Yes	1774 (20.2)	788 (24.8)	
Missing	1265 (14.4)	128 (4.0)	
Mitosis			< 0.001
No	738 (8.4)	39 (1.2)	
Yes	4828 (54.9)	112 (3.5)	
Missing	3229 (36.7)	3029 (95.3)	

Values in parentheses are percentages unless indicated otherwise;
*values are median (i.q.r.).
ǂχ2 test; P could not be determined for continuous variables as the authors had no access to raw data for the EORTC cohort.

Recurrence

In the Dutch cohort, with a median duration of follow-up of 6·0 (i.q.r. 3·7–10·2) years, 1297 (14·7 per cent) of the 8795 patients developed metastases. Locoregional recurrence occurred in 973 of these patients (75·0 per cent) and distant recurrence in 324 (25·0 per cent). The median time to first recurrence was 2·7 (i.q.r. 1·4–4·6) years. Metastases occurred after 5 years in 271 patients (20·9 per cent), and after 10 years in 53 (4·1 per cent). The RFS rates at 5 and 10 years were 85·9 (95 per cent c.i. 85·1 to 86·7) and 80·1 (79·1 to 81·3) per cent respectively.

External validation: discrimination and validation

The C-statistic of the externally validated EORTC model was 0·70 (95 per cent c.i. 0·68 to 0·71) for RFS. Fig. 1 shows Kaplan–Meier curves for the low-, intermediate- and high-risk groups for recurrence following a negative SN biopsy. The calibration plot indicated that the EORTC nomogram was well calibrated in the Dutch data, with an excellent linear correlation between predicted and observed probabilities of SN positivity (R2 = 0·99) (Fig. 2). Furthermore, the P value for the Hosmer–Lemeshow test was 0·999, confirming the excellent agreement between observed and predicted probabilities overall and within subgroups of participants.

Figure 1. Kaplan-Meier plots of recurrence-free survival for groups at low, intermediate and high risk of recurrence after a negative sentinel node biopsy according to the EORTC nomogram applied to Dutch patients with sentinel node-negative melanoma.

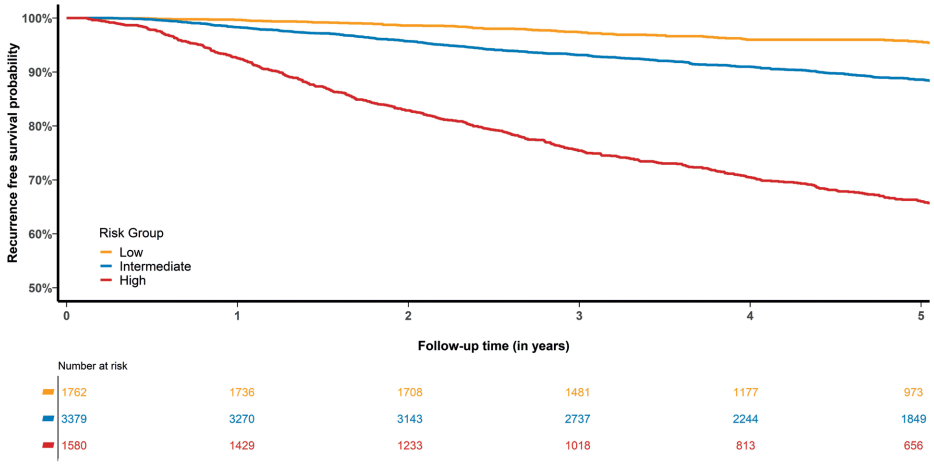
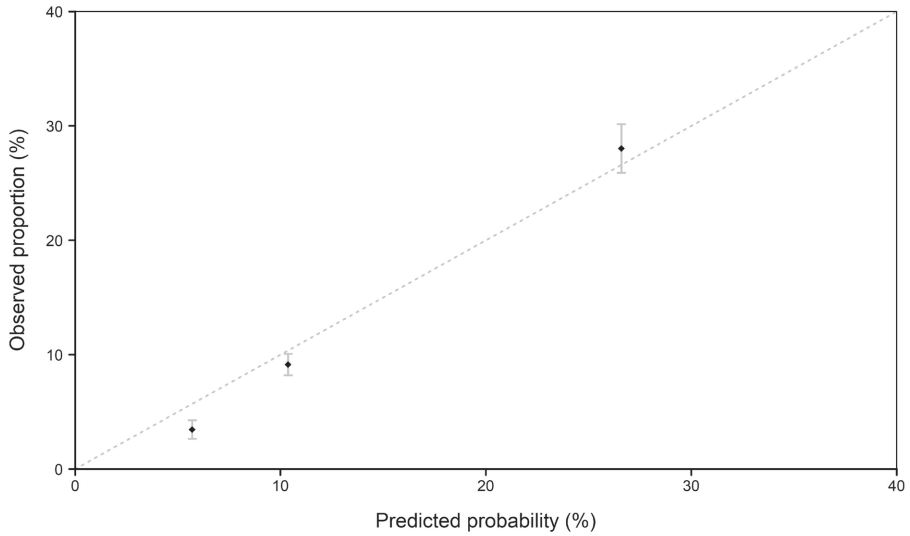


Figure 2. Calibration plot for the EORTC model in predicting recurrence in Dutch patients with sentinel node-negative melanoma.



DISCUSSION

This large, population-based study validated the EORTC model for RFS in patients with SN-negative melanoma. Model performance in the Dutch population-based cohort, as reflected by the C-statistic, was 0·70 (95 per cent c.i. 0·68 to 0·71). This compares well with the C-statistic for recurrence in both the original EORTC model (0·74, 0·71 to 0·76) and the MIA validation cohort (0·69, 0·67 to 0·71)[6]. It demonstrates that the relatively simple EORTC nomogram provides a good prediction of outcome in population-based data.

The EORTC model contains only three parameters: Breslow thickness, ulceration status and anatomical location. Using MIA data, Ipenburg and colleagues[6] sought to improve the model by adding several other prognostic factors. When sex, age, melanoma subtype and tumour mitotic rate were included, the C-statistic increased from 0·69 to 0·71 (95 per cent c.i. 0·68 to 0·74), which led the authors to conclude that the addition of more prognostic factors only marginally improved the model. The recurrence rate of 14·7 per cent in the present cohort of Dutch patients with SN-negative disease is in line with previously reported rates of 14·6–28·6 per cent in studies with at least 5 years of follow-up[5,11–14]. Only 79·1 per cent of recurrences in the present study occurred during the first 5 years of follow-up, whereas 20·9 per cent occurred after 5 years, which is in line with previously published data[6]. Identifying these patients is important, yet some guidelines recommend discontinuing routine follow-up after 5 years[8,15].

This population-based validation of a nomogram for predicting outcome in patients with SN-negative melanoma provides evidence of the generalizability of the EORTC model. The present study, however, has several limitations. There were some missing data, although this was the case in only 14·4 per cent of the cohort and mainly related to missing ulceration status. Furthermore, development of the EORTC model was initially based on patient data from four European centres, two of which were in the Netherlands. Therefore, some of these patients are also included in the current validation set. As deidentified data were used, these patients could not be excluded from the analyses. Given the large number of patients in the data set, it appears unlikely that this would have significantly altered the results as the two Dutch EORTC centres included only 1145 patients in total. In addition, the data used in the present study were population- rather than institution-based. A final limitation of the study is that the Dutch Cancer Registry does not have access to cause of death and thus melanoma-specific survival could not be investigated.

CONCLUSIONS

This population-based validation study has confirmed the value of the EORTC nomogram in predicting relapse-free survival in patients with SN-negative melanoma. The EORTC nomogram could be used in clinical practice to personalize follow-up and to select high-risk patients with SN-negative disease for trials of adjuvant systemic therapy.

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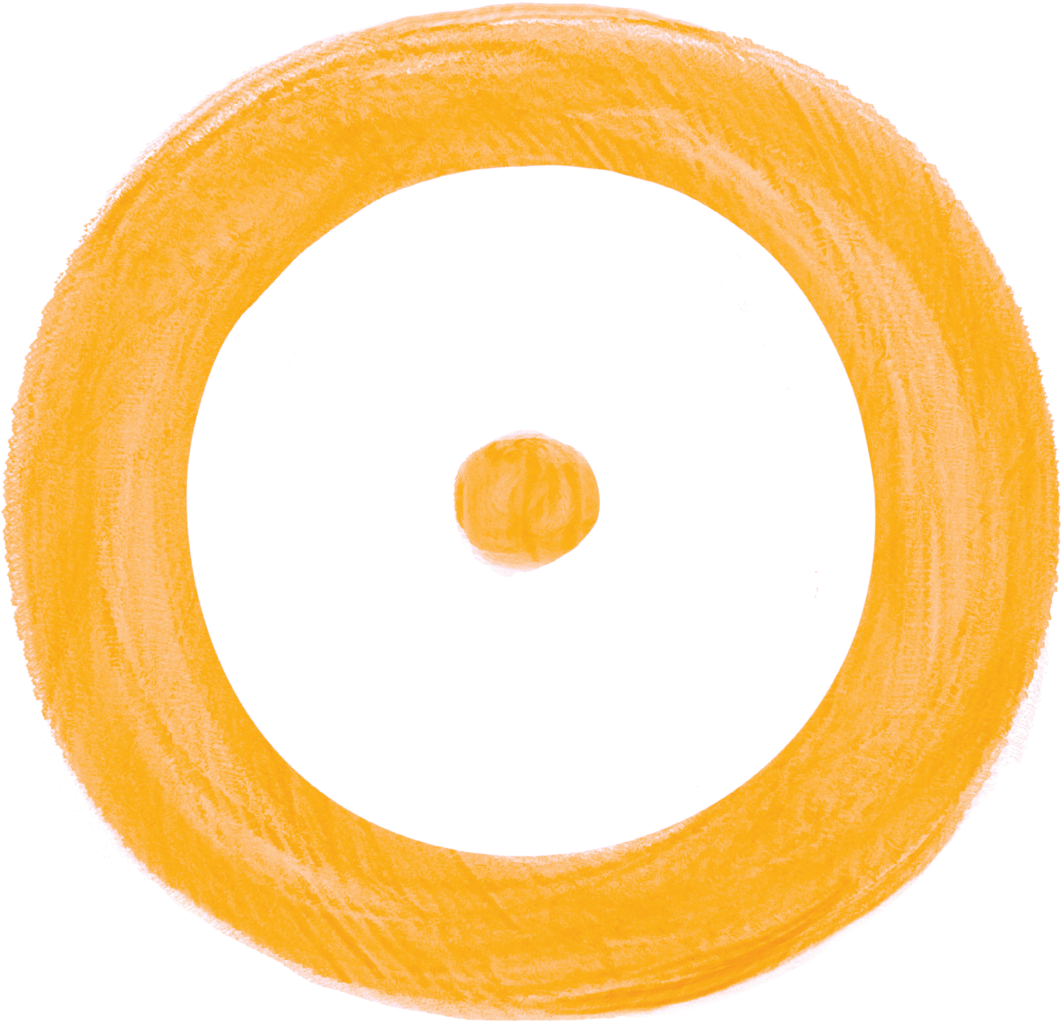

CHAPTER 16

Predicting sentinel node positivity in patients with melanoma: external validation of a risk-prediction calculator (the Melanoma Institute Australia nomogram) using a large European population-based patient cohort

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ABSTRACT

Background: A nomogram to predict sentinel node (SN) positivity [the Melanoma Institute Australia (MIA) nomogram] was recently developed and externally validated using two large single-institution databases. However, there remains a need to further validate the nomogram's performance using population-based data.

Objectives: To perform further validation of the nomogram using a European national patient cohort.

Methods: Patients with cutaneous melanoma who underwent SN biopsy in the Netherlands between 2000 and 2014 were included. Their data were obtained from the Dutch Pathology Registry. The predictive performance of the nomogram was assessed by discrimination (C-statistic) and calibration. Negative predictive values (NPVs) were calculated at various predicted probability cutoffs.

Results: Of the 3049 patients who met the eligibility criteria, 23% (691) were SN positive. Validation of the MIA nomogram (including the parameters Breslow thickness, ulceration, age, melanoma subtype and lymphovascular invasion) showed a good C-statistic of 0·69 (95% confidence interval 0·66–0·71) with excellent calibration ($R2 = 0·985$, $P = 0·40$). The NPV of 90·1%, found at a 10% predicted probability cutoff for having a positive SN biopsy, implied that by using the nomogram, a 16·3% reduction in the rate of performing an SN biopsy could be achieved with an error rate of 1·6%. Validation of the MIA nomogram considering mitotic rate as present or absent showed a C-statistic of 0·70 (95% confidence interval 0·68–0·74).

Conclusions: This population-based validation study in European patients with melanoma confirmed the value of the MIA nomogram in predicting SN positivity. Its use will spare low-risk patients the inconvenience, cost and potential risks of SN biopsy while ensuring that high-risk patients are still identified.

INTRODUCTION

Sentinel node (SN) status is an important predictor of survival outcome in patients with melanoma.[1] Together with ulceration status and Breslow thickness, it is used for staging according to the 8th edition of the American Joint Committee on Cancer staging manual.[2] The indications for SN biopsy have been well defined in national and international melanoma management guidelines, with overall SN positivity ranging from 16% to 27% in large series.[3, 4] Although SN biopsy is a minimally invasive procedure, it can sometimes lead to complications such as infection, seroma and lymphoedema.[5] Consequently, there is the need for a more tailored approach to selecting patients for SN biopsy, to ensure that those most likely to be SN positive undergo the procedure, while those most likely to be SN negative are not.

Various prediction models have been proposed to help improve patient selection and identify those unlikely to be SN positive who could reasonably forgo SN biopsy, reducing both morbidity and costs. The recently published Melanoma Institute Australia (MIA) nomogram for predicting SN status[6] (available at www.melanomarisk.org.au) has been shown to be more accurate than the previously published online calculator of risk of SN positivity that was developed at the Memorial Sloan Kettering Cancer Center more than 15 years ago.[7] The improvement afforded by the new nomogram was achieved by replacing body site and Clark level with mitotic rate, melanoma subtype and lymphovascular invasion status. It was externally validated using data from the MD Anderson Cancer Center.[6] However, patients from two large, tertiary referral institutions may not be representative of general melanoma populations. Therefore, a need to further validate the MIA nomogram using population-based data was identified. Hence, the aim of this study was to externally validate the MIA nomogram using a nationwide population-based dataset from a third continent and to evaluate the potential reduction in the rate of SN biopsy if the nomogram was utilized to assist decision making by clinicians and patients in relation to the use of this procedure.

METHODS

Collection of data

Data for this retrospective study were obtained from the Dutch Pathology Registry (PALGA). Since 1991, this registry has prospectively collected comprehensive nationwide information for all cases of primary cutaneous melanoma diagnosed in the Netherlands, through mandatory notifications from all pathology laboratories in the country.[8] The PALGA data were encoded and used anonymously. Ethical approval for the use of the data was granted by the board of PALGA.

Study population

For this cohort study, the pathology reports of all patients ≥ 18 years of age with invasive melanomas diagnosed in the Netherlands between 1 January 2000 and 31 December 2014 and who underwent SN biopsy were analysed. The eligibility criteria were the same as those utilized in the study for the development of the MIA nomogram.[6] Included melanoma subtypes were superficial spreading melanoma (SSM), nodular melanoma, lentigo maligna melanoma, acral lentiginous melanoma and pure desmoplastic melanoma. Unclassified melanomas and other subtypes were excluded.

For each patient, the following information was collected: date of diagnosis, age at time of diagnosis, gender, Breslow thickness, presence of ulceration (yes or no), presence of mitoses (yes or no), melanoma subtype, presence of lymphovascular invasion (yes or no), site of the primary melanoma (head and neck, trunk or extremity) and SN status (positive or negative). Only patients with data on all predefined variables available were selected.

Statistical analysis

Categorical variables were presented as numbers and percentages. Continuous variables were presented as medians with ranges. In order to compare the Dutch cohort with the MIA cohort, univariate parameters were analysed using the $\chi 2$-test or the Mann–Whitney U-test.

The MIA nomogram was used to calculate the probability of SN positivity for each patient in the Dutch dataset. As the Dutch cohort did not include the exact mitotic rate count and this was an optional parameter in the MIA nomogram, we primarily validated the MIA nomogram without mitotic rate. The MIA model was externally validated by estimating its discrimination and calibration.[9, 10] Discrimination was assessed by plotting a receiver operating characteristics curve, based on the estimated individual probability of the patient being SN positive, and calculating the area under the curve, also known as the C-statistic.

To assess calibration, observed and predicted risks for SN positivity were plotted against each other for all individuals from the Dutch cohort, and the logistic calibration line was calculated. In a perfect calibration, the intercept and the calibration slope are 0 and 1, respectively.[11]

To assess clinical relevance, the negative predictive value (NPV) was calculated, along with sensitivity, specificity, SN reduction rate and error rate. This was done for different minimum predicted probability cutoffs of SN positivity. The 10% cutoff was used as the main outcome, as this is the level above which the National Comprehensive Cancer Network (NCCN) recommends that SN biopsy be offered routinely (with 'consideration' of the procedure if the estimated risk is 5–10%).[12] Cases were defined as true positive (TP, when SN positivity was predicted by the nomogram and the SN was indeed positive), true negative (TN, when SN negativity was predicted by the nomogram and the SN was indeed negative), false positive (FP, when SN positivity was predicted by the nomogram but the SN was negative) or false negative (FN, when SN negativity was predicted by the nomogram but the SN was positive). NPV was calculated as [TN / (TN + FN)], sensitivity as [TP / (TP + FN)] and specificity as [TN / (FP + TN)]. The SN reduction rate was calculated as [(TN + FN) / (TN + FN + TP + FP)] and indicates the percentage of patients classified as negative by the nomogram and thus selected to forgo a SN biopsy. The error rate was calculated as [FN / (TP + TN + FP + FN)], indicating the percentage of patients incorrectly predicted to be negative by the nomogram, although they did have a positive SN biopsy.

An additional sensitivity analysis was performed to investigate the performance of the MIA nomogram if mitotic rate was coded as absent or present, and was externally validated by estimating its discrimination and calibration using the Dutch cohort.

All statistical analyses were performed using R version 3.6.1 (R Core Team, Vienna, Austria). Two-sided p-values < 0·05 were considered statistically significant.

RESULTS

Between 2000 and 2014, 38% of patients with melanoma in the PALGA registry were eligible for SN biopsy and underwent the procedure. Of these 12 181 patients, 3049 had no missing pathological information required to meet the study eligibility criteria (Figure S1; see Supporting Information).

Comparison of cohorts

An overview of the clinicopathological variables for the two cohorts is given in Table 1. There was no statistically significant difference in the SN positivity rate for the Dutch and the MIA cohorts (22·7% vs. 21·0%, P = 0·09). In total, 49·5% of Dutch patients were female, compared with 39·6% of the MIA patients. Although SSM was the most common melanoma subtype in both cohorts, followed by nodular melanoma, SSM was significantly more frequent in the Dutch cohort (P < 0·001). Mitoses were present in 93·9% of MIA patients, compared with 70·5% of Dutch patients (P < 0·001). Ulceration was present in 29·6% of MIA patients and in 26·8% of Dutch patients (P = 0·01). The most common anatomical location of melanoma in both cohorts was on an extremity, followed by trunk, and head and neck; however, the head and neck site was almost three times more common in the MIA population (15·4%) than in the Dutch cohort (5·5%, P < 0·001).

Clinicopathological features for patients who were SN positive and SN negative are detailed in Table 2. As expected, SN-positive patients were more likely to have melanomas that had a greater Breslow thickness, were ulcerated and had lymphovascular invasion. Melanoma subtype was also statistically significantly different for SN-positive and SN-negative patients. SN-positive patients were significantly younger than the SN-negative patients in the MIA dataset only.

Table 1. Patient and melanoma characteristics in the Melanoma Institute Australia (MIA) and Dutch datasets.

Characteristic	MIA (n=3477) Development set	Dutch (n=3049) Validation set	p-value
SN, positive (n (%))	729 (21.0)	691 (22.7)	0.09
Gender, female (n (%))	1377 (39.6)	1510 (49.5)	<0.001
Age at diagnosis in years (median (range))	59 (18 - 102)	56 (18-92)	<0.001
Breslow thickness in mm (median (range))	2.0 (0.4 - 47.0)	1.8 (0.1-32.0)	<0.001
Breslow thickness in mm (n (%))	376 (10.8)	370 (12.1)	<0.001
≤1.0	1478 (42.5)	1427 (46.8)	
1.1-2.0	1118 (32.2)	922 (30.2)	
2.1-4.0	505 (14.5)	330 (10.8)	
>4.0			
Body site (n (%))	536 (15.4)	168 (5.5)	<0.001
Head and neck	1395 (40.1)	1398 (45.9)	
Trunk	1546 (44.5)	1409 (46.2)	
Extremities	0 (0)	74 (2.4)	
Missing			
Ulceration, present (n (%))	1030 (29.6)	816 (26.8)	0.01
Mitoses, present (n (%))	3266 (93.9)	2151 (70.5)	<0.001
Subtype (n (%))	1870 (53.8)	2177 (71.4)	<0.001
Superficial spreading melanoma	1370 (39.4)	816 (26.8)	
Nodular melanoma	94 (2.7)	22 (0.7)	
Lentigo maligna melanoma	93 (2.7)	31 (1.0)	
Acral lentiginous melanoma	50 (1.4)	3 (0.1)	
Desmoplastic melanoma			
Lymphovascular invasion, present (n (%))	201 (5.8)	184 (6.0)	0.66

Table 2. Melanoma Institute Australia (MIA) and Dutch melanoma dataset characteristics, stratified by sentinel node (SN) status.

	MIA (n=3477) Original set			Dutch (n=3049) Validation set		
Characteristic	**SN + (n=729)**	**SN – (n=2748)**	**p-value**	**SN + (n=691)**	**SN – (n=2358)**	**p-value**
Age at diagnosis in years (median (range))	56 (18 – 93)	60 (18 – 102)	<0.001	55 (19 – 88)	55 (18 – 92)	0.533
Breslow thickness in mm (median (range))	2.6 (0.6 – 13.0)	1.8 (0.4 – 47.0)	<0.001	3.1 (0.6 – 20.0)	2.1 (0.1 – 32.0)	<0.001
Ulceration, present (N (%))	301 (41.3)	729 (26.5)	<0.001	264 (38.2)	552 (23.4)	<0.001
Lymphovascular invasion, present (N (%))	118 (16.2)	83 (3.0)	<0.001	86 (12.4)	98 (4.2)	<0.001
Subtype (N (%))			<0.001			<0.001
Superficial spreading	386 (52.9)	1484 (54.0)		438 (63.4)	1739 (73.7)	
Nodular	290 (39.8)	1080 (39.3)		245 (35.5)	571 (24.2)	
Lentigo maligna	9 (1.2)	85 (3.1)		0 (0)	22 (0.9)	
Acral lentiginous	45 (5.9)	50 (1.8)		8 (1.2)	23 (1.0)	
Desmoplastic	1 (0.1)	49 (1.8)		0 (0)	3 (0.1)	

External validation: discrimination

Mitotic rate data were not included in the Dutch dataset, therefore we validated the MIA nomogram without including mitotic rate (which was an optional parameter for estimating SN positivity in the MIA nomogram). The reduced MIA nomogram included Breslow thickness, ulceration status, age, melanoma subtype and lymphovascular invasion. The C-statistic for the reduced MIA nomogram was 0·74 [95% confidence interval (CI) 0·72–0·76] using the MIA dataset (development set) and 0·69 (95% CI 0·66–0·71) using the Dutch dataset (validation set) (Figure 1).

External validation: calibration

The calibration plot indicated that the nomogram was well calibrated in the Dutch data, with a good linear correlation between predicted and observed probabilities of SN positivity (Figure 2). There was good agreement between observed and predicted probabilities both overall and within subgroups of participants.

Clinical relevance

Table 3 shows different NPVs for different nomogram-predicted probability cutoff values. Using a 10% predicted probability cutoff of being SN positive, SN biopsy would not have been offered to 497 (16·3%) of 3049 Dutch patients, giving an NPV of 90·1%. Using this threshold, a total of 48 patients (1·6%) would have been incorrectly classified as SN negative. This would have resulted in an overall increase in the SN-positivity rate from 22·7% to 27·3% in the Dutch population.

Sensitivity analysis

The MIA nomogram using mitotic rate as present or absent provided a C-statistic of 0·74 (95% CI 0·72–0·76), the same as in the model including mitotic rate per mm2. The C-statistic in the external validation slightly improved to 0·70 (95% CI 0·68–0·74) (Figure 1).

Figure 1. Receiver operating characteristics curve showing the accuracy of the Melanoma Institute Australia (MIA) nomogram to predict sentinel node positivity when applied to the Dutch melanoma dataset. AUC, area under the curve; CI, confidence interval.

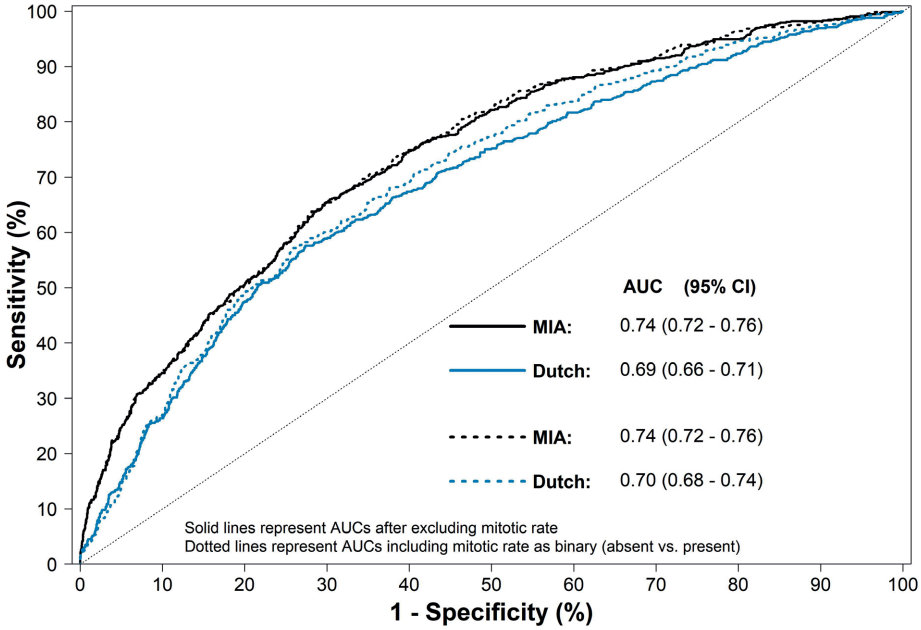

Figure 2. Calibration plot of the Melanoma Institute Australia (MIA) nomogram without mitotic rate (left panel) and with mitotic rate as a binary parameter (absent or present) (right panel) to predict sentinel node positivity applied to Dutch patients with melanoma who underwent sentinel node biopsy. A = calibration-in-the-large calculated as the logistic regression model intercept given that the calibration slope equals 1. B = calibration slope in a logistic regression model with the linear predictor as the sole predictor. C = C-statistic indicating discriminative ability. Triangles represent deciles of subjects grouped by similar predicted risk.

Table 3. Analysis of negative predictive value (NPV), sensitivity and specificity for different Melanoma Institute Australia (MIA) nomogram cutoffs to predict sentinel node (SN) positivity for the Dutch melanoma validation cohort.

Nomogram predicted probability (cut-off) (%)	NPV (%, 95% CI)	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	SN reduction rate (%, 95% CI)	Error rate (%, 95% CI)
<3	100 (NA)	100 (NA)	0.1 (0.01-0.3)	0.06 (0.008-0.2)	0.0 (NA)
<4	85.7 (42.2-99.6)	99.9 (99.2-100)	0.3 (0.09-0.6)	0.2 (0.09-0.05)	0.0 (NA)
<5	91.3 (72.0-98.9)	99.7 (99.0-100)	0.9 (0.6-1.4)	0.7 (0.5-1.1)	0.1 (0.01-0.2)
<6	89.3 (78.1-96.0)	99.1 (98.1-99.7)	2.1 (1.6-2.8)	1.8 (1.3-2.4)	0.2 (0.07-0.4)
<7	92.5 (85.8-96.7)	98.8 (97.7-99.5)	4.1 (3.4-5.1)	3.5 (2.9-4.2)	0.3 (0.1-0.5)
<8	91.2 (86.3-94.7)	97.4 (95.9-98.5)	7.8 (6.8-9.0)	6.6 (5.8-7.6)	0.6 (0.4-0.9)
<9	91.7 (88.2-94.3)	95.7 (93.9-97.1)	13.7 (12.5-15.3)	11.6 (10.6-12.9)	1.0 (0.7-1.4)
<10	90.1 (87.0-92.5)	92.8 (90.6-94.6)	18.9 (17.5-20.7)	16.3 (15.1-17.7)	1.6 (1.2-2.0)
<11	89.8 (87.0-91.8)	90.2 (87.7-92.3)	24.7 (23.1-26.6)	21.4 (20.0-22.9)	2.2 (1.7-2.8)
<12	89.0 (86.5-90.9)	86.4 (83.6-88.9)	31.5 (29.7-33.5)	27.5 (25.9-29.1)	3.0 (2.5-3.8)
<13	88.8 (86.5-90.5)	83.5 (80.5-86.2)	37.4 (35.6-39.5)	32.7 (31.1-34.5)	3.7 (3.1-4.5)
<14	88.2 (85.9-89.7)	79.9 (76.7-82.8)	42.9 (40.9-44.9)	37.8 (36.0-39.5)	4.5 (3.9-5.4)
<15	87.7 (85.6-89.2)	76.4 (73.1-79.5)	48.3 (46.3-50.3)	42.8 (40.9-44.5)	5.3 (4.6-6.2)
<16	87.3 (85.2-88.7)	73.3 (69.8-76.5)	52.7 (50.6-54.7)	46.9 (45.0-48.6)	6.0 (5.2-7.0)
<17	86.9 (84.8-88.3)	70.0 (66.5-73.4)	56.8 (54.7-58.8)	50.8 (48.9-52.5)	6.7 (5.9-7.7)
<18	86.5 (84.4-87.8)	67.0 (63.4-70.5)	60.3 (58.3-62.3)	54.2 (52.4-55.9)	7.3 (6.6-8.5)
<19	86.1 (84.1-87.4)	64.3 (60.6-67.8)	63.3 (61.3-65.2)	57.2 (55.3-58.8)	8.0 (7.2-9.1)
<20	85.9 (84.0-87.2)	61.9 (58.2-65.6)	66.6 (64.6-68.5)	60.2 (58.4-61.9)	8.5 (7.7-9.7)

DISCUSSION

This study of Dutch patients with melanoma validated the MIA nomogram for prediction of SN positivity using population-based data, a process recommended by the authors of the original nomogram.[6] Such validation is of critical importance to assess the generalizability of a model before it is appropriate to recommend its widespread use in clinical practice.[13] SN positivity was recently proposed as a biomarker to select patients who may benefit from adjuvant systemic drug therapy treatment.[14] This gives new impetus to perform SN biopsy procedures in patients with melanoma, because SN positivity now provides more accurate prognostic information and also has important therapeutic implications. The accuracy of predictions of SN positivity has therefore assumed even greater importance than previously.

The performance of the model was somewhat lower in the validation cohort than in the original cohort. This lower performance can be explained by the difference in the types of data that were utilized: institutional data (used in the development set) vs. population-based registry data (used in the validation set).[13] In addition, a well-known property of the C-statistic is that a higher value is usually obtained in the development set compared with the validation set due to overoptimism and overfitting during model development.[15] In the current study, the C-statistic for SN positivity was 0·69 (95% CI 0·66–0·71) in the validation cohort, compared with 0·74 (95% CI 0·72–0·76) in the development cohort. For the two previously mentioned reasons, a C-statistic of 0·69 using population-based data is considered good and was achieved even though Dutch patients significantly differed in almost all the parameters used in the nomogram (Table 1). These results emphasize the general applicability of the MIA nomogram to the wider melanoma population.[10]

Even though the baseline risk of melanoma differs for European and Australian patient populations,[16] the present study shows that the risk factors for SN spread in the two melanoma populations were similar. This is an important finding, indicating that the MIA nomogram is robust and can be applied to European patients with melanoma as well as Australian patients. Its applicability to a US population was previously demonstrated in the initial validation using data from a large US institution (the MD Anderson Cancer Center).[6] In addition, our results show that the MIA nomogram can significantly reduce the number of patients for whom SN biopsy is recommended, while retaining high NPVs and low error rates. This is important, as when current selection criteria in international guidelines are applied, only about 16–27% of SN biopsies reveal metastatic melanoma.[3, 4]

As the goal of the various international melanoma management guidelines is to help clinicians determine which patients are most likely to benefit from SN biopsy, principally based on the Breslow thickness, the MIA nomogram provides refinement of

this concept by also utilizing five other important parameters to derive a personalized risk of a positive SN biopsy. This enables the MIA nomogram to be used in clinical practice internationally, to counsel patients and to preoperatively discuss their risk of SN positivity. This supports the concept of discussing SN biopsy with patients deemed to have a 5–10% risk and routinely offering it to those with > 10% risk, as outlined in the NCCN guidelines, rather than having an absolute threshold.[12] This can be especially helpful in elderly patients and those with significant comorbidities. A low predicted probability of a positive SN biopsy can help make a decision to avoid the procedure in some cases, and in others a high predicted probability can provide encouragement to perform it. However, when the decision to forego a SN biopsy is contemplated on the basis of the nomogram prediction, it is important to note that the sensitivity of the model decreases for higher cutoff values of predicted probability (see Table 3).

A strength of this study is that it is the first population-based study to validate the MIA nomogram for predicting SN positivity in patients with melanoma, confirming its general applicability. A limitation is that the Dutch dataset did not include the number of mitoses in the primary melanoma, only their presence or absence. For this reason, we decided to validate the MIA nomogram without including mitotic rate, as this was an optional parameter in the original MIA nomogram.[6] Our sensitivity analysis using mitotic rate as absent or present showed a slight increase in the C-statistic in the validation cohort. In all three scenarios – using mitotic rate as either count,[6] categorical (present or absent) or excluded (optional) – the MIA nomogram showed its validity in terms of discrimination and calibration indices.

CONCLUSIONS

This external validation using a large European population-based dataset confirmed the ability of the MIA nomogram to predict SN positivity. The MIA nomogram can thus be recommended for clinical practice internationally to guide clinical decision making and counsel patients by informing them whether or not an SN biopsy procedure is likely to provide useful information that may influence management. The information may also guide follow-up recommendations.

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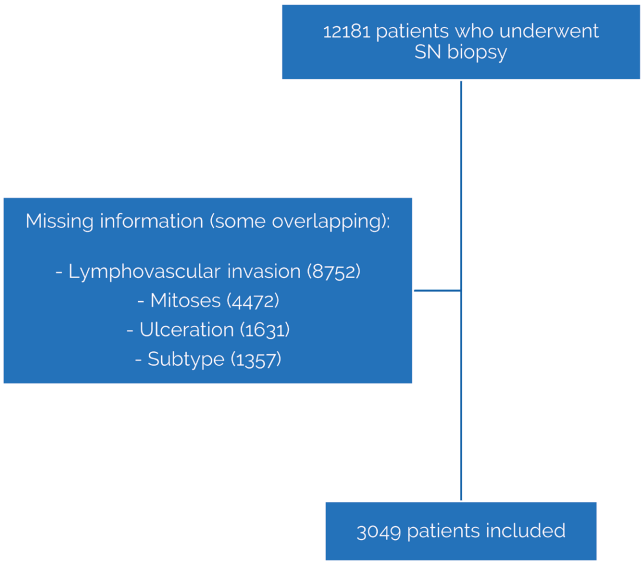
Supplementary Figure 1. Flowchart of patient selection for the Dutch cohort.



* In 1370 of the 4472 patients with missing values for mitoses, the pathologist was not able to assess mitoses, and in the remaining cases, mitoses were not reported. For ulceration, the pathologist was not able to assess the feature in 24 of the 1631 missing cases, and in the remaining cases, ulceration was not reported. Lymphovascular invasion and melanoma subtype were missing because the pathologist did not report it.

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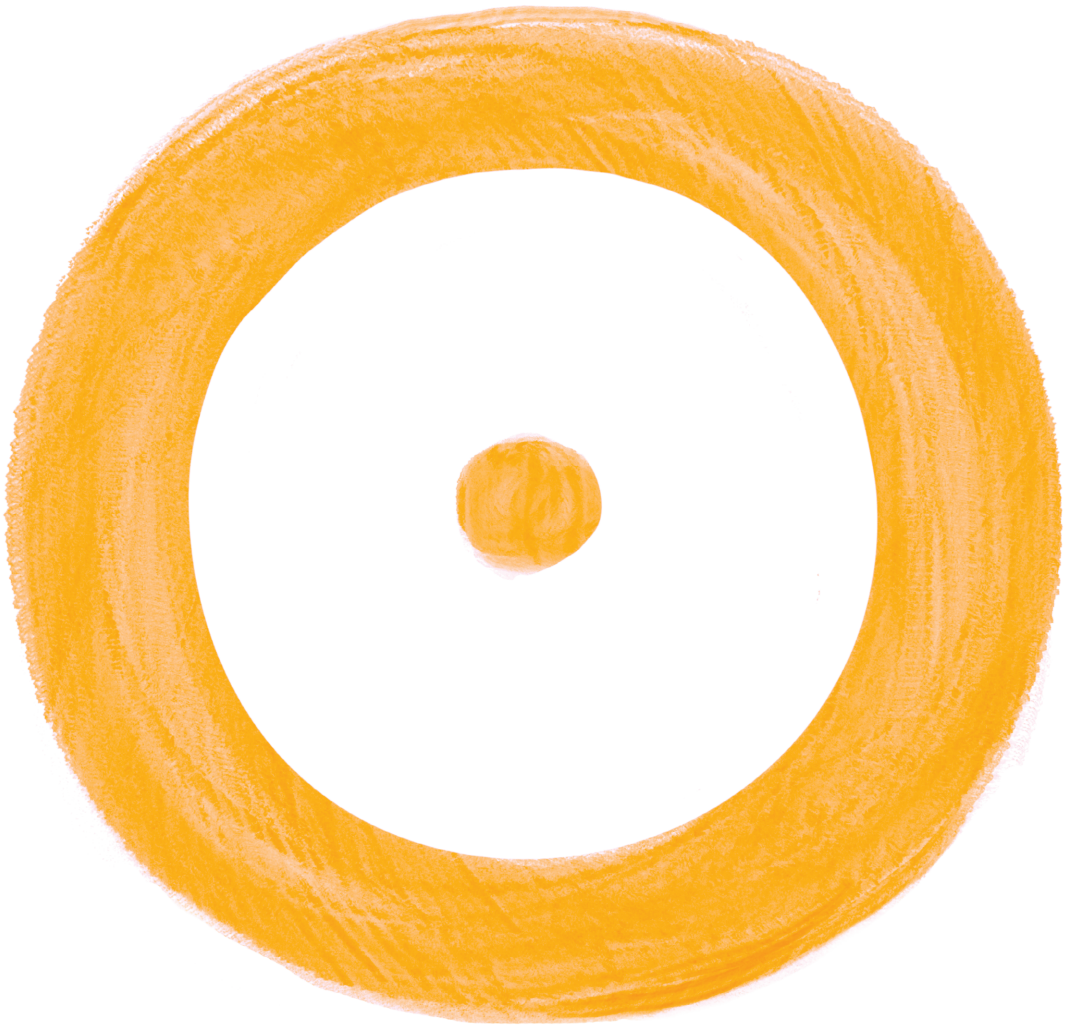

CHAPTER 17

Summary

SUMMARY

Part I – Sentinel node biopsy

The subject of **Part I** of this thesis describes the enactment, yield, concordance between pathologists and the incremental value of sentinel node (SN) biopsy in patients with melanoma. In **Chapter 2**, we assessed the trend of SN enactment in the Netherlands from 2003 to 2014 according to the American Joint Committee on Cancer (AJCC) guidelines at the time (6th and 7th). In practice, only 39.7% of all 24,603 eligible patients underwent SN biopsy, with an increasing trend from 39.1% in 2003 to still only 47.8% in 2014. Factors that were associated with non-enactment of SN biopsy were female sex, higher age and melanoma located on the head and neck. In **Chapter 3**, we retrospectively calculated the probability of SN-positivity in relation to T-stage according to the 8th AJCC in the same set of patients. SN-positivity increased from 3.4% in patients with stage T1nos to 47.4% in patients with stage T4b. Even within the group of patients with T1 melanomas, there was substantial variation in SN-positivity, ranging from 3.4% in in patients with stage T1nos to 19.3% in patients with stage T1b when ulceration was present. Thus, we concluded there is room for a more tailored approach in selecting patients eligible for SN biopsy.

Chapter 4 answers the long-standing question if knowledge on SN status in patients with melanomas improves accuracy of the prognostic estimate that can be obtained from standard clinicopathological assessment of the primary tumor. In order to do so, data from a Dutch population-based cohort of melanoma patients (n=9272) and from a validation cohort from Melanoma Institute Australia (MIA) (n=5644) were analyzed. Survival models showed a statistically significant improvement in predictive accuracy when SN status was included in the model in addition to Breslow thickness, sex, age, site, mitoses, ulceration, regression and melanoma subtype. The C-statistic in the two independent cohorts increased by 3% for overall survival (OS) when SN status was included in the model. For recurrence-free survival (RFS), there was an increase of 2% in the Dutch cohort and a 4% increase in the MIA cohort when SN status was included. A 4% increase for melanoma-specific survival (MSS) was seen in the MIA cohort. The sensitivity of 3-year overall survival predictions increased with 10-12% by including SN-status. In addition, a net benefit increase was observed across all threshold probabilities for all three survival outcomes and in the two independent cohorts, indicating that patients were more accurately identified as having a high-risk melanoma when SN status was included in the model compared to a model without SN status. Despite all these statistics showing the improvements in accuracy, cost and potential morbidity of SN biopsy have to be taken into account as well. This led us to conclude that the magnitude of the prognostic improvement must be considered while weighing advantages and disadvantages of SN biopsy.

In **Chapter 5** we selected histopathological slides of a group of 322 SN-positive melanoma patients. Review by an expert melanoma pathologist resulted in a downgrade in the diagnosis from melanoma metastasis to nodal nevus in 38 (11.8%) patients. Considering the inclusion criteria of phase 3 adjuvant trials, at least 4.3% of patients would have falsely qualified for adjuvant therapy. This led us to recommend that SN biopsies that are suspected to contain metastases should be reassessed by an expert melanoma-pathologist, especially when adjuvant treatment is considered.

Because the optimal timing of SN biopsy after melanoma diagnosis is unknown, we have examined its association with SN-positivity and survival in **Chapter 6**. We included 7660 Dutch and 3478 Australian patients. Patients were included if the SN biopsy was performed within 100 days after initial diagnosis. We found no significant association between time to SN biopsy and SN-positivity within this time frame. There was also no significant association between time to SN biopsy and survival outcome. In **Chapter 7** we included the same set of patients and assessed the association of timing on SN biopsy of SN tumor size. The SN metastasis diameter increased with delayed biopsy in the Dutch cohort but not the MIA cohort, indicating that further investigation is required.

Part II – Individual clinicopathological variables for survival

In **Part II** we detailed several clinicopathological variables related to survival in patients diagnosed with melanoma. **Chapter 8** explored the influence of the presence of regression on survival (RFS and OS) in a Dutch and Australian cohort of stage I and II melanoma patients. In both cohorts (17271 Dutch patients and 4980 Australian patients, respectively), survival outcomes were better for patients with disease regression. Hazard ratios (HRs) for those with disease regression were 0.55 for RFS and 0.87 for OS; for the Australian patients, the HRs were 0.61 for RFS and 0.73 for OS. Subgroup analyses showed that the presence of regression improved survival especially in those with thin and intermediate thickness tumors and those with superficial spreading melanoma (SSM) subtype. **Chapter 9** deals with the effect of melanoma histologic subtype on overall survival. All 48361 patients diagnosed with stage I, II or III melanoma in the Netherlands between 2000 and 2014 were included. When HRs were calculated for each melanoma subtype, and adjusted for Breslow thickness, ulceration status, age, sex, stage, and localization, we found that patients with acral lentiginous melanoma showed worse survival than SSM patients (HR 1.26). Among patients with melanomas that were thin (≤1.0mm), nodular melanoma (NM) subtype patients also showed worse survival than SSM. NM patients with tumors thicker than 1.0mm did not show worse survival than SSM patients with tumors thicker than 1.0mm. We showed that among melanoma patients, the subtype of the melanoma is an independent predictor for survival, and that NM subtypes especially showed worse survival among melanomas that were thin. Therefore, subtype was incorporated in the model described in Chapter 14.

The comparison of overall survival of 54645 Dutch melanoma patients with a single melanoma to that of 2284 Dutch patients with multiple melanomas is shown in **Chapter 10**. To prevent immortal time bias for patients with multiple primary melanomas, we performed Cox regression analysis with a time-varying covariate and found worse overall survival among patients with multiple primary melanomas compared with patients with a single primary melanoma (HR 1.31). The median Breslow thickness was 0.90mm for the first melanoma and 0.65mm for the second melanoma. More than 27% of second melanomas developed after 5 years of follow-up. We therefore recommend that more intensive follow-up strategies are applied for patients with multiple primary melanomas.

A very small group of melanoma patients are diagnosed with ultra-thick melanomas, which we defined as ≥15mm in **Chapter 11**. Survival tends to be lower as the Breslow thickness of a primary cutaneous melanoma is higher, however, the prognostic value of Breslow thickness in patients with very thick melanomas is uncertain. To allow meaningful analyses for the modelling analysis, we pooled a cohort of 4107 Dutch patients and 1488 Australian patients diagnosed with melanomas ≥4.0mm in thickness. A total of 183 patients were diagnosed with ultra-thick melanomas. We found that in patients with melanomas ≥15mm in thickness, the progressive relationship between increasing Breslow thickness and decreasing survival is lost. This novel information can be discussed with this selected group of patients.

Building on this, in **Chapter 12** we draw attention to a group of melanoma patients that seem to be forgotten: patients with thick melanomas with a negative SN biopsy. Whereas adjuvant systemic therapy is now available for patients with a positive SN biopsy (stage III), this is not the case for patients with a thick melanoma and a negative SN biopsy. We know, however, that in daily practice, these patients have worse prognosis than patients with stage IIIA disease. We therefore compared survival of those patients to patients with an intermediate or thin melanoma and a positive SN biopsy. In a Dutch cohort of 648 patients with thick melanomas (defined as >4.0mm) with a negative SN biopsy and 2018 Dutch patients with melanomas ≤4.0mm with a positive SN biopsy, we found a five-year OS of 70.5% and 71.5%, respectively. This difference was not statistically significant. This lends support to the current strategy of also including thick melanomas with negative SN biopsy in ongoing adjuvant therapy studies.

Chapter 13 reports on the survival difference between male and female patients with melanomas in the Netherlands. A total of 23879 men and 30766 women were included. They showed a median Breslow thickness of 1.0mm and 0.8mm, respectively. Men more often had melanomas localized on the trunk or head and neck. After correcting for age, Breslow thickness, anatomic location, presence of ulceration and melanoma subtype, the multivariable relative excess risks for males for

dying was 1.37. Although until today gender difference in survival among melanoma patients remains apparent and not fully understood, the recognition that older males (especially those with a melanoma located on the head and neck) have poorer prognosis is important. Campaigns with special focus on this subgroup at risk for melanoma may prevent or at least lead to early detection of melanoma.

Part III – Nomogram-based predictions: a multicontinental approach

Part III of this thesis focuses on nomogram-based predictions, using a multi-continental approach using Dutch and MIA patients. In **Chapter 14** we aimed to address the great clinical need to identify patients with T1 melanomas that will develop metastases. Although the prognosis of patients with melanomas ≤1mm in Breslow thickness is generally very good, a subset develops recurrent disease and because patients with thin melanomas constitute such a high proportion of all patients diagnosed with melanoma, in absolute numbers, more people ultimately die from T1 melanomas than from T2, T3, or T4 melanomas. Therefore, we developed and validated three nomograms to predict disease recurrence (local, regional, and distant) in patients with T1 primary melanomas. A total of 25,930 Dutch patients and 2968 Australian patients were included for the development cohort and validation cohort, respectively. All three nomograms were able to accurately identify T1 patients at greatest risk of either local, regional or distant recurrence, using only basic and readily available clinicopathological variables. C-statistics of 0.79 for local RFS, 0.77 for regional RFS, and 0.80 for distant RFS were obtained for the development model. External validation showed C-statistics of 0.80, 0.76, and 0.74, respectively. The nomograms were also able to identify T1a patients who were at high-risk of local, regional, or distant recurrence. SN biopsy is not generally recommended for these patients, but they might benefit from the procedure. We have made a freely available online tool at www.melanomarisk.org.au so that these nomograms can be directly integrated into current clinical practice to guide the management of patients with thin melanomas and ultimately improve their survival.

Chapter 15 aimed to validate a European model to predict recurrence in patients with a negative SN biopsy. Data from 8795 Dutch melanoma patients was used to validate the model developed by the European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group for RFS. One of the strengths of the model is its simplicity: it is based on just three variables (Breslow thickness, ulceration status and anatomical location). Validation showed a C-statistic of 0.70, with excellent calibration, leading us to conclude that the EORTC nomogram could be used in clinical practice for personalizing follow-up and selecting high-risk patients for trials of adjuvant systemic therapy.

Building further on our findings that we described in Chapter 3, in **Chapter 16** we aimed to validate the MIA nomogram to predict SN status more accurately. The

current clinical practice is to only use T-stage to determine eligibility for SN biopsy. By doing so, overall SN-positivity ranges from 16% to 27%. To answer the need for a more tailored approach to selecting patients with primary melanoma for SN biopsy, MIA recently developed a nomogram to predict SN status. The model was subsequently successfully validated using data from the MD Anderson Cancer Center in the United States of America. The model was based on 6 readily available clinicopathological variables: Breslow thickness, ulceration, age, melanoma subtype, lymphovascular invasion and mitotic rate. We externally validated the MIA model using European, population-based data from 3049 Dutch melanoma patients. We found a good C-statistic of 0.70, with excellent calibration. A negative predictive value of 90.1% was found at a 10% predicted probability cut-off from having a positive SN biopsy, implying that by using the nomogram, a 16.3% reduction in the rate of performing a SN biopsy could be achieved, with an error rate of only 1.6%. We therefore concluded that the MIA nomogram can well be used to predict SN-positivity in a European population. Its use may spare low-risk patients the inconvenience, cost and potential risks of SN biopsy while ensuring that high-risk patients are still identified. This nomogram is also available online at www.melanomarisk.org.au.

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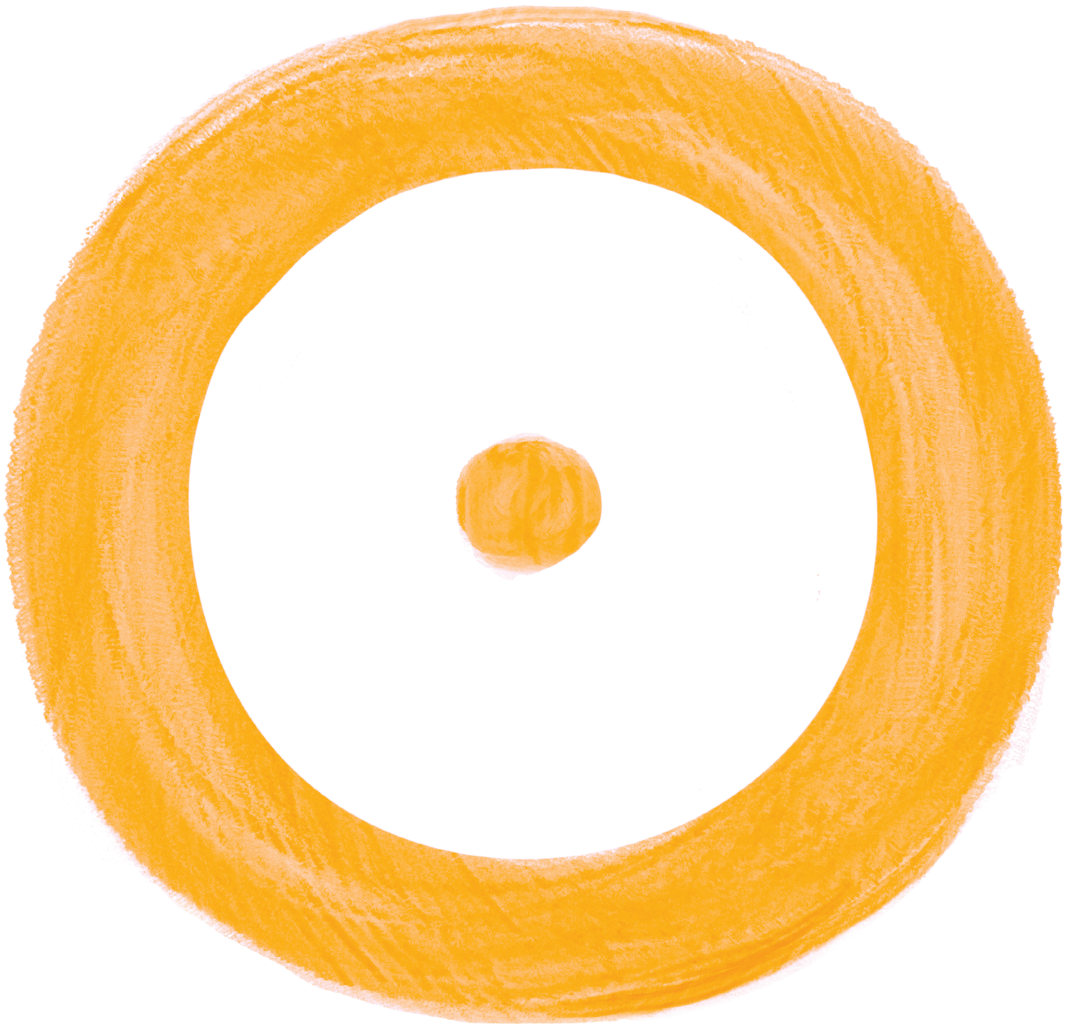

CHAPTER 18

General Discussion and Conclusions

GENERAL DISCUSSION

This thesis contributes to the ongoing work on improving predictions of individuals facing the difficult diagnosis of cutaneous melanoma. Its main focus is on predictions in survival and sentinel node (SN)-positivity, two important aspects in the management of patients diagnosed with melanoma.

A MULTICONTINENTAL APPROACH

By successfully using data from two different continents as development and external validation set, as was done in the current thesis, the generalizability of the results is increased.[1] Whereas internal validation refers to the concept of reproducibility, external validation addresses transportability.[2-5] In this thesis, we have focused on geographic external validation. Geographic validation is defined by assessing the generalizability of the predictive performance of a model to other institutes, countries or – in our case – continents.[1-5] Due to the often bigger differences in case-mix, this type of external validation provides a more stringent proof of concept than other forms of external validation like temporal validation, where the external validation set may be from the same institution but in a different (usually later) time period.[1,4] In an era where we strive for personalized risk estimates, it is imperative that clinical decisions are made on accurate prediction models. Therefore, before a model can be implemented into clinical practice, its external validation is pivotal. Research should focus more on externally validating promising risk models that already exist, and possibly updating it, rather than developing yet another similar prediction model.

Another important point is that prediction models should be as accessible as possible for actual and proper implementation in clinical care. Instead of prediction models existing solely on paper, research can literally "come alive" by developing online available nomograms that are (freely) accessible to both patients and clinicians (www.melanomarisk.org.au). Another advantage of digitalization of nomograms is their visibility and accessibility, which hopefully also encourage further external validation, rather than the development of a similar prediction model. We are currently working on further externally validating and optimizing both online nomograms that have been described in this thesis.

FIILLING IN THE GAPS AND CLINICAL IMPLICATIONS

Predicting sentinel node positivity

The result of SN biopsy is a key eligibility criterion for the indication of adjuvant systemic therapy for patients with stage III melanoma.[6-9] As previously mentioned, patients staged T1b or higher are eligible for SN biopsy, leading to an overall SN-positivity range from 16% to 27%.[10,11] It has been claimed that the use of SN status

alone to accept and stratify patients into clinical trials or to receive adjuvant systemic treatment is "not rational".[12] However, other methods of selecting high-risk patients are limited. This is why tools that predict SN-positivity, like the (externally validated) Melanoma Institute Australia (MIA) nomogram, are urgently needed.[13,14] These tools can help to improve patient selection, particularly by indicating those who are very unlikely to be SN-positive and in whom SN biopsy can therefore reasonably be omitted. Biomarkers based on gene expression profiling that predict outcome are also available, but their accuracy has not been shown to be better than that provided by SN biopsy and reliable validation of their predictive ability is in any case still required.[15-18] Until these methods of predicting or detecting nodal metastasis are validated, SN biopsy remains the best method of identifying actual lymph node metastases, predicting those patients who may benefit from adjuvant systemic therapy and those higher-risk patients who may be eligible for enrolment in clinical trials of systemic therapy. The external validation that we have performed (Chapter 16) is of critical importance to assess the generalizability of the model before it is appropriate to recommend its widespread use in clinical practice. The C-statistic for SN positivity was 0.69 in the validation cohort (population-based data), compared with 0.74 in the development cohort (institutional data). A C-statistic of 0.69 is therefore considered good and was achieved even though Dutch patients significantly differed in almost all the variables used in the nomogram. These results emphasize the general applicability of the MIA nomogram to the wider melanoma population. Even though the baseline risk of melanoma differs for European and Australian patient populations,[19] the results shows that the risk factors for SN spread in the Dutch and Australian melanoma populations are similar. Its applicability to a US population was previously demonstrated in the initial validation using data from a large US institution (the MD Anderson Cancer Center).[13] This enables the MIA nomogram to be used in clinical practice internationally, to counsel patients and to preoperatively discuss their risk of SN positivity. This supports the concept of discussing SN biopsy with patients deemed to have a 5-10% risk and routinely offering it to those with >10% risk, as outlined in the NCCN guidelines, rather than having an absolute threshold.[20] This can be especially helpful in elderly patients and those with significant comorbidities. A low predicted probability of a positive SN can help make a decision to avoid the procedure in some cases, and in others a high predicted probability can provide encouragement to perform it. However, when the decision to forego a SN biopsy is contemplated on the basis of the nomogram prediction, it is important to note that the sensitivity of the model decreases for higher cut-off values of predicted probability.

Additional prognostic value of sentinel node biopsy status

The second role of SN biopsy that has been questioned, is its additional prognostic value over known predictors such as Breslow thickness and ulceration. The current American Joint Committee on Cancer (AJCC) staging system considers only Breslow thickness, ulceration status and SN status to predict survival in patients

with melanomas.[6] However, as we have shown in Chapters 8-13, other individual variables such as sex, regression and subtype predict survival as well. Identification of individual predictors in large datasets is important, because it forms the basis for the development of prediction models where multiple predictors are included. The additional prognostic value of SN biopsy over that of other predictors was a discussion that we aimed to put to rest. We have gained similar results as Mitra et al., who showed an area under the curve (AUC) increase from 0.70 to 0.74 for overall survival (OS) when SN status was added to their model that included Breslow thickness, mitotic rate, ulceration, vascular invasion, site, age and sex.[21] As noted by Faries et al. in their recent editorial accompanying our publication, perhaps more revealing is that this question needed to be revisited at all. A critical note that they make, is that we used a binary approach, "which does not capture the dynamic range and overall richness that SN biopsy information can provide".[22] This is a valid point, which mainly concerns the size of the metastasis in the SN (the so-called "SN tumor burden") and its localization. We were not able to analyze SN tumor burden, because the data was not available in a relatively large number of patients.

LOOKING DEEPER INTO SENTINEL NODE BIOPSY

Sentinel node tumor burden

SN tumor burden has been categorized according to the Rotterdam criteria, which were identified by van Akkooi et al. in a Dutch cohort of 262 stage I and stage II patients who underwent a SN biopsy, and of whom 77 had a positive SN.[23] SN tumor burden is measured as the maximum dimension of the largest deposit of confluent neoplastic cells in the SN[24,25] and is stratified into three categories: <0.1mm, 0.1-1.0mm and >1.0mm.[23,26] A high SN tumor burden appears to be significantly associated with worse survival.[26-28] An interesting group of patients are those with <0.1mm SN tumor burden. It is unclear if they should be considered SN-negative, or that these patients should be considered as stage III. While some studies have shown that these patients have no statistically significantly different survival compared to SN-negative patients[23,29], two studies found that these patients have worse survival.[30,31] A probable explanation for these conflicting results is that patients <0.1mm SN tumor burden are relatively rare, as in the largest study up to date less than 10% of SN-positive patients had a SN tumor burden of <0.1mm (n=146), compared to 43% who had a SN tumor burden of 0.1-1.0mm and 47% who had a SN tumor burden of >1.0mm.[28] Regulatory approval and funding of adjuvant systemic therapy for patients with stage IIIA patients currently differs from country to country. The assessment of SN tumor burden is of clinical importance, because in most cases adjuvant drug therapies are only approved or reimbursed for stage IIIA patients with a SN tumor burden of >1.0mm.[32] Assessing survival of patients with a SN tumor burden <0.1mm therefore warrants further investigation, by merging data and cohorts, resulting in sufficient numbers of events.

The EORTC Melanoma Group defines SN tumor burden as *"a single measurement of the maximum diameter of the largest lesion in any direction"*.[24,33] Although a definition and recommendations on how to measure SN tumor burden have been clearly proposed, in daily practice clinically relevant discrepancies remain to exist. Literature assessing the inter-observer agreement for measuring SN tumor burden is sparse. In the study by Murali et al., 7 experienced pathologists reviewed 44 at random selected SN biopsies containing metastatic melanoma. The authors found an excellent interobserver agreement (ICC=0.88) for measuring the maximal size of largest tumor deposits.[34] We, however, know from clinical experience that these findings do not reflect our daily clinical practice. We are therefore currently performing a study to assess the inter-observer agreement for measuring SN tumor burden between European pathologists, to enable uniform and reproducible measurements.

Identification and assessment of sentinel node biopsy

Another relevant issue is the correct identification of the SN itself. A study performed by Jansen et al. in 2000 examined the reproducibility of lymphoscintigraphy in assessing the location and number of SN in 25 patients. When the procedure was re-performed in the same patient and by the same physician, the authors found a difference in number of SNs in 3 patients (12%).[35] Above that, we (Chapter 5) and others have shown discordance rates in the assessment of SN-positivity itself of 8.8% up to 11.8%.[36,37] This main reason for this discordance is the confusion of melanoma metastasis with a nodal nevus. In most cases, this differentiation is straightforward, based on location and cytomorphological features of the melanocytic cells in the lymph node. However, in a subset of cases, nodal nevi may be difficult to discriminate from melanoma metastasis.[38] In our study, we have now shown that 44 out of 1000 patients might receive unjustified adjuvant therapy, leading to unnecessary costs and relatively frequent side-effects, which are occasionally fatal.[39,40] We therefore strongly advocate for a change in clinical practice: when adjuvant treatment is considered in stage III melanoma patients, SNs suspected to contain metastases should be reassessed by an expert melanoma-pathologist.

LOOKING BEYOND SENTINEL NODE BIOPSY: TWO FORGOTTON GROUPS

Stage IIB/C patients

In 2003 in the Netherlands, 40% of all eligible patients (n= 24,603) underwent SN biopsy[41], which increased to still only 65% in 2016[42], with significant variation per region.[43] Selecting only the most appropriate patients for early adjuvant systemic therapy is of great importance. To achieve this, patients with melanomas with a high Breslow thickness and a negative SN biopsy (stage II(B/C)) may be considered for adjuvant therapy irrespective of their SN status, as these patients have a poor prognosis comparable to that of patients with thinner melanomas who are SN-

positive – a "forgotten group".[6,44] We have highlighted their poor survival in Chapter 12. Trials of adjuvant systemic therapies for patients with stage II melanomas are currently in progress and will demonstrate whether these patients derive as much benefit from adjuvant therapy as patients with a positive SN biopsy.[45,46] If these trials will show a survival benefit in this selected group of patients, another new era will begin: the indication for adjuvant therapy will no longer solely depend on SN status.

Patients with a thin melanoma

Patients without an indication for a SN procedure (i.e. most patients diagnosed with a thin melanoma; 58% to 81% of all melanoma patients) with a high risk of recurrence, should not be overlooked. The proposed individualized risk models described in this thesis (Chapter 14) can help to answer to these clinical needs. By using only basic, readily available clinicopathological variables, the C-statistics for the models presented in Chapter 14 for local, regional and distant recurrence-free survival (RFS) ranged from 0.77-0.80. It is generally accepted that a C-statistic of 0.7 is acceptable, 0.8 is good, and 0.9 is excellent.[47] The nomogram can be employed at various stages of a patient's treatment. It is not meant to substitute for existing guidelines regarding the indications to perform a SN biopsy. As the majority of T1 patients are not eligible for SN biopsy (because they have a melanoma <0.8mm Breslow thickness without ulceration), the nomogram using the SN biopsy "not performed" status will thus be applicable to most patients. In patients who are eligible for SN biopsy, however, the nomogram can be used to determine the risk of recurrence prior to SN biopsy, and also after it has been performed. A suggestion for future improvements of the model is its external validation in other populations, as well as evaluating if the model can be improved by including other prognostic variables, such as regression, and lymphovascular invasion. We did not include the latter two histopathological variables because they were missing too frequently in the Dutch dataset.

FUTURE PERSPECTIVES

Biomarkers

Gene expression profile (GEP) testing intends to predict survival and / or SN-positivity in patients with melanoma based on expression patterns of a panel of genes from the primary melanoma. Several GEP tests are currently (commercially) available.[48-50] Three GEP testing platforms are currently available: the 31-GEP test, the 8-GEP test, and the clinicopathological & GEP platform (CP-GEP) test.[17] The latter two tests are designed to predict survival, the first test to predict SN-positivity. For all three tests reliable validation of their predictive ability is still required.[17,18] The 31-GEP test has been validated most often; two studies have externally validated the 31-GEP test based on retrospective data[51,52] and 3 studies have done so using prospective data[53-56]. However, it is unclear if GEPs are additive in predicting their intended outcome,

because in none of the studies their accuracy has been evaluated against all known clinicopathological variables.

Circulating tumor DNA (ctDNA) is an example of a liquid biopsy, or blood-based genomic biomarker, in which DNA fragments are measured that are released into the bloodstream by apoptotic or necrotic cancer cells. Because of this, ctDNA can be used as a biomarker for patients with metastatic disease in lymph nodes (stage III) or at distant sites (stage IV), whereas GEPs are mainly used for patients with stages I and II melanoma. ctDNA can predict response to immunotherapy, because its amount is related to tumor stage and prognosis.[57-61] It shows promise as a biomarker for the clinical guidance of patients with stage III or IV melanoma. Another promising tumor marker is tumor mutational burden (TMB), which measures the number of somatic mutations in a tumor. Martincorena and Campbell compared the mutational burden in 20 tumor types, which clearly showed that melanoma had the highest mutational burden of all cancer types, followed by lung cancer.[62] Although conflicting results exist for this biomarker in predicting survival in patients with most solid tumors treated with immunotherapy, it seems a promising biomarker for patients with melanoma.[63,64]

It is likely that, after further evaluation, future daily practice will include some of the aforementioned biomarkers. However, which ones, their exact place, and if they will increase the C-statistic of current models that include basic clinicopathological variables or completely substitute them, remains to be determined. Until then, "*the modestly invasive, clearly prognostic, potentially therapeutic, and always controversial test that is SLNB*" will – and should be – employed.[65]

Artificial intelligence (AI) and imaging techniques

The first efforts to predict sentinel node status based on H&E slides from routine histology of the primary melanoma using deep learning methods have been undertaken.[66] Although the AUC was only 55.0% (±3.5%), larger studies are warranted to determine its additive value, and if AI will be a tool to help pathologists in their assessment of SN status. However, as we have shown that more than 10% of originally positive SN biopsies of patients with melanoma concern misclassified nodal nevi[37], there seems more urgency for AI algorithms that discriminate melanoma metastases in SNs from nodal nevi based on H&E slides from the SN. No studies in the melanoma literature assessing this have been published. Another interesting development is the initiation of automated digital volume measurement[67] and even high-resolution 3D imaging techniques[68] to assess SN tumour volume. SN metastasis volume – rather than diameter – might be an even more accurate measure of SN metastasis size, leading to a better classification of high- and low-risk patients.

Neoadjuvant therapy

Although adjuvant therapy has now become standard of care for patients with stage III melanoma, the survival of a subset of these patients – namely, patients with macrometastases (i.e. a clinically palpable positive SN) – remains poor. For stage IIIB and IIIC patients (7th AJCC) treated with nivolumab 4-year RFS rates are 60.0% and 46.1%, respectively,[69] and for those treated with pembrolizumab 3-year RFS rates are 65.7% and 54.3%, respectively[70]. These patients might benefit from neoadjuvant therapy, where immunotherapy is given before surgery (and in most cases, further adjuvant therapy after surgery). The theory behind this concept is the induction of a broader immune response when immunotherapy is started when the complete tumor is still present. Surgery of the affected nodes can either show pathological complete response (pCR), near pCR, pathological partial response (pPR) or pathological no response (no pNR). Menzies et al. recently pooled the current six neoadjuvant trials, including 192 patients in total, of whom 141 received immunotherapy and 51 received targeted therapy.[71] pCR occurred in 40% of patients and correlated with improved RFS and OS (for RFS: pCR 2-year 89% versus no pCR 50%, p<0.001, and for OS: pCR 2-year 95% versus no pCR 83%, p=0.03). This led the authors to conclude that "*pathological response should be an early surrogate endpoint for clinical trials and a new benchmark for development and approval in melanoma*". It is envisioned that in the foreseeable future, neoadjuvant therapy will become standard of care for a subset of patients diagnosed with melanoma.

CONCLUSIONS

Melanoma is a quickly moving landscape, full of opportunities. Primary tumor characteristics remain vital for accurate prognostic evaluation. There is room for further optimization of SN biopsy enactment itself, which remains the first priority to correctly stage patients diagnosed with melanoma and to identify those that are eligible for further treatment. Studies in the near-by future will indicate if patients with a high Breslow thickness and a negative SN biopsy can benefit from adjuvant therapy. The development of personalized, integrated risk models that incorporate multiple clinicopathological factors, and ultimately molecular and immune-related factors, seems to be the era ahead. In this era of digitalization, and in a world that is figuratively speaking becoming smaller and smaller, we have to find a way to streamline, optimize and uniform the worldwide data that is available to us, to combine and learn from it. To optimally inform our patients on their course of disease collaborations between institutes to validate predictors across continents is vital. Without such collaborations, the sun may go down on progress in melanoma prediction.

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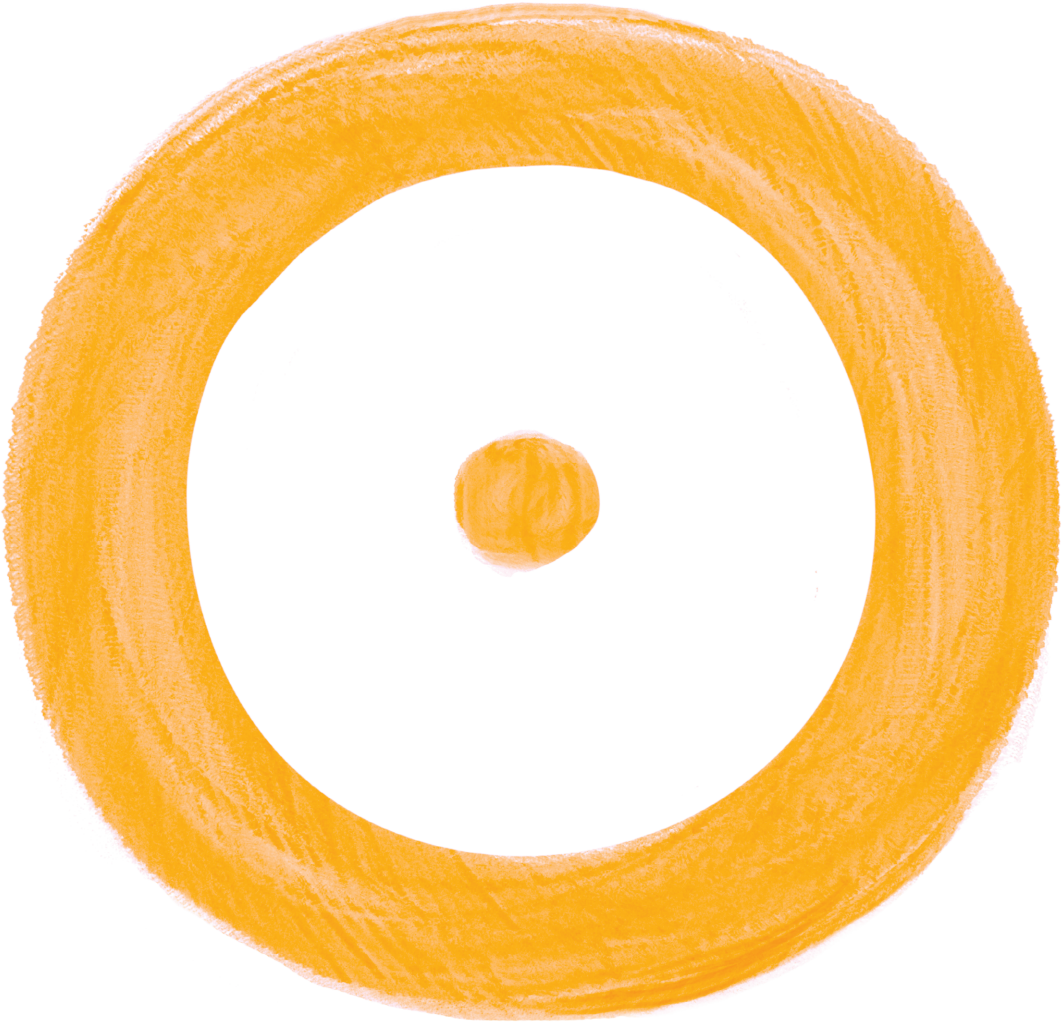

CHAPTER 19

Nederlandse samenvatting | Summary in Dutch

NEDERLANDSE SAMENVATTING

Huidkanker – een algemene introductie

Grof gezegd bestaan er drie soorten huidkanker: het basaalcelcarcinoom, het plaveiselcelcarcinoom en het melanoom. Al deze drie vormen van kanker ontstaan in de huid, en bij elk van hen is de voornaamste oorzaak overmatige blootstelling aan UV-licht. Het melanoom ontstaan echter uit andere type cellen dan het basaalcelcarcinoom en het plaveiscelcarcinoom en is daarmee dus een geheel andere vorm van huidkanker. Basaalcel- en plaveiselcelcarcinomen ontstaan uit cellen die keratinocyten worden genoemd, terwijl het melanoom voortkomt uit pigmentcellen, ookwel: melanocyten. Een "gewone" moedervlek bestaat ook uit pigmentcellen, maar in dat geval zijn de pigmentcellen rustig en vertonen ze geen verdachte kenmerken als de patholoog de moedervlek onder de microscoop bekijkt. Vaak wordt gedacht dat melanomen ontstaan uit reeds bestaande moedervlekken. Dit is inderdaad het geval bij ongeveer 1 op de 3 melanomen. Twee op de 3 melanomen ontstaan echter als een nieuwe plek, waar voorheen geen moedervlek zichtbaar was. Met name op nieuwe moedervlekken die na de leeftijd van 40 jaar ontstaan moet gelet worden. Ongeveer 1 op de 5 Nederlanders krijgt in zijn of haar leven te maken met huidkanker. Meestal gaat het om een basaalcelcarcinoom, omdat dit 75% van alle vormen van huidkanker betreft. In zo'n 15% van de gevallen gaat het om een plaveiselcarcinoom en in 10% van de gevallen om een melanoom. Het melanoom is dus niet de meest voorkomende vorm van huidkanker, maar is wel verantwoordelijk voor 70-90% van al het huidkanker-gerelateerd overlijden. Melanomen komen meestal voor op de aan de zon blootgestelde huid. Ze kunnen echter ook voorkomen in de slijmvliezen (omdat daar ook pigmentcellen zitten), zoals in de neus, de schaamlippen en vagina, de slokdarm, of in het netvlies van het oog. Dit proefschrift focust zich op het melanoom in de huid.

Deel I – Schildwachtklierbiopsie

Deel I van dit proefschrift gaat over de schildwachtklierbiopsie in patiënten met een melanoom. Melanoomcellen kunnen zich verspreiden via het lymfevocht. De schildwachtklier is de lymfeklier die als eerste het lymfevocht uit het melanoom opvangt. Deze lymfeklier wordt ook wel de schildwachtklier of poortwachtersklier genoemd. De lokalisatie van de schildwachtklier hangt af van de plek waar het melanoom heeft gezeten. Het vinden van de schildwachtklier wordt ook wel de schildwachtklierprocedure of schildwachtklierbiopsie genoemd. Deze procedure wordt uitgevoerd bij patiënten met een melanoom dat 0.8mm of dikker is, of als er zweervorming is in het melanoom. In eerste instantie wordt de verdachte moedervlek alleen de gepigmenteerde plek in de huid krap weggesneden. Als dit na onderzoek door de patholoog een melanoom blijkt te zijn, dan wordt er rondom het litteken nogmaals weefsel weggesneden (zodat de kans kleiner wordt dat het melanoom terugkeert doordat er achtergebleven cellen zijn). Vervolgens wordt– afhankelijk

van de dikte van het melanoom – een schildwachtklierbiopsie uitgevoerd. Hierbij wordt met behulp van radio-nucleaire technieken de eerste klier, de schildwachtklier, (in sommige gevallen klier*en*) waar het melanoom op uitkomt opgespoord, en chirurgisch verwijderd. De uitslag van deze schildwachtklierprocedure kan positief zijn (bevat uitzaaiingen) of negatief zijn (bevat geen uitzaaiingen), en zegt iets over de overlevingskansen van een patiënt. Verder zijn ook de dikte van het melanoom (Breslow dikte) en de aan- of afwezigheid van zweervorming (ulceratie) van belang voor het bepalen van het stadium van de ziekte. Het is ook mogelijk dat er sprake is van uitzaaiingen op afstand (in andere organen), maar deze groep patiënten wordt in dit proefschrift buiten beschouwing gelaten. In **Hoofdstuk 2** is in kaart gebracht hoe vaak schildwachtklierprocedures in Nederland worden uitgevoerd in de periode van 2003 tot en met 2014. Het bleek dat in de praktijk gemiddeld maar 39.7% van alle 24,603 patiënten die hiervoor in aanmerking komt deze procedure ondergaan heeft, met een stijging van 39.1% in 2003 tot nog steeds maar 47.8% in 2014. De kans dat de schildwachtklierbiopsie niet was verricht was groter bij vrouwen, hogere leeftijd en bij patiënten met een melanoom in het hoofdhalsgebied. Een mogelijke verklaring voor dit relatief lage percentage is dat de reden om een schildwachtklierprocedure uit te voeren over de laatste jaren sterk gewijzigd is. Een positieve of negatieve schildwachtklier betekende tot 2018 alleen iets voor de prognose; deze was beter bij een negatieve schildwachtklier. Echter, sinds 2018 is er een behandeling gekomen voor patiënten met een positieve schildwachtklier die alles heeft veranderd. Patiënten met een positieve schildwachtklier komen sinds 2018 namelijk in aanmerking voor behandeling met adjuvante systeemtherapie. Deze behandeling vindt plaats via pillen (targeted therapie) of een infuus (immunotherapie). De overleving van patiënten met een positieve schildwachtklier is hiermee met 15-20% gestegen (ter illustratie: de 5-jaars overleving steeg van 36% naar 52% in één studie en van 44% naar 64% in een andere studie). Er is hiermee een nieuwe reden bijgekomen om een schildwachtklierprocedure uit te voeren. In **Hoofdstuk 3** is de kans uitgerekend dat de schildwachtklierbiopsie positief is (dus: uitzaaiingen van het melanoom bevat) en waar dit van afhangt. Uit eerdere literatuurstudies blijkt dat gemiddeld genomen 1 op de 5 schildwachtklierprocedures positief is. De data uit Hoofdstuk 3 laat zien dat de kans op een positieve schildwachtklier van 3.4% in het laagste stadium (T1a) tot 47.4% in het hoogste stadium (T4b) liep. Binnen de groep van patiënten met een zogenaamd "dun" melanoom (waarbij de dikte van het melanoom 1mm of kleiner is (gemiddeld zo'n 70% van alle patiënten met een melanoom)) varieerde de kans op een positieve schildwachtklierbiopsie aanzienlijk. Dit liep van 3.4% bij patiënten met een dun melanoom waarbij het onbekend was of er zweervorming was tot 19.3% bij patiënten met een dun melanoom met zweervorming. Hieruit is geconcludeerd dat er ruimte is voor een meer gepersonaliseerde aanpak om patiënten te selecteren voor een schildwachtklierprocedure.

In de literatuur wordt soms de aanvullende waarde van de schildwachtklierprocedure in twijfel getrokken. **Hoofdstuk 4** beantwoordt daarom de vraag of de uitslag van de schildwachtklierprocedure (de schildwachtklierstatus) de nauwkeurigheid van de voorspelling van de overleving verbetert. Normaal gesproken wordt deze verkregen op basis van alleen standaard bekende karakteristieken van de patiënt en van het melanoom. Daarom is de data van een Nederlandse en Australische groep patiënten geanalyseerd. Dit betrof respectievelijk 9272 en 5644 patiënten met een melanoom. De overleving kon veel beter voorspeld worden wanneer schildwachtklierstatus in het model werd opgenomen naast de dikte van het melanoom, leeftijd, lokalisatie, aan- of afwezigheid van celdelingen en zweervorming, regressie en type melanoom. Dit gold voor beide datasets. Dit heeft ons doen concluderen dat de grootte van de nauwkeurigheidsverbetering tegen de nadelen van een schildwachtklierbiopsie moet worden afgewogen. In **Hoofdstuk 5** is het klierweefsel van 322 patiënten met een positieve schildwachtklierbiopsie opnieuw beoordeeld door een patholoog met expertise op het gebied van melanoom. Bij een positieve schildwachtklier worden kwaadaardige cellen in de lymfeklier gezien. Er kunnen echter ook goedaardige pigmentcellen in een lymfeklier voorkomen. In 38 patiënten (11.8%) werd de diagnose van kwaadaardig naar goedaardig bijgesteld. Patiënten met een positieve schildwachtklier worden normaal gesproken behandeld met adjuvante systeemtherapie. Onze berekening liet zien dat een deel (4.3%) van de patiënten hier ten onrechte mee behandeld zou worden. Hierom wordt in Hoofdstuk 5 aanbevolen dat schildwachtklierbiopsiën die ervan verdacht worden uitzaaiingen te bevatten, moeten worden geëvalueerd door een patholoog met expertise op het gebied van melanoom, zeker wanneer adjuvante therapie wordt overwogen.

Het is onbekend wat de optimale timing is van het uitvoeren van de schildwachtklier-procedure nadat de diagnose melanoom is gesteld. Daarom wordt de relatie tussen tijd tot schildwachtklierprocedure en schildwachtklierstatus en overleving geanalyseerd in **Hoofdstuk 6**. In totaal werden 7660 Nederlandse en 3478 Australische patiënten bestudeerd. Gemiddeld zat er een maand tussen de diagnose van het melanoom en het uitvoeren van de schildwachtklierprocedure. Patiënten bij wie de schildwachtklierprocedures werden uitgevoerd binnen 100 dagen na het stellen van de diagnose werden geselecteerd. Dit waren vrijwel alle patiënten. Er werd geen verband gevonden tussen tijd tot schildwachtklierprocedure en de schildwachtklierstatus binnen deze tijdsperiode. Ook was er geen verband tussen tijd tot schildwachtklierprocedure en de overleving. In **Hoofdstuk 7** is bij dezelfde groep patiënten nagegaan wat de relatie tussen tijd tot schildwachtklierprocedure en de grootte van de uitzaaiing was. Er was geen verband tussen interval tot de schildwachtklierprocedure en de grootte van de uitzaaiing in de schildwachtklier in de Australische groep. In de Nederlandse groep werd wel een grotere uitzaaiing gezien bij een later uitgevoerde schildwachtklierprocedure. Om dit goed uit te zoeken, is verder onderzoek nodig.

Deel II – Individuele voorspellers voor overleving

In **Deel II** worden verschillende eigenschappen van de patiënt of hun melanoom voor het voorspellen van overleving onderzocht. In **Hoofdstuk 8** wordt de invloed van "regressie" onderzocht in een Nederlandse en Australische set van patiënten. Regressie is een fenomeen dat kan optreden bij verschillende soorten kanker. In het geval van een melanoom, betekent dit dat het melanoom (deels) wordt opgeruimd door het eigen afweersysteem. Dit kan soms ook aan de buitenkant zichtbaar zijn; een deel van het melanoom lijkt dan te verdwijnen. Tot nog toe was het onduidelijk of dit fenomeen de overleving van patiënten met een melanoom beïnvloedt. Dit is in twee verschillende groepen patiënten onderzocht (17271 Nederlandse en 4980 Australische patiënten). Het bleek dat patiënten met regressie in hun melanoom een betere overleving hadden. Dit was vooral het geval bij mensen met een dun melanoom (≤1mm) of een melanoom van gemiddelde dikte (1-4mm), en in patiënten met een superficieel spreidend melanoom (een bepaald subtype van een melanoom). In **Hoofdstuk 9** wordt gekeken of het hebben van een bepaald subtype van melanoom invloed heeft op de overleving van een patiënt. Grof gezegd bestaan er 4 soorten melanomen: superficieel spreidend melanoom, nodulair melanoom, lentigo maligna melanoom en acrolentigineus melanoom. De verdeling hoe vaak deze voorkomen is respectievelijk 70%, 20%, 5-10% en 1-2%. In 48361 Nederlandse patiënten met een melanoom werd (rekening houdend met de dikte van het melanoom, zweervorming, leeftijd, geslacht, stadium van de ziekte en lokalisatie) gevonden dat patiënten met een acrolentigineus melanoom een slechtere overleving (26% meer kans op overlijden) hadden dan patiënten met een superficieel spreidend melanoom. Binnen de groep van patiënten met een dun melanoom (1.0mm of kleiner), hadden patiënten met een nodulair melanoom een slechtere overleving dan patiënten met een superficieel spreidend melanoom. In het geval van een melanoom van 1.0mm of dikker, werd dit verschil niet meer gezien. Het subtype melanoom heeft dus invloed op de overleving van de patiënt.

De vergelijking op de kans op overleving tussen 54645 Nederlandse patiënten met één melanoom en 2284 Nederlandse patiënten met meerdere melanomen staat beschreven in **Hoofdstuk 10**. Ongeveer 4% van alle patiënten in Nederland met een melanoom ontwikkelt een tweede melanoom. Het bleek dat de kans op overleving van patiënten met meerdere melanomen slechter was dan die van patiënten met één melanoom (waarbij rekening gehouden werd met o.a. de dikte van het melanoom en de aanwezigheid van zweervorming). De gemiddelde Breslow dikte van het eerste melanoom was 0.9mm, en van het tweede melanoom 0.65mm. In meer dan 27% van de gevallen ontstond het tweede melanoom meer dan 5 jaar na de eerste. Intensievere follow-up wordt dan ook aanbevolen voor patiënten met meerdere melanomen.

Een zeer kleine groep van patiënten met een melanoom wordt gediagnosticeerd met een ultra-dik melanoom. Hiervoor is in **Hoofdstuk 11** als definitie 15mm of meer gehanteerd. In het algemeen geldt dat de overleving afneemt, naarmate de dikte van het melanoom toe neemt. Echter, de voorspellende waarde van de Breslow dikte bij patiënten met een erg dik melanoom is onzeker. Omdat het weinig patiënten betreft, zijn de datasets van 4107 Nederlandse en 1488 Australische patiënten samengevoegd. Zij hadden allen een melanoom met een dikte van 4.0mm of meer, waarvan 183 patiënten een melanoom van 15mm of meer hadden. Hieruit bleek dat in deze groep patiënten (met een melanoom van 15mm of meer), de relatie tussen toenemende Breslow dikte en afnemende overleving verloren gaat. Deze nieuwe bevinding kan besproken worden met patiënten met een melanoom van 15mm of meer.

In **Hoofdstuk 12** wordt aandacht gevraagd voor een groep patiënten die vergeten lijkt te zijn: patiënten met een dik (meer dan 4.0mm) melanoom en een negatieve schildwachtklierbiopsie. Adjuvante systeemtherapie is, zoals eerder gezegd, vandaag de dag beschikbaar voor patiënten met een positieve schildwachtklier. Echter, dit is niet beschikbaar voor patiënten met een dik melanoom en een *negatieve* schildwachtklier, hoewel we uit de dagelijkse praktijk weten dat deze patiënten een slechtere prognose hebben dan een deel van de patiënten met een positieve schildwachtklier. Daarom is in dit hoofdstuk de overleving van de patiënten in deze "vergeten groep" nader in kaart gebracht en vergeleken met de overleving van patiënten met een dik melanoom en een positieve schildwachtklier, en ook met de overleving van patiënten met een dunner melanoom (4.0mm of minder) met een positieve schildwachtklier. Zoals verwacht, was de overleving van patiënten met een dik melanoom en een positieve schildwachtklier het slechtst (5-jaars overleving 48%). Echter, de overleving van patiënten met een dik melanoom (meer dan 4.0mm) en een negatieve schildwachtklierbiopsie en die van patiënten met een dunner melanoom (4.0mm of minder) was nagenoeg gelijk; 5-jaars overleving 71.5% en 70.5%, respectievelijk. Deze data laat zien dat het meer dan terecht is dat patiënten uit deze vergeten groep sinds kort mee mogen doen met studies naar adjuvante systeemtherapie.

Hoofdstuk 13 brengt de verschillen in de kans op overleving van mannen en vrouwen met een melanoom in Nederland in kaart. In totaal betrof dit 23879 mannen en 30766 vrouwen. Mannen hadden een gemiddelde dikte van het melanoom van 1.0mm, en vrouwen 0.8mm. Bij mannen werd het melanoom vaker gevonden op de romp en in het hoofdhalsgebied. Mannen hadden een hoger risico op overlijden dan vrouwen, ook wanneer rekening gehouden werd met verschillen in leeftijd, dikte van het melanoom, lokalisatie, aanwezigheid van zweervorming, en subtype melanoom. Hoewel dit verschil vandaag de dag nog steeds niet goed begrepen wordt, is het wel belangrijk te erkennen dat oudere mannen (zeker diegene met een melanoom in het

hoofdhalsgebied) een slechtere prognose hebben. Campagnes met speciale focus op deze subgroep kunnen mogelijk melanomen voorkomen, of tenminste leiden tot een eerdere diagnose.

Deel III – Nomogram-gebaseerde voorspellingen: een multi continentale aanpak

Deel III van dit proefschrift focust op voorspellingen op basis van een nomogram. Een nomogram is een simpel, tweedimensionaal diagram van een rekenmodel waaruit een voorspelling kan worden berekend. Voor het ontwikkelen van de nomogrammen in dit deel van het proefschrift is gebruik gemaakt van Nederlandse en Australische data. In **Hoofdstuk 14** wordt gehoor gegeven aan de urgente behoefte om erachter te komen welke patiënten met een dun melanoom (1.0mm of dunner) uitzaaiingen ontwikkelen. Ondanks dat de overleving van patiënten met een dun melanoom doorgaans erg goed is (5-jaar overleving 89% tot 100%), ontwikkelt een deel van hen toch uitzaaiingen. En omdat patiënten met een dun melanoom zo'n grote groep vormen (gemiddeld zo'n 70% van alle patiënten met een melanoom) overlijden er – in absolute aantallen – meer mensen aan dunne melanomen dan aan dikkere melanomen. Om die reden zijn drie nomogrammen ontwikkeld die uitzaaiingen voorspellen; in de huid, in de lymfeklier, en op afstand. In totaal werd data van 25930 Nederlandse en 2968 Australische patiënten gebruikt. Deze nomogrammen zijn ontwikkeld op basis van de Nederlandse data, en extern gevalideerd (een proces waarbij wordt gekeken hoe goed het ontwikkelde model werkt in de nieuwe dataset) met Australische data. Alle drie de nomogrammen konden goed de patiënten met het hoogste risico op uitzaaiingen identificeren, met enkel de standaard beschikbare kenmerken van de patiënt en het melanoom. De nomogrammen konden zelfs patiënten met een dun melanoom zonder zweervorming (T1a) identificeren, die in de regel niet in aanmerking komen voor een schildwachtklierbiopsie, maar wel een hoog risico op uitzaaiingen hebben. Er is een gratis, online tool ontwikkeld op www.melanomarisk.org.au, zodat er in de dagelijkse praktijk gemakkelijk gebruik kan worden gemaakt van deze nomogrammen om de begeleiding van patiënten en hun overleving te verbeteren.

Het doel van **Hoofdstuk 15** is om een Europese model te valideren dat gemaakt is om uitzaaiingen bij patiënten met een negatieve schildwachtklierbiopsie te voorspellen. Er is gebruik gemaakt van data van 9785 Nederlandse patiënten met een melanoom om het model wat eerder ontworpen was door de *European Organisation for Research and Treatment of Cancer* (EORTC) Melanoom Groep te valideren. Eén van de sterktes van dit model is de eenvoud: het is maar op drie variabelen gebaseerd (dikte van het melanoom, zweervorming en lokalisatie). De validatie liet een waarde van 0.70 zien (ter illustratie: 0.5 is gelijk aan een gok, 1.0 is perfect). Dit onderzoek laat zien dat het EORTC-nomogram in de dagelijkse praktijk kan worden gebruikt om hoog-risico patiënten met een negatieve schildwachtklier

te selecteren voor onderzoeken met adjuvante systeemtherapie. In **Hoofdstuk 16** is het Australische nomogram dat de schildwachtklierstatus voorspelt, gevalideerd met Nederlandse data. Zoals eerder geschreven wordt in de huidige praktijk alleen de dikte van het melanoom en de zweervorming meegenomen in de beslissing om wel of geen schildwachtklierprocedure uit te voeren. Met gebruik van deze twee indicatoren is gemiddeld 20% van alle schildwachtklierbiopsiën positief. Om een betere selectie van patiënten te maken die in aanmerking kunnen komen voor een schildwachtklierprocedure, heeft het Melanoma Institute Australia (MIA) een nomogram ontwikkeld om de schildwachtklierstatus te voorspellen. Het doel is om de procedure niet te verrichten bij patiënten met een hoge waarschijnlijkheid op een negatieve schildwachtklier, en tegelijkertijd degene die zeer waarschijnlijk een positieve schildwachtklier hebben niet uit het oog te verliezen. Het MIA nomogram is eerder gevalideerd met data van het gerenommeerde MD Anderson Cancer Center in de Verenigde Staten. De data die nodig is voor het model is de dikte van het melanoom, zweervorming, leeftijd, melanoom subtype, lymfovasculaire invasie, en aantal celdelingen. Omdat het model tot nu toe gebaseerd was op die van twee specialistische centra in Australië en de Verenigde Staten, zou het zo kunnen zijn dat het model minder goed werkt in de algemene melanoom populatie en in patiënten van andere delen van de wereld. Om die reden is het MIA nomogram met Europese, nationale data van 3049 Nederlandse patiënten gevalideerd. Deze validatie liet een waarde van 0.70 zien. Door het nomogram te gebruiken, kunnen er 16.3% minder schildwachtklierprocedures worden verricht, met een foutmarge van maar 1.6%. Dit onderzoek laat zien dat het MIA nomogram goed kan worden gebruikt in de Europese populatie. Het kan patiënten met een laag risico de ongemakken, kosten en potentiële risico's van een schildwachtklierbiopsie besparen en tegelijkertijd ervoor zorgen dat patiënten met een hoog risico worden geïdentificeerd. Dit nomogram is eveneens online toegankelijk via www.melanomarisk.org.au.

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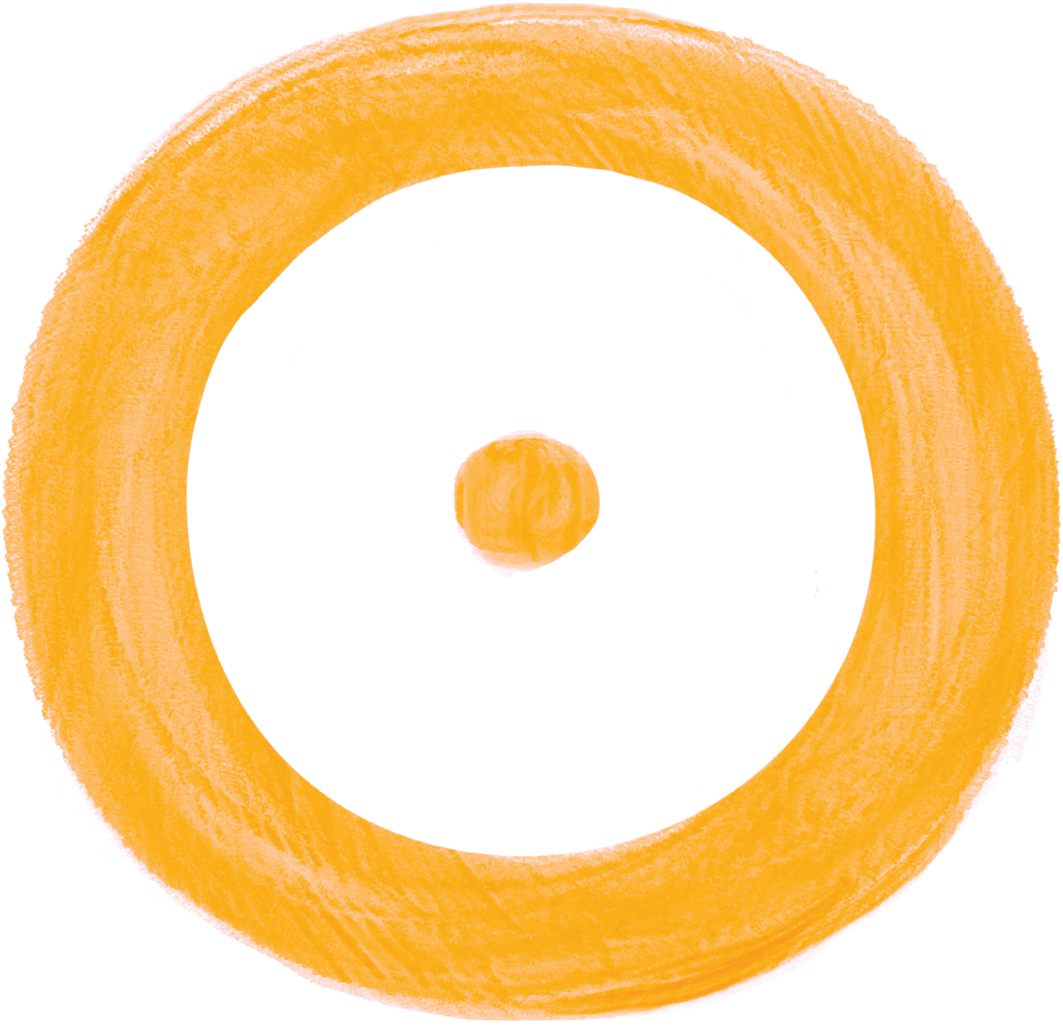

APPENDICES

Abbreviations
Contributing authors
List of publications
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ABBREVIATIONS

AJCC	American Joint Committee on Cancer
ALM	Acral lentiginous melanoma
AUC	Area under the curve
BAD	British Association of Dermatologists
BT	Breslow thickness
CI	Confidence interval
CLND	Completion lymph node dissection
DRFS	Distant recurrence-free survival
EORTC	European Organisation for Research and Treatment of Cancer
ESMO	European Society of Medical Oncology
H&E	Haematoxylin and eosin
H&N	Head and neck
HR	Hazard ratio
IKNL	Comprehensive Cancer Organization of the Netherlands
IQR	Interquartile range
LMM	Lentigo maligna melanoma
LRFS	Local recurrence-free survival
MIA	Melanoma Institute Australia
MPM	Multiple primary melanoma
MSLT	Multicenter Selective Lymphadenectomy Trial
MSS	Melanoma-specific survival
NCCN	National Comprehensive Cancer Network
NCR	Netherlands Cancer Registry
NM	Nodular melanoma
NPV	Negative predictive value
NRI	Net reclassification index
OR	Odds ratio
OS	Overall survival
PALGA	Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief / the Dutch Pathology Registry
RER	Relative excess risk
RFS	Recurrence-free survival
ROC	Receiver operating characteristic
RRFS	Regional recurrence-free survival
RS	Relative survival
SD	Standard deviation
SEER	Surveillance, Epidemiology, and End Results
SN	Sentinel node
SNB	Sentinel node biopsy
SLNB	Sentinel lymph node biopsy

SPM	Single primary melanoma
SSM	Superficial spreading melanoma
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TRIPOD	Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis
UV	Ultraviolet

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A

CURRICULUM VITAE

Mary-Ann El Sharouni was born on the 26th of December 1988 in Heemstede, the Netherlands. She participated in the Dutch national judo championship in 2002. She attended high school at the Christelijk Lyceum Zeist, the Netherlands, finishing cum laude in 2007, whilst also receiving her international baccalaureate English. She commenced her study medicine at the Utrecht University the same year. During this period, she was an active member in several student committees and started to work as a research student at the department of Pathology at the University Medical Center Utrecht. After obtaining her medical degree in 2014, she continued to work on what would later become her PhD thesis under the supervision of Prof. dr. Paul van Diest, whilst completing her internships and, later, her work as a surgical resident at the Diakonessenhuis Utrecht, the Netherlands. After a year of working as a qualified educational teacher at the department of Dermatology at the University Medical Center Utrecht, she started her Dermatology residency in training at the University Medical Center Utrecht on January 1st 2016. She continued to work on her PhD thesis, whilst commencing a post-graduate master in Clinical Epidemiology, which led to the supervision of her second PhD supervisor, Prof. dr. Carla van Gils. With financial support of the European Academy of Dermatology and Venereology she completed an 8-month research fellowship in 2020-2021 at Melanoma Institute Australia, Sydney, Australia, to collaborate with the research group of Prof. dr. John Thompson, Prof. dr. Richard Scolyer and Associate Prof. Serigne Lo, and she attended dermatology clinics under the supervision of Prof. dr. Pascale Guitera. Mary-Ann is deeply passionate about the care for skin cancer patients, especially those with melanoma.