Prognostic factors in distinct melanoma types

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CHAPTER 1

General introduction

EPIDEMIOLOGY

Cutaneous melanoma is a malignant tumor of melanocytes, the pigment producing cells residing in the skin. Melanomas also occur in other sites, such as the eye, meninges and mucosa. Over two hundred years ago, the first case of cutaneous melanoma was described.[1,2] In recent decades, the incidence of melanoma has increased across the globe.[3–5] In 2018, 6709 individuals were diagnosed with melanoma in the Netherlands and almost 800 died of the disease.[6]

Individual risk factors for developing melanoma encompass host factors and environmental factors. The most important environmental cause of melanoma is sun exposure. Ultraviolet radiation causes DNA damage. More than 90% of melanomas are attributed to sun exposure.[7–9] Especially intermittent sun exposure, such as sunbathing, is associated with an increased melanoma risk.[10,11] Individuals with large congenital nevi, dysplastic nevi or a high number of melanocytic nevi are at increased risk of developing melanoma.[10,12–15] Other host factors that are associated with an increased melanoma risk are fair skin, red hair, old age, history of skin cancer, and a family history of melanoma.[16–19]

CLINICAL DIAGNOSIS AND DIAGNOSTIC EXCISIONAL BIOPSY

Melanoma most often presents as a new or changing pigmented skin lesion (Figure 1). Several aspects of the lesion are assessed by the dermatologist. Lesions that are different from the other pigmented lesions in the patient, also called ugly duckling sign, should raise suspicion for melanoma.[20] The ABCDE criteria (**A**symmetry, **B**order irregularity, **C**olor variation, **D**iameter > 6mm, **E**volving) are frequently used to evaluate suspicious pigmented lesions with the naked eye.[21] Dermoscopy is essential in the clinical diagnosis of melanoma (Figure 2). It is more accurate than visual inspection alone.[22] The clinical and dermoscopic appearance varies between melanoma subtypes. Superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, and acral lentiginous melanoma are the four main histological subtypes of melanoma.[21] Nodular melanomas are in general more difficult to detect and have more aggressive characteristics.[21,23]

Excisional biopsy with narrow (1-3mm) margins is the recommended initial management for suspicious pigmented skin lesions.[24–26] However, melanomas are frequently diagnosed by partial biopsy, such as punch, shave or incisional biopsy.[27] In Australia, more than 25% of all melanomas is diagnosed by partial biopsy.[28]

Figure 1. Clinical picture of cutaneous melanoma.

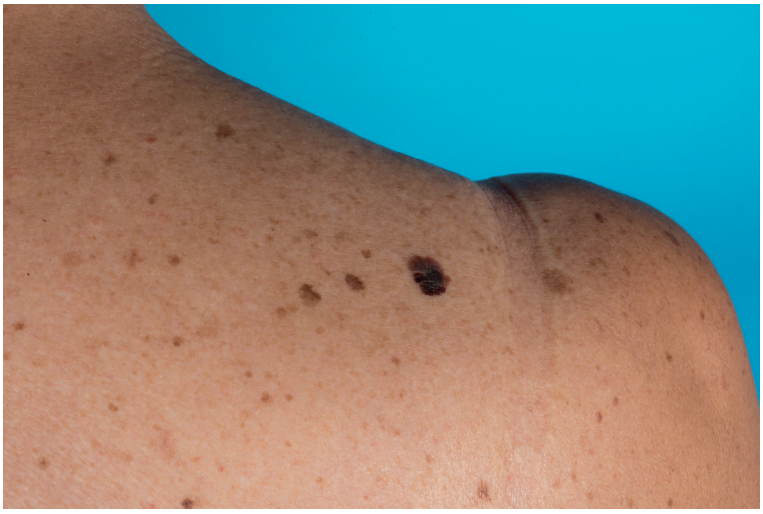
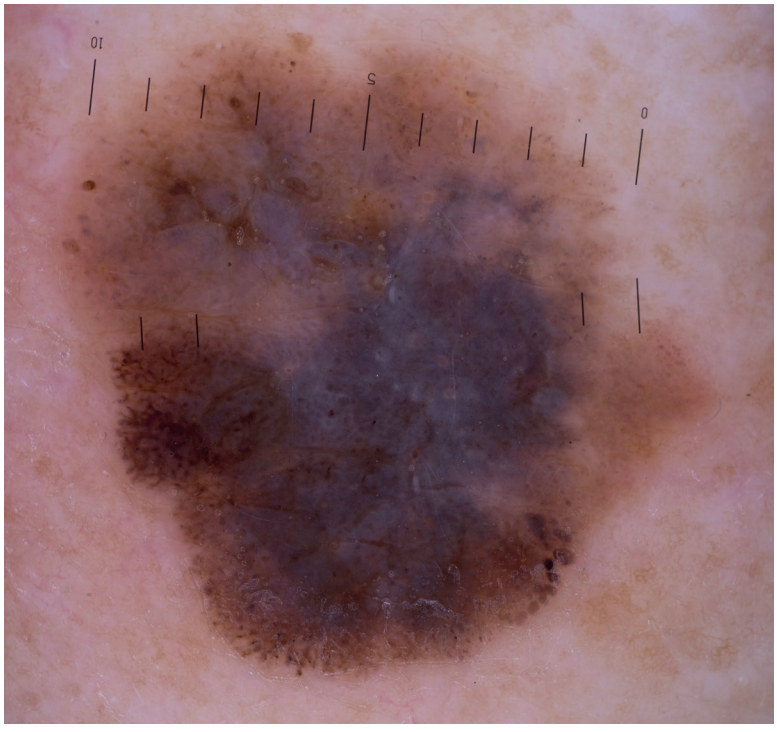


Figure 2. Dermoscopy of cutaneous melanoma.



Histopathological misdiagnosis is more common for melanocytic lesions assessed with partial than with excisional biopsy.[29] Partial biopsies are associated with several pitfalls. Sampling of only the benign part of the lesion might result in misdiagnosis. Partial biopsy of a melanocytic nevus may result in regenerative changes that overlap with the histological features of melanoma. This can lead to overdiagnosis of melanoma. Tumor implantation and inaccurate assessment of important pathological features, such as Breslow thickness, are other potential problems.[28,29]

HISTOPATHOLOGY AND STAGING

Histopathological tumor characteristics are assessed by the (dermato)pathologist on the excisional biopsy specimen. These characteristics are essential in the staging process. All patients are staged using the American Joint Committee on Cancer/Union Internationale Contre le Cancer (AJCC/UICC) melanoma staging classification (Table 1 and 2).[30] Management decisions and prognostic information are derived from this classification.

The thickness of the primary melanoma is an important prognostic feature of a clinically localized melanoma.[31–33] It was first described by Alexander Breslow in 1970 and is therefore also known as Breslow thickness.[34] Tumor thickness is measured from the top of the granular layer of the epidermis to the deepest malignant cells invading the dermis. Initially a cut off thickness of ≤ 0.75mm was used to define thin melanomas with a good prognosis. In the 6th and 7th editions of the AJCC melanoma staging classification, melanomas with a tumor thickness of ≤ 1.0mm were classified as thin.[35,36] A recent study showed that 0.8mm is a clinically important cut-off.[37] This is reflected in the most recent 8th edition of the AJCC/UICC melanoma staging classification.[30]

Ulceration has been part of the melanoma staging system for decades.[30,35,36] Ulcerated tumors have a higher risk of disease recurrence and melanoma-related death.[31,33,38] Although not part of the staging classification, the extent of ulceration is of prognostic significance. Extensively ulcerated melanomas have a worse outcome than minimally ulcerated tumors.[39]

Allen and Spitz were the first to describe the poorer survival of patients having a primary melanoma with many mitoses.[40] Tumor mitotic rate has since been validated as an independent prognostic factor in numerous studies.[41–46] It was incorporated in the 7th edition of the AJCC staging classification but has been removed as a staging parameter in the most recent staging system.[30,36]

Table 1. TNM staging categories.[30]

Tumor (T)	**Tumor thickness**	**Ulceration**
T1a	< 0.8mm	Without ulceration
T1b	< 0.8mm	With ulceration
	0.8 – 1.0mm	With or without ulceration
T2a	>1.0 – 2.0mm	Without ulceration
T2b	>1.0 – 2.0mm	With ulceration
T3a	>2.0 – 4.0mm	Without ulceration
T3b	>2.0 – 4.0mm	With ulceration
T4a	> 4.0mm	Without ulceration
T4b	> 4.0mm	With ulceration
Node (N)	**No. of tumor involved regional lymph nodes**	**Type of metastasis***
N0	0	
N1a	1	Clinically occult
N1b	1	Clinically detected
N1c	0	In-transit, satellite and/or microsatellite metastasis
N2a	2-3	Clinically occult
N2b	2-3	Clinically detected
N2c	1	In-transit, satellite and/or microsatellite metastasis
N3a	≥ 4	Clinically occult
N3b	≥ 4	Clinically detected
N3c	≥ 2	In-transit, satellite and/or microsatellite metastasis
Metastasis (M)	**Site**	
M0	No distant metastasis	
M1a	Skin, soft tissue and/or nonregional lymph node	
M1b	Lung	
M1c	Non-CNS visceral sites	
M1d	CNS	

*Clinically occult lymph node metastases are detected by sentinel node biopsy and without clinical or radiographic evidence of regional lymph node metastasis. Clinically detected nodal metastases are identified by clinical, radiographic or ultrasound examination.

Table 2. AJCC clinical and pathological prognostic stage groups (8th edition).[30]

	Clinical stage				Pathological stage		
	T	**N**	**M**		**T**	**N**	**M**
IA	T1a	N0	M0	IA	T1a	N0	M0
					T1b	N0	M0
IB	T1b	N0	M0	IB	T2a	N0	M0
	T2a	N0	M0				
IIA	T2b	N0	M0	IIA	T2b	N0	M0
	T3a	N0	M0		T3a	N0	M0
IIB	T3b	N0	M0	IIB	T3b	N0	M0
	T4a	N0	M0		T4a	N0	M0
IIC	T4b	N0	M0	IIC	T4b	N0	M0
III	Any T	≥ N1	M0	IIIA	T1-T2a	N1a or N2a	M0
				IIIB	T1-T2a	N1b/c or N2b	M0
					T2b/T3a	N1a-N2b	M0
				IIIC	T1a-T3a	N2c or N3	M0
					T3b/T4a	≥ N1	M0
					T4b	N1a-N2c	M0
				IIID	T4b	N3	M0
IV	Any T	Any N	M1	IV	Any T	Any N	M1

WIDE LOCAL EXCISION AND SENTINEL NODE BIOPSY

If an invasive primary cutaneous melanoma is diagnosed, wide local excision (WLE) of the lesion or biopsy site is indicated to reduce the risk of local recurrence. WLE surgical margins depend on tumor thickness.[24–26] The recommended safety margins are 1cm for melanomas with tumor thickness < 2mm and 2cm for thicker melanomas.[24]

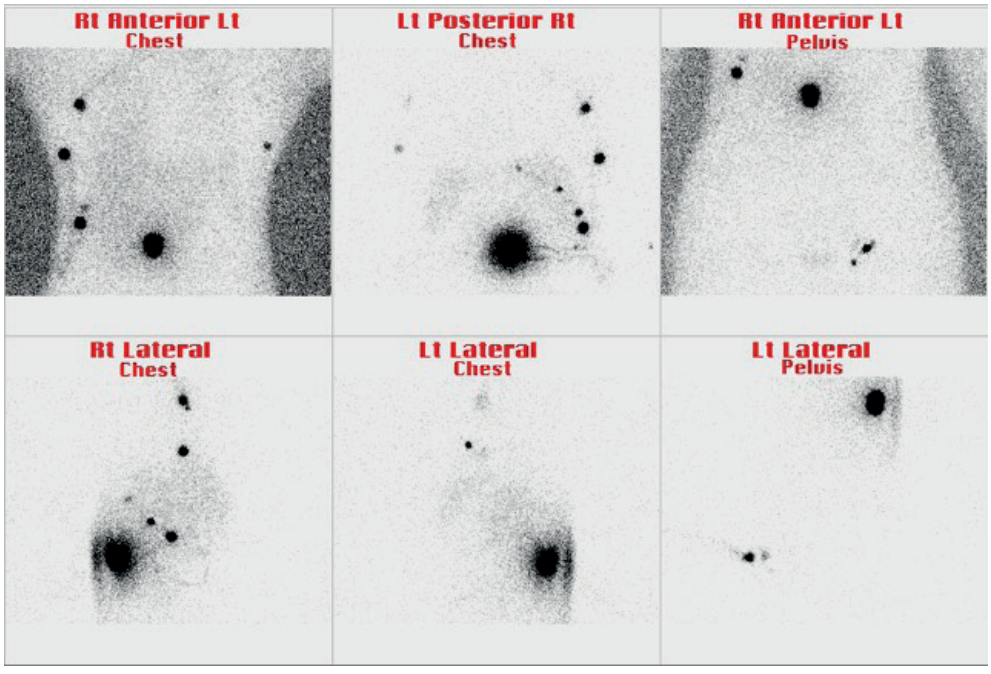
For many years, excision of the primary tumor was often combined with prophylactic regional lymph node dissection.[47] Since only 20% of the clinically localized melanoma patients has involved lymph nodes, many patients could not have any benefit from such a procedure. Prophylactic lymph node dissections were abandoned after studies showed that routine use of this procedure did not improve survival.[48–51]

In 1992, the sentinel node (SN) concept was introduced by Morton and Cochran.[52] A SN is defined as any node on a direct lymphatic drainage pathway from the primary tumor.[53] Multiple drainage pathways and thus multiple SNs can be present in one patient.[54–57] Sentinel node biopsy (SNB) can establish the tumor-status of the entire regional lymph node field.[58,59] Only patients with an involved SN underwent immediate removal of the remaining regional

lymph nodes, the so-called completion lymph-node dissection (CLND). Before the introduction of this procedure in melanoma, the term *sentinel node* was already mentioned in studies of penile cancer, parotid cancer, testis and omentum.[60–64]

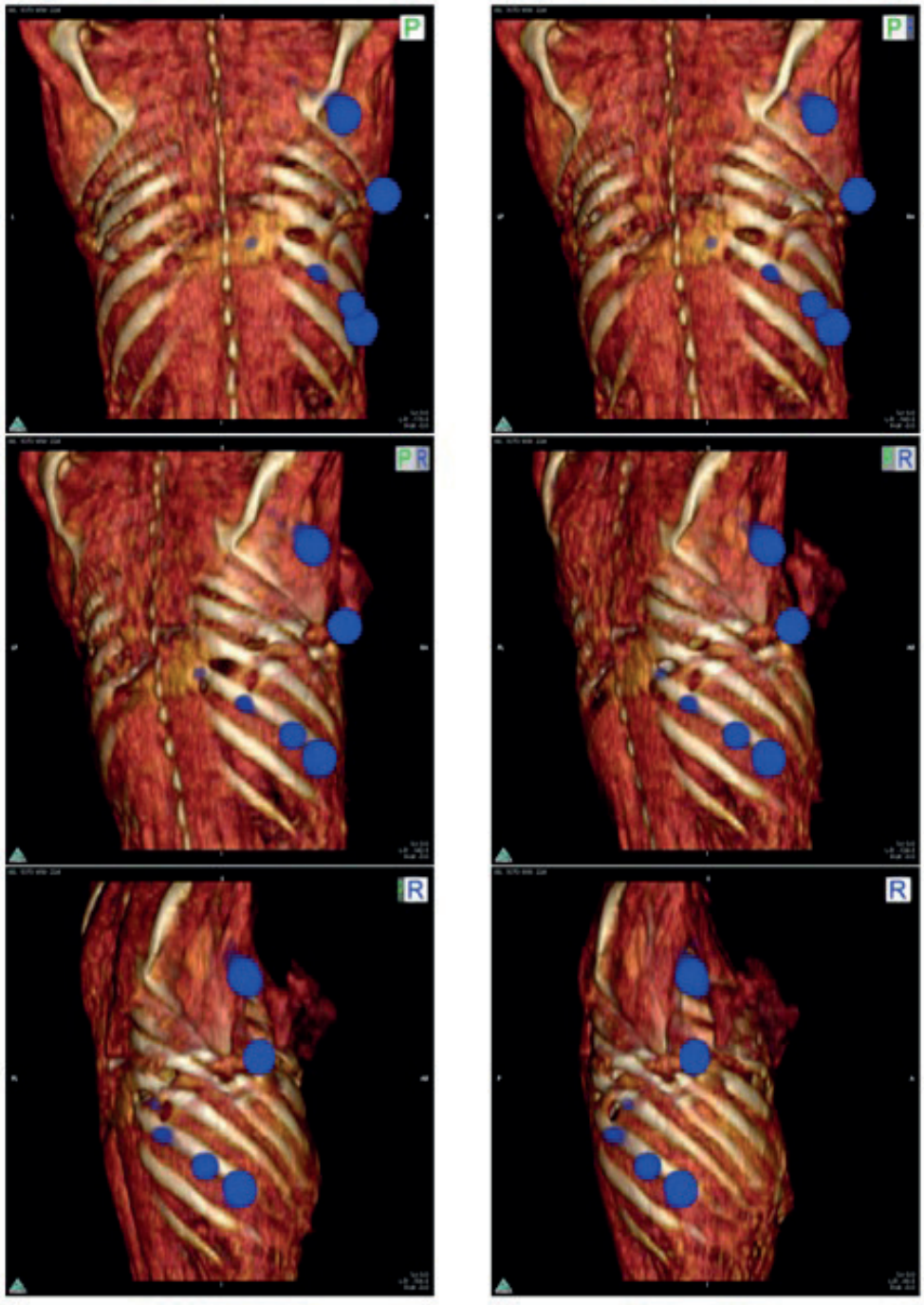
The nuclear medicine physician is of great importance in the identification of these SNs. Technetium-99m colloid is injected at the primary melanoma site. The tracer flows from the primary tumor through the afferent lymph vessel to the lymph nodes. Dynamic and static lymphoscintigraphy visualize the SNs (Figure 3).[65] Single photon emission computed tomography with integrated computerized tomography (SPECT/CT) is added to show the SNs exact anatomical location (Figure 4).[66–69] Non-palpable metastases can be detected by ultrasound (US) after which fine needle biopsy is performed.

Figure 3. Lymphoscintigrams in a patient with a melanoma on the mid back show sentinel nodes in the axillae, on the right chest wall and in the left groin.



The surgeon uses a gamma ray detection probe to locate the SNs. Intra-operatively, patent blue dye is injected intradermally at the primary tumor site. The blue travels the same route as the radiopharmaceutical. The blue-stained afferent lymph vessel can also guide the surgeon to the SNs. Only these lymph nodes are removed and assessed for the presence or absence of metastases.

Figure 4. Single photon emission computed tomography with integrated computerized tomography (SPECT/CT) displays sentinel nodes of melanoma located on the chest.



Histopathological examination is performed on multiple sections stained with hematoxylin and eosin and immunohistochemical markers, such as S100, HMB45, MelanA and SOX10.[70,71]

The SN status is the most important prognostic factor in patients with a clinically localized melanoma.[58,72–75] Patients with a positive SN have a worse prognosis than SN-negative patients. The first Multicenter Selective Lymphadenectomy Trial (MSLT-I) proved the importance of this staging procedure and showed that patients who underwent SNB had fewer recurrences than patients who underwent WLE and nodal observation.[58] SNB combined with CLND also improved melanoma-specific survival (MSS) of patients with an intermediate-thickness melanoma (1.2 – 3.5mm) who had occult nodal metastases.[58] SN-positivity is rare (<5%) in melanomas < 0.8mm in thickness. Melanomas with a tumor thickness of 0.8 – 1.0 mm have a 8 to 12% change of having spread to a SN.[76–79] Therefore, SNB is recommended for patients with a clinically localized melanoma that has a thickness ≥0.8mm or if ulceration is present (T1b or higher).[24,25] SNB seems reliable when performed after WLE, but concomitant WLE and SNB is preferred.[80]

SPECIAL POPULATIONS

Elderly

Elderly people have the highest melanoma incidence and mortality.[81–83] Between 1989 and 2015, the incidence in Dutch men aged ≥ 70 years has increased with more than 500%.[84] Compared to younger patients, primary melanomas of older patients are on average thicker, more often ulcerated and have more dermal mitoses.[38,85,86] Nodular melanomas are also more frequent.[86,87] While their melanomas are more aggressive, the SN-positivity rate is lower in these patients.[38,88,89] Age-related lymphatic dysfunction might be an explanation for this inverse correlation.[90]

Melanoma guidelines are also applicable to elderly patients.[24,25] However, studies show substandard surgical treatment in this group of patients.[87,91] Incisional biopsies and suboptimal excision margins are common.[86,87,91] SNB is less frequently performed in older patients with clinically localized melanoma.[86,91,92] Clinical decision-making in the elderly is complicated by several factors, of which frailty, medical comorbidities and reduced life-expectancy are examples.

Children and adolescents

Pediatric melanoma is arbitrarily defined as melanoma diagnosed below the age of 20 years.[21] It is the most common type of skin cancer in children and adolescents.[93] Pediatric melanoma is frequently associated with pre-existing conditions such as large congenital melanocytic nevi and xeroderma pigmentosum.[94] While within most age groups melanoma incidence has increased, a declining incidence of pediatric melanoma is observed.[3,95–97]

Only 0.1% of the melanoma cases occur in children and adolescents. Due to the rarity, melanoma is often not considered in this age group.[93] The clinical features are also frequently atypical and do not follow the conventional ABCDE criteria.[98,99] Modified ABCD criteria (**A**melanotic, **B**leeding or **B**ump, **C**olor uniformity, **D**e novo and any **D**iameter) have therefore been proposed.[99] Children and adolescents have been excluded from randomized controlled trials studying different aspects of melanoma management.[33,58,100] Currently, adult melanoma guidelines are applied to pediatric melanoma patients. SNB is also performed in pediatric melanoma patients. Paradoxically, pediatric patients have a higher incidence of SN-metastasis but a more favorable survival rate than adults.[38,101,102]

Familial melanoma

Approximately 10% of patients diagnosed with melanoma have a positive family history.[103,104] Genes implicated in familial melanoma include cyclin-dependent kinase inhibitor 2A (*CDKN2A*), cyclin-dependent kinase inhibitor 4 (*CDK4*), BRCA1-associated protein-1 (*BAP1*), protection of telomeres 1 (*POT1*), telomerase reverse transcriptase (*TERT*), ACD shelterin complex subunit and telomerase recruitment factor (*ACD*), telomeric repeat-binding factor 2-interacting protein (*TERF2IP)* and microphthalmia-associated transcription factor (*MITF*).[104–106] Genetic testing is recommended for patients who meet the criteria for familial melanoma, which are defined as the occurrence of three or more melanomas in multiple members of a family, at least two of which are diagnosed in first-degree relatives.[107] Clinical genetic consultation is also advised when two-first degree relatives are diagnosed with melanoma, families in which melanoma and pancreatic cancer are diagnosed, patients with three or more melanomas, patients with melanoma diagnosed before the age of 18 years, patients with multiple BAP1-deficient melanocytic nevi and patients with a combination of melanoma and pancreatic cancer or uveal melanoma.[107] Patients with hereditary breast and ovarian cancer syndrome (*BRCA1* and *BRCA2*), Li-Fraumeni syndrome (*TP53*), xeroderma pigmentosum, and PTEN hamartoma tumor syndromes (*PTEN)* are also at increased risk of developing melanoma.[105] Germline mutations in *CDKN2A* are found in about 20-40% of

melanoma families.[108,109] In the Netherlands, the most prevalent *CDKN2A* germline mutation is the p16-Leiden mutation (c.225-243del19). This specific founder mutation probably originated from an endogamous population.[110,111] The high penetrance gene *CDKN2A* encodes two different tumor suppressor proteins: p16INK4A (p16) and p14ARF (p14). These patients have a life-time melanoma risk of about 70% and frequently at a young age.[104,105,112] *CDKN2A* mutation carriers also have an increased risk of developing pancreatic cancer, head and neck tumors, and lung cancer.[113] Recent studies on survival of *CDKN2A* germline mutation carriers with melanoma showed conflicting results.[114–116] In a Swedish cohort, these melanoma patients had a worse survival than sporadic melanoma patients.[114,115] However, an Italian group found no survival difference.[116]

AREAS OF UNCERTAINTY

Clinically localized melanoma has been extensively studied. However, several clinical questions are still unanswered.

SNB has become a routine staging procedure in patients with clinically localized melanoma. However, SNB may be less attractive in some categories. SNB is sometimes omitted in patients with advanced age, substantial comorbidities or if SNB is likely to be technically challenging. Instead of SNB, preoperative lymphoscintigraphy followed by focused US of the identified SNs is performed at each follow-up visits. It is unknown whether focused US of the lymph nodes is an acceptable alternative for SNB in these special populations.

Due to the rarity of melanoma in children and adolescents, little is known on prognostic factors in these young patients. In adult melanoma patients, tumor mitotic rate is one of the strongest predictors of survival. Previous studies showed that tumor mitotic rate is lower in pediatric melanomas than in other age groups. However, the prognostic significance of mitotic rate in clinically localized pediatric melanoma is uncertain.

The biology of melanoma in familial melanoma patients carrying the *CDKN2A* germline mutation seems to be more aggressive. As mentioned, previous studies showed conflicting results regarding a survival difference between *CDKN2A* mutation carriers and sporadic melanoma patients. The frequency of SN-positivity and its prognostic significance are also uncertain.

Individual prognostic factors can be combined into a prognostic model enabling personalized follow-up and treatment of individual patients. The European Organisation for Research

and Treatment of Cancer (EORTC) built a prognostic model and nomogram for recurrence and melanoma-specific mortality in SN-negative melanoma patients. Currently, it is not known how applicable and accurate this prognostic model is to other populations. External validation is essential to ensure the applicability to other melanoma populations.

AIM AND OUTLINE OF THIS THESIS

This thesis describes prognostic factors and management of special melanoma populations.

Chapter two describes patients who underwent lymphoscintigraphy but did not undergo SNB because of advanced age and/or comorbidities. Instead, they were monitored with focused US of their SNs at each follow-up visit. Survival outcomes of this group were compared to patients who did undergo SNB. The aim of this study was to assess whether lymphoscintigraphy with focused US follow-up of SNs is a reasonable management alternative to SNB in patients who are elderly and/or have substantial comorbidities.

Chapter three concerns a cohort study of patients with clinically localized melanoma in whom the intended SNB was canceled after preoperative lymphoscintigraphy. Demographics and melanoma characteristics of this group were compared to patients in whom SNB was performed. The study in chapter three sought to determine if lymphoscintigraphy with focused US follow-up of SNs is an acceptable alternative for patients in whom a SNB procedure is likely to be challenging.

Chapter four describes children and adolescents diagnosed with melanoma. The aim of the study was to assess the prognostic value of tumor mitotic rate in these young patients.

Chapter five compares the characteristics and survival of *CDKN2A* mutation carriers with sporadic melanoma patients. This study aimed to assess whether presence of a pathogenic *CDKN2A* germline mutation was associated with survival in melanoma patients.

Chapter six reports the characteristics and outcome of hereditary melanoma patients carrying germline *CDKN2A* mutations who underwent SNB. The goal of this study was to assess the frequency and predictive value of SN-positivity in *CDKN2A* mutation carriers.

Chapter seven describes the external validation of a prognostic model, including Breslow thickness, ulceration and primary tumor site, to predict survival of patients with SN-negative melanoma. The secondary aim of the study was to assess whether the prognostic model could be improved by adding other prognostic factors.

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