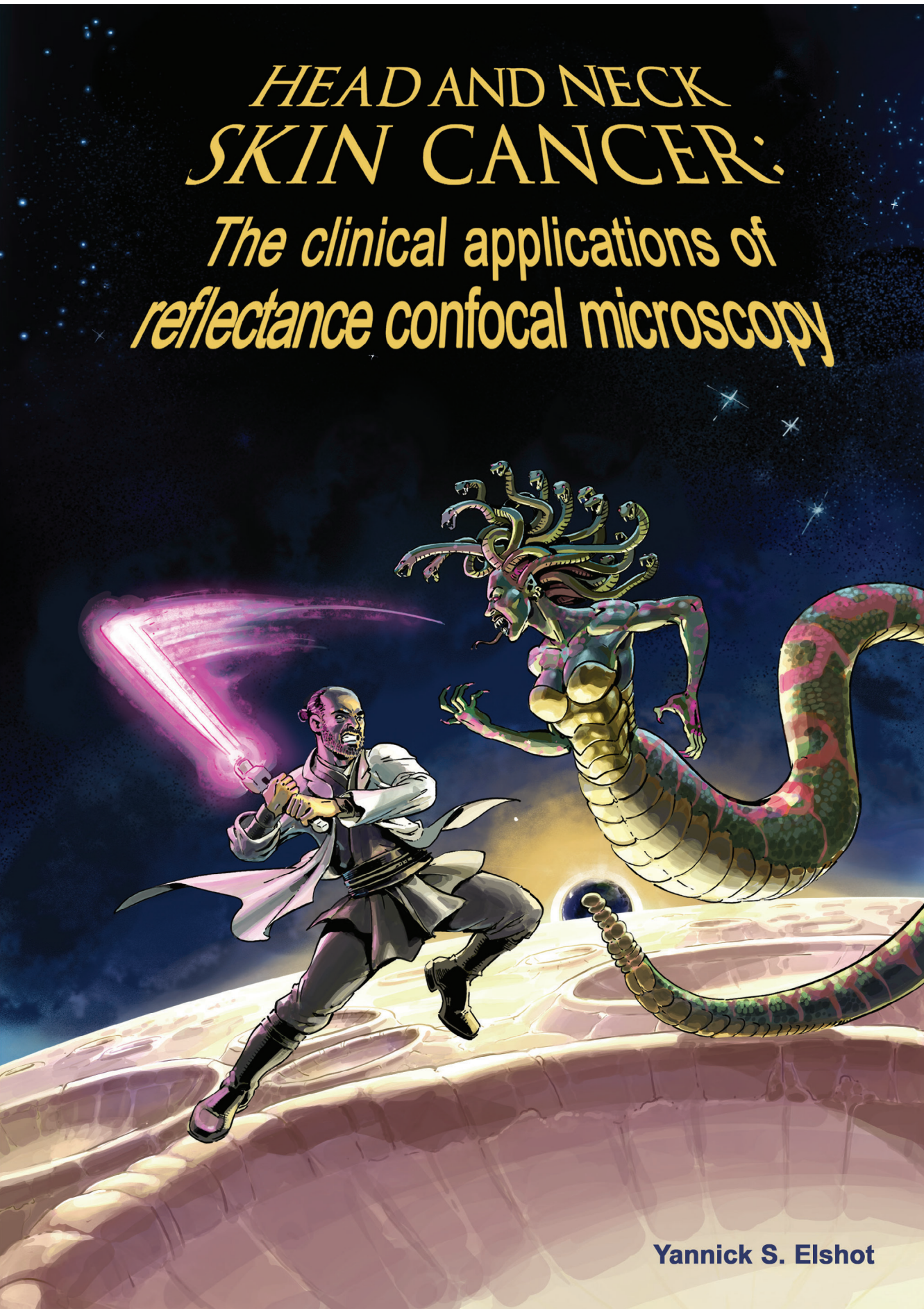
HEAD AND NECK SKIN CANCER:

The clinical applications of reflectance confocal microscopy



Yannick S. Elshot

HEAD AND NECK SKIN CANCER

THE CLINICAL APPLICATIONS OF REFLECTANCE CONFOCAL MICROSCOPY

Yannick Stephen Elshot

COLOFON

Head and neck skin cancer: The clinical applications of reflectance confocal microscopy

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HEAD AND NECK SKIN CANCER

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ACADEMISCH PROEFSCHRIFT

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Yannick Stephen Elshot
geboren te Amsterdam

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About the cover

While the cover art of this thesis may appear self-explanatory, a deeper exploration of its symbolism and connection to the research content may prove enlightening, particularly for those less familiar with the topics. For nearly a decade, I've heard patients exclaim, "It looks like the moon's surface!" when evaluating their confocal images. This recurring observation inspired the lunar theme of my thesis cover. When capturing certain histological structures in a horizontal orientation, the black-and-white confocal microscopy images evoke the appearance of the moon's surface. These structures include the interruption of the epidermis by dermal papillae at the level of the dermo-epidermal junction and adnexal structures (Introduction: **FIGURE 8**). The front cover represents the primary focus of my thesis: lentigo maligna (melanoma). It features a white-robed figure wielding the handheld device used in this thesis (Introduction: **FIGURE 13**). The near-infrared laser (830nm) is reimagined as a lightsaber, adding a touch of science fiction to scientific reality. Opposing this figure is Medusa, the mythological Gorgon. Her serpentine hair serves as a metaphor for the dendritic processes of melanocytes, the cells involved in melanoma. Furthermore, in 2014, de Carvalho et al. first described "medusa head-like" structures visible through confocal microscopy in cases of lentigo maligna. These structures represent the lentiginous proliferation projecting from hair follicles, a histological feature characteristic of this melanoma subtype (Introduction: **FIGURE 14**). The back cover illustrates the subject of **CHAPTER 7**: basal cell carcinoma. The most prevalent basal cell carcinoma subtypes form tumor nodules with peripheral cells arranged in a palisading pattern, surrounded by retraction clefts from the adjacent tumor stroma (Introduction: **FIGURE 3**). This histological arrangement is represented by moat-surrounded palisaded forts containing stormtroopers engaged in conflict with jet-pack-wearing warriors. The purplish hue of this scene mimics the appearance of tissues as seen on the most widely used histopathology stains: hematoxylin and eosin. The jet packs worn by the figures are the larger arm-mounted confocal device (Introduction: **FIGURE 13**), typically employed when evaluating melanocytic nevi. These devices bear the logos of the collaborating institutions: The Netherlands Cancer Institute - Antoni van Leeuwenhoek & Amsterdam University Medical Center. Last but not least, while this cover art serves as a visual representation of the topic of this thesis, it was also just an excuse to see myself depicted as a Jedi.

About the artist

Born in Colombia, Pablo found true happiness at an early age by drawing characters from his favorite movies and TV shows. He discovered his first comic book when he was 12 years old, and he was instantly captivated by the art form. With the advent of the X-Men animated TV series and the Image Comics revolution, Pablo's passion for drawing comics only grew. Despite his passion, he went to college to become an engineer. In 2007, Pablo and his wife moved to Argentina to pursue a career in science. While studying, he attended comic book conventions and workshops with great Argentinian masters such as Horacio Lalia, Eduardo Risso, and Ariel Olivetti. After earning his PhD in physics in 2012, Pablo decided to leave his chosen academic path behind and instead moved to Chile to pursue his lifelong dream of becoming a comic book artist. Today, he and his wife, Angélica, create stories on a daily basis from their lovely home by the Pacific Ocean.

“Do. Or do not. There is no try.” – Master Yoda

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LIST OF ABBREVATIONS

AAD	American Association of Dermatology
AJCC	American Joint Committee on Cancer
AM-RCM	Arm-mounted reflectance confocal microscopy
BAD	British Association of Dermatology
BCC	Basal cell carcinoma
iBCC	Infiltrating BCC
nBCC	Nodular BCC
sBCC	Superficial BCC
CI	Confidence interval
CSD	Cumulative solar damage
DRFS	Distant recurrence-free survival
DM	Desmoplastic melanoma
DS-HH-RCM	Dermoscopy-handheld reflectance confocal microscopy
ESMS	European Society for Mohs Micrographic Surgery
FN	False-negative
FP	False-positive
FNAC	Fine needle aspiration cytology
HH-RCM	Handheld reflectance confocal microscopy
ICD-O	International Classification of Diseases for Oncology
IHC	Immunohistochemistry
IMQ	Imiquimod
IQR	Interquartile range
LM	Lentigo maligna
LMM	Lentigo maligna melanoma
LN	Lymph node
MMS	Mohs micrographic surgery
MSS	Melanoma-specific survival
MINORS	Methodological Index for Non-Randomized Studies
MIS	Melanoma in-situ
NNE	Naked-eye examination
NPV	Negative predictive value
NKI-AVL	The Netherlands Cancer Institute – Antoni van Leeuwenhoek

NM	Nodular melanoma
NNT	Number-needed-to-treat
NVDV	Nederlandse Vereniging voor Dermatologie & Venereologie
OCT	Optical coherence tomography
OR	Odds ratio
OS	Overall survival
PALGA	Nationwide network and registry of histo- and cytopathology in the Netherlands
PPV	Positive predictive value
PRAME	Preferentially expressed antigen in melanoma
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
PRISMA-P	PRISMA for Protocols
PROSPERO	International Prospective Register of Systematic Reviews
RCM	Reflectance confocal microscopy
RR	Relative risk
RRFS	Regional recurrence-free survival
SD	Standard deviation
SE	Staged excision
SEER	Surveillance, Epidemiology, and End Results
SLNB	Sentinel lymph node biopsy
SSM	Superficial spreading melanoma
TN	True-negative
TP	True-positive
UV	Ultraviolet
WHO	World Health Organization
WLE	Wide local excision


CHAPTER
1

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

SKIN CANCER

Skin cancer has become the most common malignancy among light-skinned populations worldwide, with incidence rates continuing to rise rapidly.[1] Currently, keratinocyte carcinomas constitute the majority (90%) of skin cancer patients. Melanoma accounts for approximately 10% of all skin cancer cases. Several other types of skin cancers, including Merkel cell carcinoma and adnexal carcinomas, are exceedingly less common.[2-5]

The Netherlands Cancer Registry has been collecting national data for all patients newly diagnosed with skin cancer since 1989. Before 2016, basal cell carcinoma (BCC) data were extrapolated based on the population composition from two Dutch regions. However, since 2016, the national registration of all the first and subsequent BCC cases has allowed for a comprehensive understanding of skin cancer epidemiology in the country. Annually, 70,000 new cases of skin cancer are diagnosed in the Netherlands. Based on direct hospital care costs, skin cancer ranks fourth among the most expensive cancers in the Netherlands.[6] Although a modest decline in skin cancer incidence has been observed among younger individuals, the overall rates are predicted to increase owing to the persistently aging population. In addition to the aging population, increased UV exposure, improved registration, and increased patient awareness contribute to an increased incidence of skin cancer. By 2027, the incidence of skin cancer in The Netherlands is projected to increase by 35% compared with that in 2019. This anticipated rise will considerably influence national healthcare expenditures and intensify healthcare professionals' demand for early detection, intervention, and follow-up care.

Skin cancer has a multifactorial etiology, with various phenotypic and genotypic predispositions that interact with environmental risk factors. Patient phenotype characteristics associated with the highest risk of developing skin cancer include increasing age, light eye color, red or blond hair color, lighter skin types, and male sex.[7] Ultraviolet (UV) radiation from solar and artificial sources, such as tanning devices, are the primary environmental risk factors for cutaneous malignancies. UV radiation can cause skin cancer through several mechanisms.[8]

Three categories of UV exposure are recognized and distinguished based on intensity, duration, and frequency: i) acute, ii) intermittent, and iii) chronic UV exposure. Acute UV exposure refers to brief, high-intensity exposure resulting in erythema and sunburn. Intermittent UV exposure is generally of medium to high intensity and occurs sporadically (e.g., during outdoor activities, holidays, or artificial sources such as indoor tanning). Chronic UV exposure involves prolonged and continuous exposure, leading to cumulative skin damage and is prevalent among individuals who work outdoors or reside in regions with high UV exposure.

The head-and-neck region is particularly susceptible to developing skin cancer as it is exposed to all forms of UV radiation. The primary challenges in treating skin cancer in this region are the complexity of anatomy and cosmesis. Surgery is often the preferred treatment, but it may not always be feasible owing to potential functional or cosmetic consequences. Multidisciplinary decision-making involving dermatologists, head-and-neck surgeons, plastic surgeons, radiotherapists, medical oncologists, and pathologists is often necessary to provide the best possible outcomes for patients with (advanced) skin cancer of the head-and-neck.

The research presented in this thesis focuses on head-and-neck skin cancer, with a specific emphasis on i) lentigo maligna (melanoma), a subtype of melanoma that typically presents in this anatomical region, and ii) basal cell carcinoma, the most common form of skin cancer, also with a predilection for the head-and-neck.

LENTIGO MALIGNA (MELANOMA)

Epidemiology

Lentigo maligna (melanoma) (LM/LMM) comprises approximately 4-15% of all melanomas and 10-26% of melanomas in the head-and-neck.[9] Like all skin cancers, the annual incidence of LM and LMM is increasing. According to Dutch national statistics, from 1998 to 2013, the European standardized rate (ESR) for both LM and LMM increased from 0.72 to 3.84 and 0.24 to 1.19 per 100,000 person-years, respectively.[10] The maximum incidence of 60/100,000 person-years is observed in individuals aged 80-84, with a relatively modest increase in incidence for those born after 1955.[11] Parallel trends are also noted in the United States, where the proportion of LMM seems to be rising faster than that of other invasive melanoma subtypes.[12]

Etiology & Risk Factors

First identified by Sir Jonathan Hutchinson in 1980 as an “infective senile freckle”, lentigo maligna (LM) was initially believed to have an infectious origin due to its slow growth. However, in 1912 Dubreuilh classified it as a precancerous melanosis common in the elderly.[13] Today, its universally accepted terminologies are melanoma in-situ, lentigo maligna type, and lentigo maligna melanoma (LMM) for its invasive counterpart.

Historically, the subtyping of cutaneous melanoma has relied solely on morphological criteria. However, a new classification system that considers molecular profiles was proposed by the World Health Organization (WHO) in 2018.[14] Two melanoma pathways were identified

based on their etiological relationship with cumulative solar damage (CSD). Non-CSD melanomas, including Spitz, acral, and mucosal melanomas, exhibit little to no association with UV exposure. Within CSD-associated melanoma, a further distinction is made between low-CSD (e.g., superficial spreading) and high-CSD melanoma, encompassing LM/LMM and desmoplastic melanoma. High-CSD melanomas typically present on chronically sun-damaged skin and carry specific NRAS, NF1, KIT, and non-V600E BRAF mutations.[15, 16] Due to their more robust association with high UV exposure, they exhibit a higher mutational load than other melanoma subtypes.

Clinical Presentation & Histology

The average age at presentation for patients with LM/LMM is between 65 and 70 years, and patients are typically a decade older than those with other melanoma subtypes.[17] The gender ratio appears to have a slight male predominance.[9] Due to the association with chronic UV exposure, LM/LMM typically develops in the head-and-neck region, with an increased density in the central facial area, ears, and scalp.[18]

Clinically, LM/LMM presents as an asymmetric, varied pigmented macule with an irregular and often poorly demarcated border (**FIGURE 1**). Less common, hypo- or amelanotic lesions also occur, especially following destructive therapies such as cryotherapy or laser treatment. Up to 13% of patients report a history of cosmetic treatment, such as laser and cryotherapy, before diagnosis.[19, 20] Given that the radial growth phase can persist for decades, there seem to be both patient and diagnostic delays, leading to larger lesion sizes at presentation. Dermal invasion with vertical growth can result in the development of a papule or nodule. Nonetheless, several studies indicate that clinical features, including lesion size, frequently fall short in predicting (micro)invasive dermal invasion, although lesions on the scalp or neck might have an increased risk of invasion at presentation.[21, 22]

On histopathology, LM exhibits a lentiginous proliferation of atypical nevoid to small epithelioid melanocytes along the dermo-epidermal junction, which is (focally) confluent (**FIGURE 2**).[23] Follicular involvement is common (96%) and typically confined to the infundibulum. However, extension to sebaceous lobules has also been observed.[24] More advanced lesions reveal nesting and upward pagetoid scatter.[25, 26] Dermal invasion is often characterized by a proliferation of spindle cells, which can appear as individual cells, bundles, or, more rarely, in a desmoplastic fashion.[27]

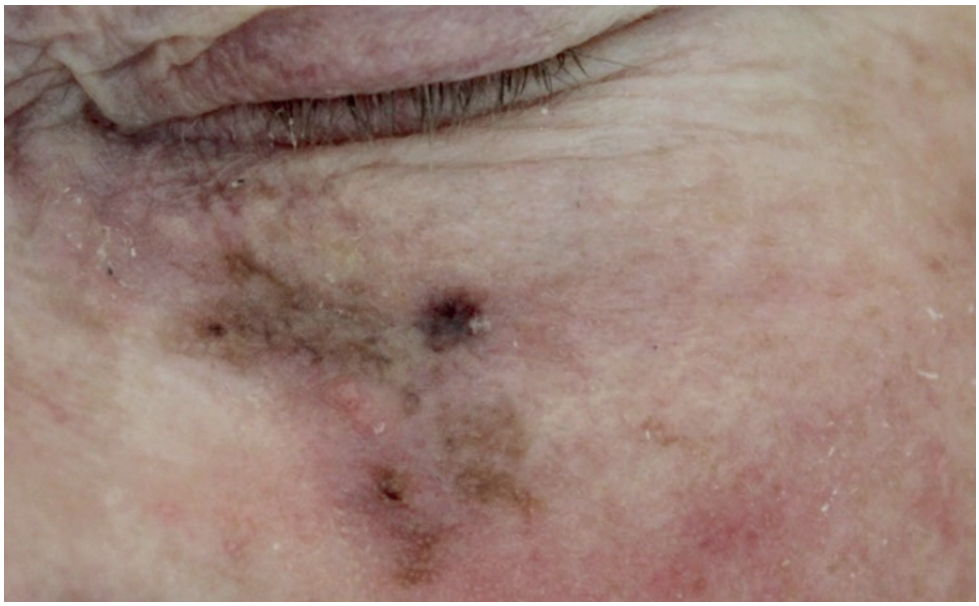

FIGURE 1. *Lentigo maligna presenting as an asymmetric, irregularly pigmented, poorly demarcated macule below the left eye.*

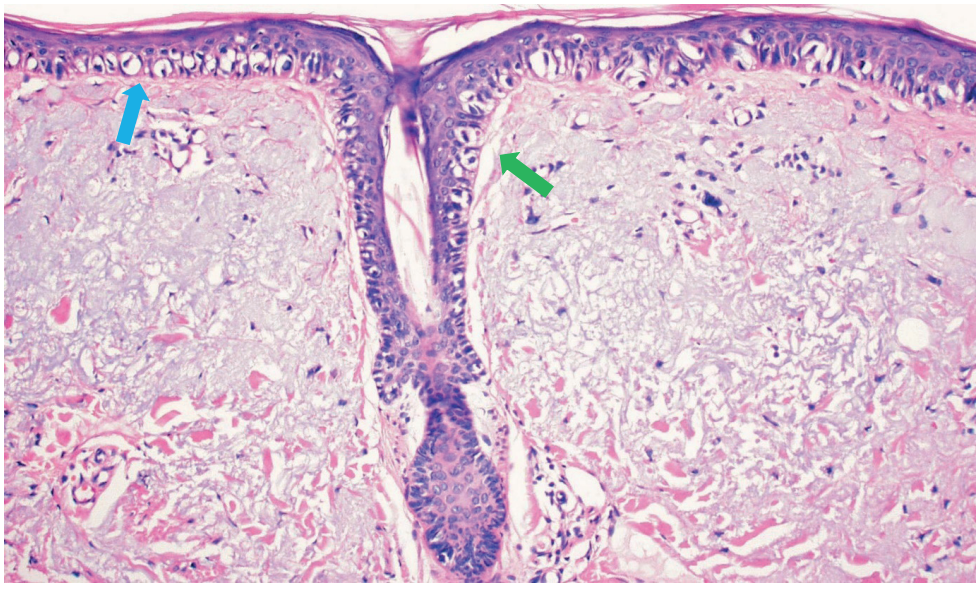

FIGURE 2. *Histology of lentigo maligna with a lentiginous proliferation of atypical melanocytes along the dermo-epidermal junction (blue arrow) with flattening of the rete ridges (red dashed line) and extension along the follicular epithelium (green arrow). [@Copyright Prof Peter Heenan]*

Identifying atypical intraepidermal melanocytes within chronically sun-damaged skin can pose a diagnostic challenge, particularly in low-grade LM or margin assessment.[28] This can be attributed to several factors: i) actinic melanocytic hyperplasia and ii) melanized (atypical) keratinocytes, as found in pigmented actinic keratosis and solar lentigo. Furthermore, "skipped lesions" may be present due to a discontinuous growth pattern/ field cancerization.[29] Numerous melanocyte markers have been established to assist in diagnostic and margin evaluation and are utilized in immunohistochemical staining to aid in the histological diagnosis.[28, 30, 31]

BASAL CELL CARCINOMA

Epidemiology

Basal cell carcinoma (BCC) is a significant public health concern worldwide, with the highest incidence in Australia, where one in two inhabitants is diagnosed with BCC.[1] It accounts for approximately 70% of all skin cancers in the Netherlands. Currently, 1 out of 5-6 in the Dutch population develops one or more BCC in their lifetime, with a 1,5-2:1 male-to-female ratio. The annual incidence of first-time BCC diagnoses has seen a marked increase, from 17.000 in 2001 to 49.000 in 2019, highlighting the importance of effective prevention strategies such as awareness campaigns and early identification of patients at increased risk.[32] Furthermore, 25% of patients with an initial BCC diagnosis develop one or more subsequent BCCs within three years.[33] However, these numbers likely represent an underestimation as up to 24% of BCC are not histologically confirmed and are directly treated by nonsurgical treatment modalities.[34, 35] Current projections indicate that within the next 10 years, the incidence of BCC will increase by 30% for men and 25% for women. This expected growth, however, is confined to patients aged 50 years or older, as recent data suggest that incidence rates have stabilized or even declined among <60-year age groups, possibly reflecting the effectiveness of public health campaigns to increase awareness of skin cancer prevention by UV exposure protective measures.[32]

Etiology & Risk Factors

Basal cell carcinoma was first described by Sir Arthur Jacob, an Irish surgeon and ophthalmologist, in 1827 as an "ulcus rodens". The Hedgehog signaling pathway's pivotal role in BCC development was published in 1996 after its discovery in the 1980s.[36] The primary involved genes are the tumor suppressor gene PTCH1 and SMO gene, which induce cell activation and proliferation. In sporadic BCC, 67–90% show somatic inactivating mutations in PTCHT1 and 10–20% show activating mutations in SMO. In addition, 40% to

65% of sporadic BCC contain mutations in the TP53 suppressor gene, often with UV signature mutations (e.g., C-T substitutions at a dyprimidine site).[37]

Intermittent and acute UV exposure seems to be the most vital environmental risk factors, as 72% of BCC mutations have a UV-induced signature.[38] Indoor tanning devices typically produce UVA and are an independent risk factor with a relative risk (RR) of 1.24 (95% CI 1.00–1.55). This effect increases further when considering early-onset BCC (<50 years of age) (RR 1.79), exposure at ≤20 years of age (RR 1.86), and >10 tanning sessions a year (RR 1.46).[39] The population-attributable risk fraction of indoor tanning is estimated to be around 4.0% for BCC.[40]

Occupational (e.g., pilots) or medically administered ionizing radiation is an additional risk factor, leading to a threefold increased risk and a latency of >20 years.[38] Although cutaneous squamous cell carcinoma is the most common malignancy in solid organ transplant patients receiving chronic immunosuppressants, the incidence of BCC is also increased (10-fold) in this population and increases significantly over time.[38, 41] Other risk factors include arsenic exposure and extremely high-risk genodermatoses, such as xeroderma pigmentosum (autosomal recessive DNA repair mutation), basal cell nevus syndrome (autosomal dominant mutation in the sonic hedgehog signaling pathway), and albinism (genetic disorders leading to disturbed melanin synthesis).

Clinical Presentation & Histology

Basal cell carcinoma can be an indolent or locally invasive tumor that results in tissue destruction. Although metastatic BCC is rare (<0.1%), locally aggressive BCC can cause considerable morbidity when left untreated, particularly in the head-and-neck.[42,43] Consequently, early detection and treatment of BCC are crucial for appropriate BCC management. Several clinical BCC subtypes have distinct histological correlates (**TABLE 1)** and additional less common subtypes have been described in the literature.[44] However, based on growth patterns, behavior, and therapeutic consequences, BCC can be divided into three subgroups: superficial, nodular, and infiltrating subtypes.

Overall, 40% of all BCC develop in the head-and-neck, with the highest relative tumor density (i.e., proportion of tumors to proportion of skin surface area) in the most sun-exposed areas of the facial region.[45] Diagnosis of the correct histological subtype is essential for BCC management, as it allows for i) choice between surgical and nonsurgical treatment and ii) determination of the optimal surgical margins and technique. The most common BCC subtype (estimated 50-80%) is nodular BCC (nBCC) (**FIGURE 3**), of which 90% occur in the head-and-neck.[44] Similarly, while less common (estimated 6%), the majority (95%)

of high-risk infiltrating BCC (iBCC) subtypes (**FIGURE 4-5**) also occur in the head-and-neck. Superficial BCCs (sBCC) (**FIGURE 6**), on the other hand, are far more common on the trunk and extremities (60%).[46] One should consider, however, that 18-74% of BCC are of mixed histology with multiple histological subtypes.[47]

TABLE 1. Clinical characteristics and histopathological correlates of basal cell carcinoma subtypes.[23, 44]

Subtype	Clinical features	Histopathology
Low-risk		
Superficial	Well-circumscribed, erythematous plaque or patch with scaling and slightly raised borders	Superficial lobules of basaloid cells that project from the epidermis into the papillary dermis, surrounded by retraction spaces and loose myxoid stroma
Nodular [A]	Shiny, pearly, elevated papule or nodule, often with raised borders that may become ulcerated	Dermally located islands of basaloid cells with peripheral palisading and surrounded by retraction spaces. It may consist of smaller basaloid lobules connected by fibromucinous stroma.
High-risk [B]		
Infiltrative	Poorly defined, indurated, flat or depressed, pale to erythematous plaque	Variably sized, jagged nests of basaloid cells with scant cytoplasm without fibrosis.
Micronodular	Erythematous or whitish macules or thin papules/plaques	Small round basaloid nodules that appear to be separated by normal collagen.
Morpheaform	Infiltrated, shiny, scar-like plaque with poorly defined borders	Thin cords of tumor embedded in a dense sclerotic collagenous stroma.
Basosquamous	Basal cell carcinoma with squamous differentiation (i.e., keratin mass, surface scaling, white structureless areas)	Islands of basaloid cells with atypical squamous cells.

[A] Variants: keratotic, (nodulo)cystic, adenoid.
[B] Can extend irregularly into the deep dermis and subcutis.
Other histopathological subtypes include adnexal differentiation, fibroepithelial, and sarcomatoid.

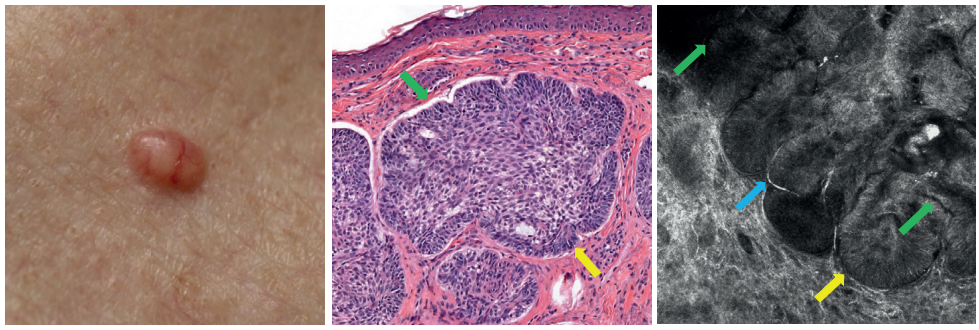

FIGURE 3. Nodular basal cell carcinoma (low risk). *Clinically (left) presenting as a shiny, pearly, elevated papule with visible telangiectasia. On histology (middle), this subtype consists of dermally located islands of basaloid cells with peripheral palisading (yellow arrow) surrounded by retraction spaces (green arrow). Correlating image on reflectance confocal microscopy (right) showing large (>300 um) basaloid nests with peripheral palisading (yellow arrow), retraction clefts (green arrow), and peritumoral collagen bundles (blue arrow).*

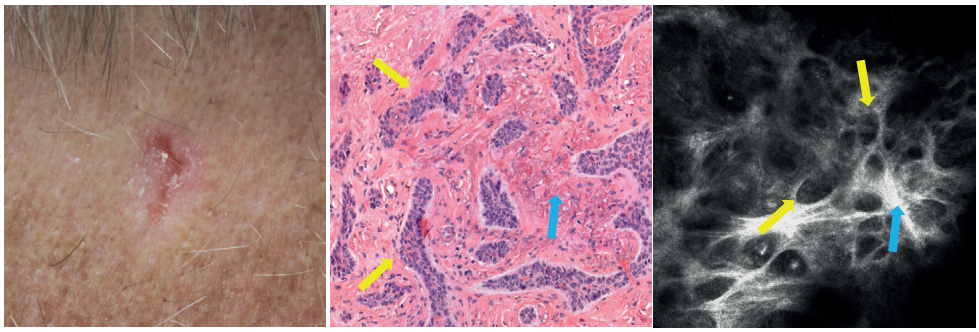

FIGURE 4. Infiltrative basal cell carcinoma (high risk). *Clinically (left) presenting as a poorly defined, indurated, depressed, pale to erythematous plaque. On histology (middle), this subtype consists of dermally located variably sized, jagged nests of basaloid cells (yellow arrow) with scant cytoplasm without fibrosis (blue arrow). Correlating image on reflectance confocal microscopy (right) showing small (<300 um) dermally located angulated dark silhouettes (yellow arrow) bordered by dense collagen bundles (blue arrow).*

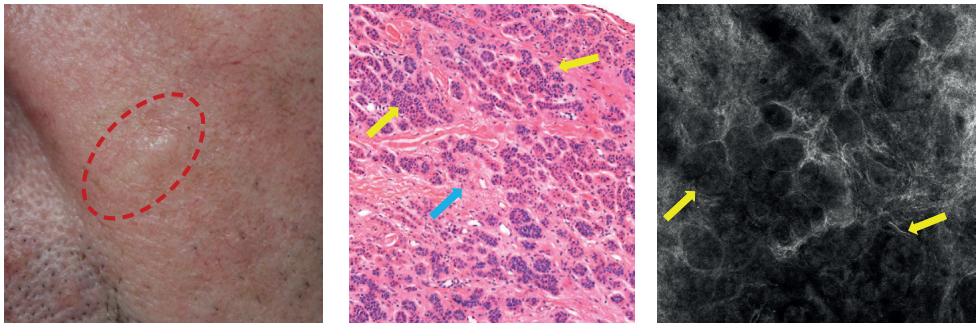

FIGURE 5. Micronodular basal cell carcinoma (high risk). *Clinically (left) presenting as poorly defined, indurated, whitish plaque. On histology (middle), this subtype consists of dermally located small and roundish basaloid nodules (yellow arrow) that appear to be separated by normal collagen (blue arrow). Correlating image on reflectance confocal microscopy (right) showing small (<300 um) dermally located round, grouped, dark silhouettes (yellow arrow).*

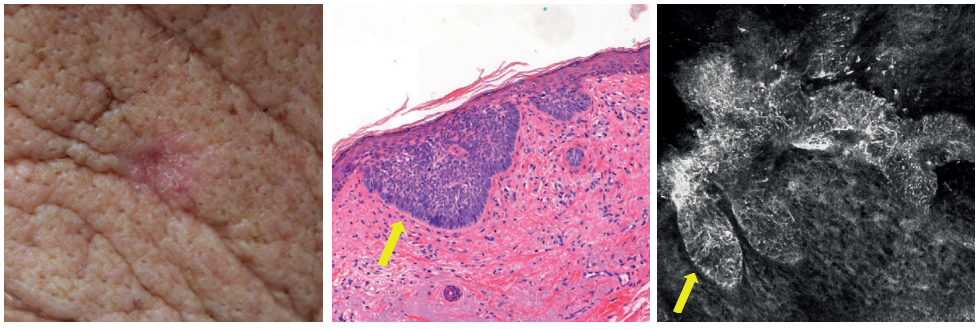

FIGURE 6. Superficial basal cell carcinoma (low risk). *Clinically (left), presenting as a well-circumscribed, erythematous plaque with scaling and slightly raised borders. On histology (middle), this subtype consists of superficial lobules of basaloid cells (yellow arrow) that project from the epidermis into the papillary dermis. The correlating image on reflectance confocal microscopy (right) shows large (>300 um), pigmented, multilobular basaloid nests connected to the epidermis with peripheral palisading (yellow arrow).*

DIAGNOSTICS - LOCAL

Dermoscopy

The technique. The clinical "naked eye" examination is the first diagnostic step during lesion screening for skin cancer. The refractive index of the skin's surface layer (stratum corneum) reflects light, resulting in surface glare. This reflection limits the assessment of deeper morphological features. After its introduction in the 90s, dermoscopy has become the foundation of skin cancer diagnostics in dermatologic practice.[48] Dermoscopy is a noninvasive, handheld diagnostic device with a magnification lens (x10-20) (**FIGURE 7**). More importantly, it reduces the refractive index, allowing horizontal visualization of epidermal and papillary dermal colors and invisible structures to the naked eye. This reduction in surface glare is achieved by either i) classic contact dermatoscopes using an immersion liquid or ii) more recently introduced dermatoscopes using a polarized light source that does not require direct contact with the skin. As both techniques result in different qualitative images, they are considered complementary. Colors seen in dermoscopy depend on the type of chromophores (e.g., melanin, keratin, collagen, blood) present in the skin and their localization.[49] The resulting patterns provide an invaluable increase in diagnostic information that often has direct histological correlates.[49] Implementing dermoscopy into clinical practice has significantly increased diagnostic accuracy for both pigmented and nonpigmented skin cancer detection compared to visual inspection alone: it lowers the threshold for early detection (sensitivity: 92% vs. 75%) while reducing the number of benign biopsied lesions (specificity: 95% vs. 75%).[50, 51]

FIGURE 7. *A handheld dermatoscope (DermLite® DL5) with a 10x magnification lens and cross-polarized and nonpolarized LED illumination. [©Copyright (2024) with permission from DermLite LCC].*

Lentigo maligna (melanoma). The accuracy of dermoscopy in diagnosing facial pigmented lesions in sun-damaged skin continues to pose a substantial clinical challenge. Conventional dermoscopic melanoma criteria are rarely present, primarily due to the flattening of the rete ridges (epithelial extensions projecting into the dermis) in the facial skin, resulting in a so-called "pseudonetwork" consisting of structureless pigmentation interrupted by openings of the adnexal structures (**FIGURE 8**). Following the classic 'Stolz criteria' publication in 2000, various other LM/LMM have been described.[52-54] However, most of these features can also be present in benign non-melanocytic lesions such as pigmented actinic keratosis, solar lentigo, and lichen planus-like keratosis.[55-57] Nonetheless, a dermoscopic progression model was proposed to diagnose LM/LMM (**FIGURE 9**).[54] Initially, early lesions usually exhibit very subtle and easily overlooked dermoscopic features, leading to the expert recommendation that diagnosis should not rely solely on the presence of LM-specific features but instead on the absence of definitive benign traits ('inverse approach').[58] More advanced lesions will eventually exhibit structureless black areas with dermoscopic obliteration of the adnexal structures, a dermoscopic feature highly indicative of LM/LMM. However, it has been demonstrated that this characteristic does not directly correlate with histological dermal invasion. Therefore, histopathological confirmation remains essential.

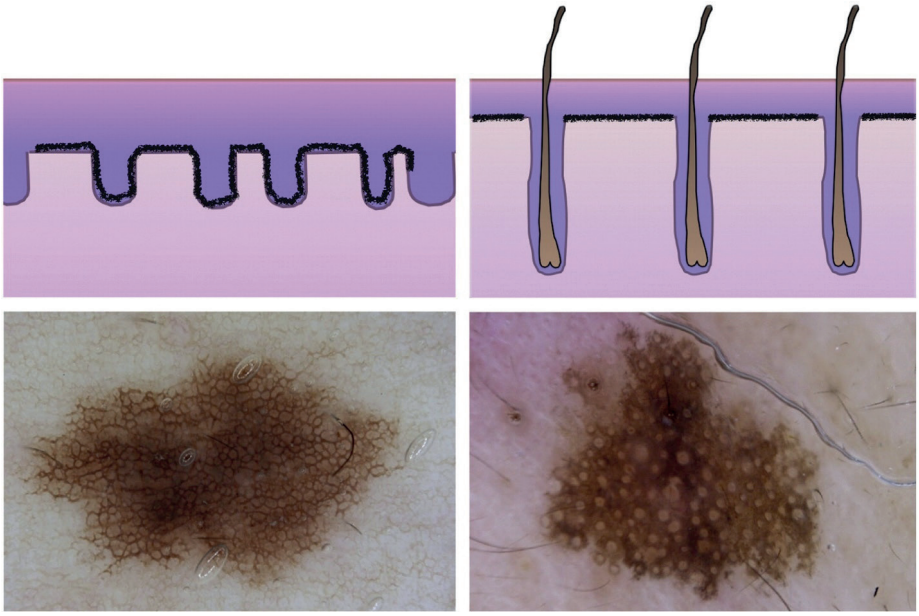

FIGURE 8. *Dermatoscopic-histopathologic correlates of the pigment network (left upper and lower images) and facial pseudonetwork (right upper and lower images). The true pigment network (left) correlates with pigmentation along elongated rete ridges (green arrow), whereas the pseudonetwork (right) results from structureless brown pigmentation (green arrow) interrupted by nonpigmented follicular openings. [Reprinted with permission from the publisher of Clinics in Dermatology, Vol 32 / Issue 1, Lallas A, Argenziano A, Moscarella E, et al. Diagnosis and management of facial pigmented macules. Page 94-100. Copyright (2014) with permission from Elsevier.]*

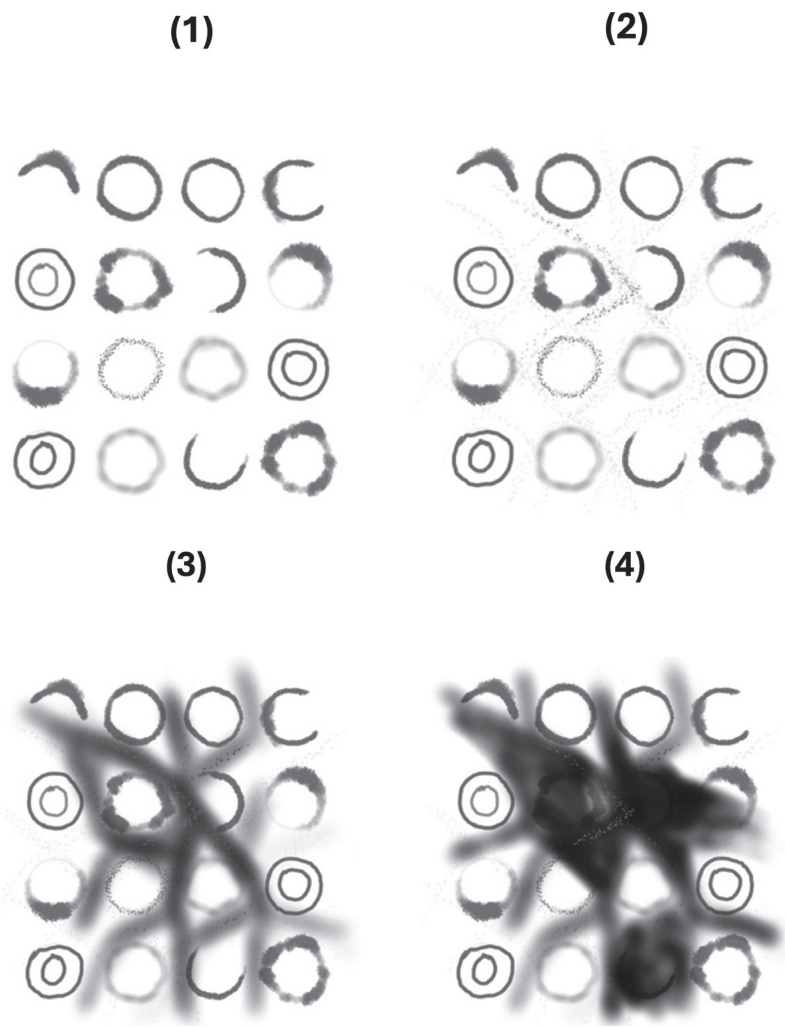

FIGURE 9. *Dermoscopic progression model for lentigo maligna.*[54]

The progression model is shown from left to right, each containing follicular openings (circles) surrounded by varying degrees of (asymmetric) pigmentation, resulting in a pseudonetwork as shown in FIGURE 8 (right side). From left to right:

(1) Hyperpigmented follicular openings: several patterns of (a)symmetrical pigmented circles surrounding the adnexal openings can be observed: fine circles, semi-circles, signet ring-like circles, irregular circles, and double circles. Histopathology: nonuniform infiltration of atypical melanocytes along the follicular epithelium.

(2) Annular-granular pattern: addition of fine brown or blue-gray dots aggregated around the adnexal openings and short polygonal lines around and in between the adnexal structures. Histopathology: aggregates of atypical melanocytes and small nests of melanocytic nests at the dermo-epidermal junction between the adnexal openings (brown) and dermal melanophages (blue-gray). The polygonal lines correspond with confluent junctional nests and aggregates of melanocytes.

(3) Pigmented rhomboidal structures: elongation, broadening, and merging of the polygonal line result in more complex polyhedral-shaped structures due to increased pigmentation. Histology: more extensive infiltration of the dermo-epidermal junction by confluent nests and aggregates of melanocytes.

(4) Dark blotches and obliterated hair follicles: structureless/homogenous pigmented areas initially with sparing of the follicular openings and eventually with obliteration. Histology: further proliferation of follicular involvement and/or dermal invasion.

Basal cell carcinoma. Over 10 dermoscopic BCC criteria have been identified (**FIGURE 10**).[59-61] Comparative studies have shown that dermoscopy increases sensitivity from 67% to 85% compared to naked eye examination alone, with an overall sensitivity and specificity of 91% and 95%, respectively.[62] Despite the varying prevalence of the dermoscopic criteria among BCC subtypes, no single criterion is completely unique to any specific subtype.[63] It is worth noting that high-risk and mixed-type BCC often exhibit even less defined and consistent dermoscopic criteria.[64] As a result, punch biopsies are routinely performed in the head-and-neck region for definite subtyping.

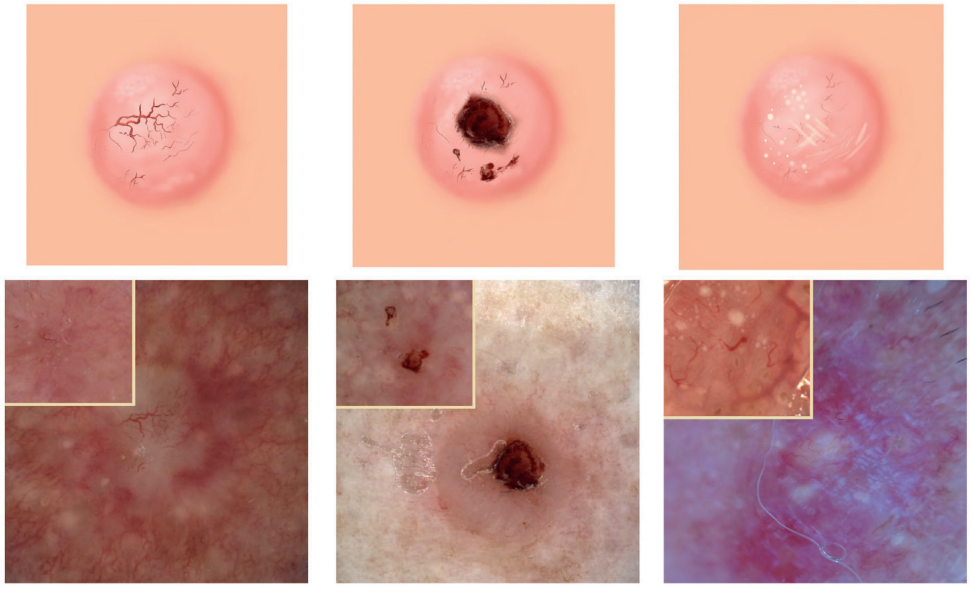

FIGURE 10. *Schematic (top) and dermatoscopic (bottom) images of the main basal cell carcinoma diagnostic features. Left: arborizing and linear vessels (box). Middle: ulceration and multiple small erosions (box). Right: polarized white streaks and may globules (box). [©LoesVos – FOKS design]*

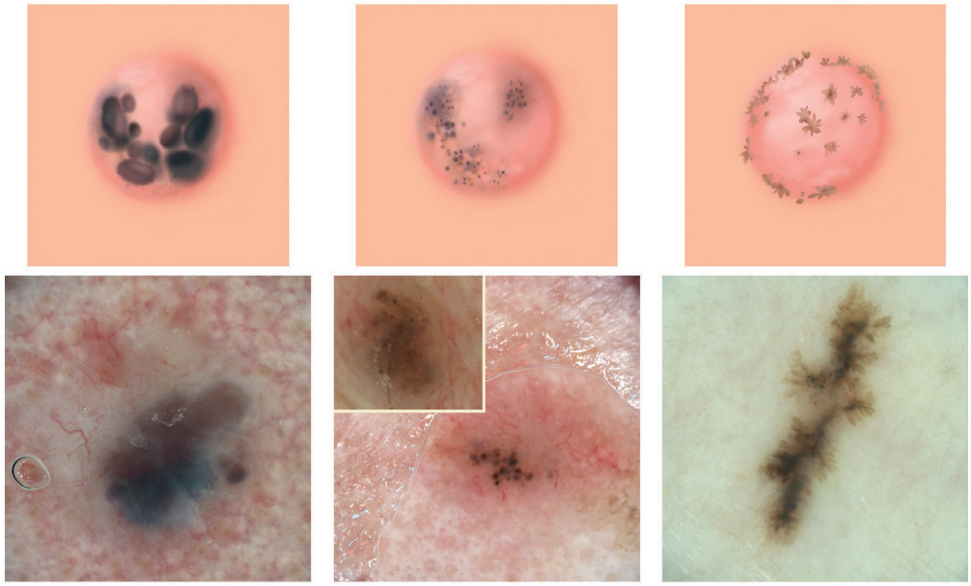

***FIGURE 10 (continued)**. Left: blue-gray ovoid nests. Middle: multiple blue-gray globules and in-focus dots (box). Right: spoke wheel/maple leaf-like areas and concentric structures. [©LoesVos – FOKS design]*

Reflectance confocal microscopy

Despite significant improvements in diagnostic accuracy brought about by dermoscopy, a diagnostic grey area with reduced sensitivity or specificity still exists, for which histological examination has long been the only solution. Various skin imaging devices are increasingly being advocated to bridge this diagnostic gap, including reflectance confocal microscopy (RCM), optical coherence tomography, and line-field confocal optical coherence tomography.[65, 66] RCM is primarily used as an add-on diagnostic tool in the diagnostic workflow to evaluate clinical/dermoscopic equivocal lesions to decrease the false-positive and false-negative rates, enhance diagnostic confidence, and enable noninvasive detection of subclinical disease.[67]

The technique. RCM is a noninvasive imaging technique that allows the visualization of cutaneous structures in vivo at a resolution that resembles histology. It generates images in the horizontal plane, reaching the superficial reticular dermis (250 μm depth), allowing for a direct correlation between dermoscopy and histopathology (**FIGURE 11**). This technique uses a low-power near-infrared laser (830 nm) to penetrate the skin. The reflected light is filtered through a pinhole to reject out-of-focus planes (confocal principle), based on the refractive index of the cellular components at the desired focal point. This process produces grayscale horizontal images, offering a field of view ranging from to 500-750 square μm

(equivalent to 350-550x magnification), depending on the device. The optical resolution is <1.25 μm in the horizontal plane and <5 μm in the vertical plane (**FIGURE 12**).

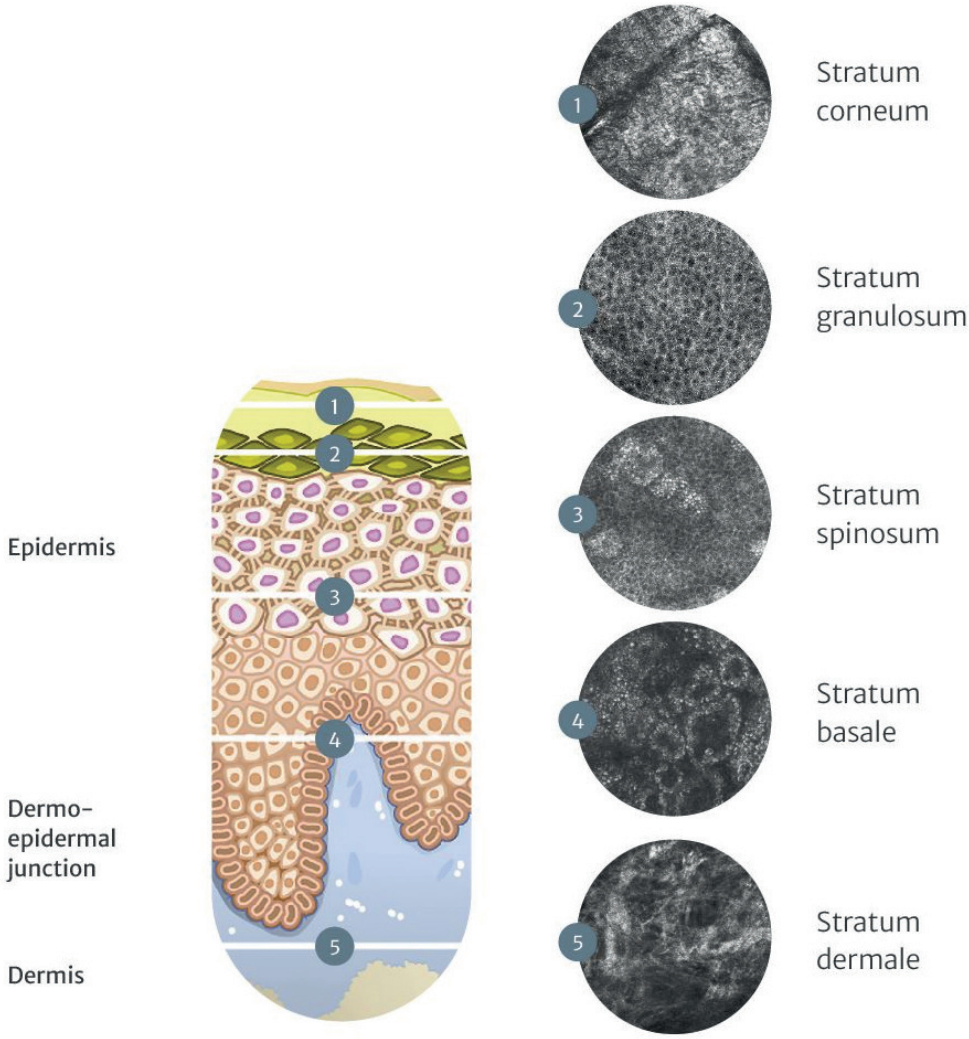

FIGURE 11. *Schematic overview of the skin up to the level of the superficial dermis with corresponding horizontal cross-sectional reflectance confocal microscopy images of the different layers (#1-5). [©VivaScope GmbH Confocal Microscopy / Oliver Gündisch].*

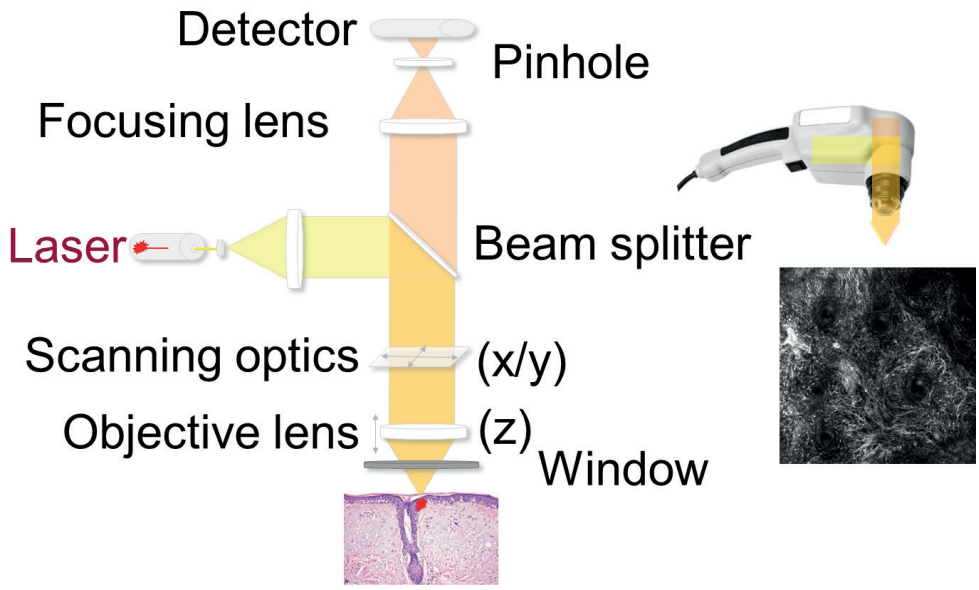

FIGURE 12. *Schematic overview of the reflectance confocal microscopy principle. The red dot in the image represents the current focal point, resulting in a black-and-white image (beige arrow) on the horizontal plane at the level of the dermo-epidermal junction.*

The traditional device (**FIGURE 13**, left) includes an arm-mounted probe (AM-RCM; VivaScope®1500; VivaScope GmbH, München, Germany) for automated image acquisition; however, it requires proper patient positioning and is relatively time-consuming. Additionally, its application in concave areas of the head-and-neck is limited because of the need for fixation using an adhesive ring. The handheld-RCM (HH-RCM) device (**FIGURE 13**, right) features a smaller probe that is not fixated (VivaScope®3000; VivaScope GmbH, München, Germany). This handheld device offers greater flexibility, enabling faster evaluation and improved access to concave areas such as the nose and periorbital region.

FIGURE 13. *Reflectance confocal microscopy system (©VivaScope 2024) with the arm-mounted probe (left) (VicaScope 1500®), handheld device (right) (VivaScope 3000®), and digital dermatoscope (bottom-left) (VivaCam®) [©VivaScope GmbH Confocal Microscopy / Oliver Gündisch].*

Lentigo maligna (melanoma). After identifying diagnostic RCM features (**FIGURE 14**), Guitera et al. proposed a diagnostic algorithm (**TABLE 2**) to differentiate LM/LMM from other equivocal pigmented facial macules.[68] This algorithm has demonstrated 85% sensitivity and 76% specificity. It has been proven to be equally effective when applied to amelanotic or hypomelanotic lesions.[69] RCM increases diagnostic confidence when directly compared to dermoscopy and is more sensitive (80% vs. 61%) but has a slightly lower specificity (81% vs. 92%). When combining dermoscopy with RCM, however, the highest overall diagnostic accuracy of 84% is achieved, compared to 75% and 80% for dermoscopy and RCM alone, respectively.[70]

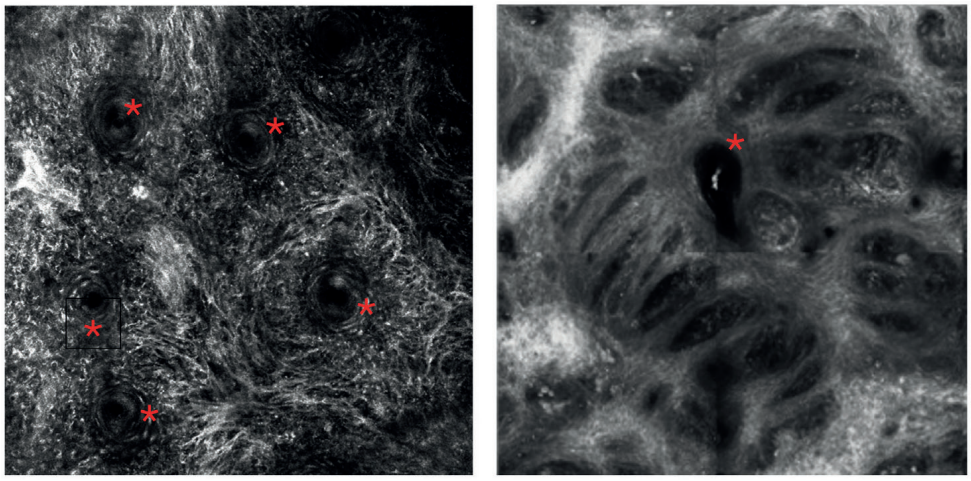

FIGURE 14. Lentigo maligna as seen on reflectance confocal microscopy. *Proliferation of perifollicular (*) atypical dendritic cells at the level of the dermo-epidermal junction (left) and atypical junctional thickening arranged around a hair follicle (*) giving rise to a "medusa head-like" appearance (right).*

TABLE 2. In vivo reflectance confocal microscopy diagnostic features for facial lentigo maligna

LM Score[68]	**Major features (+2)** ▪ Non-edged dermal papillae ▪ Round & large (>20 µm) pagetoid cells **Minor features (+1)** ▪ >3 atypical cells at the dermo-epidermal junction in 5 images ▪ Follicular localization of pagetoid/atypical cells ▪ Nucleated cells within the dermal papillae **Minor negative feature (-1 point)** ▪ Broadened honeycomb pattern
Margin assessment[71, 72]	▪ Single large round or dendritic perifollicular cells ▪ Atypical dendritic cell (any size) continuing from the trailing edge

LM: lentigo maligna
Score of ≥ 1: sensitivity 93% and specificity 51%
Score of ≥2: sensitivity 85% and specificity 76%

In contrast to other melanoma subtypes, LM/LMM diagnosis often relies on the partial sampling of the lesion. Smaller biopsy samples can pose a challenge to pathologists because of the significant histopathological heterogeneity within a single lesion. RCM, being able to evaluate the entire lesion, is a valuable tool in selecting the most appropriate biopsy site featuring the most representative histopathologic diagnostic features.[73] Detection of subclinical LM is notoriously unreliable, given the proven inaccuracy of naked eye and dermoscopic examinations in this context.[74] However, RCM has demonstrated its ability to detect subclinical disease beyond dermoscopic visible margins.[75] Therefore, RCM can provide crucial in vivo diagnostic data on the presence and extent of subclinical disease.[76, 77]

Basal cell carcinoma. Approximately 40% of basal cell carcinomas (BCCs) are comprised of two or more subtypes. A systematic review by Kadouch et al. revealed a considerable inconsistency between biopsy and excisional specimens, with a more aggressive BCC subtype detected in 15% of excisional specimens.[47] This was further supported by a prospective study comparing biopsy with Mohs' excisional specimens, wherein 20% of cases failed to identify a high-risk BCC subtype in the initial biopsy. Consequently, there is a risk of underestimating BCC subtypes through punch biopsy because the sample represents only a portion of the tumor. In a recent meta-analysis, the pooled sensitivity and specificity of RCM for diagnosing primary BCC were determined to be 92% (95% CI, 87-95) and 93% (95% CI, 85-97), respectively.[78] The pooled sensitivity was similar when directly comparing RCM to dermoscopy, but dermoscopy resulted in a significantly lower specificity (53% vs. 80%).[79] Only a few studies have described criteria for distinguishing between different BCC subtypes (**TABLE 3**)[80, 81]; therefore, more extensive prospective studies are required to validate these criteria and determine the optimal placement of RCM in the diagnostic workflow in BCC management.

TABLE 3. Dermoscopic features for basal cell carcinoma subtype classification[13, 14, 16]

I. Superficial basal cell carcinoma
Non-pigmented ▪ Superficial fine branching vessels ▪ Multiple small erosions ▪ White to red structureless areas Pigmented ▪ Radial lines converging to a central dot/clod ▪ Radial lines connected to a common base ▪ Concentric structures (Absence of: arborizing telangiectasia, ulceration, blue-gray clods)
II. Nodular basal cell carcinoma
Non-pigmented ▪ "Classic" branched vessels ▪ Ulceration ▪ White (perpendicular) lines [A] Pigmented ▪ Blue/gray large clustered clods ▪ Multiple blue/gray clods
III. Infiltrative basal cell carcinoma
▪ "Unfocused" branched vessels [B] ▪ Ulceration ▪ White to red structureless areas ▪ Stellate pattern
IV. Morpheaform basal cell carcinoma
▪ "Unfocused" branched vessels[2] ▪ Ulceration ▪ White structureless areas

[A] Only visible with polarized dermoscopy.
[B] Lower caliber and less branching compared to the "Classic" branching vessels.

DIAGNOSTICS - REGIONAL

Following histological confirmation, melanoma is currently staged according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging system.[82] Preliminary staging is based on the Breslow thickness, presence of ulceration, and clinical assessment of the regional lymph nodes (LN).[83, 84] In cases without clinical evidence of LN metastases, sentinel lymph node biopsy (SLNB) is indicated for melanoma staged ≥T1b.[82] As regional draining LNs are the most common sites of metastatic melanoma, metastases in the lymphatic drainage area (stage III melanoma) are of major prognostic significance. Definite staging is subsequently based on histology of the (re)excision of the primary melanoma, histological confirmation of (sentinel) lymph node metastases, and/or imaging data.

Skin inspection for satellite or in-transit metastases and physical neck examination by palpation of the regional lymph nodes are fundamental yet simple first steps in the routine diagnostic workup. However, physical examination on its own fails to detect the majority of lymph node (LN) metastases (false-negative). Furthermore, not all palpable LN necessarily correspond to metastases (false-positive). Compared to ultrasound, palpation has a significantly lower discriminatory power, with an odds ratio (95% confidence interval) of 21 (95% CI 4-111) compared to 1755 (95% CI 726-4238) ($P<0.001$).[85]

Ultrasound with fine-needle aspiration cytology. Ultrasound is an inexpensive, noninvasive, and rapid diagnostic tool that can guide fine-needle aspiration cytology (FNAC) of suspected LN. Although SLNB can be bypassed following positive ultrasound-guided FNAC, there remains a risk of overlooking clinically occult microscopic metastases. A Cochrane systematic review yielded a pooled sensitivity of 18% (95% CI 3.6%-56.5%) and specificity of 99.8% (95% CI 99.1%-99.9%) for ultrasound-guided FNAC compared to histology (either SLNB or completion LN dissection).[86]

Sentinel lymph node biopsy. SLNB was introduced by Morton et al. in 1992, based on the concept of tracing lymphatic drainage from the primary melanoma site. The first LN involved by a tumor in the draining basin is considered the sentinel node. Since its inception, SLNB has undergone several technological evolutions, leading to increased SN detection.[87] Sentinel lymph node biopsy in the head-and-neck remains complex because of variable (bilateral) lymphatic drainage patterns and complex anatomic structures. The reported head-and-neck average SLNB-positivity rate for melanoma is 15% with a false negative rate of 20%.[88] It's worth noting that more experienced centers often report higher accuracy of the SLNB procedure (NKI-AVL experience: 21.3% SLNB and 11.9% false-negative rate).[87] Despite the lack of clear evidence demonstrating a survival advantage for patients undergoing SLNB, it remains a powerful prognostic tool and has increasingly important consequences for (neo) adjuvant-targeted or immunotherapy treatment strategies.[89, 90]

TREATMENT

Lentigo maligna (melanoma)

The management of LM is primarily aimed at mitigating its progression to LMM and is associated with higher local recurrence rates than other melanoma subtypes. Surgical excision is the recommended primary treatment for LM, as it allows complete histological evaluation for potential subclinical invasive disease and assessment of the histological margins.[91] In the case of LMM, surgical excision is advised according to international melanoma guidelines.[83] While numerous nonsurgical alternatives are available, it is difficult to compare their efficacy due to the lack of randomized controlled trials.

The most effective surgical approach for LM/LMM treatment remains controversial. International guidelines typically recommend wide local excision (WLE) with surgical margins of 5 mm for LM and 10 mm for LMM.[91, 92] Nevertheless, these margins have often proven insufficient, resulting in a 6-20% recurrence rate.[93] The increased risk of local recurrence can be attributed to subclinical spread, discontinuous growth, and transition to melanocytic hyperplasia towards the lesion periphery. Surgical margins of up to 10 mm are advocated for LM to increase tumor clearance.[92] Following excision, the tissue is analyzed using the "bread loaf" sectioning method, which involves systematic vertical sections of the central area, and both ends of the tissue specimen (**FIGURE 15**). However, as this method evaluates less than 0.5% of the resection margin, it poses a risk of false negative margin outcomes.[94] Data from Crouch et al. supports this, with local recurrence rates following WLE of 55% for incomplete excisions, 27% for lesions with a cleared histological margin $<$3mm, and 2.6% for those with $\geq$3mm.[95]

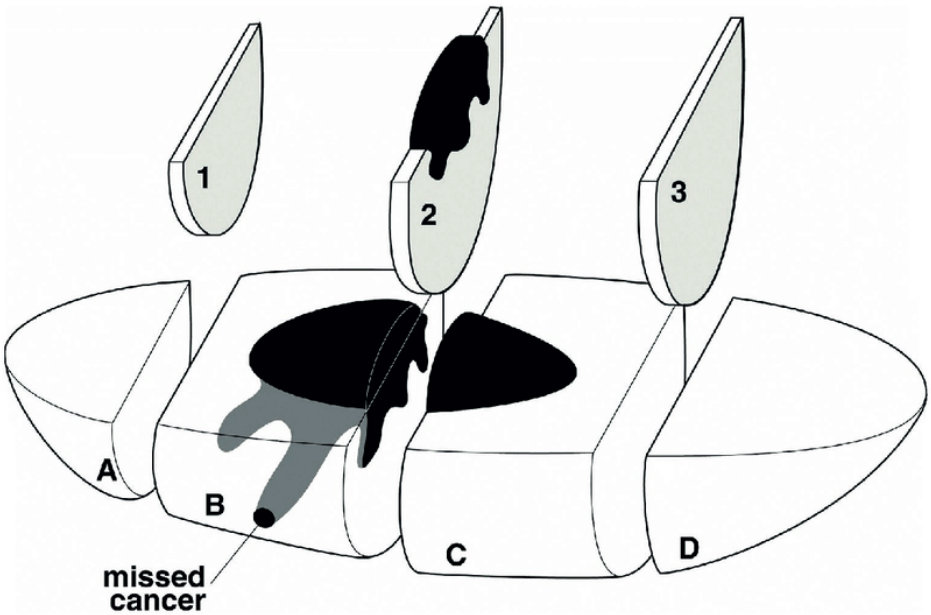

FIGURE 15. Vertical histological transverse sectioning of the tumor specimen ("bread loaf" method). *Due to limited sectioning, subclinical tumor extension at "B" is missed.*

[From Mohs Micrographic Surgery, Second Edition, edited by Stephen N. Snow and George R. Mikhail. Reprinted by permission of the University of Wisconsin Press. © 2005 by the Board of Regents of the University of Wisconsin System. All rights reserved.]

Several microscopically controlled staged excision (SE) techniques have been used in the surgical management of LM/LMM to optimize local control and minimize the risk of local recurrence. The foundational principle of these techniques allows evaluation of the entire peripheral margin by radial or longitudinal sectioning, thereby facilitating histology-guided surgery (**FIGURE 16**). To date, numerous variations have been published, such as Mohs' micrographic surgery, the "Square procedure," the "Spaghetti technique," and the "Breuninger method".[96-100] Despite the evident advantage of these techniques over WLE, there is no consensus on which iteration is the most effective as they all differ in tissue processing, sectioning, and availability of immunohistochemistry staining. Furthermore, in the context of LMM, it is unclear which surgical method offers the best survival benefit for disease-free, overall, or melanoma-specific survival. Lastly, in vivo, peri-operative reflectance confocal microscopy can be considered in detecting subclinical extension and assisting in determining surgical excision margins (as discussed in this thesis).[72, 76, 77, 101, 102]

For cases where surgery is not a viable option, including patients with advanced age, comorbidity, or specific lesion characteristics (such as size or localization), or when the patient prefers a nonsurgical approach, alternative treatment options may be considered.[91]

The second-line treatments are recommended based on empirical evidence include radiotherapy (RT) and topical imiquimod. Due to insufficient evidence supporting their effectiveness, destructive therapies like cryotherapy and laser therapy are generally not advised.[91, 103]

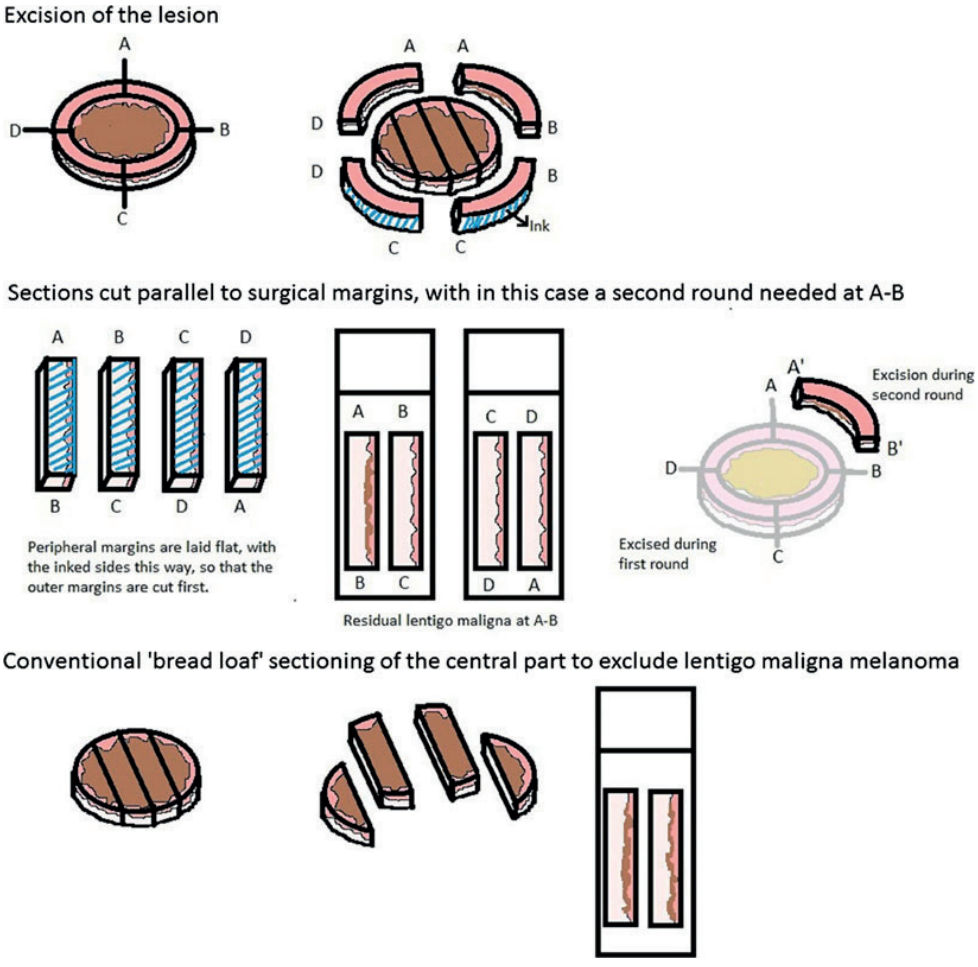

FIGURE 16. Micrographically controlled staged surgical margin excision of lentigo maligna with central debulking. *Sectioning and visualization of the peripheral margins and central portions of lesions. [Reprinted with permission from the British Journal of Dermatology, Vol 174 / Issue 3, de Vries K, Greveling K, Prens LM, et al. Recurrence rate of lentigo maligna after micrographically controlled staged surgical excision. Page 588-593. Copyright (2016) with permission from the Oxford University Press.]*

Radiotherapy (RT) can be used as a monotherapy or adjuvant treatment in cases with positive histological margins. Specific recommendations for RT are complex, as RT encompasses various techniques with different penetration depths, dosages, and fractionation.[104] A systematic review that included 1243 lesions reported recurrence rates of up to 30%.[105]

A recent study using Grenz ray (ultrasoft X-ray) treatment resulted in a 3% recurrence rate after a 10-year follow-up.[106] The cosmetic outcome of RT is considered favorable; however, induced hypo- or hyperpigmentation can make clinical follow-up more challenging.

Topical imiquimod, a Toll-like receptor 7/8 agonist, is increasingly being utilized as an off-label treatment modality for LM, inducing an innate and cytotoxic T cell-mediated immune response. However, treatment dosage, duration, outcome assessment, and follow-up heterogeneity make direct data comparisons difficult. Clinical clearance rates of 80% have been reported for monotherapy[107], with higher response rates in adjuvant or neoadjuvant settings.[108-110] These rates should be interpreted cautiously, as they primarily rely on clinical/dermoscopic evaluation and not on histological outcome, in addition to limited follow-up. Consequently, current data is possibly overestimating the efficacy as the accuracy of detecting residual LM by dermoscopy is low, with a sensitivity of 60-80% and specificity of 56-67%.[111, 112] Nevertheless, the daily application of imiquimod for 5-7 days per week for 12 weeks with sufficient inflammation is currently considered the optimal treatment regime, yielding excellent cosmetic outcomes.[107] The limitation of this treatment is the potential for severe inflammation, sterile conjunctivitis, flu-like symptoms, and fatigue.[113] A multicenter-randomized trial (NCT02394132) comparing imiquimod to RT is nearing completion and will provide new insights into the nonsurgical treatment of LM.[104, 113]

In conclusion, selecting the most appropriate treatment requires a tailored approach, considering lesion attributes, comorbidity, and patient preference.[114, 115] Frequently, this requires a multidisciplinary approach including dermatologists, (head-and-neck) surgeons, and radiation oncologists.

Basal cell carcinoma

The first-line treatment for BCC is surgical excision with the primary aim of completely removing the lesion while minimizing the risk of local recurrence. The risk of recurrence increases with high-risk histological subtypes, increased tumor size, poorly defined clinical margins, and prior recurrence. Surgical excision is the gold-standard treatment for nBCC and iBCC. As stipulated in the Dutch BCC guidelines, a surgical margin of 3 mm is recommended for low-risk BCC ≤10 mm in size, while a 5 mm margin is recommended for infiltrative BCC, size >10 mm, or recurrent cases.[116] Evaluating these excision specimens involves histological assessment of serial cross-sectionally cut formalin-fixed and paraffin-embedded tissue. This processing technique significantly reduces sensitivity for detecting potential asymmetric subclinical tumor extension.[117] While this method is effective and economical for low-risk BCC[94], Mohs' micrographic surgery (MMS) is the preferred treatment

for high-risk BCC located in the head-and-neck, particularly for lesions in the central facial area or 'H-zone.' Similar to other micrographically controlled surgical techniques, MMS allows for a comprehensive histological examination of the entire surgical margin, ensuring complete tumor removal and sparing as much healthy tissue as possible (**FIGURE 17**). This precision makes MMS particularly beneficial for preserving cosmetic and functional outcomes in areas in which tissue preservation is vital. The advantage of MSS is that it incorporates intraoperative frozen sections obtained in multiple stages within a single day, allowing same-day closure and reconstruction. In the context of high-risk BCCs, the 5-year relative risk of recurrence is 0.61 compared to conventional surgery, with an overall recurrence rate of 2%. [118, 119] Despite the high cure rates, it is essential to consider that MMS requires specialized training and is relatively time-consuming and labor-intensive compared to conventional or staged surgical excisions.

Various non-surgical alternatives are available for treating sBCC, including topical 5-fluorouracil, topical imiquimod, and photodynamic therapy. These treatment modalities have shown good-to-excellent cosmetic results. Optimizing patient compliance and maximizing response rates are crucial when considering topical treatment. Presently, topical imiquimod is regarded as the most effective regarding the 5-year recurrence rates compared to 5-fluorouracil and photodynamic therapy, with a risk ratio of 0.62 and 0.42, respectively.[119] In the case of sBCC or small low-risk nBCC, destructive techniques such as cryosurgery, curettage, and coagulation can also be considered. However, these techniques lack standardization and histological control, so the recurrence rates can be considerably higher.[120]

Radiotherapy is indicated when surgical excision is not a viable treatment option for non-sBCCs. It is worth mentioning that radiotherapy consists of different techniques and dosage schemes and may carry a higher risk of recurrence.[119] Nevertheless, studies have reported a 3.7% 5-year recurrence rate following orthovoltage treatment (n=232), which included 39% of high-risk BCC cases.[121] Thus, radiotherapy remains a viable alternative in specific scenarios.

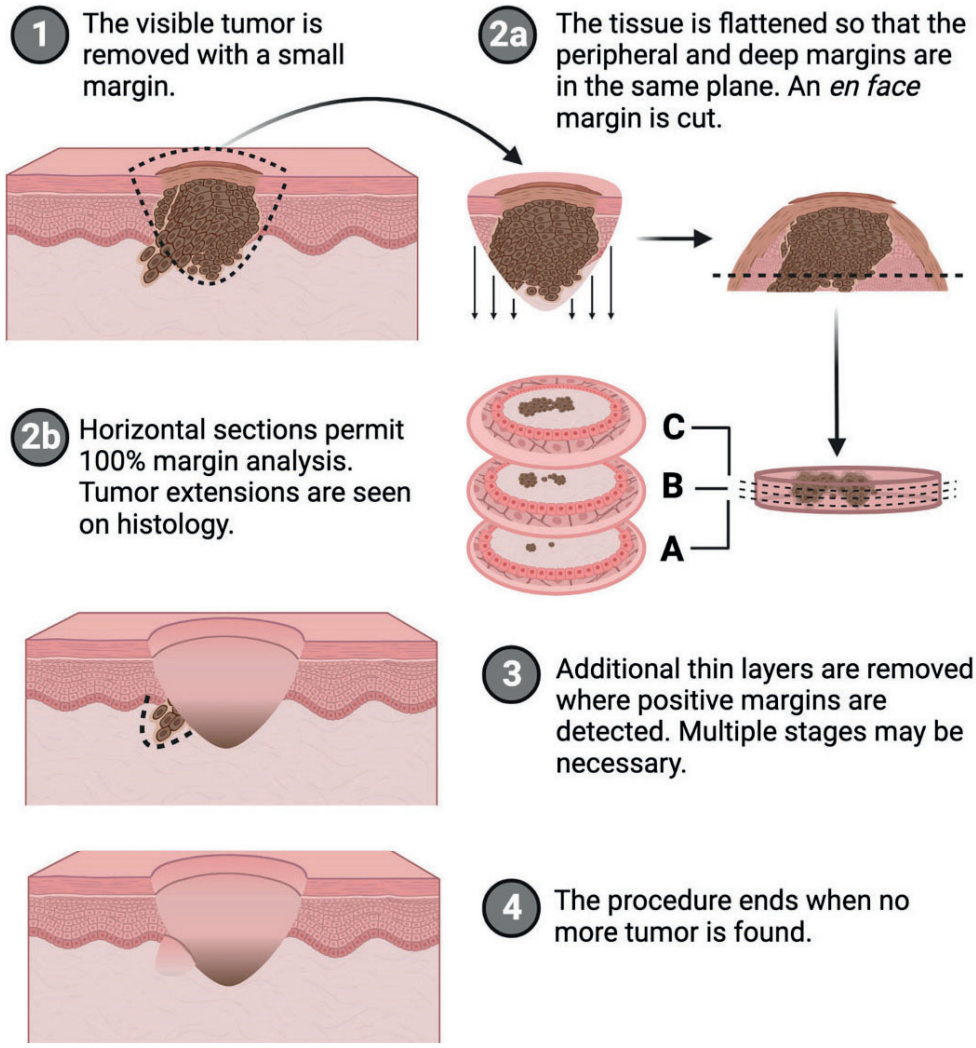

FIGURE 17. Overview of conventional Mohs micrographic surgical technique. *[Reprinted with permission from Frontiers in Oncology, Vol 13, Torres VC, Hodge S, Levy JJ, et al. Paired-agent imaging as a rapid en face margin screening method in Mohs micrographic surgery. Page 01-12. Copyright (2023) under the terms of the Creative Commons Attribution License (CC BY)]*

AIMS AND OUTLINE OF THIS THESIS

This thesis explored the diagnostic and therapeutic challenges associated with head-and-neck skin cancers. This thesis was divided into three parts, focusing on the surgical treatment of lentigo maligna (melanoma) (LM/LMM) and the role of handheld reflectance confocal microscopy (HH-RCM) (**PART I**), sentinel lymph node biopsy (SLNB) procedure for LMM (**PART II**), and the management of basal cell carcinoma (BCC) using handheld reflectance confocal microscopy (**PART III**).

PART I: TREATMENT OF LENTIGO MALIGNA (MELANOMA)

In **CHAPTER 2**, we performed a retrospective study on a cohort of primary head-and-neck melanoma to determine whether the unique clinical, diagnostic, and management challenges associated with LM/LMM affect the prognosis in terms of disease-free and survival outcomes. **CHAPTER 3** comprises of a pilot study conducted to assess the viability and efficacy of in vivo HH-RCM-guided surgery for LM/LMM of the head-and-neck. Following the pilot study, HH-RCM was introduced as the standard of care at our center. **CHAPTER 4** evaluates the effect of HH-RCM integration in the daily management of LM/LMM and examines the long-term follow-up outcomes of the patients included in Chapter 3. To better understand the optimal surgical technique and use of RCM in the surgical management of LM/LMM, we performed a systematic review and meta-analysis in **CHAPTER 5** on the margin, recurrence, and survival outcomes.

PART II: REGIONAL DIAGNOSTICS OF LENTIGO MALIGNA MELANOMA

Whether to perform a staging procedure using SLNB in patients with LMM remains a matter of ongoing discussion. In **CHAPTER 6**, we analyzed 29 years of Dutch national pathology data to determine whether SLNB should be routinely performed according to current melanoma guidelines and whether there are high-risk clinicopathological characteristics for SLNB positivity in patients with LMM.

PART III: DIAGNOSTICS OF BASAL CELL CARCINOMA

CHAPTER 7 reports the outcomes of a prospective study aimed at establishing the optimal HH-RCM position in the diagnostic pathway for BCC management in the head-and-neck.

In conclusion, **CHAPTER 8** summarizes the research findings, and **CHAPTER 9** discusses the main thesis outcomes and offers insights into future perspectives on these subjects.

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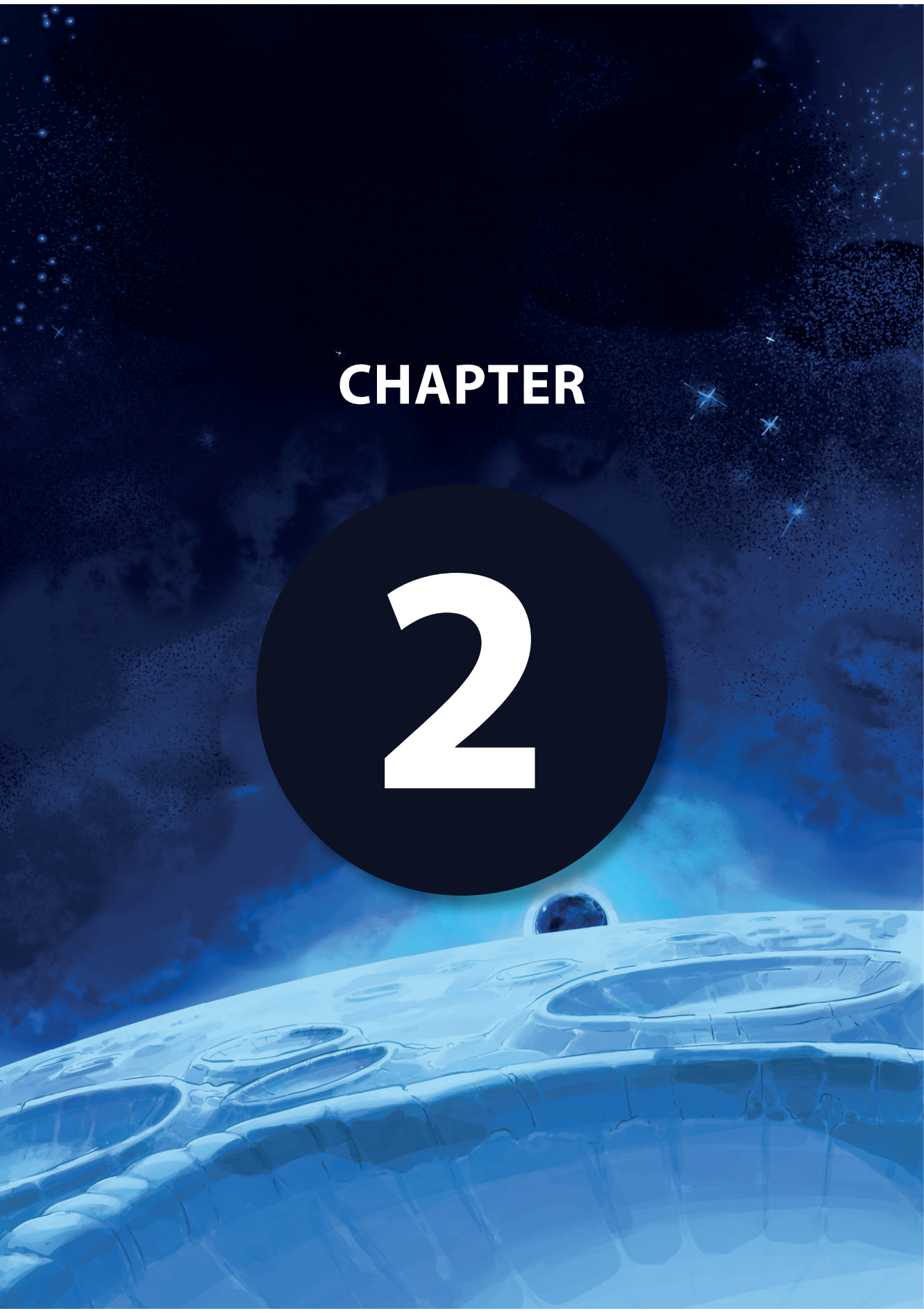
PART I

TREATMENT OF LENTIGO MALIGNA (MELANOMA)


CHAPTER
2

A COHORT ANALYSIS OF SURGICALLY TREATED PRIMARY HEAD-AND-NECK LENTIGO MALIGNA (MELANOMA): PROGNOSTIC VALUE OF MELANOMA SUBTYPE AND NEW INSIGHTS IN THE CLINICAL VALUE OF GUIDELINE ADHERENCE

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ABSTRACT

Background. Knowledge of lentigo maligna (melanoma) (LM/LMM) and its associated prognostic clinicopathological characteristics is limited compared with that of non-LM/LMM subtypes. This study aimed to determine the clinical relevance of the LM/LMM subtype and its influence on recurrence and survival outcomes.

Methods. All consecutive cases of primary cutaneous head-and-neck LM/LMM treated with wide local excision over a ten-year period were retrospectively reviewed and compared with non-LM/LMM. Clinical outcomes and prognostic factors were assessed using cumulative incidence and competing risk analysis.

Results. In total, 345 patients were identified. Specific clinicopathological characteristics such as lower median Breslow thickness (1.6 mm versus 2.1 mm; P=0.013), association with diagnostic sampling errors (17.3% versus 5.2%; P=0.01), and increased risk of local recurrences due to incomplete resection (18.7% versus 2.3%; P<0.001), were significantly associated with LM/LMM. Guideline adherence was similar between the two groups. The positive nodal status at baseline for LMM was lower than that for non-LM/LMM (4.2% vs. 17.9%, P=0.037). The LMM subtype, facial localization, and reduced surgical margins (i.e., guideline non-adherence) were not shown to be independent prognostic factors for disease-free, melanoma-specific, or overall survival after correction for competing risks, such as patient age and Breslow thickness.

Conclusions. The LMM subtype was not shown to be prognostically different from non-LM/LMM when corrected for other variables, such as patient age and Breslow thickness. Reduced resection margins did not seem to affect disease-free and melanoma-specific survival, and warrant LM/LMM-specific guidelines. Further research is required to evaluate the value of sentinel lymph node biopsy in patients with LMM.

INTRODUCTION

Because of the association with chronic sun damage, lentigo maligna (LM) and lentigo maligna melanoma (LMM) typically present as pigmented macules in the head-and-neck.[1-3] The diagnosis of LM/LMM is often based on partial instead of excisional biopsies since the clinical distinction from several benign facial lesions can sometimes be difficult.[4-5] However, partial biopsies are associated with diagnostic sampling errors, with unexpected invasive melanoma in 5-23% of completely excised LM.[6-11] Consequently, surgical excision remains the preferred treatment modality for LM/LMM, as it allows for full histological assessment.[12-13]

The guideline-recommended surgical margins for LM/LMM often result in incomplete resection. Consequently, reported local recurrence rates following wide local excision (WLE) range from 6 to 20%.[14] The recurrence rate might be further increased due to functional and cosmetic considerations in the head-and-neck, which makes it sometimes necessary to deviate from the guideline.

There are conflicting reports about the prognosis of LM/LMM compared to other head-and-neck melanoma.[15-18] With an estimated 2-5% lifetime risk of LM progressing to LMM[19-20], no clear picture emerges from the literature if, in addition to established melanoma prognostic factors, clinicopathological characteristics such as the LM/LMM subtype, incomplete resection margins, guideline adherence, and facial localization might determine the clinical outcome of LM/LMM patients.

To elucidate whether prognostic similarities existed between LM/LMM and other melanoma subtypes, we retrospectively compared the clinicopathological characteristics of patients with primary cutaneous head-and-neck melanoma treated with WLE.

MATERIALS AND METHODS

Following Institutional Review Board approval, we performed a retrospective cohort study, which included a consecutive series of primary head-and-neck LM/LMM and cutaneous melanoma treated with WLE at the Netherlands Cancer Institute (NKI-AVL) (2003–2014). All cases were identified through local Tumor Registration and pathology databases from the NKI-AVL. Patients with an additional ≥T2 classified melanoma in their medical history, histologically inconclusive melanoma subtypes, and patients referred with stage III/IV disease were excluded. In addition, we excluded patients with a follow-up of fewer than two years, as local recurrences of LM/LMM typically present after at least 2-5 years.[21]

The extracted data included the age at diagnosis, sex, melanoma subtype, clinicopathological characteristics, diagnostic procedures, and surgical and histological margins. Melanoma

subtypes were classified according to the 2018 WHO Classification of Skin Tumors. In addition to LM (ICD-O 8742/2) and LMM (ICD 8743/2), non-LM/LMM subtypes included melanoma in-situ (MIS) (ICD-O 8720/2), superficial spreading melanoma (SSM) (ICD-O 8743/3), nodular melanoma (NM) (ICD-O 8721/3) and desmoplastic melanoma (DM) (ICD-O 8754/3). In terms of anatomical localization, the head-and-neck region was divided into two sites: facial (forehead, nose, periorbital and perioral regions, cheeks, and chin) and extra-facial (scalp, ears, and neck). The staging was performed using the 8th edition of the American Joint Committee on Cancer (AJCC).[22]

According to the standard of care in the NKI-AVL, all patients were assessed by both a board-certified dermatologist and a head-and-neck surgeon. All the histological slides were reviewed by experienced melanoma dermatopathologists. In the case of a diagnosis based on a partial biopsy, a diagnostic excisional biopsy was considered before WLE with a surgical margin of 2 mm for full histological assessment. The routine workup of invasive melanoma included ultrasound examination of the neck, followed by fine-needle aspiration cytology (FNAC) in cases of suspected lymph nodes. For all >T1a classified melanomas, sentinel lymph node biopsy (SLNB) was discussed with the patient in case of negative ultrasound and/or FNAC. Following definite staging, WLE was performed according to the melanoma guideline-recommended margins, if not limited by anatomic or cosmetic constraints.[22] All SLNBs were performed following WLE in the same session. A false-negative SLNB was defined by a regional lymph node recurrence during follow-up in patients with a prior negative SLNB.[23]

We defined a diagnostic sampling error as an unexpected (i) invasive LMM, (ii) desmoplastic component, or (iii) increase in the Breslow thickness warranting additional surgical margins. Excised melanomas were processed perpendicular to the long axis (i.e., bread loafing). Additional immunohistochemical stains for Melan-A, sox10, or S100 were used where necessary.

Clinical outcomes included local, regional, and distant recurrences.[24] A local recurrence was defined as recurrence within the surgical scar due to incomplete excision of the tumor. Regional recurrence included satellite (within 2 cm of the surgical scar), in-transit (between the primary tumor and lymph node basin and at least 2 cm from the surgical scar), and regional lymph node metastases. Local recurrence was assessed for both in-situ and invasive melanomas. Regional recurrence-free survival (RRFS), distant recurrence-free survival (DRFS), overall survival (OS), and melanoma-specific survival (MSS) were only assessed for invasive melanoma.

Statistical analysis. Absolute and relative frequencies were described for all study variables. Differences in clinicopathological characteristics between the patient groups at baseline were identified using $\chi 2$ or Fisher's exact tests for categorical variables and the unpaired *t*-test or Mann-Whitney *U* test for continuous variables, where appropriate. For all survival

outcomes, the time to an event was calculated from the date of diagnosis, except for local recurrences, where the date of surgery was used. In the case of multiple occurrences of a specific event (e.g., regional metastases), patients were censored for further events of the same type, and only the date of the first event was used in the analysis.

Univariate and multivariate analyses were performed to examine the association of clinicopathological variables with RRFS, DRFS, OS, and MSS using a Fine-Gray competing risk Cox proportional hazard analysis. The competing risk was non-melanoma-related death in the MSS analysis and all-cause mortality in the other analyses. The proportional hazards assumption of the Cox model was assessed using Schoenfeld residuals and the Therneau and Grambsch non-proportionality test.[25] Melanoma prognostic factors included Breslow thickness, ulceration status, sex, age, nodal status, as well as melanoma subtype, anatomic localization, local recurrence, and guideline adherence. Kaplan-Meier (local recurrence) and cumulative incidence plots (RRFS, DRFS, and MSS) were compared with the log-rank stratified by melanoma subtype (i.e., LM/LMM vs. non-LM/LMM) or Gray's test [Gray RJ: A class of K-sample tests for comparing the cumulative incidence of a competing risk], respectively. Statistical significance was set at $P < 0.05$. Data were analyzed using SPSS (version 28.0) for Windows (IBM Corp, Armonk, NY, USA) and R version 4.02 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

A total of 345 patients met the inclusion criteria (**SUPPLEMENT I**). The clinicopathological characteristics of 75 LM/LMM and 270 non-LM/LMM patients are summarized in **TABLE 1**. The complete results of the surgical outcomes and competing risk analyses can be found in **SUPPLEMENT II-III**.

Surgical outcome

The guideline-recommended margin resulted in negative histological margins in 76% (n=52) of LM/LMM cases compared to 94.8% (n=256) of non-LM/LMM cases (P<0.001). However, the invasive component of LMM (n=48) was completely removed in all cases with a median (Q1-Q3) histological margin of 6.5 mm (1.9-10). Following re-excision, the total rate of negative margins was 93.3% (n=70) for LM/LMM and 98.9% (n=267) for non-LM/LMM (P=0.014). The median (Q1-Q3) histological margin was 1.5 (1.0-3.0) and 4.1 (1.0-10.0) mm for LM and LMM, respectively, and 12.0 (10.0-15.0) mm for non-LM/LMM (P<0.001). Adherence to the guideline-recommended surgical margins did not significantly differ between LM/LMM and non-LM/LMM (61.3% vs. 68.9%) (P=0.137) or between the <70 and ≥70-year age groups (68.3% vs. 64.6%) (P=0.298) (**SUPPLEMENT II**).

TABLE 1. Baseline clinicopathologic characteristics of included primary cutaneous head-and-neck melanoma (n=345)

	Lentigo maligna (melanoma)			Non-lentigo maligna (melanoma)					
No (%)	**LM 27 (7.8)**	**LMM 48 (13.9)**	**Total 75 (100.0)**	**DM[A] 21 (6.1)**	**MIS 8 (2.3)**	**SSM 159 (46.1)**	**NM 82 (23.8)**	**Total 270 (100.0)**	**P-value***
Age (years)									
Mean (range)	71.1 (52-91)	69.6 (45-93)	70.2 (45-93)	64.5 (43-83)	61.5 (31-81)	54.6 (18-88)	57.7 (16-90)	56.7 (16-90)	<0.001
<70	11 (40.7)	25 (52.1)	36 (48.0)	9 (42.9)	5 (62.5)	132 (83.0)	64 (78.0)	210 (77.8)	<0.001
≥70	16 (59.3)	33 (47.9)	39 (52.0)	12 (57.1)	3 (37.5)	27 (17.0)	18 (22.0)	60 (22.2)	
Sex									
Male	15 (55.6)	23 (47.9)	38 (50.7)	14 (66.7)	5 (62.5)	94 (59.5)	52 (62.7)	163 (60.4)	NS
Female	12 (44.4)	25 (52.1)	37 (49.3)	7 (33.3)	3 (37.5)	64 (40.5)	31 (37.3)	107 (39.6)	
Localization									
Facial	25 (92.5)	33 (68.8)	58 (77.3)	10 (47.6)	4 (50.0)	66 (41.8)	31 (37.3)	111 (41.1)	<0.001
Extra-facial	2 (7.4)	15 (31.3)	17 (22.7)	11 (52.4)	4 (50.0)	92 (58.2)	52 (62.7)	159 (58.9)	
Prior cosmetic treatment									
Yes	6 (22.2)	4 (8.3)	10 (13.3)	2 (9.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)	<0.001
No	21 (77.8)	44 (91.7)	65 (86.7)	19 (90.5)	8 (0.0)	158 (100.0)	83 (100.0)	268 (99.3)	
Diagnostic procedure									
Partial sample	21 (77.8)	38 (79.2)	59 (78.7)	12 (57.1)	2 (25.0)	36 (22.6)	22 (26.8)	72 (26.7)	<0.001
Excisional	6 (22.2)	10 (20.8)	16 (21.3)	9 (42.9)	6 (75.0)	123 (77.4)	60 (73.2)	198 (73.3)	
Breslow (mm) (median; Q1-Q3)	-	1.6 (0.6-3.2)	1.6 (0.6-3.2)	5.5 (3.7-7.0)	-	1.5 (1.0-2.2)	3.5 (2.1-5.0)	2.1 (1.3-3.9)	0.013
T classification[B]									
Tis	27 (100.0)	-	27 (36.0)	-	8 (100.0)	-	-	8 (3.0)	<0.001
T1	-	19 (39.6)	19 (25.3)	0 (0.0)	-	46 (28.9)	2 (2.4)	48 (17.8)	
T2	-	10 (20.8)	10 (13.3)	0 (0.0)	-	61 (38.4)	17 (20.7)	78 (28.9)	
T3	-	12 (25.0)	12 (16.0)	9 (42.9)	-	39 (24.5)	31 (37.8)	79 (29.3)	
T4	-	7 (14.6)	7 (9.3)	12 (57.1)	-	13 (8.2)	32 (39.0)	57 (21.1)	
Ulceration									
Present	-	11 (22.9)	11 (22.9)	4 (19.0)	-	21 (13.2)	32 (39.0)	57 (21.8)	NS
Absent	-	37 (77.1)	37 (77.1)	17 (81.0)	-	138 (86.8)	50 (61.0)	205 (78.2)	
Nodal status									
Positive	-	2 (4.2)	2 (4.2)	3 (14.3)	-	32 (20.1)	12 (14.6)	47 (17.9)	0.037
Negative	-	33 (68.8)	33 (68.8)	8 (38.1)	-	105 (66.0)	45 (54.9)	158 (60.3)	
Unknown	-	13 (27.3)	13 (27.3)	10 (47.6)	-	22 (13.8)	25 (30.5)	57 (21.8)	

LM= lentigo maligna; LMM= lentigo maligna melanoma; DM= desmoplastic melanoma; MIS= in-situ melanoma; SSM= superficial spreading melanoma; NM= nodular melanoma; Q = Quartile 1-3; NS = non-significant; T= tumor | [A] Including lentigo maligna-associated desmoplastic melanoma (n=5) | [B] According to 8th AJCC
*P-value for the difference between LM/LMM and non-LM/LMM from independent samples *t*-test or Mann-Whitney *U* test for continuous variables, and chi-squared test or Fisher's exact test for categorical variables where appropriate.

In 18.7% (n=9) of LMM cases, the invasive component was missed at diagnosis, compared to 2.3% (n=6) of non-LM/LMM cases (P=0.001). Three of 21 (14.3%) desmoplastic melanomas were initially misdiagnosed as LM. Furthermore, 6.2% (n=3) of LMM cases and 8.8% (n=23) of non-LM/LMM cases were upstaged in their T classification following full histological assessment (P=0.403). In almost all upstaged cases (97.6 %), the initial diagnostic procedure was a single 3 mm punch biopsy.

Local recurrences

Local recurrence was significantly associated with LM/LMM (P<0.001) (**FIGURE 1**). In the competing risk analysis (**SUPPLEMENT III-A/B/C/D**), local recurrence was significantly associated with the development of regional and distant metastases. In a subset analysis of LM/LMM, there was only a single (1/13) invasive recurrence (Breslow 0.5 mm without ulceration), whereas the remaining patients had in-situ recurrences. When considering LMM patients with local recurrence (n=8), 2/8 developed regional and 0/8 developed distant metastases within the follow-up period.

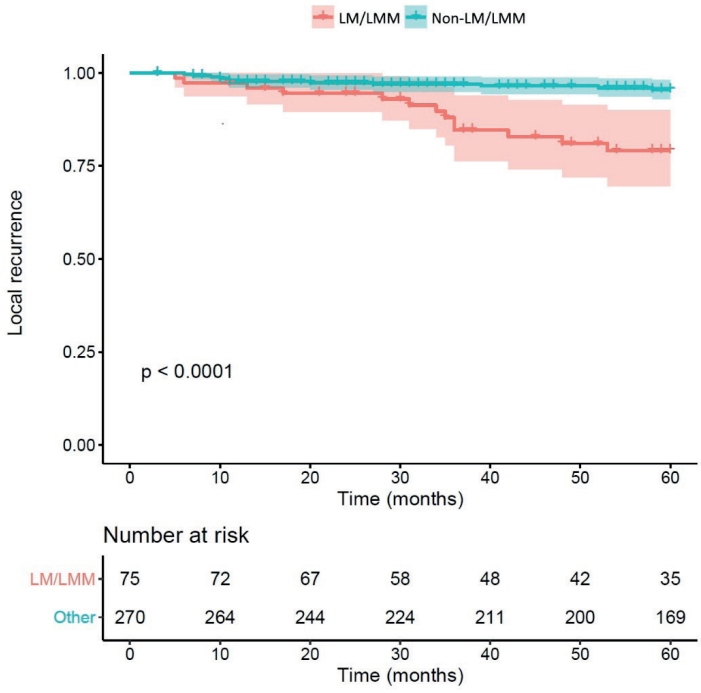

FIGURE 1. Kaplan-Meier curves for the appearance of local recurrences comparing lentigo maligna (melanoma) (LM/LMM) to in-situ and invasive non-lentigo maligna (melanoma) (non-LM/LMM) subtypes. *The LM/LMM subtypes were significantly associated (P<0.0001) with local recurrence due to incomplete resection compared to the non-LM/LMM subtypes. Both lines are shown with 95% confidence intervals.*

Survival outcomes

TABLE 2 summarizes the recurrence and survival outcomes. The median (Q1-Q3) follow-up period was 64 (49-78) months for LM/LMM and 69 (54-99) months for non-LM/LMM (P=0.076). LMM was not shown to be an independent prognostic factor for RRFS, DRFS, or MSS in multivariate analysis. Similarly, guideline adherence and lesion localization did not affect the RRFS, DRFS, or MSS. Death due to other or unknown causes was significantly correlated with LMM (P=0.004); however, when excluding the <70-year-old patient group, this difference was no longer significant. There was no difference in RRFS, DRFS, or MSS between the study groups (**FIGURE 2-3**).

Sentinel lymph node biopsy

SLNB was performed in 17 out of 48 (35.4%) LMM cases and 151 out of 262 (57.6%) invasive non-LM/LMM cases, with a positive SLNB in 2 (11.8%) and 30 (19.9%) patients, respectively. Furthermore, FNAC was positive in 17 invasive non-LM/LMM. This resulted in a total positive nodal status at baseline of 4.2% (n=2) for LMM and 17.9% (n=47) for invasive non-LM/LMM (P=0.037). There was no significant difference in nodal status at baseline between the <70 and ≥70-year age groups (P=0.339). In the LMM group, one SLNB appeared to be a false negative, whereas in the invasive non-LM/LMM group, 11 were found to be false-negatives. None of the LMM patients with positive nodal disease at baseline (n=2) developed distant metastases.

TABLE 2. Clinical and survival outcomes of primary cutaneous head-and-neck melanoma patients. (n=345)

	Lentigo maligna (melanoma)			Non-lentigo maligna (melanoma)					
	LM	LMM	Total	DM [A]	MIS	SSM	NM	Total	P-value*
No. (%)	**27 (7.8)**	**48 (13.9)**		**21 (6.1)**	**8 (2.3)**	**159 (46.1)**	**82 (23.8)**		
Recurrence [No/total (%)]									
Local	5/27 (18.5)	8/48 (16.7)	13/75 (17.3)	5/21 (23.8)	0 (0.0)	6/159 (3.8)	3/82 (3.7)	14/270 (5.2)	0.001
Regional [B]	-	7/48 (14.6)	7/48 (14.6)	6/21 (28.6)	-	29/159 (18.2)	18/82 (22.0)	53/262 (20.2)	NS
Regional lymph node	-	3/48 (6.3)	3/48 (6.3)	2 (9.5)	-	13/159 (8.2)	12/82 (14.6)	27/262 (10.3)	NS
Distant	-	5/48 (10.4)	5/48 (10.4)	3/21 (14.3)	-	37/159 (23.3)	21/82 (25.6)	61/262 (23.3)	NS
Patient outcome [No/total (%)]									
Local disease [B]	27 (100.0)	38 (79.2)	65/75 (86.7)	17 (81.0)	8 (100.0)	109 (68.6)	53 (64.6)	187/270 (69.3)	0.02[C]
Regional recurrence [D]	-	5 (10.4)	5/48 (10.4)	4 (19.0)	-	15 (9.4)	8 (9.8)	25/262 (9.5)	NS
Distant recurrence	-	5 (10.4)	5/48 (10.4)	3 (14.3)	-	37 (23.3)	21 (25.6)	61/262 (23.3)	NS
Patient status									
Alive	16 (59.3)	28 (58.3)	44 (58.7)	10 (47.6)	5 (62.5)	112 (70.4)	54 (65.9)	181 (67.0)	NS
Died, melanoma	0 (0.0)	4 (8.3)	4 (5.3)	3 (14.3)	0 (0.0)	26 (16.4)	17 (20.7)	46 (17.0)	0.009
Died, other	8 (29.6)	2 (4.2)	10 (13.3)	5 (23.8)	3 (37.5)	16 (10.1)	10 (12.2)	34 (12.6)	NS
Died, unknown	3 (11.1)	14 (29.2)	17 (22.7)	3 (14.3)	0 (0.0)	5 (3.1)	1 (1.2)	9 (3.3)	<0.001

LM= lentigo maligna; LMM= lentigo maligna melanoma; DM= desmoplastic melanoma; MIS= in-situ melanoma; SSM= superficial spreading melanoma; NM= nodular melanoma

*P-value for the difference between LM/LMM and non-LM/LMM by Fisher's exact test.

[A] Including lentigo maligna associated desmoplastic melanoma (n=5).

[B] Defined a recurrence within the surgical scar due to incomplete excision of the primary tumor.

[C] Not significant when only assessing invasive melanoma (79.2% versus 68.3%)

[D] Satellite, in transit and regional lymph node metastases.

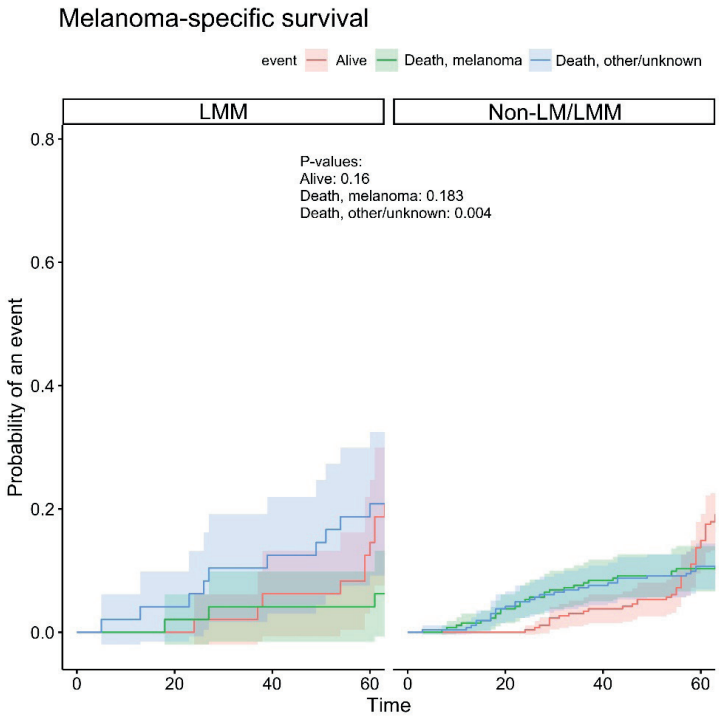

FIGURE 2. Overall and melanoma-specific survival cumulative incidence curves over time for lentigo maligna melanoma (LMM) and invasive non-lentigo maligna melanoma subtypes. *No significant differences were observed in melanoma-associated mortality (P=0.183). However, other/unknown causes of mortality were significantly higher in the LMM patient group (P=0.004). All the lines are shown with their respective 95% confidence intervals.*

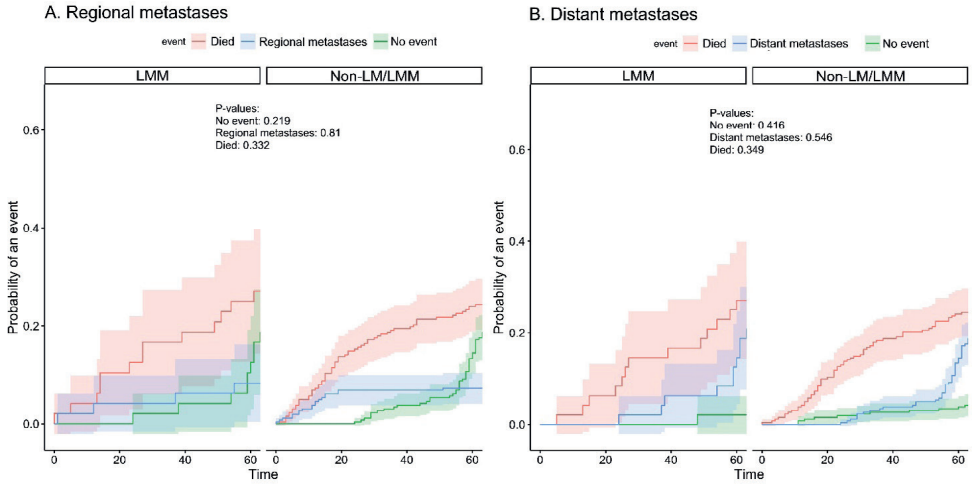

FIGURE 3. Cumulative incidence curves over time for regional (A) and distant (B) metastases for lentigo maligna melanoma (LMM) and invasive non-lentigo maligna melanoma subtypes (non-LM/LMM). *No significant difference was found between the two study groups in the development of regional (P=0.81) or distant (P=0.546) metastases. All the lines are shown with their respective 95% confidence intervals.*

DISCUSSION

In this study, we evaluated whether the LMM subtype and associated clinicopathological characteristics can be used as independent prognostic factors for DFS or MSS in a cohort of primary cutaneous head-and-neck melanoma patients, consisting of LM/LMM and non-LM/LMM patients.

Survival outcomes. The LMM subtype did not emerge as an independent prognostic factor for DFS, MSS, or OS. This is similar to what was found in other studies, where histological subtype did not remain an independent prognostic factor after correcting for variables such as Breslow thickness and ulceration, even when comparing patients with similar Breslow thickness.[16, 26-27] Nonetheless, in our study, the median Breslow thickness was significantly lower for the LMM subtype, possibly correlated with early detection of LMM in the facial region.[17, 28] Although a significant number (36%) of LMM patients died due to unrelated or unknown causes compared to non-LM/LMM patients, this difference was no longer significant when excluding the <70-year-old patient group. Consequently, our data show that patient age should be considered instead of melanoma subtype when managing patients with LM/LMM.

Local recurrences. While LM/LMM was significantly associated with local recurrence ($P<0.001$), it did not affect MSS, as the majority consisted of in-situ melanoma. Furthermore, even though the guideline-recommended margin was insufficient in one-fourth of the LM/LMM cases, the invasive component was removed in all cases.

Nonetheless, the local recurrence rate was 17.3%, which was more than double than expected from the 6.7% of cases with positive histological margins at baseline. This is likely the result of bread-loaf sectioning used in WLE, resulting in an underestimation of the rate of positive histological margins.[11, 34] To reduce this risk of local recurrence in patients treated by WLE, a histological margin of at least 3.0 mm is advised.[21] While staged excision techniques (e.g., Slow Mohs or spaghetti technique) or Mohs' micrographic surgery can be used to evaluate nearly 100% of the histological margin, they remain time-consuming. Moreover, using frozen sections can result in artefactual and fixational changes in the excision specimens.[35]

To reduce the risk of local recurrence, larger surgical margins are not always feasible in the head-and-neck due to cosmetic and/or functional considerations. Based on our experience, we are inclined to advise limited resection margins in the case of LMM, as local recurrences seem to have a limited effect on DFS and MSS. The lack of effect of either reduced or wider (>/= 2 cm) resection margins on DFS and MSS, as shown by Rawlani and Rusking et al.,

seems supportive of this proposal.[36-37] Moreover, if available, preoperative in vivo mapping by reflectance confocal microscopy (RCM) should be considered to allow for complete resection with personalized surgical margins.[38-39]

Sentinel lymph node biopsy. Only two LMM patients (4.2%) with nodal disease were identified using SLNB at baseline. Similar results were reported by Frolich et al., who did not find any positive SLNB in LMM patients.[18] Others have also reported a low yield of positive SLNB in facial melanoma, seemingly due to the overrepresentation of the LMM subtype in the facial region.[17] Furthermore, this limited yield even seems to persist in T4 classified LMM[29], possibly pointing towards an initial hematological metastatic pathway rather than a lymphatic one.[1] This is especially important to take into account considering the increased use of SLNB due to targeted and immunotherapies in the adjuvant setting for stage III melanoma.[30-31] Another explanation could be false negative SLNBs.[32-33] In our study, there was a 28.6% (12/42) overall false-negative rate of SLNBs. However, this study included only a single false-negative SLNB LMM case, making it less likely to be of influence in our cohort.

Finally, as surgical excision is not always feasible due to patient age or lesion size, topical treatment with imiquimod is increasingly being advocated for treating LM.[40] However, when opting for non-surgical treatment, one should be aware of the inherent risk of sampling errors by partial biopsies. In the current study, almost 19% of the LMM cases were initially misdiagnosed as LM. This percentage is within the reported range of unexpected invasive melanoma of 5-23% in surgically treated LM.[6-11, 21] In addition, three out of five desmoplastic melanoma cases were initially diagnosed as LM, which is not surprising, as up to 24% of DM cases are reported to be associated with LM/LMM.[2] Therefore, the authors advise aiming for multiple punch biopsies, an incisional or shave biopsy when an excisional biopsy is not an option.

Limitations. Due to the retrospective nature of our study, there were several limitations. First, we attempted to identify the specific LM/LMM clinicopathological prognostic factors. However, as 36% of LM/LMM patients died due to unrelated/unknown causes, our sample of 48 LMM was limited compared to the 262 non-LM/LMM cutaneous melanoma patients. This limited sample size, combined with a lower median Breslow thickness for LMM, could also have affected the rate of lymph node metastases at baseline. Furthermore, as the proportion of included LM/LMM was significantly lower than that reported in prior studies, we cannot rule out melanoma subtype misclassification as a potential source of bias.[41-42] Second, the cause of death was unknown in 22.7% of cases. Finally, during the inclusion period, the 7th edition of the AJCC was introduced, influencing the recommendation on when to perform SLNB.

CONCLUSIONS

In this single-center cohort, we could not show the LM/LMM subtype to be an independent prognostic factor for predicting the outcome of cutaneous head-and-neck melanoma. Nonetheless, the LM/LMM subtype is associated with unique clinicopathological characteristics, such as anatomic localization in a cosmetic and functional sensitive area, older age at presentation, and a low positive yield of SLNB. Therefore, the LM/LMM subtype poses specific diagnostic and surgical challenges regarding excision margins and the use of SLNB.

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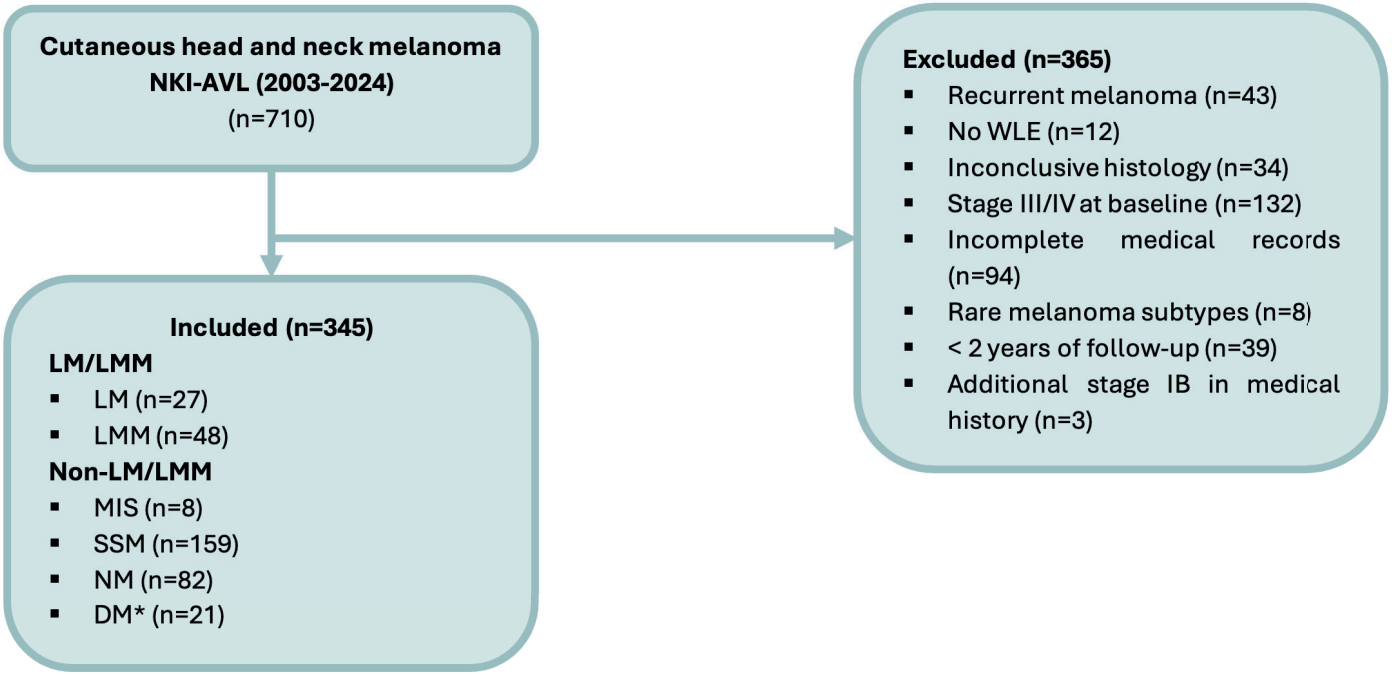
SUPPLEMENTS



SUPPLEMENT I. Inclusion flowchart.

DM = desmoplastic melanoma; LM = lentigo maligna; LMM = lentigo maligna melanoma; MIS = melanoma in-situ; NKI-AVL = Netherlands Cancer Institute – Antoni van Leeuwenhoek; NM = nodular melanoma; SSM = superficial spreading melanoma; WLE = wide local excision

* Includes lentigo maligna-associated desmoplastic melanoma (n=5)

SUPPLEMENT II. Surgical outcome of included primary cutaneous head-and-neck melanoma (n=345)

	Lentigo maligna (melanoma)			Non-lentigo maligna (melanoma)					
	LM	LMM	Total	DM [A]	MIS	SSM	NM	Total	P-value*
No. (%)	**27 (7.8)**	**48 (13.9)**	**75 (100.0)**	**21 (6.1)**	**8 (2.3)**	**159 (46.1)**	**82 (23.8)**	**270 (100.0)**	
Guideline adherence									
Yes	15 (55.6)	31 (64.6)	46 (61.3)	13 (61.9)	7 (87.5)	122 (76.7)	44 (53.7)	186 (68.9)	NS
No	12 (44.4)	17 (35.4)	29 (38.7)	8 (38.1)	1 (12.5)	37 (23.3)	38 (46.3)	84 (31.1)	
Histological clearance									<0.001
Yes	21 (77.8)	34 (70.8)	55 (73.3)	16 (76.2)	8 (100)	150 (94.3)	77 (93.3)	251 (93.0)	
No	6 (22.2)	12 (25.0)	18 (24.0)	3 (14.3)	0 (0.0)	8 (5.0)	3 (3.7)	14 (5.2)	
Unknown	0 (0.0)	2 (4.2)	2 (2.7)	2 (9.5)	0 (0.0)	1 (0.6)	2 (2.4)	5 (1.9)	
Sampling error									
Invasive component									
Yes	-	9 (18.7)	9 (18.7)	3 (14.3)	-	3 (1.9)	0 (0.0)	6 (2.3)	<0.001
No	-	39 (81.3)	39 (81.3)	18 (85.7)	-	156 (98.1)	82 (100.0)	256 (97.7)	
Upstaged	-				-				
Yes	-	3 (6.2)	3 (6.2)	6 (28.6)	-	10 (6.3)	7 (8.5)	23 (8.8)	NS
No		45 (93.8)	45 (93.8)	15 (71.4)		149 (93.7)	75 (91.5)	239 (91.2)	
Surgical margin (mm) Median (Q1-Q3)	5 (2.0-5.0)	10 (10.0-15.0)	10 (5.0-12.0)	20 (17.5-22.0)	7 (5.0-7.0)	12 (12.0-17.0)	12 (12.0-20.5)	12 (12-20)	<0.001
Histological margin (mm) Median (Q1-Q3)	1.5 (1.0-3.0)	4.1 (1.0-10.0)	2 (1.0-6.0)	10.5 (4.5-17.2)	3.8 (2.1-6.9)	11.5 (10-15)	12 (10-20)	12 (10.0-15.0)	<0.001
Negative margins									
Yes	25 (92.6)	45 (93.8)	70 (93.3)	20 (95.2)	8 (100)	157 (98.7)	82 (100)	267 (98.9)	0.014
No	2 (7.4)	3 (6.3)	5 (6.7)	1 (4.8)	0 (0.0)	2 (1.3)	0 (0.0)	3 (1.1)	
Follow-up Median (Q1-Q3)	63.0 (35-76)	65.0 (49.5-82)	64 (49-78)	57.0 (25.5-78.5)	71.5 (50.3-86.5)	70.0 (58-104)	70.5 (45.5-96.5)	69 (54-99)	NS

LM= lentigo maligna; LMM= lentigo maligna melanoma; DM= desmoplastic melanoma; MIS= in-situ melanoma; SSM= superficial spreading melanoma; NM= nodular melanoma; Q1-Q3 = Quartile; NS = non-significant.

* P-value for the difference between LM/LMM and non-LM/LMM from independent samples T-Test or Mann-Whitney U test for continuous variables, and chi-squared test or Fisher's exact test for categorical variables where appropriate.

[A] Including lentigo maligna-associated desmoplastic melanoma (n=5);

SUPPLEMENT III-A. Competing risk analyses of regional recurrence in primary cutaneous head-and-neck melanoma patients.

	Univariable		Multivariable (I)		Multivariable (II)		Multivariable (III)	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age group								
< 70	Reference	NS	Reference	NS	Reference	NS	Reference	NS
≥ 70	1.02 (0.41-2.57)		1.07 (0.4-2.85)		0.9 (0.35-2.32)		0.94 (0.37-2.4)	
Sex								
Female	Reference	0.024	Reference	NS	Reference	0.027	Reference	0.042
Male	3.44 (1.18-10.03)		2.44 (0.79-7.55)		3.32 (1.14-9.66)		3.13 (1.04-9.39)	
Breslow (mm)	1.99 (1.26-3.16)	0.003	1.76 (1.05-2.93)	0.031	1.96 (1.18-3.23)	0.009	1.87 (1.15-3.05)	0.011
Ulceration								
Absent	Reference	NS	Reference	NS	Reference	NS	Reference	NS
Present	1.98 (0.84-4.66)		0.99 (0.45-2.21)		1.13 (0.45-2.83)		1.18 (0.48-2.89)	
Subtype								
Non-LM/LMM [A]	Reference	NS	Reference	NS	Reference	NS	-	-
LMM	0.88 (0.3-2.54)		0.62 (0.18-2.12)		0.57 (0.18-1.82)		-	-
Localization [B]								
Facial	Reference	0.027	Reference	NS	-	-	-	-
Extra-facial	3.06 (1.14-8.21)		2.77 (0.97-7.92)		-	-	-	-
Guideline adherence								
Yes	Reference	NS	Reference	NS	-	-	-	-
No	1.37 (0.6-3.15)		1.39 (0.6-3.21)		-	-	-	-
Local recurrence								
No	Reference	0.006	Reference	0.019	-	-	-	-
Yes	3.97 (1.49-10.58)		3.05 (1.2-7.78)		-	-	-	-

HR= hazard ratio; CI = confidence interval; NS=non-significant; LMM= lentigo maligna melanoma

[A] Non-LM/LMM includes superficial spreading, nodular, and desmoplastic melanoma

[B] Anatomic localization: facial (forehead, nose, peri-orbital, peri-oral, and chin) and extra-facial (scalp, ear, or neck)

SUPPLEMENT III-B. Competing risk analyses of distant recurrence in primary cutaneous head-and-neck melanoma patients.

	Univariable		Multivariable (I)		Multivariable (II)		Multivariable (III)	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age group								
< 70	Reference	NS	Reference	NS	Reference	NS	Reference	NS
≥ 70	0.29 (0.04-2.27)		0.19 (0.01-3.87)		0.27 (0.03-2.71)		0.29 (0.04-2.44)	
Sex								
Female	Reference	NS	Reference	NS	Reference	NS	Reference	NS
Male	3.15 (0.68-14.53)		2.15 (0.44-10.56)		3.3 (0.77-14.05)		3.22 (0.71-14.59)	
Breslow (mm)	2.39 (1.49-3.83)	<0.001	1.85 (0.89-3.81)	NS	2.1 (1.03-4.3)	0.004	2.05 (1.08-3.89)	0.03
Ulceration								
Absent	Reference	NS	Reference	NS	Reference	NS	Reference	NS
Present	3.03 (0.93-9.83)		1.57 (0.56-4.38)		2.02 (0.56-7.26)		2.05 (0.6-7.07)	
Nodal status [A]								
Negative	Reference	0.003	Reference	0.023	Reference	0.002	Reference	0.02
Positive	6.23 (1.8-21.53)	NS	5.69 (1.26-25.71)	NS	4.76 (1.32-17.1)	NS	4.65 (1.22-17.11)	NS
Unknown	0.68 (0.08-6.07)		0.62 (0.1-4.09)		0.54 (0.06-5.02)		0.57 (0.07-4.63)	
Subtype								
Non-LM/LMM [B]	Reference	NS	Reference	NS	Reference	NS	-	-
LMM	1.85 (0.24-14.23)		1.25 (0.03-59.19)		0.67 (0.06-7.49)		-	-
Localization [C]								
Facial	Reference	0.044	Reference	NS	-	-	-	-
Extra-facial	8.17 (1.05-63.31)		4.47 (0.46-43.79)		-	-	-	-
Guideline adherence								
Yes	Reference	NS	Reference	NS	-	-	-	-
No	0.79 (0.21-2.95)		1.02 (0.34-3.08)		-	-	-	-
Local recurrence								
No	Reference	0.015	Reference	0.017	-	-	-	-
Yes	5.13 (1.36-19.29)		8.62 (1.46-50.7)		-	-	-	-

HR= hazard ratio; CI = confidence interval; NS=non-significant; LMM= lentigo maligna melanoma

[A] Regional lymph node status at baseline

[B] Non-LM/LMM includes superficial spreading, nodular, and desmoplastic melanoma

[C] Anatomic localization: facial (forehead, nose, peri-orbital, peri-oral, and chin) and extra-facial (scalp, ear, or neck)

SUPPLEMENT III-C. Competing risk analyses of overall survival of primary cutaneous head-and-neck melanoma patients.

	Univariable		Multivariable (I)		Multivariable (II)		Multivariable (III)	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age group								
< 70	Reference	<0.001	Reference	0.004	Reference	0.006	Reference	0.004
≥ 70	2.53 (1.56-4.1)		2.12 (1.27-3.55)		2.03 (1.22-3.38)		2.08 (1.25-3.45)	
Sex								
Female	Reference	NS	Reference	NS	Reference	NS	Reference	NS
Male	0.9 (0.56-1.46)		0.9 (0.54-1.5)		0.92 (0.57-1.51)		0.91 (0.56-1.48)	
Breslow (mm)	2.23 (1.63-3.06)	<0.001	1.75 (1.2-2.55)	0.004	1.84 (1.28-2.64)	<0.001	1.8 (1.26-2.57)	0.001
Ulceration								
Absent	Reference	0.01	Reference	NS	Reference	NS	Reference	NS
Present	1.95 (1.17-3.25)		1.22 (0.69-2.17)		1.17 (0.67-2.04)		1.21 (0.69-2.09)	
Nodal status [A]								
Negative	Reference	0.001	Reference	0.04	Reference	NS	Reference	NS
Positive	2.72 (1.49-4.97)	0.002	1.98 (1.02-3.83)	NS	1.8 (0.94-3.44)	NS	1.77 (0.93-3.37)	NS
Unknown	2.35 (1.34-4.11)		1.56 (0.86-2.83)		1.57 (0.88-2.81)		1.58 (0.88-2.82)	
Subtype								
Non-LM/LMM [B]	Reference	NS	Reference	NS	Reference	NS	-	-
LMM	0.84 (0.45-1.56)		0.68 (0.35-1.32)		0.75 (0.39-1.44)		-	-
Localization [C]								
Facial	Reference	NS	Reference	NS	-	-	-	-
Extra-facial	1.15 (0.71-1.87)		1.22 (0.71-2.1)		-	-	-	-
Guideline adherence								
Yes	Reference	0.009	Reference	0.02	-	-	-	-
No	1.89 (1.17-3.05)		1.88 (1.12-3.15)		-	-	-	-
Local recurrence								-
No	Reference	NS	Reference	NS	-	-	-	-
Yes	0.78 (0.29-2.16)		0.64 (0.22-1.87)		-	-	-	

HR= hazard ratio; CI = confidence interval; NS=non-significant; LMM= lentigo maligna melanoma

[a] Regional lymph node status at baseline

[b] Non-LM/LMM includes superficial spreading, nodular, and desmoplastic melanoma

[c] Anatomic localization: localization: facial (forehead, nose, peri-orbital, peri-oral, and chin) and extra-facial (scalp, ear, or neck)

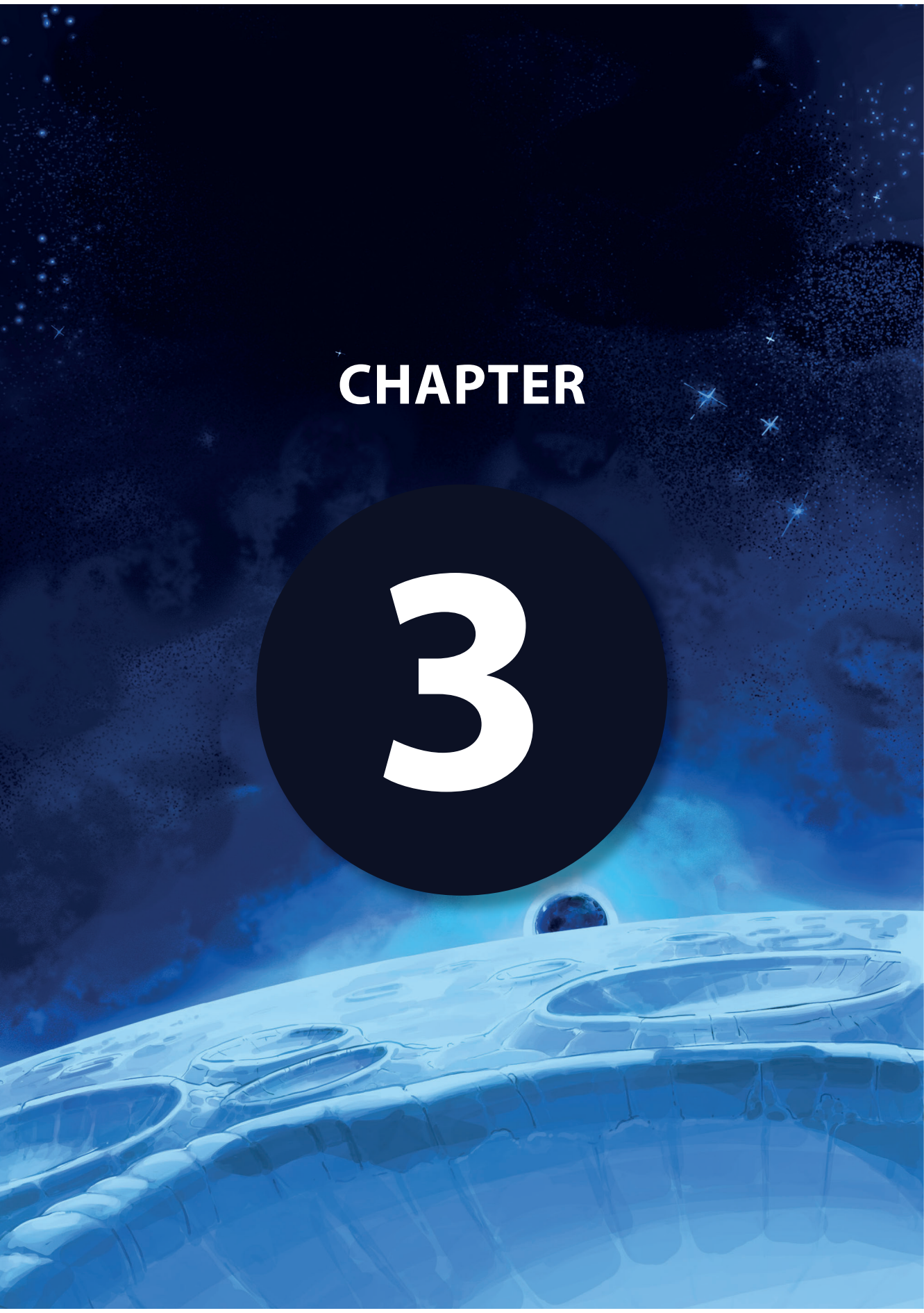
SUPPLEMENT III-D. Competing risk analyses of melanoma-specific survival of primary cutaneous head-and-neck melanoma patients.

	Univariable		Multivariable (I)		Multivariable (II)		Multivariable (III)	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age group								
< 70	Reference	NS	Reference	NS	Reference	NS	Reference	NS
≥ 70	0.33 (0.1-1.07)		0.26 (0.06-1.04)		0.25 (0.06-1.01)		0.24 (0.06-1.01)	
Sex								
Female	Reference	NS	Reference	NS	Reference	NS	Reference	NS
Male	0.98 (0.47-2.05)		0.81 (0.39-1.68)		0.82 (0.39-1.72)		0.83 (0.39-1.76)	
Breslow (mm)	2.66 (1.68-4.2)	<0.001	2.31 (1.2-4.44)	0.012	2.34 (1.23-4.42)	0.009	2.36 (1.24-4.47)	0.008
Ulceration								
Absent	Reference	0.002	Reference	NS	Reference	NS	Reference	NS
Present	3.06 (1.48-6.31)		1.97 (0.77-5.06)		2.1 (0.9-4.91)		2.09 (0.9-4.89)	
Nodal status [A]								
Negative	Reference	<0.001	Reference	0.034	Reference	0.028	Reference	0.027
Positive	4.36 (2.04-9.31)	NS	2.66 (1.07-6.59)	NS	2.62 (1.11-6.21)	NS	2.67 (1.12-6.38)	NS
Unknown	0.62 (0.18-2.2)		0.53 (0.12-2.27)		0.55 (0.14-2.22)		0.54 (0.14-2.13)	
Subtype								
Non-LM/LMM [B]	Reference	NS	Reference	NS	Reference	NS	-	-
LMM	2.53 (0.6-10.66)		1.15 (0.25-5.27)		1.31 (0.31-5.51)		-	-
Localization [C]								
Facial	Reference	NS	Reference	NS	-	-	-	-
Extra-facial	2.21 (0.98-4.96)		1.39 (0.58-3.33)		-	-	-	-
Guideline adherence								
Yes	Reference	NS	Reference	NS	-	-	-	-
No	1.13 (0.53-2.43)		1.27 (0.53-3.02)		-	-	-	-
Local recurrence								
No	Reference	NS	Reference	NS	-	-	-	-
Yes	0.96 (0.23-4.06)		0.93 (0.16-5.53)		-	-	-	-

HR= hazard ratio; CI = confidence interval; NS=non-significant; LMM= lentigo maligna melanoma

[A] Regional lymph node status at baseline; [B] Non-LM/LMM includes superficial spreading, nodular, and desmoplastic melanoma

[C] Anatomic localization: localization: facial (forehead, nose, peri-orbital, peri-oral, and chin) and extra-facial (scalp, ear, or neck)


CHAPTER
3

HANDHELD REFLECTANCE CONFOCAL MICROSCOPY: PERSONALIZED AND ACCURATE PRESURGICAL DELINEATION OF LENTIGO MALIGNA (MELANOMA)

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ABSTRACT

Background. Surgical treatment of lentigo maligna melanoma is associated with a high rate of local recurrence. Handheld reflectance confocal microscopy (HH-RCM) allows for in vivo presurgical detection of subclinical lentigo maligna (melanoma) (LM/LMM).

Methods. This single-center retrospective study was conducted between December 2015 and July 2017. The frequency and extent of negative surgical margins and diagnostic accuracy of presurgical mapping using HH-RCM were determined.

Results. Twenty-six consecutive patients with LM/LMM were included in the study. In 45.8% of cases, HH-RCM detected subclinical LM with a sensitivity of 0.90 and specificity of 0.86. Management was changed in two (7.7%) patients. Of the 24 remaining lesions, 95.8% were excised with negative histological margins, with a mean histological margin of 3.1 mm and 5.3 mm for LM and LMM, respectively. At a mean follow-up of 36.7 months, one (4.8%) patient had confirmed recurrence.

Conclusions. Our method of presurgical delineation by HH-RCM appears to be a reliable method for the surgical treatment of LM/LMM with a limited rate of overtreatment.

INTRODUCTION

Lentigo maligna (LM) is an in-situ melanoma with a predilection for the head-and-neck.[1] The treatment of LM is aimed at preventing the progression into lentigo maligna melanoma (LMM). While the risk of progression is low, the incidence of both LM and LMM is currently on the rise.[2,3]

The treatment of LM/LMM is associated with high rates of local recurrence[4] due to the frequent subclinical spread of atypical melanocytes.[1,5] The optimal approach to surgical treatment remains a topic of significant debate, especially given the presentation of the lesion in a cosmetically and functionally sensitive area. While international guidelines currently recommend surgical margins of 5mm for LM, this has been reported to be insufficient in up to 62.7% of cases, with recurrence rates ranging from 6% to 20%.[6]

A dermatoscope is a handheld device that allows the magnification and visualization of skin morphology that is not visible to the naked-eye and is widely used by dermatologists to diagnose pigmented lesions, including LM/LMM.[7] Even so, the usefulness of dermatoscopy for detecting LM beyond the clinical margin remains limited, as it seems unable to detect individual atypical melanocytes at the lesion's periphery.[8] Several surgical techniques are used to minimize tissue excision while still achieving local control, including Mohs micrographic surgery (MMS) and staged excision.[6] While MMS allows for a complete (100%) surgical margin assessment, it requires extensive training. However, it is not universally accepted for the treatment of melanocytic lesions, because frozen sections can result in artifactual and fixational changes. This issue can be circumvented using rushed permanent paraffin-embedded sections or staged excision techniques in which the entire peripheral margin is assessed in several stages without the need for special training or equipment. While offering a definite advantage over conventional excision in achieving local control, these techniques remain time intensive.

Reflectance confocal microscopy (RCM) is a noninvasive imaging technique that allows for in vivo visualization of cutaneous structures at the cellular level, up to the level of the papillary dermis. Past studies have shown that RCM is well suited for identifying subclinical disease beyond the margin delineated using dermatoscopy.[9-11] In a prospective study using traditional arm-mounted RCM (AM-RCM), the resulting surgical area was 40% larger on average than when determined by dermatoscopy alone.[11] More recently, a handheld RCM (HH-RCM) device consisting of a smaller, non-fixated probe was introduced. This flexibility allows for more rapid evaluation and access to more concave areas in the head-and-neck.

In this retrospective pilot study, we developed a new in vivo presurgical delineation method for LM/LMM of the head-and-neck using HH-RCM-assisted conventional excision.

MATERIALS AND METHODS

Consecutive patients with histopathologically confirmed LM or LMM at the Netherlands Cancer Institute (NKI-AVL) between December 2015 and July 2017 were eligible for this study. All cases were identified using the local Tumor Registration Database. The inclusion criteria were (i) primary/recurrent LM or LMM ≤ T2 classification according to the 7th edition of the American Joint Committee on Cancer (AJCC) guidelines, (ii) localization in the head-and-neck, and (iii) HH-RCM-assisted surgery. Patients with (i) T3-4 classification LMM (AJCC 7th edition), (ii) receiving diagnostic excisions (2 mm surgical margin), or (iii) non-surgical treatment were excluded. According to the standard of care in the NKI-AVL, all patients were assessed by both a board-certified dermatologist and a head-and-neck surgeon. All the histological slides were reviewed by experienced melanoma dermatopathologists. For invasive LMM, routine workup included ultrasound examination followed by fine needle aspiration cytology (FNAC) in cases of suspected macroscopic lymph node metastases. For cT1b or higher classified melanomas, sentinel lymph node biopsy (SLNB) was discussed with the patient in case of negative ultrasound and/or FNAC. All SLNBs were performed simultaneously with HH-RCM-assisted WLE. Confocal imaging and analyses were performed by a single investigator (Y.E.) who had 14 months (1 day/week) of experience with HH-RCM before embarking on this study. The study protocol was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. Following review by the NKI-AVL Institutional Review Board, the ethics committee's approval was waived.

Presurgical mapping procedure. According to the standard of care, the clinical border was identified using non-contact polarized dermatoscopy (DermLite DL4, 3Gen, Inc. San Juan Capistrano, CA, U.S.A.), followed by delineation of the surgical margin with a margin of 5 or 10 mm according to the 7th edition of the AJCC guidelines.[12] Handheld RCM imaging was performed using the commercially available VivaScope 3000 device (VivaScope GmbH, München, Germany). Surgical treatment was performed directly following HH-RCM imaging and analyses. Therefore, the investigator (Y.E.) was blinded to histopathological outcomes. Before margin delineation, the central area of the lesion was assessed to determine its predominant architecture. If invasive melanoma was suspected, the lesion was excised with a 2 mm margin for appropriate staging.

All mapping procedures were performed using commercially available circular adhesive rings, according to the following procedure[13] (**FIGURE 1 A-D**):

1. Adhesive rings (inner diameter 5,5 mm; border width: 4 mm) were applied, overlapping and bordering the entire circumference of the outer margin.

2. The inner areas of the adhesive rings were examined. The margin was considered positive for LM/LMM if epidermal round large/pleomorphic pagetoid cells and follicular or dermo-epidermal junction localization of atypical cells (round or dendritic) were present **(FIGURE 2**). The margin was redrawn depending on its extension from the previous margin (i.e., 1-2 fields of view at the midline and 2+ fields of view at the distal inner border).

3. New adhesive rings were placed at the new margins and repeated until negative margins were achieved.

4. The margin was preserved in the absence of the LM/LMM criteria.

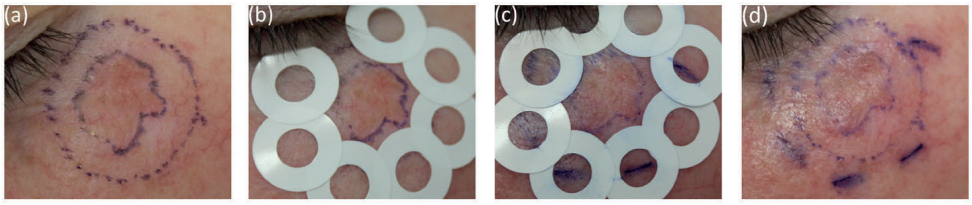

FIGURE 1. *Circumferential mapping procedure by handheld reflectance confocal microscopy.*

Step 1: Lentigo maligna located on the left zygoma delineated by a surgical marker, including the 5 mm surgical margins.

Step 2: Circumferential placement of adhesive rings (5.5 mm diameter and 4 mm inner area) bordering the surgical margin.

Step 3: Evaluation of the inner areas of the adhesive rings. In the presence of lentigo maligna criteria, a new margin was drawn, and the margin persevered in the absence of subclinical lentigo maligna.

Step 4: New adhesive rings were placed at the new margins, and steps 1 to 3 were repeated until the entire circumference was negative for lentigo maligna criteria on HH-RCM examination

Clinical characteristics, including age, sex, localization, extent of pigmentation, and histopathological diagnosis, were recorded. Histological margin assessment was used as the reference standard. All histological examinations were performed by experienced melanoma pathologists who were blinded to the RCM margin delineation outcomes. Excisions were fixated in 4% formaldehyde overnight, followed by ink application to secure the resection borders. Two ink colors were applied to the resection margins to divide the long axis of the excision into two halves. Thereafter, 3 mm thick slides were cut perpendicular to the long axis, resulting in a sequence of slices that all contained epidermis with lesions in relation to the colored 3- and 9-hour skin resection margins and the deep dermal/subcutaneous

resection margin. The 12- and 6-hour tops were prepared separately to identify potential spread along the long axis, and, where necessary, deeper cuts of the tops in the direction of the resection margin were performed until no lesion was visible. When the deepest cut at the top was still positive, the resection margin at that site was considered positive. Next, the material was paraffin-embedded, stained with standard hematoxylin and eosin staining, and, where necessary, additional immunohistochemistry for Melan-A, sox10, or S100 to stain melanocytes was performed. Histological data included the histopathological diagnosis, margin status, extent of the free margins, and Breslow thickness.

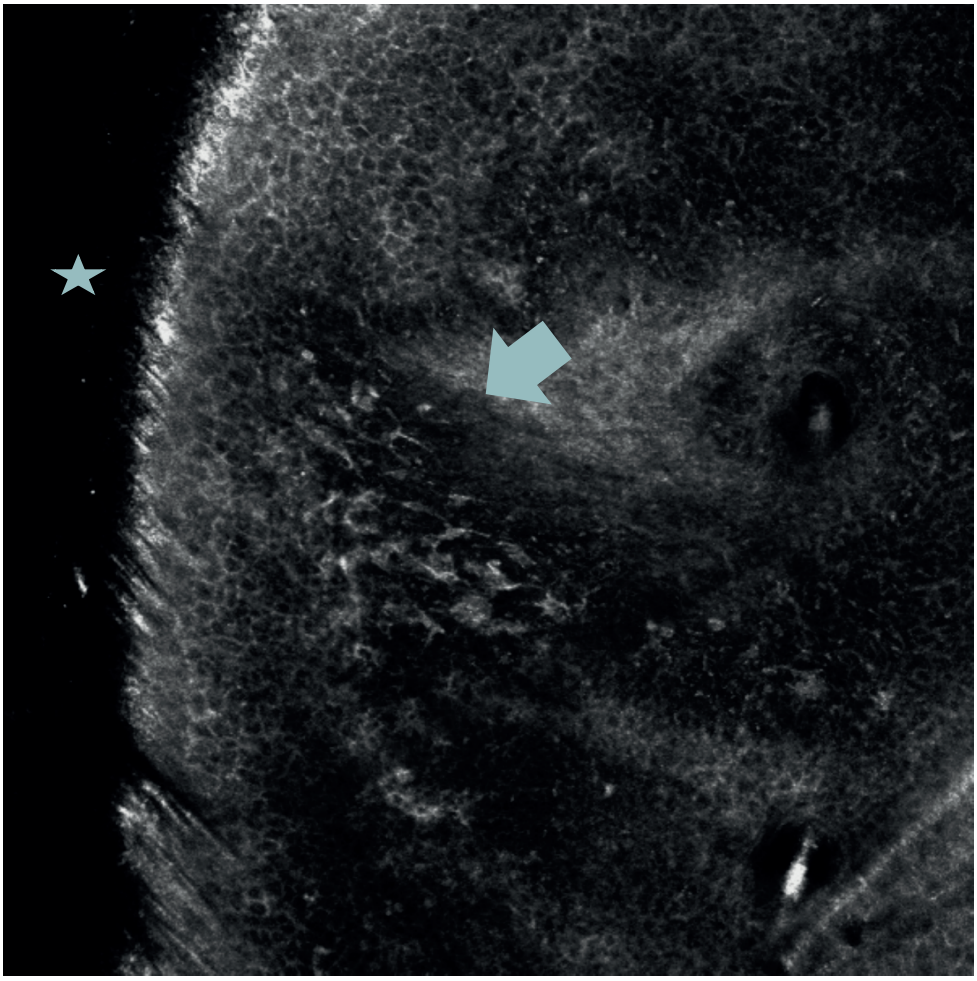

FIGURE 2. *Handheld reflectance confocal microscopy of the inner area of an adhesive ring (star) showing atypical dendritic cells at the level of the dermo-epidermal junction (arrow)*

Statistical Analysis. Statistical analysis was performed using the SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA). Absolute and relative frequencies are described for all study characteristics. The diagnostic outcome of HH-RCM was evaluated by assessing the frequency and extent (in mm) of negative margins, as reported in the final pathology report. In addition, a 2 × 2 contingency table (**TABLE 2**) was created to evaluate the accuracy of our method in detecting subclinical disease beyond the surgical margin compared with the histopathological margin outcomes. Histological margins were deemed acceptable as ≤5mm and ≤10mm for LM and LMM, respectively. Based on these parameters, larger histological margins were considered surgical overtreatments. The outcomes of diagnostic accuracy were defined as follows: (i) true-positive, subclinical LM on HH-RCM with acceptable histological margins; (ii) false-positive, subclinical LM on HH-RCM with surgical overtreatment; (iii) true-negative, no subclinical LM on HH-RCM and negative histological margins; and (iv) false-negative, no subclinical LM on HH-RCM and positive histological margins.

RESULTS

Between December 2015 and July 2017, 51 consecutive patients with histopathologically confirmed LM/LMM in the head-and-neck were diagnosed in the NKI-AVL (**FIGURE 3**). The clinical characteristics of the 26 patients included are shown in **TABLE 1**. Twenty-one (41.2%) patients met the exclusion criteria: T3/T4 classified LMM (n=12; 41.2%), diagnostic excision before wide local excision (n=4), and non-surgical treatment (n=5). In addition, 4 (7.8%) patients had lesions not accessible by HH-RCM (n=4), so they were not included in further analysis.

Following HH-RCM imaging, lesion management was changed based on the RCM evaluation in 2 patients. In the first patient, subclinical LM extended > 10 mm into the skin of the lower eyelid. Subclinical extension was confirmed using a 3 mm punch biopsy, and the patient was subsequently treated with topical imiquimod. In the second patient, cerebriform nests were observed during RCM examination, which is a rare but highly specific RCM structure correlating with invasive melanoma.[14] Following a diagnostic excision (2 mm margin), the lesion was upstaged to pT1a LMM (Breslow thickness 0,5 mm without ulceration/dermal mitoses).

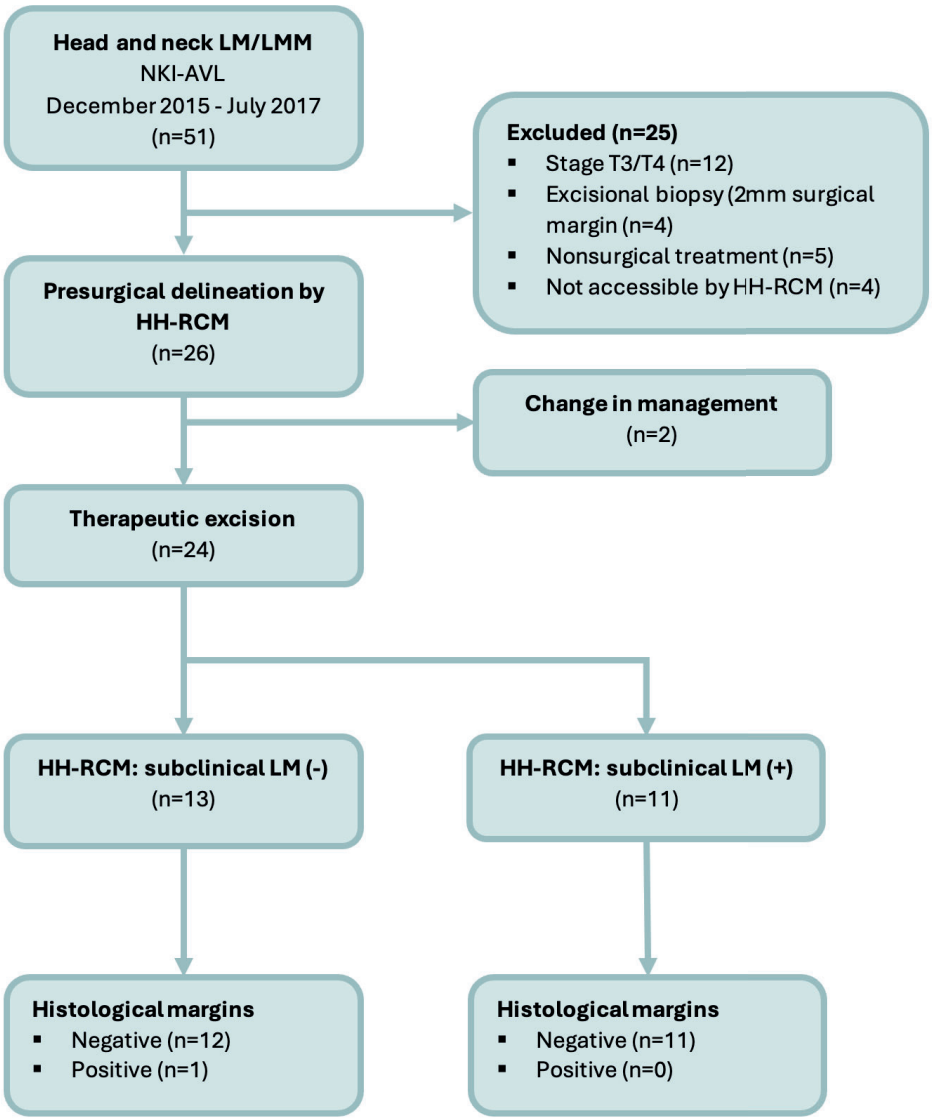

FIGURE 3. *Inclusion flowchart*

NKI-AVL = The Netherlands Cancer Institute; LM = lentigo maligna; LMM = lentigo maligna melanoma; HH-RCM = handheld reflectance confocal microscopy

TABLE 1. Clinical data of the included lentigo maligna (melanoma)

Characteristic	Descriptive data No. (%)	
Patient characteristics		
Age (SD, range) years	69.5±10.1 (48-90)	
Sex		
Male	9 (34.6%)	
Female	17 (65.4%)	
Lesion characteristics		
Anatomic localization		
Cheek	12 (46.2%)	
Periorbital	5 (19.2%)	
Scalp	3 (11.5%)	
Nasolabial	2 (7.7%)	
Other [A]	4 (15.4%)	
Pigmentation [B]		
Lightly pigmented	12 (46.2%)	
Pigmented	11 (42.3%)	
Amelanotic [C]	3 (11.5%)	
Diagnostic modality		
Punch biopsy (3mm)	21 (80.8%)	
Incisional biopsy	1 (3.8%)	
Excisional biopsy	4 (15.4%)	
Histological diagnose	Primary	Recurrent
LM	14 (53.9%)	6 (23.1%)
LMM (Breslow mean; range) (0.6mm; 0.1-1.2)	3 (11.5%)	3 (11.5%)

LM = lentigo maligna; LMM = lentigo maligna melanoma
[A] Nose/Forehead/Ear/Neck
[B] According to Menzies, et al[26]
[C] Defined as an erythematous macule/patch

TABLE 2. Subclinical LM detection by HH-RCM beyond the surgical margins compared to the histological margin outcome A

	Histology +	Histology -	
HH-RCM +	9 (37.5%) (TP)	2 (8.3%) (FP)	11 (45.8%)
HH-RCM -	1 (4.2%) (FN)	12 (50.0%) (TN)	13 (54.2%)
	10 (41.7%)	14 (58.3%)	

LM: lentigo maligna; HH-RCM: handheld reflectance confocal microscopy
[A] Positive (+) outcome was defined by subclinical LM on HH-RCM and histological margins ≤0-5/10mm (TP) or positive (FN). Negative (-) outcomes were defined by no subclinical LM on HH-RCM and histological margins >5/10mm (FP) or negative margins (TN).
LM = lentigo maligna; FN = false-negatives; FP = false-positive; HH-RCM = handheld reflectance confocal microscopy; TN = true negative; TP = true-positive

The remaining 24 evaluable lesions were excised following the presurgical mapping by HH-RCM and consisted of 18 (75.0%) LM and 6 (25.0%) LMM with a median (IQR) Breslow thickness of 0,5mm (0,1-0,5). The LMM consisted of pT1a (n=5) and pT2a (n=1) melanoma,

according to the 7th AJCC staging system. The included lesions' median (IQR) diameter was 19.0 mm (12.3-32.3).

Overall, 95.8% (23/24) of the patients had negative margins following excision, including all (15/15) primary lesions and 88.9% (8/9) recurrent lesions. HH-RCM detected subclinical LM beyond the guideline-recommended margin (**FIGURE 4**) in 45.8% (11/24) of the lesions; in 9.1% (1/11) of these cases, HH-RCM did not detect the full extent of LM, resulting in positive histological margins. No subclinical LM was detected on HH-RCM in the remaining lesions (13/24). However, in 1 of these 13 cases (7.7%), the HH-RCM outcome was considered a false negative, resulting in positive histological margins (**TABLE 2**). This was the only case (4.2%) with positive surgical margins and concerned a patient with recurrent LM.

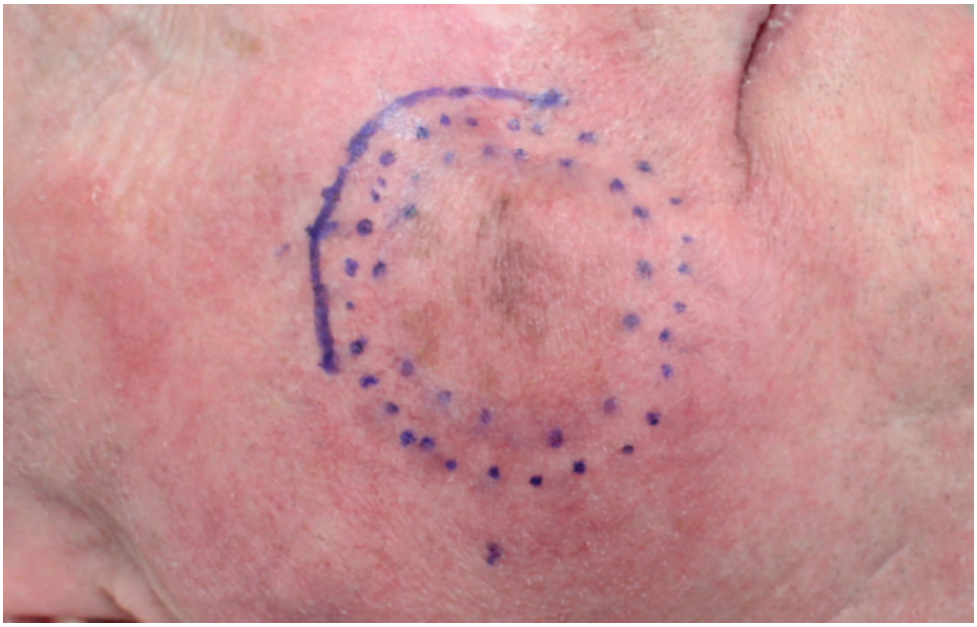

FIGURE 4. *A case of lentigo maligna on the right cheek with subclinical spread (solid line)*

The median (SD; range) of histological margins were 3.3 mm (±2; 0-7) and 5.3 mm (±3.4; 1-10) for LM and LMM, respectively. For LMM, the mean (SD; range) histological margin for the invasive component was 9.0mm (±2.0; 7-12).

The overall accuracy of subclinical disease detection (**TABLE 3**) was 87.5% (95% CI 67.4-97.3), with a sensitivity of 0.90 (95% CI 0.55-1.00), specificity of 0.86 (95% CI 0.57-0.98), and a PPV and NPV of 0.82 (0.55-0.94) and 0.92 (0.65-0.99), respectively. For primary lesions, the sensitivity was 1.00 (95% CI 0.48-1.00), with a specificity of 0.80 (95% CI 0.44-0.97).

TABLE 3. Diagnostic accuracy of subclinical LM detection by HH-RCM

Lesion type	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
All (n=24)	0.90 (0.55-1.00)	0.86 (0.57-0.98)	0.82 (0.55-0.94)	0.92 (0.65-0.99)	87.5% (67.4-97.3)
Primary (n=15)	1.00 (0.48-1.00)	80.00 (0.44-0.97)	0.71(0.42-0.90)	1.00	86.7% (59.4-98.3)
Recurrent (n=9)	0.80 (0.28-0.99)	1.00 (0.40-1.00)	1.00	0.80 (0.40-0.96)	89.9% (51.7-99.7)

HH-RCM: handheld reflectance confocal microscopy; PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval

Surgical wounds were closed using primary intention (n=9), full-thickness graft (n=9), split-skin grafts (n=5), and a single reconstructive skin flap (n=1). A single lesion was upstaged to LMM (pT1a; Breslow thickness, 0.5 mm) without ulceration/dermal mitoses. Furthermore, the Breslow thickness increased in 3 (12.5%) lesions, resulting in a single LMM being upstaged from pT2a to pT3a melanoma. No lymph node metastases were detected by ultrasound-guided FNAC (0/5) or SLNB (0/2) in any patient.

Four (16.6%) patients died during follow-up due to unrelated causes, with no signs of local recurrence at a median (IQR) follow-up of 20.5 months (14.5-37.8). There was one (4.2%) histologically confirmed recurrence at 10 months of follow-up in the single patient with positive margins following surgical treatment. The patient was subsequently treated with topical imiquimod and showed no signs of recurrence at 17 months of follow-up.

The remaining 19 (79.2%) patients had a mean follow-up of 36.7 months (range 26-49) without local recurrence. One patient with LM developed distant metastasis at 21 months of follow-up, with no prior history of invasive melanoma. All prior histological specimens were retrospectively reviewed without signs of invasive melanoma. The patient was treated with an anti-PD-1 checkpoint inhibitor (pembrolizumab) and achieved (stable) complete remission at 38 months of follow-up.

DISCUSSION

Although dermatoscopy plays a significant role in diagnosing LM/LMM, its efficacy is insufficient for full-margin control.[7,8] Reflectance confocal microscopy therefore has the potential to play a role in the (surgical) management of LM/LMM by detecting atypical melanocytes beyond the margins defined by dermatoscopy. In this study, we evaluated the accuracy of presurgical delineation by HH-RCM in a consecutive series of patients with head-and-neck LM/LMM; negative margins were achieved in 95.8% of the patients, including all primary (n=18) and amelanotic (n=3) cases. Our rate of negative histological margins compares favorably with several other successful approaches using RCM in the surgical

treatment of LM/LMM.[10,15-16] Champin et al. used HH-RCM-assisted staged excision (i.e., Spaghetti technique) of LM/LMM using two operators and identified subclinical LM beyond the guideline-recommended margins in all included lesions.[16] Following the first surgical stage, negative margins were achieved in 85% of the cases. In a follow-up study, Couty et al. were able to fully excise 88% of the lesions after one surgical stage with no reported local recurrences at an average of 44 months of follow-up.[10]

As we detected subclinical LM in 45.8% of our patients, these patients would most likely have had positive surgical margins following excision in a setting without HH-RCM. This number is comparable to published data, where the guidelines recommended surgical margins as being insufficient in up to 24-45% of LM treated by wide local excision.[6] Two lesions were upstaged to invasive melanoma following surgical excision, highlighting the need for caution when opting for non-surgical treatment modalities. Surprisingly, one of the four patients who developed distant metastasis had no history of invasive melanoma. It is striking that this patient was surgically treated six times before being referred to our center. Therefore, it is not illogical to assume that an invasive component might have been missed during histological assessment. However, an unknown primary with complete regression cannot be excluded either.

While the HH-RCM device allows for faster evaluation, one of its disadvantages is the lack of standardized imaging, which makes it more observer- and experience-dependent. Yélamos et al. attempted to overcome this limitation by converting videos into larger images using a custom-made algorithm.[17] The estimated surgical margins by HH-RCM were an average of 0.76 mm smaller than the actual surgical margins. Pellacani et al. also used video assessment, using superficial epidermal cuts as a reference point during imaging.[16] With this technique, they achieved negative margins in 93% of the lesions. The video assessment of the margins had fair/moderate inter-rater agreement with a sensitivity and specificity of 92% and 57%, respectively. While highly accurate and reproducible, epidermal cuts are semi-invasive, so one must consider the fact that the current commercially available HH-RCM device does not allow for adequate post-treatment sterilization without modifications.

Given the above facts, despite the lack of standardized imaging, HH-RCM appears to be a reliable alternative to traditional AM-RCM. Nonetheless, in the current study, we were unable to determine the inter-rater reliability because a single investigator performed all imaging and analyses.

Owing to its localization in the head-and-neck region, limiting surgical overtreatment of LM/LMM should also be considered. Handheld RCM overestimated the extent of subclinical LM in two cases (8.3 %). Dendritic cells are a potential source of false-positive outcomes, as both

melanocytic hyperplasia and Langerhans cells can result in hyperreflective dendritic cells on RCM.[17,18] To our knowledge, only one other study reported overestimation of the surgical margin using HH-RCM, which was found in 9.8% of lesion quadrants.[17] Nevertheless, the reported risk of overtreatment by RCM seems limited. Guitera et al. performed 185 punch biopsies before the excision of LM/LMM.[11] By using traditional AM-RCM, the dermatoscopic false negative rate was brought down from 65.0% to 8.3%, with a comparable false positive rate for both techniques (2.4% and 3.2%, respectively). However, compared to HH-RCM, the use of AM-RCM is increasingly time-consuming for larger lesions because of the need for fixation to the skin in several consecutive areas.

There are several limitations to our study, including the limited sample size. Ideally, only primary LM/LMM should be included, with the aim of preventing local recurrences. However, the inclusion of more complex cases from a tertiary oncologic referral hospital could have led to an underestimation of our results.

Prior to mapping with HH-RCM, the lesions were delineated using dermatoscopy. While the use of dermoscopy could be considered the standard of care for dermatologists, it could have led to a source of possible bias in our results. The importance of dermatoscopy was shown in a study by Robinson, who found that in all 26 cases, the total delineated surface area by visual inspection was significantly less than dermatoscopic delineation.[8] Nonetheless, even though dermatoscopy improves the delineation of LM/LMM compared to the naked eye, it still results in a general underestimation, leading to incomplete excisions.

Many surgical techniques have been used to achieve margin control in excised LM/LMM.[19-20] We combined the presurgical mapping of LM/LMM by HH-RCM with conventional excision to provide a time-saving alternative compared to staged excision or MMS. This immediately highlights a possible disadvantage of our technique, as it is comparable to in vivo mapping using punch biopsies. Consequently, we did not evaluate 100% of the histological margins, which could have led to an overestimation of the negative margin rate. Nevertheless, with a mean follow-up of 36.7 months (range 26-49) and only one local recurrence, the results seem to indicate that HH-RCM is a good alternative to staged excision.

Finally, although our study's low recurrence rate is comparable to rates reported in other studies, our follow-up period was limited. A recent study showed that at least 4–5 years of follow-up is needed to detect all LM/LMM recurrences following surgical treatment.[21] Accordingly, a longer follow-up period is needed to confirm the accuracy of our method.

CONCLUSION

To our knowledge, this is the first study to evaluate the accuracy of detecting subclinical LM using HH-RCM by evaluating the histological margins while remaining mindful of potential overtreatment. Compared to other approaches, we feel the strength of our method is its reproducibility, fully noninvasive use of RCM, and performance by a single operator. We believe that the low rate of overtreatment is acceptable; consequently, RCM could help to define potential tissue-sparing surgical margins in the head-and-neck. Furthermore, HH-RCM can be used as an alternative to time-consuming staged excisions with several stages of permanent-section processing. Moreover, HH-RCM findings changed the management of 8.3% (n=2) of patients. In a study by Guitera et al., 43% of the patients were treated non-surgically following RCM examination.[11] Combined with the fact that studies have also shown RCM to be a valuable tool in the follow-up of non-surgically treated LM[22-24], RCM could potentially play a role in both personalized surgical and non-surgical management of LM/LMM.[25]

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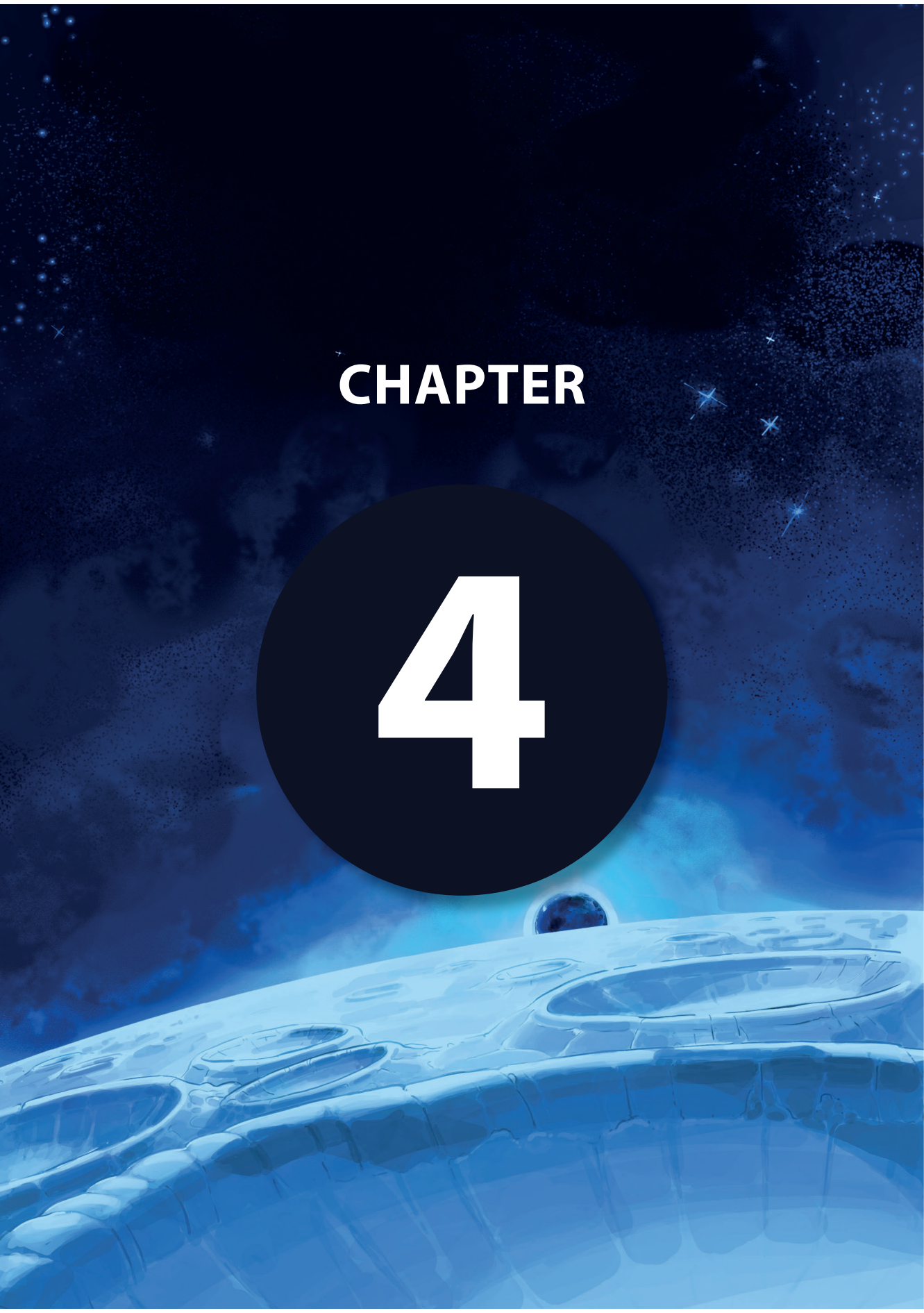
CHAPTER
4

SUCCESSFUL IMPLEMENTATION OF HANDHELD REFLECTANCE CONFOCAL MICROSCOPY AS THE STANDARD OF CARE IN THE (SURGICAL) MANAGEMENT OF LENTIGO MALIGNA (MELANOMA)

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ABSTRACT

Background. Reflectance confocal microscopy (RCM) has shown promise in predicting surgical outcomes by noninvasive detection of subclinical lentigo maligna (melanoma) (LM/LMM).

Objectives. To assess the effects of presurgical mapping using handheld RCM (HH-RCM) on surgical treatment, follow-up outcomes, and management decisions.

Methods. A total of 117 consecutive LM/LMM cases (2015-2023) were included in this study. The diagnostic accuracy of HH-RCM in detecting subclinical LM and invasive components was evaluated. The primary endpoints included histological margins and changes in management, based on the outcomes of the HH-RCM mapping procedure. Margin and follow-up outcomes were compared to a historical cohort before HH-RCM was introduced (n=94) (2003-2014).

Results. HH-RCM detected subclinical LM in 60% (n=70) of cases. The median mapping duration was 14 min (range 4-50). In 28% (n=33) of cases, the mapping procedure resulted in a change in lesion management, with the majority consisting of limited surgery with adjuvant imiquimod (n=15) or imiquimod monotherapy (n=14). The remaining patients (n=84) underwent HH-RCM-assisted wide local excision. Histological margins were cleared in 98% of the patients with a median histological margin of 3.0 mm, which was significantly higher than the 81% in the historical cohort (median 2.0 mm) (P=0.001). The sensitivity and specificity for detecting the extent of subclinical LM were 94% (95% CI 80.4-99.3) and 84% (95% CI 70.3-92.7), respectively. The negative predictive value for the detection of LMM was 94% (95% CI 84.4-97.7), and 75% of the initially missed LMM (n=12) were identified during the HH-RCM mapping procedure. The study cohort had a 1.6% local recurrence rate compared with 25% in the historical cohort.

Conclusions. Integrating HH-RCM as the standard of care could lead to more personalized treatment strategies for LM/LMM and allow the selection of patients suitable for nonsurgical treatment.

INTRODUCTION

Surgical excision is the recommended treatment for head-and-neck lentigo maligna (melanoma) (LM/LMM).[1,2] However, lesion delineation remains challenging because of the underestimation of the lesion extent by clinical examination using dermoscopy and Wood's lamp examination.[3-5] This can result in incomplete excisions and contribute to an increased risk of local recurrence.[6-8] To address this issue, various staged excisional techniques have successfully reduced the local recurrence risk.[9] Even so, these techniques do not predict the definitive size of the surgical defect, which is particularly crucial in the head-and-neck owing to cosmetic and functional considerations. Nonsurgical options such as topical imiquimod (IMQ) and radiotherapy can be considered in cases where surgical excision is not feasible.[10-13]

Reflectance confocal microscopy (RCM) is a noninvasive diagnostic device that has been shown to improve the detection of subclinical LM, leading to reduced rates of involved surgical margins and recurrence in wide local excision (WLE) and staged surgical techniques.[9, 14-17] A pilot study in our center has demonstrated the reliability of handheld reflectance confocal microscopy (HH-RCM) for presurgical mapping.[18] In this study, we aim to evaluate the impact of HH-RCM following its introduction as the standard of care in our center and assess its effect on management decisions and long-term follow-up outcomes.

MATERIALS & METHODS

This research paper expands on a pilot study (2015-2017) conducted in our center[18] and includes consecutive patients with head-and-neck LM/LMM referred to the Department of Dermatology of the Netherlands Cancer Institute (NKI-AVL) with no contraindications for surgical excision (2018-2023). A historical cohort of consecutive head-and-neck LM/LMM treated with WLE (n=92) before HH-RCM was introduced was used as the control group (2003-2014).[19] The researchers followed ethical guidelines and received approval from the institutional review board. A single investigator (Y.E.) performed all imaging/analyses with 33 months of experience with HH-RCM at the start of the inclusion period. The study data were recorded in a standardized form for the extension cohort. Staging was performed according to the 8th edition of the American Joint Committee on Cancer (AJCC).

The HH-RCM mapping procedure was followed by a multidisciplinary consultation with a dermatologist and a head-and-neck surgeon, with the patient present and used in the management decision-making process. The standard of care in the NKI-AVL for LM/LMM consists of HH-RCM-guided wide local excision (WLE) with a 5–10 mm surgical margin. Excised tissue was cut perpendicular to the long axis (i.e., bread loafing), and immunohistochemical

staining was used when necessary for Melan-A, sox10, S100, or PRAME. Diagnostic sampling errors were defined as LM being reclassified as LMM after surgery.

The lesions were analyzed for morphological characteristics, and subclinical invasion missed at the initial diagnosis. The clinical border was determined using polarized dermoscopy (DermLite DL4, 3Gen, Inc., San Juan Capistrano, California, USA). The planned surgical margin was delineated by using a surgical pen. Adhesive rings were placed around the entire circumference of the margin and examined for subclinical LM. The margin was extended in case of atypical dendritic cells, and a new ring was applied. This process was repeated until the entire lesion was mapped and no subclinical LM was found beyond the proposed surgical margins (**FIGURE 1**). The mapping procedure has been described in detail elsewhere.[18] Deviation from the standard of care was discussed with the patient based on the extent of the subclinical component with potential functional or cosmetic consequences and/or suspicion of invasive LMM. The diagnostic accuracy of HH-RCM in detecting subclinical LM and invasive components was compared with that of the histological outcome (reference standard). The HH-RCMs predicted defect size and the duration of the mapping procedure were recorded.

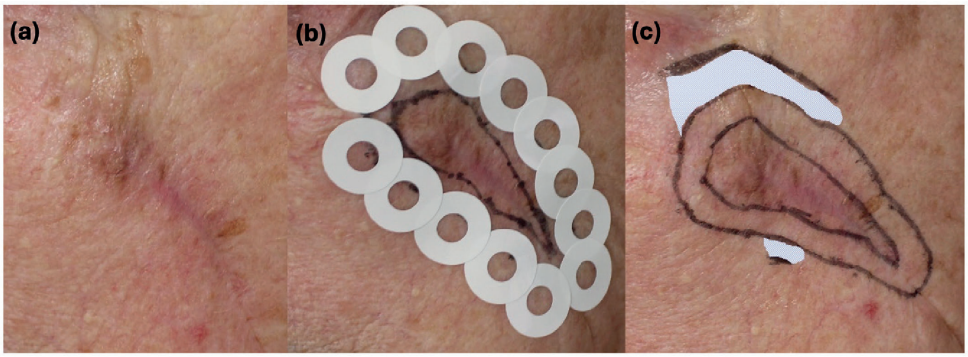

***FIGURE 1**. Recurrent lentigo maligna on the cheek (left) with presurgical margin delineation by handheld reflectance confocal microscopy using circumferential adhesive rings (middle). The final result (right) shows subclinical spread beyond the planned surgical margin.*

The primary endpoint of the study was the surgical outcome or changes in management based on HH-RCM results. Modified lesion management options consisted of imiquimod (IMQ) monotherapy or limited surgery, followed by adjuvant IMQ. Topical IMQ was applied daily for 12 weeks, with intensity reduction (up to a minimum of five days per week) in case of side effects. Six months after completing the topical IMQ treatment, the entire lesion area was assessed using HH-RCM, and a biopsy was performed when residual LM was suspected. The secondary outcome was the recurrence rate following surgical excision in

patients with at least six months of follow-up. The follow-up outcomes of the pilot study were retrospectively updated. The surgical and follow-up outcomes of HH-RCM-mapped patients were compared with those of the historical cohort.

Statistical analysis. Absolute and relative frequencies were described for all study variables. Differences in baseline clinicopathological characteristics between the study groups were identified using X^2 or Fisher's exact tests for categorical variables and the unpaired t-test or Mann-Whitney U test for continuous variables, where appropriate. The time to local recurrence was calculated from the date of surgical treatment. In the case of IMQ treatment, recurrence time was calculated from the time of topical treatment completion. Statistical significance was set at $P < 0.05$. Data was analyzed using SPSS 29.0 for Windows (IBM Corp, Armonk, NY)

RESULTS

Clinicopathological characteristics

One hundred-seventeen patients, 26 (22%) from the pilot cohort and 91 (78%) from the extension cohort, were included (**TABLE 1**). The predominant method of initial diagnosis involved a single 3 mm biopsy (n=88; 75%). At baseline, 70% (n=82) were classified as LM, and 30% as LMM (n=35), with a median Breslow thickness at diagnosis of 0.7mm (range 0.1-2.5). Following surgical excision, the proportion of LMM increased to 44% (n=51), as 24% (16/67) of surgically treated LM were histologically reclassified as LMM (median Breslow thickness 0.5mm; range 0.2-1.2). No LMM showed signs of histological ulceration. Thirteen out of 15 indicated cases underwent SLNB, and isolated tumor cells were found in 2 of the 13 patients (i.e., N1a, according to the AJCC 8th edition). In the historical cohort, 12 of 53 (23%) LM were reclassified as LMM.

TABLE 1. Clinicopathological characteristics of lentigo maligna (melanoma) included in the study cohort (HH-RCM mapping) and historical cohort.

	Study cohort (n=117)					
No (%)	**Pilot cohort (n=26)**	**Extension cohort (n=91)**	**Total (n=117)**	**P-value**	**Historical cohort (n=92)**	**P-value**
Patient characteristics						
Age (years) Mean (range)	69.5 (48-90)	72.0 (34-91)	71.0 (34-91)	NS	70.1 (45-93)	NS
Sex				NS		NS
Female	17 (65.4)	55 (60.4)	72 (61.5)		49 (53.3)	
Male	9 (34.6)	36 (39.6)	45 (38.5)		43 (46.7)	
Lesion characteristics						
LM	18 (69.2)	48 (52.7)	66 (56.4)	NS	48 (52.2)	NS
Primary | Recurrent	12 (46.2) | 5 (19.2)	30 (33.0) | 18 (19.8)	42 (35.9) |23 (19.7)	NS	34 (37.0) | 14 (15.2)	NS
LMM	8 (30.8)	43 (47.3)	51 (43.6) [A]	NS	44 (47.8)	<0.001
Primary | Recurrent	5 (19.2) | 4 (15.4)	33 (36.3) | 10 (11.0)	38 (32.5) | 14 (12.0)		41 (44.6) | 3 (3.3)	
Breslow thickness (mm) Median (range)	0.5 (0.3-2.2)	0.6 (0.3-3.6)	0.5 (0.2-3.6)		1.5 (0.1-7.0)	
Localization				NS		NS
Facial	21 (80.8)	76 (83.5)	97 (82.9) [B]		72 (78.3)	
Ear	1 (3.8)	7 (7.7)	8 (6.8)		4 (4.3)	
Scalp	3 (11.5)	7 (7.7)	10 (8.5)		14 (15.2)	
Neck	1 (3.8)	1 (1.1)	2 (1.7)		2 (2.2)	
Pigmentation				NS	NA	NA
Pigmented	9 (34.6)	39 (42.9)	48 (41.0)			
Lightly pigmented	10 (38.5)	16 (17.6)	26 (22.2)			
Partly pigmented	3 (11.5)	15 (16.5)	18 (15.4)			
Amelanotic	2 (7.7)	8 (8.8)	10 (8.5)			
Scar	2 (7.7)	13 (14.3)	15 (12.8)			
Demarcation				NS	NA	NA
Sharp	3 (11.5)	15 (16.5)	18 (15.4)			
Moderate	8 (30.8)	32 (35.2)	40 (34.2)			
Unsharp	15 (57.7)	44 (48.4)	59 (50.4)			
Diameter (mean; range)						
Long axis (mm)	18 (6-55)	16 (4-48)	17 (4-55)	NS	17 (5-57)	NS
Short axis (mm)	-	8.5 (2-40)	10.5 (2-40)	NA	NA	NA

AJCC = The American Joint Committee on Cancer; HH-RCM = handheld reflectance confocal microscopy; LM = lentigo maligna; LMM = lentigo maligna melanoma; NA = not available; NS = nonsignificant

[A] T-stage according to AJCC 8: T1a (n=34; 66.7%), T1b (n=4; 7.8%), T2a (n=7; 13.7%), T3a (n=4; 7.8%); Tx (n=2; 3.9%)

[B] Facial (n=97) included cheek (n=49), (peri)nasal (n=18), periorbital (n=14), forehead (n=10), nasolabial (n=4) and chin (n=2)

Effect of HH-RCM mapping procedure on LM/LMM management

The diagnostic accuracy outcomes are summarized in **TABLE 2**. The median mapping duration was 14 min (range 4-50). Subclinical atypical cells beyond the initial surgical margin were detected in 60% of cases (n = 70). For cases of subclinical LM, the median diameter increased from 20 mm (range, 4–55 mm) to 33 mm (range, 16–60 mm) for the longitudinal axis and from 14 mm (range, 2–40 mm) to 25 mm (range, 10–50 mm) for the transverse axis. Subclinical LM was significantly associated with increased lesion size (long-axis, P=0.04; short-axis, P<0.001). No significant differences were found in the lesion type, subtype, localization, demarcation, pigmentation, or Breslow thickness.

TABLE 2. Diagnostic accuracy of the HH-RCM procedure for detecting subclinical LM and invasive components compared to the histological outcome.

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
Subclinical, presence	97.0 (84.2-99.9)	84.6 (71.9-93.1)	80.0 (67.8-88.4)	97.8 (86.4-99.7)	89.4 (80.9-95.0)
Subclinical, extent [A]	94.3 (80.8-99.3)	82.0 (68.6-91.4)	78.6 (66.9-86.9)	95.3 (84.1-98.7)	87.1 (78.0-93.4)
Invasive component	80.0 (52.0-95.7)	88.2 (76.1-95.6)	66.7 (47.5-81.5)	93.7 (84.4-97.7)	86.4 (75.7-93.6)

CI = confidence interval; HH-RCM = handheld reflectance confocal microscopy; NPV = negative predictive value; PPV = positive predictive value

[A] In cases with detected subclinical LM, histological margins >5mm for LM and >10mm for LMM were considered false-positive (overtreatment); positive histological margins were considered false-negative.

Sixteen of 51 LMM cases (31%) were initially missed at diagnosis, of which 12/16 (75%) were identified during the HH-RCM procedure. The suspicion of the invasive component was based on the presence of cerebriform nests (n=2), atypical (i.e., sparse) melanocytic nests at the dermo-epidermal junction/papillary dermis (n=3), epidermal/junctional disarray (n=4), large melanocyte size (n=6), pleomorphic pagetoid/atypical cells (n=1), and widespread atypical cobblestone pattern (n=1). The 5 false-positive cases were based on a large melanocyte size (n=2), a disarranged pattern (n=2), and a single case with a widespread atypical cobblestone pattern (n=1).

Modifications in lesion management occurred in 27% (n=32) of cases. The remaining patients (n=85) underwent HH-RCM-guided WLE (**FIGURE 2**). The primary reason for modified management was the extent of the subclinical component (n=23; 72%), followed by suspicion of an invasive component (n=1, 3%), suspicion of an invasive component combined with the extent of the subclinical component (n=5, 16%), or refusal of (further) surgery (n=3, 9%). In the modified management group, the longitudinal (P=0.049) and transverse (P=0.008) diameters were notably larger at baseline. There was no significant

difference in the median age (P=0.748) or Breslow thickness (P=0.95). The median (IQR) age of patients treated by topical IMQ mono- or adjuvant therapy (n=26) was 70.5 (65.0-76.0). Most lesions (n=10; 39%) were localized on the cheek, whereas perinasal and orbital lesions represented 35% (n=9) and 15% (n=4), respectively.

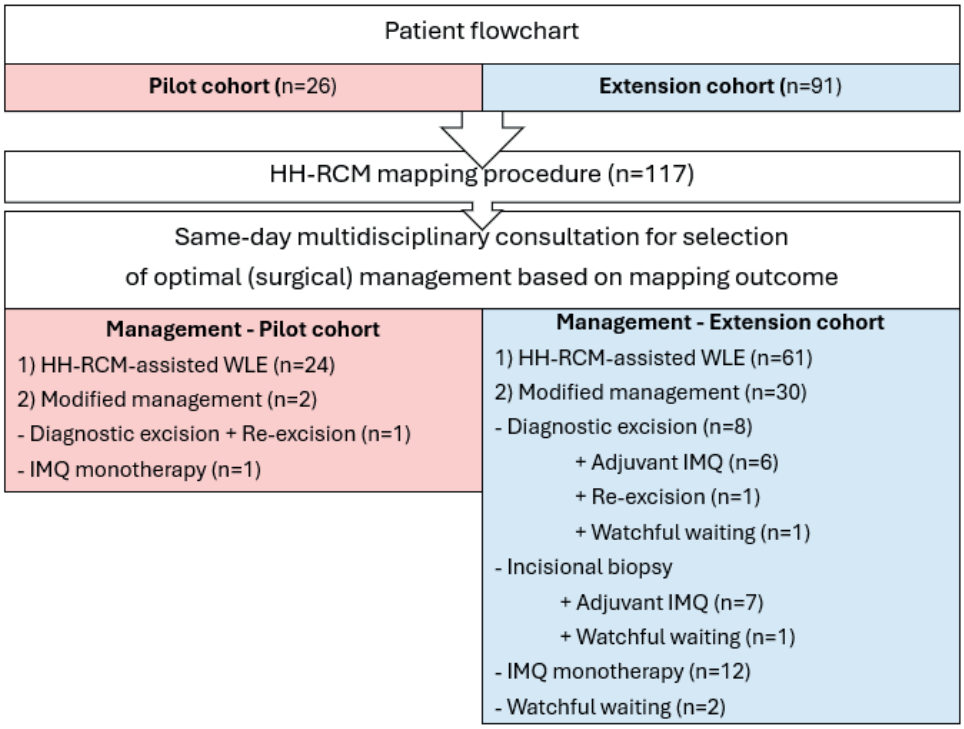

FIGURE 2. *Inclusion flowchart of included lentigo maligna (melanoma) and management outcomes following the HH-RCM mapping procedure. HH-RCM = handheld reflectance confocal microscopy; IMQ = imiquimod; WLE = wide local excision*

Surgical outcome

The resection margins were cleared in 98% of the HH-RCM-guided group with a median histological margin of 3.0 mm (IQR 2.0-5.0), compared to 81% (P=0.001) in the historical cohort with a median histological margin of 2.0 mm (IQR 1.0-6.0). There was no difference in the median histological margins between patients with and without subclinical LM detected using HH-RCM (3.0 vs. 2.9; P=0.643). The invasive component's median (IQR) histological margin was 7.0 (4.9-10.0). For the LMM cases (n=12) that were treated by diagnostic excisional/incisional biopsy followed by adjuvant treatment, the invasive component's median (IQR) histological margin was 5.0 (4.1-7.3). No residual invasive LMM was found in the excisional specimen in 33% (n=17) of LMM cases. The median (range) Breslow thickness for these cases at diagnosis was 0.9mm (0.2-3.6).

In the HH-RCM-guided WLE group, closure consisted of primary intention (n=40, 47%), full-thickness grafts (n=31, 37%), split skin grafts (n=11, 13%), and local flaps (n=3, 3%). There was a trend in the rate of primary closure based on the presence or absence of subclinical LM (45% vs. 55%) (P=0.5)

Follow-up - HH-RCM-assisted wide local excision (n=84)

Four patients died of unrelated causes, and one patient was lost to follow-up. **TABLE 3** shows the follow-up outcomes for patients with at least six months of follow-up (n=70; 82%). In two cases, HH-RCM-assisted WLE resulted in positive margins. In the first case, a scalp LM with three previous local recurrences before HH-RCM mapping resulted in the only local recurrence at 11 months of follow-up. The patient was treated with IMQ monotherapy and remained recurrence-free at 52 months. The second case involved a patient with recurrent amelanotic LMM of the cheek who refused further surgical treatment and showed no signs of local recurrence after 35 months of follow-up.

TABLE 3. Comparison of the local recurrence rates in LM/LMM after the introduction of HH-RCM-assisted WLE*

	HH-RCM-assisted WLE			
	Pilot (n=24)	**Extension (n=46)**	**Total (n=70)**	**Historical cohort (n=92)**
Inclusion period	2015-2017	2018-2023	2015-2023	2003-2014
Follow-up (months) median (IQR)	62.0 (41.0-76.7)	21.0 (12.7-30.5)	24.5 (16.7-57.3)	61.0 (35.1-76.0)
Recurrence rate No (%)	1 (4.2)	0 (0.0)	1 (1.4)	23 (25.0)

* Only cases with at least 6 months of follow-up were included (70 out of 84).
HH-RCM = handheld reflectance confocal microscopy; IQR = interquartile range; LM = lentigo maligna; LMM = lentigo maligna melanoma; WLE = wide local excision

Among the 51 patients with LMM, 1 (1.6%) progressed to stage IIIC and remained recurrence-free 32 months after adjuvant nivolumab. Two patients with LM developed non-LMM-related stage IV melanomas. The first patient had a history of 5 prior invasive melanomas and died at 19 months due to metastatic disease. The second patient, with an unknown primary tumor, was treated with pembrolizumab (anti-PD-1) and was in complete remission after 78 months of follow-up.

Follow-up – Modified management due to HH-RCM mapping procedure (n=33)

A single patient (3%) died from an unrelated cause. Six-month follow-up was available for 75% (n=24) of patients with modified management with no local recurrences at a median

follow-up of 18.5 months (IQR 9.0-32.5). For patients treated with IMQ monotherapy (n=10) or adjuvant IMQ (n=9), the median follow-up after completion of the IMQ treatment was 18 months (IQR 9.0-23.0). Two patients showed dendritic cells during HH-RCM evaluation after the completion of IMQ treatment. Residual LM was confirmed in one (4%) of these cases by HH-RCM-guided biopsy, while the other did not have histological signs of LM. A focal sparse dermal nest was observed in a single patient, which was histologically confirmed as a small dermal nevus. A single patient declined adjuvant IMQ and progressed to LMM at 16 months of follow-up (Breslow thickness 0.4 mm without ulceration). One patient with IMQ-treated LM developed regional lymph node metastases, which were attributed to an unknown primary melanoma owing to the absence of clonality. The patient was alive and recurrence-free at 34 months of follow-up.

DISCUSSION

Our study demonstrated that implementation of HH-RCM-guided surgery improved the histological clearance rate from 81% to 98% (P=0.001) while maintaining long-term local recurrence-free survival in the pilot cohort. The mapping procedure also changed management strategies in over 25% of patients and identified 75% of LMM cases that were missed at the initial diagnosis.

Our study's 96.7% histological clearance rate of HH-RCM-guided WLE is notably higher than the reported 78-83% range for LM/LMM in a recent systematic review when using guideline-recommended WLE surgical margins.[9] Negative histological margins do not always correlate with recurrence-free survival, however. Only a limited portion of the surgical margin is evaluated due to transversal tissue processing ('bread-loafing').[20] In this regard, the extent of the histological margin is the strongest predictor of local recurrence.[21] A 3 mm cut-off point has been proposed as a histological margin $<$3 mm is associated with a 27% risk of local recurrence, compared to 2.6% for $\geq$3 mm.[21-22] Although the extension cohort's follow-up period was limited, our study's median histological margin of 3 mm aligns with prior data with a limited risk of local recurrence.[21-22] In support of our data, Yélamos et al. have shown that the HH-RCM-estimated surgical defect using video mapping correlates well with the eventual surgical outcome.[23]

Approximately one-fourth of surgically treated LM were upstaged to LMM. As HH-RCM has a penetrative depth limited to the level of the papillary dermis in the horizontal plane, it can be challenging to determine the invasion depth of atypical cells.[24] Nonetheless, HH-RCM successfully identified 75% of invasive cases that were initially missed. To our knowledge, the study by Melhoranse et al. is the only study evaluating the diagnostic accuracy of RCM in detecting subclinical invasion components in LMM.[25] A total of 229 LM/LMM were evaluated,

and the invasive component was detected in 89% of the cases with a sensitivity of 63% (95% CI 52-78%) and a specificity of 79% (95% CI 74-88%). The three features most predictive of an invasive LMM component are epidermal/junctional disarray, melanocytic nests, and large melanocyte size. Including only these three predictive criteria would remove two cases from our analysis and result in a modest change in sensitivity from 80% to 79% and specificity from 88% to 90%. These proposed features align with an observational RCM study showing that melanocytic nests presenting as lentiginous perifollicular (i.e., medusa-head-like structures) and dermal nests were significant RCM features that distinguished LM from LMM. [26-27] A possible explanation for false-positive outcomes may be the pagetoid cells. Although they have been shown to predict invasive LMM in histology[28], they are not predictive RCM features for LMM, possibly because intraepidermal Langerhans cells and melanocytic hyperplasia also have dendritic morphology.[25, 29] False-negative outcomes are likely the result of the horizontal orientation of the imaging and limited penetration depth. Finally, similar to our data, the NPV (99%) was higher than PPV (46%). The negative predictive value is a critical diagnostic outcome when selecting LM cases to be treated using nonsurgical modalities.

Over one-fourth of the cases had their management strategy changed because of the outcome of the HH-RCM mapping procedure. Most patients underwent limited surgical excision followed by adjuvant IMQ or IMQ monotherapy. A recent Delphi Consensus paper endorsed topical IMQ as the primary alternative to surgical intervention in monotherapeutic and adjuvant contexts.[1] The clinical clearance rates based on data from systematic reviews of IMQ monotherapy range from 63-79%. Still, the heterogeneity of the data should be considered when interpreting the reported efficacy of topical IMQ.[30-31] The treatment effectiveness of IMQ highly depends on the intensity, duration, and presence of local inflammation. In line with the standard of care in our center, the greatest odds for clearance are found with 6-7 applications per week with at least 60 applications, with a significant decrease in efficacy when used less than 5 days per week.[10, 30]

Clinical clearance rates following IMQ treatment may overestimate histological clearance rates due to limited partial sampling by single-punch biopsies. To facilitate the early detection of treatment failure, all patients undergoing topical IMQ treatment were evaluated using HH-RCM six months after completing the treatment. Of the 28 patients, only one case of residual LM was histologically confirmed by HH-RCM-guided biopsy. Previous studies have indicated that RCM is more accurate in detecting treatment failure than clinical-dermoscopic evaluation, with 100% sensitivity and specificity exceeding 92%.[32-33] More than half of the IMQ-treated patients received adjuvant treatment, which appears to increase clearance rates compared to monotherapy and has recurrence rates comparable to micrographically controlled surgical techniques, supporting our low residual LM rate.[34-35]

Limitations. This retrospective study has several limitations. First, the limited follow-up may have led to underestimation of the recurrence rate. Previous research suggests that a considerable proportion of post-treatment recurrences occur after a median duration of three years or more.[22, 36-38] This underestimation is likely minimal since our pilot's long-term follow-up data showed a persistently low recurrence rate. The current 1.6% recurrence rate is slightly lower than the 2.6% expected from the median 3 mm histological margin.[21-22] In the case of topical IMQ treatment, we systematically mapped the affected area six months post-treatment. There is a potential for selection bias, as lesions with larger subclinical components were more frequently treated with IMQ monotherapy or adjuvant therapy. Furthermore, although we compared the clinicopathological features between the historic and study cohorts, additional histological or clinical features or management decisions in the historic cohort could have led to bias in our outcomes.

In line with the recent Delphi consensus statement, our center's primary alternative to surgical treatment was topical IMQ. However, the lack of randomized controlled data on the comparative effectiveness of IMQ and radiotherapy and the optimal usage of topical IMQ (i.e., mono-, adjuvant-, or neoadjuvant therapy) still exists.[39-40] Given the complexity of treatment options and the paucity of predictive prognostic data, shared decision-making is essential for patients with LM/LMM.[41] Although patients were involved in the multidisciplinary consultation, no pre- or post-consultation questionnaires were administered, preventing us from assessing the impact of the HH-RCM mapping process on decision-making. Finally, the mapping was conducted by a single confocal user in a tertiary hospital setting; therefore, the generalizability of our results to other patient populations is unclear.

CONCLUSION

Our findings support the efficacy of HH-RCM in precisely identifying surgical margins, reducing recurrence rates, and detecting subclinical LMM. This allows for informed selection of patients suitable for nonsurgical treatment. Although staged micrographically controlled surgical techniques have demonstrated a clear advantage in minimizing local recurrence compared to WLE, they fall short in predicting the final defect size.[9] Topical IMQ or radiotherapy may be considered when surgical excision is not feasible.[10, 12] However, nonsurgical treatment requires caution given that clinical features are unreliable in predicting invasive LMM. Implementing HH-RCM as the standard of care could help resolve diagnostic challenges, enabling more tailored treatment approaches for LM/LMM patients.[40] To confirm our findings, a prospective, preferably multi-center, matched-controlled study comparing HH-RCM-assisted excision to micrographically controlled surgery for LM/LMM with long-term follow-up and survival outcomes is needed.

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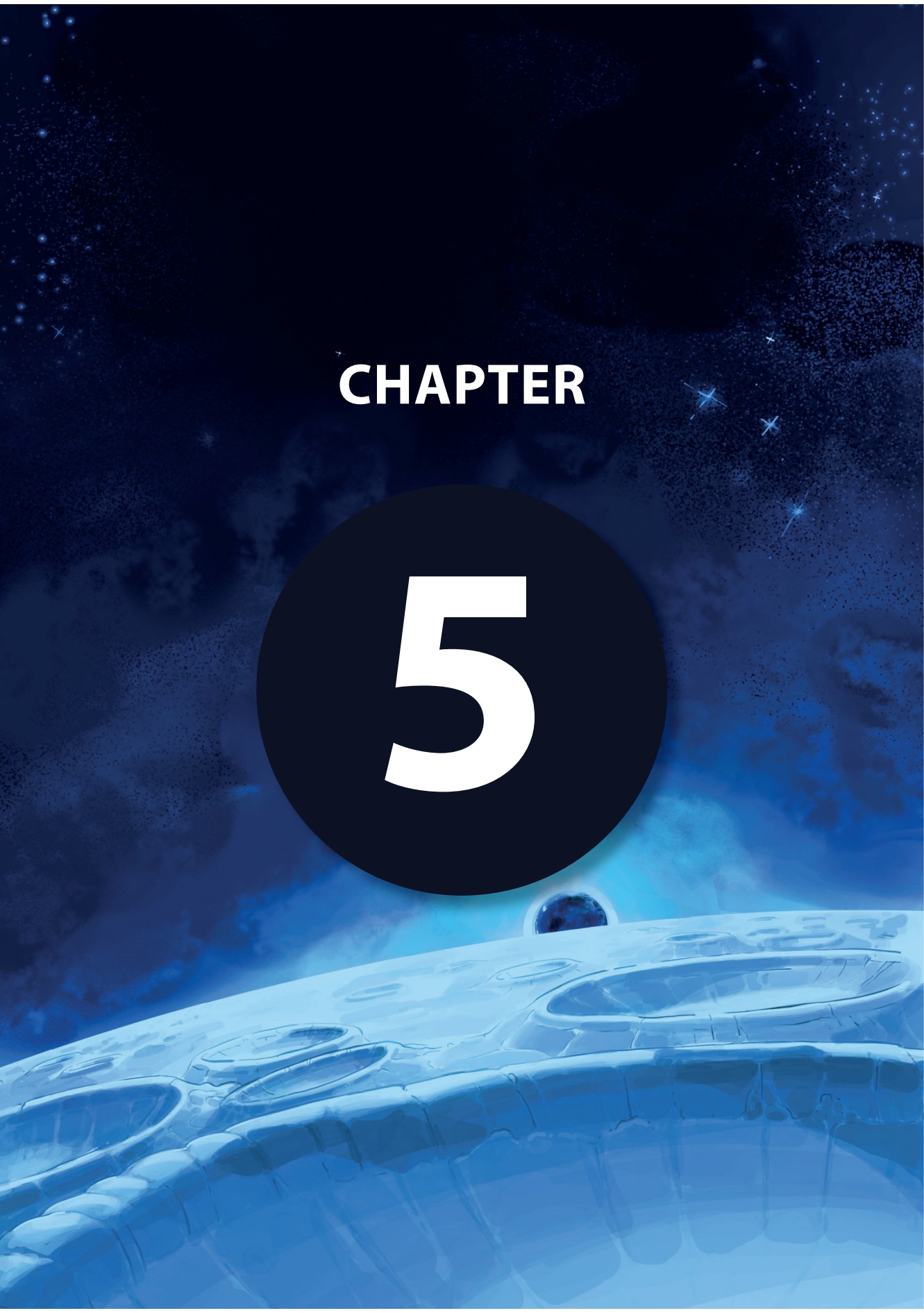
CHAPTER
5

LENTIGO MALIGNA (MELANOMA): A SYSTEMATIC REVIEW AND META-ANALYSIS ON SURGICAL TECHNIQUES AND PRESURGICAL MAPPING BY REFLECTANCE CONFOCAL MICROSCOPY

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ABSTRACT

Due to the increased risk of local recurrence following surgical treatment of lentigo maligna (melanoma) (LM/LMM), the optimal surgical technique remains a matter of debate. We aimed to evaluate the effects of different surgical techniques and reflectance confocal microscopy (RCM) on local recurrence and survival outcomes. We searched the MEDLINE, Embase, and PubMed databases until May 20th, 2022. Randomized and observational studies with ≥10 lesions were eligible for inclusion. Bias assessment was performed using the Methodological Index for Non-Randomized Studies instrument. A meta-analysis was performed for local recurrence, as there were insufficient events for other clinical outcomes. We included 41 studies with 5059 LM and 1271 LMM. Surgical techniques included wide local excision (WLE) (n=1355), staged excision (n=2442), and Mohs micrographic surgery (MMS) (n=2909). Six studies included RCM. The guideline-recommended margin was insufficient in 21.6%-44.6% of LM/LMM cases. The local recurrence rate was lowest for patients treated with MMS combined with immunohistochemistry (<1%; 95% CI, 0.3%-1.9%) and highest for WLE (13%; 95% CI, 7.2%-21.6%). Depending on the surgical technique, the mean follow-up period varied from 27 to 63 months, with moderate to high heterogeneity for MMS and WLE. Handheld-RCM decreased both the rate of positive histological margins ($P<0.0001$) and the necessary surgical stages ($P<0.0001$). Most regional (17/25) and distant (34/43) recurrences occurred in the patients treated with WLE. Melanoma-associated mortality was low (1.5%; 32/2107), and more patients died due to unrelated causes (7%; 107/1608). This systematic review showed a clear reduction in local recurrences using microscopically controlled surgical techniques over WLE. The use of HH-RCM showed a trend of reducing incomplete resections and local recurrences, even when combined with WLE. Due to selection bias, heterogeneity, low prevalence of stage III/IV disease, and limited survival data, it was not possible to determine the effect of different surgical techniques on survival outcomes.

INTRODUCTION

The surgical treatment of lentigo maligna (LM) and lentigo maligna melanoma (LMM) is associated with an increased risk of local recurrence compared to other melanoma subtypes.[1] These recurrences are the result of incomplete resections due to the often poor clinical demarcation, likely because of clinically undetectable field cancerization.[2] Reported recurrence rates vary from 6-20% following wide local excision (WLE) using the guideline-recommended 5 mm surgical margins for melanoma in-situ.[3-4] As a result, international LM guidelines acknowledge that larger margins (i.e., 5-10 mm) may be necessary to achieve local control, while for LMM, the larger surgical margins are defined according to the tumor thickness.[5-8] However, these safety margins are not always feasible in the head-and-neck due to cosmetic and functional limitations.

Consequently, the optimal surgical management and margins for LM/LMM are still a matter of debate.[3] Mohs' micrographic surgery (MMS) allows intra-operational en-face assessment of the surgical margins. Unfortunately, MMS is not only limited by requiring extensive training, but more importantly, frozen sections are not universally accepted in treating melanocytic lesions as they can result in artifactual and fixational changes in the excisional specimens.[9] To circumvent this issue (rushed), permanent paraffin-embedded sections (i.e., "Slow" Mohs) or frozen sections with immunohistochemistry (IHC) staining are increasingly being used.[10] Several alterations of staged excision (SE) techniques are used as an alternative where the entire peripheral margin can be assessed by permanent sections without the need for special training or equipment.[11] While microscopically controlled surgical techniques allow for layer-by-layer assessment of the histological margin, they remain relatively time-consuming. A more recent approach is using in vivo presurgical mapping by reflectance confocal microscopy (RCM), as it can identify subclinical LM beyond the recommended surgical margins.[12-13]

While the use of MMS and SE can reduce the risk of local recurrence, the long-term benefits of these surgical techniques are less clear.[14] This systematic review aimed to evaluate the effect of the different surgical techniques used in managing LM/LMM on local recurrences and survival outcomes, as well as the effect of RCM on resection margins and recurrence rates.

METHODS

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 statement.[15] The study protocol was developed according to the PRISMA for Protocols 2015 (PRISMA-P 2015)[16] and registered

with the International Prospective Register of Systematic Reviews (PROSPERO) on September 12th, 2020 (registration number CRD42020204013). All studies with patients with LM/LMM who were surgically treated with or without the preceding use of reflectance confocal microscopy, were eligible for inclusion. Lentigo maligna (ICD-O 8742/2) and LMM (ICD 8743/2) were defined according to the 2018 World Health Organization (WHO) classification of skin tumors. The outcomes of interest included histopathological margin assessment, local/regional/distant recurrences, progression rates, and survival outcomes. Local recurrence was defined as LM/LMM recurrence adjacent to or within the surgical scar owing to incomplete resection.

Literature search

A medical information specialist (JL) performed a comprehensive search of the MEDLINE (OVID), Embase (OVID), and PubMed (non-MEDLINE subset only) electronic databases. The core search strategy consisted of controlled terms (e.g., MESH) and text words for surgery or excision, as well as LM/LMM (see **SUPPLEMENT I** for the provisional core MEDLINE search). No language restrictions, limits related to the date, or methodological search filters were applied. Animal studies were safely excluded by double negation. To avoid missing relevant studies that did not specifically mention LM/LMM in the title and abstract, we also performed a second broader search of MEDLINE and EMBASE for melanoma and surgical treatment. The search was re-run on May 20th, 2022, just before the final analyses. Citations were imported and de-duplicated using the EndNote® software (version X7; ©2014 Thomson Reuters). References of included studies were checked for additional relevant studies.

Eligibility criteria

All randomized controlled trials, case-controlled trials, case series, and prospective and retrospective cohorts were eligible for inclusion. Cohort studies and case series were defined according to the definition by Mathes et al.[17] Reviews, editorials, letters to the editors, and conference reports were excluded, as well as case series with less than 10 cases to avoid small study effects and bias. Further exclusion criteria were as follows: i) melanoma in-situ of the superficial spreading (MIS) type, ii) localization limited to the trunk and extremities, or iii) non-surgical or destructive therapies, including (neo)adjuvant topical imiquimod combined with surgical treatment. Studies for which full texts were unavailable after contacting the corresponding author(s) were also excluded.

Study selection

Study screening was performed by evaluating study titles and abstracts when available. Abstracts of all titles, including the term lentigo maligna (melanoma) or undefined melanoma subtypes, were screened. Full texts of all abstracts, including any form of surgical treatment or articles with missing abstracts, were screened. By assessing the full texts, eligible studies were selected based on the predefined eligibility criteria stated above. Title, abstract, and full-text screening was performed in duplicate and independently by two reviewers (Y.E. & D.T./A.H.) and performed using the Rayyan platform (http://rayyan.qcri.org).[18] Consensus was reached by discussion between the reviewers. The reasons for the exclusion of full texts were registered.

Data extraction

A standardized data extraction form was developed and the data were extracted in duplicate (Y.E. & D.T.) Data for each study included study characteristics (first author, year, country, study design, and center), patient characteristics (age and sex), lesion characteristics (sample size, type, staging, localization, and lesion size), surgical technique (delineation, immunohistochemistry, and margins), surgical outcome (histological margin and number of stages for negative histological margins), and clinical outcomes (follow-up in months, local recurrence, invasive local recurrence, regional metastases, distant metastases, death due to melanoma, and death due to other causes). Staged excisions (SE) were grouped according to margin assessment (i.e., complete en-face or partial radial/bread loaf sectioning). Regional metastases included satellite metastases (within 2 cm of the surgical scar), in-transit metastases (between the primary tumor and lymph node basin and at least 2 cm from the surgical scar), and regional lymph node metastases.

Risk of bias assessment

As no randomized controlled trials were identified, bias assessment was performed using the Methodological Index for Non-Randomized Studies (MINORS) instrument, specifically developed with non-randomized (non-)comparative surgical studies in mind. The methodological quality is assessed by scoring 8-12 items using a 3-point scale (0, not reported; 1, reported inadequately; 2, reported adequately), with an optimal total score of 16 and 24 points for non-comparative and comparative studies, respectively (**SUPPLEMENT II**).[19]

Summary measures and statistical analysis

A systematic narrative review summarizes and explains the characteristics and findings of the included studies. A meta-analysis was only performed for local recurrence, as there were insufficient events for other clinical outcomes. Meta-regression random-effects models were used to pool the rates with proportions and 95% confidence intervals (95% CI). Heterogeneity was assessed using the I^2 index with values of 25%, 50%, and 75% indicating low, medium, and high heterogeneity, respectively.[20] Egger's tests were used to evaluate publication bias[21], and a *p*-value$<$0.05 was considered statistically significant. Statistical analysis was performed using the SPSS software (version 27.0; SPSS Inc., Chicago, IL, USA), and meta-analysis was performed using the *meta* package in R version 4.2.0.

RESULTS

Search results

Our electronic database search identified 1722 hits. After de-duplication, 1171 studies were screened for inclusion (**FIGURE 1**). Of the 1171 studies, 1023 were excluded based on the title (n=841) or abstract (n=182). The available full texts of 145 studies were screened according to our eligibility criteria, of which 92 did not meet our inclusion criteria. Several additional studies were excluded because no distinction was made between MIS of the superficial spreading melanoma type or MIS of the LM-type (n=9)[22-30] or reported on the same patient population as more recent publications (n=3).[31-33]

Study characteristics

A summary of the clinical data of the 41 included studies is shown in **TABLE 1** (full details are available in **SUPPLEMENT III**). Seven of the included studies were comparative cohort studies[34-40], while the 34 remaining studies were single-arm cohort studies. To date, no randomized controlled trials have been conducted. Depending on the surgical technique, approximately half of the studies (46% to 57%) used dermoscopy or Wood's lamp for margin delineation, whereas in the remaining studies, it was limited to naked-eye examination (**SUPPLEMENT III**). Presurgical mapping by RCM was used in 15% (6/41) of the studies: 5 studies used the handheld RCM (HH-RCM) device (n=190) and a single study arm-mounted RCM (n=15).[41] Mean (range) global bias score according to the MINORS instrument was 8.3 (4-12/16) for non-comparative studies and 13 (10-18/24) for comparative studies (full details available in **SUPPLEMENT IV**).

Clinical characteristics

TABLE 2 provides the clinical characteristics grouped by surgical technique. The proportion of LMM was the lowest in MMS-IHC and the highest in patients treated with WLE (4% vs. 24%). The WLE group also had the highest weighted mean Breslow thickness of 1.4 mm. The percentage of head-and-neck lesions was ≥85% for all surgical techniques except for patients treated with MMS-IHC (74.1%). At least 74% of all surgical techniques involved primary lesions. Clinical characteristics associated with increased margins were head-and-neck localization[28, 42], particularly localization on the cheek[42], ill-defined or partly hypopigmented lesions[43], increased lesion surface area[44, 45] or lesion diameter[28, 43, 46-48], recurrent lesions[36, 43], the LMM subtype[46, 49, 50], and a history of prior destructive therapy (e.g., cryotherapy)[36].

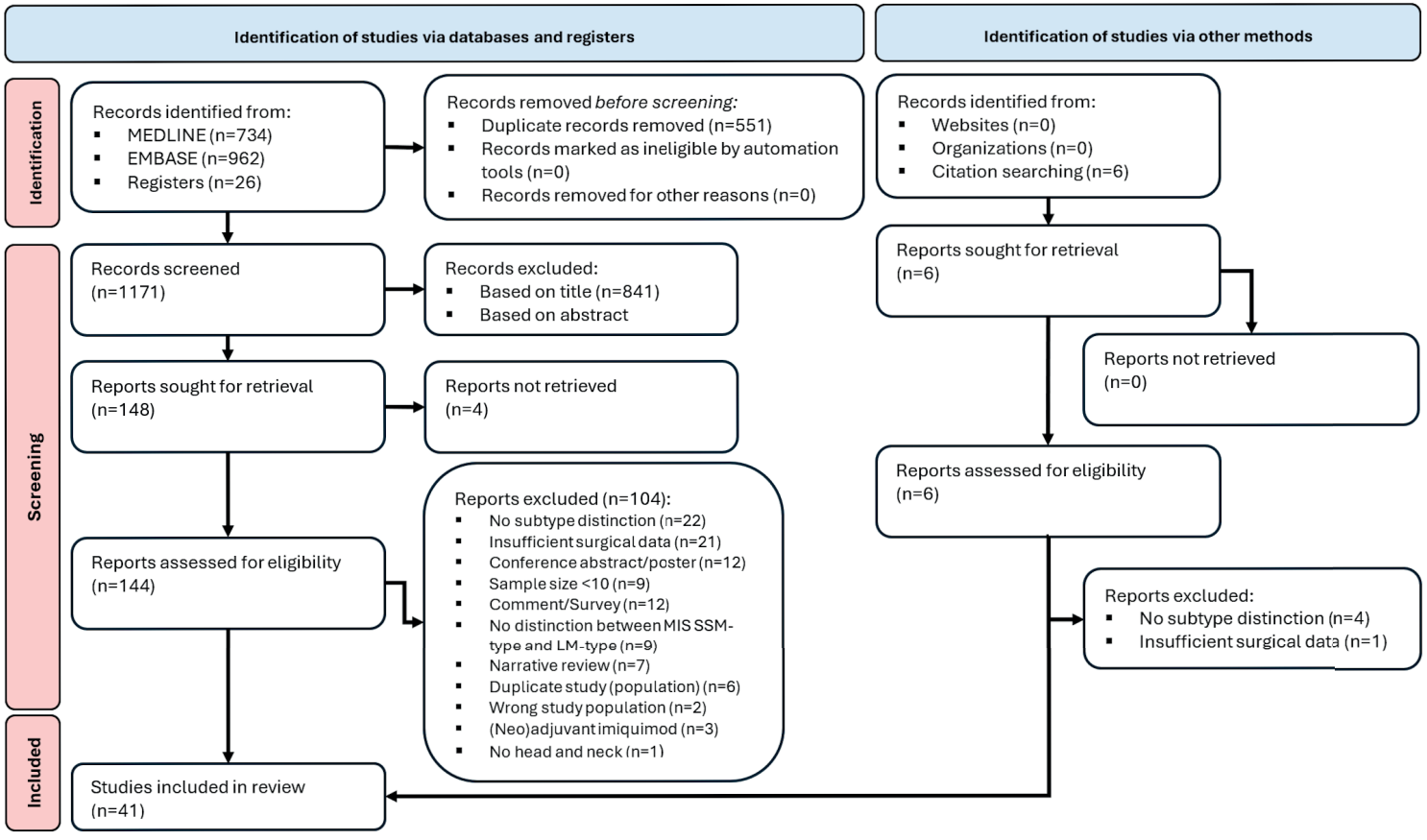

FIGURE 1. *PRISMA flowchart. Screening and selection process of included studies. From Page et al.* [15]

TABLE 1. Clinical data summary of included studies (n=41)

	No. (%)
Patient characteristics (n=6278)	
Patients/study	
Median (Q1-Q3; range)	64 (42.0-144.5; 11-1506)
Age (years)	
Mean/median (range) [A]	66.6 / 72.0 (59-75)
Sex	Male 2618 (41.7) | Female 1811 (28.9) | Unknown 1849 (29.4)
Lesion characteristics (n=6330)	
Primary	4075 (64.4)
Primary, incomplete	89 (1.4)
Recurrence	364 (5.7)
Not reported	1802 (28.5)
Subtype	LM (in-situ) 5059 (79.9) | LMM (invasive) 1271 (20.1)
Localization	HN 5177 (81.8) | TE 953 (15.1) | Unknown 200 (3.1)
Lesion size	
Surface area (mean; range)	12.2cm^2 (range: 1.2-16.0)
Max diameter	Mean 20.4mm | Median 18.0mm (range: 12.8-30.0)
Not reported	3387 (54.4)
Breslow thickness (mm)	
Mean/median (range)	0.8/ 0.6 (0.2-2.2) (n=1048)
Not reported	223 (17.5)
Unexpected LMM [B]	177/1713 (10.3) [C]
Not reported	1512/4617 (32.7)
Surgical technique	
WLE	1355 (21.4)
SE, partial margin	1115 (17.6)
SE, complete margin	1327 (21.0)
MMS	380 (6.0)
MMS, IHC	1920 (30.4)
MMS, Slow	229 (3.6)

HN = head-and-neck; IHC = immunohistochemistry; LM = lentigo maligna; LMM = lentigo maligna melanoma; MMS = Mohs' micrographic surgery; SE = staged excision; TN = trunk and extremities; WLE = wide local excision.
[A] Weighted mean/median was used.
[B] Invasive component missed at initial biopsy.
[C] Weighted mean (range) Breslow thickness: 0.5mm (0.2-0.8) (n=18; 159 missing)

TABLE 2. Clinical characteristics grouped by surgical technique (%; number/total)

Surgical technique	Primary	Head-and-neck	Lesion size Diameter (mm) (mean/median±SD [range]) Surface area (mean; cm²)	Invasive melanoma	Breslow thickness (mm) (mean/median±SD [range])	Follow-up (months)
WLE (n=1355)	95.4 (1293/1355) NR: 0.0 (0/1355)	92.7 (758/818) NR: 39.6 (537/1355)	Mean: 14.7±5.0 (10.5-21.0) (n=693) Median: 18±0.3 (18-19) (n=293) Surface area: 1.4 (n=89) NR: 31.9 (432/1355)	24.1 (326/1355)	Mean: 1.4±0.6 (0.6-2.2) (n=297) Median: - NR: 8.9 (29/326)	Mean: 57.6 (n=398) Median: 31 (n=672) Lost: 2.4 (33/1355) NR: 29.7 (403/1355)
SE, Partial [A] (n=1115)	90.6 (1010/1115) NR: 0.0 (0/1115)	89.3 (996/1115) NR: 0.0 (0/1115)	Mean: 12.6±3.2 (11.4-23.0) (n=783) Median: - Surface area: 2.2±0.9 (1.5-3.5) (n=171) NR: 14.4 (161/1115)	23.9 (267/1115)	Mean: 0.4±0.0 (0.3-0.5) (n=195) Median: 0.3 (n=162) NR: 27.0 (72/267)	Mean: 62.1 (n=391) Median: 139 (n=127) Lost: 0.6 (7/1115) NR: 64.3 (717/1115)
SE, complete (n=1327)	90.8 (946/1042) NR: 21.5 (285/1327)	76.9 (864/1124) NR: 15.3 (203/1327)	Mean: 23.2±5.0 (18-30) (n=208) Median: 17.0±4.6 (15-25) (n=161) Surface area: 8.5±4.9 (1.6-16) (n=195) NR: 60.4 (801/1327)	36.1 (479/1327)	Mean 0.5±0.1 (0.2-0.7) (n=223) Median: 0.7 (n=179) NR: 16.1 (77/479)	Mean: 35.4 (n=655) Median: 30 (n=514) Lost: 6.9 (92/1327) NR: 23.7 (315/1327)
MMS, Classic (n=380)	85.8 (326/380) NR: 0.0 (0/380)	92.0 (81/88) NR: 76.8 (292/380)	Mean: 17.0 (n=45) Median: - Surface area: 1.2 (n=18) NR: 83.4 (317/380)	30.5 (116/380)	Mean: 0.3±0.1 (0.2-0.6) (n=87) Median: - NR: 25.0 (29/116)	Mean: 27.0 (n=373) Median: - Lost: 1.8 (7/380) NR: -
MMS, IHC (n=1920)	99.0 (1900/1920) NR: 0.0 (0/1920)	74.1 (1308/1766) NR: 8.0 (154/1920)	Mean: 25.1 (n=154) Median: 23.0 (n=154) Surface area: 3.81 (n=260) NR: 78.4 (1506/1920)	3.5 (67/1920)	Mean: 0.9 (n=67) Median: - NR: 0.0 (0/67)	Mean: 63.3 (n=1766) Median: - Lost: 1.2 (4/1920) NR: 8.0 (154/1920)
MMS, Slow (n=229)	87.8 (201/229) NR: 0.0 (0/229)	96.5 (221/229) NR: 0.0 (0/229)	Mean: 17 (n=74) Median: - Surface area: N/A NR: 67.7 (155/229)	7.0 (16/229)	Mean: - Median: - NR: 100.0 (16/16)	Mean: 33.7 (n=50) Median: - Lost: 0.9 (2/229) NR: 77.7 (178/229)

DM = distant metastases; IHC = immunohistochemistry; LMM = lentigo maligna melanoma; LR = local recurrence; MMS = Mohs' micrographic surgery; NR = not reported; RCM = reflectance confocal microscopy; RM = regional metastases; SE = staged excision; WLE = wide local excision

[A] Includes bread loaf and radial sectioning

Surgical margins

Lentigo maligna. A 5mm surgical margin resulted in negative histological margins in 83% (950/1100) following WLE or SE with partial margin assessment (i.e., bread loaf or radial sectioning).[36, 37, 39, 51-55] When the complete histological margin was assessed (e.g., SE or MMS), the margins were more often found to be involved, with a 5mm surgical margin resulting in negative margins in 59% (350/598).[36, 39, 47, 48, 56-61] The weighted mean ± SD (range) surgical margin necessary for negative histological margins was 7.7±2.0 mm (6.6-13.2) (n=568).[42, 43, 46, 47, 49, 56, 61, 62]

Lentigo maligna melanoma. A 10mm surgical margin resulted in negative histological margins in 78% (40/51) following WLE or SE with partial margin assessment.[42, 52, 53] Similar to LM, this percentage decreased (55%; 144/260) in the case of SE or MMS with complete margin assessment.[39, 56, 63] The weighted mean±SD (range) surgical margin necessary for negative histological margins was 9.3±2.6mm (6.3-13.0) (n=405).[38, 42, 46, 49, 56]

Presurgical mapping of LM/LMM by HH-RCM before WLE resulted in a recurrence rate of 2% (2/84)[52, 64] compared with 27% (4/15) in a single study using arm-mounted RCM[41]. No histological margin data were available for lesions mapped using arm-mounted RCM.

Surgical stages

In the subgroup analysis, the weighted mean±SD (range) stages needed for histological clearance in patients treated by SE was 1.7±0.1 (1.4-2.1) (n=728) [40, 42, 44, 46, 49, 56, 62, 65, 66], compared to 1.1±0.03 (1.1-1.2) when HH-RCM (n=103) was used prior to SE[58, 67] (P<0.0001). Negative histological margins were obtained after the first stage in 86% (89/103) of patients using HH-RCM prior to SE[58, 67], compared to 50% (217/433) of patients without HH-RCM [47, 53, 57, 61, 66, 68] (P<0.0001). For MMS-treated patients (n=894), the weighted mean±SD (range) stages for histological clearance was 1.6±0.3 (1.4-2.3) [34, 35, 37, 40, 43, 69-71], compared to 1.3 with HH-RCM mapping prior to MMS (n=21)[35] (P<0.001). Negative histological margins were obtained after the first stage in 81% (17/21) of patients using HH-RCM prior to MMS [35], compared to 77% (1333/1723) of patients without HH-RCM [35, 36, 43, 59, 70, 72] (P=0.69).

A single comparative study directly compared HH-RCM-assisted MMS to classic MMS, with clearance rates of 81% (17/21) and 62% (16/26) after the first stage, respectively.[35] Even though this difference was not significant, HH-RCM significantly reduced the time to closure (14 vs. 27 days; P=0.038). In addition, the non-HH-RCM-assisted MMS group consisted of more challenging perinasal lesions. Walling et al. directly compared SE to MMS, and no difference in the number of stages for cleared margins was found.[40]

Clinical outcome

Clinical outcome data are shown in **TABLE 3**. Follow-up data were available for 81.0% (5130/6330) of the lesions. The weighted mean follow-up was at least 57 months in patients treated with WLE, SE with partial margin assessment, and MMS-IHC and at most 33 months for classic and slow-MMS (**TABLE 2**). The overall recurrence rate was 4% (n=230), with a weighted mean±SD (range) time to recurrence of 39.1±14 (8-96) months. Of the local recurrences, 7% (16/213) were invasive LMM with a weighted mean (range) Breslow thickness of 0.9mm (0.2-1.5).

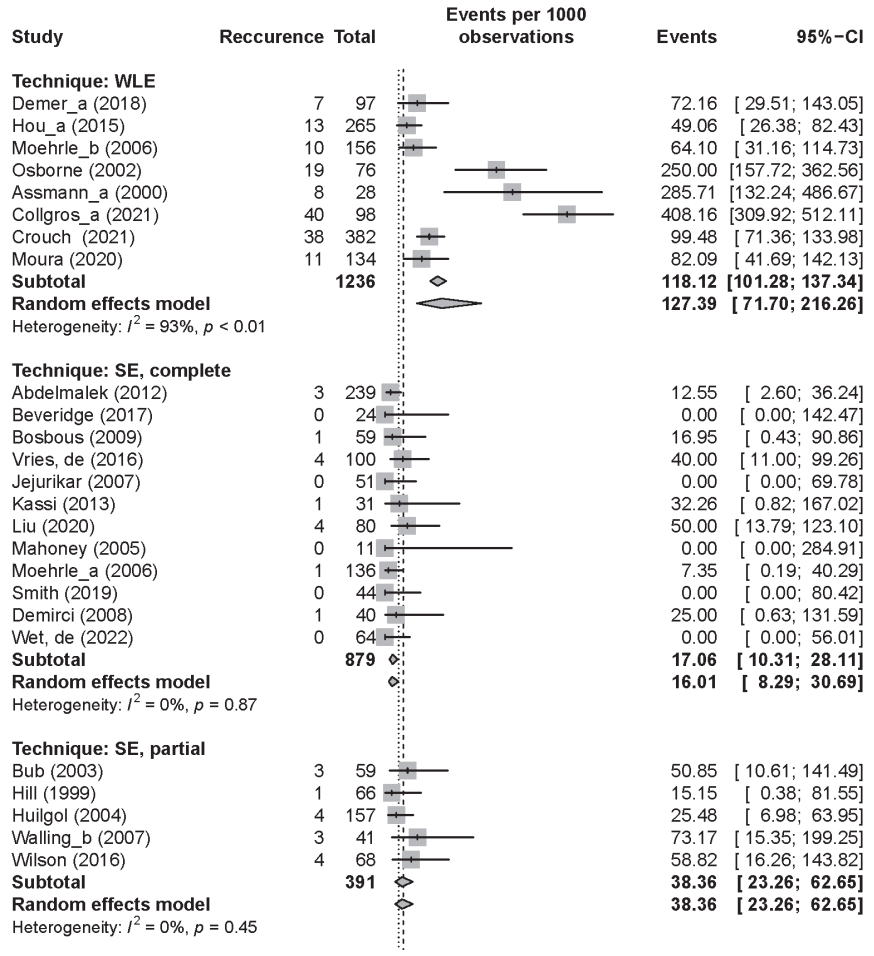

FIGURE 2. *Forest plot showing the random effects model of local recurrences of lentigo maligna (melanoma) grouped by surgical technique and presurgical mapping by handheld reflectance confocal microscopy.*

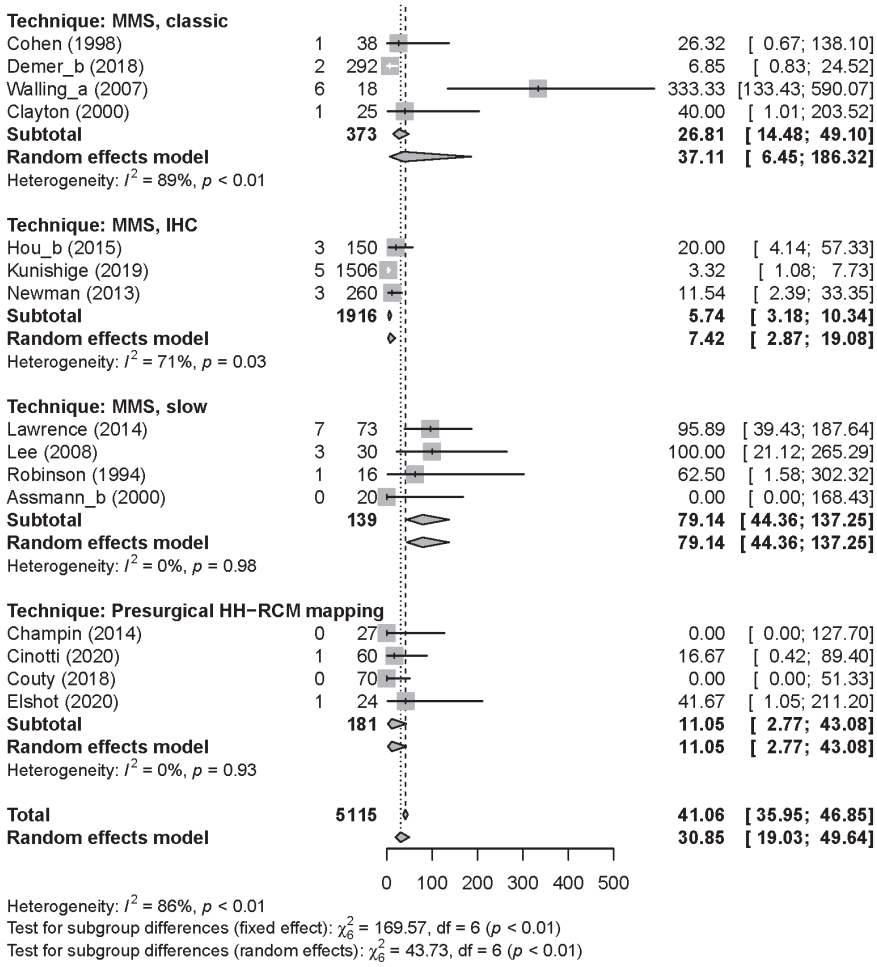

FIGURE 2. *(Continued)*

FIGURE 2 shows forest plots of local recurrence grouped by surgical technique. The local recurrence rate was lowest for patients treated with MMS-IHC (1%; 95% CI, 0.3%-1.9%) and highest for those treated with WLE (13%; 95% CI, 7.2%-21.6%).[34, 36-39, 41, 51, 54, 55] Heterogeneity was high for WLE and classic MMS, and moderate for MMS-IHC. The use of HH-RCM mapping before WLE resulted in a 2% (2/84) local recurrence rate at a weighted mean±SD (range) follow-up of 51.3±11.3 (36.7-60) months.[52, 64] In the single study using arm-mounted RCM, the recurrence rate was 27% (4/15).[2] No local recurrences (0/97) were reported following HH-RCM usage before SE with complete margin assessment, a weighted mean±SD (range) follow-up of 33.1±15.9 (10-44) months[58, 67], compared to 2% (29/1270) without the use of HH-RCM[38, 47, 49, 56, 60-62, 66, 68, 73, 74]. We could not compare the recurrence rates of LM and LMM separately, as the initial lesion subtype was reported in only 35% (n=74) of local recurrences.

TABLE 3. Clinical outcome by surgical technique (%; no/total) *

Technique			Local recurrence [A]		Metastases		Cause of death	
		RCM-	RCM+		Regional	Distant	LMM	Other
			HH	AM				
WLE	**All**	**11.8 (146/1236)**	**2.4 (2/84)**	**26.7 (4/15)**	**2.4 (19/800)**	**4.5 (36/800)**	**5.7 (28/494)**	**14.1 (34/241)**
	5-20mm	10.9 (127/1160)	2.4 (2/84)	26.7 (4/15)	2.4 (19/800)	4.5 (36/800)	6.7 (28/418)	10.9 (15/138)
	2mm	25.0 (19/76)	-	-	-	-	0.0 (0/76)	25.0 (19/76)
SE	**All** [B]	**2.3 (29/1270)**	**0.0 (0/97)**	-	**0.5 (3/634)**	**0.2 (1/634)**	**0.0 (0/882)**	**7.4 (47/636)**
	Complete	1.6 (14/879)	0.0 (0/97)	-	0.6 (3/468)	0.2 (1/468)	0.0 (0/557)	6.8 (24/351)
	Partial [C]	3.8 (15/391)	-	-	0.0 (0/166)	0.0 (0/166)	0.0 (0/325)	8.1 (23/285)
MMS	**All**	**1.3 (32/2428)**	-	**-**	**0.4 (3/635)**	**0.9 (6/635)**	**0.5 (4/731)**	**3.6 (26/731)**
	Classic	2.7 (10/373)	-	-	0.9 (3/355)	1.7 (6/355)	0.9 (3/348)	1.7 (6/348)
	IHC	0.6 (11/1916)	-	-	0.0 (0/260)	0.0 (0/260)	0.1 (1/260)	0.0 (0/260)
	Slow	7.9 (11/139)	-	-	0.0 (0/20)	0.0 (0/20)	0.0 (0/123)	16.3 (20/123)
Total		**4.2 (207/4934)**	**1.1 (2/181)**	**26.7 (4/15)**	**1.2 (25/2069)**	**2.1 (43/2069)**	**1.5 (32/2107)**	**6.7 (107/1608)**

* All pairwise outcome comparisons of 3 main surgical techniques (i.e., WLE, SE, and MMS), including the use of RCM, were statistically significant (P<0.05) except for the occurrence of distant metastases between WLE and MMS.

AM = arm-mounted; DM = distant metastases; HH = handheld; IHC = immunohistochemistry; LMM = lentigo maligna melanoma; LR = local recurrence; MMS = Mohs' micrographic surgery; RCM = reflectance confocal microscopy; RM = regional metastases; SE = staged excision; WLE = wide local excision

[A] Defined by recurrence due to incomplete resection; 7.5% (16/213) of recurrences were invasive. Weighted mean (range) Breslow thickness: 0.9mm (0.2-1.5)

[B] Staged excision with complete or partial histological margin assessment.

[C] Includes bread loaf and radial sectioning.

Three comparative studies directly compared WLE and MMS techniques (i.e., classic, IHC, and slow MMS), with a pooled recurrence rate of 8% (30/390) and 1% (5/462), respectively,.[34, 37, 39] Similarly, in a comparative study of WLE and SE with a complete margin assessment, the patients treated with WLE had a higher recurrence rate (6%; 10/156) than the SE group (1%; 1/136).[38] Finally, a single study compared SE with partial margin assessment to classic MMS, with 7% (3/41) and 33% (6/18) recurrence rates, respectively.[40] While preoperative lesion size was similar in both study arms (P=0.51), the MMS group had a significantly larger proportion of LMM (29% vs. 8%).

The reported survival data are limited (**TABLE 3**). Regional and distant recurrences occurred in 1% (25/2069) and 2% (43/2069) of patients, respectively. Most of these regional (17/25) and distant (34/43) recurrences occurred in patients treated with WLE in the studies by Demer and Moehrle et al., in which both WLE patient groups had a significantly higher mean Breslow thickness than the other surgical techniques.[34, 38] Reported melanoma-associated mortality was low (1,5%; 32/2107), and more patients died from unrelated causes (7%; 107/1608).

DISCUSSION

This systematic review of the surgical management of LM/LMM clearly shows a reduction in local recurrence using microscopically controlled surgical techniques (i.e., SE or MMS) over WLE, with MMS-IHC having the lowest recurrence rate of less than 1%. The use of HH-RCM tended to reduce the risk of incomplete resection and subsequent local recurrence, even when used with WLE. Owing to the low prevalence of stage III/IV disease and melanoma-associated mortality, it was not possible to determine the effect of different surgical techniques on survival outcomes.

Our data showed that the current guideline-recommended margin was insufficient in 27% of LM cases, and this proportion increased to almost half when considering only LMM. Furthermore, it has been reported that 24% of histologically cleared LM/LMMs locally recur, showing that our 27% guideline-recommended margin insufficiency is likely an underestimation.[41] Unfortunately, we could not perform separate recurrence analyses for either LM or LMM, as in approximately two-thirds of the cases, the initial lesion subtype was not reported.

The histological margin processing method also appeared to influence the local recurrence rate. In the study by Crouch et al., the local recurrence rate was 27% in cases with histological margins <3.0 mm, and this number dropped to 3% in cases with a histological margin of ≥ 3 mm.[51] This dichotomy results from the tissue processing (i.e., bread loafing) used during

WLE, as the entire peripheral margin was not assessed. The complete histological margin assessment used in the SE and MMS techniques can largely avoid this issue, reducing the risk of incomplete excisions. En-face processing does not entirely reduce this risk, as local recurrence can also result from field cancerization (i.e., discontinuous growth pattern).[2]

Even though the sample size of 181 HH-RCM-mapped patients was limited, all five studies showed a reduced rate of positive histological margins, a reduction in necessary stages, and fewer local recurrences, even in patients treated by WLE.[13, 52, 58, 64, 67] This might indicate a beneficial effect of presurgical mapping by HH-RCM combined with any surgical technique. Nonetheless, these data should be considered cautiously, as one comparative study by Goa et al. did not demonstrate a significant reduction of necessary surgical stages.[35] Even so, HH-RCM did allow for better presurgical planning with a significant reduction in time to closure, and significant results might be achieved by more extensive comparative studies and increased experience of HH-RCM users. This is supported by the study by Yélamos et al., in which the estimated surgical margins by HH-RCM were only a mean 0.8mm smaller than found in the final staged excision defect.[75]

When comparing all surgical techniques, MMS with IHC was shown to have the lowest recurrence rate, especially considering that it had the largest proportion of recurrent lesions, a larger mean lesion size, and the most extended follow-up compared to other surgical techniques. However, immunostained frozen sections do not entirely negate the risk of overtreatment of melanocytic hyperplasia using en-face sectioning, compared to conventional vertical cross-sections towards the tissue edge.[76] This issue is further highlighted in the study by Punchihewa et al., where there was a 13% disagreement compared to traditional permanent paraffin-embedded sections for LM treated by MMS.[9] Finally, the follow-up period of classic MMS, slow-MMS, and to a lesser extent SE with complete margin assessment, was limited or in some studies not reported. Long-term follow-up data by Collgros et al. showed that half of the recurrences occurred 4 years after treatment [41], this could have led to an underestimation of the recurrence rates.

Off-label use of topical imiquimod, a toll-like receptor 7 agonist, is increasingly being advocated in the primary management of LM as an alternative to surgical excision[77-78] Similarly, imiquimod has also been shown to be of value in reducing final defect size.[79, 80] In the study by Sampson et al., all 24 patients who completed imiquimod treatment had negative margins using a 2 mm surgical margin, resulting in a 71% reduction in the required surgical margin [80]. Opting for non-surgical treatment should be done cautiously, as partial biopsy is associated with diagnostic sampling errors, as shown by 11% of the cases in this study harboring unexpected LMM after full histological assessment.

Of all surgical techniques, the highest local recurrence rate (12%), most of stage III (2%) and stage IV (55%) disease, and melanoma-associated mortality (6%) were found in the WLE subgroup. This is likely the result of selection bias, as WLE had the highest proportion (20%) of LMM, in combination with the highest weighted mean Breslow thickness.

Limitations

Our data are limited by the fact that there was considerable variation between studies in the clinical characteristics of the included lesions with study heterogeneity for some of the surgical techniques. As certain clinical features have been reported to be associated with increased surgical margins, a head-to-head comparison of surgical techniques remains limited, as most studies did not correct for these possible confounders. Additionally, dermoscopy and Wood's lamp were used for lesion delineation in approximately half (54 %) of the studies. As both have been shown to increase the visible margin [81, 82] this could have affected the surgical outcome. Another limitation was that the follow-up data were inconsistent. Finally, as most thicker invasive melanomas were treated by WLE and there were no randomized controlled trials available, our data do not allow us to assess the real difference in clinical outcomes between the different surgical techniques. Survival outcomes could have been further confounded by the introduction of novel systemic treatments, such as immune checkpoint inhibitors [83] during the inclusion period of our review, as well as in patients with multiple melanomas.

CONCLUSION

This is the first meta-analysis aimed at the surgical management of LM/LMM, in which we compared WLE to micrographically controlled surgical techniques (i.e., SE and MMS), in addition to the value of presurgical mapping by RCM. In addition to local recurrence, we also considered the effect of different surgical techniques on other important clinical outcomes, including regional and distant recurrence. Our data supports the use of MMS combined with IHC over other surgical techniques when considering local recurrence as the primary outcome. Nonetheless, clinical demarcation remains problematic, resulting in a relatively time-consuming procedure requiring substantial training [84], nor is there consensus on the optimal use of immunostaining (**SUPPLEMENT III**). Based on well-designed studies, it is reasonable to assume that HH-RCM can significantly influence the presurgical assessment of the real extent of the lesions, resulting in a reduction in surgical stages and more adequate surgical treatment planning. Handheld RCM could potentially support the choice for non-surgical treatment modalities and allow non-invasive longitudinal monitoring when surgical excision is not feasible. As the effect of local recurrence on survival seems limited, more data on managing high-risk LMM is needed to assess the effect of different surgical techniques on survival outcomes.

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SUPPLEMENTS

SUPPLEMENT I. Search strategy

Database(s): **Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R)** 1946 to Present		
#	**Searches**	**Results**
1	Hutchinson's melanotic freckle/	580
2	(melanoma/ or lentigo/) and carcinoma in situ/	216
3	(lentig* adj3 (melanom* or malign* or Hutchinson*)).tw,kf.	1502
4	Hutchinson* melano*.tw,kf.	46
5	((Hutchinson* or melanotic or melanocyt*) adj3 freckl*).tw,kf.	77
6	Dubreuilh.tw,kf.	41
7	((melanom* or melanos*) adj6 (in situ or precancer* or pre-cancer*)).tw,kf.	1571
8	**or/1-7 [LENTIGO MALIGNA]**	**3154**
9	(exp animals/ not humans/) or (animal* or experimental or veterinar* or opossum* or zebra or zebras or dog or dogs or pup or pups or canine or rodent* or rat or rats or ((mouse or murine) not (antibod* or monoclon*)) or mice or B16 or hamster* or gerbil* or chick* or minipig* or pig or pigs or guinea).ti.	4887254
10	**8 not 9 [HUMAN LENTIGO MALIGNA]**	**3112**
11	"margins of excision"/	763
12	second look surgery/	668
13	microsurgery/	25214
14	Mohs surgery/	2616
15	(margin or margins or perimeter* or peri-meter*).tw,kf.	89568
16	(contoured or (CME and (excis* or resect* or procedur* or staged)) or coll#ret* or spaghett* or bread-loaf* or breadloaf*).tw,kf.	3834
17	((excis* or reexcis* or resect* or re-sect* or surg* or resurg* or presurg* or operat* or reoperat* or preoperat* or cut or cutting or remov* or wide or wider or width* or larger or deep or depth* or size or sizes or narrow* or extensiv* on nonextensiv* or nonradical* or non-radical* or limited or control or controlled or safety or safe or secure or security or clinical* or subclinic* or delimitat* or delineat* or determin* or evaluat* or assess* or map* or confocal* or RCM or dermascop* or vivascop* or microscop* or positive or free or negative or clear or clearance or mm or 3mm* or 5mm* or 10mm* or 1-cm or 1cm or 1?0-cm or 1?0cm or 3D or 3-D or 3-dimension*) adj5 (border or borders or boundar* or bounds or rim or rims or margin* or periphery or peripheries or stripe or stripes or circumferential)).tw,kf.	77609
18	((RCM* or microscop* or vivascop* or confoc* or dermascop* or immun* or histo* or lentigo or l?esion* or LM or LMM) adj3 (delineation* or demarcation* or contour* or outline or outlines)).tw,kf.	2489
19	((RCM* or vivascop* or confoc* or microscop* or dermascop*) adj4 (map* or clearance)).tw,kf.	1955
20	(((wide or wider or width* or narrow* or squar* or staged or stages or multistag* or phases or 2-phas* or two-phas* or multiphas* or multistep or multi-step or mapped) adj6 (excis* or reexci* or surg* or resurg* or section* or resect* or re-sect*)) or WLE*).tw,kf.	29768
21	((excision or reexcision or resection or re-section or removal or cutting or delineat*) adj2 (techni* or method* or approach* or procedur*)).tw,kf.	16502
22	((excis* or reexcis* or resect* or re-sect* or remov* or surg* or cutting or cut or delineat*) adj3 (geometric* or fusiform*)).tw,kf.	282
23	((squar* or staged) adj3 (procedur* or technique* or method* or approach or Johnson*)).tw,kf.	11055
24	(staged adj3 (squar* or serial or geometric*)).tw,kf.	29
25	((serial or en-face) adj2 (excis* or reexci* or surg* or resurg* or map*)).tw,kf.	523
26	(Moh or Mohs*).tw,kf.	4283
27	(micro-surg* or microsurg* or micro-exc* or microexc* or micro-graph* or micrograph*).tw,kf.	39323
28	((conservativ* or conventional or standard or traditional*) adj2 (excis* or reexcis* or resect* or re-sect* or surg* or cut or cutting or remov*) adj9 (lentig* or LM or LMM or (melanom* adj1 "in situ") or MIS)).tw,kf.	84
29	**or/11-28 [MARGIN / EXCISION METHOD]**	**236638**
30	**10 and 29 [LENTIGO MALIGNA + MARGIN / EXCISION METHOD]**	**463**
31	**remove duplicates from 30 [LENTIGO MALIGNA + MARGIN / EXCISION METHOD - DEDUPLICATED]**	**461**

SUPPLEMENT II. Bias scoring according to the Methodological Index for Non-Randomized Studies (MINORS) method

(Items 8-12 were only used for comparative studies)

1. **A clearly stated aim: the question addressed should be precise and relevant in the light of the available literature.**
   0: Aim not reported
   1: Aim reported but not precise
   2: Aim is precise (e.g., includes an analysis of histological margins or local recurrence)

2. **Inclusion of consecutive patients: all patients potentially fit for inclusion (satisfying the criteria for inclusion) have been included in the study during the study period (no exclusion or details about the reasons for exclusion).**
   0: Inclusion not reported
   1: Inclusion reported but not consecutive
   2: Inclusion of consecutive patients, or reasons for exclusion were reported

3. **Prospective collection of data: data were collected according to a protocol established before the beginning of the study.**
   0: Timing of the writing of the protocol in relation to the collection of data not reported / no protocol available
   1: Timing of the writing of the protocol in relation to the collection of data was reported but not prospective
   2: Prospective collection of data

4. **Endpoints appropriate to the aim of the study: unambiguous explanation of the criteria used to evaluate the main outcome, which should be in accordance with the question addressed by the study. Moreover, the endpoints should be assessed on an intention-to-treat basis.[A]**
   0: Endpoints not reported
   1: Clinical endpoint of histological margin status, but not local recurrence / regional recurrence and survival data in case in LMM.
   2: The endpoints used are histological margin status and local recurrence / regional recurrence and survival data available in case of LMM

5. **Unbiased assessment of the study endpoint: blind evaluation of objective endpoints and double-blind evaluation of subjective endpoints. Otherwise, the reasons for not blinding should be stated.[B]**
   0: Evaluation of endpoints not blinded or not reported
   1: Blinded histological examination of surgical margins, but indirect evaluation of outer outcomes (e.g., phone interview or heteroanamnesis) or not reported
   2: Blinded histological examination of surgical margins and direct clinical examination of other endpoints

6. **Follow-up period appropriate to the aim of the study: the follow-up should be sufficiently long to allow the assessment of the main endpoint and possible adverse events.[C]**
   0: Follow-up period not reported or no follow-up period
   1: Follow-up period reported but less than mean/median five years
   2: Follow-up period mean/median 5 years or longer

7. **Loss to follow-up less than 5%: all patients should be included in the follow-up. Otherwise, the proportion lost to follow-up should not exceed the proportion experiencing the major endpoint.[D]**
   0: Loss to follow-up not reported
   1: Loss to follow-up 5% or more
   2: Loss to follow-up less than 5% / The number of patients lost to follow-up should not exceed the proportion experiencing the major endpoint

8. **Prospective calculation of the study size: information on the size of the detectable difference of interest with a calculation of 95% confidence interval, according to the expected incidence of the outcome event, and information about the level for statistical significance and estimates of power when comparing the outcomes.**[E]
   0: Study size was not calculated or not reported
   1: Study size was calculated, but the actual study size was smaller than the calculated size
   2: Study size was calculated, and the actual study size was equal to or larger than the calculated size

9. **An adequate control group: having a gold standard diagnostic test or therapeutic intervention as the optimal intervention according to the available published data.**
   0: Characteristics of control group not reported
   1: Control group assessed as inadequate by the authors
   2: Control group assessed as adequate by the authors

10. **Contemporary groups: control and study group should be managed during the same time-period (no historical comparison).**
    0: Not reported if groups were contemporary or not
    1: Reported but not contemporary groups
    2: Contemporary groups

11. **Baseline equivalence of groups: the groups should be similar regarding the criteria other than the studied endpoints. Absence of confounding factors that could bias the interpretation of the results.**
    0: Baseline equivalence of groups not reported
    1: Baseline equivalence of groups was not met (e.g., lesion size, demarcation, Breslow thickness, lesion localization), but relevant clinicopathological variables were reported
    2: Baseline equivalence of groups was observed. Alternatively, if statistical methods were employed to adjust for possible confounders, this would award two points

12. **Adequate statistical analyses: whether the statistics were in accordance with the type of study with calculation of confidence intervals or relative risk.**
    0: No statistical analyses were performed
    1: Statistical analyses were performed, but no p-value was presented, or statistics did not adjust for the relevant potential confounders in the event of unequal baseline characteristics
    2: Relevant statistical analyses were performed, and a p-value was presented. If baseline equivalence was not met between the groups, the statistical analysis had to consider relevant potential confounders

[A] The intention-to-treat aspect was deemed irrelevant for the majority of the included studies and was therefore not considered in order to avoid bias.
[B] A study was considered to be blinded as long as some part of the treatment was blinded; the surgery per se did not need to be blinded.
[C] If the mean follow-up is not reported, the minimum follow-up is used instead.
[D] Only used when a major endpoint was clearly stated
[E] Any calculation of study size was accepted. The calculation of study size had to be performed for at least one of the outcomes, but it was not necessary for all outcomes.

SUPPLEMENT III. Characteristics of included studies/study arms organized by surgical technique.

Author (Year)	Country	Type	Lesions	Breslow (mm) (mean)	Primary [A] (%)	HN (%)	Surgical margin (mm)	Demarcation	IHC	Follow-up (months)
Wide local excision (bread loaf sectioning)										
Assmann [B] (2000)	Germany	R, C	7 LM 21 LMM	< 0.8 (n=11) 0.9-1.5 (n=5) 1.6-4.0 (n=5)	100	100	5-30	Naked eye	S-100 HMB-45 Melan-A	86.4 mean Lost: n=0
Collgros (2021)	Australia	R	70 LM 47 LMM	1.8	67.5	64.1	5-20	Dermoscopy	NR	90 mean Lost: 16
Crouch (2021)	Australia	R	382 LM	-	100	100	5	Naked eye	SOX10 MiTF	31 median Lost: NR
Demer [B] (2018)	USA	R, C	8 LM 89 LMM	2.2	97.9	100	5-20	Wood's lamp	Mel-5	25 mean Lost: NR
Hilari [B] (2011)	Spain	R, C	17 LM	-	76.5	100	5	Naked eye	Melan-A HMB-45	NR
Hou[b] (2015)	USA	R, C	269 LM	-	100	53.2	5	Wood's lamp	NR	94.8 mean Lost: n=4
Moehrle [B] (2006)	Germany	P, C	156 LMM	0.8	100	78.8	11 median	Naked eye	HMB-45 S-100	81 median Lost: NR
Moura (2020)	UK	R	134 LM	-	89.5	90.3	5	Naked eye	Melan-A	8 median; IQR 3-30 Lost: NR
Osborn (2002)	UK	R	89 LM	-	100	94.4	2	Naked eye	NR	44.9 mean Lost: n=13
Wide local excision, handheld RCM-assisted (bread loaf sectioning)										
Cinotti (2020)	France	P	37 LM 5 LMM	NR	94.6	100	5	Dermoscopy	Melan-A	60 mean Lost: NR
Elshot (2020)	The Netherlands	R	19 LM 5 LMM	0.6	66.7	100	5	Dermoscopy	Melan-A SOX10, S-100	36.7 mean Lost: n=0

Author (Year)	Country	Type	Lesions	Breslow (mm) (mean)	Primary [A] (%)	HN (%)	Surgical margin (mm)	Demarcation	IHC	Follow-up (months)
Staged excision, complete margin sectioning										
Abdelmalek (2012)	USA	R	225 LM 68 LMM	0.4	100	67.6	Stage: 3-5	Wood's lamp	Melan-A	32.3 mean (2-96) Lost: n=64
Agarwal (2002)	USA	P	92 LM	-	96	75	Stage: 5	Wood's lamp	NR	NR
Beveridge (2017)	Canada	R	24 LM	-	NR	NR	Stage: 3 Additional: 3	Wood's lamp Dermoscopy	None	18 mean (1-63) Lost: NR
Bosbous (2009)	USA	R	49 LM 10 LMM	NR	78	98.3	Stage (LM): 5-10 Stage (LMM): 10	Naked eye	None	27 median (0-122) Lost: NR
Breuninger (1999)	Germany	R	179 LMM	0.7 median	NR	NR	Initial: 5 Stage: 5-10	Naked eye	HMB-45, S-100	NR
Demirci (2008)	USA	R	26 LM 12 LMM	NR	100	100	Initial: 7-12 Stage: 5	Naked eye	NR	49 mean; 30 median (9-112) Lost: n=0
Jejurikar (2007)	USA	R	42 LM 9 LMM	0.7	NR	100	Initial (LM): 7 Initial (LMM): 12 Stage: 7	Naked eye	None	31.3 mean (15.6-45.1) Lost: NR
Kassi (2013)	Ivory Coast	P	31 LM	-	NR	100	Initial: 7 Stage: 5	Naked eye	Undefined	31 mean (3-60) Lost: NR
Liu (2020)	Canada	R	102 LM	-	90.2	100	Stage: 7	Naked eye	Undefined	46.3 median (8.5-57.7) Lost: n=22
Mahoney (2005)	Canada	R	11 LM	-	27.3	90.9	Initial: 7 Stage: 3-4	Wood's lamp	HMB-45 S-100	4.7 mean (1.0-13.4) Lost: NR
Moehrle [B] (2006)	Germany	P, C	136 LMM	0.6	100	92.6	Stage: 5	Naked eye	HMB-45 S-100	44 median Lost: NR
Smith (2019)	UK	R	38 LM 6 LMM	NR	77.3	100	Initial: 7-8 Stage: 2-3	Wood's lamp	NR	NR
de Vries (2016)	The Netherlands	R	100 LM	-	88	97	Initial: 3 Stage: 5	Naked eye	Melan-A S-100	60 mean Lost: NR
de Wit (2022)	South Africa	R	60 LM 4 LMM	0.2	92.2	100	Initial: 6 Stage: 3	Naked eye	NR	23.5 mean Lost: NR

Author (Year)	Country	Type	Lesions	Breslow (mm) (mean)	Primary [A] (%)	HN (%)	Surgical margin (mm)	Demarcation	IHC	Follow-up (months)
Staged excision (partial margin sectioning)										
Bub (2003)	USA	R	55 LM 7 LMM	0.5	81	92	Stage: 2-3	Wood's lamp	None	57 mean (9-139) Lost: n=3
Hazan (2008)	USA	R	91 LM 26 LMM	0.3	92.3	95	Initial: 5-10 Stage: 3-10	Wood's lamp	Melan-A	NR
Hill (1999)	Australia	R	45 LM 21 LMM	0.2-8 >1: 16/21	54.6	100	Stage: 5	Wood's lamp	None	25 mean (10-48) Lost: NR
Huigol (2004)	Australia	P	125 LM 36 LMM	≤1: 32/36	72.8 66.7	100	Stage: 5	Wood's lamp	None	38 mean (3-100) Lost: n=4
Navarrete (2020)	USA	R	438 LM 162 LMM	0.4	100	93.7	Stage: 5-7	Wood's lamp Dermoscopy	NR	NR
Walling [B] (2007)	USA	P, C	36 LM 5 LMM	NR	90.2	65.9	Initial: 2-4 Stage: 2-3	Naked eye	None	96 mean (60-240) Lost: NR
Wilson (2016)	USA	R	58 LM 10 LMM	≤0.7: 9/10	94.1	70.6	Stage: 2-3	Naked eye	None	138 mean (37-330) Lost: n=0
Staged excision, handheld RCM-assisted (complete margin sectioning)										
Champin (2014)	France	P	27 LM 6 LMM	0.5	63.6	100	Stage: 2-7 LMM: +10	Dermoscopy	Melan-A	10 mean (4-25) Lost: n=6
Couty (2018)	France	P	59 LM 11 LMM	<1: 10/11	68.2	98.5	Stage: 2-7	Dermoscopy	Melan-A	44 mean Lost: NR
Mohs' micrographic surgery, classic (complete margin sectioning)										
Clayton (2000)	USA	R	25 LMM	0.3-2.4	20	96	Stage: 3-5	Wood's lamp	None	NR
Cohen (1994/1998)	USA	R	29 LM 16 LMM	0.6 (0.2-1.3)	71	91.1	Stage: 3-4 Additional: 1-2	Naked eye	None	57.1 mean (15-106) Lost: n=7
Gao [B] (2020)	UK	R, C	26 LM	-	100	100	Stage: 1-2	Dermoscopy	NR	NR
Walling [B] (2007)	USA	P, C	14 LM 4 LMM	NR	88.9	88.9	Initial: 2-4 Stage: 2-3	Naked eye	None	117.5 mean (61-157) Lost: NR

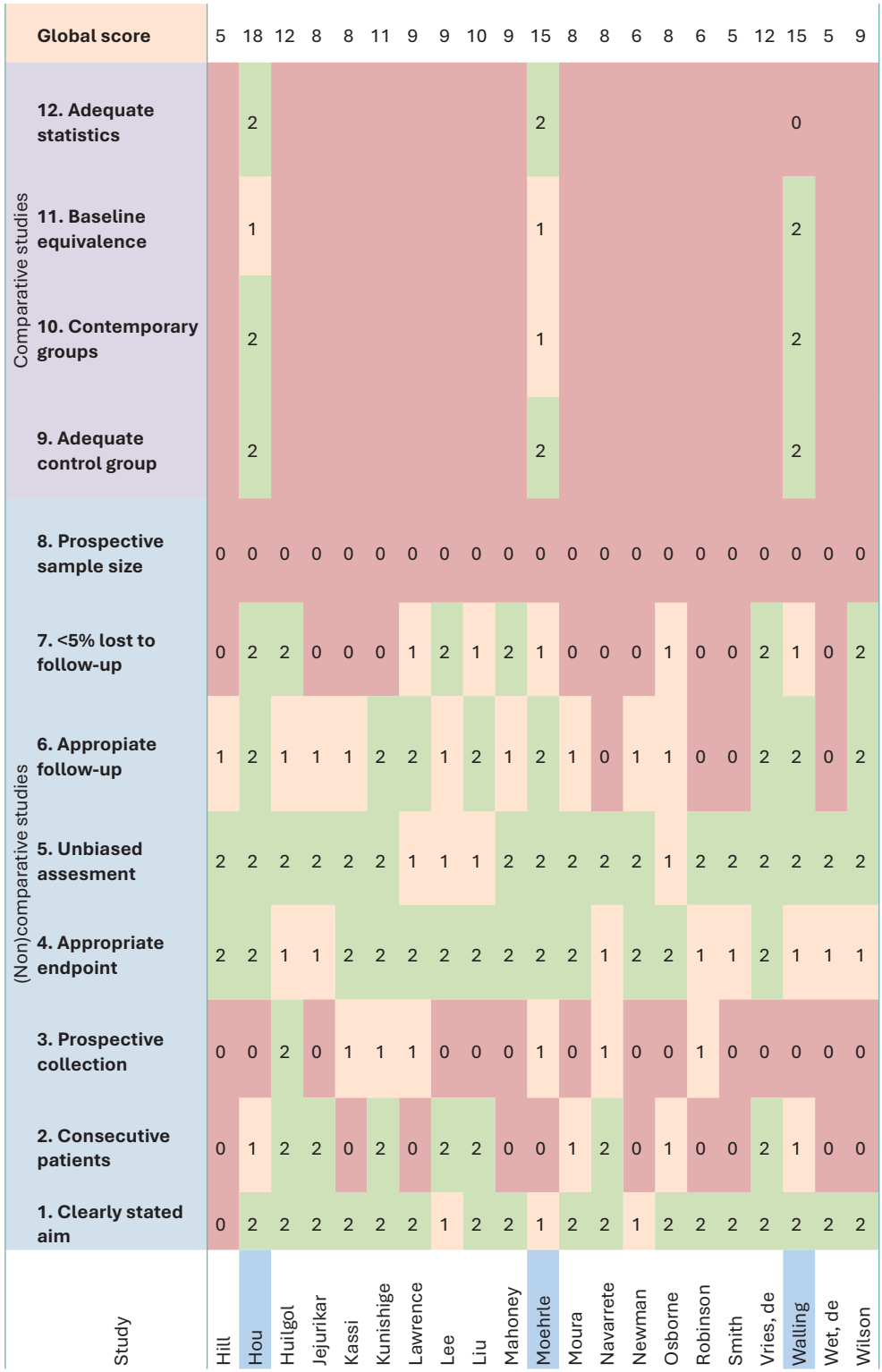
Author (Year)	Country	Type	Lesions	Breslow (mm) (mean)	Primary [A] (%)	HN (%)	Surgical margin (mm)	Demarcation	IHC	Follow-up (months)
Mohs' micrographic surgery, classic (complete margin sectioning)										
Clayton (2000)	USA	R	25 LMM	0.3-2.4	20	96	Stage: 3-5	Wood's lamp	None	NR
Cohen (1994/1998)	USA	R	29 LM 16 LMM	0.6 (0.2-1.3)	71	91.1	Stage: 3-4 Additional: 1-2	Naked eye	None	57.1 mean (15-106) Lost: n=7
Gao [B] (2020)	UK	R, C	26 LM	-	100	100	Stage: 1-2	Dermoscopy	NR	NR
Walling [B] (2007)	USA	P, C	14 LM 4 LMM	NR	88.9	88.9	Initial: 2-4 Stage: 2-3	Naked eye	None	117.5 mean (61-157) Lost: NR
Mohs' micrographic surgery, IHC staining (complete margin sectioning)										
Bhardwaj (2006)	USA	R	158 LM 42 LMM	Stage I: 78.6% Stage II: 21.4%	75.3 76.2	83.5 88.1	Stage: 2-3	Wood's lamp	Mel-5	38.4 mean (6-58) Lost: NR
Demer [B] (2018)	USA	R, C	221 LM 71 LMM	0.2	93.5	100	Initial: 3-5 Stage: 2-3	Wood's lamp	Mel-5	18 mean Lost: NR
Hou[b] (2015)	USA	R, C	154 LM	-	100	96.1	Stage: 1-2	Wood's lamp	MART-1 (37%)	94.8 mean Lost: n=4
Kunishige (2019)	USA	P	1506 LM	-	NR	73.1	Initial: 6 Stage: 3	Naked eye	HMB-45 MART-1	68.4 mean (2.4-282) 54 median Lost: NR
Newman (2013)	USA	R	193 LM 67 LMM	0.9 (0.1-4.2)	93.3 89.5	80.3 77.6	Stage: 2-3	Wood's lamp	Mel-5	34 mean Lost: NR

Author (Year)	Country	Type	Lesions	Breslow (mm) (mean)	Primary [A] (%)	HN (%)	Surgical margin (mm)	Demarcation	IHC	Follow-up (months)
Mohs' micrographic surgery, slow (complete margin sectioning)										
Assmann [B] (2000)	Germany	R,C	7 LM 13 LMM	< 0.8 (n=4) 0.9-1.5 (n=3) 1.6-4.0 (n=6)	100	100	Initial: 5-20 Stage: 3	Naked eye	S-100 HMB-45 Melan-A	21.4 mean Lost: n=0
Hilari [B] (2011)	Spain	R, C	41 LM	-	80.5	100	Stage: 5	Naked eye	Melan-A HMB-45	NR
Lawrence (2014)	UK	P	74 LM	-	89.2	93.2	Initial: 4 Stage: 2	Magnifying loop	None	Alive: 60-114 Died: 3.8-65.3 Lost: n=0
Lee (2008)	Australia	R	31 LM	-	51.6	87.1	Stage: 5	Wood's lamp	None	42 mean (12-89) Lost: n=1
Robinson (1994)	USA	P	16 LM	-	100	100	Stage: 3-4	Wood's lamp	HMB-45 S-100	60-108 mean Lost: NR
Mohs' micrographic surgery, handheld RCM-assisted (complete margin sectioning)										
Goa [B] (2020)	UK	R, C	21 LM	-	100	100	Stage: 1-2	Dermoscopy	NR	NR

HN = head-and-neck; IHC = immunohistochemistry; NR = not reported; P = prospective; R = retrospective; RCM = reflectance confocal microscopy.
[A] Including primary incomplete excisions
[B] Study arm of comparative study

SUPPLEMENT IV – Bias assessment scores according to MINORS method (see SUPPLEMENT II)

	Study	Abdelmalek	Agarwal	Assmann	Beveridge	Bosbous	Breuninger	Bub	Champin	Cinotti	Clayton	Cohen	Collgros	Couty	Crouch	Demer	Elshot	Gao	Hazan	Hilari
	Global score	10	9	10	7	9	4	8	10	10	5	8	11	12	11	14	10	11	6	10
Comparative studies	**12. Adequate statistics**			0									0			2		0		0
	11. Baseline equivalence			1									0			1		1		0
	10. Contemporary groups			1									1			2		2		2
	9. Adequate control group			2									0			2		2		2
(Non)comparative studies	**8. Prospective sample size**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	7. <5% lost to follow-up	1	0	1	0	2	0	2	1	2	1	0	1	2	0	0	1	0	0	0
	6. Appropiate follow-up	1	1	1	1	1	0	2	0	2	1	2	2	2	2	1	1	0	0	0
	5. Unbiased assesment	2	2	2	2	2	2	1	2	2	1	2	2	2	2	1	2	2	2	2
	4. Appropriate endpoint	2	1	0	2	1	1	1	2	1	1	2	1	1	2	2	2	1	1	1
	3. Prospective collection	0	1	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0
	2. Consecutive patients	2	2	0	0	1	0	0	2	0	0	0	2	2	2	1	2	1	1	1
	1. Clearly stated aim	2	2	2	2	2	1	2	2	2	1	2	2	2	2	2	2	2	2	2



	Study	Hill	Hou	Huilgol	Jejurikar	Kassi	Kunishige	Lawrence	Lee	Liu	Mahoney	Moehrle	Moura	Navarrete	Newman	Osborne	Robinson	Smith	Vries, de	Walling	Wet, de	Wilson
	Global score	5	18	12	8	8	11	9	9	10	9	15	8	8	6	8	6	5	12	15	5	9
Comparative studies	12. Adequate statistics		2									2								0		
Comparative studies	11. Baseline equivalence		1									1								2		
Comparative studies	10. Contemporary groups		2									1								2		
Comparative studies	9. Adequate control group		2									2								2		
(Non)comparative studies	8. Prospective sample size	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(Non)comparative studies	7. <5% lost to follow-up	0	2	2	0	0	0	1	2	1	2	1	0	0	0	1	0	0	2	1	0	2
(Non)comparative studies	6. Appropiate follow-up	1	2	1	1	1	2	2	1	2	1	2	1	0	1	1	0	0	2	2	0	2
(Non)comparative studies	5. Unbiased assesment	2	2	2	2	2	2	1	1	1	2	2	2	2	2	1	2	2	2	2	2	2
(Non)comparative studies	4. Appropriate endpoint	2	2	1	1	2	2	2	2	2	2	2	2	1	2	2	1	1	2	1	1	1
(Non)comparative studies	3. Prospective collection	0	0	2	0	1	1	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0
(Non)comparative studies	2. Consecutive patients	0	1	2	2	0	2	0	2	2	0	0	1	2	0	1	0	0	2	1	0	0
(Non)comparative studies	1. Clearly stated aim	0	2	2	2	2	2	2	1	2	2	1	2	2	1	2	2	2	2	2	2	2

SUPPLEMENT V. PRISMA checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Title page
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Page 110
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Page 111
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page 111
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Page 112-113
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 112-113
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	SUPPLEMENT I
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page 113
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Page 113
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Page 113-114
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Page 113
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Page 113
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Page 114

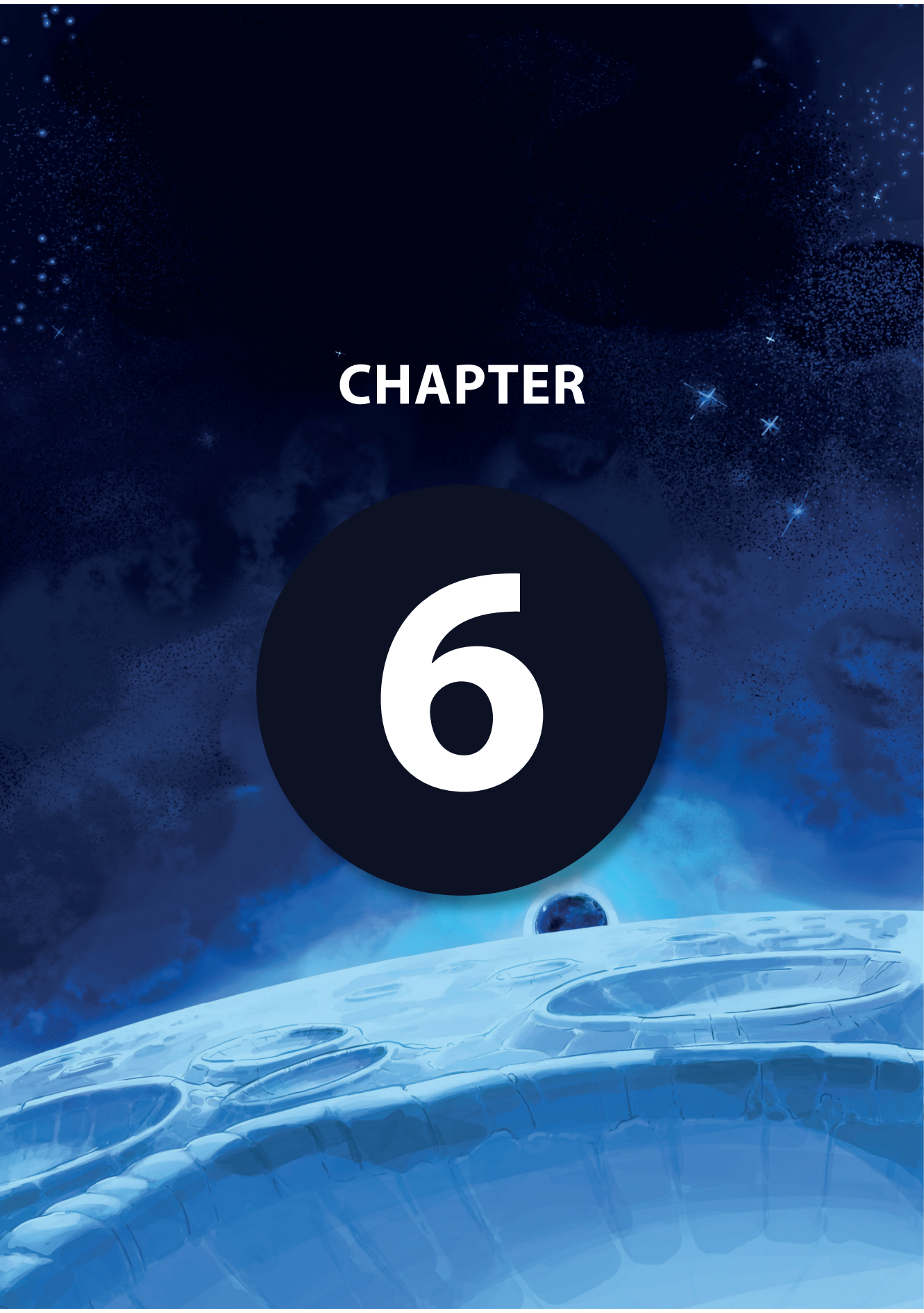
Section and Topic	Item #	Checklist item	Location where item is reported
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page 114
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Not applicable
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Figure 2.
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Page 114
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Page 114
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Not applicable
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Not applicable
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Not applicable
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Page 114 FIGURE 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Page 114
Study characteristics	17	Cite each included study and present its characteristics.	Page 114 FIGURE 1-2 SUPPLEMENT III
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Page 114 SUPPLEMENT IV
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	FIGURE 2 Page 115 Page 116
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Not applicable
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	FIGURE 2
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	FIGURE 2
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Not applicable

Section and Topic	Item #	Checklist item	Location where item is reported
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Not applicable
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Not applicable
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Page 123-125
	23b	Discuss any limitations of the evidence included in the review.	Page 125
	23c	Discuss any limitations of the review processes used.	Page 125
	23d	Discuss implications of the results for practice, policy, and future research.	Page 125
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Page 112
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Page 111-112
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Not applicable
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Page 109
Competing interests	26	Declare any competing interests of review authors.	Page 109
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Not available

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

PART II

REGIONAL DIAGNOSTICS OF LENTIGO MALIGNA MELANOMA


CHAPTER
6

THE LIMITED VALUE OF SENTINEL LYMPH NODE BIOPSY IN LENTIGO MALIGNA MELANOMA: A NOMOGRAM BASED ON THE RESULTS OF 29 YEARS OF THE NATIONWIDE DUTCH PATHOLOGY REGISTRY (PALGA)

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ABSTRACT

Background. Lentigo maligna melanoma (LMM) predominantly presents in the head-and-neck of elderly individuals. The value of sentinel lymph node biopsy (SLNB) for patients with LMM remains to be determined, as the reported average yield of positive lymph nodes is less than 10%. In this nationwide cohort study, we aimed to identify patients with LMM at an increased risk of SLNB positivity.

Methods. LMM with an SLNB indication according to the 8th AJCC melanoma guidelines were retrospectively identified from the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA). Penalized logistic regression analysis was performed to determine the optimal combination of clinicopathological factors for predicting positive SLNB.

Results. Between 1991 and 2020, 1989 LMM patients met our inclusion criteria. SLNB was performed in 16.7% (n=333) of the patients and was positive in 7.5% (25/333). The false-negative rate was 21.9%. Clinically detectable regional lymph node (LN) metastases were found in 1.3% of the patients (n=25). Clinicopathological characteristics best predictive for SLNB-positivity (Odds ratio; 95% CI) were age (0.95; 0.91-0.99), ulceration 1.59 (0.44-4.83), T4-stage (1.81; 0.43-6.2), male sex (1.97; 0.79-5.27), (lymph)angioinvasion (5.07; 0.94-23.31), and microsatellites (7.23; 1.56-32.7) (C-statistic 0.75). During follow-up, regional LN recurrences were detected in 4.2% (83/1989) of the patients, of which the majority (74/83) had no evidence of regional LN metastases at baseline.

Conclusion. Our findings confirmed the limited SLNB positivity in patients with LMM. Based on the identified high-risk clinicopathological features, a nomogram was developed to predict SLNB risk.

INTRODUCTION

Lentigo maligna melanoma (LMM) is a high cumulative solar damage (CSD)-associated melanoma subtype, predominantly present in the head-and-neck of the elderly.[1] Sentinel lymph node biopsy (SLNB) remains one of the most critical prognostic tools.[2] While SLNB is performed as indicated by melanoma guidelines[3-5], The value of SLNB for LMM patients remains uncertain, as the reported yield of positive lymph nodes (LN) is less than 10%.[6-8] It is unclear whether this is due to more favorable tumor characteristics (e.g., reduced Breslow thickness)[9] or age at presentation, as increased age is associated with lower SLNB-positivity.[10-12] In addition to patient age at presentation, using SLNB for head-and-neck LMM is further complicated by unpredictable and bilateral lymphatic drainage patterns.[13]

The number of melanoma patients receiving SLNB has been increasing[14-16] and is expected to increase further due to the availability of adjuvant therapies in stage III disease.[17, 18] In this perspective, evaluating whether SLNB should routinely be performed in LMM patients is essential, provided the primary tumor characteristics fulfill the current SLNB criteria.[3-5]

This nationwide cohort study aimed to investigate the value of SNLB in LMM patients by evaluating the yield of SLNB procedures in these patients and by identifying LMM patients at increased risk of positive SLNB.

MATERIALS AND METHODS

Patient data were retrospectively obtained after approval from the privacy and scientific committee (application # LZV2020-175) of the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA).[19] Once all patients with LMM were identified between 1991 and 2020, a second search was performed for all patients with subsequent lymph node (LN) histology and cytology reports. PALGA provided anonymized excerpts of the conclusions and microscopy of the pathology reports, including patient age at diagnosis and sex.

Inclusion criteria consisted of an unequivocal LMM diagnosis with i) performed SLNB or ii) an SLNB indication according to the current staging of the 8th edition of the American Joint Committee on Cancer (AJCC) (i.e., T1b or higher).[20] Cases with i) a Breslow thickness <0.8 with missing ulceration status, ii) unclear/unknown melanoma subtypes, or iii) missing data were excluded. A false-negative SLNB result was defined as regional lymph node recurrence during follow-up in patients with a prior negative SLNB in the same region. The FN rate was calculated by dividing the number of patients who present with a regional LN recurrence following a negative SLNB by the sum of those with a true-positive SN (TP) and those with a regional nodal recurrence (FN/[TP+FN]).[21, 22]

Extracted data included anatomic (sub)localization, Breslow thickness, T-stage according to the 8th AJCC staging system, and histological characteristics. The head-and-neck were divided into facial and nonfacial regions. The latter includes the scalp/postauricular, ear, and neck regions. Lymph node data included SLNB outcomes, fine-needle aspiration cytology (FNAC), excisional lymph node biopsies, and (selective) neck dissection. Finally, all the regional LN recurrences were evaluated.

Statistical analysis. Absolute and relative frequencies were described for all study variables. Differences in clinicopathological characteristics between patient groups at baseline were identified using $\chi 2$ or Fisher's exact tests for categorical variables and the unpaired *t*-test or Mann-Whitney *U* test for continuous variables, where appropriate. The time to regional LN recurrence was calculated from the date of histopathological diagnosis of LMM.

Univariate logistic regression analysis was used to estimate the odds ratio (OR) for positive SLNB outcomes. Clinicopathological factors included age, sex, Breslow thickness, localization, and histological characteristics (e.g., ulceration and microsatellites). Dermal mitoses were also included in the analysis as they were still considered a prognostic factor in AJCC staging during the inclusion period. We subsequently performed a penalized logistic regression analysis with 10-fold cross-validation to obtain a parsimonious combination of clinicopathological factors that predict positive SLNB. For factors with multiple options, we created dummy variables that had these options present or absent (e.g., T-stage was recoded into four variables: T1/T2/T3/T4). Once identified, the number needed-to-treat (NNT) was calculated for each identified clinicopathological predictive factor for SLNB positivity, and each variable was included to develop a nomogram for SLNB outcome prediction.

Two-sided P-values <0.05 were considered statistically significant. Data were analyzed using SPSS (version 28.0) for Windows (IBM Corp, Armonk, NY, USA) and R version 4.02 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Between 1991-2020, 6061 patients were diagnosed with LMM in the Netherlands (**FIGURE 1**). Six hundred fifty-seven patients were excluded because of equivocal melanoma subtype (n=335) or missing study data (n=322). In addition, LMM with a Breslow thickness <0.8 mm with missing ulceration status were also excluded from further analysis (n=633). A total of 1989 cases fulfilled the SLNB criteria and were included in the analysis. Due to the anonymized nature of the data, there was no information on which center the SLNB was performed.

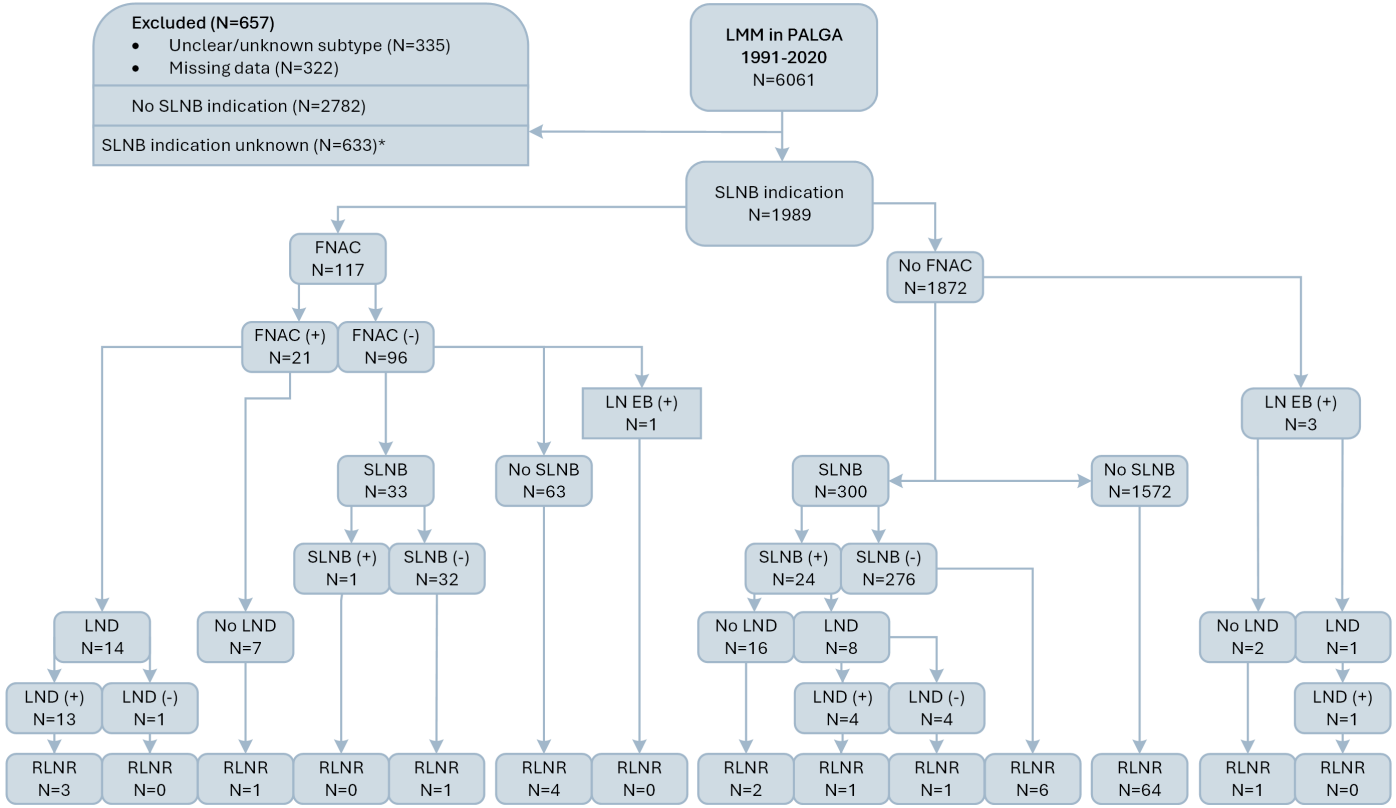

FIGURE 1. *Inclusion flowchart of all lentigo maligna melanoma identified between 1991 and 2020 in the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA). * Breslow thickness < 0.8mm with missing ulceration status*

FNAC = fine needle aspiration cytology; LMM = lentigo maligna melanoma; LND = lymph node dissection; LN EB = lymph node excisional biopsy; PALGA = nationwide network and registry of histo- and cytopathology in the Netherlands; RLNR = regional lymph node recurrence; SLNB: sentinel lymph node biopsy

Baseline patient and lentigo maligna melanoma characteristics

The clinicopathological characteristics of the 1989 cases are presented in **TABLE 1**. In 31.3% (n=618) of cases, the initial diagnosis was based on partial lesion sampling (i.e., punch, incisional, or shave biopsy). Lentigo maligna melanoma cases diagnosed by partial sampling were upstaged in 36.0% (n=222) following complete excision. The median (Q1-Q3) Breslow thickness of the included LMM was 1.3 mm (0.9-2.2). When dividing the inclusion period into decades, the median Breslow thickness did not change over time (P=0.126).

Clinically detected metastases & sentinel lymph node biopsy results

At baseline, 1.3% (n=25) of the patients with LMM had clinically detectable regional metastases. Metastases were diagnosed using FNAC (n=21) and excisional LN biopsies (n=4). Overall, FNAC was performed in 5.9% (n=117) of the LMM study population (n=1989), and 21 of 117 patients (17.9%) had positive cytology. Four positive excisional LN biopsies were performed following an uncertain outcome of the ultrasound-guided (FNAC) procedure.

Sentinel lymph node biopsy (SLNB) was performed in 16.7% (n=333) of patients, with a positive SLNB found in 7.5% (n=25). The median (Q1-Q3) number of detected sentinel nodes was 2 (1-3). Twenty (92.4%) patients had a single positive LN, whereas the remaining five had two positive SLNBs. None of the excised non-sentinel nodes (n=54) had clinically occult metastases. The median (Q1-Q3) maximum metastasis diameter was 1.5mm (0.1-9.0). In almost half (23/50) of the patients with positive LN status at baseline (2.5%; n=50), an additional (selective) neck dissection was performed, and 2 patients underwent parotidectomy with selective neck dissection. Eighteen additional positive LN were detected following neck dissection (+/- parotidectomy). Extranodal growth was observed in 16.0% (8/50) of all LN metastases.

Predictive factors for patients at high risk of SLNB-positivity

A significantly increased risk for positive SLNB (odd ratio; 95% confidence interval [CI]) was seen in the T4-stage (4.11; 1.0-17.9) (P=0.048), (lymph)angioinvasion (9.59; 2.31-36.27) (P<0.001), and microsatellites (15.15; 3.93-58.83) (P<0.001) (**TABLE 2**).

Age was inversely associated with SLNB positivity (P=0.007), with a decreasing OR (95% CI) of 0.1 (0.01-0.51) (P>0.001) for the 51-60 age group to 0.09 (0.09-0.59) (P=0.032) for the >80 age group. Because none of the patients with a positive SLNB had neck localization (n=10), histological regression (n=41), or a desmoplastic component (n=13), we were unable to include these variables in the analysis. Histological missing values were considered absent, as a sensitivity analysis including missing and absent values as included parameters did not affect the OR in the univariable analyses (**TABLE 2**).

TABLE 1. Clinicopathological characteristics of LMM grouped by baseline regional nodal status (n=1989)

	No SLNB performed	Clinically detected LN metastases [A]	SLNB performed			Total
			SLNB (+)	SLNB (-)	P-value*	
N (%)	**1631 (82.0)**	**25 (1.3)**	**25 (1.3)**	**308 (15.5)**		**1989 (100.0)**
Age at diagnosis (years) (median; Q1-Q3)	77.0 (69.0-84.0)	77.0 (71.0-82.5)	65.0 (51.5-72)	70.0 (63.0-76.0)	0.026	76 (68.0-82.0)
Sex						
Female	881 (54.0)	6 (24.0)	8 (32.0)	150 (48.7)	NS	1045 (52.5)
Male	750 (46.0)	19 (76.0)	17 (68.0)	158 (51.3)		944 (47.5)
Localization						
Head-and-neck	1301 (79.8)	24(96.0)	18 (72.0)	197 (64.0)	NS	1540 (77.4)
Trunk & extremities	294 (18.0)	0 (0.0)	7 (28.0)	111 (36.0)		412 (20.7)
Unknown	36 (2.2)	1 (4.0)	0 (0.0)	0 (0.0)		37 (1.9)
Breslow (mm) (median; Q1-Q3)	1.3 (0.9-2.2)	2.3 (1.5-4.1)	2.0 (1.1-3.7)	1.5 (1.1-2.2)	NS	1.3 (1.3-2.2)
T classification [B]						
T1	597 (36.6)	2 (8.0)	4 (16.0)	69 (22.4)	NS	672 (33.8)
T2	581 (35.6)	9 (36.0)	10 (40.0)	146 (47.4)		746 (37.5)
T3	324 (19.9)	8 (32.0)	6 (24.0)	72 (23.4)		410 (20.6)
T4	129 (7.9)	6 (24.0)	5 (20.0)	21 (6.8)		161 (8.1)
Histological characteristics [C]						
Ulceration	192 (11.8)	4 (16.0)	5 (20.0)	30 (9.7)	NS	231 (11.6)
Missing	302 (18.5)	1 (4.0)	1 (4.0)	25 (8.1)	<0.001	329 (16.5)
Microsatellites	40 (2.5)	4 (16.0)	5 (20.0)	5 (1.6)	<0.001	54 (2.7)
Missing	447 (27.4)	5 (20.0)	4 (16.0)	40 (13.0)	NS	496 (24.9)
(Lymph)angio-invasion	27 (1.7)	3 (12.0)	4 (16.0)	6 (1.9)	NS	40 (2.0)
Missing	973 (59.7)	8 (32.0)	10 (40.0)	140 (45.5)	NS	1131 (56.9)
Dermal mitoses	610 (37.4)	13 (52.0)	12 (48.0)	136 (44.2)	NS	771 (38.8)
Missing	660 (40.5)	7 (28.0)	5 (20.0)	120 (39.0)		792 (39.8)
Regression	121 (7.4)	0 (0.0)	0 (0.0)	41 (13.3)		162 (8.1)
Missing	732 (44.9)	5 (20.0)	5 (20.0)	76 (24.7)		818 (41.1)
Perineural growth	59 (3.6)	1 (4.0)	3 (12.0)	15 (4.9)		78 (3.9)
Missing	1247 (76.5)	12 (48.0)	16 (64.0)	211 (68.5)		1486 (74.7)
Desmoplastic component [D]	24 (1.5)	1 (4.0)	0 (0.0)	13 (4.2)		38 (1.9)

LN = lymph node; LMM = lentigo maligna melanoma; SLNB = sentinel lymph node biopsy;
*P-values: t-test or Mann-Whitney *U* test for continuous variables, and chi-squared test or Fisher's exact test for categorical variables where appropriate.
[A] Positive lymph node fine needle aspiration cytology (n=21) & selective excisional lymph node biopsy (n=4)
[B] According to the 8th edition of the American Joint Committee on Cancer (AJCC) TNM classification
[C] Absent histological features are not described
[D] Median (Q1-Q3) Breslow thickness 3.9mm (3.0-5.6)

The multivariable model identified the most parsimonious predictive clinicopathological characteristics for SLNB-positivity (OR; 95% CI): age (0.95; 0.91-0.99)(P=0.018), ulceration 1.59 (0.44-4.83)(P=0.445), T4-stage (1.81; 0.43-6.2)(P=0.379), male sex (1.97; 0.79-5.27) (P=0.155), (lymph)angioinvasion (5.07; 0.94-23.31)(P=0.043), and microsatellites (7.23; 1.56-32.7)(P=0.009). Dermal mitosis was not a predictor of SLNB positivity. The C-statistic for this model was 0.75, indicating a good level of discrimination between positive and negative SLNB cases.

TABLE 2. Logistic regression analysis for a positive sentinel lymph node biopsy in lentigo maligna melanoma patients.

	Univariable model		
	OR	**95% CI**	**P-value**
Age	0.95	0.91-0.99	0.007
≤ 50	Reference		w
51-60	0.1	0.01-0.51	0.009
61-70	0.2	0.06-0.68	0.008
71-80	0.17	0.05-0.58	0.003
>80	0.09	0.09-0.59	0.032
Sex			
Male	Reference		
Female	0.5	0.2-1.15	NS
Localization			
Trunk & extremities	Reference		
Head-and-neck	1.45	0.61-3.83	NS
Facial [A]	1.16	0.43-3.28	NS
Ear	4.33	0.84-18.19	NS
Scalp	2.03	0.57-6.74	NS
T-stage [B]			
T1	Reference		
T2	1.19	0.38-4.43	NS
T3	1.44	0.39-5.83	NS
T4	4.11	1.0-17.9	0.048
Histological characteristics			
Ulceration			
Absent/missing	Reference		
Present	2.32	0.73-6.21	NS
Microsatellites			
Absent/missing	Reference		
Present	15.15	3.93-58.83	<0.001
(Lymph)angioinvasion			
Absent/missing	Reference		
Present	9.59	2.31-36.27	<0.001
Dermal mitoses			
Absent/missing	Reference		
Present	1.17	0.51-2.65	NS
Perineural growth			
Absent/missing	Reference		
Present	2.66	0.59-8.86	NS

CI = confidence interval; NS=non-significant; OR = odds ratio; T=tumor stage
[A] Facial localization: forehead, nose, peri-orbital, peri-oral, and chin
[B] According to the 8th edition of the American Joint Committee on Cancer (AJCC) TNM classification

The NNT for each predictive factor is presented in **TABLE 3**. The nomogram for the risk assessment of SLNB outcome is shown in **FIGURE 2**.

TABLE 3. Number needed-to-treat for a single case of SLNB-positivity in LMM patients with an indication for SLNB biopsy according to the 8th edition of the AJCC [A]

Clinicopathological risk factors	NNT
Age (<50)	4.8
Ulceration	32.0
T4-stage [B]	23.6
Male sex	25.4
(Lymph)angioinvasion	5.9
Microsatellites	4.3

[A] Variables were identified using a multivariable model.
[B] Tumor stage according to the 8th edition of the AJCC TNM classification
AJCC = American Joint Committee on Cancer; NNT = number needed-to-treat; SLNB = sentinel lymph node biopsy

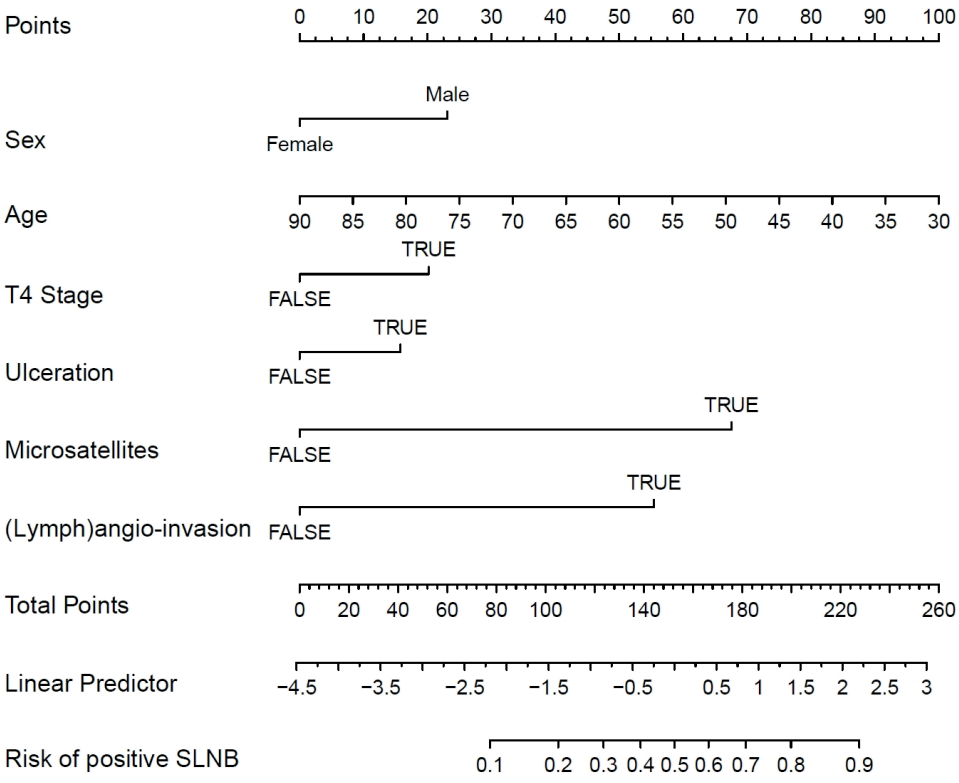

FIGURE 2. *Nomogram for sentinel lymph node biopsy outcome prediction for lentigo maligna melanoma patients. A line is drawn upwards to determine the points for each variable. The sum of these points should be marked on the Total Point line at the bottom with a straight line drawn downwards to determine the "Risk of positive SLNB". T4 stage according to the TNM staging of the AJCC 8th edition; SLNB: sentinel lymph node biopsy*

Regional lymph node recurrences

A minimal 2- and 5-year follow-up period was available for 85% (n=1701) and 64.4% (n=1281) of patients, respectively. The median (Q1-Q3) time to recurrence was 15 months (7.7-38.5). Regional LN recurrence was detected in 4.2% (n=83) of the patients. Most patients (n=74; 89.2%) had no evidence of regional LN metastases at baseline. Of the regional LN recurrences, 7 patients had a prior negative SLNB, resulting in a 21.9% FN SLNB rate (78.1% sensitivity). Regional LN recurrence rates were 5.3% (n=2) and 3.7% (n=6) for LMM with desmoplastic components and regression, respectively.

DISCUSSION

With the increased therapeutic relevance of SLNB with the arrival of adjuvant therapies[17,18], this study aimed to determine the value of the SLNB procedure in LMM patients. To our knowledge, we evaluated the most extensive known cohort study on LMM using the nationwide network and registry of histo- and cytopathology (PALGA) in the Netherlands, which included 1989 patients. While we identified high-risk clinicopathological factors for SLNB positivity in the study population, our findings show a limited positive yield of SNLB in patients with LMM according to the current melanoma guideline-advised SLNB indications. Based on our data, we developed a nomogram to predict SLNB outcomes.

Compared to other available validated nomograms, our predictive variables are aimed explicitly at LMM patients.[23, 24] The current nomogram is based on data from SLNBs performed in a population of 333 LMM patients, compared with 94 in the nomogram by Lo et al.[23] This might have affected the threshold of the relevance of certain variables. For example, unlike the nomogram of Lo et al., dermal mitoses were not associated with SLNB positivity in patients with LMM. Furthermore, based on our data ≤T3-stage LMM were at a significantly reduced risk of SLNB positivity. Consequently, T4-stage instead of Breslow thickness, was included as a relevant predictive variable.

The highest OR for SLNB positivity was associated with the presence of microsatellites. The definition of microsatellites was updated in the 8th edition of the AJCC melanoma staging system and is considered to be the result of intralymphatic or possibly angiotropic tumor spread.[20, 25] In a matched control study, microsatellites were the only independent predictor of SLNB positivity in the multivariate analysis.[25] Riquelme-Mc Loughlin et al. further confirmed the relevance of microsatellites, with the lowest survival outcome related to the presence of microsatellites combined with SLNB-positivity.[26] Even so, as shown in the current study, the diagnosis of LMM is often based on partial sampling. Consequently, excisional biopsy should be considered before wide local excision to identify all histological features of the tumor.

The 7.5% positive SLNB rate for LMM patients contrasts with head-and-neck melanoma in general, with reported average identification of positive SLNBs at 21%.[27-31] However, the low yield in the case of LMM is in line with other studies. Fröhlich et al. evaluated 150 cases of primary LMM, of which 1/5 patients underwent SLNB, and none showed evidence of clinically occult metastases.[8] The lack of LN metastases in their study could also be explained by the low prevalence of known histological risk factors: less than 8% of the cases presented with a Breslow thickness > 1.5 mm, and in 1.6% of the cases, ulceration was histologically present.

Comparable results were found by Ettl et al., who evaluated the predictive clinical and pathological characteristics of LN metastases following neck dissection in head-and-neck melanoma.[9] Although the LMM was the most frequent subtype (n= 155; 46.7%), no LN metastases were found in their series. Similar to the study by Fröhlich et al., most (95.6%) of the LMM cases were classified as T1-T2. While the reduced Breslow thickness and lack of ulceration could be seen as the most likely explanation, the remarkably reduced yield of positive SLNB seems to persist even in thick (>4mm) LMM, as shown by Boada et al. at 4.3%.[6]

While we could not include histological regression in our analysis, it could be a relevant predictive factor for survival outcomes.[32] Regression is hypothesized to be an immunological response directed against melanocyte-associated antigens, resulting in (partial) destruction of melanoma cells.[33] In our cohort, no LMM patients with LN metastases at baseline showed signs of histological regression. Additionally, the regional LN recurrence rate for LMM with regression was low (3.7%). A meta-analysis demonstrated a reduced risk of positive SLNB in melanoma patients with histological regression with an OR of 0.56 (95% CI 0.41-0.77).[34] A more recent multivariate analysis showed that histological evidence of regression combined with tumor-infiltrating lymphocytes predicted a negative SLNB outcome.[35]

Similar to histological regression, several previous studies have reported that older age also appears to be an independent prognostic factor with a decreased risk of SLNB-positivity.[10 11, 36] As our data show, increasing age was inversely related to SLNB positivity. Consequently, age is an essential factor to consider because LMM is typically associated with older age at presentation compared to other head-and-neck melanomas.[7, 37] This phenomenon might be explained by differences in the tumor microenvironment and decreased lymphatic flow with increasing age.[38, 39] Nonetheless, a small percentage (4.2%) of the included patients developed regional lymph node metastases during follow-up.

The reported low yield of SLNB in LMM patients[6-8] and our findings might be a good reason to consider alternative options for early detection of regional metastases. With a focus on lymph nodes at risk, ultrasound surveillance of LN following lymphoscintigraphy could be considered a valuable alternative in selected patients. This approach was described by

Ipenburg et al. and appeared not to have any impact on melanoma-specific and distant recurrence-free survival while detecting 1/3 of regional LN recurrences before becoming clinically detectable.[40]

The limited yield of positive SLNB for LMM may also indicate a different metastatic pathway. This possible difference is highlighted in the study by Conrad et al., who compared the progression pattern between false-negative SLNB (n=61) and patients with negative SLNB and progressive disease at distant sites (n=48). Surprisingly, all LMM and spindle cell melanomas belong to the second group. Similar to LMM, the value of SLNB in desmoplastic melanoma (DM) is also considered controversial. In a systematic review, SLNB-positivity was limited to 6.5% of DM patients.[41] In our cohort, no LMM patient with a desmoplastic component (n=38) had a positive SLNB, even though the DM component's median Breslow thickness was significantly higher than the LMM component (3.9 mm vs. 1.3 mm). Both LMM and DM are considered chronically sun-damaged (CSD) melanoma in the 2018 WHO classification, associated with a distinct genetic profile consisting of NRAS, NF1, KIT, and non-V600E BRAF mutations, potentially leading to differences in biological behavior.[42]

Finally, even though the regional LN recurrence rate was limited (4.2%), approximately 1/5th of patients with a negative SLNB developed recurrence in the regional LN basin. Consequently, FN SLNBs also affected the SLNB positivity rate. However, our study's 22% FN rate is significantly higher than the 6-12% reported for head-and-neck melanoma in general.[27-31] This is likely not only the result of the complex anatomy and variable lymphatic drainage system in the head-and-neck but also related to differences in the experience level of the performing institutions.[43, 44]. However, we could not include this variable in our analysis because of the anonymized nature of the PALGA data. Furthermore, LMM often presents as an invasive focus in larger in-situ LM lesions. Therefore, an unclear macroscopic demarcation of the invasive component could also affect the reliability of the SLNB procedure.

Limitations. Due to the retrospective nature of our study, there were several limitations. First, melanoma subtypes have historically been diagnosed based purely on partly subjective morphological criteria, including those in our cohort. With recent advances in molecular and genetic understanding and inherent changes in the classification of melanoma subtypes, our study has a risk of misclassification. Similarly, as the atypical melanocytes of lentigo maligna also proliferate along the adnexal epithelium, tangential histological sectioning could also result in the misclassification of LM as LMM (i.e., false-positive invasive component).[45] Second, over 600 LMM with a Breslow thickness below 0.8 mm and missing ulceration status were excluded from the analysis, which likely included patients with an SLNB indication. In addition, SLNB was performed in 17% of the indicated cases, according to the current

melanoma guidelines. Because the regional LN recurrence rate was low, the effect of these missing cases on SLNB outcomes should be limited. Third, 2- and 5-year follow-up data were available for 85% and 64.4% of patients, respectively, which could have influenced the number of LN recurrences. However, the limited number of recurrences in our series might mitigate this effect. Data correction for patients with multiple melanomas is likely to further reduce the number of regional LN recurrences. Finally, we included all the national data on SLNB in patients with LMM between 1991 and 2020. Several technological advances in SLNB were made during the inclusion period. It remains to be seen to what extent technical improvements were implemented as knowledge is lacking on the participation of head-and-neck surgeons experienced in performing SNLB.

CONCLUSION

Our findings indicate a limited positive yield of SLNB in LMM patients when performed according to current melanoma guideline-recommended indications, especially with increasing age. Using data from the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA), we identified clinicopathological characteristics of LMM patients at high risk for SLNB positivity, including age < 60 years, male sex, T4 stage, ulceration, presence of (lymph)angio-invasion, and microsatellites. Based on these variables, we developed a nomogram aimed explicitly at the LMM patient group with indications for SLNB. However, microsatellites (N1c) are classified as stage IIIB, according to the current 8th edition of the AJCC. Consequently, their presence would already make the SLNB procedure redundant.[18] As the diagnosis of LMM is often based on partial sampling, certain histological features might be missed at diagnosis. A diagnostic excisional biopsy, if cosmetically acceptable, should be considered before evaluating whether SLNB is indicated. Finally, future research should be focused on validating the predictive value of our nomogram and determining its value compared to currently available tools.[23]

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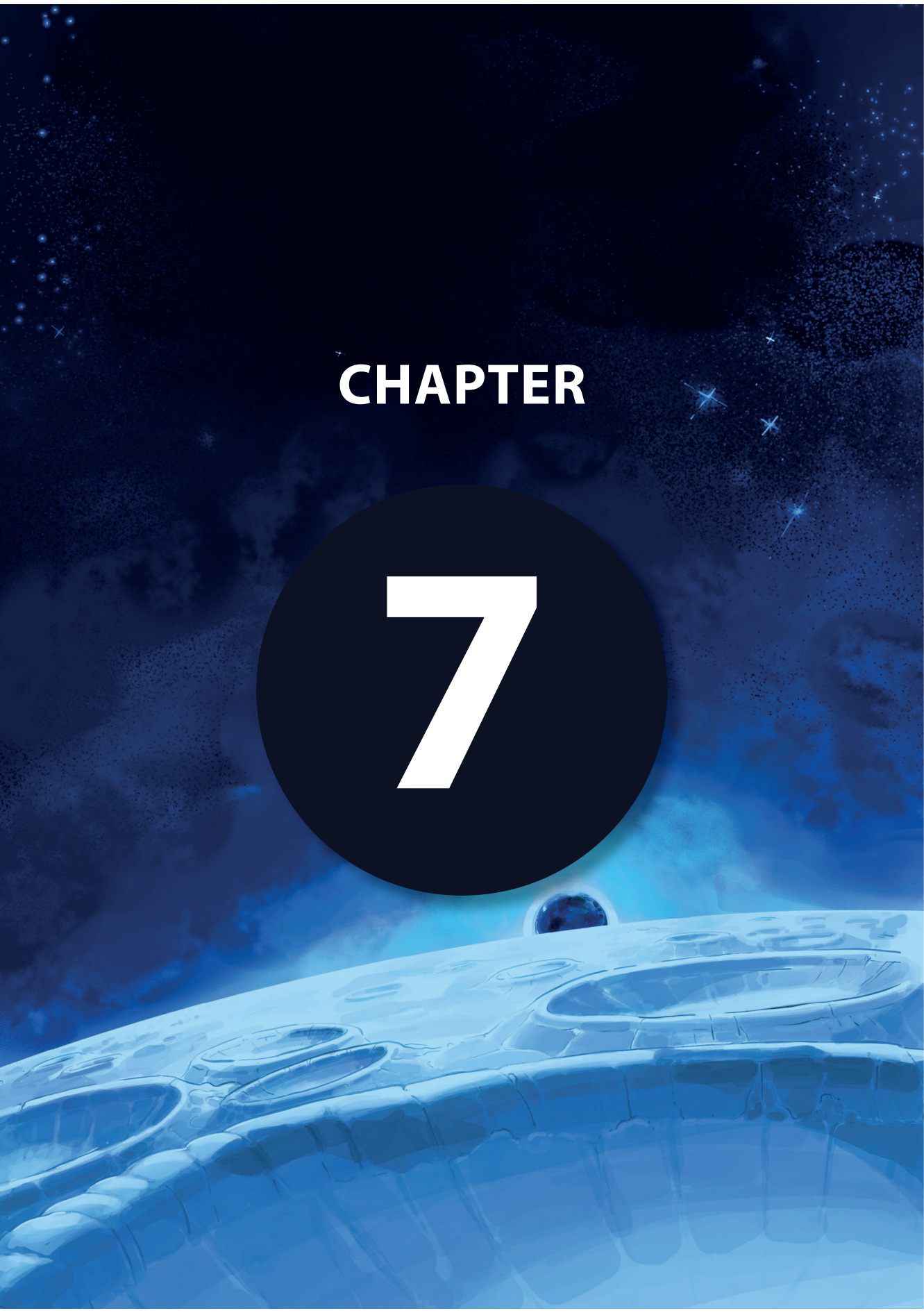
PART III

DIAGNOSIS OF BASAL CELL CARCINOMA


CHAPTER
7

NONINVASIVE DIAGNOSTICS AND SUBTYPING OF BASAL CELL CARCINOMA IN THE HEAD-AND-NECK BY DERMOSCOPY AND HANDHELD REFLECTANCE CONFOCAL MICROSCOPY

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Conflicts of interests: none

ABSTRACT

Background. Because clinical subtyping is unreliable, the management of head-and-neck basal cell carcinoma (BCC) is highly reliant on diagnostic biopsies. Nonetheless, there is a substantial discordance between biopsy and excisional specimens. Handheld reflectance confocal microscopy (HH-RCM) can reduce the need for invasive biopsy for diagnosis and subtyping.

Objective. To assess the diagnostic accuracy of combined dermoscopy-HH-RCM for diagnosing and subtyping primary head-and-neck BCC and determine the outcome of this diagnostic pathway when applying different international Mohs micrographic surgery (MMS) criteria.

Methods. This was a prospective, single-center, fully paired diagnostic comparison study. Diagnostic confidence was recorded using a 3-point Likert scale (low, medium, and high) for all outcomes.

Results. A total of 340 consecutive lesions with clinical suspicion of BCC on naked-eye examination were included. The biopsy subtype was inconsistent with the final excision in 33%. Dermoscopy combined with HH-RCM had the highest proportion of cases with high diagnostic confidence. The diagnostic accuracy resulted in a sensitivity (95% confidence interval [CI]) of 97.5% (95.0-99.0) and specificity of 85.2% (72.9-93.4), which increased to 98.8 (96.5-99.7) and 94.1 (80.3-99.3) for cases with high diagnostic confidence. The sensitivity/specificity for detecting non-superficial BCC (i.e., nodular and infiltrative) and infiltrative BCC were 95.5 (92.9-98.1) / 44.8 (26.7-62.9) and 58.8 (45.3-72.3) / 79.6 (74.3-84.9), respectively. Several international MMS criteria were applied to our study cohort in cases with high diagnostic confidence (n=281). This hypothetical scenario would have resulted in the excision of ≤0.7% (n=2) of benign lesions.

Limitations. Single-center, non-randomized study

Conclusions. Dermoscopy combined with HH-RCM diagnosis significantly reduces the rate of unnecessary diagnostic biopsies and could potentially replace diagnosis-confirming biopsies in managing head-and-neck BCC when considering micrographically controlled surgery.

INTRODUCTION

Basal cell carcinoma (BCC) is the most prevalent malignancy among light-skinned populations, with an increasing burden on healthcare systems worldwide.[1–3] Current estimates suggest that one in 3-5 individuals will develop BCC in their lifetime, with a 25% subsequent risk of developing additional BCCs.[4]

The head-and-neck region accounts for approximately 40% of BCCs, with an increased risk of morbidity due to the potential cosmetic and functional consequences of treatment.[5,6] The histological subtype is the most crucial factor in BCC management, as treatment options vary according to the subtype. Current guidelines differentiate between low/medium-risk subtypes (superficial and nodular BCC) and high-risk infiltrating subtypes (infiltrative, micronodular, and sclerosing).[7,8]

Although dermoscopy has improved BCC diagnostics compared to naked-eye examination alone[9], histology remains the gold standard due to unreliable clinical subtyping.[10] Nonetheless, there is substantial discordance between biopsy and excisional specimens, with more aggressive subtypes identified in 15-20% of cases following surgical treatment, compared with initial biopsy.[11]

Accurate BCC subtyping is essential for managing BCC, as superficial BCC (sBCC) may be amenable to nonsurgical approaches. By contrast, nodular BCC (nBCC) and infiltrating BCC (iBCC) require surgical excision. Infiltrating BCC necessitates larger surgical margins to ensure complete removal.[7,8,12] In selected cases, radiotherapy may serve as an effective alternative to surgery.[13] In addition to the infiltrative BCC subtype, several other clinical factors, such as size and localization, can increase the risk of local recurrence. In these high-risk cases, micrographically controlled surgical techniques, such as Mohs micrographic surgery (MMS), offer a tissue-sparing alternative with reduced recurrence rates.[14–16] Nonetheless, confirmatory biopsies are needed to avoid the surgical excision of benign lesions.

Handheld reflectance confocal microscopy (HH-RCM) has emerged as a promising noninvasive imaging modality that allows in vivo visualization of the skin at a cellular resolution up to the papillary dermis.[17] This technology could provide a noninvasive alternative to diagnostic biopsies for BCC management. With the current limitations of the diagnostic practices highlighted above, this study aimed to evaluate the diagnostic accuracy of HH-RCM in conjunction with dermoscopy. We specifically assessed its efficacy in diagnosing and subtyping head-and-neck BCC and explored its potential to reduce the need for diagnostic biopsies.

METHODS

This prospective, single-center, fully paired diagnostic comparison study was conducted at the Department of Dermatology of the Netherlands Cancer Institute between 2017-2022 (Trialsearch. who.int; NL-OMON27500). The study protocol adhered to established ethical guidelines and was approved by the Institutional Review Board (CFMPB540). Consecutive patients aged ≥ 18 years with suspected primary head-and-neck BCC identified by naked-eye examination (NEE) by dermatologists with >10 years of experience were eligible for inclusion. Written informed consent was obtained from all the participants. Patients with recurrent BCC, inaccessible or fully ulcerative lesions, previously irradiated skin, genetic syndromes associated with an increased BCC risk, and those ineligible for surgical excision were excluded.

Diagnostic procedures. A single investigator (Y.E.) performed all the study procedures. All lesions underwent bedside examinations using dermoscopy and handheld reflectance confocal microscopy (HH-RCM). Dermoscopic images were captured using polarized (DermLite DL4, 3Gen, Inc., San Juan Capistrano, California, USA) and digital contact dermoscopy (VivaCam D200, VivaScope GmbH). HH-RCM imaging (VivaScope 3000; VivaScope GmbH) was performed using a standardized protocol with over three years of hands-on experience at the initiation of the study. Horizontal, consecutive, optical sections (z-stacks) with a field of view of 750 µm × 750 µm were obtained at 5.21 µm intervals from the stratum corneum to the papillary dermis (200-250µm depth) in any area of interest. The examined areas included the central portion of the lesion and at least two additional areas at the lesion border. A 3 mm punch biopsy was performed in the most clinically elevated area of the lesion border.

All biopsy-proven BCCs were excised according to Dutch BCC Guidelines: 3 mm for low-risk BCC and 5 mm or Mohs micrographic surgery (MMS) for high-risk BCC. In the case of MMS, diagnostic tumor debulking was performed before the first round of frozen sections. Blinded expert evaluations were conducted for all dermoscopy images (G.A.; G.B.; N.K.) separately from the HH-RCM images (Y.E.; S.G.; M.J.) to assess the diagnosis, subtype, and scoring of predefined diagnostic criteria (**SUPPLEMENT I & II**).[18] Diagnostic confidence was recorded using a 3-point Likert scale (low, medium, and high) for all outcomes.

Two expert pathologists (M.B.; L.J.), blinded to all other outcomes, performed histopathological evaluations of all biopsies and excisional specimens. Discrepancies were resolved by a majority vote by a third pathologist (E.C.). All subtypes were registered in the case of mixed-type BCC. The most aggressive subtype found in either the biopsy or the excisional specimen was considered the reference standard. In cases in which no residual BCC was found, the biopsy outcome was maintained.

Data analysis. The diagnostic performance of dermoscopy-HH-RCM (DS-HH-RCM) evaluation in the outpatient clinic (study investigator) and blinded expert evaluation of the dermoscopic and HH-RCM images were assessed by calculating the accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) compared to histology. The diagnostic accuracy was determined for two subgroups: (i) sBCC vs. non-superficial (i.e., n/iBCC) and (ii) s/nBCC vs. iBCC. Interobserver agreement was measured using the observed agreement and positive and negative specific agreements.[19,20] The agreement between the different expert pairings (i.e., range) was also calculated. The 95% confidence interval (95% CI) was calculated using bootstrap resampling. Hypothetical sensitivity and specificity calculations for MMS indications were performed in the study cohort. For the MMS analysis, only cases with high diagnostic confidence based on the DS-HH-RCM BCC outcome were included using criteria from various international dermatological societies (**SUPPLEMENT III**). Each lesion's RCM BCC score was calculated based on Longo et al.'s predictive model.[21]

A sample size of 258 confirmed BCCs was determined to achieve 80% power for the study objectives, considering an expected false-positive rate of 10-20% by naked eye examination. This calculation employs one-sided binomial tests with a significance level of 5%. All statistical analyses were conducted using SPSS 29.0 for Windows (IBM Corp., Armonk, NY, USA) and R version 4.4.1.

RESULTS

In total, 340 consecutive lesions from 270 patients were included in this study (**FIGURE 1**). Of these, 286 (84.1%) were biopsy-proven BCCs, resulting in a 16% false-positive rate for NEE (**TABLE 1**). The median age (range) at diagnosis was 72 years (24-90), and 47% (n=127) of the patients were male. The Fitzpatrick skin phototypes were distributed as follows: Type I (14.8%), Type II (64.1%), and Type III (21.1%). A history of BCC, squamous cell carcinoma, or melanoma was present in 71.9% (n=194), 19.6% (n=53), and 41.9% (n=113) of patients, respectively.

Confirmed BCCs cases were treated using conventional surgery (87.8%, n=251) and MMS (12.2%, n=35). The proportion of cases with high diagnostic confidence was 33.5% for NEE, which significantly increased to 49.1% ($P<0.001$) and 87.9% ($P=0.001$) for blinded dermoscopy and HH-RCM expert evaluations, respectively. Dermoscopic-HH-RCM BCC diagnosis was classified as having high confidence in 82.6% (n=281) of the cases ($P<0.001$).

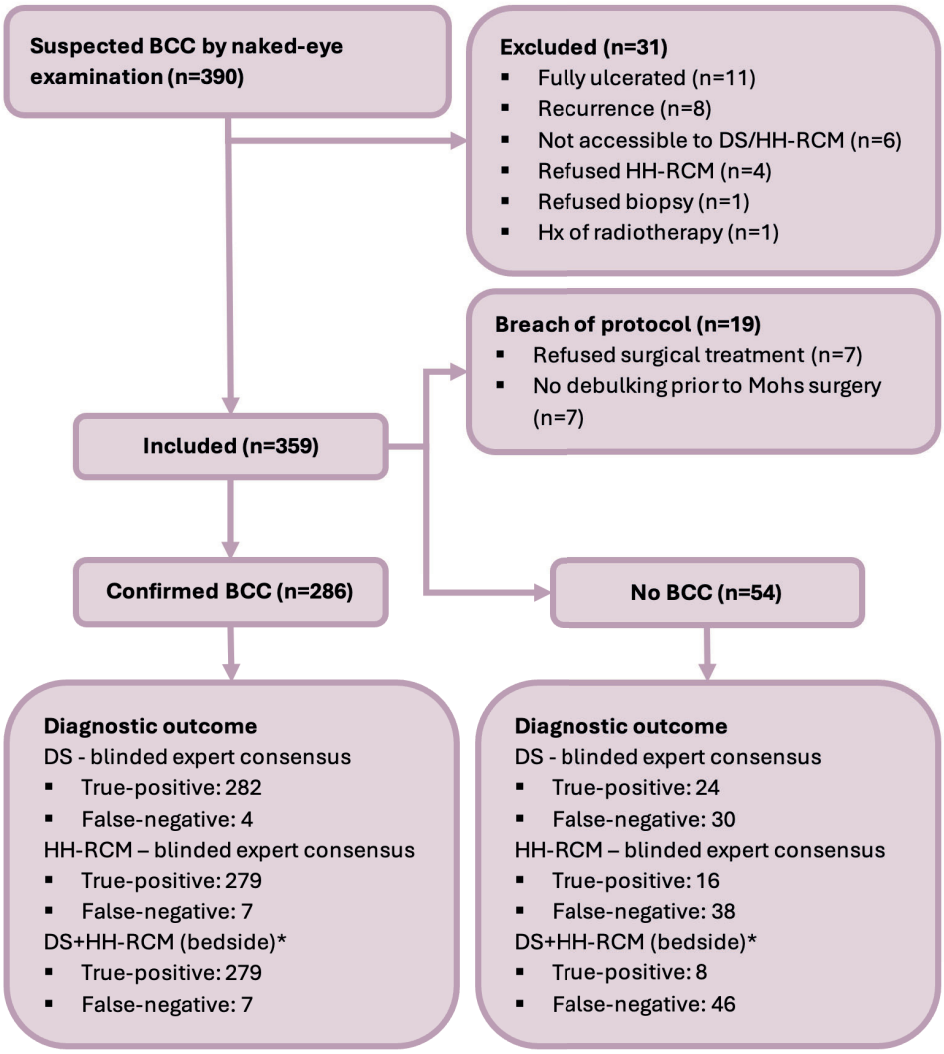

***FIGURE 1**. Lesion inclusion flowchart with diagnostic outcomes of dermoscopy, handheld reflectance confocal microscopy, or combined dermoscopic-HH-RCM head-and-neck basal cell carcinoma diagnosis according to the reporting standard of the STARD statement. * Study investigator*

BCC, basal cell carcinoma; DS, dermoscopy; RCM, reflectance confocal microscopy; STARD, Standard for Reporting Diagnostic Accuracy.

TABLE 1. Clinical features of included head-and-neck lesions suspected to be BCC by naked-eye examination.

Clinical features N (%)	sBCC 22 (6.5)	nBCC 190 (55.9)	iBCC 74 (21.7)	Non-BCC[A] 54 (15.9)	Total 340 (100.0)	P-value[B]
Localization						NS
H-zone	9 (40.9)	100 (52.6)	44 (59.5)	32 (59.3)	185 (54.4)	
M-zone	13 (59.1)	90 (47.4)	30 (40.5)	22 (40.7)	155 (45.6)	
Demarcation						NS
Poorly defined	8 (36.4)	50 (26.3)	29 (39.2)	16 (29.6)	103 (30.3)	
Well defined	14 (63.6)	140 (73.7)	45 (60.8)	38 (70.4)	237 (69.7)	
Lesion size (mm) (Median; range)						
Long axis	6.5 (4-25)	6.0 (2-30)	7.0 (3-20)	5 (2-15)	6 (2-30)	0.012
Short axis	5.0 (3-15)	4.0 (2-12)	5.0 (2-16)	4 (2-10)	4 (2-16)	0.001
Macroscopic						
Ulceration/crusts	5 (22.7)	52 (27.4)	32 (43.2)	17 (31.5)	106 (31.2)	0.03
Pigmentation	1 (4.5)	20 (10.5)	3 (4.1)	3 (5.6)	27 (7.9)	NS
Lesion profile						<0.001
Flat	11 (50.0)	29 (15.3)	11 (14.9)	14 (25.9)	65 (19.1)	
Slightly elevated	8 (36.4)	47 (24.7)	31 (41.9)	12 (22.2)	98 (28.8)	
Papule/nodule	3 (13.6)	114 (60.0)	32 (43.2)	28 (51.9)	177 (52.1)	
Lesion border						
Pearly	20 (90.9)	174 (91.6)	66 (89.2)	48 (88.9)	308 (90.6)	NS
Raised border	3 (13.6)	26 (13.7)	14 (18.9)	9 (16.7)	52 (15.3)	NS
Subclinical pigmentation	4 (18.2)	41 (21.6)	15 (20.3)	4 (7.4)	64 (18.8)	NS
Dermoscopic pigmentation						NS
Non/Lightly (<30%)	21 (95.5)	174 (91.6)	71 (93.0)	53 (98.1)	319 (93.8)	
Pigmented (30-70%)	1 (4.5)	11 (5.8)	3 (4.1)	1 (1.9)	16 (4.7)	
Heavily (>70%)	0 (0.0)	5 (2.6)	0 (0.0)	0 (0.0)	5 (1.5)	

BCC = basal cell carcinoma; iBCC = infiltrating basal cell carcinoma; nBCC = nodular basal cell carcinoma; sBCC = superficial basal cell carcinoma

[A] Actinic keratosis (n=14), dermal nevus (n=11), squamous cell carcinoma (n=7), sebaceous hyperplasia (n=6), folliculitis (n=5), squamous cell carcinoma in-situ (n=4), lichen planus-like keratosis (n=2), melanoma in-situ (n=1), benign (other) (n=5).

[B] Significant differences between histological BCC subtype groups

Fourteen (4.9%) of 286 BCCs (4 sBCC, 7 nBCC, and 3 iBCC) were missed by dermoscopy or HH-RCM and were excluded from the subtype analysis. The proportion of BCC subtyping cases with high diagnostic confidence significantly increased for dermoscopy (66.2%, n=180) compared to NEE (41.9%, n=114; P=0.001). While both HH-RCM (84.2%, n=229) and DS-HH-RCM diagnoses (67.6%, n=184) had a higher proportion of high-confidence subtyping than NEE, this difference was not significant compared with dermoscopy alone (all P>0.05).

Histological outcome

After the initial pathologist's assessment, a consensus on the most aggressive histological BCC subtype was reached in 89.2% (n=255) of the biopsies and 87.8% (n=251) of the excisions. A third pathologist's assessment resolved any remaining discrepancy.

Two or more histological subtypes (mixed-type BCC) were found in 37.8% of the punch biopsies, which further increased to 59.4% after excision. The most aggressive subtype of punch biopsy was consistent with the final excisional specimens in 60.5% (n=173) of the cases, with discrepancies of 33.2% (n=95). Of these, 9.4% (n=27) were downstaged and 23.8% (n=68) were upstaged (8 nBCC and 60 iBCC). In the remaining 6.3% (n=18) of cases, only scar tissue was found in the excisional specimen. One-third (n=11; 33.3%) of biopsy-proven sBCC were upstaged following excision.

Basal cell carcinoma diagnosis and subtyping

TABLE 2 shows the diagnostic criteria grouped according to subtype. **FIGURE 2** shows a new diagnostic feature for nonpigmented BCC: light brown-grey ("rusty") globules/clods corresponding to nodular dermally localized basaloid nests.

The observed, positive, and negative specific agreement (range; 95%CI) for BCC diagnosis was 68% (55-92; 95% CI 58-79), 80% (69-96; 95% CI 71-89), and 24% (15-39; 95% CI 14-34) for dermoscopy; and 90% (89-93; 95% CI 84*-97), 95% (94-96; 95% CI 89-100), and 61% (52-74; 95% CI 50-72) for HH-RCM. For non-superficial BCC (nBCC/iBCC) subtyping, the outcomes were 85% (80-89; 95% CI 76-93), 92% (89-94; 95% CI 85-98), and 24% (12-39; 95% CI 16-36) for dermoscopy, and 89% (88-90; 95% CI 81-96), 94% (94%; 95% CI 88-99), and 50% (47-53; 95% CI 38-61) for HH-RCM. Finally, for iBCC subtyping, the outcomes were 72% (70-72; 95% CI 61-82), 26% (11-35; 95% CI 16-37), and 82% (81-84; 95% CI 73-91) for DS, and 78% (76-81; 95% CI 68-88), 64% (56-70; 95% CI 53-75), and 84% (82-86; 95% CI 76-93) for HH-RCM.

TABLE 2. Dermoscopic and RCM basal cell carcinoma features grouped according to most aggressive histological subtype [A]

Histological subtype; N (%)	sBCC	nBCC	iBCC	Total	P-value	Agreement % (range between expert pairings | 95% CI)		
Dermoscopic criteria	19 (7.0)	184 (67.6)	69 (25.4)	272 (100.0)		A_o	P_{pos}	P_{neg}
Vascular structures								
Branched	12 (63.2)	161 (87.5)	62 (89.9)	235 (86.4)	0.016	79 (79-80 | 70-89)	87 (87-88 | 79-95)	47 (39-59 | 35-59)
Branched (in focus)	2 (10.5)	97 (52.7)	37 (52.7)	136 (50.0)	0.002	69 (65-74 | 59-80)	65 (62-69 | 354-76)	73 (67-79 | 62-83)
Branched (low caliber)	11 (57.9)	120 (65.2)	47 (68.1)	178 (65.4)	NS	47 (32-64 | 36-59)	51 (31-74 | 40-63)	43 (34-54 | 31-54)
Short-fine	6 (31.6)	12 (6.5)	1 (1.4)	19 (7.0)	<0.001	76 (73-80 | 66-86)	25 (21-28 | 15-35)	86 (83-89 | 78-94)
Stellate	1 (5.3)	7 (3.8)	5 (7.2)	13 (4.8)	NS	92 (91-95 | 86-99)	39 (29-50 | 28-50)	96 (95-97 | 91-100)
Basaloid nests								
Ovoid nests	0 (0.0)	9 (4.9)	2 (2.9)	11 (4.0)	NS	93 (91-95 | 87-99)	36 (35-37 | 25-47)	96 (95-97 | 92-100)
Blue globule	0 (0.0)	37 (20.1)	9 (13.0)	46 (16.9)	0.04	83 (80-86 | 74-92)	49 (44-55 | 38-61)	90 (88-92 | 83-97)
In-focus dots	0 (0.0)	6 (3.3)	0 (0.0)	6 (2.2)	NS	96 (95-97 | 92-100)	38 (32-42 | 26-49)	98 (97-98 | 95-100)
Spoke-wheel	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-	99 (99-100 | 98-100)	0 (0 | 0)	100 (100 | 98-100)
Maple leaf-like	0 (0.0)	3 (1.6)	0 (0.0)	3 (1.1)	NS	97 (97 | 93-100)	24 (15-29 | 14-34)	98 (98-98 | 95-100)
Concentric globule	0 (0.0)	2 (1.1)	0 (0.0)	2 (0.7)	NS	96 (94-99 | 91-100)	15 (8-33 | 7-24)	98 (97-99 | 94-100)
Clods, “rusty” brown	0 (0.0)	13 (7.1)	2 (2.9)	15 (5.5)	NS	87 (85-89 | 79-95)	18 (0-38 | 9-27)	93 (92-94 | 87-99)
Miscellaneous								
Ulceration	1 (5.3)	42 (22.8)	24 (34.8)	67 (24.6)	0.018	86 (82-90) | 78-94	69 (61-74 | 58-80)	91 (88-94 | 84-98)
Multiple small erosions	2 (10.5)	10 (5.4)	3 (4.3)	15 (5.5)	NS	90 (89-91 | 83-97)	33 (28-37 | 22-44)	95 (94-95 | 89-100)
Mayflower globules	1 (5.3)	10 (5.4)	8 (11.6)	19 (7.0)	NS	84 (79-92 | 76-93)	26 (22-30 | 16-36)	91 (88-96 | 70-89)
Structureless, white-to-red	11 (57.9)	50 (27.2)	22 (31.9)	83 (30.5)	0.021	71 (70-74 | 61-82)	55 (50-57 | 43-66)	79 (78-81 | 70-89)
Lines/blotches, polarized	5 (26.3)	50 (27.2)	20 (29.0)	75 (27.6)	NS	70 (66-77 | 59-80)	40 (32-52 | 28-51)	80 (77-85 | 85-98)
Stellate pattern[B]	0 (0.0)	3 (1.6)	0 (0.0)	3 (1.1)	NS	97 (96-97 | 92-100)	15 (0-30 | 7-23)	98 (98-99 | 95-100)

Histological subtype; N (%)	sBCC	nBCC	iBCC	Total	P-value	Agreement % (range between expert pairings | 95% CI)		
Dermoscopic criteria	19 (7.0)	184 (67.6)	69 (25.4)	272 (100.0)		A_o	P_{pos}	P_{neg}
Reflectance confocal microscopy criteria								
Epidermal changes								
Atypia	5 (26.3)	9 (4.9)	5 (7.2)	19 (7.0)	0.009	56 (39-90 | 45-68)	22 (18-43 12-32)	69 (51-95 | 59-80)
Streaming	9 (47.4)	77 (41.8)	21 (30.4)	107 (39.3)	NS	50 (36-74 | 38-62)	43 (40-47 | 31-54)	56 (33-83 | 44-67)
Shadowing	6 (31.6)	63 (34.2)	15 (21.7)	84 (30.9)	NS	59 (51-74 | 48-71)	38 (36-42 | 27-50)	70 (60-84 | 59-80)
Basaloid structures	18 (94.7)	176 (95.7)	65 (94.2)	259 (95.2)	NS	67 (56-87 | 56-78)	77 (68-92 | 68-87)	41 (32-56 | 29-52)
Large (≥300µm)	14 (73.7)	169 (91.8)	50 (72.5)	233 (85.7)	<0.001	49 (28-83 | 37-61)	56 (27-90 | 45-68)	39 (29-58 | 28-50)
Small (≤300µm)	1 (5.3)	25 (13.6)	21 (30.4)	47 (17.3)	0.003	75 (74-77 | 65-86)	45 (40-43 | 34-57)	84 (83-85 | 76-93)
Epidermal cords	12 (63.2)	38 (20.7)	13 (18.8)	63 (23.3)	<0.001	76 (75-76 | 66-86)	47 (37-57 | 35-58)	84 (83-85 76-93)
Dark silhouettes	3 (15.8)	21 (11.4)	25 (36.2)	49 (18.0)	<0.001	80 (75-76 | 71-89)	45 (37-57 | 33-56)	88 (86-89 | 80-95)
Tumor island features								
Clefting	8 (42.1)	143 (77.7)	38 (55.1)	189 (69.5)	<0.001	63 (56-75 | 52-74)	69 (67-73 | 58-80)	54 (35-77 | 43-66)
Peripheral palisading	18 (94.7)	176 (95.7)	56 (81.2)	250 (91.9)	0.001	81 (76-87 | 72-90)	88 (83-92 | 80-95)	62 (53-67 | 51-73)
Peritumoral fibrosis	11 (57.9)	160 (87.0)	62 (89.9)	233 (85.7)	0.004	70 (65-79 | 60-81)	79 (75-86 | 69-88)	51 (40-57 | 39-63)
Dendritic cells	3 (15.8)	47 (25.5)	18 (26.1)	68 (25.0)	NS	75 (71-80 | 65-85)	49 (46-51 | 38-61)	83 (80-87 | 74-92)
Infiltrate								
Small bright particles	11 (57.9)	119 (64.7)	37 (53.6)	167 (61.4)	NS	61 (57-63 | 50-72)	64 (59-69 | 52-75)	58 (54-64 | 46-69)
Plumb bright cells	3 (15.8)	53 (28.8)	19 (27.5)	75 (27.6)	NS	71 (69-72 | 60-81)	50 (47-53 | 38-61)	79 (77-81 | 70-89)

[A] Excluding 14 BCC cases missed by dermoscopy or HH-RCM; [B] White lines, skin folds

A_o = absolute agreement; P_{pos} = positive specific agreement; P_{neg} = negative specific agreement

BCC = basal cell carcinoma; CI = confidence interval; nBCC=nodular BCC; sBCC = superficial BCC; iBCC = infiltrating BCC

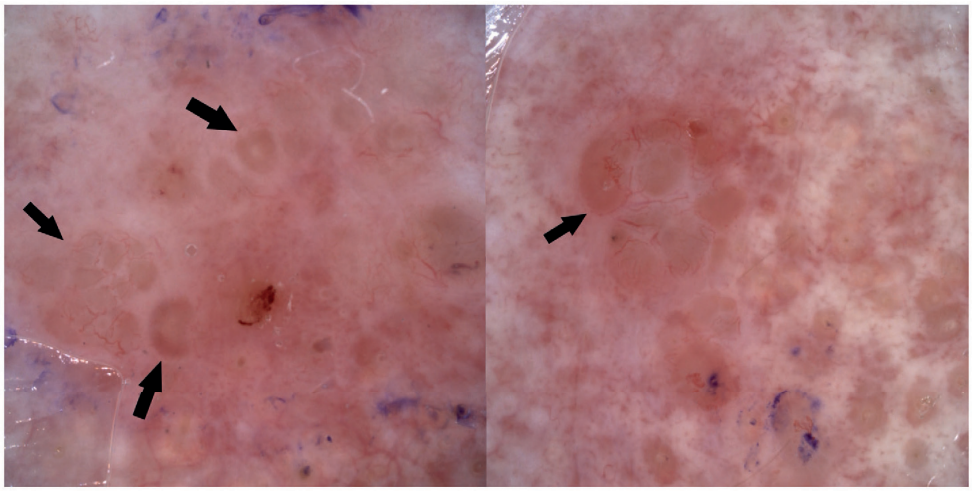

***FIGURE 2**. Digital contact dermoscopy images (VivaCam D200, ©VivaScope GmbH) at 15x magnification of two basal cell carcinomas displaying several gray-brown or "rusty" globules/clods (black arrows) corresponding to nonpigmented dermally located large basaloid nests on histology.*

The sensitivity and specificity for diagnosing all BCC subtypes were highest for punch biopsy (**TABLE 3**). The sensitivities of BCC diagnosis and subtyping were consistent across the three noninvasive diagnostic pathways. However, DS-HH-RCM diagnosis showed increased sensitivity in detecting iBCC compared to dermoscopy alone. Specificity was significantly higher for DS-HH-RCM BCC diagnosis and detection of non-superficial BCC (nBCC/iBCC). DS-HH-RCM (79.6%) and dermoscopic (81.9%) diagnoses demonstrated a higher specificity than HH-RCM (71.5%) for iBCC detection.

High-confidence dermoscopy-HH-RCM diagnostic outcomes

For BCC diagnosis (95% CI), the sensitivity and specificity increased to 98.8 (96.5-99.7) and 94.1 (80.3-99.3), respectively. Superficial BCC subtyping showed comparable sensitivity (92.5% vs. 95.5%) but an increase in specificity from 44.8% (26.7-62.9) to 66.7% (38.4-88.2). For iBCC, there was a reduction in sensitivity (52.3% vs. 58.8%) but an increase in specificity from 79.6% (74.3-84.9) to 89.0% (82.7-93.6). The predictive BCC score (median; Q1-Q3) was 28.7 (23.9-31.2) for BCC and 11.5 (9.0-15.2) for non-BCC ($P<0.001$), corresponding to a 95% and 10% probability, respectively.

TABLE 3. Diagnostic accuracy of dermoscopy, HH-RCM, and combined dermoscopic-HH-RCM diagnostics in the diagnosis (n=320) and subtyping (n=272) of head-and-neck BCC.

	DS	HH-RCM	DS+HH-RCM	P-value	Diagnostic biopsy [A]
BCC Diagnosis	% (95%CI)	% (95%CI)	% (95%CI)		
Sensitivity	98.6 (96.5-99.6)	97.5 (95.0-99.0)	97.5 (95.0-99.0)	NS	N.A.
Specificity	55.6 (41.4-69.1)	70.4 (56.4-82.0)	85.2 (72.9-93.4)	<0.005*/**	N.A.
PPV	92.2 (89.7-94.1)	94.6 (92.0-96.3)	97.2 (94.8-98.5)	<0.005**/***	N.A.
NPV	88.2 (73.4-95.3)	84.4 (71.9-92.0)	86.8 (75.8-93.2)	NS	N.A.
Accuracy	91.8 (88.3-94.5)	93.2 (90.0-95.7)	95.6 (92.8-97.5)		N.A.
BCC Subtype Nodular/infiltrating					
Sensitivity	97.1 (95.0-99.2)	95.9 (93.4-98.4)	95.5 (92.9-98.1)	NS	95.6 (92.3-97.8)
Specificity	17.2 (3.5-31.0)	31.0 (14.2-47.9)	44.8 (26.7-62.9)	<0.05***	61.1 (43.5-76.9)
PPV	90.8 (87.3-94.3)	92.1 (88.8-95.4)	93.5 (90.5-96.6)	<0.05***	94.5 (91.9-96.3)
NPV	41.7 (13.8-69.6)	47.4 (24.9-69.8)	54.2 (34.2-74.1)	NS	66.7 (51.5-79.0)
Accuracy	88.6 (84.2-92.1)	89.0 (84.6-92.4)	90.1 (85.9-93.4)		91.3 (87.4-94.3)
BCC subtype Infiltrating					
Sensitivity	43.1 (29.5-56.7)	49.0 (35.3-62.7)	58.8 (45.3-72.3)	<0.05***	67.2 (54.0-78.7)
Specificity	81.9 (76.8-87.0)	71.5 (65.5-77.4)	79.6 (74.3-84.9)	<0.001**/****	93.2 (90.3-96.9)
PPV	35.5 (23.6-47.4)	28.4 (19.0-37.8)	40.0 (28.9-51.1)	<0.001**	75.9 (64.4-84.6)
NPV	86.2 (81.5-90.9)	85.9 (80.8-90.9)	89.3 (85.0-93.6)	0.02**	91.4 (88.1-93.8)
Accuracy	74.6 (69.0-79.7)	67.3 (61.4-72.8)	75.7 (70.2-80.7)		88.5 (84.2-91.9)

BCC = basal cell carcinoma; CI = confidence interval; DS = dermoscopy; HH-RCM = handheld reflectance confocal microscopy; NPV = negative predictive value; NS = not significant; PPV = positive predictive value

[A] Biopsy compared to excision outcome

* Significant increase for RCM and combined dermoscopic-HH-RCM compared to DS

** Significant increase for combined dermoscopic-HH-RCM compared to HH-RCM

*** Significant increase for combined dermoscopic-HH-RCM compared to DS

**** Significant increase for DS compared to HH-RCM

Indications for Mohs micrographic surgery

The proportion of cases with an indication for MMS was 98.6% (n=282) according to the USA Taskforce, 78.0% (n=223) for the American/British Association of Dermatology (AAD/BAD), 69.2% (n=198) for the European Society for MMS (ESMS) and 31.1% (n=89) for the European Society for MMS (ESMS). For high-confidence DS-HH-RCM cases (n=281; 82.6%), the sensitivity and specificity (95%CI) outcomes were 98.8% (96.4-99.7) / 92.1% (78.6-98.3) for the USA Taskforce, 99.5% (97.1-100.0) / 86.0% (77.3-92.3) for the AAD/BAD, 96.4% (92.4-98.7) / 86.7% (79.1-92.4) for the ESMS, and 95.8% (88.1-99.1) / 97.6% (94.5-99.2) for the NVDV. Based on these results, applying the USA Taskforce and AAD/BAD criteria would have led to the surgical excision of two benign lesions. In contrast, using the ESMS and NVDV criteria did not result in unnecessary excision of benign lesions.

DISCUSSION

This prospective study evaluated the combination of DS-HH-RCM diagnosis and head-and-neck BCC subtype. Earlier studies have shown limited accuracy of BCC subtyping by dermoscopy[10] and substantial discordance between the outcomes of diagnostic biopsies and excisional specimens.[11,22,23] Using HH-RCM both as a standalone diagnostic tool and in conjunction with dermoscopy showed a significant increase in specificity while maintaining the high sensitivity of dermoscopy (all $P>0.05$). Furthermore, HH-RCM enhanced the diagnostic confidence in BCC detection compared with dermoscopy alone ($P<0.001$). Although DS-HH-RCM provided increased specificity for detecting non-superficial BCC (nBCC/iBCC) and increased sensitivity for iBCC detection, there was a clear trade-off between sensitivity and specificity. In clinical scenarios with other high-risk BCC features, diagnosis using DS-HH-RCM could provide a non-invasive alternative to diagnostic biopsies when considering MMS.

Currently, preoperative biopsy is considered the gold standard for the diagnosis of BCC. However, histological BCC subtyping based on punch biopsy appears to have a limited reliability. Our data demonstrated a notable discrepancy in 33% of biopsy outcomes, scored by experienced pathologists, which were inconsistent with the corresponding excisional specimens, with 24% of cases being upstaged. This aligns with a recent systematic review, which reported a 31% disconcordance rate between biopsy and excisional or MMS specimens.[11] In some cases, this can be avoided by more extensive sectioning, which has been shown to yield more aggressive subtypes, especially in the head-and-neck.[22,24] Discordance is likely related not only to limited sampling but also to the size and quality of the biopsies, resulting in poor quality biopsies for the pathologist to assess. In 11-12% of

cases, a third pathologist was consulted to achieve a consensus on the most aggressive subtype. Consequently, a simplified classification has been suggested because of the moderate inter-rater reliability of pathologists for non-superficial BCC (nBCC/iBCC).[25,26]

The 55.6% specificity of dermoscopy in BCC diagnosis was significantly lower than that in a previous meta-analysis, with a pooled specificity of 95%.[9] This is likely due to 94% of the included cases lacking pigmented dermoscopic structures, as the accuracy decreased in this setting. Furthermore, our cases were limited to clinically suspected head-and-neck BCC based on NEE, and not dermoscopic equivocal BCC. The dermoscopic accuracy in our study was similar to a recent prospective multicenter study in which the addition of HH-RCM to the dermoscopic diagnosis was evaluated.[21] Although BCC inclusion by Longo et al. was not limited to the head-and-neck, their HH-RCM sensitivity (97.8% vs. 97.5%) and specificity (86.2% vs. 85.2%) were consistent with our findings. When only cases with high diagnostic confidence were included, the sensitivity and specificity of HH-RCM increased further to 98.8% and 94.1%, respectively. These outcomes are within the reported range of a 2019 systematic review[27] and compare favorably to a randomized clinical trial comparing RCM to punch biopsies, which resulted in a BCC diagnosis sensitivity of 99% and specificity of 59%.[28]

Several dermoscopic criteria have been published to aid the diagnosis of BCC with different prevalence among subtypes.[29–31] Nonetheless, dermoscopic subtyping remains problematic, as no feature is exclusive to a specific subtype.[10] Even though DS-HH-RCM had the highest specificity (45%) in diagnosing non-superficial BCC (nBCC/iBCC); this still resulted in a significant false-positive rate. However, the clinical relevance of sBCC in the head-and-neck is less clear, as only a limited proportion (7%) of our study consisted of sBCC, and one-third was upstaged after excision. As sBCC in the head-and-neck region has a higher risk (OR 13.15) of presenting as mixed-type, nonsurgical treatment should be considered cautiously.[24,32] In our study, almost 60% of the included BCC consisted of two or more subtypes, likely explaining the overlap of dermoscopic features between nBCC and iBCC.[23] The highest sensitivity for diagnosing iBCC (59%) was achieved by DS-HH-RCM. Nevertheless, DS-HH-RCM still leads to suboptimal selection of surgical margins when considering conventional surgery.

When conventional excision is considered, the histological subtype determines the surgical margins. In the case of MMS, additional clinical criteria such as localization, size, and demarcation are applied in the decision-making process. Considering all these factors, DS-HH-RCM could potentially reduce the need for presurgical biopsies in cases of MMS owing to the limited risk of excision of benign lesions in high-confidence cases.

The limited accuracy of BCC subtyping by HH-RCM likely results from the mixed histology, limited penetration depth (250µm), and horizontal orientation of RCM imaging. Although RCM appears to have a higher accuracy in detecting BCC than optical coherence tomography (OCT), [33–36] the increased penetration depth (1 mm) of OCT results in more reliable detection of sBCC.[35,37] Line-field confocal OCT and a combined RCM-OCT device are new techniques with promising results. They bridge the gap between RCM and OCT by acquiring 3D images with cellular resolution.[38–41] However, the accuracy of both devices in detecting mixed-type iBCC remains unclear. The inter-rater agreement for BCC diagnosis and subtyping was higher than previously reported by Kadouch et al. [42] Due to the horizontal orientation of the HH-RCM, it can be difficult to determine the depth of the basaloid nests. This is likely why the negative specific agreement was lower in the case of non-superficial BCC (i.e., ruling out sBCC). In contrast, positive agreement was lower for iBCC. The limited penetration depth of HH-RCM and lack of clearly defined diagnostic thresholds for iBCC are the most likely explanations for this finding. Finally, the variation in specific agreement on the diagnostic criteria also depends on the prevalence of the specific criteria. The reliability of the subtype-specific BCC RCM criteria should be validated in future studies on a cohort of non-mixed-type BCC.

Limitations

The single-center design may limit generalizability to other settings, especially because of the low prevalence of dermoscopic pigmented features in our study. Although the diagnostic accuracy was increased for blinded expert evaluations and the study investigator over dermoscopy, the HH-RCM assessment remains highly user-dependent. Blinded RCM experts depended on the images acquired by the study investigator. The prevalence of individual diagnostic criteria also affects the observed and specific agreement used in this study and does not correct for chance agreement. Finally, lesion inclusion was based on NEE, whereas the typical RCM workflow includes dermoscopic equivocal lesions. Nonetheless, this study aimed to identify the potential role of HH-RCM in the head-and-neck as a replacement for diagnostic biopsies. Although HH-RCM has an increased accuracy in BCC subtyping compared to dermoscopy, it is insufficient to replace biopsies in all clinical scenarios, such as conventional surgery, where the histological subtype determines the surgical margin.

A direct comparison between punch biopsy and excision outcomes was limited by incorporation bias. While the histological outcome resulted in a significant number of discrepancies between pathologists, it remains the gold standard, with biopsies outperforming dermoscopy and RCM. Nonetheless, there remains a lack of consensus on precisely defining specific BCC subtypes.[26] In addition, we did not correct for the quality

and depth of the diagnostic biopsies, which likely affects the reliability of BCC subtyping in some cases. Deeper sectioning could also have identified more aggressive BCC subtypes than performed during standard care.[22,24] Finally, BCC has several rare benign histological mimics (e.g., benign follicular tumors).[43] While none were diagnosed in this study, they are unlikely to be differentiated from BCC based on DS-HH-RCM findings in clinical practice, potentially resulting in overtreatment.[44]

CONCLUSIONS

The discrepancies between punch biopsy and excisional specimens underscore the limitations of relying solely on punch biopsies for the management of head-and-neck BCC. HH-RCM has the clear benefit of reducing unnecessary biopsies with higher confidence than dermoscopy alone. Although HH-RCM has an increased diagnostic accuracy compared to dermoscopy, it remains insufficient to completely replace diagnostic punch biopsies when management relies solely on BCC subtyping. Future studies should focus on clinical scenarios such as MMS, where management relies on additional clinical features (e.g., size and localization) in addition to subtypes. In conclusion, although HH-RCM could potentially replace presurgical biopsies in specific clinical scenarios such as MMS, this must be confirmed in a randomized study. Such studies should include both cost-effectiveness analyses and patient preferences.

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SUPPLEMENTS

SUPPLEMENT I. Description of dermoscopic basal cell carcinoma features.

Dermoscopic feature	Description
I. Neovascularisation	
Branched vessels*	Horizontal, bright-red, sharply in focus/unfocused, large or thick-diameter vessels dividing into smaller vessels *Metaphoric term: "Arborizing vessels".*
Short fine linear vessels	Irregularly distributed, short, fine, linear vessels with relatively few branches. Usually located in a white to red structureless background.
II. Tumor Nests	
Blue/Gray large clustered clods	Well-circumscribed ovoid structures with confluent or near confluent blue-gray pigmentation. *Metaphoric term: "Blue-gray ovoid nests".*
Blue clods	Blue to gray well-circumscribed solid round to oval clods. *Metaphoric term: "Blue globules".*
In-focus dots	Blue to gray loosely arranged well-defined small dots, which appear to be sharply in focus. To be differentiated from multiple blue/gray unfocused dots representing melanophages *("Peppering").*
Radial lines converging to a central dot/clod*	Well-circumscribed radial projections, usually light brown (sometimes blue or gray) in color meeting at a central darker clod that has a dark brown, black (or blue) color. *Metaphoric term: "Spoke wheel area"*
Concentric clods	Irregularly shaped circumscribed solid round to oval clods with a central darker structure. Variant of the radial converging lines. *Metaphoric term: "Concentric globules".*
Radial lines connected to a common base	Brown to gray/blue discrete linear or bulbous structures coalescing at a common off-center base. *Metaphoric term: "Maple Leaf-like areas".*
Aggregated multiple yellow-white globules	Yellow to white well-circumscribed solid round globules arranged in clusters (polarized and nonpolarized) *Metaphoric term: "May globules".*
III. Dermal fibrosis	
White to red structureless area	Translucent to opaque white to red structureless areas. *Metaphoric terms: "Milky-red structureless areas"*
"Polarized" structures	
White perpendicular lines	Straight, short, and sometimes perpendicular white lines only seen by polarized dermoscopy. *Metaphoric terms: "Shiny white streaks" (former synonyms: chrysalis, chrysalids, crystalline)*
White clods	Discreet white clods of variable size only seen by polarized dermoscopy. *Metaphoric terms: "White blotch"*

SUPPLEMENT II. Description of reflectance confocal microscopy basal cell carcinoma features

Confocal feature	Description
Mild keratinocyte atypia	Loss of regular "honeycomb pattern" with pleomorphic keratinocytes with atypical nuclei and scant cytoplasm.
Epidermal polarization of nuclei	Keratinocytes that appear to be focally elongated and distorted along the same axis ("Streaming").
Epidermal shadowing	Large featureless area with blurred border disrupting the normal epidermis, corresponding to horizontal "retraction clefts" (see below).
Basaloid tumor islands	Round to oval, cord-like, or lobulated structures at the level of the DEJ or superficial dermis.
Large	≥ 300μm basaloid tumor islands not connected to the epidermis, with/without peripheral palisading.
Small	< 150μm basaloid tumor islands not connected to the epidermis, with/without peripheral palisading.
Grape-shaped	Grouped small basaloid tumor islands not connected to the epidermis, with/without peripheral palisading.
Epidermal cords	Sharply demarcated basaloid cords connected to the epidermis, with/without peripheral palisading
Dark Silhouettes	Hyporeflective areas outlined by bright collagen bundles in the surrounding dermis. Corresponding to non-pigmented basaloid tumor islands/strands.
Tumor Island Features	
Retraction clefts	Peritumoral dark spaces.
Peripheral palisading	Peripheral palisading of nuclei of basaloid cells.
Fibrosis	Highly refractive bundles of dermal collagen surrounding the basaloid tumor islands.
Dendritic cells	Highly refractive bright, delicate, branching structures within the tumor islands, corresponding to melanocytes or Langerhans cells.
Increased vascularization	Thickened, elongated or tortuous dark structures, oriented in the horizontal plane in relation to the skin surface, containing moving small, round bright particles (caliber scored as +, ++ or +++).
Solar elastosis	Moderate to highly refractive bundles of collagen.
Small bright particles	Bright, highly refractive small round cells representing an inflammatory leukocyte infiltrate.
Plump bright cells	Large (>20μm) irregularly shaped bright cells with ill-defined borders and usually no visible nucleus.

DEJ: Dermo-epidermal junction

SUPPLEMENT III. International Mohs Micrographic Surgery (MMS) indications

Society	Indications
USA 2012 Taskforce[1]	• Area H, any subtype or size • Area M, any subtype or size
American Association of Dermatology (AAD)[2]	• Area L ≥ 20 mm • Area M ≥ 10 mm • Area H • Poorly defined • Recurrent • Immunosuppression • Site of prior radiation therapy • Aggressive growth pattern
British Association of Dermatology (BAD)[3]	• Area H • Area M >10 mm • Poorly defined borders • Recurrent • Immunosuppression • Site of prior radiation therapy • Infiltrative (infiltrating, morphoeic, micronodular) • Basosquamous differentiation (with or without lymphovascular invasion) • Beyond subcutaneous fat • Level of invasion >6 mm • Perineural invasion • Histological margins < 1mm
European Society for MMS (ESMS)[4]	• Localization in the central face, around the eyes, nose, lips, or ears (regardless of the size). • Poor clinical definition of tumor margins. • Recurrent or incompletely excised lesions. • Aggressive histopathological subtypes (e.g., morphoeic, infiltrative, micronodular, and basosquamous). • Perineural or perivascular involvement. • Size >2 cm. • When tissue sparing is of great importance (regardless of location).
Dutch Society of Dermatology and Venerology (NVDV)[5]	• Primary BCC >10 mm in area H • Primary BCC >5 mm near the eyelids or nasal tip • Primary BCC outside area H infiltrative >10 mm or nodular >15 mm • Recurrent BCC • Residual BCC in the face

[1] Kim JYS, Kozlow JH, Mittal B, et al. Guidelines of care for the management of basal cell carcinoma. J Am Acad Dermatol. 2018 Mar;78(3):540-559.

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[4] https://www.esms-mohs.eu/fileadmin/user_upload/ESMS_Society_web/Resources_PDF/ESMS_Position_Paper_-_WEB.pdf

[5] https://storage.googleapis.com/alii-ea36b.appspot.com/images/6966f9db-3da0-49d7-bf4e-54b205063d9a.pdf

SUPPLEMENT III. Standards for Reporting of Diagnostic Accuracy Studies (STARD) 2015 checklist

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	168
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	168
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	169
	4	Study objectives and hypotheses	169
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	170
Participants	**6**	Eligibility criteria	170
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	170
	8	Where and when potentially eligible participants were identified (setting, location and dates)	170
	9	Whether participants formed a consecutive, random or convenience series	170
Test methods	**10a**	Index test, in sufficient detail to allow replication	170
	10b	Reference standard, in sufficient detail to allow replication	170
	11	Rationale for choosing the reference standard (if alternatives exist)	170
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	-
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	-
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	170-171
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	170
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	171
	15	How indeterminate index test or reference standard results were handled	-
	16	How missing data on the index test and reference standard were handled	171
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	-
	18	Intended sample size and how it was determined	171

Section & Topic	No	Item	Reported on page #
RESULTS			
Participants	**19**	Flow of participants, using a diagram	FIGURE 1
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	21a	Distribution of severity of disease in those with the target condition	171
	21b	Distribution of alternative diagnoses in those without the target condition	TABLE 1
	22	Time interval and any clinical interventions between index test and reference standard	-
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	FIGURE 1
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	TABLE 3
	25	Any adverse events from performing the index test or the reference standard	-
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	181-182
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OTHER INFORMATION			
	28	Registration number and name of registry	-
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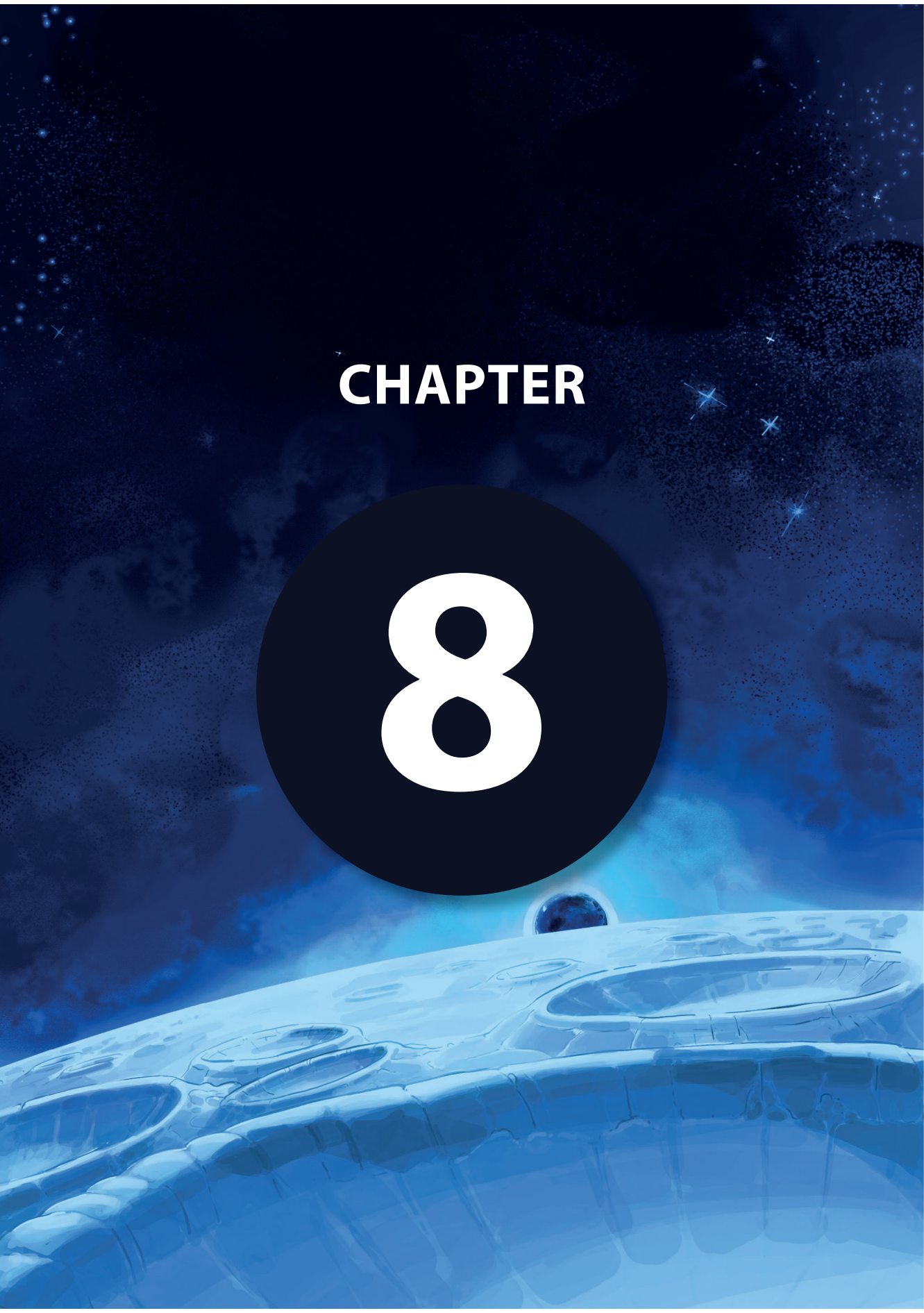
PART IV

CONCLUSION


CHAPTER
8

SUMMARY

Skin cancer has become the most common malignancy among light-skinned populations worldwide, with incidence rates continuing to rise rapidly. The head-and-neck region is particularly susceptible to developing skin cancer as it is exposed to all forms of UV radiation. The primary challenges in treating skin cancer in this region are the complexity of anatomy and cosmesis. The research presented in this thesis focuses on head-and-neck skin cancer, with a specific emphasis on i) lentigo maligna (melanoma) (LM/LMM), a subtype of melanoma that typically presents in this anatomical region, and ii) basal cell carcinoma (BCC), the most common form of skin cancer, with a predilection for the head-and-neck.

PART I - TREATMENT OF LENTIGO MALIGNA (MELANOMA)

Lentigo maligna (melanoma) has a distinct clinicopathological presentation compared to other melanoma subtypes and is typically diagnosed in elderly patients. It is unclear whether the distinct features of LM/LMM affect the prognosis. In **CHAPTER 2**, we retrospectively compared consecutive primary LM/LMM (n=75) and non-LM/LMM (n=270) melanoma subtypes in the head-and-neck treated with wide local excision (WLE) (2003-2014). Patients with additional stage ≥ T2 melanoma or stage III/IV disease at baseline were excluded. Survival outcomes and prognostic factors were assessed using cumulative incidence and competing risk analysis. Lower Breslow thickness at diagnosis, upstaging to invasive melanoma, and an increased risk of local recurrence due to incomplete resection (18.7% vs. 2.3%) were significantly associated with LM/LMM. There was no significant difference in guideline adherence with respect to surgical margins. Nonetheless, a significant proportion of the LM/LMM cases (24% vs. 5%) had positive histological margins. The positivity rate of sentinel lymph node biopsy (SLNB) for the LMM subtype (12% vs. 20%) was lower. LMM was not an independent prognostic factor for disease-free or melanoma-specific survival. Similarly, guideline adherence did not affect survival outcomes.

Between December 2015 and July 2017, we performed a pilot study using handheld reflectance confocal microscopy (HH-RCM) to improve histological margin control and reduce the risk of local recurrence (**CHAPTER 3**). Twenty-four patients, 18 with LM and 6 with LMM (stage ≤T2a), were treated with HH-RCM-guided WLE. We obtained negative histological margins in 96% (n=23) of patients. The in vivo mapping procedure resulted in a sensitivity of 90% and a specificity of 86%. A single recurrent LM with positive histological margins presented with local recurrence at 21 months of follow-up.

After completing the pilot study, HH-RCM was introduced as the Netherlands Cancer Institute's (NKI-AVL) standard of care. The main aim of this follow-up study (**CHAPTER 4**) was to confirm the efficacy of HH-RCM-assisted WLE in terms of surgical outcomes and

recurrence rates in a larger population. In addition, we aimed to evaluate the accuracy of HH-RCM in detecting subclinical dermal invasion (i.e., invasive LMM), as well as the effect of the mapping procedure on management decision-making. A total of 117 consecutive patients with LM/LMM (n = 117) were enrolled between 2015 and 2023. The surgical outcomes were compared to those of a historical cohort (2003-2014) before HH-RCM was introduced in the NKI-AVL. The median mapping duration was 14 minutes. In 60% of cases, HH-RCM detected subclinical LM beyond the guideline-recommended margin. Of the 16 LMM missed at initial diagnosis, 75% were identified during the mapping procedure, with a sensitivity and specificity of 80% and 88%, respectively. The negative predictive value for the detection of LMM was 94%. Owing to the outcome of the mapping procedure, management was changed to 27% (n=32). In these cases, the treatment of choice mainly consisted of topical imiquimod monotherapy (n=14) or limited surgery followed by adjuvant imiquimod (n=15). The remaining patients (n=84) were treated with HH-RCM-assisted WLE. Histological margins were cleared in 97% of the patients with a median histological margin of 3.0 mm, which was significantly higher than the 81% in the historical cohort (median 2.0 mm). The mapping procedure's high diagnostic accuracy in the pilot study was maintained with 94% sensitivity and 84% specificity. The recurrence rate of HH-RCM-assisted WLE was limited to 1.5% compared with 25% in the historical cohort.

Owing to the increased risk of local recurrence of LM/LMM, several staged and micrographically controlled surgical techniques have been proposed to reduce this risk. However, the long-term effects of the various techniques on survival outcomes remain unclear. In **CHAPTER 5**, we performed a systematic review to evaluate the effect of the different surgical techniques used in managing LM/LMM on local recurrence and survival outcomes as well as the effect of RCM on resection margins and recurrence rates. Forty-one studies with 5059 LM and 1271 LMM were included. Surgical techniques included WLE (n = 1355), staged excision (n = 2442), and Mohs micrographic surgery (MMS) (n = 2909). The guideline-recommended margin was insufficient in 22%–45% of LM/LMM cases. The local recurrence rate was the lowest in patients treated with MMS combined with immunohistochemistry (<1%) and the highest in those treated with WLE (13%). Handheld RCM decreased both the rate of positive histological margins and the number of necessary surgical stages. Due to selection bias, heterogeneity, low prevalence of stage III/IV disease, and limited survival data, it was impossible to determine the effects of different surgical techniques on survival outcomes.

Main conclusions - Part I

- Although LM/LMM has a significantly increased risk of local recurrence, it appears to have a limited effect on survival outcomes.
- LM/LMM has an increased risk (10%) of harboring subclinical invasive components owing to the partial sampling used in the diagnostic process.
- LMM was not shown to be prognostically different from non-LMM invasive melanoma when corrected for other variables such as patient age and Breslow thickness. Reduced resection margins did not appear to affect disease-free or melanoma-specific survival rates.
- Handheld reflectance confocal microscopy provides an accurate (sensitivity 90%) method for in vivo presurgical delineation of LM/LMM with a limited rate of overtreatment (specificity 86%).
- HH-RCM-assisted WLE results in a high rate of negative histological margins and low local recurrence rates.
- HH-RCM allows for a more personalized approach for patients with LM/LMM by mapping the full extent of the lesion before selecting optimal (surgical) management.
- The high negative predictive value of HH-RCM in detecting occult invasive melanoma allows the selection of patients who are more suitable for nonsurgical treatment, such as topical imiquimod.
- Our systematic review showed a clear reduction in local recurrences using microscopically controlled surgical techniques compared with WLE. The use of HH-RCM reduces the risk of incomplete resection and local recurrence even when used in conjunction with WLE.

PART II – REGIONAL DIAGNOSTICS IN LENTIGO MALIGNA MELANOMA

Following the low yield of SLNB in patients with LMM in our retrospective study (**CHAPTER 1**), we conducted a national study using a nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA) (**CHAPTER 6**) to confirm our findings. We identified all patients with LMM between 1991 and 2020 with subsequent regional lymph node histology or cytology reports. We included all patients with reported SLNB and all lesions with an indication for SLNB according to the current 8th edition of the American Joint Committee on Cancer (AJCC) (i.e., T1b or higher) (n=1989). Clinicopathological characteristics were extracted, and penalized logistic regression analysis was performed to determine the optimal combination of clinicopathological factors to predict positive SLNB. Sentinel lymph node biopsy was performed in 17% (n=333) of cases, with a positivity rate of 7.5 % (n = 25). An additional 25 patients had clinically detected regional lymph node metastasis.

The best predictive clinicopathological characteristics (odds ratio) for SLNB positivity were age (0.95), ulceration (1.59), T4-stage (1.81), male sex (1.97), (lymph)angioinvasion (5.0), and microsatellites (7.23). The C-statistic for this model was 0.75, indicating a good level of discrimination between positive and negative SLNB cases. Nonetheless, the number needed-to-treat for a single case of SLNB positivity remained high, even for T4 (23.6) staged LMM or the presence of ulceration (32.0). Age was inversely associated with SLNB positivity.

During follow-up, regional LN recurrences were detected in 4.2% of cases, resulting in a false-negative rate of 22% for SLNB. Based on these findings, a nomogram was developed to predict the risk of SLNB-positivity.

Main conclusions - Part II

- Based on nationwide data, the yield of sentinel lymph node biopsy (SLNB) in patients with LMM was confirmed to be limited when current guidelines to perform SLNB were applied.
- A nomogram was developed based on the clinicopathological characteristics that best predicted SLNB positivity (age, ulceration, T4-stage, [lymph]angioinvasion, and microsatellites).

PART III - DIAGNOSTICS OF BASAL CELL CARCINOMA

As LM/LMM is relatively rare, our focus in **CHAPTER 7** was on the diagnosis of the most common type of skin cancer in the head-and-neck, basal cell carcinoma. The current management of head-and-neck BCC relies heavily on diagnostic biopsies. The reason for this is twofold: i) the clinical differential diagnosis contains several potential benign mimics and ii) the BCC subtype determines the best surgical management. In this prospective study, we aimed to assess the diagnostic accuracy of the combined dermoscopy-HH-RCM (DS-HH-RCM) diagnosis and BCC subtyping. The secondary aim was to determine the outcome of the DS-HH-RCM diagnostic pathway when applying several international Mohs micrographic surgery (MMS) criteria to our study cohort.

We performed a fully paired diagnostic comparison study of 340 consecutive lesions with a clinical suspicion of BCC on naked-eye examination. The diagnostic accuracy of DS-HH-RCM was evaluated by the study investigator and blinded expert evaluations of dermoscopic and RCM images. Diagnostic confidence was recorded using a 3-point Likert scale (low, medium, and high) for all diagnostic steps. All histologically confirmed BCC cases were surgically treated. Histological diagnosis was based on a consensus between blinded expert pathologists, and the most aggressive subtype found in either the biopsy or excisional specimen was considered the reference standard.

Two or more histological subtypes (mixed-type BCC) were found in 59% of the BCC cases. The most aggressive subtype of punch biopsy was consistent with the final excisional specimens in 61% (n=173) of cases, with discrepancies of 33% (n=95). The sensitivities of BCC diagnosis were consistent (>97%) across the three diagnostic pathways. Dermoscopy with DS-HH-RCM had the highest specificity for all outcomes and the highest proportion of cases with a high diagnostic confidence. The diagnostic accuracy resulted in a sensitivity of 97% and a specificity of 85%, which increased to 99% and 94%, respectively, in cases with high diagnostic confidence. The sensitivity/specificity for detecting non-superficial BCC (i.e.,

nodular and infiltrative) and infiltrative BCC were 95%, 45%, and 59% / 80%, respectively. When applying several international MMS criteria to our study cohort, only cases with high diagnostic confidence (n=281) would have resulted in excision of 0-2 benign lesions.

Main conclusions - Part III

- BCC diagnosis: The combination of dermoscopy with handheld reflectance confocal microscopy (DS-HH-RCM) significantly reduced the rate of unnecessary biopsies compared with dermoscopy alone (specificity: 85% vs. 56%).
- BCC diagnosis: The specificity further increased when only including cases with high diagnostic confidence (94%) while maintaining excellent sensitivity (99%).
- BCC subtyping: Although DS-HH-RCM showed the highest sensitivity and specificity for detecting non-superficial BCC (superficial vs. nodular/infiltrative) and infiltration BCC (superficial/nodular vs. infiltrating), this outcome was not sufficient to replace diagnostic biopsy in all clinical scenarios.
- When considering Mohs micrographic surgery, DS-HH-RCM could potentially replace diagnostic biopsies when only cases with high diagnostic confidence are included.

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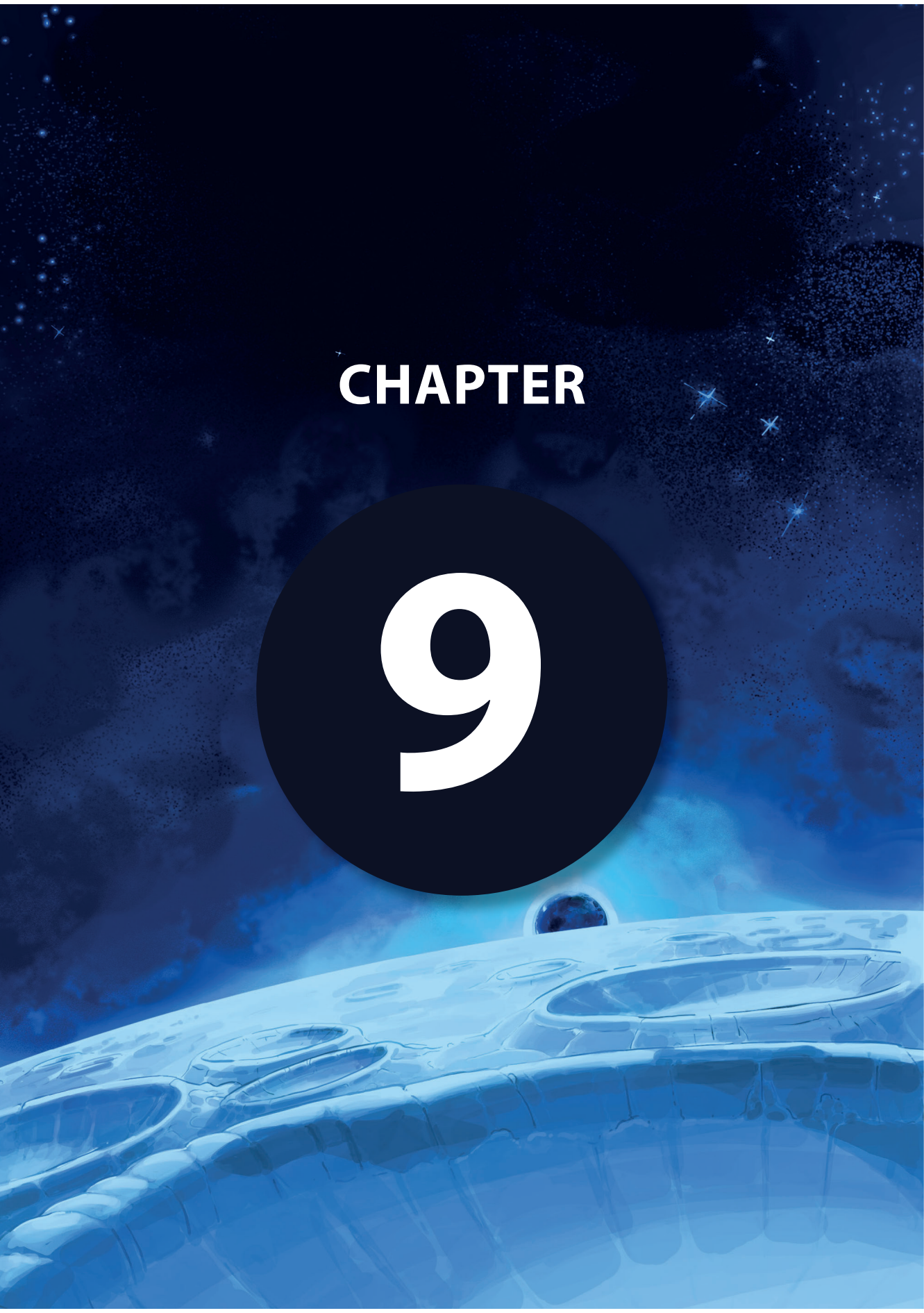
CHAPTER
9

GENERAL DISCUSSION AND CONCLUDING REMARKS

INTRODUCTION

The studies in this thesis focused on two types of skin cancers with a predilection for the head-and-neck: lentigo maligna (melanoma) (LM/LMM) and basal cell carcinoma (BCC). The head-and-neck region was chosen as the region of interest in this thesis because managing both types of skin malignancies poses a particular challenge: the localization of these skin malignancies can lead to potential cosmetic and functional consequences of diagnostic and therapeutic interventions. Current international LM/LMM guidelines offer only general advice on diagnostic and therapeutic management[1,2], which might be inadequate for the individual, often elderly, LM/LMM patient due to clinical heterogeneity on the one hand and lack of predictive tools on the other hand. In contrast, management guidelines for BCC are based on a foundation of robust evidence-based research.[3–8] The scientific challenge lies more in the increasing incidence and associated diagnostic burden on the healthcare system.

The past decade has seen an increased use of imaging techniques to assist in the noninvasive diagnosis of different types of skin cancers.[9–12] In this thesis, we investigated the clinical applications of handheld reflectance confocal microscopy (HH-RCM) in the diagnostic management of LM/LMM and BCC in the head-and-neck. In **PART I** and **PART II,** we evaluated the effect of HH-RCM in reducing diagnostic sampling errors and its impact on (surgical) management of LM/MM. In **PART III,** the focus was on the potential role of in vivo diagnosis and subtyping using HH-RCM for BCC diagnosis.

LENTIGO MALIGNA (MELANOMA)

Guideline recommendations: One size does not fit all.

Current international guidelines recommend surgical excision as the first-line therapy for LM. The central therapeutic debate concerning patients with LM should focus on the relevant clinical endpoint: complete resection or avoidance of progression to invasive melanoma or potentially metastatic disease. Lentigo maligna typically has a prolonged radial growth phase.[13] The most cited epidemiological study was published by Weinstock et al. in 1987.[14] They estimated an annual risk of progression of 0.03% and 0.14% for the 45-64 and 65+ age groups, respectively. This annual progression risk is significantly lower than that of more recent prospective data, which report an annual risk of 3.5% (95% confidence interval: 2.5-5.0).[15] Based on national data from the Netherlands and the United States, the absolute 10-year cumulative risk of progression to LMM after treatment of LM is reported to be between 1.0% and 2.6%.[16,17] The lack of longitudinal prospective studies on the progression rate of untreated LM means that the actual rate remains unknown; nonetheless,

all currently available data points towards an overall limited progression risk. Therefore, as the peak incidence of LM/LMM occurs in the 7th decade[16–18], patient age should be included in the decision-making process when discussing the risk of progression (**CHAPTER 1)**. Once invasive, the diagnostic and therapeutic management of LMM is generally performed according to general melanoma guidelines.

The principle of wide local excision (WLE) of cutaneous melanoma is threefold: i) local control, ii) regional control, and iii) ultimately resulting in a survival benefit. However, the real (survival) benefit of WLE is currently under scrutiny.[19,20] Surgical treatment for LMM should be approached similarly. International guidelines acknowledge that wider margins are often needed for LM/LMM due to unpredictable subclinical extension.[21] However, increased surgical margins are not always feasible in the head-and-neck for cosmetic and functional reasons.[22,23] Furthermore, the use of HH-RCM has shown that subclinical extension is often asymmetric and wide circumferential margins could result in unnecessary excision of too much healthy tissue.

Local control. The increased risk of incomplete resection after WLE is a unique clinicopathological feature of the LM/LMM subtype (**CHAPTER 1**). Except for desmoplastic melanoma, which shares its etiology with LM/LMM[24–26], other melanoma subtypes are likely to be completely resected after diagnostic excisional biopsy. Regarding excision margins of LM/LMM, it is important to consider that positive histological margins generally consist of an in-situ component, whereas the invasive component is completely removed in most cases (**CHAPTER 2)**. When local recurrence occurs, a minority of patients present with invasive recurrence and often have a good prognosis based on the Breslow thickness (median 0.9 mm) (**CHAPTER 5**).

A unique clinicopathological feature of the LM/LMM subtype supporting surgical treatment is an increased risk of diagnostic sampling errors (**CHAPTER 1**). As clinical and dermoscopic characteristics are often insufficient to detect LMM accurately[27–30], approximately 10% of surgically treated LM are upstaged to invasive melanoma when completely excised (**CHAPTER 5**). Consequently, when opting for non-surgical treatment modalities, there is the potential to undertreat invasive melanoma missed at the initial diagnosis.[31]

Regional control. In the 8th edition of the AJCC, microsatellites were included as an important prognostic factor, resulting in a stage III classification regardless of nodal status.[32,33] The rationale for WLE in this context is to excise these microscopic deposits, as they represent intralymphatic or possibly angiotropic spread, thereby reducing the chance of locoregional recurrences. The presence of microsatellites is one of the strongest independent predictors of sentinel lymph node biopsy (SLNB) positivity.[32–35] However, the

incidence of microsatellites seems low compared to that of other melanoma subtypes.[33] Similarly, data from the Dutch National Pathology database (PALGA) show that less than 3% of LMM with an SLNB indication are diagnosed with microsatellites present in the excisional tissue (**CHAPTER 6**), which are likely diagnosed after WLE has already been performed due to diagnosis based on partial sampling.

Sentinel lymph node biopsy is currently recommended for ≥T1b staged melanoma for prognostic stratification.[36,36] When applying this threshold to perform SLNB, the rate of SLNB positivity seems lower for LMM than for other melanoma subtypes. Consequently, the number needed-to-treat is higher, even in higher T-staged LMM (**CHAPTERS 1 & 5**).[37–40] Given the current adjuvant treatment options, the outcome of SLNB remains essential. Adjuvant anti-PD-1 has been shown to have similar benefits in recurrence-free survival in patients aged ≥65 years with fewer than three comorbidities compared with younger patients.[41] Increasing age and the possible effect on the performance of SLNB likely play a role in the reduced yield in LMM patients (**CHAPTER 5**). Next to the altered lymphatic drainage in the elderly, however, it could be a biological difference with a higher rate of hematogenous metastatic pattern spread[40,42]

Survival benefit. In daily clinical practice, deviation from the surgical guidelines for the head-and-neck is not uncommon, resulting in reduced resection margins. In **CHAPTER 2**, we performed a competing risk analysis, and guideline nonadherence was not shown to be an independent prognostic factor for survival outcomes in head-and-neck melanoma. This is in line with data supporting that reduced surgical margins in head-and-neck melanoma are not associated with decreased survival.[43–46] Based on the available survival data in our systematic review (**CHAPTER 5**), we could not determine whether there was a difference in survival outcomes between WLE and micrographically controlled surgical techniques. This might be due to the low number of reported events (i.e., development of stage III/IV and melanoma-associated deaths). Even so, the majority (>75%) of reported deaths were due to unrelated causes. A recent study using the US Surveillance, Epidemiology, and End Results (SEER) database by Mitchell et al. concluded that LMM patients have an increased MSS when treated with MMS over WLE.[47] However, their data showed a clear risk of selection bias, as 29% of WLE-treated LMM patients had a Breslow thickness >1.00 mm compared with 6.2% in the MMS group, and ulceration status was not included in the analysis. In addition, 5% of WLE cases were diagnosed with stage III disease at baseline, compared to 1% of MMS cases. There seems to be a tendency to treat low-risk melanoma with MMS, making direct comparisons misleading due to the different baseline risk factors for recurrence and survival.[48]

In summary, increased surgical margins have a limited positive survival benefit for most patients with LM/LMM and are more likely to result in cosmetic and functional ramifications in elderly patients. In addition, the current threshold for performing SLNB has limited yield when applied to all patients with LMM. Consequently, a more personalized, multidisciplinary, collaborative approach would be more suitable for this patient group than a one-size-fits-all approach.

Personalized management – Numbers tell the tale

Although micrographically controlled surgical techniques offer the lowest recurrence rates (**CHAPTER 5**), a major limitation is that they do not predict defect size. This is problematic, as this information is crucial for patients to make informed decisions when deciding between different treatment options.[49] For example, in a retrospective cohort of recurrent or residual LM, surgical staged excision was interrupted in 11% of the cases due to patient request or increasing proximity to critical anatomic sites.[50]

Diagnostic criteria for LM/LMM have previously been established for RCM[51], with additional features identified for margin assessment.[52,53] This has resulted in a significantly more accurate and noninvasive method for detecting the subclinical lesion border.[21,54] One of the first studies published on this topic was the study by Guitera et al., who detected subclinical disease beyond the guideline-recommended margin in 59% of the cases using the arm-mounted RCM device.[55] Our outcome with the handheld device (HH-RCM) verified these findings, as we detected subclinical LM in 60% of our population (**CHAPTER 4**). The estimated defect size of HH-RCM seems to correlate well, albeit slightly smaller, with those found in micrographically controlled surgical techniques (mean 0.76 mm smaller [95% confidence interval 0.67-0.84 mm]).[53,56] Our data supports this, which shows that HH-RCM significantly reduces the surgical stages necessary for negative histological margins (**CHAPTER 5**) and, consequently, the time needed to repair.[57]

By mapping the full extent of the lesion, we can improve the decision-making process for both the patients and healthcare providers. Based on the extent of subclinical disease, it can be concluded that non-surgical treatment or less extensive surgical intervention followed by adjuvant topical treatment is more appropriate on a case-by-case basis. Another critical factor to consider is the possibility of occult dermal invasion. Although the penetration depth of RCM is limited (250 µm) and provides a horizontal orientation, it allows for image-guided skin biopsies, resulting in a more robust histopathological correlation.[58–61] As of this writing, a single study has evaluated the accuracy of RCM in detecting dermal invasion, with an area under the curve (AUC) value of 74% (CI 95% 64-85%).[62] Using three independent

features predictive of LMM in the background of LM, Gouveia et al.[62] correctly identified 89% of LMM cases. These criteria included a large atypical melanocyte size, epidermal/junctional disarray, and melanocytic nests.

Due to the HH-RCM findings, management was changed in over 25% of cases in our center (**CHAPTERS 3 & 4**), which is significantly lower than previously reported.[55] The most likely explanation for this difference is the clinical characteristics of the included lesions or the imaging procedure itself (i.e., the device used and the radial vs. circumferential approach). The high negative predictive value of HH-RCM when assessing dermal invasion also allows for the selection of patients suitable for nonsurgical treatment. However, the follow-up of nonsurgical treatments (radiotherapy and topical imiquimod) can be problematic because of the risk of false-negative clinical examination results. In this clinical scenario, RCM is highly suitable for detecting residual disease because of its significantly increased sensitivity compared with dermoscopic evaluation.[63,64] The follow-up of our LM patients treated with topical imiquimod in our cohort was limited (median 18.5 months) (**CHAPTER 5**). In a systematic review of imiquimod-treated patients (n=471), less than 2% of LM progressed to LMM at a mean of 4 months (range 0-11)[65], which is significantly lower than the reported 10% of occult dermal invasion in surgically treated LM. It can be hypothesized that micro-invasive LMM responds to the pro-inflammatory and T-cell mediated response initiated by topical imiquimod.[66] Conversely, histological diagnostic pitfalls, such as tangential cut adnexal involvement or incidental dermal melanocytes, can result in false-positive "occult melanoma" outcomes.[67,68] Nonetheless, the risk of diagnostic sampling errors has to be considered, paving the way to more conservative surgery (**CHAPTER 4**) combined with adjuvant or neoadjuvant use of topical imiquimod.[69–74]

Finally, the prognostic use of SLNB has shifted in recent years, as it can identify patients with stage III melanoma suitable for adjuvant treatment with PD-1 or BRAK-MEK inhibitors.[75–77] As an overall survival benefit has yet to be shown for adjuvant treatments[78–80], it is essential to identify those LMM patients who are at increased risk of disease progression.[42] Several nomograms have been developed to predict SLNB outcomes more accurately.[81–83] However, the discriminatory ability highly depends on the clinicopathological features of the population of interest. A recent study concluded that the risk of SLNB-positivity was consistently underestimated when applied to a cohort with thinner melanomas and a different proportion of melanoma subtypes.[84] Similarly, at lower risk thresholds (5-10%), the clinical benefit of the nomograms was deemed insufficient, which led to over-performing SLNB for all patients.[85] With these two limitations in mind, the question arises if the Melanoma Institute of Australia (MIA) nomogram is sufficiently reliable for LMM patients, as less than 3% (n=93) of the melanoma included in the development set consisted of LMM.[82]

BASAL CELL CARCINOMA

Basal cell carcinoma diagnostics – Sometimes less, is more.

The high and increasing number of BCC diagnoses will remain a significant burden on healthcare and healthcare-related costs due to the high volume.[5,86] To alleviate this burden, skin imaging techniques could play a role in the non-invasive and fast-track management of BCC. Histological confirmation for diagnosis and subtyping is considered the current gold standard for BCC management. However, diagnostic punch biopsies should be regarded as the reference standard rather than the golden standard[87] because of the 39% discordance between biopsy and excision specimens (**CHAPTER** 7).[88–90] Nonetheless, with the current limitations in skin imaging techniques, a trade-off remains between the already limited penetration depth and image resolution. Consequently, current skin imaging devices cannot directly compete with full-thickness dermal biopsies.[91] This was confirmed in **CHAPTER 7,** where BCC subtyping in the head-and-neck region was challenging owing to the high rate of mixed-type BCC. However, in specific clinical scenarios, confirmation of the diagnosis is sufficient.

While diagnostic pitfalls, in addition to rare histological BCC mimics, remain[92], the question at hand is whether these cases, medicolegal issues aside, should result in maintaining the current diagnostic workflow. For example, while cutaneous melanoma is considered a high-risk tumor compared to BCC, a large (n=3165) multicenter trial with a follow-up of at least 10 months showed that adjunctive use of RCM reduced the number-needed-to-excise for melanoma by 43% with all delayed definitive melanoma diagnosis having a Breslow thickness < 0.5 mm.[93]

Outside the head-and-neck, RCM has already been shown to be non-inferior using a one-stop-shop concept in terms of histological margin outcome compared to the standard of care.[94] Similarly, a non-inferiority trial using optical coherence tomography (OCT) showed that OCT-guided diagnosis and treatment (excluding the “H-zone”) were non-inferior to punch biopsies when considering recurrent or residual BCC at 12 months post-treatment as the primary endpoint.[95] Patient preference is also important to consider, as patients prefer RCM over punch biopsy because of its convenience.[94,96] In conclusion, in managing head-and-neck BCC, it is essential to identify those clinical scenarios where adapted diagnostic strategies are feasible to meet the increasing demands.

LIMITATIONS

One limitation of LM/LMM’s body of evidence (and this thesis) is the lack of randomized controlled trials (RCT) comparing different treatment modalities. The results of the RADICAL

Trial (NCT02394132) comparing radiotherapy to topical imiquimod will represent the first RCT performed on LM patients. Head-to-head comparisons between different (surgical) treatment modalities can also be problematic because of the sizable clinicopathological heterogeneity of LM/LMM lesions. These clinicopathological variations (e.g., size, localization, and lesion demarcation) can represent potential confounders, which most studies do not correct for. Solving this issue would require a multicenter international endeavor to allow for matched cohort studies or RCTs.

Although RCM can provide significant benefits in the clinical management of skin cancer in general, its efficacy is highly user dependent because of the subjective nature of the evaluation. Mapping procedures, in particular, lack standardization, resulting in the publication of several methods. A limited number of studies have evaluated the learning curve involved in RCM assessment, but the learning curve seems limited in the case of BCC diagnosis.[97,98] Although a recent prospective study in the UK showed high sensitivity and specificity for BCC diagnosis after a 6-day training course and controlled evaluation of 100 online cases, the learning curve for BCC subtyping is less clear.[99] On the technical side, unlike the arm-mounted device, the handheld RCM device lacks direct dermoscopic correlation, which hampers the direct translation of the images to the patient. Furthermore, limited penetration depth and lack of immunohistochemical staining are further limitations of RCM diagnostics. Consequently, HH-RCM should be viewed as a "super-dermatoscope" to evaluate dermoscopic equivocal lesions and, at most, as a noninvasive alternative to superficial shave biopsy instead of a replacement for histological examination. For example, although the high sensitivity of RCM for the detection of subclinical LM has been established[56,100], the dendritic morphology of atypical melanocytes, as observed in LM, can result in false-positive outcomes. Activated Langerhans cells share morphological characteristics with dendritic melanocytes.[101,102] Similarly, it is well known that LM can gradually transition into melanocytic hyperplasia towards the lesion periphery. As melanocytic hyperplasia also has a dendritic morphology, it can be difficult to assess where LM stops, and melanocytic hyperplasia starts.[103,104]

The orientation in the horizontal plane and depth (250 µm) of the RCM images allowed a complete view of the epidermis and subsequent assessment of the lateral margins in the case of LM/LMM. With a mean invasion depth of 2.3mm for BCC[105], the limited penetration depth of RCM poses a more significant limitation in diagnosing BCC as invasion depth is increased for mixed-type BCC, especially in the presence of an infiltrative component.[106,107]

FUTURE PERSPECTIVES

Owing to the functional and cosmetic importance of the head-and-neck, guidelines for LM/LMM resection and appropriate margins should be developed separately from other melanoma subtypes and localizations. However, the current literature is limited by a high risk of bias in patient and treatment selection, clinical heterogeneity, and a limited number of prospective studies. To improve the consistency and quality of LM/LMM research, it is essential to register relevant clinicopathological features in a standardized, reliable, and feasible manner. Therefore, a consensus on a core set of domains and domain items (i.e., what to measure) would allow for better data comparisons between different treatment modalities. Therefore, a large and well-coordinated international consortium is required.

In the absence of LM-specific prognostic tools to predict malignant potential, advising the individual LM patient on the best therapeutic management remains challenging.[49] While our nomogram for SLNB outcome still needs to be validated in an external cohort, the threshold to perform SLNB in LMM should likely be higher than other melanoma subtypes. Another approach could be stratifying the overall risk profile of LM/LMM based on molecular biology, such as gene expression profiles[108–110] or circulating microRNA signatures.[111,112]

In conclusion, before the widespread implementation of skin imaging devices such as HH-RCM, it is crucial to determine their exact role in the healthcare system by performing cost-effectiveness and patient preference studies. Implementing an adequate reimbursement system is essential to cover the cost of the device and time required for imaging and analysis. More objective assessment can be achieved with the assistance of deep learning systems, which have already been shown to improve diagnostics, determine tumor depth, and have high accuracy in evaluating resection margins.[113–116]

CONCLUDING REMARKS

This thesis is comprised of three parts. The studies aimed to investigate a more personalized approach for managing patients with LM/LMM (**PART I**). The optimal surgical technique remains unclear when considering survival outcomes other than local recurrence. Nonetheless, we were able to use HH-RCM to guide the(surgical) management with less extensive surgical intervention, identification of occult dermal invasion, and persistent long-term local control. To allow for a more widespread implementation of this technique in high-volume settings, reimbursement is essential. Future studies should focus on identifying patients with LM who are at a high risk of progression. Similarly, the current threshold for SLNB must be specifically tailored to the LMM subtype, as the current number needed-to-treat for SLNB positivity remains high (**PART II**). Finally, skin imaging devices, such as HH-RCM, offer new possibilities for managing BCC, allowing bedside diagnostics and a more patient-centered approach (**PART III**).

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APPENDICES

A

NEDERLANDSE SAMENVATTING | DUTCH SUMMARY

Huidkanker is wereldwijd de meest voorkomende kanker onder de populaties met een licht huidtype, waarbij de incidentiecijfers blijven stijgen. Het hoofd-halsgebied is bijzonder vatbaar voor het ontwikkelen van huidkanker, omdat het in verhouding mee wordt blootgesteld aan alle vormen van UV-straling. De primaire uitdagingen bij de behandeling van huidkanker in dit gebied zijn de complexiteit van de anatomie en de cosmetische aspecten. Het onderzoek gepresenteerd in dit proefschrift richt zich op huidkanker in het hoofd-halsgebied, met specifieke nadruk op: i) lentigo maligna (melanoom) (LM/LMM), een subtype van melanoom dat typisch in deze anatomische regio voorkomt, en ii) basaalcelcarcinoom (BCC), de meest voorkomende vorm van huidkanker, eveneens met een voorkeur voor het hoofd-halsgebied.

DEEL I - BEHANDELING VAN LENTIGO MALIGNA (MELANOOM)

Lentigo maligna (melanoom) heeft een unieke klinisch-pathologische presentatie en wordt vooral gediagnosticeerd bij oudere patiënten. Het is onduidelijk of de specifieke clinicopathologische kenmerken van LM/LMM de prognose beïnvloeden. In **HOOFDSTUK 2** hebben we in een retrospectieve studie (2003-2014) LM/LMM (n=75) met niet-LM/LMM (n=270) melanoom subtypes in het hoofd-hals gebied met elkaar vergeleken. Dit cohort bestond uit primaire melanomen die allemaal behandeld zijn met conventionele excisie. Patiënten met 1 of meer ≥ T2 melanomen in de voorgeschiedenis of stadium III/IV ziekte bij aanvang werden geëxcludeerd. Overlevingsuitkomsten en prognostische factoren werden beoordeeld met cumulatieve incidentie en 'competing risk' analyse. Lagere Breslow-dikte bij diagnose, onverwachts invasief melanoom en verhoogd risico op lokaal recidief door onvolledige resectie (18,7% vs. 2,3%) waren significant geassocieerd met het LM/LMM subtype. Er was geen significant verschil in richtlijn-adherentie met betrekking tot chirurgische marges. Een significant deel van de LM/LMM patiënten (24% vs. 5%) had positieve histologische marges. De schildwachtklierprocedure was minder vaak positief in het geval van LMM (12% vs. 20%). Lentigo maligna melanoom was geen onafhankelijke prognostische factor voor ziektevrije of melanoom-specifieke overleving. Het afwijken van de richtlijn-geadviseerde chirurgische marges beïnvloedde de overlevingsuitkomsten niet.

Tussen december 2015 en juli 2017 voerden we een pilotstudie uit met handheld reflectie confocale microscopie (HH-RCM) om de histologische uitkomsten van de resectiemarges te verbeteren en het risico op lokaal recidief te verminderen (**HOOFDSTUK 3**). Vierentwintig patiënten, 18 LM en 6 LMM (stadium ≤T2a), werden behandeld met HH-RCM-geleide conventionele excisie. In 96% (n=23) van de patiënten was er sprake van radicaliteit. De 'mapping' procedure had een sensitiviteit van 90% en een specificiteit van 86%. Er was één patiënt met een lokaal recidief.

Na afronding van de pilotstudie werd de HH-RCM geïntroduceerd als standaardzorg in het Antoni van Leeuwenhoek ziekenhuis (AVL). In een vervolgstudie (**HOOFDSTUK 4**) was het hoofddoel om de effectiviteit van HH-RCM-geassisteerde conventionele excisie te bevestigen in een grotere populatie. Daarnaast wilden we de nauwkeurigheid van HH-RCM evalueren bij het detecteren van subklinische invasie en het effect van de 'mapping' procedure op het beleid. Tussen 2015 en 2023 werden 117 opeenvolgende patiënten met LM/LMM (n=117) geïncludeerd. De chirurgische uitkomsten werden vergeleken met die van een historisch cohort (2003-2014) van voor de introductie van HH-RCM in het AVL. De mediane duur van de 'mapping' procedure was 14 minuten. In 60% van de gevallen detecteerde HH-RCM subklinische LM buiten de door de richtlijn aanbevolen marge. Van de 16 LMM die bij de initiële diagnose waren gemist, werd 75% geïdentificeerd tijdens de 'mapping' procedure, met een sensitiviteit en specificiteit van respectievelijk 80% en 88%. De negatief voorspellende waarde voor de detectie van LMM was 94%. Als gevolg van de de uitkomst van de mapping procedure werd het beleid in 27% (n=32) gewijzigd. In deze gevallen bestond de behandeling van keuze voornamelijk uit topicale imiquimod monotherapie (n=14) of beperkte chirurgie gevolgd door adjuvante imiquimod behandeling (n=15). De overige patiënten (n=84) werden behandeld met HH-RCM-ondersteunde coventionele excisie. Radicaliteit werd bereikt in 97% van de patiënten met een mediane histologische marge van 3,0 mm, wat significant hoger was dan de 81% in het historische cohort (mediaan 2,0 mm). De diagnostische accuratesse van de 'mapping' procedure in de pilotstudie werd gehandhaafd, met 94% sensitiviteit en 84% specificiteit. Het recidiefpercentage van HH-RCM-geleide excisie was beperkt tot 1,5% vergeleken met 25% in het historische cohort.

Vanwege het verhoogde risico op lokaal recidief van LM/LMM worden verschillende micrografisch gecontroleerde chirurgische technieken toegepast om dit risico te verminderen. De langetermijneffecten van de verschillende technieken op overlevingsuitkomsten blijven echter onduidelijk. In **HOOFDSTUK 5** voerden we een systematische review uit om het effect te evalueren van de verschillende chirurgische technieken die worden gebruikt bij de behandeling van LM/LMM op lokaal recidief en overlevingsuitkomsten, evenals het effect van RCM op histologische resectiemarges en recidiefpercentages. Eénenveertig studies met 5059 LM en 1271 LMM werden geïncludeerd. De chirurgische technieken omvatten conventionele excisie (n = 1355), micrografisch gecontroleerde chirurgie met paraffine coupes (n = 2442), en Mohs micrografische chirurgie (MMC) (n = 2909). De door de richtlijn aanbevolen chirurgische marge was onvoldoende in 22%–45% van de LM/LMM gevallen. Het lokale recidiefpercentage was het laagst bij patiënten behandeld met MMC gecombineerd met immunohistochemie (<1%) en het hoogst bij behandeling met conventionele excisie (13%). Handheld RCM verminderde zowel het percentage irradicale excisies als het aantal

rondes bij micrografisch gecontroleerde chirurgische technieken. Vanwege selectiebias, heterogeniteit, lage prevalentie van stadium III/IV ziekte, en beperkte overlevingsgegevens was het niet mogelijk om het effect van verschillende chirurgische technieken op overlevingsuitkomsten te bepalen.

Belangrijkste conclusies - DEEL I

- Hoewel LM/LMM een aanzienlijk verhoogd risico op lokaal recidieven heeft, lijkt het een beperkt effect te hebben op overlevingsuitkomsten.
- LM/LMM heeft een verhoogd risico (10%) op subklinische invasief melanoom bij patiënten gediagnosticeerd met huidbiopten in het diagnostische proces.
- LMM bleek prognostisch niet anders te zijn dan niet-LMM invasief melanoom wanneer gecorrigeerd voor andere variabelen zoals leeftijd en Breslow-dikte. Verminderde chirurgische marges leken geen invloed te hebben op ziektevrije of melanoom-specifieke overleving.
- 'Handheld' reflectie confocale microscopie biedt een nauwkeurige (sensitiviteit 90%) niet-invasieve methode om de uitgebreidheid van de subklinische component van LM/LMM met een beperkt percentage overbehandeling (specificiteit 86%).
- HH-RCM-ondersteunde conventionele excisie resulteert in een hoog percentage radicaliteit en lage lokale recidiefpercentages.
- HH-RCM maakt een meer gepersonaliseerde aanpak mogelijk voor patiënten met LM/LMM door de volledige omvang van de laesie in kaart te brengen en op basis hiervan het verdere beleid te bepalen.
- De hoge negatief voorspellende waarde van HH-RCM bij het detecteren van occult invasief melanoom maakt het mogelijk patiënten te selecteren die geschikter zijn voor niet-chirurgische behandeling, zoals topicaal imiquimod.
- Onze systematische review toonde een duidelijke vermindering van lokale recidieven bij gebruik van microscopisch gecontroleerde chirurgische technieken in vergelijking met conventionele excisie. Het gebruik van HH-RCM vermindert het risico op irradicaliteit en lokale recidieven, zelfs wanneer de techniek gebruikt wordt in combinatie met conventionele excisie.

DEEL II - REGIONALE DIAGNOSTIEK BIJ LENTIGO MALIGNA MELANOOM

Na het opvallend lage percentage positieve schildwachtlymfklieren bij patiënten met LMM in onze retrospectieve studie (**HOOFDSTUK 1**), hebben we een landelijke retrospectieve studie uitgevoerd met behulp van het Pathologisch Anatomisch Landelijk Geautomatiseerd Archief (PALGA) (**HOOFDSTUK 6**) om de uitkomsten van de SLNB-procedure verder te onderzoeken. We identificeerden alle patiënten met LMM tussen 1991 en 2020 alsmede regionale lymfeklier histologie of cytologie. We includeerden alle patiënten met een gerapporteerde schildwachtklierprocedure en alle LMM met een indicatie voor deze ingreep volgens de huidige 8e editie van de 'American Joint Committee on Cancer' (AJCC) (d.w.z. T1b of hoger) (n=1989). Klinisch-pathologische kenmerken werden geëxtraheerd en een gepenaliseerde logistische regressieanalyse werd uitgevoerd om de optimale combinatie van klinisch-pathologische factoren te bepalen voor het voorspellen van een positieve uitslag. Schildwachtlymfeklierbiopsie werd uitgevoerd in 17% (n=333) van de gevallen,

waarvan 7,5% (n = 25) positief waren voor metastasen. Nog eens 25 patiënten hadden klinisch of echografisch detecteerbare regionale lymfekliermetastasen.

De beste voorspellende klinisch-pathologische kenmerken (odds ratio) voor positiviteit waren leeftijd (0,95), ulceratie (1,59), T4-stadium (1,81), mannelijk geslacht (1,97), (lymf-)angioinvasie (5,0) en microsatellieten (7,23). De C-statistiek voor dit model was 0,75, waarmee een goed onderscheid tussen positieve en negatieve uitkomsten gemaakt kan worden ten opzichte van louter toeval. Toch bleef het aantal patiënten dat behandeld moest worden voor één geval van een positieve schildwachtlymfklier hoog, zelfs voor T4 (number-needed-to-biopsy 23,6) gestadieerd LMM of de aanwezigheid van ulceratie (number-needed-to-biopsy 32,0). Leeftijd was omgekeerd geassocieerd met positieve uitslag. Tijdens follow-up werden regionale lymfklier recidieven gedetecteerd in 4,2% van de gevallen, wat resulteerde in een fout-negatief percentage van 22% voor de procedure. Op basis van deze bevindingen werd een nomogram ontwikkeld om het risico op een positief schildwachtlymfklierbiopt te voorspellen.

Belangrijkste conclusies - DEEL II

- Op basis van landelijke gegevens werd bevestigd dat de opbrengst van een schildwachtlymfklierbiopsie (SLNB) bij patiënten met LMM beperkt is wanneer de huidige richtlijnen voor het uitvoeren van schildwachtklierprocedure worden toegepast.
- Er werd een nomogram ontwikkeld op basis van de klinisch-pathologische kenmerken die schildwachtlymfeklier positiviteit het best voorspelden (leeftijd, ulceratie, T4-stadium, (lymf-) angioinvasie en microsatellieten).

DEEL III - DIAGNOSTIEK VAN HET BASAALCELCARCINOOM

Ons focus lag in **HOOFDSTUK 7** op de diagnose van het meest voorkomende type huidkanker in het hoofd-halsgebied: het basaalcelcarcinoom (BCC). De keuze van het beleid in de behandeling van BCC is sterk afhankelijk van de uitkomsten van het diagnostisch biopt. De reden hiervoor is tweeledig: i) de klinische differentiaaldiagnose bevat verschillende benigne laesies, en ii) het BCC-subtype bepaalt de (chirurgische) behandeling. In deze prospectieve studie wilden we de diagnostische nauwkeurigheid bepalen van de gecombineerde dermatoscopie-HH-RCM (DS-HH-RCM) diagnostiek naar BCC-diagnose en subtypering. Het secundaire doel was om de uitkomst van het gebruik van DS-HH-RCM te bepalen bij toepassing van verschillende internationale criteria voor Mohs micrografische chirurgie (MMC) op ons studiecohort.

We voerden een volledig gepaarde diagnostische vergelijkingsstudie uit van 340 opeenvolgende laesies met een klinische verdenking op BCC op basis van het blote oog. De diagnostische nauwkeurigheid van DS-HH-RCM werd geëvalueerd door de onderzoeker

en geblindeerde expertbeoordelingen van de afzonderlijke de dermatoscopische en RCM-beelden. Diagnostisch vertrouwen werd geregistreerd met behulp van een 3-punts Likertschaal (laag, gemiddeld en hoog) voor alle diagnostische stappen. Alle histologisch bevestigde BCCs werden chirurgisch behandeld. De histologische diagnose was gebaseerd op de consensus tussen geblindeerde expertpathologen. Het meest agressieve BCC-subtype in ofwel het biopt of het excisiepreparaat werd beschouwd als de referentiestandaard.

Twee of meer histologische subtypen (gemengd-type BCC) werden gevonden in 59% van de BCCs. Het meest agressieve subtype van het diagnostisch biopt kwam in 61% (n=173) overeen met de uiteindelijke excisiepreparaten. Er was in 33% (n=95) sprake van discrepanties. De sensitiviteit van BCC-diagnosen was consistent hoog (>97%) voor de drie vormen van diagnostiek. Dermatoscopie gecombineerd met HH-RCM had de hoogste specificiteit voor alle uitkomsten en het hoogste aandeel gevallen met hoog diagnostisch vertrouwen. Er was sprake van een sensitiviteit van 97% en specificiteit van 85%, die verder toenamen tot respectievelijk 99% en 94% in de gevallen met hoog diagnostisch vertrouwen. De sensitiviteit/specificiteit voor het detecteren van niet-superficiële BCCs (d.w.z. nodulair en infiltratief) en infiltratief BCC waren respectievelijk 95% / 45% en 59% / 80%. Bij toepassing van verschillende internationale MMC-criteria op ons studiecohort, op alleen de gevallen met hoog diagnostisch vertrouwen (n=281), zou hebben geresulteerd in de excisie van 0-2 benigne laesies.

Belangrijkste conclusies - DEEL III

- BCC-diagnose: De combinatie van dermatoscopie met handheld reflectantie confocale microscopie (DS-HH-RCM) verminderde het aantal onnodige diagnostische biopsieën aanzienlijk in vergelijking met alleen dermatoscopische beoordeling (specificiteit: 85% vs. 56%).
- BCC-diagnose: De specificiteit van DS-HH-RCM neemt verder toe wanneer alleen gevallen met hoog diagnostisch vertrouwen worden meegenomen (94%) terwijl de uitstekende sensitiviteit (99%) behouden blijft.
- BCC-subtypering: Hoewel DS-HH-RCM de hoogste sensitiviteit en specificiteit toonde voor het detecteren van niet-oppervlakkig BCC (oppervlakkig vs. nodulair/infiltratief) en infiltratief BCC (oppervlakkig/nodulair vs. infiltrerend), was deze uitkomst niet betrouwbaar genoeg het om diagnostisch biopt in alle klinische scenario's te vervangen.
- Bij het overwegen van Mohs micrografische chirurgie zou DS-HH-RCM potentieel diagnostische biopsieën kunnen vervangen wanneer er sprake is van een hoog diagnostisch vertrouwen.

A

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A cohort analysis of surgically treated primary head-and-neck lentigo maligna (melanoma): prognostic value of melanoma subtype and new insights in the clinical value of guideline adherence.

- Conceptualization and design: AB, MC, YE, MK, PL, WO, MR, BZ
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Handheld reflectance confocal microscopy: personalized and accurate presurgical delineation of lentigo maligna (melanoma)

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Lentigo maligna (melanoma): a systematic review and meta-analysis on surgical techniques and presurgical mapping by reflectance confocal microscopy.

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The limited value of sentinel lymph node biopsy in lentigo maligna melanoma: a nomogram based on the results of 29 years of the nationwide Dutch Pathology Registry (PALGA)

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Noninvasive diagnosis and subtyping of head-and-neck basal cell carcinoma using dermatoscopy and handheld reflectance confocal microscopy: A prospective fully paired direct comparison study.

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LIST OF PUBLICATIONS

Publications related to this thesis

[1] **Elshot YS**, Ouwerkerk W, Zupan-Kajcovski B, Bol M, de Cuba EMV, Jaspars LH, Briatico G, Argenziano G, Kukutsch NA, Gonzalez S, Jain M, Crijns MB, Balm AJM, Klop WMC, Bekkenk MW, de Rie MA. Noninvasive diagnosis and subtyping of head and neck basal cell carcinoma using dermatoscopy and handheld reflectance confocal microscopy: A prospective fully paired direct comparison study. (**SUBMITTED**)

[2] **Elshot YS**, Lasso Peña DJP, Zupan-Kajcovski B, Bekkenk MW, Balm AJM, Klop WMC, de Rie MA. Successful implementation of handheld reflectance confocal microscopy as the standard of care in the (surgical) management of lentigo maligna (melanoma). J Eur Acad Dermatol Venereol. 2024 Jun 26. Epub ahead of print.

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[6] **Elshot YS**, Zupan-Kajcovski B, Ouwerkerk W, Klop WMC, Lohuis PJFM, Bol M, Crijns MB, Bekkenk MW, de Rie MA, Balm AJM. A cohort analysis of surgically treated primary head and neck lentigo maligna (melanoma): Prognostic value of melanoma subtype and new insights in the clinical value of guideline adherence. Eur J Surg Oncol. 2023 Apr;49(4):818-824.

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[7] Van der Waa JD, **Elshot YS**. Anti-PD1 inhibitor-induced oral lichen planus. 2024 (**SUBMITTED**)

[8] Ortiz-Brugués A, Fattore D, Boileau M, Forsea A, Apalla Z, Nikolaou V, Stojkovic-Filipovic J, Freites-Martinez A, Kaminska-Winciorek G, **Elshot Y**, Baltas E, Torre A, Riganti J, Anadkat M, Bang A, Fida M, Richert B, Kraehenbuehl L, Avitan E, Preto-Gomes N, Hassel J, Doolan B, Kluger N, Pagès C, Guillon B, Lacroix N, Lacouture M, Sibaud V. International survey on training of residents in dermatology in supportive oncodermatology: the rescue study. 2024 (**SUBMITTED**)

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PhD PORTFOLIO

Name PhD student:	Yannick Stephen Elshot
PhD period:	Augustus 2014 - November 2024
Supervisors	prof. dr. M.W. Bekkenk prof. dr. M.A. de Rie
Co-supervisors	prof. dr. A.J.M. Balm dr. M.B. Crijns

1. PhD training

General courses	Year	ECTS
▪ Electronic Basic Course Legislation and Organization for Clinical Researchers (eBROK)	2024	1.5
▪ NVDV GRADE course	2022	0.1
▪ NVDV GRADE course	2020	0.1
▪ Re-registration Basic Course Legislation and Organization for Clinical Researchers (BROK)	2019	0.5
▪ Medical Literature: Citation Analysis and Impact Factors	2016	0.1
▪ Computing in R	2016	0.4
▪ Scientific Writing in English for Publication	2016	1.5
▪ Medical Literature: Endnote	2016	0.1
▪ Project Management	2016	0.6
▪ Basic Course Legislation and Organization for Clinical Researchers (BROK)	2015	0.9
▪ Practical Biostatistics	2015	1.1
▪ Oral Presentation (English)	2015	0.8
▪ Clinical Data Management	2015	0.2
▪ Medical Literature: Zoeken voor een CAT	2015	0.1
▪ Medical Literature: Embase/Medline via Ovid	2015	0.1
▪ Clinical Epidemiology: Randomized Clinical Trials	2014	0.9
▪ Clinical Epidemiology: Systematic Reviews	2014	0.7
▪ Medical Literature: Searching for a Systematic Review	2014	0.1
▪ Expert Management of Medical Literature	2014	0.3
	Total	**10.1**

Specific courses	Year	ECTS
▪ "Advanced Expert Training In Vivo Reflectance Confocal Microscopy" University of Modena and Reggio Emilia - Modena, Italy	2017	1.0
▪ "1st International Course Ex Vivo Confocal Microscopy " Hospital Clinic of Barcelona - Barcelona, Spain	2015	1.0
▪ "Basic Expert Training In Vivo Reflectance Confocal Microscopy" University of Modena and Reggio Emilia - Modena, Italy	2014	1.0
	Total	**3.0**

Presentations	Year	ECTS
▪ V&VN Symposium Immuuntherapie, Utrecht (The Netherlands) Oral presentation: "Immuungemedieerde toxiciteit: Dermatologie"	2024	0.1
▪ 33rd European Association of Dermatology & Venereology (EADV) Congress Oral presentation: "Classification of cutaneous melanoma"	2024	0.5
▪ Immunotherapie gerelateerde bijwerkingen symposium (VUmc), Amsterdam (The Netherlands) Oral presentation: "Immunotherapie gerelateerde bijwerkingen op dermatologie gebied - More than meets the eye"	2024	0.5
▪ Dermatopathology Update 2024, Dutch Society of Dermatopathology, Groningen (The Netherlands) Oral presentation: "Cutaneous adverse events of novel cancer therapies. Clinical presentation and histology"	2024	0.5
▪ 20th European Association of Dermato Oncology (EADO) Congress, Paris (France) Oral presentation: "Free communication 1: Successful implementation of handheld reflectance confocal microscopy as the standard of care in the (surgical) management of lentigo maligna (melanoma)"	2024	0.5
▪ Refereeravond Dermatologie regio Utrecht (St Antonius Ziekenhuis), Bunnik (The Netherlands) Oral presentation: "Dermatologische bijwerkingen tijdens systemische antikankerbehandelingen"	2024	0.5
▪ Boerhaave nascholing: De cursus over huidkanker 2023 (LUMC), Leiden (The Netherlands) Oral presentation: "Dermatoscopie: Basisprincipes en toepassing in de diagnostiek in de dagelijkse praktijk."	2023	0.5

▪ Refereeravond UMCG, Groningen (The Netherlands) Oral presentation: “Bulleuze dermatosen tijdens immuun- en doelgerichte therapie”	2023	0.5
▪ EADV in Oranje, Berlin (Germany) Oral presentation: “Lentigo maligna”	2023	0.5
▪ 32nd European Association of Dermatology & Venereology (EADV) Congress, Berlin (Germany) Oral presentation: “(Neo)adjuvant therapies in melanoma. 2022-2023 UPDATE”	2023	0.5
▪ European Association of Dermatology & Venereology (EADV) Congress, Berlin (Germany) Oral presentation: “Free communications: the limited value of sentinel lymph node biopsy in lentigo maligna melanoma: results of 29 years of the nationwide Dutch Pathology Registry (PALGA)”	2023	0.5
▪ Brugge Dagen, Den Bosch (The Netherlands) Oral presentation: “Het veranderend landschap van het melanoom. Van Diagnostiek tot behandeling”	2023	0.5
▪ The 15th International Netherlands Cancer Institute Head and Neck Symposium: Diagnosis and treatment of head and neck cancer (NKI-AVL), Amsterdam (The Netherlands) Oral presentation: “The value and indication of reflectance confocal microscopy for head and neck skin cancer”	2023	0.5
▪ AVL Huisartsensymposium, Amsterdam (The Netherlands) Oral presentation: “Dermatoscopie van het Melanoom”	2022	0.5
▪ Dutch Working Group on Immunotherapy of Oncology (WIN-O) conference, Ede (The Netherlands) Oral presentation: “Lentigo maligna (melanoma): the role of reflectance confocal microscopy”	2022	0.5
▪ Nascholing Dermato-oncologie Stichting Nederlandstalige Nascholing voor Dermatologie en Venereologie (SNNDV), Brugge (Belgium) Oral presentation: “Dermatologische huidtoxiciteit”	2022	0.5
▪ Dutch Dermatology Days (‘Dermatologendagen’) by the Dutch Society of Dermatology & Venereology (NVDV), Amsterdam (The Netherlands) Oral presentation: “Dermatologic Side effects of immunotherapy.”	2022	0.5
▪ Dutch Working Group on Immunotherapy of Oncology (WIN-O) conference, Ede (the Netherlands) Oral presentation: “Rash: more than meets the eye? Toxiciteit van immunotherapie: behandeling door orgaan- of ziektespecialist?”	2021	0.5

▪ 10th World Congress of Melanoma / 17th European Association of Dermato Oncology (EADO) Congress, Rome (Italy) Poster presentation: "Poster-045: Surgical outcome of head and neck lentigo maligna (melanoma): time for a new guideline proposal?"	2021	0.5
▪ Symposium Melanoom. Van theorie naar praktijk in 2020 (Virtual meeting) Oral presentation: "Lentigo maligna (melanoom): in vivo margebepaling met handheld reflectie confocale microscopie"	2020	0.5
▪ Dermatopathology Update 2019, Dutch Society of Dermatopathology, Maastricht (The Netherlands) Oral presentation: "Dermatologic adverse events during immune checkpoint inhibition"	2019	0.5
▪ JEUK! Bij de oudere patiënt Oral presentation: "Jeuk als bijwerking van doelgerichte en immunotherapie"	2019	0.5
▪ Dutch Dermatology Days ('Dermatologendagen') by the Dutch Society of Dermatology & Venereology (NVDV, Utrecht (The Netherlands) Oral presentation: "Mucocutane bijwerkingen van doelgerichte en immunotherapie"	2019	0.5
▪ Boerhaave Huisartsen nascholing 'De cursus over huidkanker' (LUMC), Leiden (The Netherlands) Oral presentation: "Dermatoscopie – Stap 2"	2019	0.5
▪ 20th Annual Scientific Meeting of the Dutch Society of Experimental Dermatology (NVED), Lunteren (The Netherlands) Poster presentation: "Diagnostic accuracy of handheld reflectance confocal microscopy in the presurgical mapping of lentigo maligna (melanoma): a retrospective pilot study"	2019	0.5
▪ World Congress of Confocal Microscopy, Rome (Italy) Oral presentation: "Diagnostic accuracy of handheld reflectance confocal microscopy in the presurgical mapping of lentigo maligna (melanoma)"	2018	0.5
▪ Therapietrouw bij orale oncolytica: "tijd van starten, tijd van stoppen", Symposium (VUmc), Amsterdam (The Netherlands) Oral presentation: "Huidreacties bij orale oncolytica: huidtoxiciteit of niet?"	2017	0.5
▪ Dutch Working Group on Immunotherapy of Oncology (WIN-O) conference, Ede (The Netherlands) Oral presentation: "Reflectie Confocale Microscopie in de Diagnostiek van het Melanoom"	2016	0.5

▪ Regionaal IKNL Symposium 'Het melanoom actueel' Oral presentation: "Reflectie Confocale Microscopie in de Diagnostiek van het Melanoom"	2016	0.5
▪ 22ste AVL-Symposium 'Oncologie in perspectief – Maatwerk' (NKI-AVL), Amsterdam (The Netherlands) Oral presentation: "Niet-invasieve Diagnostiek in de Dermato-oncologie"	2016	0.5
▪ EADV Review Oral presentation: "Diagnostiek in de Dermatologie".	2015	0.5
▪ The 12th International Netherlands Cancer Institute Head and Neck Symposium – Diagnosis and Treatment of Skin Cancer of the Head and Neck (NKI-AVL), Amsterdam (The Netherlands) Oral presentation: "Lentigo Maligna: The role of reflectance confocal microscopy"	2015	0.5
	Total	**16.0**

(Inter)national conferences	**Year**	**ECTS**
▪ 33rd European Association of Dermatology & Venereology (EADV) Congress, Amsterdam (The Netherlands)	2024	1.0
▪ Scientific Meeting of the International Confocal Group, Amsterdam (The Netherlands)	2024	0.25
▪ European Society for Micrographic Surgery Course, Geneva (Switzerland)	2024	1.0
▪ Dutch Dermatologist Days of the Dutch Society of Dermatology & Venereology, Amsterdam (The Netherlands)	2024	0.5
▪ Dermatopathology Update 2024, Dutch Society of Dermatopathology, Groningen (The Netherlands)	2024	1.0
▪ 20th European Association of Dermato Oncology (EADO) Congress, Paris (France)	2024	1.0
▪ 32nd European Association of Dermatology & Venereology (EADV) Congress, Berlin (Germany)	2023	1.0
▪ Dutch Dermatologist Days of the Dutch Society of Dermatology & Venereology, Ermelo (The Netherlands)	2023	0.5
▪ 3rd World Congress on Confocal Microscopy, Barcelona (Spain)	2023	1.0
▪ EDF Advanced Euroderm Excellence Program, Montreux (Switzerland)	2023	1.0
▪ Dutch Working Group on Immunotherapy of Oncology (WIN-O) conference, Ede (The Netherlands)	2022	0.5
▪ 31st European Association of Dermatology & Venereology (EADV) Congress, Milan (Italy)	2022	1.0

▪ Stichting Nederlandstalige Nascholing voor Dermatologie en Venereologie (SNNDV): Dermato Oncology	2022	1.0
▪ Melanoom behandeling: van palliatief naar gepersonaliseerd (LUMC), Leiden (The Netherlands)	2023	0.25
▪ Annual meeting of the American Academy of Dermatology (AAD), Boston (USA)	2022	1.25
▪ Leidse Melanoomdag (LUMC), Leiden (The Netherlands)	2022	0.5
▪ Dutch Dermatology Days ('Dermatologendagen') by the Dutch Society of Dermatology & Venereology (NVDV), Amsterdam (The Netherlands)	2022	0.5
▪ Dutch Working Group on Immunotherapy of Oncology (WIN-O) conference, Ede (The Netherlands)	2021	0.5
▪ 2nd World Congress on Confocal Microscopy (Virtual meeting)	2021	1.0
▪ 10th World Congress of Melanoma / 17th European Association of Dermato Oncology (EADO) Congress (Virtual meeting)	2021	1.0
▪ Dutch Working Group on Immunotherapy of Oncology (WIN-O) conference, Ede (The Netherlands)	2020	0.5
▪ Symposium Melanoom. Van theorie naar praktijk in 2020 (Virtual meeting)	2020	0.25
▪ Dutch Working Group on Immunotherapy of Oncology (WIN-O) conference, Ede (The Netherlands)	2019	0.5
▪ 20th Annual Scientific Meeting of the Dutch Society of Experimental Dermatology (NVED), Lunteren (The Netherlands)	2019	1.0
▪ Leidse Melanoomdag, Leiden (The Netherlands)	2019	0.5
▪ Dermatopathology Update 2019, Dutch Society of Dermatopathology, Maastricht (The Netherlands)	2019	1.0
▪ Dutch Dermatology Days ('Dermatologendagen') by the Dutch Society of Dermatology & Venereology (NVDV), Utrecht (The Netherlands)	2019	0.5
▪ 20th Annual Scientific Meeting of the Dutch Society for Experimental Dermatology, Lunteren (The Netherlands)	2019	1.0
▪ 6th AbbVie Dermatology Resident Days ('DIO-dagen'), Doorn (The Netherlands)	2018	0.5
▪ 18th EDF Euroderm Excellence Training Program, Rome (Italy)	2018	1.0
▪ 1st Word Congress on Confocal Microscopy, Rome (Italy)	2018	1.0
▪ 5th World Congress of Dermoscopy, Thessaloniki (Greece)	2018	1.0
▪ 4th AbbVie Dermatology Resident Days ('DIO-dagen'), Doorn (The Netherlands)	2016	0.5

▪ Dutch Working Group on Immunotherapy of Oncology (WIN-O) conference, Ede (The Netherlands)	2016	0.5
▪ 1st International Symposium of the International Confocal Working Group, Madrid (Spain)	2016	1.0
▪ Stichting Nederlandstalige Nascholing voor Dermatologie en Venereologie (SNNDV): Infectious diseases	2016	1.0
▪ 25th European Association of Dermatology & Venereology (EADV) Congress, Vienna (Austria)	2016	1.0
▪ 10 jaar Boerhaave Dermatoscopie Nascholing (LUMC), Leiden (The Netherlands)	2015	0.5
▪ 24th European Association of Dermatology & Venereology (EADV) Congress, Kopenhagen (Denmark)	2015	1.0
▪ 4th World Congress of Dermoscopy, Vienna (Austria)	2015	1.0
▪ 12th International Netherlands Cancer Institute Head and Neck Symposium – Diagnosis and Treatment of Skin Cancer of the Head and Neck (NKI-AVL), Amsterdam (The Netherlands)	2015	0.5
▪ 23rd European Association of Dermatology & Venereology (EADV) Congress, Amsterdam (The Netherlands)	2014	1.0
▪ Skin Cancer of the Head and Neck: Treatment and Reconstruction of Head and Neck Skin Cancer, Utrecht (The Netherlands)	2014	0.5
	Total	**33**

Miscellaneous	**Year**	**ECTS**
Chairing sessions: ▪ Melanoma session (Updates) - European Association of Dermatology & Venereology EADV Congress 2024 - Co-chair: Dr. J. Malvehy	2024	0.1
Book chapters: ▪ Elshot YS, Blok SG, Bekkenk MW, Matos TR. Chapter 16: Dermatologic autoimmunity associated with immune checkpoint inhibitors. In Translational Autoimmunity: Challenges for Autoimmune Diseases: Volume 5. Vol. 5. Elsevier. 2022. p. 311-327. (Translational Autoimmunity: Challenges for Autoimmune Diseases: Volume 5).	2022	1.5
	Total	**1.6**

2. Teaching

Lecturing	Year	ECTS
▪ Teacher national dermatology year resident training (COCOM) "Introduction in dermoscopy" (Basiscursus Systematische Diagnostiek)	2019-current	0.5
▪ Organizer & Teacher regional resident dermoscopy training Amsterdam University Medical Center	2018-current	1.4
▪ Oncologisch spectrum IKNL: Non-melanoma huidkanker	2021-2023	0.6
	Total	**2.5**

Supervising	Year	ECTS
Supervising PhD:		
▪ N. Shifai. Topic: Early diagnostics of cutaneous melanoma	2024-current	1.0
▪ T.V.M. Bruijn. Topic: Dermatologic toxicity during immune checkpoint inhibition.	2022-current	5.0
Supervising extra scientific internship:		
▪ T.V.M. Bruijn "The value of the sentinel procedure in lentigo maligna melanoma: results of 29 years of the nationwide Dutch pathology registry (PALGA)"	2021	1.0
Supervising master thesis:		
▪ T.H. Boere. "Sequential digital dermatoscopy: the Netherlands Cancer Institute Experience"	2019	1.0
▪ A. Hof "Dermatologic adverse events in treatment with immune checkpoint inhibitors in stage IV melanoma"	2019 2017	1.0 1.0
▪ W.F. ten Bolscher. "Cost-Effectiveness of the addition of reflectance confocal microscopy in the diagnostic pathway of skin cancer"		
	Total	**10.0**

Miscellaneous	Year	ECTS
▪ Organizer monthly dermatopathology dermatologic toxicity NKI-AVL Amsterdam UMC meeting	2023-current	1.0
▪ Organizer AbbVie "Dermatology Resident Days (DIO-dagen)"	2019-2022	1.5
	Total	**2.5**

3. Parameters of Esteem		
▪ Invited peer review: International Journal of Dermatology	2025	0.5
▪ Reviewing project proposal: Hanarth Foundation	2024	0.5
▪ Selected (Top 10) for the Advanced Euroderm Excellence Course, Montreux (Switzerland)	2023	N.A.
▪ Invited peer review: Australasian Journal of Dermatology (x2)	2024	1.0
▪ Invited peer review: British Journal of Dermatology	2024	0.5
▪ 1st place – 18th edition Euroderm Excellence Training Program, Rome (Italy)	2021	N.A.
▪ Reviewing project proposal: Hanarth Foundation	2022	0.5

Memberships	
▪ Dutch Association of Supportive Care in Cancer (Dutch affiliate MASCC)	2023-current
▪ Taskforce member ”Dermatology for Cancer Patients” – European Association of Dermatology & Venereology	2022-current
▪ Dermato-oncology working group of the Dutch Society of Dermatology & Venereology (NVDV)	2021-current
▪ European Association of Dermato Oncology	2021-current
▪ European Academy of Dermatology and Venerology	2021-current
▪ Study group member “Oncodermatology of the Multinational Association of Supportive Care in Cancer (MASCC)”	2021-current

A

DANKWOORD | ACKNOWLEDGEMENTS

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Anoek, **Tessa**, **Peter Top, Crazy Thomas, Friso** en **PD.** De original horeca tijgers. Door jullie heb ik soms ook het gevoel gehad dat ik in de Horeca werkte, alleen dan zittend aan de bar. **Anoek**. Een zeer grappige persoon met de meest uitgebreide dancemoves. Je hebt een fantastische man aan de haak geslagen die mij gelukkig geleerd heeft wat lineaire tv-kijken is. Dank daarvoor. **PD** ik zal je eeuwig dankbaar zijn voor je New England IPA. Hopelijk lukt het Margaux en mij om ook een keer samen op jullie Winter BBQ te komen! **Tessa,** my sister from another mother. Ondertussen voel ik me soms een soort broertje van, want je kan me zeer duidelijk toespreken als ik weer irritant ben. Je kan ontzettend hard lachen, meestal het hardst om jezelf. Dankzij jou is Snorri Sturluson in mijn leven. **Paul**, mijn tweede meest favoriete Pool: nee ik kom niet in Oostzaan wonen. Ondanks dat **Florien** daar ook woont! **Peter "Nog ééntje dan" Top**. Een van de grappigste mensen die ik ken en een man met vele kwaliteiten. Kunsthistoricus, pupquiz master en de beste biersommelier van Nederland. Living my dream: een huis in Amsterdam Noord. Vele jaren geleden vroeg je of je een vriend mocht meenemen naar mijn verjaardag. Ik zei ja en de rest is geschiedenis, daar was Ray. **Ray**, wat ben je een speciale persoon die zich helemaal kan verliezen in een specifiek onderwerp. Je bent een fantastische fotograaf en zeer getalenteerde kok. We mogen blij zijn als het voor middennacht op tafel staat, maar de smaken zijn altijd spot on. Mogelijk sta je op mijn promotiefeest foto's te maken, dan graag wel een verzoek: laat het doorsturen niet langer duren dan mijn promotieonderzoek. Ons gastro-intestinale stelsel is het hier niet helemaal mee eens, maar onze reis naar Portland met Peter was toch een van mijn

hoogtepunten. Wat een luxe waren die motels met maar 2 bedden. De bromance, **Sander** en (aangetrouwde) **Rutger**. We weten allemaal wie de betere helft is, maar laat dat de pret niet drukken. Het was een gok jullie mee te vragen naar Maui, maar het was een groot succes. Het scheelde natuurlijk dat we slippers moesten dragen, waardoor we veiliggesteld waren van modekeuzes van Rutger. Altijd lachen, goed eten en nu samen gymrats. Hopelijk zet dit door tot na ons pensioen, exclusief breukletsels. **Shoura** en **JJ**. Ook wij hebben ondertussen heel wat meegemaakt, van Oud en Nieuw vanuit je kraambed, zeer ongemakkelijke dancebattles in meshshirts tot bijna slaags raken met concurrende DJs.

Jan & **Jorijn**, mijn oudste vrienden. The three not-so-cool musketeers. After high school, we never managed to live in the same country simultaneously. As a result, we do not see each other often enough. When we are together though, it is like no days have passed. Friends for life! **Jan**, my knight in shiny armor when I was being robbed. I still clearly recall it was you falling into the dog doody though. **Jorijn,** stoer hoe je het allemaal hebt gedaan en doet. Altijd onderweg en nu ook nog met Benjamin en de honden! **Laurens** de rots in de branding voor Jorijn. Het is ontzettend fijn dat jij in haar leven bent, gezien ze, ondanks al die intelligentie die ze bezit, altijd in de meest vreemde situaties weet terecht te komen. Jullie avontuur in Parijs is bijna voorbij, dan komen jullie gelukkig met de roedel terug naar Amsterdam.

Deborah & **Rob**. I couldn't have hoped for better "schoonouders". From weekly dinners to yearly visits to the paradise called Maui. Thank you for all the support and memorable moments. Maui *Nō Ka ʻOi*!

Lieve **Mamma**, **Thomas** en **Philip**. We wonen allemaal in een ander land en zijn daarom op te weinig momenten samen. Ik ben heel blij dat iedereen gelukkig zijn eigen plekje heeft gevonden. Vaak uit het oog, maar zeker niet uit het hart! Ik hou van jullie.

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ABOUT THE AUTHOR

Born on May 26, 1983, in Amsterdam, the Netherlands, Yannick Elshot, eldest of 2 brothers, spent his formative years in various Dutch cities before moving to the picturesque town of Mol, Belgium, at the age of 9. There, he completed his secondary education, earning a European Baccalaureate from The European School Mol.

Returning to his roots in Amsterdam at 19, Yannick initially mistakenly pursued Biology at the Vrije Universiteit (VUmc) van Amsterdam for two years to ultimately be accepted into Medicine School. After a brief confusion about wanting to become a radiologist, his interest in Dermatology was confirmed during his senior medical student internship at the Dijklander Hospital in Purmerend. In 2014, he received his master's degree (MSc) in Medicine from the VUmc.

His professional career began in 2015 when he started working as a resident dermatologist (not in training) at the Dijklander Hospital. The following year, in 2016, he started working at the Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital (NKI-AVL), where he continued working as a Dermatology resident for a year and a half. From 2017 to 2022, Yannick completed his formal residency in Dermatology at the Department of Dermatology of the Amsterdam UMC.

Parallel to his full-time clinical work, Yannick embarked on his doctoral studies in 2014/2015, pursuing a PhD in Dermatology through a collaboration between Departments of Dermatology of the Amsterdam UMC/NKI-AVL and Head and Neck Oncology and Surgery of the NKI-AVL.

In 2022, he was thrilled to accept the position of dermatologist at both the Departments of Dermatology of the Amsterdam UMC and NKI-AVL. Yannick began his Mohs Micrographic Surgery fellowship in 2023. He started this specialized training at the St Antonius Hospital in Utrecht and, at the beginning of 2024, transitioned to the Dijklander Hospital to complete his fellowship, bringing his journey full circle to where he had started his career as a resident.

In the future, Yannick hopes to continue to combine clinical work and research focused on dermato-oncology and supportive care for solid and hematological cancer patients.

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