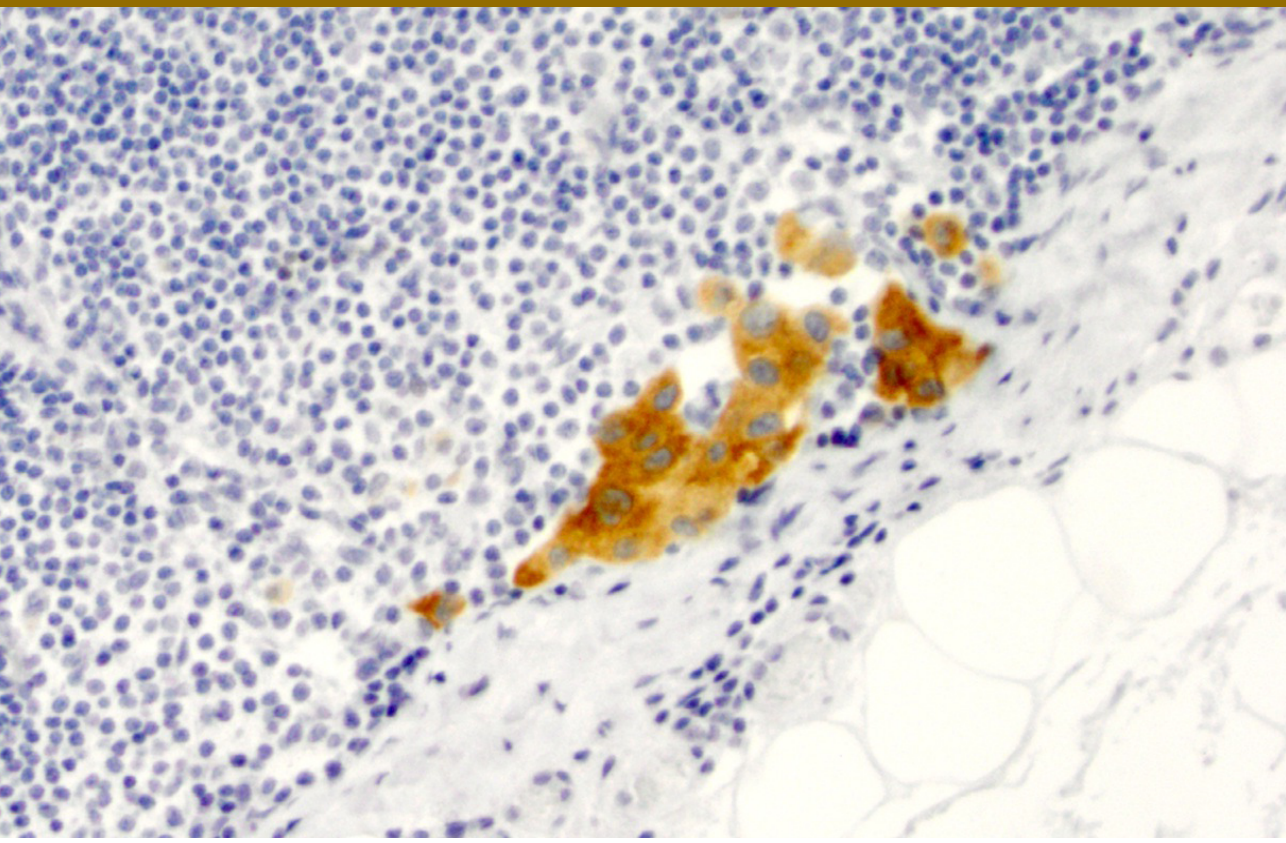
TUMOR LOAD IN LYMPH NODE POSITIVE MELANOMA

Classification systems, prognostication models and management recommendations



Stijn van der Ploeg

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This study has been performed in
The Erasmus Medical Center – Daniel den Hoed Cancer Center, Rotterdam, the Netherlands
The Melanoma Institute Australia, Sydney, Australia
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Tumor Load in Lymph Node Positive Melanoma
Classification Systems, Prognostication Models and Management Recommendations

Tumor hoeveelheid in lymfeklierpositieve melanoompatiënten
Classificatiesystemen, prognostische modellen en management aanbevelingen

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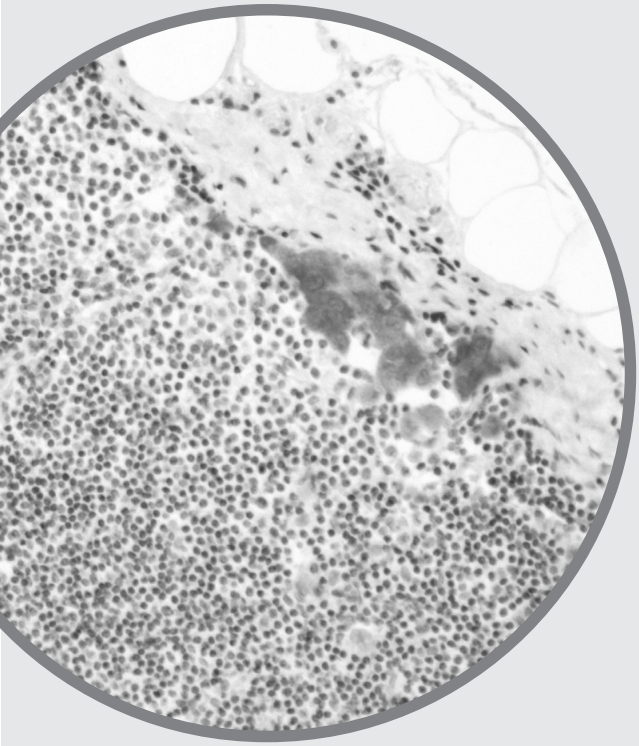
Chapter 1

Introduction and outline of this thesis



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INTRODUCTION AND OUTLINE OF THIS THESIS

The most severe, but least common skin cancer is melanoma, a malignant tumor of melanocytes. In 1787, Hunter was the first to perform surgery on a patient with melanoma.[1] In 1812, Laennec described melanoma as "cancer noire" when he found metastatic deposits in distant sites. He named the disease melanosis.[1] Norris was, in 1857, the first to suggest wide excision for a primary melanoma to prevent locoregional recurrence, which was the beginning of clinicians realizing the regional lymph nodes were at risk. A pioneer in melanoma surgery, Dr. Snow, subsequently suggested that the proper management of melanoma of the skin was removal of all regional lymph nodes in 1892.[1] For nearly a century, this approach remained to be routine management.[2]

Worldwide, the incidence and mortality of melanoma is still on the rise. In the Netherlands, the incidence rate of melanoma increased with an average of 4.1% annually in the last two decades.[3] This increase is much larger than the increase in mortality, although there have been no major improvements in melanoma therapy during this period. It is thought to be caused by early awareness in patients and doctors, an increase in excision of pigmented lesions cautious diagnosis by pathologists which upstages patients and, most importantly, due to earlier detection.[3] The rise of incidence and mortality of melanoma has also been demonstrated across Europe, however, with considerably variation.[4] Trends recently seem to decrease in northern Europe, stabilize in Western Europe, but are still increasing in eastern and southern Europe.[5] Both Australian and New Zealand cancer registries have melanoma incidence rates that were substantially above those from all other reporting registries worldwide.[6] The increased incidence worldwide is mainly caused by earlier detection due to more campaigns and prevention programs detecting more thin primary tumors.[7]

Prognosis of melanoma patients is accurately defined by the American Joint Committee on Cancer (AJCC), which determines the TNM melanoma staging system.[8, 9] The 7th edition, published in 2009, analyzed almost 60.000 patients from 17 cancer centers. Stage I – II patients (T1-4) have non-metastatic disease confined to the skin. Stage III melanoma patients (N1-3) have metastatic disease limited to the lymph nodes and stage IV patients (M1a-c) have disseminated disease distant sites and especially to the organs. The latter group (M1a-c) will not be discussed in the present thesis. The management and prognosis of stage I, II and III patients will be discussed.

PART I: STAGE I – II MELANOMA

Prognosis for stage I - II patients has a large spread. Five-year estimated survival rates differ from 95% for T1 (≤ 1.00 mm thick melanoma) patients to 50% for T4 (> 4.00 mm thick melanoma) patients.[9] For primary melanoma patients, melanoma thickness and tumor ulceration define the T category strata. Primary tumor mitotic rate, defined as mitoses/mm^2, is an important independent adverse predictor of survival as well.[9, 10]

Elective lymph node dissection

The most frequent first apparent site of melanoma spread is to the regional lymph node fields. Therefore, it was very common to perform an elective lymph node dissection (ELND) in stage I – II melanoma patients. ELND is prophylactic removal of all regional lymph nodes. Approximately 20% of patients who underwent ELND turned out to have metastatic disease in their resection specimen. Four randomized controlled trials (RCT) evaluated stage I and II primary melanoma patients randomized for either wide local excision (WLE) and ELND or WLE and delayed lymph node dissection (DLND) when regional metastatic melanoma was diagnosed.[11-16] All four RCTs and two meta-analyses could not demonstrate a survival benefit for patients who underwent ELND.[11-18] However, analyses of subgroups revealed that selected subgroups, such as intermediate thickness melanoma patients (1.0 – 2.0 mm and 0.76 – 3.99 mm), non-ulcerated or extremity melanomas, might have had a therapeutic benefit from undergoing prophylactic lymphadenectomy.[16, 18] The high morbidity and unknown therapeutic benefit of ELND made clinicians search for alternatives.

Sentinel node biopsy

Earlier developed in 1977 for penile carcinoma by Dr. Cabanas, Dr. Morton and colleagues introduced sentinel node (SN) biopsy (SNB) for patients with early stage melanoma disease in the early 1990s.[19-21] This new procedure was developed to intraoperatively identify the SN, which is the first lymph node to receive afferent lymphatic drainage from the primary.[21, 22] Lymph node metastases from melanoma can be detected early by SNB with less morbidity than a lymph node dissection.[23] Only patients with proven melanoma metastases in one or more SNs undergo an "early" completion lymph node dissection (CLND), sparing the patients without SN metastases the additional surgery. Twenty years after its introduction, SNB for stage I and II melanoma is standard staging in most cancer centers worldwide. SNB is still, however, subject of extensive debate and ongoing controversy.[24] Many have raised questions on, amongst others, the false negative rate and the therapeutic value of SNB. These issues are addressed in chapter 2 of part I of this thesis, evaluating nearly

6000 melanoma patients, who underwent SNB or were observed after undergoing wide local excision of their primary at the Melanoma Institute Australia (MIA), Sydney, Australia.

In brief, the procedure of SNB consists of three steps; (1) identification of the SN field and site with pre-operative lymphoscintigraphy using radio-labelled colloid, undertaken within 24 hours of the operation being performed, (2) perioperative use of patent blue dye and (3) a handheld gamma detection probe to detect the SN(s). A lymph node is identified as SN if stained blue, if it is located in the expected site and/or has a high in situ or ex vivo radioactivity count. With experience in this procedure, the SN(s) can be identified in nearly 100% of the patients.[25, 26] Most difficulties are present in the head and neck region, compared to the axilla or groin regions, due to the unpredictable lymphatic drainage and the anatomical difficulties.[27, 28] Pre-operative lymphoscintigraphy is of great value in the head and neck region to determine the lymphatic drainage pattern and the locations of the SNs.[29] Ear melanomas represent approximately 10% of head and neck melanomas.[30] In chapter 3, the lymphatic drainage patterns of ear melanomas are determined in 111 patients treated at the MIA, Sydney, Australia. The precise location on the ear was correlated with the location of the SNs identified by lymphoscintigraphy.

Histo-Pathologic work-up

After the surgical procedure, the SNs are sent to the pathology department for pathological examination. Pathology work-up protocols are different worldwide and no standard approach for the SNs has been adopted. In general, three protocols are being assessed. The European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group uses the protocol developed by Cook et al.[31] The Melanoma Institute Australia handles a protocol formed by Scolyer et al.[32] The John Wayne Cancer Institute uses the protocol developed by Cochran et al.[33]

The differences between protocols are where the sections in the SN are cut, how many sections are cut and how large the interval between sections is. These differences, amongst others, lead to different percentages of SN positive cases. SN positivity rates show a spread from approximately 14% to nearly 30% in literature for different patient cohorts, with other baseline prognostic values regarding the Breslow thickness and ulceration rates.[34-36] Studies have shown that the use of immunohistochemistry, an increased number of sections cut from the SN and RT-PCR increases the detection rate of melanoma cells.[31, 37-40]

Histo-pathologic analysis

As above mentioned, approximately 20% of SNB patients are diagnosed with SN tumor burden by the pathologist. Different parameters as the size, location and

penetrative depth of SN metastases provide important prognostic information.[41-47] The maximum size of largest SN tumor deposit could be categorized in different classification schemes, i.e. the Rotterdam criteria (≤ 0.1, 0.1 – 1.0, > 1.0 mm) or the Gershenwald criteria (≤ 0.5, > 0.5 – ≤ 2.0, > 2.0 – ≤ 10.0, > 10.0 mm).[43, 48] The location is originally classified by the Dewar criteria into the following categories: subcapsular, parenchymal, combined (subcapsular and parenchymal), multifocal and extensive.[45] The tumor penetrative depth is classified according to the Starz S-classification (≤ 0.3 (S1), > 0.3 – ≤ 1.0 (S2), > 1.0 (S3) mm).[46, 49]

PART II: STAGE III MELANOMA, MICROMETASTASES

In the 6th, and subsequently in the 7th TNM staging category created by the AJCC, stage III melanoma patients are divided in two different groups with lymph node metastases by the mode of detection.[8, 9] Patients with clinically occult micrometastases are diagnosed by SNB, while patients with macrometastases are defined as clinically detectable nodal metastases confirmed pathologically.[41] Patients with micrometastases are discussed in part II of this thesis, while patients with macrometastases are discussed in part III.

Management and prognosis

Outcome of SN positive patients is determined by the predictive value for non-sentinel node (NSN) status in the CLND specimen and the prognostic value for survival. The group of SN positive patients is a heterogeneous group of patients with survival rates ranging from approximately 20% to over 90% in various subgroups.[41] Patients with high volume SN tumor burden have significant worse outcome than patients with low volume tumor burden.[41-47]

In chapter 4 of this thesis, management and prognosis of 421 SNB patients from the Erasmus University Medical Center – Daniel den Hoed Cancer center, Rotterdam, the Netherlands, are evaluated. SN positive patients are compared to SN negative patients. To identify low-risk and high-risk patients, 121 SN positive patients are stratified for the maximum SN tumor size classified according to the Rotterdam criteria. In chapter 5, management and prognosis of a large group of SN positive patients is evaluated. Nine cancer centers collaborating in the EORTC Melanoma Group combined their data. The outcome of more than 1000 SN positive patients in a multicenter setting was evaluated. The predictive and prognostic values of two SN tumor burden parameters are evaluated: the maximum size classified according to the Rotterdam criteria and the intranodal location classified according to the Dewar criteria. In chapter 6, the cohort is extended with data from one European and one

Australian melanoma treatment center to the largest group of SN positive patients in the world. The prognostic significance of the Rotterdam and Dewar criteria and S-classification is evaluated in the total group of 1539 patients and, for the first time, in each individual melanoma treatment center.

Completion lymph node dissection

In most cancer centers, standard treatment for all SN positive patients is CLND. The therapeutic benefit of CLND is unknown and it attends with high morbidity.[23, 50-52] Some have hypothesized that not all SN positive patients might be indicated for immediate "early" CLND.[43, 44, 50, 53, 54] Others have reported that all SN positive patients should undergo CLND.[55-57] In chapter 7, the European cohort of over 1000 SN positive patients are evaluated in light of this issue. SN positive patients who did not undergo CLND are evaluated and compared with patients who underwent CLND. Again, patients were stratified for the above mentioned different SN tumor burden parameters.

PART III: STAGE III MELANOMA, MACROMETASTASES

Approximately 4 to 9% of all patients presenting with melanoma are diagnosed with clinically detectable nodal disease, i.e. macrometastases.[35, 58] Patients with nodal macrometastases have 5-year survival rates ranging from 30% to 50%.[41] Important prognostic factors are the number of metastatic nodes, the presence or absence of extracapsular extension, the presence or absence of ulceration and tumor thickness.[41] After excluding stage IV disease using appropriate investigations, a therapeutic lymph node dissection (TLND) is indicated. The AJCC staging system classifies melanoma patients with macroscopic metastases as stage IIIB or IIIC by considering the number of invaded nodes, presence of ulceration in the primary tumor and presence of satellitosis or in-transit metastases. Other factors of prognostic significance in stage III patients that have been described such as age or site of invasion have not been considered in current staging criteria. In **chapter 8** all prognostic factors were assessed in a cohort with patients who underwent TLND for palpable disease from Brisbane, Australia and validated in a cohort from Rotterdam. Nomograms are created to accurately predict recurrence and survival patterns in these patients.

Groin surgery

In general, palpable melanoma disease can be detected in three different lymph node fields followed by three different TLNDs, i.e. a neck dissection, an axillary dissection or a groin dissection. The appropriate management of palpable groin

metastases is very frequently discussed.[59-63] According to most publications in the literature, an ilioinguinal or combined superficial and deep groin lymph node dissection (CGD) should be performed. In practice, an inguinal or superficial groin dissection (SGD) is still performed in some patients. In chapter 9, the experience in patients with clinically detectable disease of the groin treated with TLND at the Erasmus University Medical Center – Daniel den Hoed Cancer Center is evaluated. The outcome of 121 patients treated with CGD is compared with the outcome of 48 patients who underwent SGD.

Unknown primary

Of all melanoma patients presenting with clinically detectable nodal disease, 13 to 17% has melanoma with an unknown primary site (MUP).[64-66] MUP has been defined as histologically confirmed subcutaneous, nodal or visceral metastatic melanoma with no evidence of a cutaneous or non-cutaneous primary melanoma.[67] The outcome of MUP in relation to patients with a known primary melanoma (MKP) is unknown as well as the origin of MUP. Chapter 10 describes a retrospective study performed at the Erasmus University Medical Center – Daniel den Hoed Cancer Center regarding the outcome of 47 MUP patients and 292 MKP patients who were all treated with TLND for palpable disease. The total patient population was assessed for analyses. To validate the results of chapter 9, an equivalent study has been performed at the MIA, Sydney, Australia. In chapter 11, the outcome of 287 MUP patients is compared with the outcome of a uniformly selected group of 264 MKP patients treated with TLND.

To conclude, chapter 12, and chapter 13 in Dutch, provides a general summary and conclusion of the entire thesis. In chapter 14, a general discussion is presented together with future perspectives in the field of the management and prognosis of early-stage melanoma.

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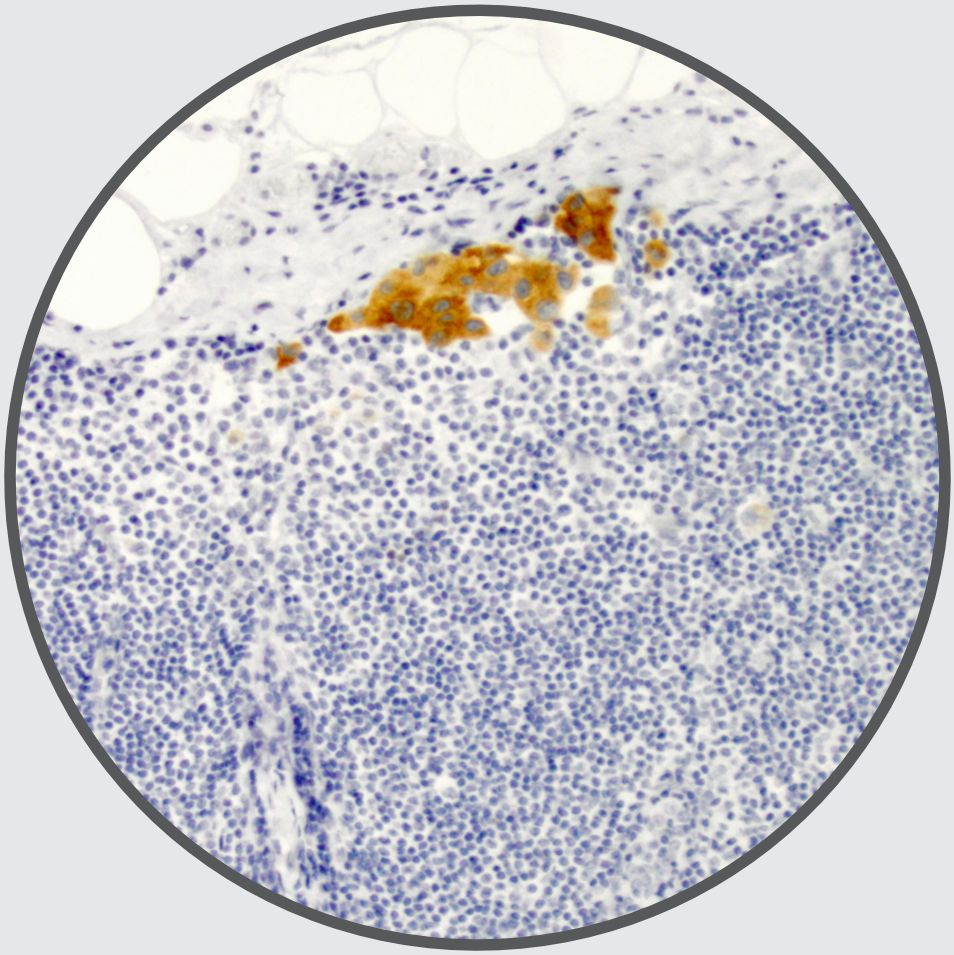


Part I

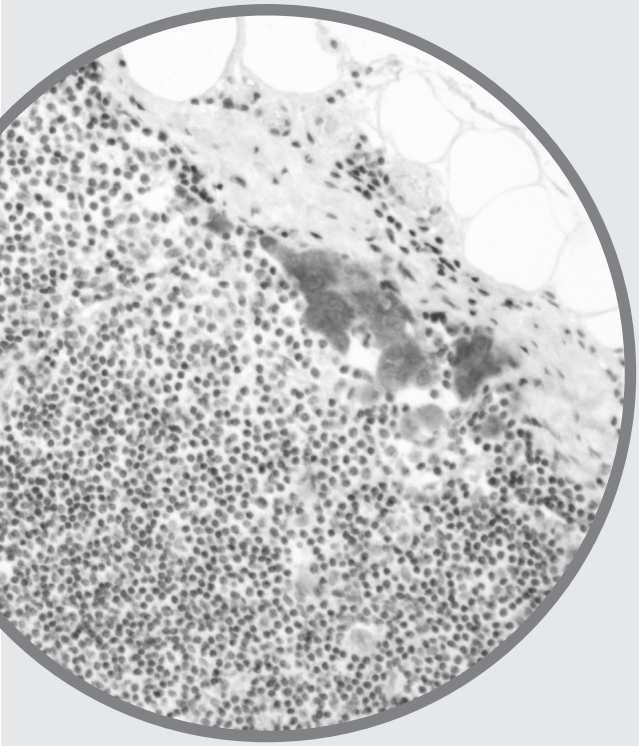
Management and prognosis of stage I – II melanoma

Chapter 2
Outcome following sentinel node biopsy versus wide local excision only for melanoma; analysis of a large single institution experience
Annals of Surgery, in press

Chapter 3
The unpredictability of lymphatic drainage from the ear in melanoma patients, and its implications for management
Annals of Surgical Oncology. 2013 May;20(5):1707-13

Chapter 2

Outcome following sentinel node biopsy versus wide local excision only for melanoma; analysis of a large single institution experience



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ABSTRACT

Worldwide, sentinel node biopsy (SNB) is now a standard staging procedure for most patients with melanomas ≥1mm in thickness, but its therapeutic benefit is not clear, pending randomized trial results. This study sought to assess the therapeutic benefit of SNB in a large, non-randomized patient cohort.

Patients with primary melanomas ≥1.00mm thick or with adverse prognostic features treated with wide local excision (WLE) at a single institution between 1992 and 2008 were identified. The outcomes for those who underwent WLE plus SNB (n=2909) were compared with the outcomes for patients in an observation (OBS) group who had WLE only (n=2931). Median follow-up was 42 months.

Melanoma-specific survival (MSS) was not significantly different for patients in the SNB and OBS groups. However, a stratified univariate analysis of MSS for different thickness subgroups indicated a significantly better MSS for SNB patients with T2 and T3 melanomas (>1.0-4.0mm thick) ($p=0.011$), but this was not independently significant in multivariate analysis. Compared with OBS patients, SNB patients demonstrated improved disease-free survival (DFS) ($p<0.001$) and regional recurrence-free survival ($p<0.001$). There was also an improvement in distant metastasis-free survival for SNB patients with T2 and T3 melanomas ($p=0.041$).

In this study, the outcome for the overall cohort after WLE alone did not differ significantly from the outcome after additional SNB. However, the outcome for the sub-group of patients with melanomas >1.0-4.0mm in thickness was improved if they had a SNB, with significantly improved disease-free and distant metastasis-free survival.

INTRODUCTION

Worldwide, sentinel node (SN) biopsy (SNB) is now a standard staging procedure for most patients with melanomas ≥1.0mm in thickness.[1-4] The American Joint Committee on Cancer (AJCC) included micrometastasis diagnosed by SNB in the two latest (6th and 7th) editions of its TNM staging system.[5, 6] Survival in patients with SN metastases is considerably better than in patients with clinically-evident metastases,[7] and SN status is the most important prognostic factor for survival in patients with early stage melanoma.[1, 8, 9]

Nevertheless, the role of SNB is still being defined, and an overall survival benefit in patients having SNB, with immediate completion lymph node dissection (CNLD) if found to be SN-positive, has yet to be demonstrated in a randomized clinical trial (RCT).[2, 10, 11] In the third interim analysis of the first Multicenter Selective Lymphadenectomy Trial (MSLT-I), which compared patients who had SNB and patients who had nodal observation (OBS), no overall survival benefit for SNB patients was demonstrated but disease-free survival (DFS) was improved.[1] However, there was substantially improved 5-year survival (72.3% vs 52.4%, p=0.004) in patients with intermediate-thickness melanomas (1.2-3.5mm) with nodal metastases who had an immediate CLND.[1]

Most retrospective studies comparing outcome between SNB and OBS patients have also shown a DFS benefit in favor of SNB, with no overall survival benefit.[12-17] However, a survival benefit has been reported in specific groups stratified by thickness.[14, 17] Several investigators have analyzed the group of patients with nodal metastases only, comparing SN-positive patients undergoing CLND with OBS patients undergoing therapeutic lymph node dissection (TLND) when regional lymph node metastasis was diagnosed clinically.[16-22] Results have been conflicting, with four studies showing a survival benefit for CLND patients and three reporting no statistical difference. Meta-analysis of six of these studies, however, did show an overall survival benefit for SNB patients undergoing immediate CLND compared with patients having a TLND for clinically evident lymph node disease.[22]

The criteria for recommending SNB are not similar in melanoma management guidelines. The most recent guideline of the American Society of Clinical Oncology (ASCO) and Society of Surgical Oncology (SSO) advocate offering SNB to patients with melanoma ≥1.0mm to 4mm. SNB may be recommended to patients with thick melanomas (> 4 mm) for staging purposes and to facilitate regional disease control. The European and Australian guidelines encourage to discuss SNB in patients with melanoma > 1.0 mm and > 1.2 mm, respectively, or when one or more adverse prognostic features is present.[23-25] Such features may be ulceration or mitotic rate ≥ 1/mm^2, especially in the subgroup of patients with melanomas 0.75 to 0.99 mm in Breslow thickness

The aims of the present study were to compare regional recurrence-free survival, distant metastasis-free survival (DMFS) and melanoma-specific survival (MSS) of SNB patients with OBS patients in a large patient cohort treated at a single institution, as well as comparing the outcomes for SNB patients undergoing early CLND with those of OBS patients undergoing a delayed TLND for recurrence.

PATIENTS AND METHODS

Patients

Prospectively-collected data were extracted from the Melanoma Institute Australia (MIA) database. Patient selection for this retrospective assessment was based on eligibility for SNB at the MIA. Between 1992 and 2008, wide local excision (WLE) was performed in 5840 patients with a single primary melanoma ≥1.0 mm in thickness or when ulceration, Clark level IV or V invasion or a tumor mitotic rate ≥1/mm^2 was recorded. SNB was discussed with nearly every patient in this study, but some chose not to have the additional procedure or were advised against SNB for reasons including medical co-morbidities and advanced age. After WLE, 2909 (49.8%) patients underwent SNB and 2931 (50.2%) did not (OBS). Of the 5840 patients, 803 (13.7%) were enrolled in the randomized first Multicenter Selective Lymphadenectomy Trial (MSLT-I)[1] and those randomized to WLE only did not have a SNB. Patients were excluded from the present study if they had multiple primary melanomas, extra-cutaneous melanoma, melanoma of the ear, incomplete follow-up, a SNB for recurrent melanoma, an elective lymph node dissection or no immediate CLND when found to be SN-positive.

Methods

WLE was performed in all patients, with surgical clearance margins based on the Australian clinical practice guidelines.[23, 26] These recommend margins of 1cm for melanomas <1.0mm thick, 1-2cm for melanomas 1.0-4.0mm thick and 2cm for melanomas > 4.0mm thick. After WLE, 49.8% of patients underwent SNB, using a standard protocol that has been described in detail previously[27]. After removal, the SNs were examined histopathologically as previously described.[28] In patients found to be SN-positive, CLND was performed. Follow-up surveillance of melanoma patients at MIA has varied over time and by clinician preference.

Statistics

Statistical analyses were performed with IBM SPSS Statistic 19.0. Variables were coded and included in statistical analyses as reported in Table 1. Survival time was

Table 1 – Baseline characteristics of patients in the sentinel node biopsy (SNB) and observation (OBS) groups and of patients with nodal metastases

	All Patients			Patients with Nodal Metastases			
	SNB	Observation		SNB Positive††	SNB False-Negative‡‡	Observation w/ Recurrence‡‡	
	n (%)	n (%)	*P*-value	n (%)	n (%)	n (%)	*P*-value
Total	2909 (49.8)	2931 (50.2%)		394 (13.5)†	89 (18.4) ‡	417 (14.2) ∫	
Follow-up (months)							
Median (IQR)	44 (20-76)	40 (18-81)	0.190	43 (22-73)	42 (25-81)	51 (27-97)	0.003
Mean±SE	53.4±0.8	54.2±0.8	0.500	52.0±1.8	59.2±4.6	64.4±2.2	<0.001
Gender							
Female	1167 (40.1)	1253 (42.7)	0.041	147 (37.3)	38 (42.7)	160 (38.4)	
Male	1742 (59.9)	1678 (57.3)		247 (62.7)	51 (57.3)	257 (61.6)	0.640
Age (yr)							
Mean±SE	56.1±0.3	60.2±0.3	<0.001	53.0±0.9	57.3±1.7	57.7±0.9	<0.001
≤ 50	1015 (34.9)	880 (30.0)	<0.001	170 (43.1)	32 (36.0)	148 (35.5)	0.069
> 50	1894 (65.1)	2051 (70.0)		224 (56.9)	57 (64.0)	269 (64.5)	
Site							
Extremity	1319 (45.5)	1157 (39.7)		165 (42.0)	41 (46.1)	171 (41.0)	
Trunk	1133 (39.1)	1025 (35.2)	<0.001	169 (43.0)	29 (32.6)	154 (36.9)	0.059
Head & Neck	445 (15.4)	731 (25.1)		59 (15.0)	19 (21.3)	92 (22.1)	
Thickness (mm)							
Median	1.8	1.5	<0.001	3	2.4	2.2	<0.001
Mean±SE	2.47±0.03	2.33±0.04	0.015	3.41±0.11	3.00±0.22	2.94±0.13	0.016
≤ 1.00 / T1	328 (11.3)	811 (27.7)		20 (5.1)	5 (5.6)	53 (12.7)	
> 1.00 – ≤ 2.00 / T2	1328 (45.7)	1055 (36.0)	<0.001	107 (27.2)	31 (34.8)	134 (32.1)	<0.001
> 2.00 – ≤ 4.00 / T3	840 (28.9)	671 (22.9)		149 (37.8)	36 (40.4)	156 (37.4)	
> 4.00 / T4	413 (14.2)	394 (13.4)		118 (29.9)	17 (19.1)	74 (17.7)	
Mitotic Rate (/mm^2)							
Median (IQR)	3 (2-7)	2 (1-6)	<0.001	5 (3-9)	6 (3-12)	5 (2-9)	0.037
Mean±SD	5.18±0.11	4.67±0.12	0.003	6.74±0.30	8.16±0.74	6.60±0.33	0.120
Clark level							
II-III	791 (27.7)	833 (29.2)		72 (18.5)	20 (22.7)	95 (23.2)	
IV	1825 (64.0)	1759 (61.6)	0.161	265 (68.1)	58 (65.9)	270 (65.9)	0.503
V	237 (8.3)	263 (9.2)		52 (13.4)	10 (11.4)	45 (11.0)	
Ulceration							
Absent	1863 (71.2)	1734 (71.0)	0.922	217 (59.0)	46 (56.8)	210 (57.4)	0.883
Present	755 (28.8)	707 (29.0)		151 (41.0)	35 (43.2)	156 (42.6)	

Table 1 (continued)

Melanoma subtype							
SSM	863 (39.4)	879 (43.1)		102 (32.4)	16 (21.3)	116 (38.4)	
NM	800 (36.5)	595 (29.2)	<0.001	147 (46.7)	29 (38.7)	118 (39.1)	0.002
Other	529 (24.1)	567 (27.8)		66 (21.0)	30 (40.0)	68 (22.5)	
Positive nodes							
Mean±SE				1.69±0.06	2.57±0.32	2.92±0.17	<0.001
1				243 (61.7)	51 (57.3)	181 (47.0)	
2-3				91 (23.1)	12 (13.5)	79 (20.5)	<0.001
>3				60 (15.2)	26 (29.2)	125 (32.5)	
First recurrence type							
Local	116 (20.9)	86 (12.7)					
Intransit	95 (17.1)	51 (7.5)	<0.001				
Nodal	111 (20.0)	367 (54.3)					
Distant	234 (42.1)	172 (25.4)					

SNB = Sentinel Node Biopsy; OBS=Observation; IQR = InterQuartile Range; SE = Standard Error of the Mean; SSM = Superficial Spreading Melanoma; NM = Nodular Melanoma
† 394 of 2909 (13.5%) of SNB patients had positive SNs.
ǂ 89 of 2515 (3.5%) patients with a negative SNB had their first recurrence in a lymph node in the SNB field. False negativity rate = false-negative SNB / (false-negative SNB + true positive SNB) = 89/483 = 18.4%
∫ 417 of 2931 patients (12.5%) with nodal observation had regional lymph node recurrence.
†† Early completion lymphadenectomy (CLND) was performed in 393 sentinel node-positive patients.
Delayed lymphadenectomy (DLND) was performed in 89 false-negative SNB patients and 389 patients with nodal observation.

measured from date of definitive primary melanoma surgery to first disease recurrence for disease-free survival (DFS), to first recurrence in the regional lymph node field for regional lymph node recurrence, to first relapse at a distant site for DMFS, and to last follow-up (censored) or death from melanoma for MSS. P-values <0.05 were considered statistically significant.

Fisher's exact test, Chi-square test, independent groups' t-test, Kruskal-Wallis test and one-way ANOVA test were performed to determine differences between the OBS and SNB groups, and the differences between the groups with nodal metastases, (SN-positive patients and SN-false-negative patients, with first recurrence in the biopsied regional lymph node field) and OBS patients with regional lymph node recurrence. Kaplan-Meier curves together with the log rank (Mantel-Cox) test were used to assess univariate survival. The Cox proportional hazards model was used for multivariate survival analysis. The proportionality assumption was inspected visually for each categorical covariate.

RESULTS

SNB vs. OBS groups – Characteristics and Recurrence Rates

The mean and median follow-up times for the 2931 OBS patients (57.2% men) were 54.2 and 40 (interquartile range (IQR) 18-81) months, respectively. The mean and median follow-up times for the 2909 SNB patients (59.9% men) were 53.4 and 44 (IQR 20-76) months, respectively. There were significant differences in baseline characteristics between the SNB and OBS groups (see Table 1). The SNB group contained more young patients and more melanomas of nodular subtype, while the OBS group contained more melanomas <1mm in thickness, with a lower mitotic rate and located in head/neck sites ($p<0.05$). The site of first recurrence in the SNB group differed significantly from the OBS group ($p<0.001$). In the SNB group, a distant metastasis (DM) was the most common first recurrence (42.1%) while in the OBS group a regional node metastasis was most common (54.3%). The median time to first recurrence was 38 (range 1-215) months for SNB patients and 31 (range 1-223) months for OBS patients.

Recurrence rates for the OBS and SNB groups stratified by three different primary tumor thickness criteria are presented in Table 2 for patients having a minimum of five years follow-up or recurrence within five years (n=2918). The three separate criteria for SNB assessed in these analyses were (1) tumors <1mm thick with ulceration, Clark level IV or V invasion, or a mitotic rate ≥1/mm^2; (2) tumors =1.0mm thick and (3) tumors with >1mm thick.

Table 2 – First recurrence data in the sentinel node biopsy (SNB) and observation (OBS) groups according to primary tumor thickness criteria for SNB*

Group	None		Local		In-transit		Regional Node		Distant		Total	
	N	%	N	%	N	%	N	%	N	%	N	%
OBS (n=1471)												
< 1 mm**	152	76.4%	6	3.0%	0	0.0%	29	14.6%	12	6.0%	199	13.5%
1 mm	107	78.7%	4	2.9%	0	0.0%	18	13.2%	7	5.1%	136	9.2%
> 1 mm	536	47.2%	76	6.7%	51	4.5%	320	28.2%	153	13.5%	1136	77.2%
SNB (n=1447)												
< 1 mm**	40	81.6%	3	6.1%	0	0.0%	2	4.1%	4	8.2%	49	3.4%
1 mm	56	83.6%	3	4.5%	0	0.0%	3	4.5%	5	7.5%	67	4.6%
> 1 mm	795	59.7%	110	8.3%	95	7.1%	106	8.0%	225	16.9%	1331	92.0%

**Including patients with at least five years of follow-up or a recurrence event within five years. In this sub-group of patients, the median Breslow thickness in the OBS group (1.7 mm) was significantly lower than the SNB group (2.0 mm) p<0.001). Similarly, the median mitotic rate in the OBS group (3/mm2) was significantly lower than that of the SNB group (4/mm2) (p<0.001). Percentage of ulcerated cases did not differ significantly between the OBS and SNB groups (31% and 30%, respectively).*

***Clark IV/V or ulceration or mitoses present*

There were significantly fewer regional node recurrences in the SNB group compared with the OBS group for the SNB criteria <1mm (p=0.047) and >1mm (p<0.001) groups, but not for the 1mm group (p=0.054; Table 2). However, the proportion of patients in the SNB and OBS groups that developed a regional node metastasis, when including positive SNs at primary presentation, was statistically the same for each primary tumor thickness criterion (<1mm, p=0.959; 1mm, p=0.743; >1mm, p=0.406).

There was no difference between the SNB and OBS groups in the proportion of DM as first recurrences for the SNB criteria of <1mm and 1mm. However, in the group of patients with melanomas >1mm thick, there were significantly more DMs as first recurrences in the SNB group (16.9%) compared with the OBS group (13.5%, p=0.018). Similarly, when considering SN-positive patients as first recurring in the regional nodes there was no difference in the proportion of DMs as first recurrences between the SNB and OBS groups for each of the SNB criteria (<1mm, p=0.586; 1mm, p=0.511; >1mm, p=0.058).

For patients with melanomas >1mm thick, there were significantly fewer experienced a recurrence of any type in the SNB group (40.3%) compared with the OBS group (52.8%, p<0.001); this was not significantly different for patients in the <1mm and 1mm SNB groups.

SNB vs. OBS groups – Disease-Free and Distant Metastasis-Free Survival

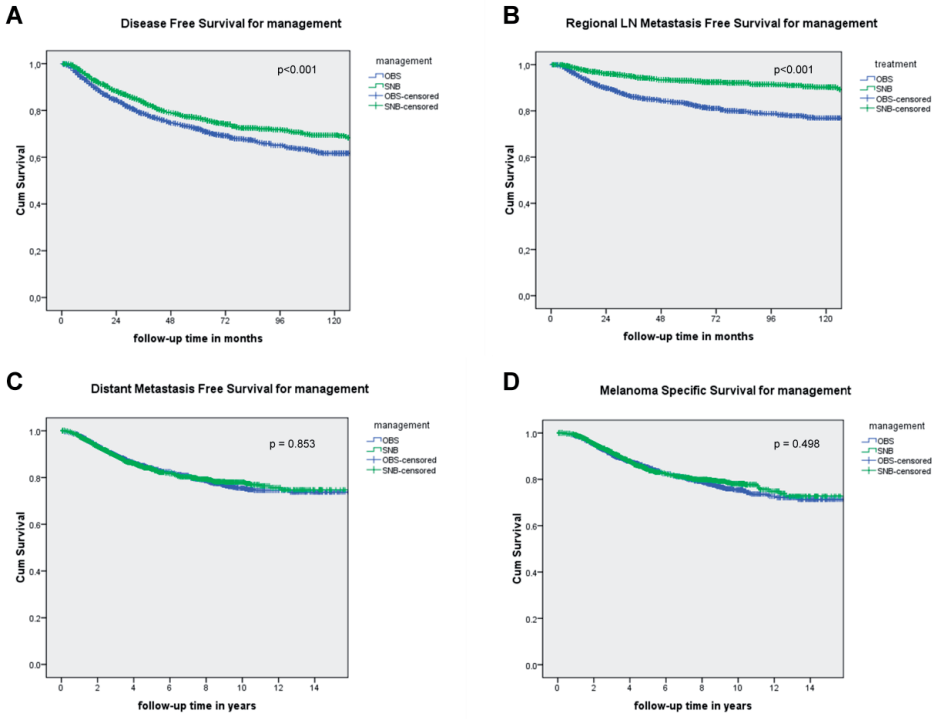
In univariate analysis, SNB patients showed improved DFS (p<0.001) and regional lymph node control (p<0.001) but no difference in DMFS (p=0.974) (Figure 1A-B). However, in the subgroup of patients with T2 and T3 melanomas (>1.0–4.0mm in thickness), SNB patients demonstrated improved DMFS compared to the OBS group (p=0.021) (Figure 1C).

After adjusting for all major prognostic factors in multivariate analysis, SNB patients continued to have significantly improved DFS compared with OBS patients (HR=1.40; 95%CI 1.23 - 1.58; p<0.001) (Table 3A). The same associations were observed with respect to regional lymph node control in the overall cohort (OBS HR=3.23, 95% CI: 2.66-3.94, p<0.001) and DMFS for T2 and T3 melanoma sub-groups (OBS HR=1.23, 95% CI: 1.01-1.50, p=0.041) as in the univariate analyses (Table S1).

SNB vs. OBS group – Melanoma-specific Survival

Univariate analysis of MSS demonstrated no significant difference when comparing all patients in the SNB and OBS groups (p=0.560). The five-year Kaplan-Meier estimates of MSS were 85.0% for SNB patients and 85.8% for OBS patients. However, a stratified analysis of MSS for different tumor thickness subgroups demonstrated a better prognosis for all patients in the SNB group with melanomas >1mm thick (p=0.012) and in those with T2 and T3 melanomas (>1.0-4.0mm thick, p=0.011)

Figuur 1 - A) Disease free survival, B) regional lymph node-free survival, C) distant metastasis-free survival and D) melanoma-specific survival in melanoma patients managed by sentinel node biopsy or nodal observation.



(Figure 1D). For the patients with T2 and T3 melanomas, 5-year MSS rates were 86.8% for the SNB group and 85.3% for the OBS group (Figure 3B). However, after adjusting for known prognostic factors in multivariate MSS analysis, no significant benefit for SNB patients was demonstrated overall (Table 3A) or for any of the tumor thickness subgroups.

SN-positive vs. SN-negative groups

In multivariate analysis of the SNB group, SN status was an independent prognostic factor for DFS (HR= 3.04; 95%CI: 2.50-3.70; p<0.001) and MSS (HR=2.97; 95%CI: 2.34-3.77; p<0.001) (Table 3B). SN-negative and SN-positive patients had estimated 5-year DFS rates of 81.4% and 51.2%, respectively and estimated 5-year MSS rates of 88.9% and 63.8% (p<0.001).

SNB with early CLND group vs. OBS with late TLND group

Of the 2909 SNB patients, 394 (13.5%) were SN-positive, and these patients subsequently received CLND. There were positive non-SNs (NSNs) in the CLND specimen

Table 3 – Multivariate analysis of disease-free survival and melanoma-specific survival in A) all patients and B) the sentinel node biopsy (SNB) group

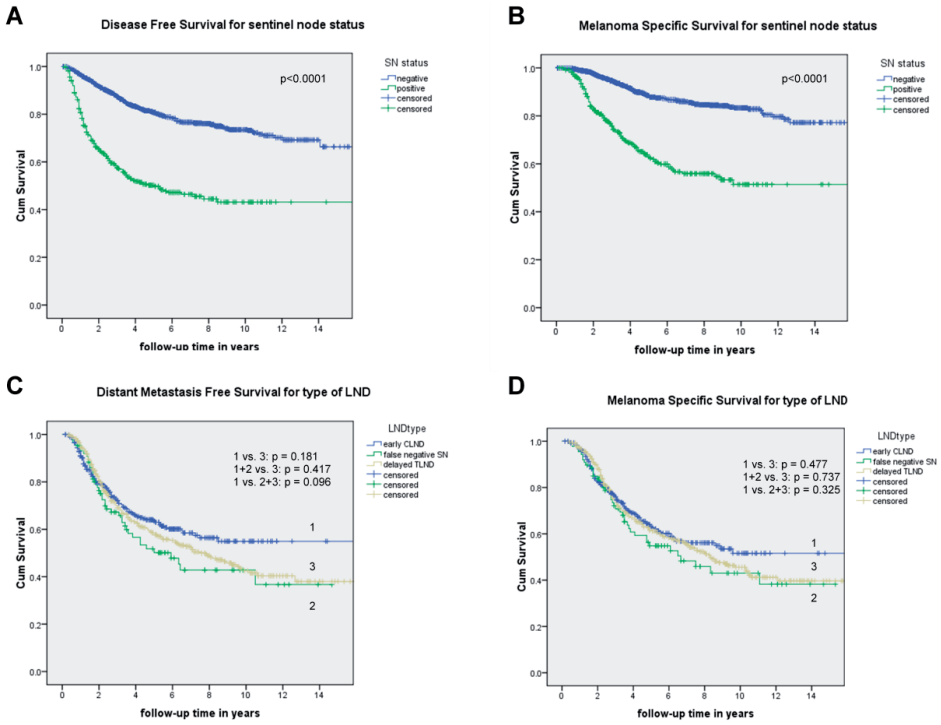
A			**DFS (n=4671)**			**MSS (n=4473)**	
Factor	**Value**	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Management*	Observation	1.40	1.23 - 1.58	<0.001	1.04	0.88 - 1.22	0.642
Gender	Male	1.17	1.02 - 1.34	0.021	1.50	1.24 - 1.80	<0.001
Age	(years)	1.01	1.00 - 1.01	0.005	1.01	1.00 - 1.01	0.003
Breslow thickness	(mm)	1.11	1.09 - 1.14	<0.001	1.15	1.11 - 1.18	<0.001
Mitotic rate	(/mm^2)	1.03	1.02 - 1.04	<0.001	1.02	1.01 - 1.04	<0.001
Clark level (ref: II-III)	IV-V	1.52	1.30 - 1.78	<0.001	1.37	1.11 - 1.68	0.003
Ulceration	Present	1.73	1.52 – 1.98	<0.001	1.82	1.53 - 2.16	<0.001
Primary site	Trunk	0.97	0.84 - 1.13	0.707	1.19	0.98 - 1.45	0.072
(ref: extremity)	Head & Neck	1.15	0.98 - 1.36	0.093	1.31	1.05 - 1.64	0.017
B			**DFS (n=2479)**			**MSS (n=2352)**	
Factor	**Value**	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Sentinel Node status	Positive	3.04	2.50 - 3.70	<0.001	2.97	2.34 - 3.77	<0.001
Gender	Male	0.96	0.82 - 1.21	0.957	1.21	0.94 - 1.57	0.148
Age	(years)	1.02	1.01 - 1.03	<0.001	1.02	1.01 -1.02	<0.001
Breslow thickness	(mm)	1.13	1.09 - 1.18	<0.001	1.16	1.11 - 1.22	<0.001
Mitotic rate	(/mm^2)	1.03	1.02 - 1.04	<0.001	1.03	1.01 - 1.04	0.001
Clark level (ref: II-III)	IV-V	1.32	1.05 - 1.67	0.018	1.22	0.92 - 1.63	0.168
Ulceration	Present	1.50	1.24 - 1.82	<0.001	1.98	1.55 - 2.52	<0.001
Primary site	Trunk	0.91	0.74 - 1.12	0.359	1.35	1.04 - 1.75	0.026
(ref: extremity)	Head & Neck	1.16	0.90 - 1.50	0.264	1.39	1.00 - 1.94	0.053

HR = Hazard Ratio, CI = Confidence Interval, DFS=Disease-free Survival, MSS= Melanoma-specific Survival
**SNB versus OBS*

in 77 (19.5%). Eighty-nine (3.5%) of the 2515 SN-negative patients had regional node recurrence as a first recurrence (false-negative SNB) and underwent a delayed lymphadenectomy. The SN false-negative rate (defined as false-negative / (false-negative + true-positive) was 18.4%.[10] In the OBS group, 417 patients (14.2%) recurred in the regional node field and 385 received a "delayed" TLND. Patients who received an early CLND had a mean number of positive nodes of 1.69, which was significantly less compared to 2.92 and 2.57, respectively, in the OBS group and the SN false-negative group (p<0.001) at the time of delayed lymphadenectomy. Furthermore, 15.2% of early CLND patients had N3 disease (> 3 involved nodes) compared to 32.5% and 29.2%, respectively, in the OBS group and the SN false-negative group (p<0.001) (Table 1).

SN-positive patients having early CLND had a significantly prolonged DMFS compared with OBS patients having a "delayed" TLND on multivariate analysis (Figure

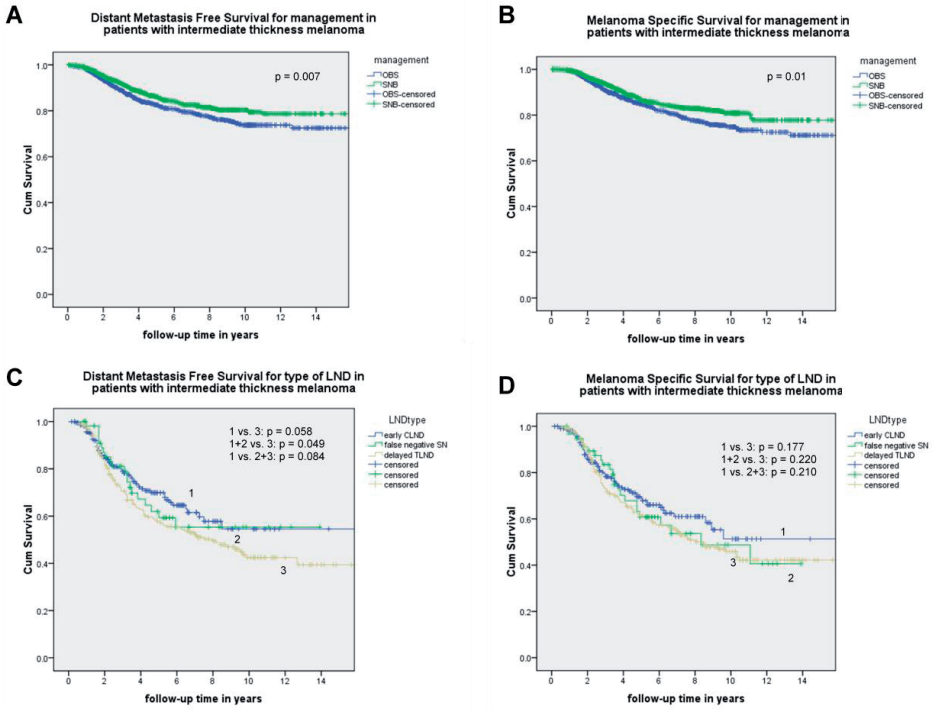
Figuur 2 - A) Disease free survival and B) melanoma-specific survival for sentinel node status and C) distant-metastasis free survival and D) melanoma-specific survival for the type of lymph node dissection, i.e. completion lymph node dissection, delayed therapeutic lymph node dissection and therapeutic lymph node dissection in patients with a false negative sentinel node



2A). In multivariate analysis, however, this result was clearly significant (OBS HR=1.36, 95% CI: 1.08-1.72, p=0.010) (Figure 2A, Table S2), but this just failed to reach statistical significance in univariate analysis (p=0.052). DMFS was not significantly different for the SN-positive (CLND) group compared with the OBS ("delayed" TLND) group for patients with T2 and T3 melanomas in univariate analysis (p=0.072), but again the difference was statistically significant on multivariate analysis (OBS HR=1.36, 95% CI: 1.01-1.84, p=0.042).

MSS was not significantly influenced by early CLND or "delayed" TLND in either univariate or multivariate analyses (Figure 2B, Table S2). Five-year Kaplan-Meier MSS estimates were 64.1% for CLND patients and 60.5% for TLND patients (p=0.144). For T2 and T3 patients, five-year Kaplan-Meier MSS estimates were 68.3% following CLND and 62.7% following "delayed" TLND, but the difference was not statistically significant in either univariate or multivariate analysis.

Figuur 3 - A) Distant metastasis-free and B) melanoma-specific survival for management in patients with T2 and T3 melanoma and C) distant metastasis-free survival and D) melanoma-specific survival for the type of lymph node dissection in patients with T2 and T3 melanoma.



DISCUSSION

In this retrospective study, the MSS of patients having WLE followed by observation (OBS) was not significantly different from the MSS of those having WLE and SNB. However, SNB patients had improved DFS and improved regional control compared with patients not undergoing SNB.

Several previous retrospective studies have also compared the outcomes of SNB patients with that of OBS patients (Table 4).[12-17] To overcome the limitations of retrospective studies, prospective RCTs are necessary. A large RCT addressing the issue of SNB or nodal observation is MSLT-I, the primary aim of which is to report the outcome following SNB or OBS in patients with intermediate thickness melanomas (1.2-3.5mm).[1] In the third interim analysis of MSLT-I, patients who underwent SNB had no improved MSS over patients in the OBS group. However, the subgroup of SN-positive patients had significantly prolonged MSS after undergoing early CLND compared to WE-only patients who had a delayed TLND for regional node recurrence.[1]

Table 4 – Published series reporting overall (OS) or melanoma-specific (MSS) and disease-free survival (DFS) for sentinel node biopsy (SNB) and observation (OBS) patients respectively, following immediate completion lymph node dissection after a positive sentinel-node biopsy (CLND) and delayed therapeutic lymph node dissection (TLND) after regional lymph node recurrence

Author (year)	**Group of patients**	**Number of patients**	**Median follow-up** (mo)	**Thickness** (mm)	**p†** (DFS)	**p†** (MSS/OS*)	**HR MSS/OS***	**5-yr MSS/ OS*** (%)
Mohrle	OBS	2617	58					NA
(2004)	SNB	271	35	Any	NA	0.37	0.80	NA
Gutzmer	OBS	377	59.7					NA
(2005)	SNB	296	35.5	Any	0.0064	0.317	0.98	NA
Morton	OBS	500	48.4					86.6
(2006)	SNB	769	48.4	1.20 - 3.50	0.009	0.58	0.92	87.1
Koskivuo	OBS	616	74					85.2
(2007)	SNB	305	16	Any	0.414	0.656	0.70	87.8
	OBS	324	74					NA
	SNB	159	16	> 1.00 - 4.00	0.499	0.646	NA	NA
Starz	OBS	61	115					±93
(2007)	SNB	87	74	0.76 - 1.00	0.01	0.03	NA	100
Leiter	OBS	440	57.6					81.5
(2010)	SNB	439	54.3	> 1.00	0.003	0.09	0.74	85.5
Satzger	OBS	377	64.0					80.3
(2010)	SNB	296	72.5	> 1.00	0.001	0.049	NA	84.8
Present	OBS	2931	40					85.8
study	SNB	2909	44	Any	<0.001	0.561	0.96	85.0
	OBS	1726	40					85.3
	SNB	2168	44	> 1.00 – 4.00	<0.001	0.011††	0.79	86.8
	OBS	2120	40					82.2
	SNB	2581	44	> 1.00	<0.001	0.012††	0.82	83.6
Morton	CLND	287						73
(2003)	TLND	287	12-360	Any	NA	<0.001	2.0	51
Kretschmer	CLND	314	32					62.5
(2004)	TLND	623	123	Any	NA	0.002	1.82	50.2
Morton	CLND	122	48.4					72.3
(2006)	TLND	78	48.4	1.20 - 3.50	NA	0.004	1.95	52.4
Van Akkooi	CLND	64	37					±70
(2007)	TLND	124	56	Any	NA	0.115	1.60	±57
Nowecki	CLND	258	35					52.5
(2008)	TLND	286	37	Any	NA	0.04	1.24	39.5
	CLND	111	35					57.2
	TLND	100	37	> 1.00 - 4.00	NA	0.0006	NA	37.9
Pasquali	CLND	100	50					68.9
(2010)	TLND	90	56	Any	NA	0.17	1.07	50.4
	CLND	58	50					71
	TLND	58	56	> 1.00 - 4.00	NA	0.49	NA	57.8
Leiter	CLND**	72	47.0					52.9
(2010)	TLND	72	48.0	> 1.00	NA	0.196‡	2.2	42.0

Table 4 (continued)

Author (year)	Group of patients	Number of patients	Median follow-up (mo)	Thickness (mm)	p† (DFS)	p† (MSS/OS*)	HR MSS/OS*	5-yr MSS/ OS* (%)
Satzger (2010)	CLND	77	72.5					NA
	TLND	147	64.0	> 1.00	NA	0.006	NA	NA
Present study	CLND	394	44					64.1
	TLND	417	40	Any	<0.001	0.146	1.18	60.5
	CLND	256	44					68.3
	TLND	290	40	> 1.00 - 4.00	<0.001	0.147	1.24	62.7
	CLND	374	44					63.1
	TLND	364	40	> 1.00	<0.001	0.149	1.19	57.9

OBS = Observation, SNB = Sentinel Node-biopsy, NA= Not applicable/not reported, CLND = early Completion Lymphadenectomy after a positive SNB, TLND = delayed Therapeutic Lymphadenectomy when melanoma recurred in the regional lymph node in follow-up

** When assessed in paper, melanoma specific survival results are shown above. Otherwise, overall survival results are shown.*

***Patients with recurrence after a negative SNB followed by a delayed TLND were added to the CLND group.*

† results of univariate analysis

†† not significant after adjusting for prognostic factors in multivariate analysis

ǂ p=0.009 in multivariate analysis

Our results also suggest that patients with intermediate thickness melanomas (defined as >1.0–4.0mm in our study) who undergo SNB and early CLND if SN-positive may have an overall survival benefit. SNB patients with melanomas ≤1mm in thickness did not have a survival benefit in our dataset. Patients with melanomas >1.0-4.0mm who underwent SNB had significantly better DMFS and MSS than OBS patients in univariate analysis. Patients with T1 melanomas (≤1.0mm) might not demonstrate a benefit from SNB because they have a very low rate of distant metastasis (Table 2). Conversely, patients with melanomas >4mm have a high rate of distant metastasis regardless of management, suggesting that they might not obtain a survival benefit from SNB[29]. Nevertheless, these patients are likely to benefit not only from the prognostic information SNB provides, but also from the improved node field control provided by early CLND.[29]

Of the 2515 SN-negative patients in the study, 89 (3.5%) had a first recurrence in the regional lymph node field. The clinical false-negative rate is conventionally reported as the rate of patients with a negative SN procedure who have a first recurrence in the same regional lymph node field as the SN procedure (false-negative / (true positive + false-negative)).[10, 30] Calculated in this way, this study has a SN false-negative rate of 18.4% (89/483). This is in line with rates reported in the literature, which range from 7% to 24.8%.[15, 31] MSLT-I had a SN false-negative rate of 17.6%.[10] The clinical false-negative rate of SNB may result from deficiencies in nuclear medi-

cine, surgery or pathology, or may be a result of biologic events such as the presence of microscopic in-transit disease at the time of SNB, or subsequent metastasis from clinically apparent or occult loco-regional recurrences.[8,32] Our results indicate that the more experience a surgeon has with SNB, the lower the number of false-negative SNs will be. Between 1992 and 2000, the false-negative rate was 23.6% (35 / (35+113)), but between 2001 and 2008 it was 16.1% (54 / (54+281)). Considering that SNB was first introduced in 1992, the reduction in the false-negative rate over time most likely reflects the learning curve of surgeons conducting a new, technically demanding procedure. Approximately one third (909/2909) of patients in the SNB group were treated during the earlier timeframe (1992-2000). A stratified analysis was conducted to assess whether changes in experience with SNB or management over time affected outcome. Separately in the patient cohorts treated in the earlier (1992-2000) and the later (2001-2008) timeframes, MSS was statistically similar, and DFS continued to be statistically different when comparing SNB and OBS groups (data not shown).

The rate of SN-positivity in this study was 13.5%, which is fairly low compared to rates reported in literature, which range from 14% to 29%.[33, 34] However, the median tumor thickness for the patients in the study was 1.70 mm, which is lower than in most other reported studies. Additional NSN positivity was 19.5% (77/394). In the OBS group, 417 (14.2%) patients recurred in the regional lymph node field (Table 1). The hypothesis being tested in MSLT-I is that SNB accurately identifies occult nodal metastases that will grow to palpable size if a "watch and wait" policy is adopted. In our study, SNB identified metastases in 13.5% of patients, with a false-negative result in 3.5% of patients (making a total of 17.0%). This is a higher percentage than the proportion of patients who subsequently had regional node metastases diagnosed clinically in the OBS group (14.2%). This difference of 2.8% requires explanation. The suggestion has been made that some patients with very low volume micrometastatic disease in a SN might never progress to clinically detectable metastatic disease in the node field[35-37] However, the MSLT-I data indicating that all SN-positive patients will eventually develop clinically detectable nodal metastases do not support this concept.[38] The lower rate of detection of metastases in the OBS group in this study is most likely due to the median follow-up time of only 42 months. Longer follow-up of the patients in the present study will undoubtedly identify more patients with nodal metastases in the OBS group if there is the same pattern of time to nodal recurrence in the OBS group as occurred in MSLT-I, where it was not until ten years of follow-up had elapsed that nodal recurrence in the OBS group reached a plateau. This was at a level that was virtually identical to the SN-positive plus false-negative value (20.5% versus 20.8%)[1, 38].

In addition to the inherent biases of any retrospective study, selection bias is an inevitable consequence of the design of the current study due to the exclusion of

patients receiving either ELND or no immediate CLND following a positive SNB. The proportion of patients receiving ELND at MIA varied significantly over time, from 15% of all regional node operations during 1992-2000 to 2% during 2001-2008. Based on the results of the aforementioned analysis stratified by timeframe, this bias likely does not significantly influence our results. In the study, 144 patients were excluded on the basis of no immediate CLND following a positive SNB, of which 113 (78%) were N1a and the remainder (31, 22%) were N2a. As a result there are 82 more N1a (than N2a) patients removed from the dataset who had a lower probability of benefit from SNB compared with N2a patients, however this small number of patients is unlikely to have significantly influenced the results of the study. Nevertheless, selection bias should be considered when interpreting its resultss.

In this large, non-randomized study, the overall outcome of patients having WLE alone was not significantly different from that of patients having additional SNB. However, the results indicate that the outcome for patients with T2 and T3 melanomas (>1.0mm – 4.0mm) may be improved if they undergo SNB, as did the interim results of MSLT-I, a large prospective trial. As well, SNB provided significantly improved regional disease control and overall disease-free survival. SN status was the most important prognostic factor for survival, with disease recurrence and death approximately three times greater for SN-positive patients.

Pending the final results of MSLT-I, it is likely that most clinicians caring for patients with melanomas ≥1.0mm in thickness will continue to recommend SNB as a staging procedure. SNB not only enables patients to be given more accurate prognostic information, but it also improves regional control and DFS. In addition there may be an improvement of MSS in node-positive patients with intermediate thickness melanomas following SNB and early CLND, but long-term follow-up of patients in randomized trials (such as MSLT-I) will be necessary to confirm this. With trials of potentially more effective systemic adjuvant therapies likely to commence soon, SNB will also be necessary for selection and stratification of high risk patients.

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Supplementary Table 1 - Multivariate analysis of regional lymph node recurrence-free survival for the entire cohort and distant metastasis-free survival for patients having T2 and T3 tumors

		Regional Lymph Node Recurrence Free Survival (n=4591)			Distant Metastasis Free Survival (n=3234)		
Factor	**Value**	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Management*	Observation	3.23	2.66 - 3.94	<0.001	1.23	1.01 - 1.50	0.041
Gender	Male	1.36	1.12 - 1.66	0.002	1.36	1.09 - 1.70	0.007
Age	(years)	1.00	0.99 - 1.00	0.134	1.00	0.99 - 1.00	0.538
Breslow thickness	(mm)	1.09	1.05 - 1.12	<0.001	1.53	1.36 - 1.73	<0.001
Mitotic rate	(/mm^2)	1.03	1.02 - 1.04	<0.001	1.02	1.01 - 1.04	0.004
Clark level (ref: II-III)	IV-V	1.41	1.13 - 1.76	0.002	1.29	1.02 - 1.63	0.035
Ulceration	Present	1.78	1.47 - 2.16	<0.001	1.56	1.26 – 1.91	<0.001
Primary site	Trunk	0.74	0.60 – 0.92	0.006	1.39	1.10 - 1.76	0.005
(ref: extremity)	Head & Neck	0.84	0.66 - 1.07	0.147	1.36	1.03 - 1.78	0.029

HR = Hazard Ratio, CI = Confidence Interval
**SNB versus OBS*

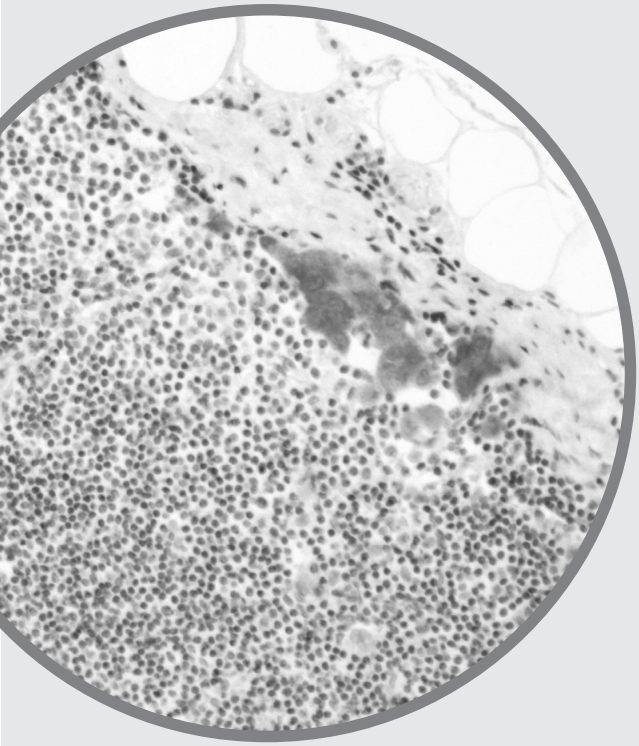
Supplementary Table 2 – Multivariate distant metastasis-free and melanoma-specific survival analyses of the sub-group of patients with nodal metastases

		Distant Metastasis-Free Survival (n=812)			Melanoma Specific Survival (n=812)		
Factor	**Value**	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Nodal Surgery	SN False-Negative	1.68	1.18 - 2.40	0.004	1.63	1.14 - 2.32	0.007
(ref: CLND)	"Delayed" TLND	1.36	1.08 - 1.72	0.010	1.26	0.99 - 1.59	0.057
Gender	Male	1.36	1.08 - 1.72	0.009	1.32	1.05 - 1.66	0.019
Breslow thickness	(mm)	1.13	1.09 - 1.18	<0.001	1.13	1.08 - 1.17	<0.001
Ulceration	Present	1.54	1.23 – 1.93	<0.001	1.71	1.37 - 2.14	<0.001

HR = Hazard Ratio, CI = Confidence Interval

Chapter 3

The unpredictability of lymphatic drainage from the ear in melanoma patients, and its implications for management



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ABSTRACT

The ear is known to have variable lymphatic drainage. The aim of the present study was to better define the lymphatic drainage patterns of the ear by correlating the location of primary tumors, classified according to the embryologically-derived anatomical subunits of the ear, with their mapped sentinel nodes (SNs) identified by lymphoscintigraphy (LS).

Lymphatic drainage data for patients with a primary melanoma of the ear were reviewed, and correlated with the precise primary melanoma site.

Between 1993 and 2010, LS was performed in 111 patients with a primary melanoma on the ear, identifying 281 SNs in 195 lymph node (LN) fields. The mean numbers of SNs and LN fields identified by LS per patient were 2.65 and 1.76. SN biopsy (SNB) was performed in 71 patients (64%). The mean number of SNs removed was 2.36. The 111 ear melanomas were mostly located on the helical rim (55.0%), followed by the lobule (24.3%). The five different primary ear sites drained mainly to SNs in level CII, level CV and the pre-auricular region. Drainage was most often to level CII (36.4%). Drainage to the contralateral neck was not observed.

Lymphatic drainage of the ear has no predictable pattern and can be to SNs anywhere within the ipsilateral neck. Most commonly drainage is to cervical level II and the preauricular and postauricular LN fields. LS defines the lymphatic drainage pattern in individual melanoma patients and is essential for accurate SN identification and reliable SNB.

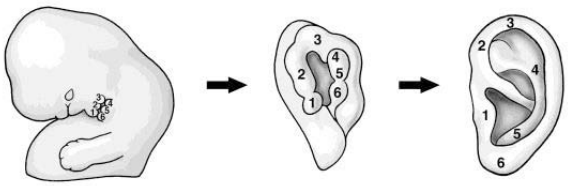
INTRODUCTION

The sentinel node (SN) biopsy (SNB) technique was introduced by Morton et al.[1] In the early 1990's, using lymphatic mapping with blue dye to identify SNs. Subsequently, lymphoscintigraphy (LS) was used to identify SNs preoperatively, and a handheld gamma probe was used intraoperatively to assist in the localization of blue-stained SNs and provide greater reliability of SN identification.[2, 3] SNB has since become a standard procedure for patients with early-stage melanoma in most melanoma treatment centers around the world.[4, 5] In 7-18% of melanoma patients, the primary tumor is located in the head and neck region, and SNB procedures in the head and neck are recognised to be more technically demanding than procedures in the axilla or groin.[6-15] The greater difficulty of SNB for head and neck melanomas is partly due to the unpredictable lymphatic drainage from tumors located in this region.[8, 16-18] Some believe that it is inappropriate to claim that metastatic patterns from head and neck melanomas are unpredictable.[19, 20] Nevertheless, there can be no doubt that preoperative LS is of great value in directing the surgical management of patients with cutaneous head and neck malignancies.[8, 21, 22]

In approximately 1% of patients with cutaneous melanomas, the primary tumor is located on the external ear.[23] The prognosis for these patients is generally believed to be similar to that of patients with melanomas at other cutaneous sites, although some have reported a worse prognosis.[9, 24] What has become clear is that the worse overall prognosis of patients with head and neck melanomas is primarily attributable to melanomas of the scalp, which have a significantly worse prognosis than melanomas arising elsewhere in the head and neck region.[25] Based on concerns about the reported complexity of lymphatic drainage patterns from the ear, the Multicenter Selective Lymphadenectomy Trial (MSLT–1), designed to validate the SN hypothesis and test the reliability of SNB as a staging procedure for melanoma, excluded patients with primary tumors on the ear.[26]

Lymphatic drainage from the skin of the external ear has been studied previously in patients with melanoma.[19, 27-32] These studies considered the ear and immediately surrounding tissues as a single site. However, the ear is comprised of anatomically defined areas related to its embryological development. The auricle arises from the

Figure 1: Embryological development of the external ear



first and second pharyngeal arches via six developmental hillocks; number 1 forms the tragus, numbers 2 and 3 the helical root and rim, number 4 the scapha and anti-helix, number 5 the concha, and number 6 the ear lobe.[33] (Figure 1) Each of these areas might be expected to have different lymphatic drainage.

The purpose of this study was to define the lymphatic drainage patterns of the ear by correlating the precise location of primary tumors, classified according to the embryologically derived anatomical subunits of the ear, with their correspondingly mapped SNs identified by LS.

PATIENTS AND METHODS

Patients

This retrospective study included patients with melanomas located on the external ear, treated at Melanoma Institute Australia (MIA) (formerly the Sydney Melanoma Unit) between 1993 and 2010, who underwent lymphatic mapping using lymphoscintigraphy. From the prospectively collected information in the MIA database, data for 111 patients were extracted, including patient and primary melanoma characteristics, pathology reports, lymphoscintigraphy reports, operation details and follow-up information.

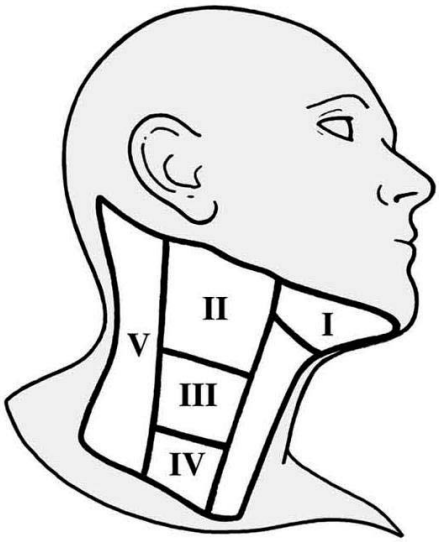
Primary melanomas

The location of each primary melanoma was documented as part of the routine recording of lymphoscintigraphy injections, using a graphics grid superimposed over the outline of an ear. X and Y co-ordinates were recorded for each primary site. The ear was subdivided into five sites: lobule, tragus, concha, scapha/anti-helix and helical rim. The helical rim included all tissue 5 mm on either side of the most lateral part of the ear margin, extending from the helical root medially and then superiorly down to the lobule. (Figure 1) Patients were grouped into these five anatomical sites according to the location of their primary tumor.

Lymphatic mapping

Lymphoscintigraphy utilizing Technetium 99 m antimony trisulfide colloid, which has a particle size of 5-40 nm, was performed using a standardized technique within the 24hr period before surgery.[34] Standard regional surface anatomy classification was used by the nuclear medicine physician to record the location and number of the SNs that were identified, i.e., the cervical levels I-V (CI-CV), postauricular, preauricular and supraclavicular lymph node regions.[35] (Figure 2) The lymphatic drainage from the five anatomical sites of the primary on the ear to the lymph node fields classified according to the surface anatomy was evaluated. All identified SNs were

Figure 2: Location of the five cervival levels



examined with high-resolution ultrasound immediately after lymphoscintigraphy to assess the internal architecture of each node for abnormalities, which if seen could represent metastatic disease. Ultrasound further assisted in the localization of each node, particularly its depth beneath the skin surface.

Sentinel node biopsy

Thirty-seven patients (33.3%) did not undergo SNB after lymphoscintigraphy. Many of these were patients undergoing wide excision only (i.e., without SNB), when SNB was considered likely to be unduly complicated (e.g., when multiple SNs in different node fields were found), when the possibility of morbidity was a concern (e.g., when intraparotid SNs were identified), or if the patient was elderly or had significant medical comorbidities. Patients who did not undergo SNB after wide excision of their primary tumor underwent serial clinical review and periodic repeat high-resolution ultrasound examination of the SNs that had been identified on the preoperative lymphoscintigram.

SNB was performed under general anesthesia utilizing conventional techniques. Pre-operatively, 0.3–1 ml of Patent Blue V dye (Guerbert, Aulnay-Sous-Bois, France) was injected intradermally close to the centre of the excision-biopsy site. Intraoperatively, a handheld gamma probe was used to confirm the location of the SNs that had been identified by LS. Wide local excision of the primary melanoma site on the ear was routinely performed before the SNB, thereby reducing radioactivity in the region of the primary tumor site and facilitating SN identification with the gamma probe.

Follow-up

Median follow-up time was 30 months (interquartile (IQR) range 13 – 60 months). Time to recurrence and survival time were calculated from the date of primary diagnosis to the date of recurrence, date of death due to melanoma, or date of last follow-up.

RESULTS

Patients

Between 1993 and 2010, 111 patients with a primary melanoma located on the ear underwent lymphatic mapping using lymphoscintigraphy at Melanoma Institute Australia (MIA), Sydney, Australia. There was a predominance of males (80.2%, n=89) compared with females (19.8%, n=22). The median age was 64 years (IQR 44 - 74 years).

Primary melanoma site

Fifty-six (50.5%) of the patients had a primary melanoma on the left ear and 55 (49.5%) a melanoma on the right ear. Median Breslow thickness was 1.90 mm (IQR 1.20 – 3.20 mm). The primary tumor was ulcerated in 32 patients (33.0%). (Table 1) The distribution of primary disease by anatomical location is shown in Table 1.

Lymphoscintigraphy

The number and location of SNs identified by lymphoscintigraphy (LS) are documented in tables 1 and 2. Although technically part of levels CIV and CV, the "supraclavicular" designation was included as a separate field, because the reporting nuclear medicine physician had specifically indicated this as the SN location. Drainage to the contralateral neck was not observed.

Table 1 – Baseline characteristics of the patient, the primary melanoma disease, lymphoscinitgraphy and sentinel node biopsy

Characteristic	N (%)	Characteristic	N (%)
Gender		**Number of SNs identified on LS**	
Female	22 (19.8)	Median (IQR)	3 (2 – 3)
Male	89 (80.2)	1	12 (11.3)
Age (years)		2	40 (37.7)
Median (IQR)	64 (44 – 74)	≥ 3	54 (50.9)
< 60	48 (43.2)	Missing	5
≥ 60	63 (56.8)		

Table 1 (continued)

Characteristic	N (%)	Characteristic	N (%)
Primary, LS and SNB side		**Drainage sites of identified SNs†**	
Left	56 (50.5)	Preauricular	39 (20.0)
Right	55 (49.5)	Cervical I	3 (1.5)
Site of primary on the ear		Cervical II	71 (36.4)
Lobule	27 (24.3)	Cervical III	3 (1.5)
Tragus	4 (3.6)	Cervical IV	3 (1.5)
Concha	14 (12.6)	Cervical V	22 (11.3)
Scapha / Anti-helix	5 (4.5)	Postauricular	51 (26.2)
Helical rim	61 (55.0)	Supraclavicular	3 (1.5)
Breslow (mm)		**Number of drainage sites**	
Median (IQR)	1.90 (1.20 – 3.20)	1	47 (42.3)
≤ 1.00	21 (19.1)	2	48 (43.2)
> 1.00 – ≤ 2.00	43 (39.1)	3	12 (10.8)
> 2.00 – ≤ 4.00	28 (25.5)	4	4 (3.6
> 4.00	18 (16.4)	**Surgery**	
Missing	1	No	37 (33.0)
Mitoses		SNB	71 (64.0)
Median (IQR)	3 (2 – 6)	LND	3 (2.7)
0	9 (9.0)	**Number of harvested SNs***	
≥ 1	91 (91.0)	Median (IQR)	2 (2 – 3)
Missing	11	1	15 (22.4)
Clark level		2	27 (40.3)
II	8 (7.5)	≥ 3	25 (37.3)
III	24 (22.6)	Missing	4
IV	56 (52.8)	**SN status***	
V	18 (17.0)	Negative	62 (92.5)
Missing	5	Positive	5 (7.5)
Ulceration		Missing	4
Absent	65 (67.0)	**SN removed / identified ratio***	
Present	32 (33.0)	Median (IQR)	1.00 (0.67 – 1.00)
Missing	14	< 1 (Incomplete SNB)	25 (38.5)
		≥ 1 (Complete SNB)	40 (61.5)
		Missing	6

IQR = Interquartile range; LS = Lymphoscintigraphy; SNB = Sentinel Node Biopsy; LND = Lymph Node Dissection

†The total number of 195 drainage site is larger than 111 since multiple drainage sites were identified in 57.3% of the patients.

** The number of 111 is not reached for these characteristics since 71 of the 111 patients underwent sentinel node biopsy*

Table 2 – Lymphatic drainage patterns from different primary melanoma sites on the ear

			Number of times an SN has been identified (%)*							
Anatomical Site on Ear	**Number of patients / primaries (%)**	**Number of LN fields with SNs (%)**	**Pre- auricular**	**CI**	**CII**	**CIII**	**CIV**	**CV**	**Post-auricular**	**Supra-clavicular**
Lobule	27 (24.3)	40 (20.5) (100.0)	6 (15.4) (15.0)	1 (33.3) (2.5)	23 (32.4) (57.5)	0 (0.0) (0.0)	0 (0.0) (0.0)	2 (9.1) (5.0)	5 (9.8) (12.5)	3 (100.0) (7.5)
Tragus	4 (3.6)	9 (4.6) (100.0)	4 (10.3) (44.4)	0 (0.0) (0.0)	3 (4.2) (33.3)	0 (0.0) (0.0)	0 (0.0) (0.0)	2 (9.1) (22.2)	0 (0.0) (0.0)	0 (0.0) (0.0)
Concha	14 (12.6)	24 (12.3) (100.0)	7 (17.9) (29.2)	2 (66.6) (8.3)	7 (9.9) (29.2)	0 (0.0) (0.0)	0 (0.0) (0.0)	1 (4.5) (4.2)	7 (13.7) (29.2)	0 (0.0) (0.0)
Scapha / anti-helix	5 (4.5)	6 (3.1) (100.0)	3 (7.7) (50.0)	0 (0.0) (0.0)	1 (1.4) (16.7)	0 (0.0) (0.0)	0 (0.0) (0.0)	2 (9.1) (33.3)	0 (0.0) (0.0)	0 (0.0) (0.0)
Helical rim	61 (55.0)	116 (59.5) (100.0)	19 (48.7) (16.4)	0 (0.0) (0.0)	37 (52.1) (31.9)	3 (100.0) (2.6)	3 (100.0) (2.6)	15 (68.2) (12.9)	39 (76.5) (33.6)	0 (0.0) (0.0)
Total	111 (100.0)	195 (100.0) (100.0)	39 (100.0) (20.0)	3 (100.0) (1.5)	71 (100.0) (36.4)	3 (100.0) (1.5)	3 (100.0) (1.5)	22 (100.0) (11.3)	51 (100.0) (26.2)	3 (100.0) (1.5)

** Explanation of the numbers of percentages between brackets;*
Vertically, the percentage within the LN field is described (= the number of times this LN field was detected corresponding to the specific location on the ear / total number of times this LN field was detected corresponding to ANY location).
Horizontally, the percentage within the primary site is described (= the number of times this LN field was detected corresponding to the specific location on the ear / total number of times this LN field was detected corresponding to this SPECIFIC location)

Surgery

Sentinel node biopsy (SNB) was performed in 71 patients (64.0%) and a lymph node dissection (LND) was performed in 3 patients (2.7%). (Table 1) No nodal surgery was performed in 37 (33.0%) patients, for the reasons outlined previously. Full details of the SN results are given in tables 1 and 3.

Eight of the 158 SNs (5.0%) that were removed contained metastatic melanoma, whereas 2 of the 52 (3.8%) NSNs that were removed were positive. In the 67 patients with known SN status, the SN positivity rate was 7.5% (n=5). An incomplete SNB procedure was recorded when less SNs were removed than were identified by LS. Using this criterion, SNB was complete in 61.5% of the patients. (Table 3).

Three patients did not undergo SNB, but underwent a complete cervical LND. The number of nodes removed was 18, 21 and 46. The number of positive nodes was 5, 0 and 1, respectively.

Table 3 – Numbers of sentinel nodes identified and removed for 65 patients

	Number of SNs removed						
Number of SNs identified	**1**	**2**	**3**	**4**	**5**	**7**	**Total Patients**
1	4			1			5
2	6	16	3	1	1		27
3	3	9	9	2		1	24
4	1	2	2	2			7
5				1			1
8				1			1
Total Patients	14	27	14	8	1	1	65

The gray area corresponds with incomplete SNBs performed i.e. when fewer SNs were removed than were identified by lymphoscintigraphy

Follow-up

Mean and median follow-up times were 41 and 30 months (IQR 13 – 60 months). Nine patients were lost to follow-up (8.1%). Of 102 patients with follow-up, 14 (13.7%) recurred, with mean and median times to first recurrence of 28 and 10 months (IQR 7–36): 12 patients had locoregional recurrence as a first recurrence; 2 patients had visceral metastases as a first recurrence.

Excluding the 3 patients who had a complete LND and no SNB, 6 of 37 (16.2%) patients without SNB recurred, whereas 8 of 71 (11.3%) SNB patients had any type of recurrence. At the time of last follow-up, 3 of the 102 patients (2.9%) had died of melanoma, whereas 91 patients were alive with no disease (89.2%). Two patients were alive with melanoma, four had died of other known causes, one was alive with unknown status and one had died of an unknown cause.

DISCUSSION

This study confirms that the lymphatic drainage pattern from melanomas on the ear is unpredictable, with drainage to eight different lymph node fields within the head and neck region. Five different primary sites on the ear were identified, based on anatomically defined areas relating to embryological development. The frequency and location of primary tumors found in this study, classified by auricular anatomical subunits, matched previous reports for melanoma.[29, 36]

Determination of lymphatic drainage patterns requires precise documentation of the primary tumor site and accurate mapping of the draining SNs. X-Y coordinates, routinely documented to record the LS injection site, were interpreted to represent the location of the primary tumor. SNs were most frequently identified in level CII (36.4%), the postauricular region (26.2%), the preauricular region (20.0%) and level CV (11.3%). Stratification by embryologically derived anatomical subunits confirmed that the lymphatic drainage pattern from each specific location was unpredictable with drainage to between three and six different LN fields from each location. (Table 2)

Lymphatic drainage of the ear has been investigated previously, either as part of an overall review of head and neck malignancy or in the context of regionally recurrent disease.[19, 27-32] Reynolds et al[32] used data from MIA to produce an elegant interactive internet-based software tool (http://www.bioeng.auckland.ac.nz/head), which depicts lymphatic drainage of the head and neck. This tool suggests that a primary located on the ear would most often be expected to drain to cervical level II (69% for the left ear and 59.1% for the right ear). As far as we are aware, the only previous study that has related the anatomical subunits of the ear to the subsequently identified SNs was by Cole et al.[29] Only 9 of their 19 patients underwent SN mapping by LS. Lymphatic drainage patterns were reported to be highly variable and unpredictable, but the site-specific drainage patterns were not evaluated.[29] Our study, specifically examining drainage from anatomical subunits on the ear, along with several other studies of head and neck malignancies, confirms the highly variable pattern of lymphatic drainage from melanoma sites on the head and neck.[8, 16-18, 28, 29]

Gray's textbook of anatomy divides the ear into three sections when discussing its lymphatic drainage: an upper lateral area that drains to superficial parotid nodes; an upper medial area that drains to postauricular and deep upper cervical nodes; and the ear lobule that drains to superficial or deep upper cervical nodes.[37] This ordered arrangement of lymphatic drainage is not what we found. (Table 2) Drainage was mostly to nodes in close proximity to the primary site but not necessarily to an immediately adjacent node. There was no contralateral node involvement, although lymphatic drainage to the contralateral neck has been reported from other cutaneous head and neck sites.[11]

In general, the establishment of lymphatic drainage follows the embryological pattern of vascular development. We have shown that 83.6% of peripherally located primaries, i.e., on the lobule or helical rim, drain directly to the posterior auricular area as would be predicted. However, the terminal branches of the post-auricular artery are well-documented perforating vessels that pass through the anterior and posterior surfaces of the ear before finally anastamosing anteriorly with branches of the superficial temporal artery.[33] Lymphatic vessels accompanying these perforating vessels are likely to explain our unexpected observation of transaural lymphatic drainage to postauricular nodes in 29% of conchal primary sites.

This study has confirmed that lymphatic drainage of the ear is highly variable; therefore, it follows that no specific selective neck lymph node dissection can be recommended for an ear primary without lymphatic mapping for that individual. However, certain observations are relevant to the planning process: up to 30% of patients displayed lymphatic drainage to SNs anterior and posterior to the ear; the lobule was the only site to drain to the supraclavicular region; levels CII, CV and preauricular nodes were the only areas to receive drainage from all sites on the ear; the lobule and concha were the only sites to drain to level CI; however, these nodes only accounted for 1.5% of all nodes retrieved and 4.5% of all nodes associated with these two sites (Table 2). Dissection of the postauricular region is not included in a standard elective node dissection in head and neck surgery.[11] However, we found that postauricular drainage occurred from the concha, helical rim and lobule in 28% of all nodes associated with these three sites, which accounted for 26% of all nodes retrieved.

Based on Table 2, this study could be used to set a threshold of identified nodes below which a specific region is not dissected, e.g., 6% for the concha would mean the dissection should include preauricular and postauricular nodes and those in levels CI and CII. Raising the threshold to 10% would mean excluding the level CI nodes. However, the numbers were small in many of the subgroups and we do not think this is a safe argument. We recommend that CLND following a positive SNB should include at least all of the levels mapped by the LS. Our study highlights those unexpected regions of drainage that could be considered for inclusion in a lymph node dissection, particularly for macroscopic disease, rather than those that should be excluded.

In conclusion, lymphatic drainage from melanomas of the ear occurs in a retrograde, antegrade or transaural pattern in our study but never contralaterally. The most frequent sites for SNs from ear melanomas were level CII and the preauricular and postauricular LN fields; this knowledge may influence surgical planning for a cervical lymph node dissection. Even in this group of patients with clearly defined anatomical boundaries, detailed primary site information and high quality lymphatic

mapping, we were unable to demonstrate any correlation between the location of the primary tumor on the ear, its embryological development and the pattern of lymphatic drainage.

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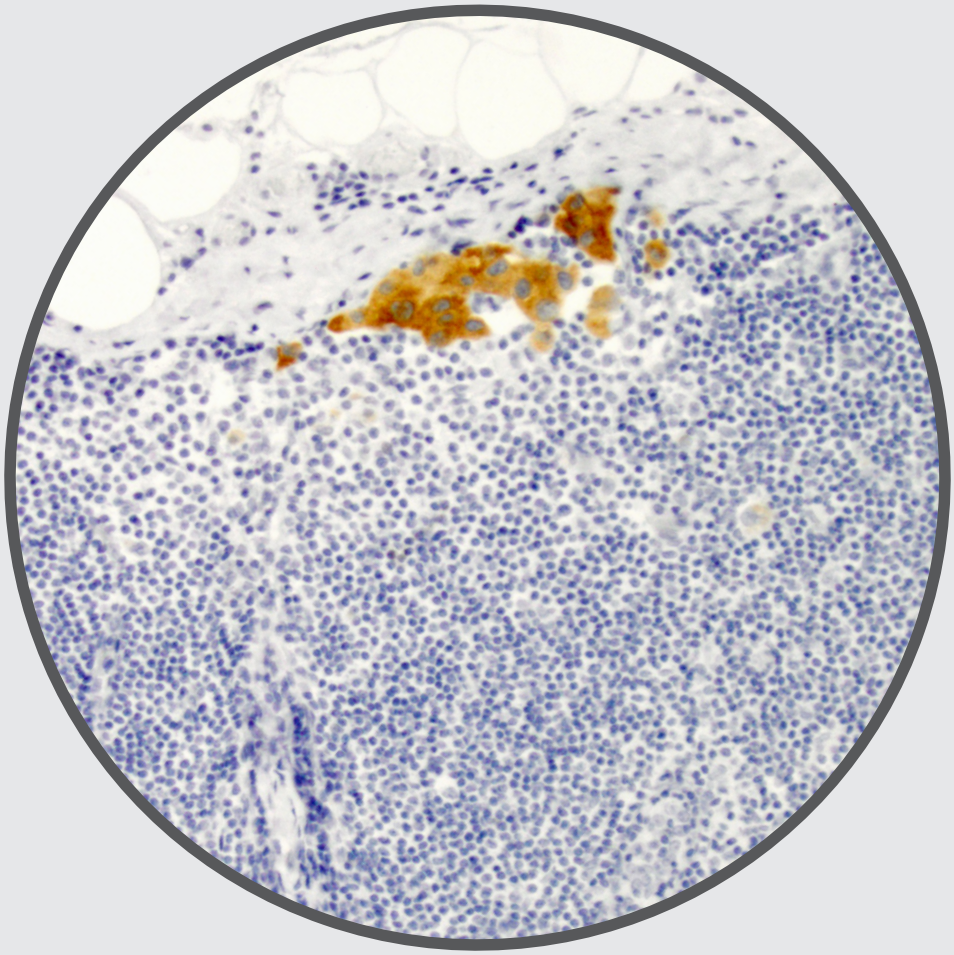


Part II

Management and prognosis of stage III melanoma; micrometastases

Chapter 4
EORTC Melanoma Group sentinel node protocol identifies high rate of submicrometastases according to Rotterdam criteria
European Journal of Cancer. 2010 Sep;46(13):2414-21

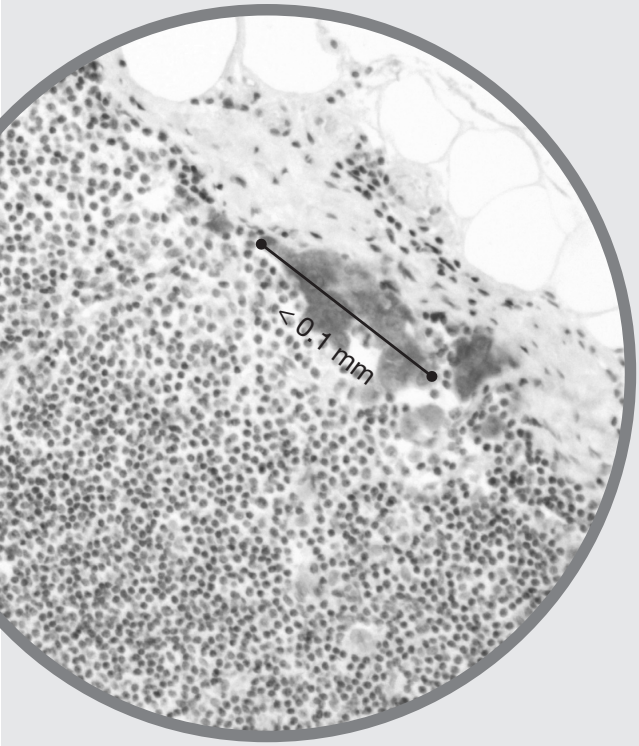
Chapter 5
Prognosis in patients with sentinel node-positive melanoma is accurately defined by the combined Rotterdam tumor load and Dewar topography criteria
Journal of Clinical Oncology. 2011 Jun 1;29(16):2206-14

Chapter 6
The prognostic significance of sentinel node tumor burden in melanoma patients: an international, multicenter study of 1539 sentinel node-positive melanoma patients
European Journal of Cancer. 2013 Sep 25

Chapter 7
Prognosis in patients with sentinel node-positive melanoma without immediate completion lymph node dissection
British Journal of Surgery. 2012 Oct;99(10):1396-405.

Chapter 4

EORTC Melanoma Group sentinel node protocol identifies high rate of submicrometastases according to Rotterdam Criteria



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European Journal of Cancer. 2010 Sep;46(13):2414-21

ABSTRACT

Sentinel node (SN) status is the most important prognostic factor for disease free (DFS) and overall survival (OS) in stage I-II melanoma. We evaluated the positive sentinel node identification rate of the EORTC Melanoma Group (MG) protocol as well as its capacity to identify minimal tumour burden, according to the Rotterdam Criteria in 421 consecutive patients. Correlations between primary tumour characteristics and SN tumor burden were investigated. The same 2 pathologists worked up all SNs according to the EORTC MG protocol and tumour burden was scored according to the Rotterdam Criteria (<0.1 mm, 0.1 – 1.0 mm and >1.0 mm for the largest diameter of the largest metastasis in the SN).

The positive SN detection rate was 28.7% with a false negative rate of 10.4% at a median Breslow thickness of 2.1 mm. The high positive identification rate of about 30% of the EORTC MG protocol has been confirmed in this study. The protocol is sensitive and identifies submicrometastases (<0.1 mm) in a high percentage (18%). The variables SN tumour load, non SN (NSN) status and ulceration of the primary, were independent prognostic factors for DFS and OS in the multivariate analysis. At a median follow up time of 4.3 years patients with minimal tumour burden (<0.1 mm) had a 5 year OS rate of 91%, virtually identical to 90% for SN negative patients. The NSN positivity rate of 0% in these patients indicates that they may be spared a completion lymph node dissection (CLND) and its morbidity.

INTRODUCTION

In the early 90s Morton and colleagues introduced a new procedure for clinical stages I-II melanoma, the sentinel lymph node (SN) procedure.[1] The SN is the first regional lymph node for tumour cells spreading from the primary tumour. Thus it may be the first site to demonstrate that a primary melanoma may have spread, as regional lymphatic spread appears in general more frequently as first dissemination site than distant haematogenous spreading sites.[1] The SN status is the most important prognostic factor for disease free (DFS) and overall survival (OS) in stage I - II melanoma.[2] Although the SN procedure has not been demonstrated to have a therapeutic effect,[2] it has become a widely accepted diagnostic procedure for patients with clinically negative lymph nodes.

Identification rates of SN positivity in patients with melanoma are described in many studies. SN positivity rates differ from 13.9%[3] to 29.4% in our institute.[4] These differences may occur due to differences in primary tumour characteristics, different populations or different protocols for histopathological workup.

The aim of this single institute retrospective study was to evaluate the positive sentinel node identification rate of the EORTC Melanoma Group (MG) protocol as well as its capacity to identify minimal tumour burden, according to the Rotterdam Criteria. Correlations between primary tumour characteristics and SN tumour burden were investigated.

PATIENTS AND METHODS

Patients

From October 1997 to December 2008, 421 patients with malignant melanomas underwent a SN procedure at our institute (Erasmus University Medical Centre, Daniel den Hoed Cancer Centre, Rotterdam, the Netherlands). Data of all patients were included in this retrospective, single institute study and were collected into a database with patient, primary tumour and follow-up data.

Median age was 49 years (range 15 – 83 years). Mean and median Breslow thickness was 2.79 mm and 2.10 mm (range 0.30 – 15.00 mm) respectively. Baseline characteristics are described in Table 1.

All patients underwent therapeutic re-excision of the melanoma before the SN procedure according to National Guidelines. Tumour-free margins of at least 1 cm were achieved in melanomas smaller than or equal to 2 mm Breslow thickness. Melanoma larger than 2 mm in Breslow thickness were excised with a tumour-free margin of at least 2 cm or less for distal acral and head and neck primaries for

Table 1 – Patient characteristics for all 421 patients.

	N	2010 (%)	2006 (%)
Gender			
Male	204	49	44
Female	217	51	56
Age			
≤ 50 yrs	222	53	52
> 50 yrs	199	47	48
Melanoma location			
Extremities	224	53	58
Trunk	174	41	35
Head & neck	23	6	7
Histology			
SSM	206	48	48
NM	138	33	34
ALM	7	2	2
Other	9	2	1
Unclassified	61	15	15
Breslow thickness			
≤ 1.00 mm	18	4	5
1.01 – ≤ 2.00 mm	182	43	45
2.01 – ≤ 4.00 mm	137	33	30
> 4.00 mm	75	18	16
Unknown	9	2	4
Clark			
II	8	2	2
III	159	38	42
IV	200	47	46
V	29	7	4
Undeterminable	25	6	6
Ulceration			
Present	119	37	28
Absent	202	63	72
SN status			
Negative	300	71.3	70.6
Positive	121	28.7	29.4
Rotterdam Criteria			
< 0.1 mm	22	18	-
0.1 – 1.0 mm	57	47	-
> 1.0 mm	42	35	-
NSN status			
Negative	95	89	85
Positive	12	11	15

SSM = Superficial Spreading Melanoma, NM = Nodular Melanoma, ALM = Acrolentiginous Melanoma, SN = Sentinel Node, NSN = Non Sentinel Node.

feasibility limits or cosmetic reasons. Finally, the defected areas were closed via primary closure or split skin graft. At the same time as wide local excision of the malignant melanoma the SN procedure was performed.

The SN procedure was offered to patients with Breslow thickness >1.0 mm or to patients with histopathological features as ulceration or Clark level IV or V (see Table 2).

Table 2 – Cox univariate regression analyses of disease-free and overall survival.

Univariate	**DFS**			**OS**		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Gender						
Female	1			1		
Male	1.31	0.90 – 1.90	0.16	1.73	1.09 – 2.76	0.02
Age						
≤ 50 yrs	1			1		
> 50 yrs	1.31	0.90 – 1.91	0.15	1.32	0.83 – 2.09	0.24
Location						
Extremities	1			1		
Head and neck/trunk	1.53	1.05 – 2.23	0.03	1.90	1.19 – 3 03	0.006
Histology						
SSM	1			1		
NM	1.70	1.13 – 2.55	0.01	2.03	1.24 – 3.32	0.004
Breslow						
≤ 2.00 mm	1			1		
2.01 – ≤ 4.00 mm	1.91	1.20 – 3.05		1.87	1.05 – 3.34	
> 4.00 mm	3.53	2.18 – 5.70	< 0.00005	3.59	2.00 – 6.46	< 0.00005
Clark						
II, III	1			1		
IV	1.13	0.74 – 1.72		0.85	0.51 – 1 41	
V	2.80	1.49 – 5.28	0.003	2.23	1.02 – 4.91	0.044
Ulceration						
Absent	1			1		
Present	2.49	1.71 – 3.64	< 0.00005	3.40	2.14 – 5.39	< 0.00005
SN status						
Negative	1			1		
Positive	3.75	2.57 – 5.47	< 0.00005	3.64	2.29 – 5.77	< 0.00005
NSN status						
Negative	1			1		
Positive	6.43	3.90 – 10.60	< 0.00005	3.92	2.11 – 7.30	< 0.00005

Table 2 (continued)

Univariate	DFS			OS		
	HR	95% CI	P	HR	95% CI	P
Rotterdam Criteria						
Negative	1			1		
<0,1	1.06	0.38 – 2.93		0.82	0.20 – 3.42	
0,1-1,0	3.90	2.45 – 6.22	< 0.00005	3.47	2.00 – 6.01	
>1,0	6.86	3.99 – 11.82		6.62	3.69 – 11.87	< 0.00005
Rotterdam Criteria						
Negative, < 0.1	1			1		
0.1 – 1.0	3.83	2.44 – 5.99		3.58	2.08 – 6.18	
> 1.0	7.21	4.45 – 11.68	< 0.00005	7.29	4.08 – 13.02	< 0.00005

SSM = Superficial Spreading Melanoma; NM = Nodular Melanoma; SN = Sentinel Node; NSN = Non Sentinel Node

Sentinel lymph node procedure

At our centre the SN is identified by the use of the triple technique, described in detail elsewhere.[4] Basically, patients are first seen at the nuclear medicine department for a pre-operative lymphoscintigraphy (LS). The LS should be undertaken within 24 h of the operation being performed, by four intradermal injections of radioactive nanocolloid around the primary tumour or the scar of the primary tumour excision. Scanning should be carried out immediately after the injection for approximately 10 – 15 min and again (=delayed) after 2 hours. Secondly, intraoperative use of a handheld gamma detection probe should be used to verify the location of the SNs. Thirdly, patent blue should be injected pre-operatively in the operating theatre, again through four intradermal injections around the primary tumour or the scar of the primary tumour excision (this does not have to be the same 4 locations). The blue is also used to verify the identity of the SNs. A lymph node was considered to be a SN if it was stained blue, or if it had an in situ radioactivity count of at least three times that of the background count, or if it had an ex vivo radioactivity count of at least ten times greater than that of the background count.

After the surgical procedure the SNs will be sent to the pathology department for examination and in positive cases to establish the SN tumour burden.

Pathological features

All SNs were worked up according to the EORTC MG protocol.[5] Within 24 h lymph nodes are placed in formalin. After fixation the SN will be bivalved through the hilum. Each half sentinel node will be examined in six serial step sections cut at 50 µm intervals. All sections are stained with H&E and S100 and/or Melan A. Spare sections are made at each level for a number of difficult cases where additional

immunochemistry is needed. Two individual specialized pathologists at our institute worked up all SNs. Tumour burden was scored according to the Rotterdam Criteria (< 0.1 mm, 0.1 – 1.0 mm and > 1.0 mm largest diameter of the largest metastasis in the SN).[6]

Follow-up

Most patients were followed in our outpatient's clinic. Some patients were followed at other hospitals by dermatologists or surgeons. Follow-up time was defined as the date between the SN procedure and the date of last follow-up or death. Recurrence sites were scored as primary relapse, in-transit metastasis, local regional lymph node metastasis, distant subcutaneous, distant lymph node metastasis or visceral metastasis.

Statistics

Univariate analyses of potential prognostic factors were performed using the Kaplan-Meier method and the logrank test. Multivariate analyses of the significant factors in the univariate analyses were performed with Cox proportional hazards regression. (Table 3 and 4)

Table 3 – Cox multivariate proportional hazard regression analyses of disease-free and overall survival

Multivariate	DFS			OS		
	HR	95% CI	P	HR	95% CI	P
Location						
Extremities				1		
Head and neck/Trunk				2.95	1.73 – 5.03	< 0.0005
Histology						
SSM				1		
NM				1.86	1.07 – 3.26	0.03
Ulceration						
Absent	1			1		
Present	2.23	1.49 – 3.33	< 0.0005	2.76	1.55 – 4.90	0.001
NSN status						
Negative	1			1		
Positive	4.80	2.75 – 8.35	< 0.0005	4.29	2.13 – 8.65	< 0.0005
Rotterdam Criteria						
Negative, < 0.1 mm	1			1		
0.1 – 1.0 mm	2.63	1.63 – 4.24		2.20	1.18 – 4.11	
> 1.0 mm	5.00	2.75 – 8.35	< 0.0005	4.27	2.27 – 8.02	< 0.0005

SSM = Superficial Spreading Melanoma, NM = Nodular Melanoma, NSN = Non Sentinel Node.

Table 4 – Non-sentinel node (NSN) positivity rates.

	SN positivity rate	# Patients SN positive	NSN positivity rate	# Patients with additional NSN tumour after positive SN(s)
Our institute	28.7%	121 (0.287*421)	11.2%	14 (0.112*121)
Other literature	20% (13.9%-29.4%)	84 (0.2*421) (58.5-123.8) (0.139*421-0.294*421)	20% (14-28%)	17 (0.2*84) (8.2-34.7) (0.14*58.5-0.28*123.8)

SN = Sentinel Node; NSN = Non Sentinel Node

Analyses were executed with the following variables: sex (female or male), age (≤ 50 or > 50 years), location of the melanoma (extremities or trunk/head&neck), histology of the melanoma (superficial spreading melanoma (SSM) or nodular melanoma (NM)), Breslow thickness (≤ 2.00 mm, 2.01 – ≤ 4.00 mm or > 4.00 mm), Clark level (II and III, IV or V), ulceration (absent or present), SN status (positive or negative), NSN status (no additional nodes or additional nodes) and Rotterdam Criteria (negative and < 0.1 mm, 0.1 – 1.0 mm or > 1.0 mm).

Disease free survival (DFS) and overall survival (OS) were calculated from the operation date of the SN procedure to the date of death or the last follow-up. Patients without such an event at their time of last follow-up were censored at that time.

All calculations were performed with STATA version 11.0 (StataCorp LP, College Station, TX, USA).

RESULTS

Patient characteristics and SN status

This study included 421 melanoma patients (217 women and 204 men) who underwent the SN procedure in a period over more than 11 years. In this group of patients, a total of 732 sentinel nodes were collected during the operations, with an average of 1.74 (range 1 – 7) lymph nodes per patient. In 255 patients, just one single SN was located and excised (60.6%) At least one SN was found in all patients, which defines a SN detection rate of 100%.

SN positivity was found in 121 patients (28.7%) after pathological examination. During pathological examination of the nodes, all were classified according to the Rotterdam Criteria. Median tumour size according to the Rotterdam Criteria was 0.6 mm. 79 of SN positive patients (65%) showed tumour load of < 1.0 mm and 22 patients (18%) showed tumour load of < 0.1 mm. (Table 1)

A total of 107 CLND were performed in the group of 121 SN-positive patients. The CLND was not performed seven times due to factors as high age, rejection of further treatment or diagnosis of distant metastases prior to undergoing CLND. Due to a change in hospital policy since 2004, 7 patients did not undergo a CLND because of presence of minimal SN tumour burden according to the Rotterdam Criteria (< 0.1 mm). The patients are followed up by ultrasound exams of the regional node basin at regular intervals and none have developed a regional nodal relapse. The median follow-up of these 7 patients is 3.1 (range 1.1 – 7.0) years. The other 15 patients with submicrometastases in the SN underwent CLND and 0% showed NSN positivity in the CLND specimen.

Of the 107 patients who underwent CLND, 12 had additional positive nodes (11.2%). Five patients (4.6%) had one additional metastatic node and 7 patients (7.6%) had multiple additional metastatic nodes.

The false negative rate for the population of SN patients at our clinic is 10.4% (14/14+121).[7] Until now, 14 of the 299 patients with a negative SN procedure had regional lymph node recurrence in the same lymphatic basin as the one of the SN procedure.

Survival

The median follow-up time for the entire group was 4.3 (range 0.1 – 11.6) years. The median follow-up time for SN-positive patients was 3.2 (range 0.1 – 10.3) years. The median follow-up time for SN-negative patients was 4.6 years (range 0.3 – 11.6) years.

The 3, 5 and 10 year estimated overall survival (OS) rate for patients undergoing the SN procedure after an excised primary melanoma were, respectively, 87%, 82% en 70%. The 3, 5 and 10 year estimated OS rate according to the SN status were respectively 94%, 90% and 76% for SN-negative and 73%, 62% and 55% for SN-positive patients (both $p<0.00005$). (Figure 1a)

The 3, 5 and 10 year estimated disease free survival (DFS) rate for patients undergoing the SN procedure were 78%, 73% and 64%. The 3, 5 and 10 year estimated DFS rate according to the SN status were, respectively, 89%, 82% and 72% for SN-negative and 52%, 51% and 44% for SN-positive patients ($p < 0.00005$) (figure 1b).

The 5 year estimated OS rates for patients in the four different categories of Breslow thickness, i.e. ≤1.0 mm, >1.0-≤2.0, >2.0-≤4.0 and >4.0, were 100%, 88%, 80% and 67% ($p<0.00005$). The 5 year estimated OS rates for patients with presence or absence of ulceration at the primary melanoma were, respectively, 65% in presence of ulceration and 88% in absence of ulceration ($p<0.00005$).

The 5 year estimated OS rates for patients with Rotterdam Criteria divided into three categories, namely, <0.1 mm, 0.1-1.0 mm and >1.0 mm, were respectively

Figure 1A: Overall Survival – SN status

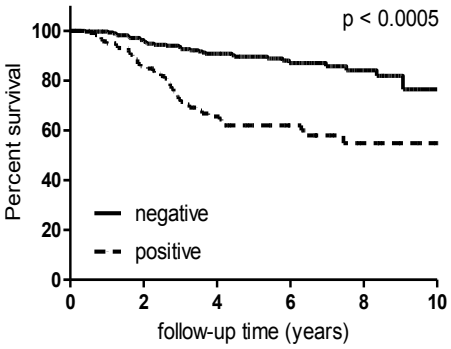
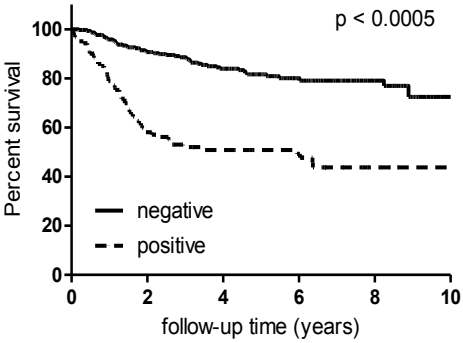


Figure 1B: Disease Free Survival – SN status



	Nr at Risk							Nr at Risk					
Negative	**300**	**247**	**178**	**89**	**41**	**3**	**Negative**	**300**	**234**	**167**	**80**	**39**	**3**
Positive	**121**	**91**	**56**	**37**	**14**	**1**	**Positive**	**121**	**62**	**45**	**32**	**14**	**1**
Total	**421**	**338**	**234**	**126**	**55**	**4**	**Total**	**421**	**296**	**212**	**112**	**53**	**4**

91%, 65% and 36% (p=0.002). The 5-year estimated OS rate was 90% for SN negative patients. (Figure 2)

Prognostic factors

Table 3 shows the Cox's univariate regression analyses for DFS and OS. All variables except age are significant for OS and all variables except age and gender are significant for DFS ($p < 0.05$).

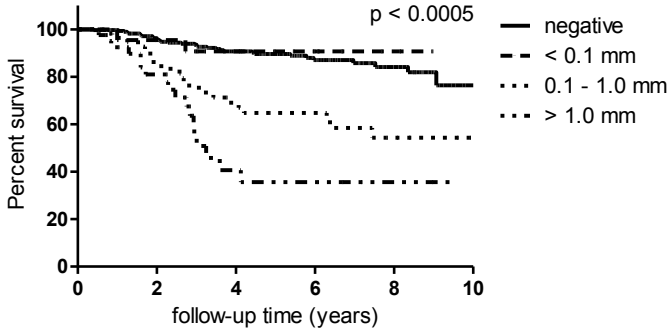
Table 4 shows the result of the multivariate proportional hazard regression analysis. The Rotterdam Criteria, ulceration and NSN status had an independent significant influence on both DFS and OS. The location and histology of the primary melanoma had a significant influence on OS. For the purpose of the multivariate analysis, SN negative and tumour burden < 0.1 mm were grouped as one, because in the univariate analysis they had a virtually identical outcome.

DISCUSSION

In this single institution study we have confirmed the high positive Sentinel Node (SN) identification rate of the EORTC Melanoma Group (MG) protocol as well as its capacity to identify minimal tumour burden, according to the Rotterdam Criteria.

This study identified a high SN positivity rate of 28.7% (121/421). SN positivity rates in literature differ from 13.9% to 29.4% with median Breslow thicknesses from 1.1 to 3.0 mm.[2-4, 7-28] A previous report from our centre identified the highest rate of

Figure 2: Overall Survival – Rotterdam criteria



Nr at Risk						
Negative	**300**	**247**	**178**	**89**	**41**	**3**
< 0.1 mm	**22**	**20**	**16**	**9**	**2**	**0**
0.1 – 1.0 mm	**57**	**45**	**32**	**23**	**10**	**1**
> 1.0 mm	**42**	**26**	**8**	**5**	**2**	**0**
Total	**421**	**338**	**234**	**126**	**55**	**4**

SN positive patients.[4] The factors ulceration rate, mean and median Breslow thickness, false negative rates, survival rates and number of patients included do not seem to be correlated with SN positive rates. For example, the current study has a lower ulceration rate, lower mean and median tumour thickness than several other studies, yet our SN positivity rate is higher.[11, 20, 22]

Reasons for the differences in SN positivity may be found in the differences in pathological assessments. We speculate that the cause of the high SN positivity rate at our institution is due to the specific pathological workup of the EORTC MG according to the examination designed by Cook, which can detect melanoma in up to 33.8% of SNs.[5, 29]

The sensitivity of the EORTC MG SN pathology protocol is apparent, because the current study demonstrated that 65% of SN positive patients have metastases < 1.0 mm and 18% has metastases < 0.1 mm in maximum diameter according to the Rotterdam Criteria.[30]

(Table 1) 7 patients with minimal SN tumour burden have not undergone a CLND, yet none of these has developed a recurrence or died due to melanoma. Furthermore, none of the 15 patients with submicrometastases who underwent a CLND showed non-SN positive lymph nodes. This indicates that in this group of patients CLND may be forfeited. Although our pathological work-up of the SN is very sensitive, the question remains if all detected tumour cells are of clinical importance. These are merely preliminary results; further prospective studies on SN tumour burden are currently ongoing to examine the clinical relevance of minimal SN tumour burden,

such as the EORTC MG MINITUB registry study or the randomized phase III MSLT-2 trial.[31-32]

The false negative rate of this study is 10.4% (14/135), which is somewhat higher than the general rate of 5% reported in the literature. Yet, false negative rates have been incorrectly calculated, which leads to an underestimation of the actual false negative rate. Re-calculations of actual false negative rates have demonstrated a range from 8.6% to 21%.[2, 4, 7-8, 10, 12, 15-17, 20-21, 23, 26-27] The false negative rate is described as the rate of patients with a negative SN procedure who had regional lymph node recurrence in the same lymph basin as the SN procedure was performed in (false negative / (true positive + false negative)).[33] Although the current study demonstrated a higher SN positivity rate with similar Breslow thickness and ulceration rates as other studies, it had not lead to a decrease in false negative rates, which suggests that failure of the SN procedure might not be due to pathological analysis of the SN. Rather, it may be due to detection failures in lymphoscintigraphy. Perhaps it might be due to the tumours' biological activity, which could not to be detected at all since it passed the sentinel node and immediately disseminated haematogenously.

The 5-year overall survival (OS) rates of the present study are 90% and 62% for SN negative and SN positive patients respectively. These rates are comparable with many other studies showing OS rates from 87.5% to 94% for SN negative patients and rates from 42.9% to 75.4% for SN positive patients.[2-4, 10-12, 15, 17-20, 23, 26-27] The 5-year disease free survival (DFS) rates of this study are 82% among those with a negative SN procedure and 51% among those with a positive SN procedure. Both survival rates are comparable with several other studies showing rates from 75.9% to 89.1% for SN negative patients and rates from 35.2% to 65% for patients with a positive SN .[2, 4, 9, 12, 15, 18-19, 23, 25-26] Compared to our previous report, the OS and DFS rates of SN negative and SN positive patients has only changed marginally, due to the increased follow-up with more events. Interestingly, the high SN positivity rate with the detection of increasingly more patients with early metastatic disease has not improved survival rates for SN positive, nor for SN negative patients, when compared to other studies. This might suggest a lack of therapeutic benefit of undergoing a SN followed by early CLND.

A benefit of the SN is that it is a minimally invasive procedure with a low complication rate compared to the morbidity and expense of a lymph node dissection.[34-37] It spares SN negative patients an unnecessary CLND. However, only 14-28% of the SN positive patients undergoing a CLND of the regional nodes has positive non-SNs.[38] Thus, approximately 80% of the SN positive patients will have undergone an unnecessary operation with the possible risks of known complications and morbidity, such as wound infections and lymph oedema.[34, 39] The current study shows an additional positive nodal rate in the CLND specimen of 11.2% (12/107), which is quite low

compared to the literature and our previous report, which demonstrated a CLND positivity rate of 14.7% (10/68). The clinical procedure regarding a CLND has not changed in recent years. Our hypothesis is that our high sensitive pathology

model could explain our low CLND positivity rate. Higher detection rate of SN minimal tumour burden correlates with more negative nodes in the CLND specimen. Moreover, our SN positivity rate is higher than others in the literature; therefore our CLND positivity rate is relatively lower than others in the literature. Yet, in absolute numbers the amount is equal. (Table 4)

SN staging has become a widely accepted and implemented routine staging procedure providing important prognostic information and in case of node positivity arguments that will play a role in determining whether to embark on adjuvant therapy with interferon (IFN). This has become evident in the light of the outcome of the two largest adjuvant trials conducted to date, i.e. EORTC 18952 regarding intermediate doses of IFN, or the EORTC 18991 regarding the role of pegylated-IFN.[40-41] These trials indicated that IFN-based adjuvant therapy was clearly more effective in the SN-positive patients than in patients with palpable nodal disease.

Adjuvant IFN-therapy is highly unlikely to have had any influence on the incidence of submicrometastases since only 9% or patients with metastases < 0.1 mm (2/22) received adjuvant IFN. In patients with 0.1 – 1.0 mm metastases this was 14% (8/57) and in patients with metastases > 1.0 mm this was 2% (1/42). Survival in these patients that received adjuvant IFN was not improved in any way.

In conclusion, this study confirms the high detection rate of nearly 30% of the EORTC MG protocol in SN positive patients, its capacity to identify minimal tumour burden according to the Rotterdam Criteria and verifies that the Rotterdam Criteria is an independent prognostic factor for survival. Further research is required to investigate which SN positive patients should be the target of CLND.

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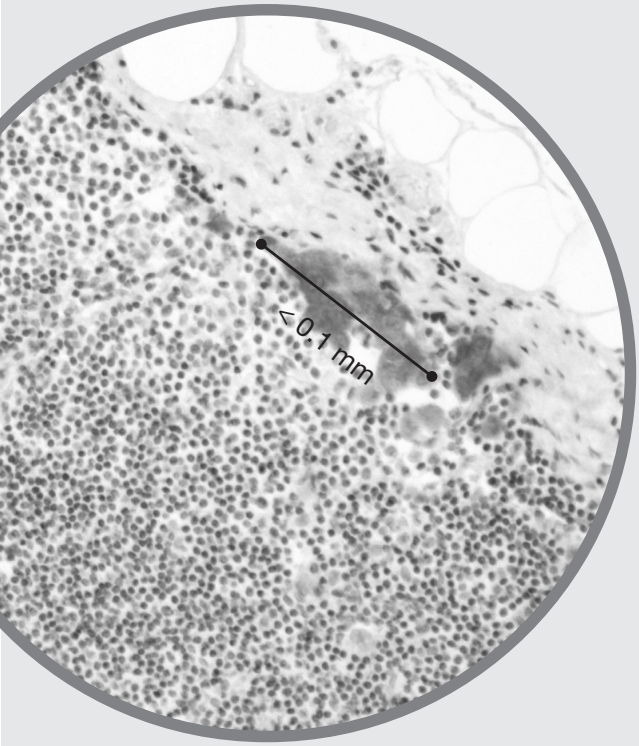
Chapter 5

Prognosis in patients with sentinel node-positive melanoma is accurately defined by the combined Rotterdam tumor load and Dewar topography criteria



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ABSTRACT

Prognosis in patients with sentinel node (SN)–positive melanoma correlates with several characteristics of the metastases in the SN such as size and site. These factors reflect biologic behavior and may separate out patients whom may or may not need additional locoregional and / or systemic therapy.

Between 1993 and 2008, 1080 patients (509 women and 571 men) were diagnosed with tumor burden in the SN in nine European Organisation for Research and Treatment (EORTC) melanoma group centers. In total, 1009 patients (93%) underwent completion lymph node dissection (CLND). Median Breslow thickness was 3.00 mm. The median follow-up time was 37 months. Tumor load and site were reclassified in all nodes by the Rotterdam Criteria for size and in 88% by the Dewar Criteria for topography.

Patients with submicrometastases (< 0.1 mm in diameter) were shown to have an estimated 5-year overall survival rate of 91% and a low nonsentinel node (NSN) positivity rate of 9%. This is comparable to the rate in SN-negative patients. The strongest predictive parameter for NSN positivity and prognostic parameter for survival was the Rotterdam-Dewar Combined (RDC) criteria. Patients with submicrometastases that were present in the subcapsular area only, had a NSN positivity rate of 2% and an estimated 5- and 10-year melanoma-specific survival (MSS) of 95%.

Patients with metastases <0.1 mm, especially when present in the subcapsular area only, may be overtreated by a routine CLND and have an MSS that is indistinguishable from SN-negative patients. Thus the RDC Criteria provide a rational basis for decision making in the absence of conclusions provided by randomized controlled trials.

INTRODUCTION

Sentinel lymp node biopsy (SNB), introduced by Morton et al,[1-2] is widely accepted as a highly accurate diagnostic method of identifying early lymph node micrometastasi in patients with melanoma.

Sentinel node (SN) tumor burden is the most important prognostic factor for patients with early-stage melanoma.[3] Prognosis in patients with SN-positive melanoma correlates with several characteristics of the metastases in the SN such as size and site. These factors reflect biologic behavior and may separate out patients who may or may not need additional locoregional and / or systemic therapy.

SN positivity rates depend on median and mean Breslow thickness of the primary, ulceration rates and the SN workup protocol and vary in the literature from 14% to 30%.[4-6] Approximately 20% of patients who are SN-positive have further nodes involved, which are demonstrated by completion lymph node dissection (CLND) findings, the so called nonsentinel node (NSN) positivity rate. Many specialists in the melanoma field have tried to identify the correct patient group to undergo a CLND and to identify those patients who can safely be spared unnecessary CLND and its associated morbidity, such as wound infections and chronic lymph edema.[6-39] Ongoing prospective multicenter studies aim to identify the group of patients who can be considered for observation instead of CLND. The two most prominent studies are the Multicenter Selective Lymphadenectomy Trial II (MSLT-II) and the European Organisation for Research and Treatment of Cancer (EORTC) MINITUB studies.[40]

In this study, two important morphometric parameters are assessed: the microanatomical location (Dewar criteria)[28] and the maximum diameter of the largest tumor lesion (Rotterdam criteria).[6, 12] The EORTC Melanoma Group (MG) recommends that all pathologists report these criteria for each SN-positive patient.[41]

The aim of this study, which uses the largest reclassified database of SN positivity, was to determine the role of tumor load and tumor site in the SN as prognostic factors for survival and as predictive factors for NSN positivity.

PATIENTS AND METHODS

Patients

Patients with a positive SNB after wide local excision of a malignant melanoma in nine major collaborating EORTC MG centers were included in this retrospective study. Participating EORTC MG centers are listed in table 1. Between 1993 and 2008, 1080 patients were diagnosed with tumor burden in the SN. A database with personal

Table 1 – Baseline Characteristics of SN–Positive Patients (N = 1080)

Characteristic	N	%
Sex		
Male	571	53
Female	509	47
Center		
DDHCC	115	11
CHUB	86	8
MMCCIO	245	23
RSCH	214	20
AVL	116	11
IGR	68	6
VU	107	10
UMCG	56	5
EIO	73	7
Age, years		
≤ 50	523	48
> 50	557	52
Location		
Extremity	643	60
Trunk	405	37
Head and neck	32	3
Histology		
SSM	401	37
NM	347	32
Other	332	31
Breslow, mm		
T1 (≤ 1.00 mm)	53	5
T2 (1.01 – ≤ 2.00 mm)	270	25
T3 (2.01 – ≤ 4.00 mm)	434	40
T4(> 4.00 mm)	323	30
Clark		
I	2	0
II	33	3
III	266	25
IV	614	57
V	117	11
Unknown	48	4
Ulceration		
Present	603	56
Absent	477	44

Table 1 (continued)

Characteristic	N	%
Rotterdam criteria		
< 0.1 mm	113	10
0.1 – 1.0 mm	457	42
> 1.0 mm	510	47
Dewar criteria		
Subcapsular	181	17
Combined	423	39
Parenchumal	154	14
Multifocal	41	4
Extensive	152	14
Unknown	129	12
NSN status		
Negative	797	74
Positive	212	20
Unknown	71	7

SN = Sentinel Node; DDHCC = Erasmus University Medical Center – Daniel Den Hoed Cancer Center, Rotterdam, the Netherlands; CHUB = the Charité, Humboldt University of Berlin, Berlin, Germany; MMCCIO = M.Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland; RSCH = Royal Surrey County Hospital, Guildford, UK; AVL = Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands; IGR = Institut de cancérologie Gustave Roussy, Villejuif, France; VU = Vrije Universiteit, Amsterdam, the Netherlands; UMCG = University Medical Center Groningen, Groningen, the Netherlands; EIO = European Insitute of Oncology, Milan, Italy; SSM = Superficial Spreading Melanoma, NM = Nodular Melanoma

information and information on previous medical history, disease and follow-up was created for these patients. Baseline characteristics are summarized in Table 1.

In general, patients underwent the SNB in the same session as the re-excision procedure, after the diagnostic excision of the primary melanoma. Common procedure was to achieve margins of 1 cm for melanomas < 2 mm and margins of 1 to 2 cm for melanomas > 2 mm. The SNB procedure was offered to patients with Breslow thickness ≥ 1.0 mm or to patients with histopathological features such as ulceration or Clark level IV or V invasion.

CLND was not performed in all SN-positive patients. In 71 patients (6.6%), CLND was not performed for several reasons: refusal of further treatment, the diagnosis of distant metastasis between SNB and CLND, or the presence of minimal tumor burden in the SN.

The triple technique

After wide local excision of the malignant melanoma, the SN surgical procedure was done by using the triple technique, described in detail elsewhere.[5, 42-43] In short, the

triple technique consists of preoperative lymphoscintigraphy, undertaken within 24 hours of the operation being performed; perioperative use of patent blue; and use of a handheld gamma detection probe to detect the SN or SNs. A lymph node is identified as an SN if it stained blue, if it had an in situ radioactivity count at least three times that of the background count, or if it had an ex vivo radioactivity count at least ten times greater than the background count.

After the surgical procedure, the SNs were sent to the pathology department for pathological examination. SN tumor burden was reviewed, for the purpose of this study, by a second pathologist, in a later phase.

Pathology

In the nine EORTC centers, all SNs were worked-up according to the EORTC MG pathology protocol designed by Cook et al.[44] First, the SNs were fixed for 24 hours in buffered formalin. Second, after fixation, the lymph nodes were halved through the hilum in its longest dimension and embedded in paraffin. From each face of the lymph node five serial step sections of 4 µm each were cut with 50 µm intervals between different numbers of sections. Finally, all sections were stained with hematoxylin & eosin and S100 and/or MelanA. There were slight local differences in the Cook protocol regarding the number and distance of step sections in different time periods; however, the main principles remained unchanged.

All SNs with tumor burden were reviewed by different members of the EORTC MG. In seven of nine EORTC Centers, SN tumor load was re-classified by van Akkooi. In two EORTC centers (Antoni van Leeuwenhoek Hospital, Netherlands Cancer Institute, Amsterdam, the Netherlands and University Medical Center Groningen, Groningen, the Netherlands), other experienced melanoma specialists reclassified the SN tumor load. SN tumor load was classified according to the Rotterdam criteria and Dewar Criteria. All positive SNs were classified according to the Rotterdam criteria.[6, 12] Dewar criteria were available for 951 patients (88%).[28]

Dewar Criteria define the micro anatomic location of the melanoma lesion.[28] Micro anatomic locations are subcapsular, parenchymal, combined, multifocal, or extensive. Because Dewar criteria showed that the subcapsular group had a better prognosis than any other, we have also grouped the locations into two groups: subcapsular and nonsubcapsular (which we called the Dewar Criteria II). The Rotterdam criteria (< 0.1 mm, 0.1 – 1.0 mm, > 1.0 mm) consists of the measurement of the maximum diameter in any direction of the largest lesion overall on a slide. Several other studies included the maximum diameter of the largest tumor lesion as a parameter of SN tumor load and used other cut-off points.[7-11, 13-17, 19-21] For this reason, we used cutoff points other than < 0.1 mm in our analyses (ie, < 0.2 mm [Rotterdam Criteria II], < 0.3 mm [Rotterdam Criteria III] and < 0.4 mm [Rotterdam Criteria IV]. We also created

a new variable after first analysis: Rotterdam-Dewar Combination (RDC) criteria (< 0.1 mm subcapsular, < 0.1 mm nonsubcapsular), combining the two most predictive and prognostic subgroups of the parameters. Patients with tumors for which it was difficult to determine the different micromorphometric parameters were discussed during EORTC MG meetings, which took place every 6 months.

Statistics

Univariate analyses for NSN positivity were performed using a χ^2 test. Univariate analyses of end points for survival were performed by using the Kaplan-Meier method and the log-rank test. Multivariate analyses to determine the prognostic value of covariates regarding melanoma-specific survival (MSS), disease-free survival (DFS) and overall survival (OS) were performed using the Cox's proportional hazards model. DFS and OS were calculated from the operation date of the SNB to the date of first disease recurrence or the date of death or the last follow-up, respectively. MSS was calculated from the operation date of the SNB to the date of death caused by melanoma disease. Follow-up time was defined as the date of last follow-up or death starting from the date of the SN procedure.

For the survival analyses and analysis for NSN status, the following variables were included: sex (male, female), centers (nine EORTC centers), age (≤ 50, > 50 years), location of the melanoma (extremities, trunk, head and neck), histology of the melanoma (superficial spreading melanoma, nodular melanoma, other), Breslow thickness (T1, T2, T3, T4), Clark level (II, III, IV, V), ulceration (absent/unknown and present), Rotterdam criteria (< 0.1 mm, 0.1 to 1.0 mm, or > 1.0 mm), Rotterdam criteria II (< 0.2 mm, 0.2 to 1.0 mm or > 1.0 mm), Rotterdam criteria III (< 0.3 mm, 0.3 to 1.0 mm, or > 1.0 mm), Rotterdam criteria IV (< 0.4 mm, 0.4 to 1.0 mm, or > 1.0 mm), Dewar criteria (subcapsular, parenchymal, combined, multifocal, extensive, unknown), Dewar criteria II (subcapsular, nonsubcapsular), RDC criteria (<0.1 mm subcapsular, <0.1 mm nonsubcapsular) and, for survival analysis only, NSN status (negative, positive, unknown). Statistics were performed with STATA version 11.1 (StataCorp LP, College Station, TX).

RESULTS

Characteristics

Baseline characteristics are summarized in Table 1. This study included 1080 patients with melanoma (509 women and 571 men) with a positive SN procedure over a 16-year period. Average age was 51 years (range, 6 to 88 years). Mean and median Breslow thicknesses were 4.00 mm and 3.00 mm (range, 0.1 to 90 mm), respectively.

Table 2 – Characteristics per EORTC Center

Characteristic	DDHCC	CHUB	MMCCIO	RSCH	AVL	IGR	VU	UMCG	EIO
Median Breslow (mm)	3.00	3.34	4.00	2.40	3.00	2.90	2.10	2.50	3.00
Ulceration percentage	45	50	64	31	40	47	25	30	49
Rotterdam criteria percentage									
< 0.1 mm	17	26	3	11	4	9	11	23	4
0.1 – 1.0 mm	48	35	33	46	32	50	49	57	52
> 1.0 mm	35	40	64	43	64	41	40	20	44
Dewar criteria percentage									
Subcapsular	30	40	4	18	34	15	15	N/A	N/A
Combined	30	29	47	50	41	50	56	N/A	N/A
Parenchymal	13	12	22	17	0	26	19	N/A	N/A
Multifocal	12	8	4	1	5	3	0	N/A	N/A
Extensive	15	12	24	13	21	6	10	N/A	N/A

DDHCC = Erasmus University Medical Center – Daniel Den Hoed Cancer Center, Rotterdam, the Netherlands; CHUB = the Charité, Humboldt University of Berlin, Berlin, Germany; MMCCIO = M.Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland; RSCH = Royal Surrey County Hospital, Guildford, UK; AVL = Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands; IGR = Institut de cancérologie Gustave Roussy, Villejuif, France; VU = Vrije Universiteit, Amsterdam, the Netherlands; UMCG = University Medical Center Groningen, Groningen, the Netherlands; EIO = European Insitute of Oncology, Milan, Italy; N/A = Not Applicable

The mean and median follow-up times for the entire group were 3.8 and 3.1 years (46 and 37 months; range, 1 to 172 months). The mean and median times to first recurrence were 3.2 and 2.3 years (38 and 27 months). At last follow-up, 336 (31%) of 1080 patients were deceased.

In Table 2, the characteristics of Breslow thickness, ulceration rate, and subgroups of the Rotterdam Criteria and the Dewar Criteria are compared among the nine EORTC MG centers. With a median Breslow thickness of 4.00 mm and an ulceration percentage of 64%, the M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology (MMCIO; Warsaw, Poland) is the center with the group of SN-positive patients who have the worst prognosis. This is reflected by the large proportion of patients with advanced SN metastases (> 1.0 mm in Rotterdam Criteria; extensive for the Dewar Criteria).

NSN status

Of the 1009 patients who underwent a CLND, 21% (212 patients) had one or more positive NSNs. Table 3 shows NSN positivity and negativity rates for all factors assessed in this study. The following factors were significant regarding NSN status: age; center; histology and location of the primary; Clark level; Breslow thickness;

Table 3 – Association between clinicopathological factors and the detection of metastases in NSNs

Predictive factor	NSN			P
	Positive N (%)	Negative N (%)	unknown n (%)	
Sex				
Female	111 (22)	360 (71)	38 (7)	
Male	101 (18)	437 (77)	33 (6)	.094
Center				
DDHCC	11 (10)	90 (78)	14 (12)	
CHUB	24 (28)	52 (60)	10 (12)	
MMCCIO	66 (27)	178 (73)	1 (0)	
RSCH	25 (12)	164 (77)	25 (12)	
AVL	15 (13)	101 (87)	0 (0)	
IGR	11 (16)	55 (81)	2 (3)	
VU	25 (24)	72 (67)	10 (9)	
UMCG	10 (18)	45 (80)	1 (2)	
EIO	25 (34)	40 (55)	8 (11)	< .001
Histology				
SSM	76 (19)	297 (74)	28 (7)	
NM	88 (25)	244 (70)	15 (4)	
Other	48 (14)	256 (77)	28 (8)	.003
Location				
Extremity	123 (19)	466 (72)	54 (8)	
Trunk	82 (20)	310 (77)	13 (3)	
Head and neck	7 (22)	21 (66)	4 (13)	.011
Age, years				
≤ 50	101 (19)	398 (76)	24 (5)	
> 50	111 (20)	399 (72)	47 (8)	.032
Clark				
II	8 (23)	25 (71)	2 (6)	
III	39 (15)	218 (82)	9 (3)	
IV	126 (21)	440 (72)	48 (8)	
V	33 (28)	75 (64)	9 (8)	
Unknown	6 (13)	39 (81)	3 (6)	.011
Breslow				
T1	7 (13)	41 (77)	5 (9)	
T2	37 (14)	210 (78)	23 (9)	
T3	74 (17)	333 (77)	27 (6)	
T4	97 (29)	546 (66)	16 (5)	< .001
Ulceration				
Absent	103 (17)	457 (76)	43 (7)	
Present	109 (23)	340 (71)	28 (6)	.052

Table 3 (continued)

Predictive factor	NSN			P
	Positive N (%)	Negative N (%)	unknown n (%)	
Rotterdam criteria, mm				
< 0.1	10 (9)	87 (77)	16 (14)	
0.1 – 1.0	73 (16)	349 (76)	35 (8)	
> 1.0	129 (25)	361 (71)	20 (4)	< .001
Rotterdam criteria II, mm				
<0.2	27 (14)	140 (73)	24 (13)	
0.2 – 1.0	56 (15)	296 (78)	27 (7)	
> 1.0	129 (25)	361 (71)	20 (4)	< .001
Rotterdam criteria III, mm				
<0.3	38 (14)	202 (75)	30 (11)	
0.3 – 1.0	45 (15)	234 (78)	21 (7)	
> 1.0	129 (25)	361 (71)	20 (4)	< .001
Rotterdam criteria IV, mm				
<0.4	43 (13)	253 (76)	38 (11)	
0.4 – 1.0	40 (17)	183 (78)	13 (6)	
> 1.0	129 (25)	361 (71)	20 (4)	< .001
Dewar criteria				
Subcapsular	12 (7)	152 (84)	17 (9)	
Combined	80 (19)	319 (75)	24 (6)	
Parenchymal	25 (16)	119 (77)	10 (7)	
Multifocal	7 (17)	29 (71)	5 (12)	
Extensive	53 (35)	93 (61)	6 (4)	
Unknown	35 (27)	85 (66)	9 (7)	< .001
Dewar Criteria II				
Subcapsular	12 (7)	152 (84)	17 (9)	
Non-subcapsular	165 (21)	560 (73)	45 (6)	
unknown	35 (27)	85 (66)	9 (7)	< .001
RDC Criteria				
<0.1 subcapsular	1 (2)	47 (80)	11 (19)	
<0.1 non-subcapsular	82 (16)	402 (77)	41 (8)	
<0.1 unknown	129 (26)	797 (74)	71 (7)	< .001

NSN = Non Sentinel Node; DDHCC = Erasmus University Medical Center – Daniel Den Hoed Cancer Center, Rotterdam, the Netherlands; CHUB = the Charité, Humboldt University of Berlin, Berlin, Germany; MMCCIO = M.Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland; RSCH = Royal Surrey County Hospital, Guildford, UK; AVL = Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands; IGR = Institut de cancérologie Gustave Roussy, Villejuif, France; VU = Vrije Universiteit, Amsterdam, the Netherlands; UMCG = University Medical Center Groningen, Groningen, the Netherlands; EIO = European Insitute of Oncology, Milan, Italy; SSM = Superficial Spreading Melanoma; NM = Nodular Melanoma; RDC Criteria= Rotterdam – Dewar Combined (criteria).

Rotterdam criteria; Rotterdam criteria II, III and IV; Dewar criteria; Dewar criteria II; and RDC criteria.

The rate of additional positive lymph nodes in the group of patients with submicrometastases (< 0.1 mm, Rotterdam criteria) was 9%, although 16% of patients with Rotterdam Criteria 0.1 to 1.0 mm had positive NSNs and 25% of patients had > 1.0 mm of SN tumor burden. NSN positivity rates for the other cut-off points were similar. Patients with < 0.2 mm, < 0.3 mm and < 0.4 mm had 14%, 14% and 13% positive NSNs, respectively. NSN positivity was 7% in patients with subcapsular metastases and 22% in patients with nonsubcapsular metastases. The subgroup of patients with the best predictivity for NSN status was the group with subcapsular metastases < 0.1 mm, which showed positive NSNs in only 2% of patients.

Survival

Results of univariate and multivariate analyses are provided in Table 4. Because of multicollinearity in multivariate analyses due to the covariates Rotterdam Criteria (with different cutoff values) and RDC Criteria, separate multivariate analyses were performed. On multivariable analyses of the covariates regarding MSS, sex, Breslow thickness (T3 and T4), ulceration, Rotterdam Criteria (with different hazard ratios for different cutoff values), RDC Criteria, and NSN status were independent prognostic factors. Dewar or Dewar II Criteria were not significant on multivariate analyses.

Table 4 – Univariate and multivariate analyses of covariates regarding melanoma specific survival

	Univariate			Multivariate		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Sex						
Female	1			1		
Male	1.38	1.10 – 1.73	.006	1.31	1.04 – 1.64	.022
Center						
DDHCC	1					
CHUB	1.75	1.02 – 3.01	.042			
MMCCIO	1.93	1.23 – 3.04	.004			
RSCH	1.53	0.95 – 2.46	.081			
AVL	1.09	0.65 – 1.84	.74			
IGR	1.07	0.51 – 2.22	.87			
VU	1.54	0.93 – 2.55	.091			
UMCG	0.83	0.43 – 1.60	.58			
EIO	1.78	1.02 – 3.10	.041			N/S

Table 4 (continued)

	Univariate			Multivariate		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Histology						
SSM	1					
NM	1.44	1.10 – 1.88	.009			
Other	1.51	1.13 – 2.01	.005			N/S
Location						
Extremity	1					
Trunk	1.07	0.85 – 1.36	.55			
Head and neck	1.18	0.66 – 2.13	.57			N/S
Age, years						
≤ 50	1					
> 50	1.24	0.99 – 1.55	.063			N/S
Clark						
II	1					
III	1.45	0.63 – 3.36	.39			
IV	2.07	0.92 – 4.66	.081			
V	3.43	1.48 – 7.99	.004			
Unknown	2.23	0.84 – 5.96	.108			N/S
Breslow						
T1	1			1		
T2	1.07	0.51 – 2.27	.85	-	-	N/S
T3	1.92	0.94 – 3.93	.075	1.53	1.10 – 2.13	.012
T4	3.74	1.83 – 7.64	< .001	2.45	1.73 – 3.45	< .001
Ulceration						
Absent	1			1		
Present	2.11	1.68 – 2.64	< .001	1.50	1.18 – 1.92	.001
Rotterdam criteria, mm						
< 0.1	1			1		
0.1 – 1.0	3.28	1.72 – 6.25	< .001	2.65	1.38 – 5.06	.003
> 1.0	5.36	2.83 – 10.13	< .001	3.30	1.73 – 6.31	< .001
Rotterdam criteria II, mm						
<0.2	1			1		
0.2 – 1.0	1.60	1.05-2.44		1.40	0.93 – 2.12	N/S
> 1.0	2.84	1.91-4.21	< .001	1.83	1.23 – 2.72	.003
Rotterdam criteria III, mm						
<0.3	1			1		
0.3 – 1.0	1.67	1.14-2.45		1.42	0.98 – 2.05	N/S
> 1.0	2.75	1.96-3.88	< .001	1.77	1.26 – 2.50	.001

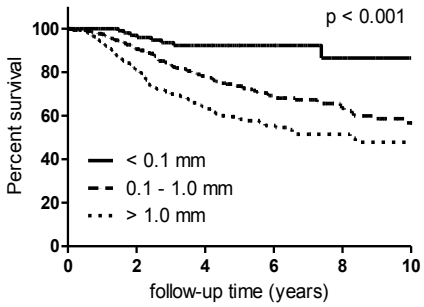
Table 4 (continued)

	Univariate			Multivariate		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Rotterdam criteria IV, mm						
<0.4	1			1		
0.4 – 1.0	1.61	1.12-2.33		1.24	0.87 – 1.76	N/S
> 1.0	2.57	1.89-3.50	< .001	1.59	1.17 – 2.17	.003
Dewar criteria						
Subcapsular	1					
Combined	1.88	1.29 – 2.76	.001			
Parenchymal	1.94	1.23 – 3.05	.004			
Multifocal	1.46	0.72 – 2.95	.297			
Extensive	3.62	2.38 – 5.51	< .001			
Unknown	1.61	1.02 – 2.56	.042			N/S
Dewar criteria II						
Subcapsular	1					
Non-subcapsular	2.04	1.43 – 2.92	< .001			N/S
RDC criteria						
<0.1 subcapsular	1			1		
<0.1 non-subcapsular	2.57	0.66 – 9.95	N/S	-	-	N/S
0.1 – 1.0 subcapsular	5.23	1.60 – 17.15	.006	4.53	1.37 – 14.91	.013
0.1 – 1.0 non-subcapsular	5.92	1.87 – 18.69	.002	5.01	1.58 – 15.88	.006
> 1.0 non and subcapsular	9.36	2.99 – 29.32	< .001	6.17	1.95 – 19.45	.002
NSN status						
Negative	1			1		
Positive	2.46	1.89 – 3.22	< .001	2.12	1.62 – 2.79	< .001
Unknown	1.45	1.09 – 1.93	.011	1.68	1.26 – 2.25	< .001

HR = Hazard Ratio; CI = Confidence Interval; DDHCC = Erasmus University Medical Center – Daniel Den Hoed Cancer Center, Rotterdam, the Netherlands; CHUB = the Charité, Humboldt University of Berlin, Berlin, Germany; MMCCIO = M.Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland; RSCH = Royal Surrey County Hospital, Guildford, UK; AVL = Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands; IGR = Institut de cancérologie Gustave Roussy, Villejuif, France; VU = Vrije Universiteit, Amsterdam, the Netherlands; UMCG = University Medical Center Groningen, Groningen, the Netherlands; EIO = European Insitute of Oncology, Milan, Italy; N/S = Not Significant; SSM = Superficial Spreading Melanoma; NM = Nodular Melanoma; NSN= Non-Sentinel Node; RDC Criteria= Combined Rotterdam and Dewar Criteria

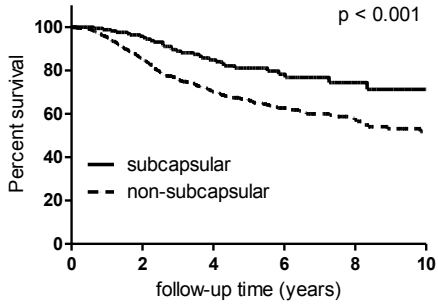
The Kaplan-Meier 5- and 10-year OS rates were 91% and 81% for patients with Rotterdam Criteria < 0.1 mm, followed by 71% and 54% in the 0.1 to 1.0 mm group, and 57% and 46% in the > 1.0 mm group. The Kaplan-Meier 5- and 10-year DFS rates were 83% and 83% for patients with Rotterdam Criteria < 0.1 mm, followed by

Figure 1A: Melanoma Specific Survival – Rotterdam criteria

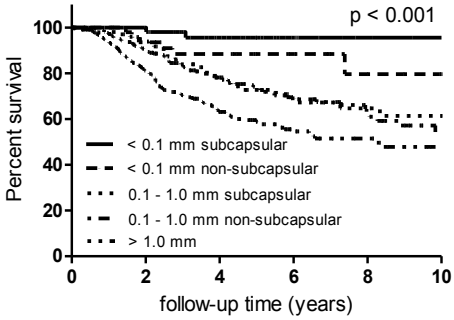


	Nr at Risk					
< 0.1 mm	113	91	64	33	14	10
0.1 – 1.0 mm	457	335	201	115	59	27
> 1.0 mm	510	328	156	64	33	15
Total	1080	754	421	212	106	52

Figure 1B: Melanoma specific survival – Dewar criteria



	Nr at Risk					
Subcapsular	181	149	97	53	27	15
Non-Subcapsular	899	604	323	158	78	35
Total	1080	753	420	211	105	50



	Nr at risk					
< 0.1 mm subcapsular	59	51	35	16	6	4
< 0.1 mm non-subcapsular	54	41	30	18	9	7
0.1 – 1.0 mm subcapsular	108	89	58	38	22	12
0.1 – 1.0 mm non-subcapsular	349	247	144	78	38	16
> 1.0 mm	510	328	156	64	33	15
Total	1080	756	423	214	108	54

61% and 49% in the 0.1 to 1.0 mm group, and 40% and 32% in the > 1.0 mm group. The Kaplan-Meier 5- and 10-year MSS rates were 92% and 87% for patients with Rotterdam Criteria <0.1 mm, followed by 74% and 57% in the 0.1 to1.0 mm group and 59% and 48% in the > 1.0 mm group. (Fig 1A)

The Kaplan-Meier 5- and 10-year MSS rates of patients with cutoff points < 0.2, < 0.3 and < 0.4 mm were 81% and 73%, 81% and 74% and 80% and 70%, respectively. The Kaplan-Meier 5- and 10-year MSS rates were 81% and 71% for patients with subcapsular metastases and 66% and 52% for those with nonsubcapsular metastases. (Fig 1B) The Kaplan-Meier 5- and 10-year MSS rates were both 95% for patients with RDC Criteria < 0.1 mm subcapsular, although the 5- and 10-year OS rates were 88% and 80% for patients with RDC Criteria < 0.1 mm nonsubcapsular. (Fig 1C)

DISCUSSION

To the best of our knowledge, this is the largest study ever performed in this field, evaluating almost three times more SN-positive patients than reports of two studies performed in the United States,[9, 20] a previous report of the EORTC MG,[6] and a report from the Melanoma Institute Australia in Sydney.[22] This study investigated prognostic factors for survival and predictive factors for NSN status by addressing two different histological parameters of classifying SN tumor load.

This study confirms that patients with submicrometastases (< 0.1 mm) had an estimated 5-year OS rate of 91% comparable with SN-negative patients.[6] The NSN positivity rate is 9%, which is comparable to a false-negative SN rate in patients who underwent SNB. The most predictive and prognostic parameter in our study was the RDC Criteria. Patients with submicrometastases present in the subcapsular area only had a NSN positivity rate of only 2% and an estimated 5- and 10-year MSS rate of 95%. It is highly unlikely that this patient group benefits from a routine CLND. We propose that they might be classified as SN-negative in the next American Joint Committee on Cancer (AJCC) classification system.

Various micromorphometric parameters for tumor load in the SN have been studied, such as SN tumor burden, tumor penetrative depth, square area, percentage area, number of metastatic foci, number of positive SNs, extracapsular spread, and capsular invasion.[14, 17-18, 20, 23-26, 32-33, 36, 39] Others combined primary melanoma and/or SN characteristics into working models for predicting survival and/or NSN status.[16-17, 20, 22, 31-32, 36, 39] Reproducibility and accuracy are important aspects in the assessment of micromorphometric parameters in the histopathologic workup and measurement of SN tumor deposits.[41, 45] Murali et al.[45] observed the agreement of assessment of histologic parameters among seven different pathologists. Quantitative

Table 5 – Overview of literature for predictive factors for NSN Involvement and 5-year estimated OS rates

Parameters for SN tumor burden	Studies	Analyzed NSN positive patients	Most prognostic subgroup of variable for NSN status	NSN Positivity rate	5-year estimated OS rate
		N (%)		(%)	(%)
Size as largest diameter of largest lesion (eg, Rotterdam criteria)	Ranieri et al. [7]	13 (14)	≤3 mm	-	86 *
	Carlson et al. [8]	15 (16)	Isolated tumor cells	-	86 *
			≤2 mm	-	90 *
	Lee et al. [9]	46 (24)	<2 mm	16	-
	Sabel et al. [10]	34 (15)	Micrometastasis	2	-
	Pearlman et al. [11]	17 (21)	≤2 mm	6	85
	van Akkooi et al. [5, 12]	10 (15)	<0.1 mm	0	100
	Govindarajan et al. [13]	20 (16)	≤0.2 mm	0	-
	Debarbieux et al. [14]	22 (22)	≤2 mm	18	±80
			≤1 mm (smallest diameter)	13	-
	Scheri et al. [15]	N/A †	≤0.2 mm	12	87
	Roka et al. [16]	18 (21)	≥2 mm	8	-
	Rossi et al. [18]	20 (21)	≤2 mm	16	-
	Satzger et al. [17]	28 (16)	<0.1 mm	0	-
			<1 mm	9	-
			<2 mm	11	-
	Guggenheim et al. [19]	22 (22)	≤2 mm	16	-
	Gershenwald et al. [20]	48 (14)	≤0.5 mm	5	-
			≤2 mm	8	-
	van Akkooi et al. [6]	91 (23)	<0.1 mm	3	91
	van der Ploeg et al. [21, 27]	15 (13)	<0.1 mm	0	100
	This study	184 (17)	<0.1 mm	9	91
Microanatomic Location (eg, Dewar criteria)	Dewar et al. [28]	24 (16)	Subcapsular	0	-
	van Akkooi et al. [5, 12]	10 (15)	Combined	9	-
	Govindarajan et al. [13]	20 (16)	Sinusoidal	0	-
	Roka et al. [16]	18 (21)	Non-extensive	13	-
	Rossi et al. [18]	20 (21)	Subcapsular	0	-
	Gershenwald et al.[20]	48 (14)	Subcapsular	10	-
	Frankel et al. [29]	29 (21)	Subcapsular	10	-
	van Akkooi et al. [6]	91 (23)	Subcapsular	8	-
	van der Ploeg et al. [21, 27]	15 (13)	Subcapsular	3	83
	This study	184 (17)	Subcapsular	7	81

NSN = Nonsentinel Node; OS = Overall Survival; SN = Sentinel Node; N/A = Not Applicable

** These rates are 3-year estimated overall survival rates*

† Only the group of patients with isolated tumor cells and known NSN status were included. Six of 52 (12%) patients with ≤ 0.2 mm had NSN positivity in this study.

parameters like the maximal size of largest SN deposit (Rotterdam Criteria), the tumor penetrative depth (S-classification) and the estimated percentage area occupied by metastasis had an excellent degree of interobserver agreement.[45] Thus, besides containing predictive and prognostic value, a measurement of SN tumor load must be simple and reproducible.

Many studies addressed cutoff points other than 0.1 mm and 1.0 mm used by the Rotterdam Criteria measuring the largest diameter of the largest lesion.[6-21] (Table 5) We scrutinized the cutoff point of 0.1 mm in this study and examined other cutoff points, for example, 0.2 mm as used for patients with SN-positive breast cancer[46-47] and as addressed in other studies,[13, 15] 0.3 mm as in the S-Classification,[24] and 0.4 mm as suggested by van der Ploeg et al.[21] NSN positivity increased rapidly and in agreement with these other cutoff points, which had positive NSNs in 13% to 14% of SN-positive patients. (Table 3) Survival rates in these groups showed similar poor outcome. Five-year MSS survival rates were 80% to 81% and 10-year MSS survival rates were 70% to 74%. Of the four different cut-off points, < 0.1 mm according to the Rotterdam Criteria had the best prognostic and predictive value. (Tables 3 and 4) The other three cutoff points addressed had worse survival than SN negative patients (80% to 81% compared to 88% to 94%), while patients with submicrometastases according to the Rotterdam Criteria had similar survival (91%).

Tumor load and topography characteristics predict NSN positivity, but whether they can play a role in identifying patients, who may or may not benefit from routine CLND is another important question. Our study strongly suggests that some patients may not benefit from routine CLND. Because almost all patients underwent CLND, the question remains as to whether these patients would have had the same outcome without a CLND. The outcome of a group of patients with good prognosis but without CLND has never been published, although two recent studies described the difference between a group of SN-positive patients without CLND after a positive SN.[48-49] There was no significant difference in locoregional control and disease-specific survival between groups, indicating that CLND may not influence survival. It seems obvious that patients who are SN-positive with no CLND with high-volume SN tumor burden have worse outcome than patients with low- volume SN tumor burden.

Obviously, all retrospective studies including this one have the traditional downside that can be overcome only by a prospective randomized controlled trial. Several prospective trials are currently under way to further investigate the possibility of reducing the 80% of unnecessary CLND operations. The two most prominent studies are the MSLT-II and the EORTC MINITUB studies.[40] When the outcomes of these studies are final, alternative options to CLND can be discussed and proposed.

Another issue is the biologic significance of minimal SN tumor burden. We pose two hypotheses: (1) These are dormant cells that will inevitably become active

metastatic cells after a long period of time. Thus, the removal of such deposits (by excising the SN) can be curative. (2) These are cells presented to the immune system that has led to an immune response to destroy circulating tumor cells; therefore, these cells will never progress to viable metastatic cells and should be considered prognostically false positive. This is difficult to study, because the SN has been removed in both cases to establish the presence of minimal SN tumor burden.

There are additional arguments for classifying tumor load in the SN because it may help identify which patients could benefit from adjuvant systemic therapy with Interferon, since the large EORTC 18952 and 18991 trials both clearly demonstrated that patients with less disease had the greater benefit.[50-51]

In conclusion, this study of the EORTC MG proposes that patients with tumor burden < 0.1 mm might safely be spared a routine CLND, especially when found in the subcapsular area only. We acknowledge that long-term follow-up is necessary, and these results need to be validated prospectively. We invite surgical oncologists to participate in studies, such as the MSLT-II or the EORTC MINITUB study. The RDC criteria provide the strongest prognostic information for survival and most accurately predict NSN positivity. The simplicity of these classification systems is an argument for their standard implementation.

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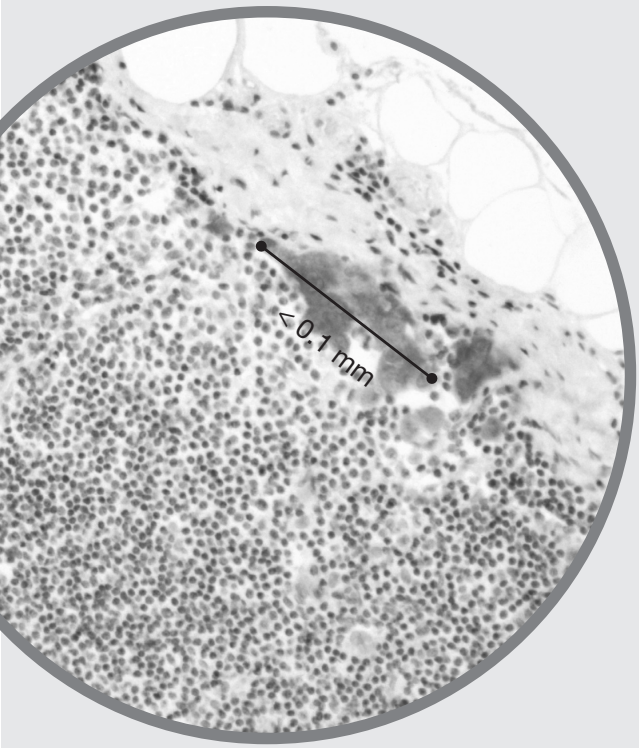
Chapter 6

The prognostic significance of sentinel node tumor burden in melanoma patients: an international multicenter study of 1539 sentinel node-positive melanoma patients



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ABSTRACT

Sentinel node (SN) biopsy (SNB) and completion lymph node dissection (CLND) when SN positive have become standard of care in most cancer centers for melanoma. Various SN tumor burden parameters are assessed to determine the heterogeneity of SN positivity. The aim of the present study was to validate the prognostic significance of various SN tumor burden micromorphometric features and classification schemes in a large cohort of SN-positive melanoma patients.

In 1539 SN-positive patients treated between 1993 and 2008 at 11 melanoma treatment centers in Europe and Australia, indices of SN tumor burden (intranodal location, tumor penetrative depth (TPD) and maximum size of SN tumor deposits) were evaluated.

Non-subcapsular location, increasing TPD and increasing maximum size were all predictive factors for non-SN (NSN) status and were independently associated with poorer melanoma-specific survival (MSS). Patients with subcapsular micrometastases <0.1mm in maximum dimension had the lowest frequency of NSN metastasis (5.5%). Despite differences in SN biopsy protocols and clinicopathologic features of the patient cohorts (between centers), most SN parameters remained predictive in individual center populations. Maximum SN tumor size >1mm was the most reliable and consistent parameter independently associated with higher non-SN positivity, poorer DFS, and poorer MSS.

In this large retrospective, multicenter cohort study, several parameters of SN tumor burden including intranodal location, TPD and maximum size provided prognostic information, but their prognostic significance varied considerably between the different centers. This could be due to sample size limitations or to differences in SN detection, removal and examination techniques.

INTRODUCTION

Twenty years ago, sentinel node (SN) biopsy (SNB) was introduced as a staging technique for patients with early-stage melanoma.[1, 2] Since then, SN status has been shown to be the strongest independent prognostic factor in patients with clinically localized primary cutaneous melanoma.[3-6]

First introduced in the 6th edition (2001) of the American Joint Commission on Cancer (AJCC)/Union Internationale Contre le Cancer (UICC) staging system for cutaneous melanoma, sentinel lymph node tumor burden is now established as an N1-2a staging criterion in the TNM staging system[7, 8]. However, specific subgroups of SN-positive patients have vastly differing survival rates, ranging from approximately 30% to over 90%.[3, 10-14] Patient characteristics, primary tumor and SN parameters, and models for risk stratification of SN-positive patients have been assessed in numerous studies with respect to prediction of non-SN (NSN) status and survival.[11-14, 16-22] Ideally, the parameters utilized for prognostic stratification must be easy and quick to assess and reproducible.[23, 24] The best validated prognostic SN tumor burden parameters to date are: tumor penetrative depth beneath the SN capsule, maximum size of SN tumor deposits and intranodal location of SN tumor.[6, 11, 13, 16-19, 22, 25-31]

In recent years, the European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group (MG) and Melanoma Institute Australia (MIA) have each gathered large independent datasets of SN-positive patients, assessed micromorphometric parameters of tumor in SNs and demonstrated the prognostic importance of these factors.[6, 16, 28] The aim of the current study was to combine the large European and Australian patient cohorts, and evaluate the prognostic significance of SN tumor burden parameters and classification schemes overall. A secondary aim was to assess and compare the predictive power of these parameters in individual melanoma treatment centers.

PATIENTS AND METHODS

Patients

Patients diagnosed between 1993 and 2008 with primary melanoma and a positive SN, at eleven melanoma treatment centers (ten EORTC MG centers in six different countries and one center, MIA, in Sydney, Australia) were studied. Patient demographics, information on previous medical history and follow-up data were collected by each center. SN tumor burden was measured and classified by at least two of the following morphometric parameters: intranodal location (9/11 centers)[17], maximum size of the largest discrete SN tumor deposit (11/11 centers)[13, 31]) and tumor penetra-

tive depth (7/11 centers;[11, 19, 30]). The RDC (Rotterdam-Dewar Combined) classification was derived from the Rotterdam classification and the modified Dewar classification (9/11 centers).[6]

Lymphatic mapping, sentinel node biopsy and completion lymph node dissection

At all centers, SNB was offered to patients with Breslow thickness ≥ 1mm or to patients with thinner tumors with adverse prognostic features such as ulceration, a high mitotic rate or Clark level IV or V invasion. SNB was performed using the triple technique identifying SNs with a combination of lymphoscintigraphy, preoperative injection of blue dye at the primary melanoma site and intraoperative use of a gamma probe. Full details have been reported previously.[14, 32-35] However, there were some differences in the procedures for identifying and removing SNs at the different centers. These included differences in the radiocolloids used for preoperative lymphoscintigraphy, the timing and planes of view utilized for lymphoscintigraphy, the type and volume of blue dye used, the type and sensitivity of the hand-held gamma probe and the criteria utilized for defining a SN, as well as the experience of the nuclear medicine physicians, radiologists and surgical oncologists performing these procedures. Excised SNs were fixed in buffered formalin and sent for pathologic examination. Subsequently, SN tumor burden was determined by histopathologic review of available tissue sections. CLND was performed in 1381 of 1539 (90%) SN-positive patients. Reasons for not performing CLND were eligibility for the EORTC 1208 (Minitub) study[36], the presence of micrometastases <0.1mm in maximum dimension since an excellent survival is to be expected, enrolment in the observation arm of the second Multicenter Selective Lymphadenectomy Trial (MSLT-II)(Clinicaltrials.gov identifier NCT00297895), patient refusal of further treatment, or when surgical and anesthetic risks associated with CLND were considered too great due to patient co-morbidities.

Pathology processing and analysis

There were also differences in the pathology processing and analysis of retrieved SNs between the eleven different centers. Generally, SNs from most of the ten EORTC MG centers were processed and assessed according to the basic principles of the EORTC MG SN pathology protocol as described by Cook et al,[14, 37] while the SNs at MIA were processed according to a different protocol.[38, 39]

Briefly, for the EORTC MG protocol, SNs were bisected through the hilum in its longest dimension after 24h placement in formalin. After cutting several sections from each SN, sections were stained with hematoxylin & eosin (H&E) and for S-100, Melan-A and/or HMB-45. Six pairs of 4µm-thick sections were cut with a 50µm

interval between them. Spare sections were made at each level for additional staining if required for interpretation. In 2003 the protocol was revised, and the distance between the step sections changed from 250µm to 50µm, but the main principles of bisecting through the hilum and cutting sections from the hilum remained the same.

At MIA, SNs were bisected along their longest axis after 24 hours fixation in 10% buffered formalin. Each half of the SN was cut in four sequential sections 5µm thick (i.e. eight sections were examined routinely). Additional sections were cut when examination of further sections was deemed necessary for the assessment of the presence or absence of metastasis or to determine the site, size or TPD of tumor deposits.

An average of 12 slides per SN was taken at the EORTC MG centers versus 8 slides at MIA. In comparison to SN samples, CLND specimens were examined routinely with a less extensive protocol at all centers, consisting of only bivalving and H&E staining of the lymph nodes. Four experienced pathologists and a non-pathologist specialist in the melanoma area reviewed SNs belonging to European patients. Two pathologists at Royal Prince Alfred Hospital (RPAH) (R.A.S. and R.M.) and a non-pathologist melanoma specialist in the field of analyzing SN slides who also reviewed the EORTC MG patients (A.C.J.v.A.) reviewed the MIA SN slides. Consensus in analyzing SN tumor burden was reached and metastases were analyzed according to equivalence principles, which are explained in another study.[24]

Statistical Methods

Statistics were performed with STATA version 11 (StataCorp LP, College Station, TX) and IBM SPSS Statistic v 19.0 (Chicago, IL). Variables were coded and assessed as described in Table 1. Pearson's chi-square, Fisher's exact and the Mann-Whitney U tests were employed where appropriate. Binary logistic regression was employed to assess factors that were predictive of NSN status. The Kaplan-Meier method together with the Log Rank test and Cox regression were executed for univariate survival analyses. Four separate Cox regression multivariate models were performed to assess disease-free survival (DFS) and melanoma-specific survival (MSS), in order to account for the correlation of SN tumor burden parameters. Disease-free survival (DFS) was measured from date of SNB to date of first recurrence and melanoma-specific survival (MSS) from date of SNB to date of death caused by melanoma, or last follow-up (censored). Two–tailed p-values less than 0.05 were considered statistically significant.

RESULTS

Between 1993 and 2008, 1539 patients diagnosed with primary melanoma were found to have a positive SN. Clinicopathologic characteristics of all SN-positive patients are summarized in Table 1. The numbers of patients from each center were: MIA (n=350), Warsaw (n=245), Guildford (n=214), Amsterdam – NKI (n=116), Rotterdam

Table 1 – Clinicopathologic factors for all sentinel node positive patients (n=1539).

Characteristic	n	%	Characteristic	n	%
Gender			Intranodal location (Dewar classification)		
Female	702	46	Subcapsular	248	18
Male	837	54	Non-subcapsular	1155	82
Age *(years)*			Missing	136	
mean±SD	52.3±15.3		Tumor penetrative depth *(mm)*		
≤ 50	693	45	median (IQR)	0.80 (0.30 – 1.95)	
> 50	846	55	S-Classification		
Location			SI	246	30
Extremity	779	51	SII	227	28
Trunk	533	35	SIII	344	42
Head&Neck	211	14	Missing	722	
Other	14	1	Tumor penetrative depth *(mm)*		
Missing	2		≤ 0.5	333	41
Melanoma subtype			> 0.5	484	59
SSM	572	48	Missing	722	
NM	536	45	Maximum size *(mm)*		
Other	89	7	median (IQR)	0.90 (0.30 – 2.50)	
Missing	342		Rotterdam classification *(mm)*		
Breslow thickness *(mm)*			< 0.1	146	10
mean±SD	3.74±0.10		0.1 – 1.0	665	43
median (IQR)	2.95 (1.80 – 4.50)		> 1.0	728	47
T1	90	6	Maximum size *(mm)*		
T2	394	26	≤ 2	1094	71
T3	616	40	> 2	445	29
T4	435	28	Maximum size *(mm)*		
Missing	4		≤ 1		
Clark level			> 1		
I-II	37	3	RDC classification		
III	350	24	< 0.1mm subcapsular	69	5
IV	921	62	< 0.1mm non-subcapsular	55	4

V	172	12	0.1 – 1.0mm subcapsular	160	11
Missing	59		0.1 – 1.0mm non-subcapsular	435	31
Ulceration			> 1.0mm	684	49
Absent	781	54	Missing	136	
Present	655	46	CLND performed		
Missing	103		no	158	10
Number of removed SNs			yes	1381	90
1	468	36	NSN status		
2	426	33	Negative	1098	80
3	411	32	Positive	283	21
Missing	234		Time SNB		
Number of positive SNs			1993 – 2002	714	46
1	1030	79	2003 – 2008	825	54
2	228	17			
≥3	53	4			
Missing	230				

SSM=Superficial spreading melanoma; NM=Nodular melanoma; IQR=Interquartile range, SD = standard deviation

(n=115), Padova (n=109), Amsterdam – VUMC (n=107), Berlin (n=86), Milan (n=73), Villejuif (n=68) and Groningen (n=56) (Table S1). Mean age was 52.3 (standard deviation (SD) ± 15.3) years. Median Breslow thickness was 2.95mm (interquartile range (IQR) 1.80-4.50mm). Ulceration was present in 46% of the melanomas. Median maximum SN tumor size was 0.90mm (IQR 0.30-2.50mm) (Tables 1 and S1). Mean and median follow-up times were 42 and 32 (IQR 20-58) months, respectively. In 1381 (90%) patients, CLND was performed.

Differences between centers

Significant differences in primary tumor and SN characteristics were observed between centers (Table S1 and Fig. 2). Median Breslow thickness was 3.00mm for the European cohort and 2.43mm for the Australian cohort (p=0.001), and ulceration was more common in the European than the Australian cohort (45% vs. 35%, p=<0.001). The median maximum SN tumor sizes for the European and Australian cohorts were not significantly different (0.95mm and 0.80mm, respectively, p=0.126).

NSN status

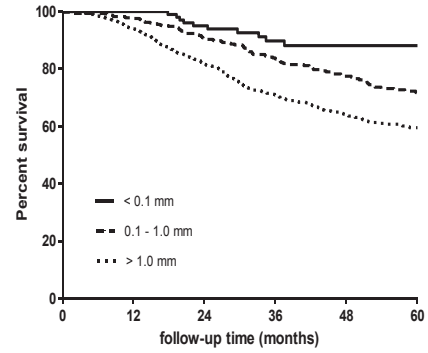
Details of NSN status were available for 1381 patients who had a CLND. Breslow thickness, ulceration, Clark level of invasion, number of SNs removed, and all micromorphometric parameters were significant predictors of NSN status (p< 0.05) (Table 2). Of the SN tumor burden parameters, the RDC classification subgroup

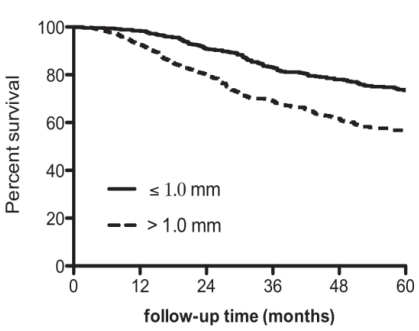
Table 2 – Factors predictive of non-sentinel node status.

Characteristic	Total	NSN-positive		p-value*
	n	n	%	
Age				
≤ 50	637	118	18.5	
> 50	744	165	22.2	NS
Breslow thickness				
T1	80	10	12.5	Ref
T2	348	61	17.5	NS
T3	549	94	17.1	NS
T4	400	117	29.3	0.003
Ulceration				
Absent	686	122	17.8	Ref
Present	600	144	24.0	0.006
Dewar classification				
subcapsular	217	19	8.8	Ref
non-subcapsular	1040	229	22.0	< 0.001
S-Classification				
SI	202	23	11.4	Ref
SII	195	35	17.9	NS
SIII	309	76	24.6	<0.001
Tumor penetrative depth *(mm)*				
≤ 0.5	274	30	10.9	Ref
> 0.5	432	104	24.1	< 0.001
Rotterdam Classification *(mm)*				
< 0.1	117	14	12.0	Ref
0.1 – 1.0	589	93	15.8	NS
>1.0	675	176	26.1	0.001
Maximum size *(mm)*				
≤ 1	706	107	15.1	Ref
> 1	675	176	26.1	<0.001
Maximum size *(mm)*				
≤ 2	964	150	15.6	Ref
> 2	417	133	31.9	< 0.001
RDC classification				
< 0.1mm subcapsular	55	3	5.5	Ref
< 0.1mm non-subcapsular	43	9	20.9	0.030
0.1 – 1.0mm subcapsular	146	16	11.0	NS
0.1 – 1.0mm non-subcapsular	379	55	14.5	NS
> 1.0mm	634	165	26.0	0.003

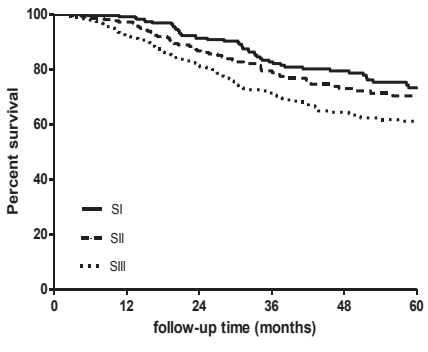
**Univariate binary logistic regression was used to calculate the significance of individual strata for each classification system in predicting NSN status; only the p-values are reported here.*
NSN=Non-sentinel node
NS=Not significant
Ref=reference category

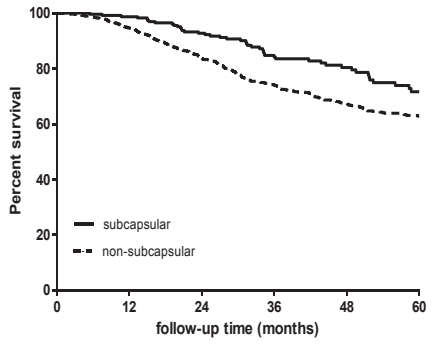
Figure 1 – Melanoma specific-survival curves for all SN-positive patients.





	Nr at Risk							Nr at Risk					
< 0.1 mm	146	146	107	73	54	33	≤ 1.0 mm	811	719	574	408	303	216
0.1–1.0 mm	665	586	467	335	249	183	> 1.0 mm	728	617	448	284	205	133
> 1.0 mm	728	617	448	284	205	133	Total	1539	1338	1112	692	508	349
Total	1593	1349	1022	692	508	349							





	Nr at Risk							Nr at Risk					
SI	246	227	187	136	104	71	Subcapsular	248	223	189	135	94	63
SII	225	207	161	120	88	67	Non-subcapsular	1155	992	735	474	345	227
SIII	396	339	259	176	128	85	Total	1403	1215	924	609	439	290
Total	867	773	607	432	320	223							

with subcapsular micrometastases <0.1mm in maximum dimension had the lowest NSN-positivity rate (5.5%). (Table 2).

As continuous variables measured in millimeters, maximum SN metastasis size (OR=1.11, 95%CI: 1.07-1.15, p<0.001) and tumor penetrative depth (OR=1.26, 95%CI: 1.15-1.38, p<0.001) were significant predictors of NSN status in the overall cohort

(Table S2). The categorization of maximum size (≤1 vs. >1mm and ≤2 vs. >2mm), TPD (≤0.5 vs. >0.5mm) and intranodal location (sub-capsular vs. non-subcapsular) resulted in a statistically significant stratification of risk for NSN-positivity (Table 2). When comparing the eleven melanoma centers, there was variation in the prediction of NSN positivity by the proposed classification systems (Table S3). Classifications of maximum SN tumor size using a 1mm or 2mm cutoff significantly stratified risk for NSN positivity in 5/11 and 6/11 centers, respectively. The Rotterdam system, RDC system, TPD, S-classification and intranodal location significantly stratified risk for NSN positivity in only 2/11, 1/9, 2/7, 2/7 and 2/9 centers, respectively (Table S3).

Survival

In univariate analysis of the overall cohort, factors significantly associated with melanoma-specific survival (MSS) were: patient sex, age, melanoma subtype, Breslow thickness, Clark level, ulceration, Dewar classification, TPD, S-classification, maximum size, Rotterdam classification, RDC classification (only in the subgroups of patients with tumor deposits >1.0mm in maximum dimension and in patients with deposits >0.1-1.0mm in maximum dimension and non-subcapsular) and NSN status (Table 3).

Table 3 – Univariate analyses for melanoma-specific survival of all sentinel node positive patients.

Characteristic	Univariate analysis		
	HR	95% CI	p-value
Gender			
Female	1		
Male	1.30	1.07-1.57	0.009
Age *(years)*			
continuous	1.02	1.01-1.02	<0.001
≤ 50	1		
> 50	1.36	1.12-1.65	0.002
Location			
Extremity	1		
Trunk	0.99	0.80-1.22	NS
Head&Neck	0.99	0.74-1.32	NS
Melanoma subtype			
SSM	1		
NM	1.59	1.27-2.00	<0.001
Other	1.87	1.27-2.75	0.002
Breslow thickness *(mm)*			
continuous	1.08	1.06-1.09	<0.001
T1	1		

Table 3 (continued)

Characteristic	Univariate analysis		
	HR	95% CI	p-value
T2	1.71	0.88-3.31	NS
T3	2.67	1.41-5.07	0.003
T4	5.09	2.69-9.63	<0.001
Clark level			
I-III	1		
IV	1.47	1.15-1.89	0.002
V	2.27	1.64-3.15	<0.001
Ulceration			
Absent	1		
Present	2.49	2.03-3.05	<0.001
Dewar classification			
subcapsular	1		
non-subcapsular	1.75	1.31-2.35	<0.001
Tumor penetrative depth *(mm)*			
continuous	1.19	1.13-1.26	<0.001
≤ 0.5	1		
> 0.5	1.77	1.34-2.35	<0.001
S-classification			
SI	1		
SII	1.58	1.09-2.28	0.016
SIII	1.93	1.38-2.70	<0.001
Maximum size *(mm)*			
continuous	1.10	1.08-1.12	<0.001
≤ 1	1		
>1	1.96	1.61-2.37	<0.001
≤ 2	1		
>2	2.17	1.79-2.64	<0.001
Rotterdam classification *(mm)*			
< 0.1	1		
0.1 – 1.0	1.99	1.21-3.28	0.007
>1.0	3.55	2.17-5.80	<0.001
RDC classification			
< 0.1mm subcapsular	1		
< 0.1mm non-subcapsular	1.17	0.43-3.24	NS
0.1 – 1.0mm subcapsular	1.80	0.84-3.86	NS
0.1 – 1.0mm non-subcapsular	2.05	1.00-4.2	0.050
> 1.0mm	3.43	1.69-6.93	0.001

Table 3 (continued)

Characteristic	Univariate analysis		
	HR	95% CI	p-value
NSN status			
Negative	1		
Positive	2.03	1.63-2.52	<0.001
CLND performed			
no	1		
yes	0.87	0.62-1.20	NS
Year of SNB			
1993 - 2002	1		
2003 - 2008	0.89	0.72-1.10	NS

HR = Hazard Ratio; CI = Confidence Interval; SE = Standard Error; SSM = Superficial Spreading Melanoma; NM = Nodular Melanoma; SN = sentinel node; NSN = Non-sentinel node; NS=Not significant

Because of multicollinearity between SN tumor burden parameters, four different multivariate models were used. Each multivariate model contained the following variables: Breslow thickness, age, NSN status, ulceration, and one of the four SN tumor burden parameters. Other significant prognostic factors at univariate analyses, i.e. sex, melanoma subtype and Clark level, were not included due to insignificance in the multivariate model. Significant prognostic factors for poorer MSS in the multivariate models included the presence of non-sub-capsular metastases, TPD >1mm, and maximum SN tumor size >1mm (Table 4). The SII sub-group of the S-classification and the 0.1-1mm sub-group of the Rotterdam classification did not vary significantly from the reference groups (SI and <0.1mm, respectively). (Table 4).

A comparison of the eleven melanoma centers revealed variation in the accuracy of survival prediction using the various classification systems (Tables S4, S5). The two classifications of maximum SN tumor size (≤ 1mm vs. > 1mm and ≤ 2mm vs. > 2mm) were the most consistently significant, distinguishing prognostic sub-groups for MSS in 6/11 and 5/11 centers and prognostic sub-groups for DFS in 7/11 and 8/11 centers. TPD and the S-classification were more frequently predictive of DFS than MSS. Non-subcapsular tumor location was significantly associated with DFS and MSS in only one of nine centers.

DISCUSSION

Micromorphometric parameters of SN tumor burden (TPD, intranodal tumor location and maximum tumor size) were all predictive factors for NSN status (Table 2), DFS (Table S5) and MSS (Table 3) in this large cohort of SN-positive patients

Table 4 – Four different stepwise multivariate Cox's hazard regression models for melanoma-specific survival for all sentinel node positive patients.

Multivariate Model* (n)	Significant	Variable for SN tumor burden	Melanoma Specific Survival		
			HR	95% CI	p-value
#1		Dewar Classification			
(n=1159)	Breslow°	Subcapsular	1		
	Age°	Non-Subcapsular	1.49	1.07-2.08	0.018
	NSN status				
	Ulceration				
#2		S-Classification			
(n=679)	Breslow°	SI	1		
	Age°	SII	1.49	0.99-2.26	NS
	NSN status	SIII	1.63	1.12-2.38	0.011
	Ulceration				
#3		Rotterdam Classification			
(n=1278)	Breslow°	< 0.1mm	1		
	Age°	0.1 - 1.0mm	1.75	0.99-3.11	NS 0.001
	NSN status	> 1.0mm	2.56	1.45-4.50	
	Ulceration				
#4		RDC Classification			
(n=1159)	Breslow°	< 0.1mm Subcapsular	1		
	Age°	< 0.1mm Non-Subcapsular	1.26	0.41-3.91	NS
	NSN status	0.1 - 1.0mm Subcapsular	1.54	0.64-3.69	NS
	Ulceration	0.1 - 1.0mm Non-Subcapsular	1.85	0.81-4.25	NS
		> 1.0mm	2.37	1.05-5.37	0.038

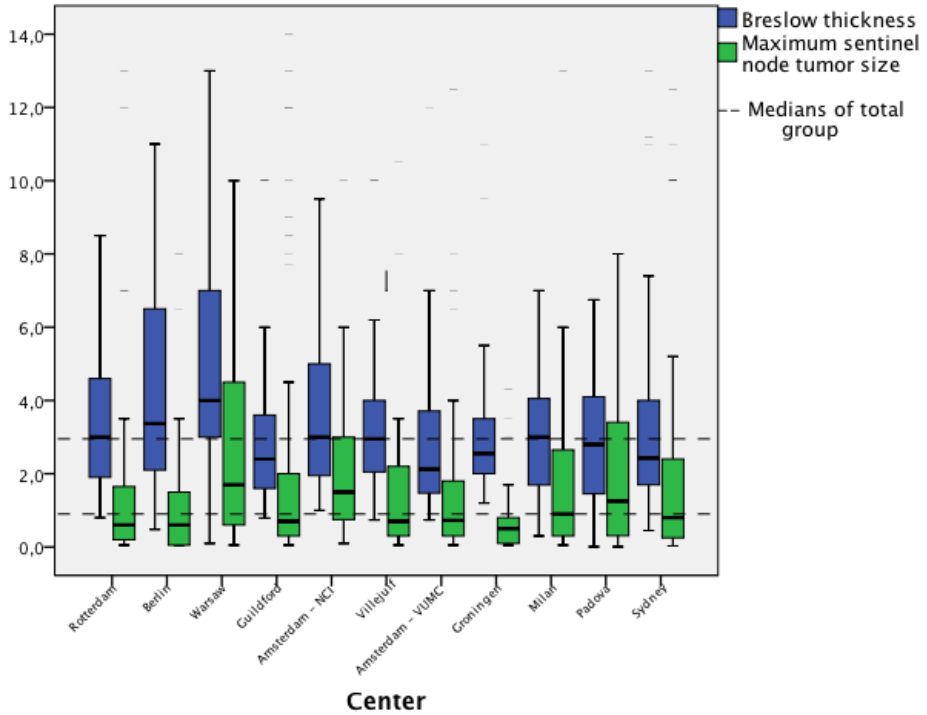
HR = Hazard Ratio; CI = Confidence Interval; NSN = Non-sentinel node
** Four different multivariate analyses were performed due to multicollinearity between the four classifications for sentinel node tumor burden, i.e. modified Dewar Classification, S-Classification, Rotterdam Classification and RDC Classification.*
° The variables Age and Breslow are continuous variables.

treated at eleven different centers. The SN classification systems assessed in the study significantly differentiated MSS outcomes for each sub-group on univariate analysis (Table 3). However, when adjusting for known prognostic factors in multivariate MSS analysis, at least one cut-off for each system (S-classification, RDC and Rotterdam classifications) failed to significantly differentiate outcome (Table 4). Similarly, not all cut-offs significantly stratified risk of NSN positivity or univariate DFS outcomes (Table 2 and S5). After adjustment for other clinico-pathologic factors, increasing age, increasing Breslow thickness, presence of ulceration and NSN status, non-subcapsular location (Dewar classification), high TPD (SIII of S-classification) and increasing maximum size of tumor (>1mm or >2mm or the Rotterdam comparison

of >1mm with <0.1mm) were independently associated with poorer MSS (Table 4). Patients within the RDC classification subgroup having micrometastases <0.1mm in maximum dimension in a subcapsular location had the lowest NSN-positivity rate (5.5%) (Table S2).

There was considerable heterogeneity between centers with regard to primary tumor characteristics and SN tumor burden features. The differences between centers are clear. Median Breslow thickness and ulceration rate ranged from 2.1mm and 25.5% in the Amsterdam – VUMC center (n=107) to 4.00mm and 67.7% in the Warsaw center (n=245) (Table S1). Patients from the Amsterdam – NCI center (n=116) had no patients with <0.1mm SN metastases, whilst 27% of the Berlin cohort had <0.1mm SN metastases. The percentage of SN-positive patients with subcapsular metastases ranged from 4% in the Warsaw cohort to 38% in the Berlin cohort. The median maximum SN tumor size for the total group of patients was 0.90mm, with a range of 0.50-1.70mm. Moreover, no clear correlation between the thickness of the primary lesion and the SN tumor burden at each center was apparent (Figure 2). These large variations may not only reflect differences in clinical presentation and management of melanoma patients in the different centers, but also differences in the methodolo-

Figure 2 - Box plot showing the median and 95 percentile of A) the Breslow thickness and B) the maximum sentinel node tumor size per center



gies used to identify, remove and examine SNs, and possibly the use of preoperative SN ultrasound screening in some centers.[40, 41]

SN tumor burden parameters were not of prognostic and predictive value in each individual center (Tables S2-S5). In the combined cohort of SN-positive patients (n=1539), all four SN tumor burden classification systems were independently prognostic in univariate analysis for MSS (Table 3, Figure 1), which is in line with previous studies of these parameters.[6, 22]. In those studies, patients with minimal SN tumor burden, i.e. < 0.1mm metastases in a subcapsular location, had an excellent estimated 5-year MSS of approximately 90%, which is equivalent to that of SN-negative patients.[6] However, in the combined cohort of the present study, patients with micrometastases <0.1mm had an 83% 5-year MSS, whilst patients with subcapsular metastases (of any size) had a 5-year MSS of 72% (Figure 1). This suggests that the excellent 5-year MSS estimate reported in the earlier studies[6] may have been influenced by lead-time bias. However, follow-up has not been extended compared to the earlier study. The only difference between earlier results and results of this study is the addition of data from two centers. It is nevertheless important to bear in mind that for patients with SN micrometastases <0.1mm in maximum dimension, 5-year survival figures may be misleading because if recurrence does occur, it is likely to be much later than recurrence in patients with larger SN metastases. This was demonstrated clearly in the AJCC Melanoma Database analysis, which highlighted the very great differences in prognosis and time to recurrence for patients with nodal micrometastases and those with nodal macrometastases[10]. When the data were analyzed by center, there were substantial differences in the prognosis for patients with minimal SN tumor burden (<0.1mm), with the MSS ranging from 54% to 100% in different centers.

In all centers, SNs were detected by preoperative lymphoscintigraphy and identified at the time of surgery with blue dye and a gamma probe.[32, 33, 42, 43] However, as detailed in the Methods section, there were some important differences in how SNs were identified in different centers. These differences may have influenced the results of this study because it is well documented that variations in any part of the SNB technique (including in nuclear medicine, surgery and pathology) can affect the accuracy of SNB.[44-46] There were also differences between protocols utilized for SN pathology assessment at MIA and most of the European melanoma centers. The main difference was the larger number of sections cut from each half of the SN in European centers. Furthermore, the EORTC protocol was altered slightly during the period of this study (as detailed in the supplementary methods). Potentially these differences could be important because examination of extra sections of SNs can increase the SN positivity rate.[14, 34, 37, 39, 47-49] The number of sections pathologically examined and the distance between the sections may also affect the reported size,

location and TPD of melanoma metastases in a SN and therefore its sub-classification according to the various proposed classification schemes. In view of this it might be predicted that an increase in the SN-positivity rate might be associated with an increase in the detection rate of minimal SN tumor burden cases.[14] Interestingly, the percentage of patients with minimal SN tumor burden (<0.1mm) in our study differed significantly between individual European centers (where a greater number of sections were cut, allegedly according to the same protocol) (Table S1, Figure 2) but was not significantly different in the Australian cohort compared to the European cohort overall.

In conclusion, primary tumor and SN tumor burden parameters assessed in this large retrospective multicenter study have been shown to provide valuable prognostic information in SN-positive patients. A maximum SN tumor size >1mm separated the cohort into two groups of similar size, and was the most consistent independent predictor of NSN positivity and poorer DFS and MSS in individual centers, and in the combined cohort. The study has provided valuable insights into the prognostic value of SN tumor burden assessment in patients with melanoma. However, prospective studies with long term follow-up are clearly required to establish a classification system for SN tumor burden that consistently and accurately stratifies patients into meaningful prognostic groups with respect to NSN-positivity and survival outcomes, and is not unduly affected by minor variations in SN identification and examination protocols.

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Table S1 – Clinicopathologic factors of sentinel node positive patients described per center.

Center	Number of patients (%)	Median Breslow thickness (mm)	Ulceration (%)	Median Maximum tumor size (mm)	Rotterdam classification			S-classification			Dewar classification		RDC classification
					< 0.1 mm (%)	0.1 – 1.0 mm (%)	> 1.0 mm (%)	SI (%)	SII (%)	SIII (%)	Subcapsular (%)	Non-subcapsular (%)	< 0.1mm subcapsular (%)
Rotterdam	115 (7.5)	3.00	51.0	0.60	18	48	35	46	35	19	30	71	10
Berlin	86 (5.4)	3.37	55.8	0.60	27	35	38	22	11	0	38	62	17
Warsaw	245 (15.9)	4.00	67.7	1.70	3	33	64	-	-	-	4	96	1
Guildford	214 (13.9)	2.40	41.1	0.70	11	46	43	-	-	-	18	82	5
Amsterdam NKI*	116 (7.5)	3.00	39.7	1.50	0	36	64	13	40	63	34	66	0
Villejuif	68 (5.3)	2.95	53.3	0.70	9	50	41	-	-	-	15	85	5
Amsterdam VUMC*	107 (6.9)	2.12	25.5	0.75	11	49	40	-	-	-	15	85	6
Groningen	56 (3.6)	2.55	30.9	0.50	23	57	20	50	30	20	-	-	-
Milan	73 (4.7)	3.00	50.7	0.90	4	52	44	25	26	49	-	-	-
Padova	109 (7.1)	2.80	53.8	1.25	8	34	58	21	26	53	11	89	5
Sydney	350 (22.7)	2.43	34.8	0.80	8	47	45	30	24	46	17	83	4
Total	1539	2.95	45.6	0.90	10	43	47	30	28	42	18	82	5

RDC = Rotterdam Dewar Combined

**Amsterdam NKI stands for the Netherlands Cancer Institute – Antoni van Leeuwenhoek hospital in Amsterdam, while Amsterdam VUMC stands for the Vrije Universiteit Medical Center in Amsterdam*

Table S2 – Maximum size and tumor penetrative depth (mm) assessed as continuous variables at each individual center and in the total cohort, for the following outcomes: non-sentinel node (NSN) status, melanoma-specific survival (MSS), and disease-free survival (DFS).

		Maximum Size (mm)				Tumor Penetrative Depth (mm)		
Centre Name	**N**	OR/HR	95% CI	P-value	**N**	OR/HR	95% CI	P-value
NSN Status								
Rotterdam	101	-	-	NS	69	-	-	NS
Berlin	77	2.07	1.33-3.20	***0.001***	29	-	-	NS
Warsaw	243	1.09	1.02-1.16	***0.009***	-	-	-	-
Guildford	189	-	-	NS	-	-	-	-
Amsterdam NKI	116	1.32	1.02-1.72	***0.035***	116	-	-	NS
Villejuif	66	1.41	1.07-1.87	***0.015***	-	-	-	-
Amsterdam VU	97	-	-	NS	-	-	-	-
Groningen	55	-	-	NS	55	-	-	NS
Milan	65	-	-	NS	65	-	-	NS
Padova	104	1.18	1.05-1.32	***0.005***	104	1.38	1.16-1.64	***<0.001***
Sydney	268	-	-	NS	268	1.44	1.18-1.75	***<0.001***
Overall	1381	1.11	1.07-1.15	***<0.001***	706	1.26	1.15-1.38	***<0.001***
MSS								
Rotterdam	113	1.13	1.02-1.26	***0.016***	80	1.23	1.01-1.51	***0.045***
Berlin	87	1.33	1.03-1.72	***0.032***	28	-	-	NS
Warsaw	238	1.06	1.02-1.10	***0.002***	-	-	-	-
Guildford	211	1.11	1.04-1.18	***0.001***	-	-	-	-
Amsterdam NKI	114	1.18	1.00-1.39	0.057	114	1.18	1.00-1.39	0.055
Villejuif	61	-	-	NS	-	-	-	-
Amsterdam VU	103	-	-	NS	-	-	-	-
Groningen	55	-	-	NS	55	-	-	NS
Milan	65	1.28	1.09-1.50	***0.003***	65	1.27	1.06-1.53	***0.01***
Padova	109	1.16	1.08-1.24	***<0.001***	109	1.18	1.07-1.29	***<0.001***
Sydney	345	1.11	1.05-1.18	***<0.001***	345	1.18	1.05-1.33	***0.005***
Overall	1533	1.1	1.08-1.12	***<0.001***	807	1.19	1.13-1.26	***<0.001***
DFS								
Rotterdam	115	1.1	1.02-1.20	***0.018***	80	1.26	1.07-1.48	***0.007***
Berlin	87	1.31	1.10-1.57	***0.003***	32	-	-	NS
Warsaw	242	1.05	1.02-1.08	***0.003***	-	-	-	-
Guildford	206	1.08	1.02-1.15	***0.009***	-	-	-	-
Amsterdam NKI	114	1.26	1.10-1.44	***0.001***	114	1.3	1.14-1.48	***<0.001***
Villejuif	67	1.18	1.00-1.38	***0.049***	-	-	-	-
Amsterdam VU	107	-	-	NS	-	-	-	-
Groningen	56	-	-	NS	56	-	-	NS
Milan	71	-	-	NS	71	-	-	NS
Padova	109	1.15	1.08-1.22	***<0.001***	109	1.16	1.07-1.25	***<0.001***
Sydney	350	1.1	1.04-1.16	***<0.001***	350	1.18	1.06-1.31	***0.002***
Overall	1539	1.09	1.07-1.11	**<0.001**	817	1.18	1.13-1.24	***<0.001***

OR=Odds ratio computed for NSN status; HR=Hazard ratio computed for MSS and DFS; NS=Not significant; NSN=Non-sentinel node; MSS=Melanoma-specific survival; DFS=Disease-free survival

Table S3 – Micromorphometric classifications that significantly predict non-sentinel node status at each individual centre.

Centre Name	Rotterdam	Berlin	Warsaw	Guildford	Amsterdam NKI	Villejuif	Amsterdam VU	Groningen	Milan
N	101	77	243	189	116	66	97	55	65
Maximum Size (mm)									
≤2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
>2	NS	***<0.001***	***<0.001***	NS	***0.016***	NS	***0.048***	NS	NS
Maximum Size (mm)									
≤1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
>1	NS	***<0.001***	***0.023***	NS	***0.032***	NS	NS	NS	NS
Rotterdam (mm)									
<0.1	Ref	Ref	Ref	Ref	-	Ref	Ref	Ref	Ref
0.1-1.0	NS	NS	NS	NS	Ref	NS	NS	NS	NS
>1.0	NS	***0.002***	NS	NS	***0.032***	NS	NS	NS	NS
Dewar									
Subcapsular	Ref	Ref	Ref	Ref	Ref	Ref	Ref	-	-
Non-subcapsular	NS	***0.002***	NS	NS	***0.043***	NS	NS	-	-
RDC									
<0.1 Subcapsular	Ref	Ref	Ref	Ref	-	Ref	Ref	-	-
<0.1 Non-subcapsular	NS	NS	NS	NS	-	NS	NS	-	-
0.1-1.0 Supcapsular	NS	NS	NS	NS	Ref	NS	NS	-	-
0.1-1.0 Non-subcapsular	NS	NS	NS	NS	NS	NS	NS	-	-
>1.0	NS	***0.011***	NS	NS	NS	NS	NS	-	-
TPD (mm)									
≤0.5	Ref	Ref	-	-	Ref	-	-	Ref	Ref
>0.5	NS	NS	-	-	NS	-	-	NS	NS

Table S3 (continued)

Centre Name	Rotterdam	Berlin	Warsaw	Guildford	Amsterdam NKI	Villejuif	Amsterdam VU	Groningen	Milan
S-Classification (mm)									
SI (≤0.3)	Ref	Ref	-	-	Ref	-	-	Ref	Ref
SII (>0.3-1.0)	NS	NS	-	-	NS	-	-	NS	NS
SIII (>1.0)	NS	***0.006***	-	-	NS	-	-	NS	NS

NS=Not significant
Ref=Reference group

Table S4 – Micromorphometric classifications that significantly influence melanoma-specific survival at each individual centre

Centre Name	Rotterdam	Berlin	Warsaw	Guildford	Amsterdam NKI	Villejuif	Amsterdam VUMC	Groningen	Milan	Padova	Sydney
N	115	87	244	214	116	68	107	56	73	109	350
Maximum Size (mm)											
≤2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
>2	NS	0.053	***0.001***	***0.003***	NS	NS	NS	NS	***0.002***	***0.012***	***<0.001***
Maximum Size (mm)											
≤1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
>1	***0.009***	NS	***0.032***	***0.002***	NS	NS	NS	NS	***0.038***	***0.008***	***0.018***
Rotterdam (mm)											
<0.1	Ref	Ref	Ref	Ref	-	Ref	Ref	Ref	Ref	Ref	Ref
0.1-1.0	NS	NS	NS	NS	Ref	NS	NS	NS	NS	NS	NS
>1.0	***0.006***	NS	NS	***0.007***	NS	NS	NS	NS	NS	NS	NS
Dewar											
Subcapsular	Ref	Ref	Ref	Ref	Ref	Ref	Ref	-	-	Ref	Ref
Non-subcapsular	NS	NS	NS	NS	***0.024***	NS	NS	-	-	NS	NS

Table S4 (continued)

Centre Name	Rotterdam	Berlin	Warsaw	Guildford	Amsterdam NKI	Villejuif	Amsterdam VUMC	Groningen	Milan	Padova	Sydney
RDC											
<0.1 Subcapsular	Ref	Ref	-	Ref	-	Ref	Ref	-	-	Ref	Ref
<0.1 Non-subcapsular	NS	NS	-	NS	-	NS	NS	-	-	NS	NS
0.1-1.0 Supcapsular	NS	NS	-	NS	Ref	NS	NS	-	-	NS	NS
0.1-1.0 Non-subcapsular	NS	NS	Ref	NS	***0.041***	NS	NS	-	-	NS	NS
>1.0	***0.009***	NS	NS	***0.025***	NS	NS	NS	-	-	NS	NS
TPD (mm)											
≤0.5	Ref	Ref	-	-	Ref	-	-	Ref	Ref	Ref	Ref
>0.5	NS	NS	-	-	0.05	-	-	NS	***0.042***	NS	NS
S-Classification (mm)											
SI (≤0.3)	Ref	Ref	-	-	Ref	-	-	Ref	Ref	Ref	Ref
SII (>0.3-1.0)	***0.032***	NS	-	-	NS	-	-	NS	NS	NS	NS
SIII (>1.0)	***0.018***	NS	-	-	NS	-	-	NS	NS	NS	NS

*Unadjusted pairwise comparisons were conducted for each strata of a Kaplan-Meier curve using the Logrank test.
NS=Not significant
Ref=Reference group

Table S5 – Micromorphometric classifications that significantly influence disease-free survival at each individual centre and the total cohort

Centre Name	Rotterdam	Berlin	Warsaw	Guildford	Amsterdam NKI	Villejuif	Amsterdam VU	Groningen	Milan	Padova	Sydney	Overall
N	115	87	244	214	116	68	107	56	73	109	350	1539
Maximum Size (mm)												
≤2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
>2	***0.01***	***<0.001***	***0.001***	***0.006***	***0.019***	NS	***0.036***	NS	NS	***0.011***	***<0.001***	***<0.001***

Table S5 (continued)

Centre Name	Rotterdam	Berlin	Warsaw	Guildford	Amsterdam NKI	Villejuif	Amsterdam VU	Groningen	Milan	Padova	Sydney	Overall
Maximum Size (mm)												
≤1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
>1	***0.001***	***0.041***	***0.008***	***0.002***	NS	***0.011***	NS	NS	NS	***0.006***	***0.005***	***<0.001***
Rotterdam (mm)												
<0.1	Ref	Ref	Ref	Ref	-	Ref	Ref	Ref	Ref	Ref	Ref	Ref
0.1-1.0	***0.042***	NS	NS	NS	Ref	NS	NS	0.056	NS	NS	NS	***0.002***
>1.0	***<0.001***	***0.008***	NS	***0.016***	NS	NS	NS	NS	NS	NS	NS	***<0.001***
Dewar												
Subcapsular	Ref	Ref	Ref	Ref	Ref	Ref	Ref	-	-	Ref	Ref	Ref
Non-subcapsular	NS	NS	NS	NS	***0.007***	NS	NS	-	-	NS	NS	***<0.001***
RDC												
<0.1 Subcapsular	Ref	Ref	-	Ref	-	Ref	Ref	-	-	Ref	Ref	Ref
<0.1 Non-subcapsular	NS	NS	-	NS	-	NS	NS	-	-	NS	NS	NS
0.1-1.0 Supcapsular	NS	***0.015***	-	NS	Ref	NS	NS	-	-	NS	NS	NS
0.1-1.0 Non-subcapsular	NS	NS	Ref	NS	NS	NS	NS	-	-	NS	NS	0.053
>1.0	***0.002***	***0.008***	***0.044***	0.059	***0.039***	NS	NS	-	-	NS	NS	***<0.001***
TPD (mm)												
≤0.5	Ref	Ref	-	-	Ref	-	-	Ref	Ref	Ref	Ref	Ref
>0.5	***0.007***	NS	-	-	***0.029***	-	-	NS	NS	NS	***0.038***	***<0.001***
S-Classification (mm)												
SI (≤0.3)	Ref	Ref	-	-	Ref	-	-	Ref	Ref	Ref	Ref	Ref

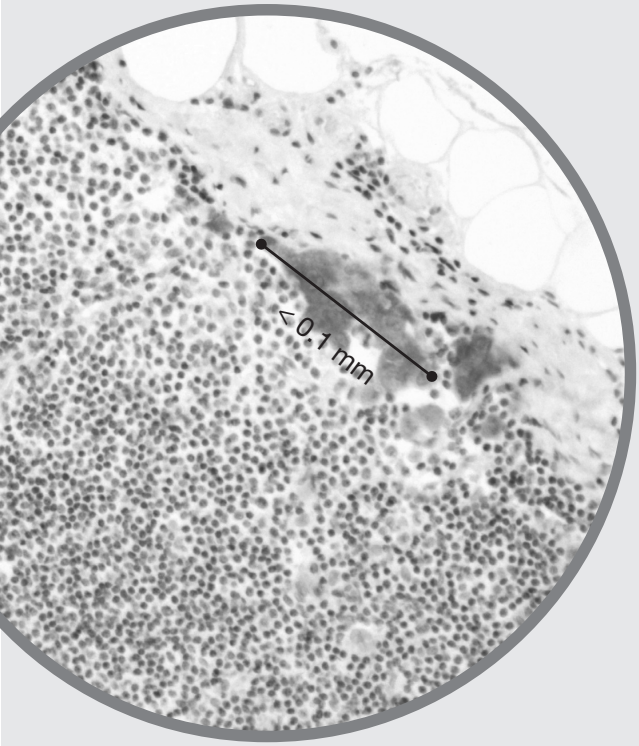
Table S5 (continued)

Centre Name	Rotterdam	Berlin	Warsaw	Guildford	Amsterdam NKI	Villejuif	Amsterdam VU	Groningen	Milan	Padova	Sydney	Overall
SII (>0.3-1.0)	***0.020***	NS	-	-	NS	-	-	NS	NS	NS	NS	NS
SIII (>1.0)	***0.002***	NS	-	-	***0.021***	-	-	NS	NS	NS	NS	***<0.001***

*Unadjusted pairwise comparisons were conducted for each strata of a Kaplan-Meier curve using the Logrank test.
NS=Not significant
Ref=Reference group

Chapter 7

Prognosis in patients with sentinel node-positive melanoma without immediate completion lymph node dissection



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ABSTRACT

The therapeutic value of immediate completion lymph node dissection (CLND) for sentinel node (SN)-positive melanoma patients is unknown. The aim of this study was to evaluate the impact of immediate CLND on the outcome of patients with SN-positive melanoma.

Patients with SN metastases treated between 1993 and 2008 at ten cancer centers from the European Organization for Research and Treatment of Cancer Melanoma Group. Maximum tumor size, intranodal location and penetrative depth of SN metastases were measured. Outcome in those who had CLND was compared with that in patients who did not undergo completion lymphadenectomy.

Of 1174 patients with SN-positive melanoma, 1113 (94.8 per cent) underwent CLND and 61 (5.2 per cent) did not. Median follow-up for the two groups was 34 and 48 months respectively. In univariable survival analysis, CLND did not significantly influence disease-specific survival (hazard ratio (HR) 0.89, 95 per cent confidence interval 0.58 to 1.37; P = 0.600). However, patients who did not undergo CLND had more favourable prognostic factors. Matched-pair analysis, with matching for age, Breslow thickness, tumour ulceration and SN tumour burden, showed that CLND had no influence on survival (HR 0.86, 0.46 to 1.61; P = 0.640). After adjusting for prognostic factors in multivariable survival analyses, no difference in survival was found.

In these two cohorts of patients with positive SN and with prognostic heterogeneity, outcome was not influenced by CLND.

INTRODUCTION

In most countries, sentinel node biopsy (SNB) has become part of the standard management of patients with early-stage melanoma. SN tumour burden is the most important prognostic factor for survival, but an overall survival benefit for patients undergoing SNB versus wide local excision (WLE) alone has not been demonstrated[1]. Approximately 80 per cent of patients with early-stage melanoma who undergo SNB have a negative SN and no further surgical treatment is indicated. Completion lymph node dissection (CLND) for patients diagnosed with one or more positive SNs is performed routinely in most centres, although therapeutic evidence for such routine treatment is lacking[2,3]. In roughly 80 per cent of patients treated with CLND no additional non-SN metastases are detected. The value of CLND could therefore be questioned. This is important because the morbidity of CLND can be significant in terms of wound infections and chronic lymphoedema.

It might not be justified to consider patients with SN-positive disease as a single group because their prognosis is heterogeneous[4]. Patients with a high SN tumour burden have a significantly worse outcome than those with a low burden[4–6]. Micrometastases located in the subcapsular space have a better prognosis than those located elsewhere[4,7]. Outcome might be based on tumour biology and not on earlier removal of low-volume nodal disease. Some authors have suggested that not all patients with a positive SN might need to undergo CLND[2,4,6,8–12], whereas others have reported that CLND is necessary in all patients with SN-positive disease[13–17].

Two retrospective studies have described no significant difference in outcome of patients with SN-positive melanoma between those who underwent CLND and those who did not[2,3]. However, heterogeneity with respect to SN tumour burden was not taken into consideration in these reports. The aim of the present study was to evaluate the influence of immediate CLND on outcome in patients with SN-positive melanoma. The maximum tumour size, intranodal location and penetrative depth of the SN metastases were considered in the analyses.

METHODS

Patients

Patients diagnosed with SN metastases after WLE of malignant melanoma between 1993 and 2008 at ten major cancer centres collaborating in the European Organization for Research and Treatment of Cancer Melanoma Group (EORTC MG) who did not undergo CLND were included in this retrospective multicentre study. Tumour size, location and penetrative depth of SN tumour were measured. The control group

comprised patients with SN-positive melanoma who were treated with immediate CLND at the same centres, within the same time frame. Data were registered in a database, including patient details, and information on the primary melanoma, SNB, SN tumour burden and follow-up.

Surgery

After diagnostic excision or biopsy of the primary melanoma, all patients underwent WLE to achieve margins of 1 cm for melanomas with a Breslow thickness of 2 mm or less and at least 2 cm for melanomas larger than 2 mm. In most patients SNB was performed simultaneously with WLE of the primary tumour. Patients were eligible for SNB if the primary tumour had a Breslow thickness of 1 mm or more, or if the tumour had other adverse prognostic factors such as ulceration or Clark level IV or V.

The SNB procedure has been described in detail elsewhere[18,19]. It consisted of preoperative lymphoscintigraphy, undertaken within 24 h of perioperative injection of patent detection probe toγblue dye near the excision mark and use of a handheld detect SNs. Lymph nodes were identified as SNs if they had an afferent blue lymph duct originating at the melanoma site, were stained blue, had an in situ radioactivity count of at least three times the background count or an ex vivo radioactivity count of at least ten times the background count[20,21].

Pathology

After SNB, the identified SNs were sent to the pathology department. The EORTC MG pathology protocol described by Cook and colleagues[22] was used for investigation of the nodes. SNs were fix in buffered formalin for 24 h, bisected through the hilum in its longest dimension and embedded in paraffin. Five serial step sections of 4 μm each were cut at 50–100-μm intervals from each side of the lymph node. All sections were stained with haematoxylin and eosin, and S100 and/or Melan-A. There were slight local differences in protocol between centres, but the main principles remained the same.

Three melanoma specialists and/or pathologists of the EORTC MG reviewed the SN slides. Patients for whom it was difficult to determine parameters of SN tumour burden were discussed during twice yearly EORTC MG meetings. The maximum size of the largest lesion was measured and classified according to the Rotterdam criteria (smaller than 0.1 mm, 0.1–1.0 mm, larger than 1.0 mm)[4,6,9], tumour penetrative depth according to the S-classification (less or equal to 0.3 mm, 0.3–1.0 mm, larger than 1.0 mm)[5], and the intranodal location of metastases according to a modified version of the Dewar criteria (subcapsular, non-subcapsular)[7]. The Rotterdam– Dewar combined (RDC) criteria were derived from the size and location of the metastases (less than 0.1 mm subcapsular, less than 0.1 mm non-subcapsular, 0.1–1.0 mm subcapsular, 0.1–1.0 mm non-subcapsular, larger than 1.0 mm)[4].

Statistics

Continuous variables are reported as median (interquartile range), unless specified otherwise. The Mann–Whitney U χtest, ^{2} test and Fisher's exact test were used to determine differences between groups. To allow for differences in group characteristics identified in the overall univariable analysis, a matched-pair analysis was performed, in which patients from the study group were matched with those in the control group with respect to age category, Breslow thickness, tumour ulceration, Rotterdam criteria, Dewar criteria, S-classification and RDC criteria.

Disease-specific survival (DSS) was calculated from date of SNB until the date of death from melanoma. Univariable survival analyses were carried out by means of Kaplan–Meier curves and log rank tests. Multivariable analyses were performed with four Cox hazard regression models owing to multicolinearity between the four micromorphometric parameters of SN tumour burden (Dewar criteria, S-classification, Rotterdam criteria and RDC criteria). A statistical measure of model fit, the Akaike information criterion index (AIC index = $-2 \times$ log likelihood of the model + $2 \times$ number of parameters included in the model) was assessed for each model[23]; lower values of the index indicated the preferred model, which included the fewest parameters and had the strongest prognostic importance. Statistical analysis was done using Stata® version 11 (StataCorp LP, College Station, Texas, USA).

RESULTS

Among a total of 1174 patients with SN-positive melanoma, 1113 (94.8 per cent) underwent immediate CLND and 61 (5.2 per cent) did not. Reasons for not having immediate completion lymphadenectomy included: refusal of further treatment or significant co-morbidity (46 patients), minimal SN tumour burden (smaller than 0.1 mm) or decision not to undergo CLND after consultation with physician(s) (15). The distribution of patients among the ten participating centres is shown in Table S1 (supporting information).

The Rotterdam criteria were determined in all 61 patients who did not undergo immediate CLND, the S-classification in 32 (52 per cent), and the Dewar and RDC criteria in 53 (87 per cent). The Rotterdam criteria were determined in all 1113 patients who underwent CLND, the S-classification in 483 (43.4 per cent), and the Dewar and RDC criteria in 989 (88.9 per cent).

The median Breslow thickness was 2.50 (1.60–4.00) mm in patients did not undergo CLND and 3.00 (1.90–4.80) mm in those who did (P = 0.121). Patients without immediate CLND were less likely to have melanoma located on the trunk and had fewer positive SNs; they more often had subcapsular micrometastases (Dewar crite-

ria), smaller tumours (Rotterdam criteria) and less penetrative depth (S-classification). The patients who underwent CLND were younger (Table 1) .

Median follow-up was 48 (25–70) and 34 (20–60) months in the CLND and no-CLND groups respectively (P = 0.030). Median follow-up among the 61 matched patients who underwent CLND was 44 months (P = 0.704).

Table 1 – Baseline characteristics of 1174 patients with sentinel node-positive melanoma

Characteristic	**CLND** *n (per cent)* 1113 (94.8)	**No CLND** *n (per cent)* 61 (5.2)	**p***
Gender			
Female	525 (47.2)	33 (54.1)	
Male	588 (52.8)	28 (45.9)	0.296
Age (years)			
Median (IQR)	51 (40 – 62)	60 (49 – 73)	< 0.001
≤ 50	518 (46.5)	16 (26.2)	
≥ 50	595 (53.5)	45 (73.8)	0.002
Site of primary			
Extremity	489 (44.5)	37 (60.7)	
Trunk	463 (42.1)	18 (29.5)	
Head & Neck	148 (13.5)	6 (9.8)	0.047
Missing	13 [1.2]	0 [0.0]	
Histology			
SSM	406 (47.9)	28 (65.1)	
NM	380 (44.8)	13 (30.2)	
Other	62 (7.3)	2 (4.7)	0.088
Missing	265 [23.8]	18 [27.7]	
Breslow thickness (mm)			
Median (IQR)	3.00 (1.90 – 4.80)	2,50 (1,60 – 4,00)	0.121
≤ 1.00	58 (5.2)	5 (8.2)	
> 1.00 – ≤ 2.00	275 (24.8)	19 (31.2)	
> 2.00 – ≤ 4.00	440 (39.8)	22 (36.1)	
> 4.00	334 (30.2)	15 (24.6)	0.449
Missing	6 [5.4]	0 [0.0]	
Clark level			
II-III	310 (29.1)	9 (15.0)	
IV	635 (59.6)	42 (70.0)	
V	121 (11.4)	9 (15.0)	0.059
Missing	47 [4.2]	1 [1.7]	
Ulceration			
Absent	516 (50.6)	31 (55.4)	

Table 1 (continued)

Characteristic	CLND *n (per cent)* 1113 (94.8)	No CLND *n (per cent)* 61 (5.2)	p*
Present	504 (49.4)	25 (44.6)	0.496
Missing	93 [8.4]	5 [8.2]	
Site of SNB			
Groin	286 (39.5)	23 (53.5)	
Axilla	384 (53.0)	15 (34.9)	
Neck	55 (7.6)	5 (11.6)	0.067
Missing	388 [34.9]	18 [29.5]	
Number of excised SNs			
Median (range)	2 (1 – 12)	2 (1 – 5)	0.778
1	359 (39.6)	20 (44.4)	
2	307 (33.9)	12 (26.7)	
≥ 3	240 (26.5)	13 (28.9)	0.976
Missing	207 [18.6]	16 [14.4]	
Number of positive SNs			
Median (range)	1 (1 – 5)	1 (1 – 3)	0.008
1	716 (78.5)	43 (95.6)	
2	157 (17.2)	1 (2.2)	
≥3	38 (4.2)	1 (2.2)	0.093
Missing	202 [18.1]	16 [14.4]	
Intranodal location ***(Dewar)***			
Subcapsular	173 (17.5)	16 (30.7)	
Non-subcapsular	816 (82.5)	36 (69.2)	0.025
Missing	124 [11.1]	9 [14.8]	
Tumor penetrative depth (mm) ***(S-classification)***			
Median (IQR)	0.80 (0.30 – 2.10)	0.40 (0.20 – 1.27)	0.024
SI	126 (26.1)	13 (40.6)	
SII	132 (27.3)	10 (31.3)	
SIII	225 (46.6)	9 (28.1)	0.092
Missing	630 [56.6]	29 [47.5]	
Maximum size (mm) ***(Rotterdam criteria)***			
Median (IQR)	1.40 (0.40 – 4.00)	0.40 (0.08 – 1.15)	< 0.001
< 0.1	101 (9.1)	15 (24.5)	
0.1 – 1.0	462 (41.5)	30 (49.2)	
> 1.0	550 (49.4)	16 (26.2)	< 0.001

Table 1 (continued)

Characteristic	CLND *n (per cent)* 1113 (94.8)	No CLND *n (per cent)* 61 (5.2)	p*
Maximum size and location *(RDC criteria)*			
< 0.1 mm subcapsular	45 (4.4)	10 (18.2)	
< 0.1 mm non-subcapsular	37 (3.6)	4 (7.3)	
0.1 – 1.0 mm subcapsular	114 (11.1)	5 (9.1)	
0.1 – 1.0 mm non-subcapsular	284 (27.6)	20 (36.4)	
> 1.0 mm	509 (53.4)	13 (29.1)	< 0.001
Missing	124 [11.1]	9 [14.8]	

SN = sentinel node; SNB=sentinel node biopsy; CLND = completion lymph node dissection; IQR = Interquartile range; RDC = Rotterdam Dewar Combined; SSM = Superficial spreading melanoma; NM = Nodular melanoma;
**p-values are calculated using the Fisher exact test, chi-square test or Mann-Whitney U test to evaluate differences between patients with CLND (n=1113) and patients without CLND (n=61)*

Survival

Univariable analyses demonstrated that the following factors were associated with DSS: sex, age, histology of the primary tumour, Breslow thickness, Clark level, tumour ulceration, number of positive SNs, Dewar criteria, S-classification, Rotterdam criteria and RDC criteria (Table 2). CLND was not a significant prognostic factor for DSS (hazard ratio (HR) 0.89, 95 per cent confidence interval (c.i.) 0.58 to 1.37; P = 0.600).

Table 2 – Univariable analysis of disease-specific survival for all patients with sentinel node-positive melanoma

	Univariable analyses		
Variable	**HR**	**95 *per cent* CI**	**p***
Gender			
Female	1		
Male	1.32	1.06 – 1.64	0.014
Age	1.14	1.06 – 1.22	0.001
Site of primary			
Extremity	1		
Trunk	1.09	0.86 – 1.38	0.485
Head and neck	1.06	0.76 – 1.48	0.738
Missing	0.97	0.40 – 2.40	0.954
Histology			
SSM	1		
NM	1.40	1.08 – 1.82	0.011

Table 2 (continued)

	Univariable analyses		
Variable	**HR**	**95 *per cent* CI**	**p***
Other	2.03	1.31 – 3.15	0.002
Missing	1.34	0.99 – 1.81	0.058
Breslow thickness (mm)			
≤ 2.00	1		
> 2.00 – ≤ 4.00	1.76	1.30 – 2.39	< 0.001
> 4.00	3.44	2.55 – 4.64	< 0.001
Clark level			
II-III	1		
IV	1.49	1.13 – 1.98	0.005
V	2.52	1.76 – 3.59	< 0.001
Missing	1.46	0.79 – 2.71	0.226
Ulceration			
Absent	1		
Present	2.49	1.96 – 3.16	< 0.001
Missing	1.54	1.01 – 2.34	0.047
Site of SNB			
Groin	1		
Axilla	1.32	1.00 – 1.75	0.051
Neck	1.33	0.84 – 2.11	0.223
Missing	0.92	0.69 – 1.22	0.544
Number of excised SNs			
1	1		
2	0.83	0.63 – 1.10	0.202
≥ 3	1.07	0.80 – 1.44	0.641
Missing	0.95	0.69 – 1.30	0.745
Number of positive SNs			
1	1		
2	1.56	1.16 – 2.10	0.003
≥3	1.08	0.56 – 2.11	0.813
Missing	1.07	0.80 – 1.44	0.646
Intranodal location *(Dewar)*			
Subcapsular	1		
Non-subcapsular	2.08	1.46 – 2.97	< 0.001
Missing	1.23	0.77 – 1.97	0.393
Tumour penetrative depth *(S-classification)*			
SI	1		

Table 2 (continued)

Variable	Univariable analyses		
	HR	**95 *per cent* CI**	**p***
SII	2.00	1.21 – 3.31	0.007
SIII	2.63	1.65 – 4.19	< 0.001
Missing	2.52	1.63 – 3.89	< 0.001
Maximum size (mm) *(Rotterdam criteria)*			
< 0.1	1		
0.1 – 1.0	2.33	1.29 – 4.23	0.005
> 1.0	4.28	2.39 – 7.67	< 0.001
Maximum size and location *(RDC criteria)*			
< 0.1 mm subcapsular	1		
< 0.1 mm non-subcapsular	2.08	0.59 – 7.38	0.256
0.1 – 1.0 mm subcapsular	2.77	0.97 – 7.88	0.056
0.1 – 1.0 mm non-subcapsular	3.49	1.28 – 9.56	0.015
> 1.0 mm	5.70	2.12 – 15.36	0.001
Missing	2.78	0.99 – 7.85	0.053
CLND			
Not performed	1		
Performed	0.89	0.58 – 1.37	0.600

HR = Hazard ratio; CI= Confidence Interval; SSM = Superficial spreading melanoma; NM = Nodular melanoma; SNB = sentinel node biopsy; SN = sentinel node; CLND = Completion lymph node dissection
**p-values are calculated using the log rank test*

The results of the matched-pairs analysis of patients with and without CLND are shown in Table 3. Median Breslow thickness was 2.50 mm in both groups, and 31 per cent of patients in each group had subcapsular metastases. Median maximum SN tumour size was 0.40 mm in both groups. In matched-pair analysis, CLND did not significantly influence DSS (HR 0.86, 0.46 to 1.61; P = 0.640).

To adjust for the prognostic imbalance in baseline factors between groups, multivariable analysis was carried out, using four different models owing to the multicolinearity of the SN tumour burden characteristics (Table 4). Factors independently associated with significantly worse DSS in all four analyses were older age, increased Breslow thickness and tumour ulceration. In all models, the SN tumour burden characteristic was also a significant prognostic factor, but CLND had no significant influence on prognosis in any of the models (Table 4). However, there was a trend towards improved outcome for patients who underwent CLND. HRs in the four models were 0.81 (95 per cent c.i. 0.52 to 1.25; P = 0.340), 0.82 (0.53 to 1.27; P = 0.377), 0.74 (0.48 to 1.16; P = 0.189) and 0.73 (0.47 to 1.14; P = 0.169) for CLND

Table 3 – Baseline characteristics of patients selected for matched-pair analysis.

Characteristic	No CLND *n (per cent)* 61 (5.2)	CLND † (matched) *n (per cent)* 61	p*
Gender			
Female	33 (54.1)	28 (45.9)	
Male	28 (45.9)	33 (54.1)	0.469
Age (years)			
Median (IQR)	60 (49 – 73)	59 (49 – 68)	0.458
≤ 50	16 (26.2)	16 (26.2)	
≥ 50	45 (73.8)	45 (73.8)	1.000
Site of primary			
Extremity	37 (60.7)	33 (54.1)	
Trunk	18 (29.5)	21 (34.4)	
Head & Neck	6 (9.8)	7 (11.5)	0.765
Missing	0 [0.0]	0 [0.0]	
Histology			
SSM	28 (65.1)	26 (56.5)	
NM	13 (30.2)	18 (39.1)	
Other	2 (4.7)	2 (4.4)	0.677
Missing	18 [27.7]	15 [24.6]	
Breslow thickness (mm)			
Median (IQR)	2,50 (1,60 – 4,00)	2,50 (1,55 – 4,00)	0.959
≤ 1.00	5 (8.2)	5 (8.2)	
> 1.00 – ≤ 2.00	19 (31.2)	19 (31.2)	
> 2.00 – ≤ 4.00	22 (36.1)	22 (36.1)	
> 4.00	15 (24.6)	15 (24.6)	1.000
Missing	0 [0.0]	0 [0.0]	
Clark level			
II-III	9 (15.0)	20 (34.5)	
IV	42 (70.0)	33 (56.9)	
V	9 (15.0)	5 (8.6)	0.042
Missing	1 [1.7]	3 [4.9]	
Ulceration			
Absent	31 (55.4)	31 (55.4)	
Present	25 (44.6)	25 (44.6)	1.000
Missing	5 [8.2]	5 [8.2]	
Site of SNB			
Groin	23 (53.5)	21 (42.0)	
Axilla	15 (34.9)	24 (48.0)	

Table 3 (continued)

Characteristic	No CLND *n (per cent)* 61 (5.2)	CLND † (matched) *n (per cent)* 61	p*
Neck	5 (11.6)	5 (10.0)	0.438
Missing	18 [29.5]	11 [18.0]	
Number of excised SNs			
Median (range)	2 (1 – 5)	2 (1 – 5)	0.176
1	20 (44.4)	28 (53.9)	
2	12 (26.7)	16 (30.8)	
≥ 3	13 (28.9)	8 (15.4)	0.552
Missing	16 [14.4]	16 [14.4]	
Number of positive SNs			
Median (range)	1 (1 – 3)	1 (1 – 2)	0.142
1	43 (95.6)	45 (86.5)	
2	1 (2.2)	7 (13.5)	
≥3	1 (2.2)	0 (0.0)	0.079
Missing	16 [14.4]	9 [14.8]	
Intranodal location ***(Dewar)***			
Subcapsular	16 (30.7)	16 (30.7)	
Non-subcapsular	36 (69.2)	36 (69.2)	1.000
Missing	9 [14.8]	9 [14.8]	
Tumor penetrative depth (mm) ***(S-classification)***			
Median (IQR)	0.40 (0.20 – 1.27)	0.45 (0.20 – 1.10)	0.989
SI	13 (40.6)	13 (40.6)	
SII	10 (31.3)	10 (31.3)	
SIII	9 (28.1)	9 (28.1)	1.000
Missing	29 [47.5]	29 [47.5]	
Maximum size (mm) ***(Rotterdam criteria)***			
Median (IQR)	0.40 (0.08 – 1.15)	0.40 (0.10 – 1.10)	0.881
< 0.1	15 (24.5)	15 (24.5)	
0.1 – 1.0	30 (49.2)	30 (49.2)	
> 1.0	16 (26.2)	16 (26.2)	1.000
Maximum size and location ***(RDC criteria)***			
< 0.1 mm subcapsular	10 (18.2)	10 (18.2)	
< 0.1 mm non-subcapsular	4 (7.3)	4 (7.3)	
0.1 – 1.0 mm subcapsular	5 (9.1)	5 (9.1)	

Table 3 (continued)

Characteristic	No CLND *n (per cent)* 61 (5.2)	CLND † (matched) *n (per cent)* 61	p*
0.1 – 1.0 mm non-subcapsular	20 (36.4)	20 (36.4)	
> 1.0 mm	13 (29.1)	16 (29.1)	1.000
Missing	9 [14.8]	9 [14.8]	

The two groups were matched for age category, Breslow thickness category, ulceration, Dewar criteria, S-classification, Rotterdam criteria and Rotterdam-Dewar combined (RDC) criteria. SN = sentinel node; SNB=sentinel node biopsy; CLND = completion lymph node dissection; IQR = Interquartile range; RDC = Rotterdam Dewar Combined

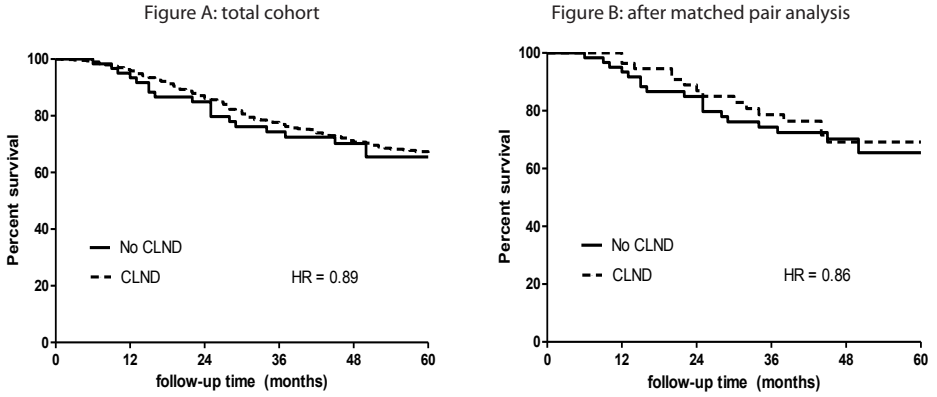
** p-values are calculated using the Fisher exact test, chi-square test or Mann-Whitney U test to evaluate differences between the matched pairs, i.e. patients with CLND (n=61) and patients without CLND (n=61)*

Table 4 Four different multivariable analyses of disease-specific survival for all patients with sentinel node-positive melanoma

Model	Target variable	HR	95 *per cent* c.i.	p	Other included (all significant) variables	AIC index *
A	**CLND**				Age	- 224.08
	Not performed	1			Breslow thickness	
	Performed	0.81	0.52 – 1.25	0.34	Ulceration	
					Intranodal location (Dewar criteria)	
B	**CLND**				Age	-223.04
	Not performed	1			Breslow thickness	
	Performed	0.82	0.53 – 1.27	0.38	Ulceration	
					Tumor penetrative depth (S-classification)	
C	**CLND**				Age	-242.86 *
	Not performed	1			Breslow thickness	
	Performed	0.74	0.48 – 1.16	0.19	Ulceration	
					Maximum size (Rotterdam criteria)	
D	**CLND**				Age	-235.86
	Not performed	1			Breslow thickness	
	Performed	0.73	0.47 – 1.14	0.17	Ulceration	
					Maximum size and intranodal location (RDC criteria)	

HR = Hazard ratio; c.i.= Confidence Interval; SN = sentinel node; CLND = Completion lymph node dissection; RDC = Rotterdam Dewar Combined;
AIC = Akaike Information Criteria
** AIC index = statistical model fit measure for aid to choosing between competing models. Lower values of the index indicate the preferred model, that is, the one with the fewest parameters and the strongest prognostic importance (Model C).*

Figure 1 – Disease specific survival for sentinel node positive patients who did and who did not undergo immediate completion lymph node dissection for A) the total group of patients and B) after matched pair analysis



	Nr at Risk							Nr at Risk						
No CLND	**61**	**57**	**49**	**40**	**31**	**22**	**No CLND**	**61**	**57**	**49**	**40**	**31**	**22**
CLND	**1113**	**990**	**773**	**529**	**396**	**287**	**CLND**	**61**	**55**	**45**	**36**	**26**	**22**
Total	**1174**	**1047**	**822**	**579**	**427**	**309**	**Total**	**122**	**112**	**94**	**76**	**57**	**44**

HR = Hazard Ratio, CLND = Completion Lymph Node Dissection

versus no CLND. The AIC index indicated that the model including the Rotterdam criteria was the preferred model (AIC index –242.86) (Table 4).

Subgroup analyses were performed in which the DSS was stratified by SN tumour burden. After correcting for age, Breslow thickness and tumour ulceration in multivariable analyses, no significant benefit of CLND was found in any subgroup (Table S2, supporting information).

Three and 5-year DSS rates were 74 and 66 per cent for patients who did not undergo CLND, compared with 76.9 and 66.9 per cent respectively for those who had immediate CLND (HR 0.89, 0.58 to 1.37; P = 0.600) (Fig. 1a). Rates for the 61 patients who underwent CLND included in the matched-pair analysis were 79 and 69 per cent (HR 0.86, 0.46 to 1.61; P = 0.640) (Fig. 1b).

DISCUSSION

In the present study patients with SN-positive melanoma who had CLND did not have better outcome than those who did not undergo completion lymphadenectomy. Estimated 5-year DSS rates were 66.9 and 66 per cent respectively. No significant differences in survival were identified by matched-pair analysis or after correcting for other significant prognostic factors in multivariable analyses.

The group that did not undergo CLND had a favourable prognosis in terms of patient characteristics, the primary melanoma and SN tumour burden, including a smaller maximum SN tumour size. Therefore, it could not be ruled out that the total group of patients with SN-positive disease could benefit from CLND. A matched-pair analysis was undertaken to evaluate the outcome, but it did not demonstrate a significant different between the two groups. However, the groups of patients were not representative of the population with SN-positive melanoma as they had less tumour burden, were older and had thinner primary tumours. Thus, selection bias occurred in the present study.

In most cancer centres, immediate CLND is standard management for melanoma with SN metastases. However, Bilimoria and colleagues[24] demonstrated, surprisingly, that only 50 per cent of patients with melanoma diagnosed with a positive SN in 2004 and 2005 underwent CLND. Therefore, it remains uncertain whether CLND is of therapeutic benefit in patients with SN-positive melanoma. Some investigators have stated that all patients with a positive SN should undergo completion lymphadenectomy[13–17], whereas other studies have suggested that some of these patients might be spared immediate CLND[2,4,6,8–10]. No additional metastases are detected in approximately 80 per cent of patients undergoing CLND. Furthermore, CLND is associated with considerable greater morbidity than SNB alone[25–30]. Results from the Sunbelt Melanoma Trial demonstrated complication rates of 23 per cent after CLND and 5 per cent for patients who underwent SNB alone[29]. Quality of life was worse in those who underwent CLND than in patients who underwent SNB alone[31].

Two retrospective studies of patients with SN-positive melanoma have reported comparisons of outcome in those who had CLND versus those who did not[2,3]. With a median follow-up of 36 and 20 months respectively, nodal recurrence-free survival and DSS were similar in both groups in the study by Wong and colleagues[3]. However, similar to the present study, selection bias was evident, a problem highlighted by Henderson[32]. In the other study, recurrence-free survival and DSS were similar in 271 patients who underwent CLND and 42 patients who did not, with median follow-up of 43 and 32 months respectively[2]. These retrospective studies did not report on SN tumour burden, although this is the most important prognostic factor for survival[6]. Differences in SN tumour burden between groups could therefore have affected outcome.

The prognosis among patients with SN-positive melanoma is highly heterogeneous. Patients with a minimal SN tumour burden, especially when located in the subcapsular area, might be spared CLND, as their 5-year DSS is 95 per cent and the chance of additional non-SN metastases is 2 per cent[4]. Therefore, subgroup analyses were performed in the present study, with comparison of outcomes stratified by SN tumour burden. In univariable analyses, the outcome of patients with microme-

tastases and/or subcapsular located metastases was not significantly different (data not shown). After correcting for age, Breslow thickness and tumour ulceration in multivariable analyses, no significant difference in outcome was demonstrated in any of the subgroups. However, all analyses were based on small subgroups with few events and should therefore be interpreted with caution. For S-classification and tumour location, HRs for melanoma-related death decreased with increased SN tumour burden. These results suggest that patients with a higher SN tumour burden might benefit more from CLND than patients with a lower burden. However, this was not seen for the Rotterdam or RDC criteria. The AIC index indicated that the multivariable model including the Rotterdam criteria was strongest, followed by that including the RDC. However, these results should again be interpreted with caution, as they are based on subgroups containing a limited number of patients and events in a retrospective, non-randomized setting.

This study has demonstrated no significant difference in outcome among patients with SN-positive melanoma between those who underwent immediate CLND and those who did not, in either in multivariable or matched-pair analysis. However, the outcome in patients who did not have CLND was better than expected because, among other factors, these patients more often had smaller micrometastases that were located subcapsularly. Ongoing prospective studies, such as the MINITUB Study and Multicentre Selective Lymphadenectomy Trial II, should provide information regarding the possible therapeutic benefit of CLND in patients with a positive SN and indicate which patients might safely be spared this procedure, eventually leading to a change in practice.

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SUPPLEMENTARY TABLES

Suppl. Table 1 – Number of patients treated in each center

Centre	CLND *n (per cent)* 1113 (94.8)	No CLND *n (per cent)* 61 (5.2)	CLND (matched) *n (per cent)* 61
DDHCC	101 (9.1)	13 (21.3)	13 (21.3)
CHUB	76 (6.8)	9 (14.8)	2 (3.3)
MMCCIO	244 (21.9)	0 (0.0)	10 (16.4)
RSCH	189 (17.0)	16 (26.2)	9 (14.8)
AVL	116 (10.4)	0 (0.0)	2 (3.3)
IGR	66 (5.9)	1 (1.6)	0 (0.0)
VUMC	97 (8.7)	9 (14.8)	8 (13.1)
UMCG	55 (4.9)	1 (1.6)	4 (6.6)
EIO	65 (5.8)	7 (11.5)	5 (8.2)
UP	104 (9.3)	5 (8.2)	8 (13.1)

CLND = Completion Lymph Node Dissection; DDHCC = Daniel den Hoed Cancer Center, Rotterdam, the Netherlands; CHUB = the Charité, Humboldt University of Berlin, Berlin, Germany; MMCCIO = M.Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland; RSCH = Royal Surrey County Hospital, Guildford, UK; AVL = Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands; IGR = Institut de cancérologie Gustave Roussy, Villejuif, France; VUMC = Vrij Universiteit Medical Center, Amsterdam, the Netherlands; UMCG = University Medical Center Groningen, Groningen, the Netherlands; EIO = European Insitute of Oncology, Milan, Italy; UP = Veneto Institute of Oncology – IRCSS University of Padova, Padova, Italy.

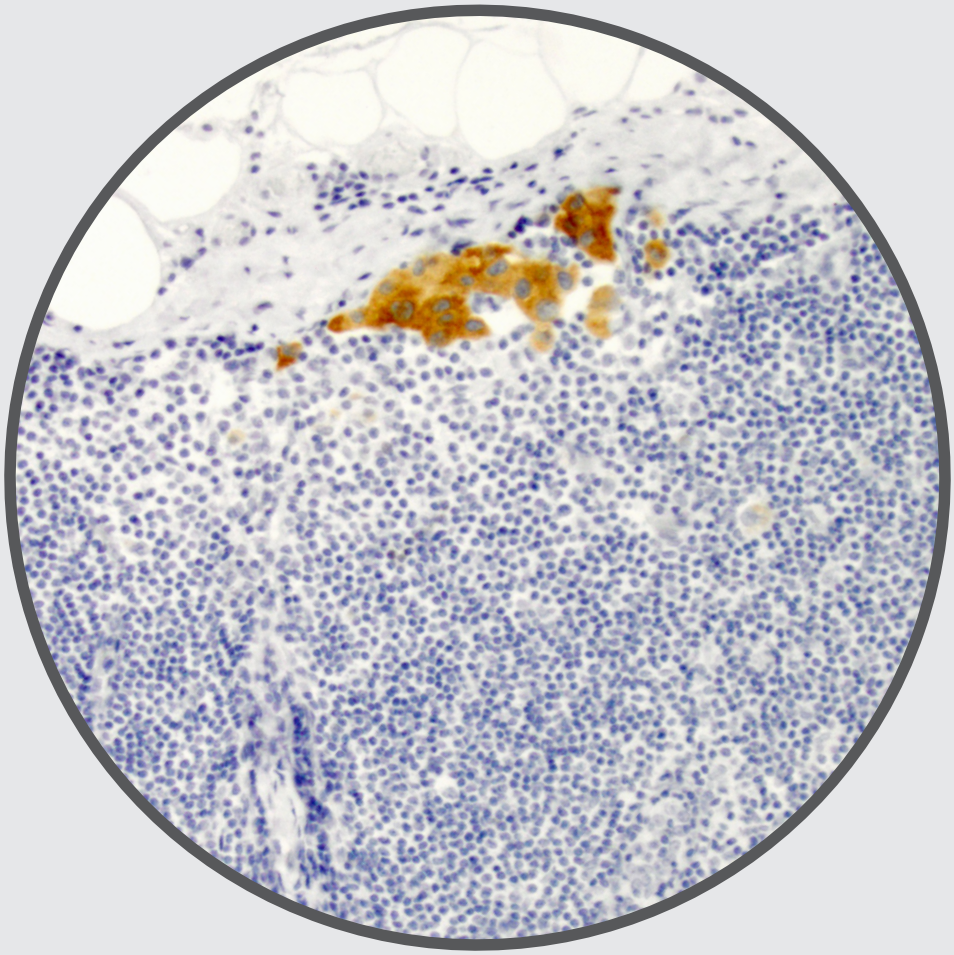
Suppl. Table 2 – Results of multivariable analysis of disease specific survival for subgroups of patients with a positive sentinel node who underwent completion lymph node dissection or not, stratified by sentinel node tumor burden

SN tumour burden characteristic within multivariable analyses			Multivariable analyses			
		CLND	n	HR	95 *per cent* c.i.	p*
Dewar criteria	**Subcapsular**	Not performed	16	1		
		Performed	173	0.84	0.52 – 1.36	0.48
	Non-subcapsular	Not performed	36	1		
		Performed	816	0.59	0.29 – 1.21	0.15
	Unknown location	Not performed	9	1		
		Performed	124	0.88	0.54 – 1.45	0.62
S-classification	**SI**	Not performed	13	1		
		Performed	126	1.02	0.61 – 1.70	0.93
	SII	Not performed	10	1		
		Performed	132	0.87	0.54 – 1.42	0.59
	SIII	Not performed	9	1		
		Performed	225	0.74	0.46 – 1.22	0.99

Suppl. Table 2 (continued)

SN tumour burden characteristic within multivariable analyses			Multivariable analyses			
		CLND	n	HR	95 *per cent* c.i.	p*
	Unknown TPD	Not performed	29	1		
		Performed	630	0.61	0.36 – 1.07	0.08
Rotterdam criteria	**< 0.1 mm**	Not performed	15	1		
		Performed	101	0.74	0.47 – 1.17	1.00
	0.1 – 1.0 mm	Not performed	30	1		
		Performed	462	0.90	0.46 – 1.77	0.76
	> 1.0 mm	Not performed	16	1		
		Performed	550	0.65	0.38 – 1.12	0.12
RDC criteria	**< 0.1 mm subcapsular**	Not performed	10	1		
		Performed	45	0.79	0.48 – 1.29	0.35
	< 0.1 mm non-subcapsular	Not performed	4	1		
		Performed	37	0.77	0.47 – 1.24	0.28
	0.1 – 1.0 mm subcapsular	Not performed	5	1		
		Performed	114	0.89	0.53 – 1.50	0.66
	0.1 – 1.0 mm non-subcapsular	Not performed	20	1		
		Performed	284	0.78	0.42 – 1.45	0.43
	> 1.0 mm with known location	Not performed	13	1		
		Performed	509	0.71	0.39 – 1.32	0.28
	Known size, unknown location	Not performed	9	1		
		Performed	124	0.79	0.49 – 1.28	0.34

Multivariable analyses included the following variables: age, Breslow, tumour ulceration and the specific subgroup of patients
HR = Hazard ratio; c.i.= Confidence Interval; SN = sentinel node; CLND = Completion lymph node dissection; N/A = Not applicable, RDC = Rotterdam Dewar Combined
** p-values are calculated using the log rank test*



Part III

Management and prognosis of stage III melanoma; macrometastases

Chapter 8
Nomograms to predict recurrence and survival in stage IIIB and IIIC melanoma after therapeutic lymphadenectomy
Submitted

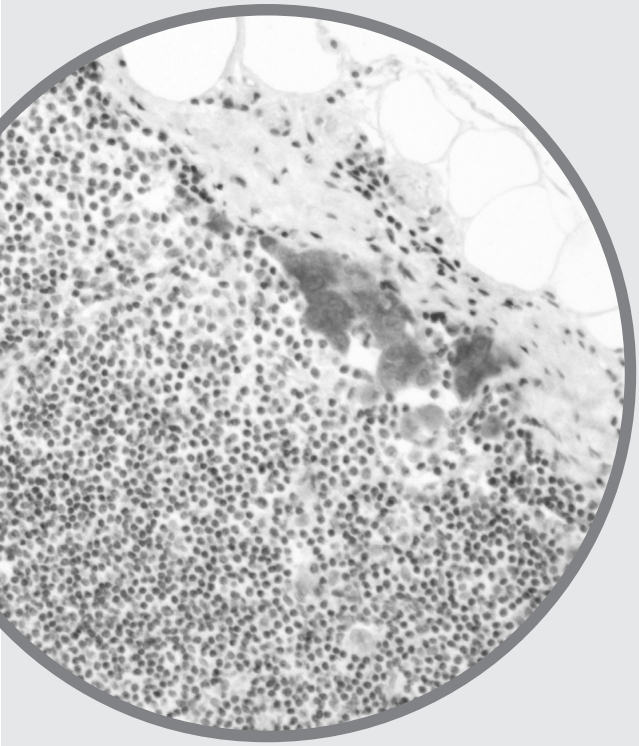
Chapter 9
Therapeutic surgical management of palpable melanoma groin metastases: superficial or combined superficial and deep groin lymph node dissection
Annals of Surgical Oncology. 2011 Nov;18(12):3300-8

Chapter 10
Outcome after therapeutic lymph node dissection in patients with unknown primary melanoma site
Annals of Surgical Oncology. 2011 Dec;18(13):3586-92

Chapter 11
Melanoma patients with an unknown primary tumor site have a better outcome than those with a known primary following therapeutic lymph node dissection for macroscopic (clinically palpable) nodal disease
Submitted

Chapter 8

Nomograms to predict recurrence and survival in stage IIIB and IIIC melanoma after therapeutic lymphadenectomy



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ABSTRACT

Current staging algorithms in melanoma patients undergoing lymph node dissection (LND) fail to accurately distinguish long term survivors from those at risk of rapid relapse. Our goal was to establish and validate nomograms for predicting both recurrence and survival after LND.

A prospective cohort of stage IIIB and IIIC melanoma patients was ascertained from a tertiary hospital in Brisbane, Australia. Failure-time multivariate analysis identified key factors that, in adjusted combinations, generated nomograms to predict 2-year recurrence and melanoma-specific survival. The predictive value of these nomograms was further tested in a separate prospective patient cohort from Rotterdam, The Netherlands.

In the 494 Australian patients, number of positive lymph nodes, extracapsular extension, nodular histopathological subtype and post-operative seroma were independent predictors of 2-year recurrence while age, number of positive nodes and extra capsular extension were the independent predictors of survival. Predictive value was confirmed in The Netherlands cohort of 331 patients. The nomograms were able to classify patients according to their 2-year recurrence and survival rates even within each stage III sub-class.

Models that include extra-capsular extension predict outcomes in patients with clinically invaded lymph nodes. This tool may help tailor treatment and monitoring of this group of patients.

INTRODUCTION

Melanoma patients with regional lymph node metastasis can have substantial variations in outcome despite proper surgical management[1-3]. Five-year survival rates vary between 40% and 59%[2], including some patients that survive long-term[4]. The current American Joint Committee on Cancer (AJCC) staging system classifies melanoma patients with macroscopic metastases as stages IIIB or IIIC by considering the number of invaded nodes (1, 2 or 3, 4 or more), presence of ulceration in the primary tumour and presence of satellitosis or in-transit metastases[2]. However, other factors of prognostic significance in stage III patients that have been described such as age or site of invasion have not been considered in the current staging criteria[5, 6]. Our objective was to identify all independent prognostic factors in a well-characterised series of patients with stage IIIB and IIIC melanoma in order to generate prediction models of recurrence and death and then to validate these nomograms in an independent cohort.

PATIENTS AND METHODS

Cohort characteristics

Patients having regional lymphadenectomy between January 2000 and August 2011 at the Melanoma Clinic of the Princess Alexandra Hospital, Queensland, Australia were included if they satisfied the following eligibility criteria: had palpable metastatic lymph-node field disease; had complete cervical, axillary, inguinal or ilio-inguinal lymphadenectomy (LND); the number of involved nodes was 1 or more; and were aged at least 18 years[7].

All patients having regional nodal surgery were prospectively documented in a database. Demographic information, site (head and neck, upper or lower limb, trunk or melanoma of unknown primary site[7] and histological characteristics of the primary melanoma when available including thickness (mm), histological classification and the presence of ulceration, satellites or regression were collected. Patients presenting with palpable nodal metastasis had the diagnosis confirmed by fine needle aspiration cytology and were staged with computerised tomography (CT) scans of the head, chest, abdomen and pelvis. From 2004 onwards fluorodeoxyglucose-positon emission tomography (FDG-PET) scanning was added to this list for routine staging in this group of patients. Other investigations were performed as required to assess and exclude stage IV disease. Regarding the regional nodal dissection the following data were collected: site (neck, axilla, inguinal, ilio-inguinal, iliac only), total number of nodes removed, number of invaded nodes, size of largest node (cm) and presence of

microscopic or gross extracapsular extension. Post-surgery complications (infection, seroma) were also recorded. Seroma was defined as a lymph collection that needed additional intervention (such as needle aspiration) beyond the routine use of drains immediately post-surgery.

Follow-up

Following nodal surgery, patients were followed every 3 months for 2 years, theevery 6 months for 2 years, then annually up to 10 years. Follow-up assessment consisted of a history and physical examination including skin surveillance. Investigations were directed to new symptoms or signs that had occurred between visits and/or were present at follow-up (unless the patient was participating in a trial that required specific investigations to be performed). Recurrence was defined as new local, regional or disseminated disease and the date of review when new disease was identified clinically was recorded.

Validation cohort

The validation cohort has been described previously[8]. Patients who were treated with LND for palpable nodal disease classified as Stage IIIB or C between 1982 and 2009 at the Erasmus University Medical Center and Daniel den Hoed Cancer Center, Rotterdam, The Netherlands, were included. Patients who underwent LND with an isolated limb perfusion were excluded. Available patient, primary and metastatic tumour characteristics were prospectively collected and details entered into a database. Clinically detectable nodal disease and the absence of visceral metastases were confirmed by either ultrasound of the lymph node fields and/or the liver, chest x-ray, cerebral magnetic resonance imaging, or CT scan of the thorax and abdomen but not ascertained by FDG-PET.

The present study was approved by the Human Research Ethics Committees of the University of Queensland, the Princess Alexandra Hospital and the Queensland Institute of Medical Research.

Outcome

The outcomes of interest were recurrence within two years and melanoma-specific survival, calculated from date of LND to date of first recurrence or to the date of death from melanoma. Patients who had a recurrence after 2 years were censored at the time of recurrence, while patients who did not have a recurrence by their last follow-up were censored at that date. For survival, patients who died of causes other than melanoma were censored at their date of death.

Statistical analysis

All potential predictors of two-year recurrence or death were screened using failure-time procedures, either by log-rank statistics for categorical variables or by univariate Cox proportional hazards methods for quantitative variables such as age, Breslow thickness, number of nodes dissected, number of positive nodes and size of largest positive node. Those that showed an association with time to recurrence or death at the 10% level of significance were included in a multivariate proportional hazards model to determine the independent predictors. Cut-points in the linear formula derived from the resultant coefficients, rounded to one decimal place, were determined by calculating the proportion of recurrences or death at each realised value of the formula and applying the pool-adjacent-violators algorithm[9, 10] to achieve a monotonic increase in the recurrence or death proportions. Proportions of individuals with recurrence within two years or death and actuarial two-year recurrence rates or five year survival rates in each band of the nomogram were then computed for the predictive and the validation cohorts.

As indications of predictive power, the Area Under the Receiver Operating Characteristic curve (AUC) was computed from the Mann-Whitney test[11] and the C index for failure-time models[12] as well as calibration curves[13]. Calibration curves were drawn by grouping patients with respect to their nomogram-predicted probabilities and plotting the mean of predicted probabilities for each group with the mean observed Kaplan-Meier 2-year estimate of recurrence-free or 5-year melanoma specific survival. As a further indication of predictive power over and above that due to the 'number of positive nodes' variable, outcomes were computed separately for patients staged IIIB and IIIC. The C index and calibration curves were calculated using 'hmisc', 'epi' and 'rms' packages in R (version 2.15, R Foundation for Statistical Computing, Vienna, Austria 2011). All analyses other than the calculation of the C index and calibration curves were performed using Statistical Analysis Software (SAS) version 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

From January 2000 to June 2011, 560 adult patients underwent regional nodal dissection, of whom 494 patients were eligible for the present study (Table 1). Patients were excluded because of absence of follow-up data (n=29); ineligible disease stage (n=33) including 10 patients with microscopic disease only; or prior radiotherapy (n=4). The mean age of males in the study cohort was 58.4 years and of females, 55.0 years and patients were followed for a median of 17.4 months (Interquartile range 32.1). Recurrent metastatic melanoma occurred in 217 patients (44%). Mean

Table 1. Demographic, clinical and histological characteristics of melanoma patients, total and with recurrence within two years and died within 5 years of lymph node dissection: Australia and The Netherlands cohorts

Characteristic	Australia					The Netherlands				
	Total (n=494)	Recurred (n=198)	P-value	Died (n=206)	P-value	Total (n=331)	Recurred (n=213)	P-value	Died (n=200)	P-value
	n	n (%)		n (%)		n	n (%)		n (%)	
Sex										
Male	319	125 (39)	0.58	141 (44)	0.13	171	106 (62)	0.35	108 (63)	0.29
Female	175	73 (42)		65 (37)		160	107 (67)		92 (58)	
Age at LND, y										
<35	52	19 (37)	0.44	21 (40)	0.029	37	24 (65)	0.40	22 (59)	0.42
35 – <45	65	30 (46)		32 (49)		51	34 (67)		35 (69)	
45 – <55	103	45 (44)		40 (39)		73	50 (68)		47 (64)	
55 – <65	89	28 (31)		31 (35)		81	56 (69)		48 (59)	
65 – <75	94	40 (43)		32 (34)		55	29 (53)		27 (49)	
75+	91	36 (40)		50 (55)		34	20 (59)		21 (62)	
mean ± sd	57.7 ± 16.6	57.1 ± 16.7	0.50	58.4 ± 17.5	0.46	54.7 ± 15.2	54.0 ± 14.7	0.25	54.0 (±15.0)	0.28
Unknown primary										
No	412	171 (42)	0.15	179 (43)	0.078	285	185 (65)	0.60	177 (62)	0.12
Yes	82	27 (33)		27 (33)		46	28 (61)		23 (50)	
Site of primary melanoma[1]										
Head and Neck	75	27 (36)	0.43	29 (39)	0.35	60	35 (58)	0.073	33 (55)	0.022
Arm	45	19 (42)		22 (49)		135[4]	84 (62)		78 (58)	
Leg	137	64 (47)		54 (39)		–	–		–	
Trunk	155	61 (39)		74 (48)		85	64 (75)		64 (75)	
Other	–	–		–		5	2 (40)		2 (40)	

Table 1. (continued)

Characteristic	Australia					The Netherlands				
	Total (n=494)	Recurred (n=198)	P-value	Died (n=206)	P-value	Total (n=331)	Recurred (n=213)	P-value	Died (n=200)	P-value
	n	n (%)		n (%)		n	n (%)		n (%)	
Breslow thickness[1]										
T1: 0.2 – 1.0 mm	89	33 (37)	0.15	37 (42)	0.069	34	20 (59)	0.49	17 (50)	0.42
T2: >1.0 – 2.0	149	56 (38)		62 (42)		79	55 (70)		51 (65)	
T3: >2.0 – 4.0	96	49 (51)		52 (54)		68	46 (68)		45 (66)	
T4: >4.0	67	28 (42)		23 (34)		72	43 (60)		44 (61)	
Histology[1]										
SSM	257	104 (41)	0.036	108 (42)	0.36	63	42 (67)	0.12	39 (62)	0.47
NM	106	52 (49)		52 (49)		85	54 (64)		52 (61)	
LMM	7	1 (14)		4 (57)		6	1 (17)		2 (33)	
Other	23	11 (48)		10 (43)		10	8 (80)		8 (80)	
Unknown	19	3 (16)		5 (26)		121	80 (66)		76 (63)	
Ulceration[1]										
No	279	116 (42)	0.98	116 (42)	0.22	211	136 (64)	0.78	128 (61)	0.40
Yes	127	53 (42)		61 (48)		74	49 (66)		49 (66)	
Satellites[1]										
No	395	164 (42)	0.40	170 (43)	0.16	–	–		–	
Yes	9	5 (56)		6 (67)		–	–		–	
Regression[1]							–		–	
No	363	152 (42)	0.78	153 (42)	0.063	–	–		–	
Yes	38	15 (39)		22 (58)		–	–		–	
Dissection site										
Neck	126	45 (36)	0.10	54 (43)	0.24	92	53 (58)	0.20	47 (51)	0.050
Axilla	185	68 (37)		79 (43)		66	50 (76)		49 (74)	

Table 1. (continued)

Characteristic	Australia					The Netherlands				
	Total (n=494)	Recurred (n=198)	P-value	Died (n=206)	P-value	Total (n=331)	Recurred (n=213)	P-value	Died (n=200)	P-value
	n	n (%)		n (%)		n	n (%)		n (%)	
Iliac[2]	7	5 (71)		4 (57)		13	9 (69)		9 (69)	
Ilio-inguinal	34	13 (38)		8 (24)		119	74 (62)		69 (58)	
Inguinal	142	67 (47)		61 (43)		41	27 (66)		26 (63)	
Number of nodes removed										
1 – 11	123	61 (50)	0.08	49 (40)	0.95	89	60 (67)	0.79	55 (62)	0.58
12 – 18	127	50 (39)		55 (43)		103	65 (63)		66 (64)	
19 – 27	123	45 (37)		52 (42)		66	43 (65)		36 (55)	
28+	121	42 (35)		50 (41)		67	40 (60)		38 (57)	
median (Q1, Q3)	18 (12, 27)	17 (10, 25)	0.54[3]	18 (12, 27)	0.69	16 (11, 26)	15 (11, 26)	0.41	15 (11, 25)	0.30
AJCC staging										
N1: 1	254	74 (29)	<0.0001	80 (32)	<0.0001	155	84 (54)	0.0004	79 (51)	0.0004
N2: 2 – 3	148	69 (47)		69 (47)		89	60 (67)		54 (61)	
N3: 4+	92	55 (60)		57 (62)		87	69 (79)		67 (77)	
Number of positive nodes										
median (Q1, Q3)	1 (1, 3)	2 (1, 4)	<0.0001	2 (1, 4)	<0.0001	592 (1, 4)	2 (1, 5)	<0.0001	2 (1, 5)	<0.0001
Size of largest node										
0.2 – 2 cm	119	47 (40)	0.32	39 (33)	0.11	59	35 (59)	0.08	33 (56)	0.62
>2.0 – 3.0	155	64 (41)		73 (47)		54	29 (54)		30 (56)	
>3.0 – 4.0	109	50 (46)		48 (44)		52	34 (65)		32 (62)	
>4.0	109	37 (34)		46 (42)		60	47 (78)		41 (68)	
Unknown	2	0 (0)		2 (0)		106	68 (64)		64 (60)	

Table 1. (continued)

Characteristic	Australia					The Netherlands				
	Total (n=494)	Recurred (n=198)	P-value	Died (n=206)	P-value	Total (n=331)	Recurred (n=213)	P-value	Died (n=200)	P-value
	n	n (%)		n (%)		n	n (%)		n (%)	
median (Q1, Q3)	3.0 (2.1, 4.0)	2.9 (2.1, 4.0)	0.42	3.0 (2.2, 4.0)	0.31	3.0 (2.0,4.5)	3.5 (2.2, 5.0)	0.0097[5]	3.5 (2.2, 4.5)[5]	0.14
Extra capsular extension										
No	267	88 (33)	0.0005	84 (31)	<0.0001	230	135 (59)	0.001	122 (53)	<0.0001
Yes	227	110 (48)		122 (54)		101	78 (77)		78 (77)	
Tumour stage										
IIIB	257	82 (32)	0.0001	83 (32)	0.0001	192	111 (58)	0.004	101 (53)	0.0006
IIIC	237	116 (49)		123 (52)		139	102 (73)		99 (71)	
Infection										
No	426	168 (39)	0.46	179 (42)	0.72	276	180 (65)	0.46	166 (60)	0.82
Yes	68	30 (44)		27 (40)		55	33 (60)		34 (62)	
Seroma										
No	396	148 (37)	0.014	152 (38)	0.003	274	176 (64)	0.92	168 (61)	0.47
Yes	98	50 (51)		54 (55)		57	37 (65)		32 (56)	

NB: for some factors, summed total may be less than number of patients due to missing values

Q1, Q3: value of first and third quartiles

[1]Available only for patients with known primary; Australian cohort: n=412, with two-year recurrence n=171, died within 5 years n=179; The Netherlands cohort: n=285, with two-year recurrence n=185, died within 5 years n= 177;

[2] Australian cohort: iliac dissections were performed subsequently to inguinal dissection; ilioinguinal dissection was performed only if pelvic region was involved on imaging.

[3] After correction for dissection site.

[4] The Netherlands data on primary site had one category for melanomas on "extremities".

[5] Calculated only for n=225; n=106 (32% of 331) patients in The Netherlands cohort with missing data on size of largest node.

time to recurrence in the study cohort was 11±0.9 months and 90% of recurrences were systemic (10% local). Among the patients who recurred, 198 (91%) had a recurrence within two years of nodal dissection. Death due to melanoma occurred in 150 patients within the first two years and in 206 patients within 5 years.

Predictors of two-year recurrence at the 10% level of significance were: extracapsular extension, whether microscopic or gross; presence of seroma (yes, no); stage (IIIB, IIIC), nodular primary melanoma, dissection site (inguinal/ilio-inguinal, elsewhere), post-operative radiotherapy (yes, no); number of nodes dissected; and number of positive nodes detected. Further assessment of the association of recurrence with the number of positive nodes indicated that after eight positive nodes no additional association was evident, so number of positive nodes was truncated at eight. Post-operative radiotherapy was judged unsuitable for the purpose of a generally applicable predictive formula as it relied already on a combination of other study variables with its use being subject to local therapeutic modes.

After multivariate proportional hazards analysis, five variables remained for inclusion in the linear predictive formula, namely the presence of extra-capsular extension, seroma, number of positive nodes, nodular subtype and dissection site. Dissection site and seroma added a negligible amount to the predictive value beyond the other variables as measured by the AUC and were therefore omitted for parsimony. Using the coefficients derived from the proportional hazards model, a formula was developed as follows: Nomogram score = 0.49 x extracapsular extension (coded 0, 1) + 0.41 x presence of nodular subtype of primary melanoma (coded 0, 1 for known primaries, 0 for unknown primaries) + 0.187 x number of positive nodes (to a maximum of 8). For individuals with missing information on primary melanoma histology, that variable was set equal to zero without significantly affecting the predictive value of the formula. This was the case for 82 patients (16.6%) with a melanoma of unknown primary and an additional 19 (4.6%) where the primary tumour histological subtype was not recorded. The AUC for this formula was 0.68 and the C index value was 0.67. Attempts to provide a separate formula for patients without information on primary histology yielded no advantage in terms of the AUC.

For the Australian cohort, proportions of two-year recurrences in each nomogram band and two year recurrence rates calculated by the Kaplan-Meier method rose steadily across the bands, from 20.5% and 24.7±4.3%, respectively, in the lowest band to 66.1% and 77.7% , respectively, in the highest band (Table 2). In addition, this same strategy when applied to Stage IIIB or to stage IIIC patients separately successfully classified patients according to their 2-year recurrence rate (Supplemental Table 1). Indeed, recurrence rates rose monotonically from the lowest to highest band of the nomogram within each stage separately.

Table 2. Nomogram ranges and associated numbers of patients, recurrences and two-year recurrence rates in the Australian and The Netherlands cohorts.

Nomogram Range	Australia				The Netherlands			
	Recurrences/ total	Percentage	Two-year recurrence rate	Standard Error	Recurrences/ total	Percentage	Two-year recurrence rate	Standard Error
≤ 0.2	25/122	20.5	24.7	4.3	40/83	48.2	53.5	5.8
0.3 – 0.6	29/92	31.5	40.5	6.0	56/87	64.4	68.1	5.2
0.7	33/85	38.8	51.4	6.5	19/28	67.9	69.5	8.9
0.8 – 1.2	56/108	51.9	63.0	5.3	45/68	66.2	70.4	5.9
1.3 – 1.4	14/25	56.0	73.7	10.8	9/14	64.3	74.7	12.8
≥ 1.5	41/62	66.1	77.7	6.1	44/51	86.3	88.5	4.6
Total	**198/494**	**40.1**	**48.5**	**2.6**	**213/331**	**64.4**	**67.9**	**2.7**

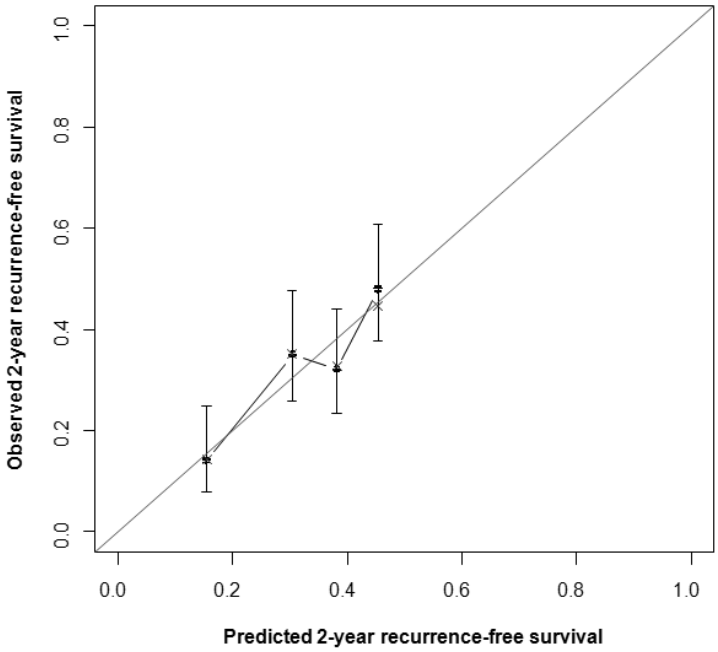
Table 3. Nomogram ranges and associated numbers of patients, deaths within five years and actuarial five-year death rates, with 95% confidence intervals (95% CI), in the Australian and The Netherlands cohorts.

Nomogram Range	Australia				The Netherlands			
	Deaths/ total	Percentage	Five-year death rate	95% CI	Deaths/ total	Percentage	Five-year death rate	95% CI
≤51	38/159	23.9	39.6	28.7 – 50.3	58/122	47.5	60.4	49.3 – 69.8
0.52 – 0.56	26/69	37.7	55.6	38.7 – 69.6	34/66	51.5	62.7	47.7 – 74.5
0.57 – 1.03	42/95	44.2	63.9	50.7 – 74.4	21/33	63.6	74.8	52.6 – 87.7
1.04 – 1.08	20/39	51.3	61.3	40.3 – 76.8	30/42	71.4	76.5	59.4 – 87.2
1.09 – 1.60	43/79	54.4	69.6	54.7 – 80.5	20/23	78.0	89.6	65.4 – 97.2
≥ 1.61	37/53	69.8	87.4	67.1 – 95.5	37/45	82.2	94.4	72.1 – 99.0
Total	**206/494**	**41.7**	**57.5**	**51.6 – 63.0**	**200/331**	**60.4**	**70.0**	**64.0 – 75.2**

The validation dataset from The Netherlands contained data from 337 patients aged 17 years or older who underwent LND (Table 1). Recurrences occurred in 246 (73%) with mean time to recurrence of 11.0± 1.0 months. Among these 216 (64%) recurred in the first two years. Six patients, three with recurrences within two years, had no information on the number of positive nodes removed, and were excluded from the validation cohort, leaving 331 individuals. Information on primary histology was missing for 167, 46 of whom had melanomas of unknown primary. The average age of patients was 55.3 years in males and 54.1 in females. Compared to the Australian cohort, The Netherlands cohort had more dissections in the groin, iliac or ilio-inguinal fields (52.3vs37.3%), more patients with greater than 4 positive nodes (26.3% v 18.3%), a larger average size of largest node (3.6±2.2 vs 3.2±2.0) but less extracapsular extension (30.5% v 45.4%).

When the above nomogram was applied to The Netherlands cohort, two-year recurrence rates also rose steadily across the nomogram bands, from 53.5±5.8% in

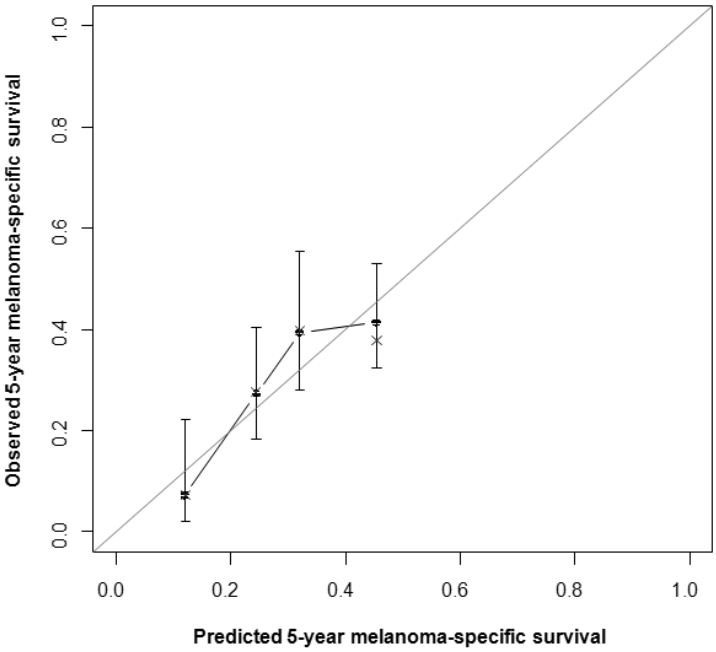
Figure 1: Calibration plots for two-year recurrence-free predictive performance in validation cohort.



the lowest band to 88.5±4.6% in the highest band (Table 2). The clinical significance of the nomogram was assessed using calibration curves comparing predicted 2-year recurrence-free probabilities with observed probabilities in this validation dataset (Figure 1). These calibration curves displayed high concordance between nomogram-predicted probabilities and the mean observed Kaplan-Meier 2-year recurrence-free survival. Within nomogram bands the recurrence rates were uniformly higher in The Netherlands cohort compared with those in the Australian cohort, particularly at lower bands. Restricting The Netherlands cohort to more recent patients (2000-2009) to take into account accuracy of imaging techniques used for stag ng did not change the difference in outcome between the cohorts. In this dataset, the AUC for the nomogram was 0.63 and the C index was 0.60. When the two-year recurrence rates were calculated separately for stage IIIB or IIIC patients recurrence rates rose overall in both groups with nomogram scores, although not monotonically (Supplemental Table 2).

A similar strategy was adopted regarding melanoma specific survival. Following the same methods a nomogram was developed that included the number of positive nodes as a categorical variable and the presence or absence of extra-capsular extension whether microscopic or macroscopic. The resulting formula gave Nomogram score = 0.52 x (0 if number of positive nodes=1; 1 if number of positive nodes=

Figure 2: Calibration plots for 5- year melanoma-specific survival predictive performance in validation cohort.



2 to 3, and 3 if number of positive nodes equal or more than 4) + 0.57 x extra-capsular extension (presence of any type=1, absence=0). No strong evidence for multi-collinearity (variance inflation factor<4) was noted between age, number of positive nodes categories and extra-capsular extension.

This nomogram resulted in a C index of 0.67 and an AUC of 0.67 in the Australian dataset. When examined only in stage IIIB patients or IIIC patients separately, the AUC was 0.64 and 0.65 respectively. In the validation cohort, this nomogram had an AUC of 0.66 and a C index at 0.62. The clinical significance of the nomogram was again assessed using calibration curves comparing predicted 5 year survival probabilities with observed probabilities in this validation dataset (Figure 2). Similarly, the AUC when considering only stage IIIB or only stage IIIC patients was 0.61 and 0.63 respectively.

DISCUSSION

Accurate predictors of melanoma outcome are needed for stage III melanoma patients undergoing lymph node dissection to orient management. In this study, we established and validated nomograms for predicting recurrence at 2 years or

melanoma-specific death. In a prospective cohort, we first conducted a failure-time multivariate analysis that identified some of the key predictive factors important in stage III melanoma. Formulas based on an adjusted combination of these factors were then used to generate nomograms that increased in value linearly with two-year recurrence and death. Each of these nomograms incorporated additional characteristics such as extracapsular extension beyond criteria already present in the AJCC staging system. They were able to classify patient outcome within each AJCC subclass and their predictive value was also validated in a second prospective cohort from The Netherlands.

Previous studies have described prognostic factors of stage III progression. In their study of 441 patients with macrometastases, Balch et al, reported that age, ulceration, anatomic site, and number of tumor-bearing lymph nodes independently predicted survival[5]. In Cox proportional hazard models, only number of nodes and age were independent predictors. None of the primary tumour attributes played an important role as predictors of survival[5]. The present study of the larger Australian cohort of stage IIIB and IIIC melanoma patients, reproduced these findings for age, number of invaded nodes and anatomic site. Similar results were also obtained in our confirmation cohort[8]. The reproducibility of these factors across multiple cohorts including ours suggests the generalizability of our study sample. We also considered additional parameters not reported by Balch et al, such as extracapsular extension. The prognostic significance of extracapsular extension has been previously reported and proved essential in terms of predictive value in our nomogram for both recurrence and death[4, 8, 14].

A major strength of this study is the use of a validation cohort[8]. Similar to ours, this cohort was composed of patients treated in a tertiary referral centre and, in many aspects, they shared general characteristics of our cohort, though on average, Dutch patients were older, had more advanced Stage III disease and consequently the recurrence rate was overall higher. Lack of staging with FDG-PET in the Netherlands patients might play a role in the differences in outcome observed between both cohorts[15]. However, the ability of the nomogram to accurately classify Dutch patients into distinct prognostic groups despite the outcome differences demonstrates its robustness.

For patients with stage III metastatic melanoma there are no clear adjuvant therapeutic options after surgery[3]. Interferon alpha may be used but its benefit is limited; postoperative radiotherapy may reduce local recurrence in high risk stage III patients[16, 17]. In our study, 90% of recurrences were systemic and therefore unlikely to be affected by radiotherapy. Others have used immunotherapy also with modest benefits[18, 19]. Clinical trials of adjuvant regimens where therapy is applied to all

stage III patients regardless of their risk of progression or response may fail due to inclusion of patients unlikely to progress and therefore unlikely to benefit from the intervention, reducing the potential difference between treated and untreated groups. They will be nevertheless exposed to the toxicity of the therapy. An important clinical application of a nomogram for stage III patients may be the capacity to analyse therapeutic responses of subgroups stratified according to the nomogram bands as described here. An example is interferon therapy where the benefit has been shown to be maximal for patients with lower tumour burden[20, 21].

In conclusion, we have developed and validated mathematical models to predict outcome in stage IIIB and IIIC melanoma patients undergoing LND. The models included prognostic factors additional to the number of positive nodes described in the AJCC staging system. Such models have not been applied to this subgroup of patients previously and may add value beyond existing staging tools for patients with lymph node metastases. Nomograms described here could become a tool for routine use in a clinical setting for the management of patients with stage IIIB and stage IIIC melanoma or in a research setting for better stratification in clinical trials.

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SUPPLEMENTARY TABLES

Supplemental Table 1. Nomogram ranges and associated numbers of patients, recurrences and two-year recurrence rates for Stage IIIB and Stage IIIC patients in the Australian cohort.

	Stage IIIB		Stage IIIC	
Range	Recurrences/total	Two year recurrence rate	Recurrences/total	Two year recurrence rate
≤ 0.2	16/89	21.8	9/33	35.9
0.3 – 0.6	16/57	37.9	13/35	45.8
0.7	17/53	43.8	16/32	63.6
0.8 – 1.2	28/50	69.7	28/58	58.5
13 – 1.4	3/6	50.0	11/19	82.6
≥ 1.5	2/2	100.0	39/60	76.6
Total	82/257	39.2	116/237	59.1

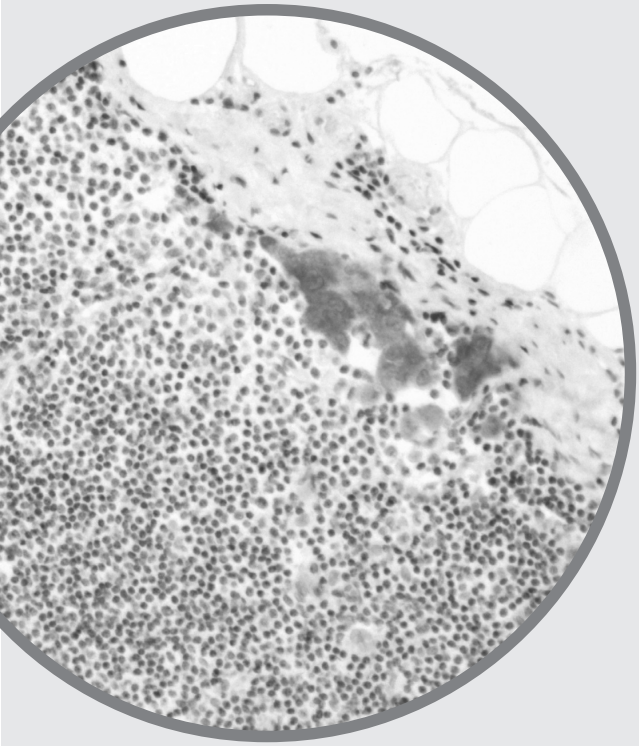
For the stage IIIB sub-group the AUC and C-index were 0.68 and 0.67 respectively; for the stage IIIC sub-group, 0.64 and 0.64 respectively.

Supplemental Table 2. Nomogram ranges and associated numbers of patients, recurrences and two-year recurrence rates for Stage IIIB and Stage IIIC patients in the Netherlands cohort.
For the stage IIIB sub-group the AUC and C-index were 0.57 and 0.54 respectively; for the stage IIIC sub-group, 0.64 and 0.58 respectively.

	Stage IIIB		Stage IIIC	
Range	Recurrences/total	Two year recurrence rate	Recurrences/total	Two year recurrence rate
≤ 0.2	36/77	51.8	4/6	77.8
0.3 – 0.6	44/66	68.9	12/21	63.0
0.7	12/18	69.7	7/10	75.0
0.8 – 1.2	17/29	64.9	28/39	75.2
13 – 1.4	1/1	100	8/13	72.8
≥ 1.5	1/1	100	43/50	88.3
Total	111/192	61.0	102/139	77.6

Chapter 9

Therapeutic surgical management of palpable melanoma groin metastases: superficial or combined superficial and deep groin lymph node dissection



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ABSTRACT

Management of patients with clinically detectable lymph node metastasis to the groin is by ilioinguinal or combined superficial and deep groin dissection (CGD) according to most literature, but in practice superficial groin dissection only (SGD) is still performed in some centers. The aim of this study was to evaluate the experience in CGD vs. SGD patients in our center.

Between 1991 and 2009, 121 therapeutic CGD and 48 SGD were performed in 169 melanoma patients with palpable groin metastases at our institute. Median follow-up was 20 and, for survivors, 45 months.

In this heterogeneous group of patients, overall (OS) and disease-free survival, local control rates and morbidity rates were not significantly different between CGD and SGD patients, however CGD patients had a trend towards more chronic lymphedema. Superficial lymph node ratio, the number of positive superficial lymph nodes and the presence of deep nodes were prognostic factors for survival. CGD patients with involved deep lymph nodes (24.8%) had estimated 5-year OS of 12% compared with 40% with no involved deep lymph nodes (p=0.001). Preoperative computed tomography (CT) scan had a high negative predictive value of 91% for the detection of pelvic nodal involvement.

This study demonstrated that survival and local control do not differ for patients with palpable groin metastases treated by CGD or SGD. Patients without pathological iliac nodes on CT might safely undergo SGD, while CGD might be reserved for patients with multiple positive nodes in the SGD and/or positive deep nodes on CT scan.

INTRODUCTION

Management of patients with clinically detectable lymph node metastasis to the groin consists of ilioinguinal or combined superficial and deep groin dissection (CGD) according to most literature.[1-7] Some surgeons are willing to do so only when there are multiple positive nodes in the groin or when there is evidence of pelvic nodal involvement on the basis of imaging information.[8] In practice, a solely inguinal or superficial groin dissection (SGD) is still being performed in some cases and/or centers. The potential survival or local control benefit of extensive surgery remains controversial in the absence of randomized data.

Prognosis and outcome of patients after a CGD is believed to correlate with the biology of the disease rather than with the extent of the operation.[4, 8-11] It is advocated that CGD should be performed when there is clinically gross involvement of the groin, when there are clinically detectable deep lymph nodes, when Cloquet's node is histologically positive or when pelvic computed tomography (CT) demonstrates pelvic lymphadenopathy.[12]

The aim of this study is to evaluate the experience in patients with clinically evident metastatic melanoma to the groin who underwent CGD versus SGD only. Postoperative morbidity, regional recurrence, preoperative CT scan, and disease-free and overall survival were analyzed. The necessity of removal of the deep iliac and obturator lymph nodes as well as prognostic factors for survival in patients with metastatic melanoma to the groin were evaluated.

PATIENTS AND METHODS

Patients

Patients in this study presented with clinically detectable metastases to the groin at the Erasmus University Medical Center – Daniel den Hoed Cancer Center, Rotterdam, the Netherlands. Patients were selected for therapeutic ilioinguinal or combined superficial and deep groin dissection (CGD) or for inguinal or superficial groin dissection (SGD). All patients underwent the operation within 2 months of detection of palpable metastasis. Patients who underwent sentinel lymph node biopsy were excluded. All patients' characteristics, tumor characteristics, postoperative morbidities, regional recurrence patterns and imaging procedures (preoperative CT scanning) were collected and sorted in a database for this retrospective single institution study. Postoperative morbidities were collected from patient's charts and divided into two categories; short-term morbidities, e.g. wound infection or necrosis, seroma, and long-term morbidities, e.g. chronic lymphedema. Chronic lymphedema

was recorded if moderate or severe swelling was present for more than 6 weeks postoperatively and the patient required therapy. Adjuvant radiotherapy was given to 16 (9.5%) patients and they were treated with doses between 15 and 80 Gy.

Surgical procedure

Four coauthors performed the majority of lymph node dissections assessed for this study (J.H. de W., A.N. van G., A.M.M.E., and C.V.). In general, patients with palpable inguinal nodes underwent CGD. Indication for SGD was based on surgeon or patient preference. Patients with significant (cardiopulmonary) co-morbidities and absence of preoperative radiological and/or clinical suspicion for involved deep lymph nodes underwent SGD. SGD was performed via a transverse inguinal incision and involved complete dissection of lymph nodes from the inguinofemoral content to the apex of the femoral triangle where the long saphenous vein joins the femoral vein. Sartorius muscle transposition to cover and protect femoral vessels was selectively performed when adjuvant radiotherapy was to be expected and/or patient's skin was at risk. When a CGD was performed, an additional incision was made approximately 3 to 5 centimeters above the line of the inguinal ligament. CGD included dissection of the inguinofemoral and external iliac nodes up to the common iliac artery (if necessary up to the aortic bifurcation) and dissection of the obturator nodes.

Statistics

Disease free survival (DFS) was calculated from the operation date of the lymph node dissection to the date of first recurrence at any site. Overall survival (OS) was calculated from the operation date of the lymph node dissection to the date of death due to any cause.

Different statistical methods were assessed when appropriate. The chi-square test, Fisher's exact test and Mann-Whitney test were assessed to investigate differences in clinicopathological features, the predictive value of the number of involved superficial nodes for deep lymph node involvement, postoperative morbidities and regional recurrence patterns in CGD and SGD patients. The log-rank test and the Kaplan-Meier method were assessed for survival analysis and the search for prognostic factors in CGD patients, SGD patients, and the total group of patients. All calculations were performed with STATA version 10.1 and 11.1 (StataCorp LP, College Station, TX, USA).

Table 1 – Clinicopathological factors

	Combined deep and superficial Groin Dissections (n=121) n (%)	**Superficial Groin Dissections (n=48) n (%)**	**P-value***
Gender			
Female	70 (57.9)	32 (66.7)	
Male	51 (42.2)	16 (33.3)	0.303
Age (years)			
≤ 50	47 (38.8)	11 (22.9)	
> 50	74 (61.2)	37 (77.1)	0.072
Site of primary			
Leg	78 (78.8)	37 (92.5)	
Trunk	21 (21.2)	3 (7.5)	0.080
Missing	22	8	
Breslow thickness (mm)			
≤ 2.00	52 (57.1)	14 (38.9)	
2.01 - ≤ 4.00	23 (25.3)	10 (27.8)	
> 4.00	16 (17.6)	12 (33.3)	0.099
Missing	30	12	
Clark level			
II-III	26 (32.1)	9 (30)	
IV	48 (59.3)	19 (63.3)	
V	7 (8.6)	2 (6.7)	0.907
Missing	40	18	
Ulceration			
Absent	89 (73.6)	34 (70.8)	
Present	32 (26.5)	14 (29.2)	0.706
Extranodal invasion			
Absent	33 (48.5)	14 (51.9)	
Present	35 (51.5)	13 (48.2)	0.823
Missing	53	21	
Largest diameter of positive superficial node (cm)			
< 3	21 (29.2)	11 (50.0)	
≥ 3	51 (70.8)	11 (50.0)	
Missing	50	26	0.002
No. positive superficial nodes			
1	57 (47.1)	26 (54.2)	
2 – 3	35 (28.9)	14 (29.2)	
> 3	29 (24.0)	8 (16.7)	0.553

Table 1 (continued)

	Combined deep and superficial Groin Dissections (n=121) n (%)	Superficial Groin Dissections (n=48) n (%)	P-value*
No. harvested superficial nodes			
Median (IQR)	15 (12-22)	8 (5-14)	< 0.001
Superficial lymph node ratio (%)			
Median (IQR)	11 (6-25)	20 (10-50)	0.0004
≤ 10	54 (45.4)	12 (25.0)	
10 – ≤ 25	37 (31.1)	18 (37.5)	
> 25	28 (23.5)	18 (37.5)	0.035
Missing	2	0	
Positive deep lymph nodes			
Absent	91 (75.2)	48 (100.0)	
Present	30 (24.8)	0 (0.0)	-
Adjuvant radiotherapy	11 (9.1)	5 (10.4)	0.776

IQR = Interquartile range

* P-values are calculated with the Fisher exact test, Chi-square test and Mann-Whitney test.

RESULTS

CGD vs. SGD

This study included 121 patients (70 women and 51 men) who underwent a therapeutic combined superficial and deep dissection (CGD) and 48 patients (32 women and 16 men) who underwent a therapeutic superficial dissection (SGD) for palpable melanoma metastases to the groin. Surgeries were performed between 1991 and 2009 at the Erasmus University Medical Center – Daniel den Hoed Cancer Center, Rotterdam, the Netherlands. Median follow-up time was 20 months for all patients and 45 months for all survivors (both, range 1 – 202 months). Median age at time of surgery was 54 (range 21 – 87) years at time of surgery. CGD patients had significantly more patients with large superficial nodes than SGD patients (p=0.002), more harvested superficial lymph nodes (p<0.001) and lower superficial lymph node ratio (p=0.0004). (Table 1)

Preoperative diagnosis

Patients were clinically diagnosed by computed tomography (CT), fine-needle aspiration cytology (FNAC) and/or ultrasound. All SGD patients were diagnosed with superficial lymph node involvement only. Of all CGD patients, 24 (19.8%) were diagnosed with superficial and deep lymph node involvement and 97 (80.2%) were diagnosed with only superficial lymph node involvement.

Table 2 – CT accuracy for pelvic lymph node involvement in CGD patients

	CT pelvic +	CT pelvic -	Total
Histology Pelvic +	10	4	14
Histology Pelvic -	7	40	47
Total	17	44	61

Sensitivity = 10/14 = 71.4%
Specificity = 40/47 = 85.1%
Positive predictive value = 10/17 = 58.8%
Negative predictive value = 40/44 = 90.9%
CT= Computed Tomography, CGD = Combined deep and superficial Groin Dissection

Preoperative CT scans could be retrieved in 61 of 121 CGD. Of the 61 radiographically evaluated CGD patients, 44 (62.1%) were diagnosed with only superficial lymph node involvement, of which 40 were histologically confirmed by the pathologist (negative predictive value for pelvic involvement 91%). Positive predictive value for pelvic metastases was 59%, sensitivity was 71%, and specificity was 85%. (Table 2)

Postoperative morbidity

Median hospital stay was 6 (range 3 – 27) days in patients with CGD and 6 (range 2 – 32) days in patients with SGD. There were no statistically significant differences in postoperative morbidities between CGD and CGD patients (all p>0.05), although

Table 3 – Post-operative morbidity en regional recurrence rates

Type of morbidities	Combined deep and superficial Groin Dissections (n=121) n (%)	Superficial Groin Dissections (n=48) n (%)	P-value†
Overall	77 (63.6)	24 (50.0)	0.119
Short-term*	60 (49.6)	19 (39.6)	0.305
Long-term**	32 (26.5)	8 (16.7)	0.229
Wound infection and/or necrosis	30 (24.8)	13 (27.1)	0.845
Chronic lymphedema	31 (25.6)	7 (14.6)	0.154
Type of recurrence			
Median time to recurrence	7.6	6.0	0.677
Regional superficial and deep lymph node recurrence	19 (15.7)	10 (20.8)	0.498
Of which: Pelvic lymph node recurrence	12 (9.9)	5 (10.4)	1.000

**Short term morbidities include wound infection and/or necrosis, seroma, post operative bleeding, urinary tract infection, pulmonary embolism or thrombosis and transient nerve damage.*
***Long term morbidities include chronic lymphedema, urinary tract damage, permanent nerve damage and loss of function*
† P-values are calculated with the Mann-Whitney test, Fisher's exact test and Chi-square test.

there was a trend towards more chronic lymphedema in the CGD group (25.6% vs. 14.6%, p=0.154). (Table 3)

Recurrence

There was no statistical difference in disease-free survival time or time to regional relapse between SGD and CGD patients, with overall recurrence rate of 73% (90/121) and 74% (35/48), respectively. At time of last follow-up, 81 of 121 patients (67%) in the CGD group and 31 of 48 patients (65%) in the SGD group were dead. Regional recurrence rates were more common in SGD than in CGD patients, i.e., 21% and 16% (p=0.498), and pelvic recurrence rates were 10% in both groups of patients (p=1.000). Median time to first recurrence was 7.6 (range 1 – 96) months for CGD patients and 6.0 (range 1 – 42) months for SGD patients (p=0.677). (Table 3)

Survival analysis

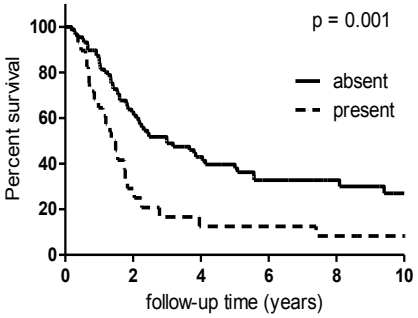
Disease-free (DFS) and overall survival (OS) in CGD patients were not better than in SGD patients (p=0.722 and p=0.647, respectively)). (Figure 1C-D) Comparison of DFS and OS of CGD patients who only had superficial nodes involved with SGD patients also showed no significant difference (p=0.421 and p=0.217, respectively).

Five-year estimated DFS and OS rates for patients who underwent SGD were 15.7% and 28.7%, respectively. Five-year estimated DFS and OS rates for patients who underwent CGD were 18.3% and 33.0% respectively. (Figure 1C-D)

On univariate analysis of prognostic factors in the total number of patients (n=169), the number of positive superficial nodes (1, 2 – 3, ≥ 4) were significant prognostic factors for DFS [≥ 4 nodes only; hazard ratio (HR) = 1.85; 95% confidence interval (CI) 1.21 – 2.84; p=0.005] and OS (HR=1.60; 95%CI 1.03 – 2.51; p=0.038 and HR=2.36; 95%CI 1.50 – 3.71; p=0.0005) as well as superficial lymph node ratio for DFS (HR=2.33; 95%CI 1.25 – 4.34; p=0.008) and OS (HR=3.16; 95%CI 1.68 – 5.94; p<0.001). Presence of involved deep lymph nodes was a prognostic factor for OS (HR=1.95; 95%CI 1.24 – 3.07; p=0.004).

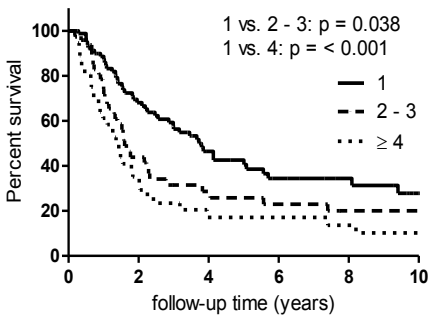
On univariate analysis of prognostic factors in SGD patients only, the largest diameter of the positive lymph node was significant for OS (HR=3.10; 95%CI 1.07 – 8.98; p=0.037), while analysis in CGD patients revealed superficial lymph node ratio, more than 3 positive superficial nodes as well as the presence of involved deep lymph nodes as poor prognostic factors for OS (HR=5.90; 95%CI 2.21 – 15.76; p<0.001, HR=2.29; 95%CI 1.34 – 3.91; p=0.002 and HR=2.25; 95%CI 1.38 – 3.66; p=0.001, respectively) and DFS (HR=4.64; 95%CI 1.70 – 12.65; p=0.003, HR =1.96; 95%CI 1.19 – 3.22; p=0.008 and HR=1.61; 95%CI 1.02 – 2.55; p=0.041, respectively). (Table 4)

Figure 1A: Overall survival – Positive deep lymph nodes



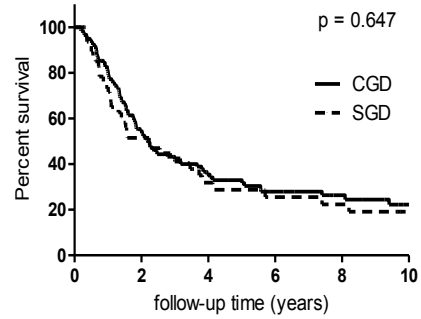
Nr at Risk							
Absent	**91**	**47**	**26**	**18**	**12**	**7**	**1**
Present	**30**	**4**	**3**	**3**	**2**	**1**	
Total	**121**	**51**	**31**	**21**	**14**	**8**	

Figure 1B: Overall survival – Number of positive superficial lymph nodes



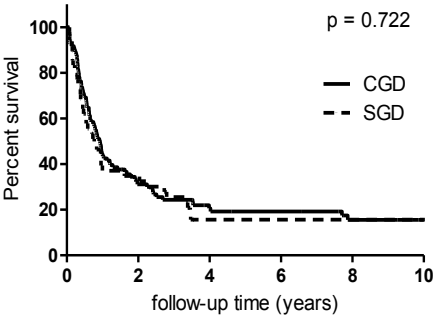
Nr at Risk							
1	**83**	**47**	**24**	**16**	**11**	**0**	
2 – 3	**49**	**18**	**10**	**8**	**0**	**0**	
≥ 4	**37**	**12**	**5**	**5**	**4**	**0**	
Total	**169**	**77**	**39**	**29**	**15**	**0**	

Figure 1C: Overall survival – Type of groin dissection



Nr at Risk						
CGD	**121**	**54**	**29**	**21**	**14**	**0**
SGD	**48**	**23**	**10**	**8**	**7**	**0**
Total	**169**	**77**	**39**	**29**	**21**	**0**

Figure 1D: Disease free survival – Type of groin dissection



Nr at Risk						
CGD	**121**	**28**	**15**	**13**	**8**	**0**
SGD	**48**	**15**	**3**	**3**	**3**	**0**
Total	**169**	**43**	**18**	**16**	**11**	**0**

Five-year estimated DFS and OS rates for positive deep lymph nodes were 9.1% and 12.5%, respectively, compared with 5-year estimated DFS and OS rates for positive superficial lymph nodes only in CGD patients of 21.5% and 39.7%. (Figure 1A) Five-year estimated DFS rates for the number of positive superficial lymph nodes was 23.7% for 1 involved node, 12.0% for 2 – 3 and 11.2% for ≥ 4 involved nodes. Five-year estimated OS rates for the number of positive superficial lymph nodes was 42.6% for 1 involved node, 25.8% for 2 – 3 and 17.1% for ≥ 4 involved nodes. (Figure 1B)

Table 4 – Cox regression univariate analysis of overall and disease-free survival for prognostic factors in CGD and SGD patients, and the total group of patients.

	Combined deep and superficial Groin Dissections (n=121)			Superficial Groin Dissections (n=48)			All Groin Dissections (n=169)		
					Disease Free Survival*				
	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P
Largest diameter of superficial node (cm)									
< 3	1			1			1		
≥ 3	1.69	0.90-3.17	0.100	2.48	0.91-6.80	0.077	1.82	1.08-3.07	0.024
No. of positive superficial nodes									
1	1			1			1		
2-3	1.53	0.93-2.51	0.092	1.29	0.63-2.66	0.494	1.40	0.93-2.11	0.103
≥4	1.96	1.19-3.22	0.008	1.85	0.77-4.41	0.167	1.85	1.21-2.84	0.005
Superficial lymph node ratio	4.64	1.70-12.65	0.003	1.64	0.66-4.08	0.283	2.33	1.25-4.34	0.008
Positive deep nodes									
Absent	1						1		
Present	1.61	1.02-2.55	0.041			N/A†	1.48	0.96-2.28	0.075
					Overall Survival*				
Largest diameter of superficial node (cm)									
< 3	1			1			1		
≥ 3	1.43	0.74-2.77	0.292	3.10	1.07-8.98	0.037	1.72	0.99-3.00	0.055
No. of positive superficial nodes									
1	1			1			1		
2-3	1.66	0.96-2.87	0.071	1.48	0.69-3.17	0.316	1.60	1.03-2.51	0.038
≥4	2.29	1.34-3.91	0.002	2.44	0.99-6.01	0.052	2.36	1.50-3.71	0.0005
Superficial lymph node ratio	5.90	2.21-15.76	<0.001	2.27	0.88-5.88	0.091	3.16	1.68-5.94	<0.001
Positive deep nodes									
Absent	1						1		
Present	2.25	1.38-3.66	0.001			N/A†	1.95	1.24-3.07	0.004

N/A= Not Applicable, CGD= Combined deep and superficial groin dissection, SGD= superficial groin dissections

**the following variables did not have any prognostic significance in all groups: gender, age, site of primary, Breslow thickness, Clark level, ulceration and extra nodal invasion.*

† Variable not assessable due to no presence of positive deep nodes

DISCUSSION

Survival in patients with palpable metastatic melanoma to the groin is poor. In the literature, estimated 5-year overall survival (OS) rates vary from 20 to 40%.[8, 11, 13] In our series of 169 patients with palpable nodes in the groin, 5-year estimated OS rates were 33% for CGD and 29% for SGD respectively. Also 5-year DFS rates were

Table 5 – Overview of literature describing survival rates in patients with positive deep nodes to the groin diagnosed after therapeutic combined deep and superficial lymph node dissection only

Institute	Reference	Year	Study period	Median follow-up (months)	No. patients with positive pelvic nodes (% of total)	5-year OS (%)
NCI/ALH	Jonk [24] * Strobbe [7]	1999	1961-1995	18	71 (20)	24
UCLA	Finck [14]	1982	1970-1980	23	24 (29)	17
MSKCC	Coit [10]	1989	1974-1984	86**	10 (7)	6
	Mann [8]	1999	1985-1994	40	21 (19)	±35
RPCI	Karakousis ‡ [2-3, 15, 25]	1996	1977-1993	±46	48 (NR)	34
UE	Meyer [13]	2002	1978-1997	20	23 (31)	21
MLUHW	Kretschmer [16]	2001	1983-1994	68**	24 (35)	6
RMH	Hughes [11]	2000	1984-1998	19	29 (40)	19
MDACC	Badgwell [26]	2007	1990-2001	90	55 (51)†	42†
DDHCC	Recent study	2010	1991-2009	20	30 (25)	12

OS= Overall Survival, NR= Not reported; NCI/ALH = Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands; UCLA = University of California, Los Angeles, California, USA; MSKCC = Memorial Sloan-Kettering Cancer Center, New York City, New York, USA; RPCI = Roswell Park Cancer Institute, Buffalo, New York, USA;
UE= University of Erlangen, Erlangen, Germany; MLU HW = Martin-Luther-Universitat Halle-Wittenberg, Halle, Germany
RMH= Melanoma and Sarcome Unit, Royal Marsden Hospital, London, UK; MDACC = M.D. Anderson Cancer Center, Houston, Texas, USA; DDHCC = Erasmus Medical Center - Daniel Den Hoed Cancer Center, Rotterdam, the Netherlands
**The 23 patients (5 year overall survival 32%) reported by Jonk et al. in 1989 are included in the study performed by Strobbe et al.*
***The median survival shown is for the patients who survived only. The median follow-up for the entire group is not reported*
‡ The patients described in the three earlier reports (1986 and two reports in 1994) of Karakousis et al. are included in the 1996 study
† The patients in this study underwent any type of lymph node dissection and not only therapeutic lymph node dissections

virtually identical, i.e.,18% for CGD and 16% for SGD. Patients with CGD with positive deep nodes have the poorest prognosis with OS ranging in the literature from 6 to 34%.[7-8, 10, 14-16] (Table 5) In our institute, patients with CGD and positive deep nodes have estimated 5-year OS and DFS rates of 12% and 9%. In contrast, for CGD patients without deep nodal involvement, we observed 40% and 22%, respectively. There were differences between the CGD and SGD patients. CGD patients had a significantly larger size of involved superficial lymph nodes than SGD patients. (Table 1) Moreover, 25% of CGD patients had involved deep lymph nodes, while there was no suspicion and no diagnosis of deep nodal involvement in SGD patients. CGD patients had unfavorable preoperative prognosis, which is apparent since selection

for the extent of surgery was based on comorbidities and the suspicion of involvement of deep lymph nodes. However, during CGD, more superficial nodes were harvested and the number of positive superficial nodes was not different, resulting in a significant lower superficial lymph node ratio. (Table 1) Lower superficial lymph node ratio is a good prognostic factor for survival.[17-19]

Based on surgery only, CGD patients were expected to have favorable prognosis. In this study, the outcome of CGD patients was virtually identical to that of SGD patients. (Figure 1C-D) Even comparison of patients with superficial involved nodes only showed no difference, indicating that the extent of groin surgery does not influence outcome (data not shown; p=0.217). Also in other studies, it has been demonstrated that the extent of groin surgery, regardless of the presence or absence of deep lymph node involvement in CGD patients, has no effect on survival.[8, 10, 16]

Preoperative CT was performed in 61 of 121 patients who underwent CGD. Positive predictive value (PPV) of CT scanning was only 59% in our experience, whilst the negative predictive value (NPV) was fairly good at 91%. Sensitivity was 71% and specificity 85% in our group of patients. (Table 2) Allen et al. found different results with PPV of 100%, NPV of 86%, specificity of 100% and sensitivity of 60%, stating CT scanning was not reliable as a tool for preoperatively assessing pelvic lymph node involvement.[4] However, both studies show that a CT-based decision on whether or not to perform a CGD could be correctly made in 9 out of 10 patients. Thus, CT scan may be used as a tool in the decision on whether or not to remove the deep lymph nodes.

Morbidity rates in the present study are divided into short- and long-term morbidities. Under-estimation of events in the morbidity data could have occurred due to the retrospective gathering of the data from medical records. However, comparison of the two groups of patients in this study remains valid since this presumed underestimation arose in both groups. Neither short- nor long-term morbidities were significantly lower in SGD than in CGD patients, being 39.6% versus 49.6% (p=0.305) and 16.7% versus 26.5% (p=0.229), respectively. (Table 3) The most debilitating morbidity is chronic lymphedema, which is difficult to define. Some authors have used measurements to define this, whilst others have opted to define chronic lymphedema as edema requiring intervention. Also debated is the minimum period of edema to define it as chronic, which we did when moderate or severe swelling was present (more than) 6 weeks after surgery and required treatment. In any case, it is a widely feared and unpleasant complication.[20-22] There was a trend towards increased chronic lymphedema in patients after a CGD (25.6%) than in patients after a SGD (14.6%), yet this difference was not statistically significant (p=0.154). This difference was not the result of an imbalance of additional radiotherapy to the groin, as 10% of SGD patients received radiotherapy versus 9% of CGD patients (p=0.776). The

assessment of one large or two small incisions for CGD has no influence on the rate of lymphedema as well.[23] Other reports also indicate that lymphedema rates after CGD (range 23 – 55%) are greater than after SGD (range 7% - 29%) albeit not always statistically significant.[8, 11, 20, 22] Faries et al. recently reported the difference in lymphedema rates between immediate and delayed lymph node dissection. In these data of the Multicenter Selective Lymphadenectomy Trial (MSLT) I, lymphedema rates for SGD patients were 21.4% when undergoing immediate dissection and 22.6% when undergoing delayed dissection (p=0.9), while CGD patients had higher lymphedema rates of 36.4% for immediate dissection and 34.2% for delayed dissection (p=0.89).[22] Unfortunately, p-values for the difference in lymph edema between SGD and CGD patients were not provided.[22]

Regarding loco-regional control in the iloinguinal region, we found no differences between CGD and SGD, as regional recurrence rates were similar. (Table 3) The frequency of pelvic recurrences was equal in both groups. Possible causes for this counterintuitive observation could be the small sample size, patient selection, i.e., SGD patients having occult pelvic disease at time of surgery, and/or the overall worse prognosis of both groups of patients compared with literature. Patients might die of distant visceral metastases before pelvic recurrence has been developed. Our results are in line with other reports in the literature. Coit et al. reported similar nodal recurrence rates for SGD and CGD patients, while Singletary et al. reported relatively more nodal recurrence in SGD patients, but attributed that to the extent of tumor burden rather than the extent of surgery.[10, 27]

Our group of patients has worse survival compared to the literature; for example, Balch et al. reported 5-year OS rates of 50% for N1b, 45% for N2b and 40% for N3 patients in the 2009 AJCC melanoma staging system analysis.[28] Patients from our center showed (in that same order) 5-year OS rates of 43%, 26%, and 17%. Because of our relatively small study population compared with the enormous AJCC databases of > 30.000 patients, a single event will have a greater impact on the estimate survival rates in Kaplan-Meier analysis, because the number at risk is smaller. Due to our relatively short median follow-up, we underestimate our long-term survival. Moreover, all patients were operated at the Erasmus University Medical Center – Daniel den Hoed Cancer Center, a tertiary center in the Netherlands for such cases. This implies that a negative selection bias is most likely. More advanced cases might lead to worse survival.

As well as the superficial lymph node ratio, the number of positive superficial nodes was a consistent prognostic variable for OS and DFS. (Table 4) This is consistent with the outcome after analysis of stage III melanoma patients by the AJCC.[28-29] With an increasing number of positive superficial lymph nodes, the chance of involvement of the deep lymph nodes increased. No patients with 1 involved superficial node

showed additional positivity in the pelvic area, while this applied to 32% of patients with 2 – 3 involved superficial lymph nodes and to 66% of patients with ≥ 4 involved superficial lymph nodes. A decision on the extent of surgery might be made on the number of involved superficial lymph nodes. A scenario based on our results of preoperative CT scanning and the number of involved superficial lymph nodes could be considered. When preoperative CT is negative for involvement in the pelvic region and only one superficial lymph node is involved, SGD might be performed. When, after pathological analyses of the SGD specimen, more than one involved superficial lymph node is found, additional dissection of the pelvic region could be considered. CGD is performed in case of suspicion of multiple positive superficial lymph nodes and/or positive deep lymph nodes.

We acknowledge that this study is retrospective and has short follow-up time. We selected patients who underwent only therapeutic groin dissection for palpable disease and excluded patients who underwent elective lymph node dissection or sentinel node biopsy. The consequence was that our clean cohort of melanoma patients underwent surgery in a period of time (1991-2009) with evolving practice and imaging. Before bringing this scenario into clinical practice, results similar to those of the present study should be reported by other retrospective studies or a randomized controlled trial.

In conclusion, the poor outcome in melanoma patients with palpable nodal disease in the groin after CGD was equal after SGD in our series and in many other reports in the literature. Patients without overt iliac nodes on CT might safely undergo SGD and be spared the greater morbidity of CGD. CGD might be reserved for patients with multiple positive nodes in the SGD and/or positive nodes on CT scan. A prospective randomized controlled trial is the only study that can overcome the classical drawbacks of this and other retrospective studies.

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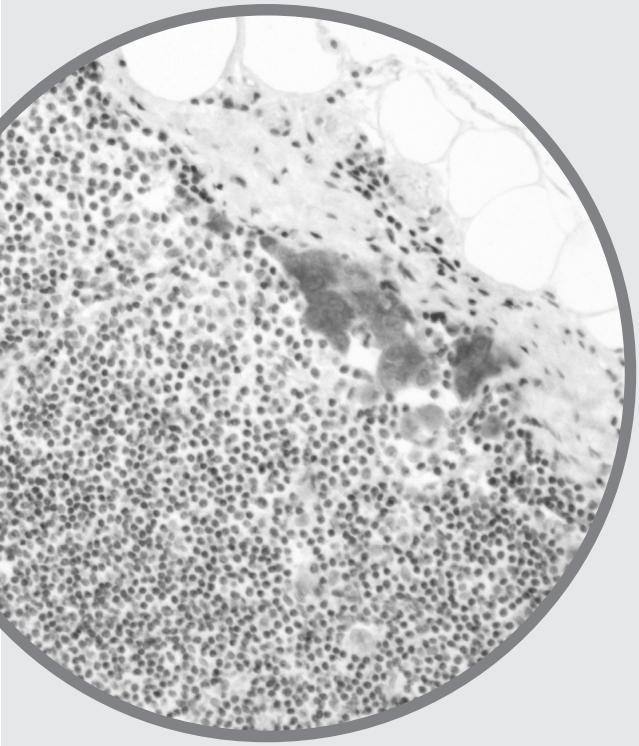
Chapter 10

Outcome after therapeutic lymph node dissection in patients with unknown primary melanoma site



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ABSTRACT

The aim of this study was to evaluate the incidence and outcome of melanoma of unknown primary site (MUP) after therapeutic lymph node dissection (TLND) of palpable nodal melanoma metastases. Disease-free (DFS) and overall survival (OS) time of MUP patients were analyzed and compared to patients undergoing a TLND for known primary melanomas (MKP).

This single institution retrospective study analyzed 342 consecutive patients who were treated with 415 TLNDs for palpable nodal disease from 1982 to 2009. Univariate and multivariate analyses included: MUP versus MKP, gender, Breslow thickness, ulceration of primary tumor, site of primary tumor, site of dissection, extracapsular extension (ECE), number of collected nodes, number of positive nodes and the node positive ratio.

A total of 47 MUP were identified in 342 patients (13.7%). In univariate analysis, a trend was seen towards better survival for MUP patients compared to MKP patients having 5-year OS rates of 40% and 27%, respectively (p=0.06). Multivariate analysis for OS showed two highly significant factors associated with worse prognosis: extracapsular extension and N3 status (both $p < 0.001$). Two factors were associated with a significant better prognosis: MUP ($p = 0.03$) and a neck dissection ($p = 0.04$).

Patients with MUP showed a significant better OS compared to patients with melanoma metastases from known primary tumors. Presence of extracapsular extension and an increased number of positive nodes are statistically significantly negative prognostic factors for OS. The absence of a primary melanoma in stage III melanoma patients does not preclude surgery.

INTRODUCTION

First presentation of palpable nodal disease in melanoma patients still occurs in spite of early recognition programs leading to a decrease in Breslow thickness.[1] Approximately 4% to 9% of all patients presenting with melanoma are diagnosed with palpable nodal disease, i.e. stage III disease.[2-3] Patients with clinically detected and histologically confirmed nodal melanoma metastases with no identification of a primary site are diagnosed as patients with melanoma of unknown primary site (MUP). In 8% to 20% of all therapeutic lymph node dissections (TLND) for regional metastatic melanoma, no primary tumor can be found.[4-5] Possible explanations for the absence of a primary tumor are spontaneous regression, unidentified primary melanoma, previous excision of what was considered a benign lesion or a malignant transformation of an ectopic nodal melanocyte.[4, 6] Whether patients with MUP have better or worse prognosis than patients with melanoma of known primary site (MKP) presented with nodal metastases is uncertain. Some studies suggest an improved survival for patients with MUP compared to MKP, whereas others report similar survival or even worse survival for MUP patients.[3-5, 7]

The aim of this study was to evaluate the incidence and outcome of MUP patients after TLND for palpable nodal disease compared to patients undergoing a TLND for palpable nodal disease with a known primary tumor. Disease-free (DFS) and overall survival (OS) were analyzed to identify prognostic factors for all patients who underwent TLND.

PATIENTS AND METHODS

Patients in this retrospective study were all treated with therapeutic lymph node dissection (TLND) for palpable nodal disease between 1982 and 2009 at the Erasmus University Medical Center - Daniel den Hoed Cancer Center in Rotterdam, the Netherlands. In 342 melanoma patients, a total of 415 TLNDs were performed. Patients who were treated with a lymph node dissection (LND) because of a positive sentinel node, patients who underwent LND with an isolated limb perfusion and patients who underwent elective LND were excluded.

There was no history of a primary melanoma and no primary tumor could be located in 47 of 342 patients (13.7%). The control group was formed by 295 patients (86.3%) with a known primary tumor. The diagnosis of unknown primary melanoma (MUP) consisted of histologically confirmed nodal metastatic melanoma and the absence of a primary tumor, confirmed after thorough examination of the skin and unusual primary sites such as urogenital, nasopahryngeal, or ocular.

All patient, primary and metastatic tumor characteristics were prospectively collected and sorted in a data base. Clinically detectable nodal disease and the absence of visceral metastases was radiographically confirmed by either ultrasonography of the lymph node fields and/or the liver, a chest X-ray, a cerebral MRI or a computed tomography scan of the thorax and abdomen.

Surgical technique

Four co-authors performed the majority of lymph node dissections assessed for this study (A.N.V.G., J.H.D.W., A.M.M.E. and C.V.) Ilio-inguinal dissections or deep groin dissections included dissection of the femoral-inguinal and external iliac nodes up to the common iliac artery (if necessary up to the aorta bifurcation) and dissection of the obturator nodes. Ilio-inguinal dissections were performed using one long vertical incision in the early stage of the study period. Two separate incisions were used in a later stage. Sartorius muscle transposition to cover and protect the femoral vessels was selectively performed when adjuvant radiotherapy was to be expected and/or patient's skin was at risk. An axillary lymphadenectomy comprised dissection for levels I-III. The modified radical neck consists of dissection of level I-V with preservation of the spinal accessory muscle, internal jugular vein and sternocleidomastoid muscle. Radical neck dissections were only performed if last mentioned structures were involved in the tumor process. In all patients vacuum drains were placed operatively and removed postoperatively if they produced less than 100 ml in 24 hours. Postoperatively the treatment protocol of all patients consisted of daily wound inspections. No pre-, peri- or post-operative antibiotic prophylaxis was routinely given. Ilio-inguinal dissection patients were ordered 3 days of bed rest postoperatively, after which they would be mobilized with the use of a support stocking. Patients received low molecule weight heparin during immobilization. The number and type of complications and duration of hospitalization were recorded.

Adjuvant therapy

In the later years of this study, several patients participated in the EORTC 18 951, 18 952 or 18 991 trials. The EORTC 18 951 trial found no clinically relevant activity for adding interleukine-2 (IL-2) to a chemo-immunotherapy combination of dacarbazine, cisplatin and interferon (IF)-α 2b.[8] The EORTC 18 952 trial evaluated the effects of adjuvant therapy with intermediate doses of IF-α 2b, and did not show a significant survival benefit for patients in the treatment group.[9] The EORTC 18 991 trial evaluated the role of long-term treatment with pegylated IF and found a significant sustained effect on recurrence-free survival.[10] Seven of 47 (14.9%) MUP patients and 46 of 295 (15.6%) of MKP patients participated in these trials.

Adjuvant radiotherapy was considered if narrow resection margins, excessive nodal involvement, i.e. more than three positive lymph nodes, extracapsular extension or simultaneous in-transit, subcutaneous or skin metastases in the operation area were present.

Statistical analysis

All descriptive and survival analyses were performed assessing the 342 patients. The Fisher exact test, chi-square test and Mann-Whitney U test were executed to determine the differences between MUP and MKP patients. Disease-Free Survival (DFS) was calculated from the first dissection date to the date of first recurrence. Overall Survival (OS) time was calculated from the dissection date to date of death. Patients without such an event at their last follow-up were censored at that time. Estimates were made according to the Kaplan Meier method and compared with the log rank score. The following factors were evaluated with a univariate Cox regression analysis: age, gender, MUP, location of the affected lymph node basin, the number of tumor positive lymph nodes, node-positive ratio (N-ratio; total affected lymph nodes / total harvested nodes), extracapsular extension (ECE) and adjuvant radiotherapy. The number of positive lymph nodes was defined by the AJCC 2009 classification, i.e. N1 (one positive lymph node), N2 (two or three positive lymph nodes) and N3 (more than three positive lymph nodes). Multivariate analysis using Cox's proportional hazards regression model was performed with all variables reaching a significance level of 10% in the univariate models. A stepwise backward algorithm was used at a level of 5% significance to exclude factors. All statistical analyses were performed using SPSS software (SPSS PASW 17.0.2).

RESULTS

A total of 342 patients were treated with therapeutic lymph node dissection (TLND). A melanoma of unknown primary (MUP) was diagnosed in 47 (13.7%) patients and 295 patients (86.3%) had a known primary tumor (MKP). Two or more dissections were performed in 59 patients making the total number of dissections 415. The following types of dissections were performed: inguinal (13%), iliacal (5%), ilio-inguinal (35%), axillary (20%) and neck (28%).

Characteristics

Patient, tumor and lymph node characteristics are summarized in Table 1. Age characteristics were very similar for MUP (median 56, interquartile range (IQR) 44 – 66 years) and MKP (median 56, IQR 46 – 68 years) patients (p=0.75). Gender was not

Table 1 – Baseline characteristics of patient, primary and metastatic melanoma for patients with a known primary site (MKP, n=295) and patients with an unknown primary site (MUP, n=47)

Characteristic	MKP		MUP		p-value*
	N	%	N	%	
Gender					
Male	149	50.5	27	57.4	
Female	146	49.5	20	42.6	0.38
Age					
Median (IQR)	56 (44 – 66)		56 (46 – 68)		0.75
Site of primary					
Head/neck	61	20.7	-	-	
Trunk	88	29.8	-	-	
Extremity	141	47.8	-	-	
Other	5	1.7	-	-	N/A
Breslow thickness					
T1	35	11.9	-	-	
T2	80	27.1	-	-	
T3	72	24.4	-	-	
T4	75	25.4	-	-	
Missing	33	11.2	-	-	N/A
Histology					
NM	89	30.2	-	-	
SSM	64	21.7	-	-	
Other	17	5.8	-	-	
Missing	125	42.4	-	-	N/A
Cark level					
II	13	4.4	-	-	
III	69	23.4	-	-	
IV	115	39.0	-	-	
V	34	11.5	-	-	
Missing	64	21.7	-	-	N/A
Ulceration					
Absent	217	73.6	-	-	
Present	78	26.4	-	-	N/A
Site of TLND					
Inguinal	39	13.2	5	10.6	
Iliacal	15	5.1	1	2.1	
Ilio-inguinal	103	34.9	17	36.2	
Axillary	58	19.7	10	21.3	
Neck	80	27.1	14	29.8	0.89

Table 1 (continued)

Characteristic	MKP		MUP		p-value*
	N	%	N	%	
Nr. of harvested nodes					
Median (IQR)	16 (11 – 26)		17 (12 – 28)		0.55
Nr. of positive nodes					
Median (IQR)	2 (1 – 4)		1 (1 – 7)		0.71
AJCC staging					
N1	124	42.0	24	51.1	
N2	86	29.2	6	12.8	
N3	70	23.7	14	29.8	
Missing	15	5.8	3	6.4	0.07
LN ratio (%)					0.38
Median (IQR)	11.6 (6.3 – 26.3)		8.2 (4.8 – 32.3)		
ECE					
no	205	69.5	29	61.7	
yes	90	31.5	18	38.3	0.31
Adjuvant radiotherapy					
no	243	82.4	33	70.2	
yes	52	17.6	14	29.8	0.07
Nr. of TLND performed					
1	295	81.7	47	87.0	
> 1	66	18.3	7	13.0	0.88

MKP = Melanoma of known primary site; MUP = Melanoma of unknown primary site; IQR = Interquartile Range
NM = Nodular melanoma; SSM = Superficial spreading melanoma; TLND = Therapeutic Lymph Node Dissection; LN = Lymph Node; ECE = extra capsular extension; N/A = Not applicable
**p-values are calculated using the Fisher exact test, Chi-square test or Mann-Whitney U test*

significantly different between the MKP group (50% male) and MUP group (57% male) (p=0.38). The site and the extent of the lymph node dissection performed was not significantly different between both groups (all p>0.05). Adjuvant radiotherapy was given in 17.6% of MKP patients and 29.8% of MUP patients (p=0.07). The mean and median follow-up for the entire population was 36 and 19 months respectively (IQR 9 – 43). The mean and median follow-up for MUP was 40 and 24 months (IQR 14 – 49) against 35 and 19 months for MKP (IQR 8 – 42), respectively.

Complications

Of all patients, 44.4% experienced at least one complication during follow-up. Most frequent complications were wound infection and/or skin necrosis (17.8%), seroma (16.9%) and chronic lymph edema (12.3%). For patients who underwent an inguinal

LND, 59.1% experienced at least one complication, while at least one complication was found in 37.5% of patients who underwent an iliacal LND, in 65.0% of patients who underwent an ilio-inguinal LND, in 26.5% of patients who underwent an axillary dissection and in 25.5% of patients who underwent a neck dissection. Chronic lymph edema was present in 11.4% of patients who underwent an inguinal LND, in 31.3% of patients who underwent an iliacal LND, in 24.2% of patients who underwent an ilio-inguinal LND and in 1.5% and 2.1% of patients who underwent an axillary and neck LND, respectively. MUP patients had at least one complication in 55.3% and chronic lymph edema was present in 12.5%. MKP patients had at least one complication in 55.6% and chronic lymph edema was present in 12.8%.

The median duration of hospitalization was 5 days (IQR 3 – 8 days) for both MUP and MKP patients (p=0.484).

Survival

Univariate analyses demonstrated that the following factors significantly impacted disease free survival (DFS): site of dissection, number of positive nodes, node positive ratio, ECE. (Table 2) Multivariate analyses for DFS showed three significant prog-

Table 2 – Univariate analyses of prognostic factors for disease free survival and overall survival

	DFS			OS		
Variable	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Gender						
Female	1			1		
Male	1.03	0.80 – 1.33	0.81	1.22	0.94 – 1.59	0.13
Age						
≤50	1			1		
>50	0.94	0.73 – 1.21	0.63	1.01	0.77 – 1.32	0.94
Site of primary						
extremity	1			1		
head/neck	0.77	0.53 – 1.11	0.16	0.81	0.55 – 1.18	0.26
trunk	1.34	0.99 – 1.82	0.06	1.30	0.95 – 1.78	0.11
other	0.54	0.22 – 2.20	0.54	0.49	0.11 – 1.97	0.31
unknown	0.73	0.63 – 1.38	0.73	0.70	0.45 – 1.08	0.10
MUP						
No	1			1		
Yes	0.92	0.64 – 1.31	0.63	0.68	0.45 – 1.03	0.07
Breslow						
T1	1			1		
T2	1.37	0.85 – 2.21	0.20	1.54	0.91 – 2.63	0.11
T3	1.78	1.10 – 2.89	0.02	1.80	1.05 – 3.07	0.03

Table 2 (continued)

	DFS			OS		
Variable	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
T4	1.18	0.72 – 1.94	0.51	1.62	0.95 – 2.76	0.08
MUP	1.21	0.72 – 2.06	0.47	1.08	0.59 – 1.97	0.81
Histology						
SSM	1			1		
NM	1.07	0.74 – 1.55	0.73	1.10	0.75 – 1.61	0.64
MUP	0.91	0.59 – 1.41	0.68	0.71	0.44 – 1.15	0.16
Other	0.70	0.36 – 1.39	0.31	0.94	0.49 – 1.82	0.85
Clark level						
II	1			1		
III	0.88	0.46 – 1.69	0.70	1.16	0.55 – 2.45	0.70
IV	0.96	0.51 – 1.80	0.90	1.11	0.54 – 2.29	0.78
V	0.85	0.41 – 1.73	0.64	1.12	0.51 – 2.51	0.77
MUP	0.83	0.42 – 1.64	0.60	0.79	0.36 – 1.74	0.55
Ulceration						
Absent	1			1		
Present	1.21	0.90 – 1.63	0.2	1.48	1.10 – 1.99	0.01
Site of TLND						
inguinal	1			1		
iliacal	0.97	0.47 – 1.98	0.93	0.96	0.49 – 1.87	0.90
ilio-inguinal	0.76	0.50 – 1.14	0.18	0.71	0.47 – 1.07	0.10
axillary	1.14	0.73 – 1.76	0.57	1.06	0.68 – 1.67	0.78
neck	0.63	0.41 – 0.97	0.04	0.61	0.39 – 0.94	0.03
Nr of harvested nodes	1.00	0.99 – 1.01	0.54	1.00	0.99 – 1.01	0.65
AJCC staging						
N1	1			1		
N2	1.34	0.98 – 2.50	0.07	1.33	0.96 – 1.84	0.09
N3	1.90	1.39 – 2.58	<0.001	1.97	1.43 – 2.72	<0.001
LN ratio	2.35	1.51 – 3.64	<0.001	2.83	1.80 – 4.45	<0.001
ECE						
Absent	1			1		
Present	1.60	1.23 – 2.08	<0.001	1.83	1.39 – 2.40	<0.001
Adjuvant radiotherapy						
No	1			1		
Yes	1.13	0.83 – 1.53	0.45	1.18	0.85 – 1.63	0.33

DFS = Disease free survival; OS = Overall survival; HR = hazard ratio; MKP = melanoma of known primary site; MUP = melanoma of unknown primary site; SSM = superficial spreading melanoma; NM = nodular melanoma; TLND = Therapeutic Lymph Node Dissection; LN = Lymph node; ECE = Extra capsular extension

Table 3 – Multivariate analyses of prognostic factors in all patients (n=342) for disease free survival and overall survival

Disease Free Survival					
Variable		**N**	**HR**	**95% CI**	**P**
AJCC staging	N1	148	1		
	N2	92	1.40	1.02 – 1.93	0.04
	N3	84	2.08	1.47 – 2.94	<0.001
ECE	No	234	1		
	Yes	108	1.57	1.16 – 2.12	0.004
Overall Survival					
Variable		**N**	**HR**	**95% CI**	**P**
ECE	No	285	1		
	Yes	130	1.69	1.25 – 2.28	0.001
MUP	No	47	1		
	Yes	295	0.62	0.40 – 0.96	0.03
AJCC staging	N1	148	1		
	N2	92	1.28	0.92 – 1.79	0.15
	N3	84	1.80	1.28 – 2.52	0.001
Dissection type	Inguinal	44	1		
	Axillary	68	0.74	0.47 – 1.16	0.19
	Neck	94	0.61	0.38 – 0.97	0.04

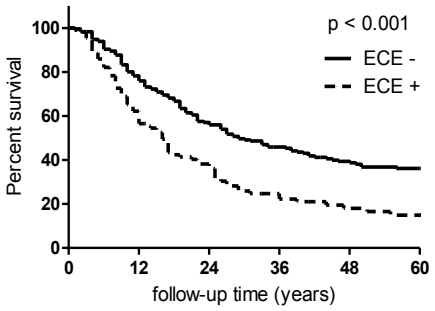
AJCC = American Joint Committee of Cancer; ECE = extracapsular extension; HR = Hazard ratio; CI = Confidence Interval; MUP = Melanoma of Unknown Primary

nostic factors: N2 (p = 0.04), N3 (p <0.001) and ECE (p = 0.004). (Table 3) Gender, age, the number of harvested lymph nodes, primary site, Clark level, histology of the primary, MUP and adjuvant radiotherapy were not significant. Hazard ratios and p-values of all analyzed factors are summarized in table 2 and 3.

As for overall survival (OS), ulceration, site of dissection, node positive ratio, number of positive nodes, ECE and MUP were significant prognostic factors. (Table 2) Ulceration was not known for MUP patients and not included in multivariate analyses. Multivariate analysis for OS revealed two highly significant factors associated with worse prognosis: the presence of extracapsular extension (ECE) and N3 status (both p < 0.001). Two factors were associated with significant better prognosis: MUP (p = 0.03) and neck dissections (p = 0.04). (Table 3)

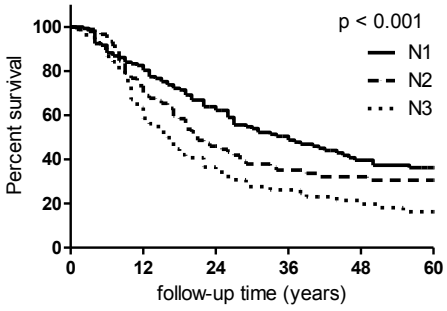
The estimated 5-year DFS rates for extracapsular extension (ECE) were an estimated 12% when present and 26% when absent (p <0.001). For nodal status, 5-year DFS rates were 31%, 17% and 9% for the respective N1, N2 and N3 categories (p < 0.001). The five-year DFS rate for MKP patients was 21%, while the five-year DFS rate for MUP patients was 25% (p=0.619).

Figure 1A: Overall Survival – Extracapsular extension



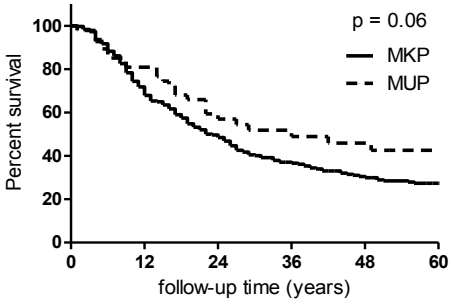
	Nr at Risk					
ECE –	234	172	111	82	62	54
ECE +	108	65	36	20	12	7
Total	342	237	147	102	74	61

Figure 1B: Overall survival – number of positive lymph nodes according to AJCC system



	Nr at Risk					
N1	148	113	79	56	36	29
N2	92	65	35	25	21	20
N3	84	52	26	17	12	8
Total	324	230	140	98	69	57

Figure 1C: Overall Survival – Melanoma with unknown and known primary site



	Nr at risk					
MUP	47	38	25	18	14	11
MKP	295	199	122	48	60	50
Total	342	237	147	102	74	61

Absence of ECE demonstrated a 5-year OS of 36% compared to 15% when present ($p < 0.001$). (Figure 1A) The estimated 5-year OS rates for the different categories of nodal status (N1, N2 and N3) were 36%, 30% and 16% respectively ($p < 0.001$). (Figure 1B) The 5-year estimated Kaplan-Meier OS rate for MUP vs. MKP showed a trend towards a better survival for MUP at 43% vs. 27% for MKP, respectively ($p = 0.06$). (Figure 1C)

DISCUSSION

In this retrospective study, 342 melanoma patients treated with a total of 415 therapeutic lymph node dissections (TLND) for palpable lymph nodes metastases were analyzed. Outcome of melanoma patients with an unknown primary site (MUP) were compared with patients with a known site of the primary melanoma lesion (MKP). After multivariate analysis, a significant overall survival (OS) benefit was found for patients with MUP over MKP patients. Five-year OS rates were 43% for MUP patients and 27% for MKP patients ($p = 0.03$).

Cormier et al. demonstrated a significant survival benefit for 71 MUP patients in a multivariate Cox proportional hazard model when adjusted for nodal status, dissection site, age, gender and adjuvant therapy as well ($p = 0.006$). The 5-year OS rates were 55% and 42% for MUP vs. MKP respectively, with a median follow-up of 92 months.[5] Lee et al. demonstrated similar results with 5-year OS rates of 55% for MUP patients (N = 262) and 44% for MKP patients (N = 1309), with a median follow up of 36 months. Again MUP was identified as a significant prognostic factor in multivariate analysis ($p = 0.0001$).[4] Where previous mentioned studies revealed a small increased 5-year OS rate compared to our results, Chang et al.[3] reported similar 5-year OS rates for both groups; 46% for MUP and 49% for MKP.

All the above-mentioned studies demonstrated higher 5-year OS rates for MKP (42% - 49%) compared to our results (27%). No significant differences were found in patient and tumor characteristics between all studies.[3-5] A reason for the worse survival of MKP patients in the present study might be the short median follow-up. Moreover, a tertiary referral center might perform surgery in patients with more advanced cases, which might lead to worse survival.

A possible hypothesis for the survival benefit seen in MUP patients is an endogenous immune response,[11] which also might have caused regression of the primary lesion. Interleukin-2 (IL-2) and interferon-α (IFN-α), have shown some therapeutic benefit, supposedly by enhancing antitumor immune responses.[10, 12] In a small study Moschos et al. treated twenty stage IIIB and IIIC patients with neoadjuvant high-dose interferon-alfa-2b. Three were diagnosed with MUP and demonstrated no evidence of disease after 7, 9 and 10 months respectively.[13] Furthermore, cytoreductive surgery (complete metastatectomy) revealed a long-term clinical benefit that depended on the host's immune response to a surgical reduction in tumor burden.[14] Causes for the effectiveness of these therapies in MUP patients might be the favorable patients' immune system. Unfortunately, no specific data is available to prove a difference between survival of MUP and MKP patients receiving any form of immunotherapy.

The rate of MUP in patients treated with TLND for palpable nodal disease was 13.7% in our institute (47/342). This is in line with other studies. In the study per-

formed by Rutkowski et al., the rate was 12.8%.[15] Lee et al. demonstrated a MUP rate of 16.7%, while the study performed by Cormier et al. had a MUP rate of 13.2%.[4-5]

Lymph node status and ECE are important significant prognostic factors for OS, which has been demonstrated in several studies.[4, 15-18] A previous study from our group demonstrated that ECE was the most important prognostic factor for OS after TLND.[16] A recent MUP study by Rutkowski et al also revealed ECE and lymph node status as significant factors for OS.[15] Balch et al. found several significant prognostic factors for stage III melanoma such as nodal micrometastases, number of tumor-containing lymph nodes, Breslow thickness, patient age, ulceration, site of the primary and primary mitotic rate. Unfortunately MUP and ECE were not analyzed in their study.[17] Disease of patients with metastatic melanoma from an unknown primary site, arising in lymph nodes, skin, or subcutaneous tissues, was clarified to be categorized as stage III rather than stage IV.[19]

The fact that patients treated with neck dissections had a survival benefit versus inguinal dissections is counter-intuitive, since head/neck melanomas are associated with worse prognosis.[2, 17, 20] It may be a chance finding as the minimal significant difference ($p = 0.04$) could be explained by the small sample size, the difference in age (median 54 vs. 62 years) and percentage of ECE (24.4% vs. 37.1%).

Comparing MUP patients with MKP patients stratified by Breslow thickness as T1, T2, T3 and T4 showed increased hazard ratio's for T2 (HR = 1.54), T3 (HR = 1.80) and T4 (HR = 1.62) versus a T1 tumor. MUP patients showed nearly the same hazard ratio (HR = 1.08) as patients with T1 tumors at univariate analysis. Increasing Clark level was of little significance for OS, as did the MUP patients with unknown Clark scores. Patients with ulcerated primaries had significant worse OS ($p = 0.01$) compared to the unknown group. Also MUP patients have a survival benefit compared to patients with a primary tumor located on an extremity or trunk. It was previously suggested that MUP patients had a worse prognosis compared to patients with known primary tumors.[3] These data suggest at least that MUP is not a significant negative prognostic factor compared to some stage III melanoma patients with known primary tumor characteristics.

A recent study in the Netherlands by Koomen et al. demonstrated that the incidence of non-cutaneous melanoma is very rare.[21] Therefore the value of nasopharyngeal examination is doubtful and could be ignored when a patient presents with a palpable lymph node without a known primary tumor. A thorough physical examination in order to locate a primary tumor is still recommended. Also close examination of the skin of the drainage area of the metastatic lymph node could be considered in order to indentify a regressive primary lesion.[22]

In conclusion, this study showed the presence of ECE, an increased number of positive nodes and patients with MKP as significantly negative prognostic factors

for OS. Patients with melanoma of an unknown primary site showed a significantly better OS after multivariate analysis compared to patients with melanoma metastases from known primary tumors. Melanoma patients with palpable nodal disease and an unknown primary melanoma should be classified as stage III disease. The absence of a primary melanoma in stage III melanoma patients does not preclude surgery.

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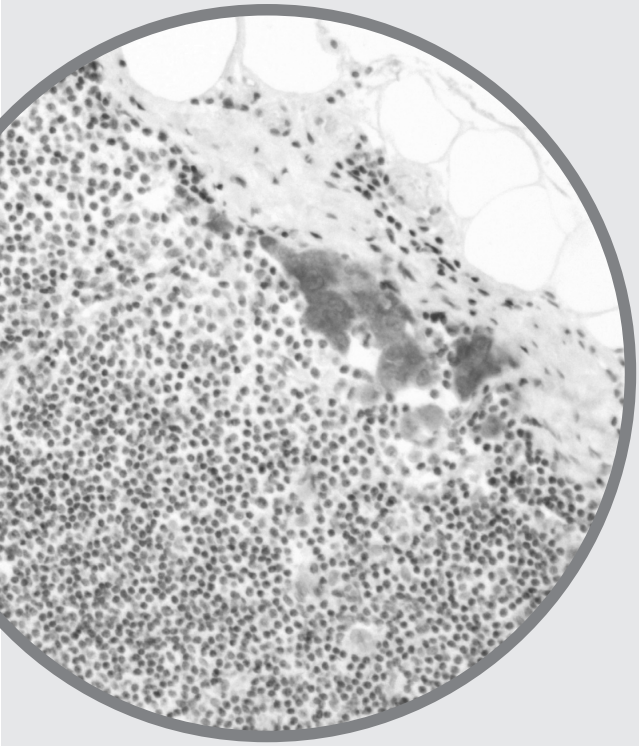
Chapter 11

Melanoma patients with an unknown primary tumor site have a better outcome than those with a known primary following therapeutic lymph node dissection for macroscopic (clinically palpable) nodal disease



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ABSTRACT

Several reports in the literature suggest a difference in outcome between melanoma patients with an unknown primary (MUP) and a known primary (MKP) with macroscopic (clinically palpable) nodal disease. The purpose of this study was to compare the outcomes for MUP and MKP patients following therapeutic lymph node dissection (TLND) for macroscopic nodal disease.

From a large, prospective, single institution database the details of melanoma patients who first presented with macroscopic nodal disease and underwent TLND between 1971 and 2010 were extracted and analyzed.

There were 287 MUP patients and 264 MKP patients who fulfilled the study selection criteria. MUP patients had better disease-free, distant metastasis-free and melanoma-specific survival after their TLND than MKP patients (all $p<0.001$). Extranodal melanoma extension, >3 positive lymph nodes and administration of adjuvant radiotherapy were all independent predictors of reduced disease-free and melanoma-specific survival (all $p<0.05$). MUP patients also had a better prognosis than MKP patients whose primary melanoma had regression ($p=0.001$).

The occurrence and improved outcome of MUP patients may be due to immune-induced total regression of the primary tumor and better immunological prevention or control of distant metastatic disease. Alternatively, in some MUP patients, melanoma may not be metastatic but may originate de novo from naevus cells in lymph nodes, with the more favorable prognosis attributable to their primary nodal origin and complete surgical resection.

INTRODUCTION

In most patients with metastatic melanoma there is a known primary tumor site.[1] However, 2-5% of all melanoma patients with metastatic disease have an unknown primary site (MUP)[2-9]. MUP has been defined as histologically confirmed subcutaneous, nodal or visceral metastatic melanoma with no evidence or history of a cutaneous or non-cutaneous primary melanoma. Explanations for the existence of MUP include an unidentified primary melanoma, spontaneous regression of a primary melanoma after it has metastasized, or melanoma arising de novo from ectopic melanocytes located within lymph nodes (such as intranodal naevus cells).[2,10-12]

The most common site of first recurrence in melanoma patients with a known primary (MKP) is in the regional lymph node (LN) field.[1,13] Of melanoma patients presenting with macroscopic nodal disease, 13-17% have no evidence of a synchronous primary tumour after thorough examination, and no history of a previous primary melanoma.[1,14-17]

Several reports in the literature suggest a difference in outcome between MUP and MKP patients. Dasgupta and colleagues published the first report on MUP in 1963. In their study survival was similar in MUP patients and MKP patients.[5] Since then, several further reports have also suggested an equivalent outcome for MUP and MKP patients.[4,6-8,12,17,18] However, others have reported that MUP patients have a worse outcome.[4,19] Yet other studies suggest that the prognosis of MUP patients is better than that of MKP patients.[2,14-16]

Two previously reported retrospective studies from our institution demonstrated better survival for MUP patients with Stage IV disease than for MKP patients with equivalent disease.[23,24] The aim of the current study was to compare the outcome for MUP patients undergoing a therapeutic lymph node dissection (TLND) for macroscopic nodal disease (AJCC stage IIIB or IIIC) with that of MKP patients diagnosed and treated similarly. Another objective was to assess the prognostic significance of primary tumor regression in the MKP cohort.

METHODS

For this retrospective, single institution study, data were extracted from the prospectively collected Melanoma Institute Australia (MIA) database, and patients who had undergone a TLND for macroscopic (clinically palpable) nodal disease at MIA between 1971 and 2010 were identified in the following manner: In the study period 26,825 patients with a single known primary melanoma were treated at MIA, and 1162 who had metastatic disease from an unknown primary site. Only patients within

these two groups who had stage III disease at the time of their initial presentation were selected for the study (n=1969). Patients who were classified as having stage III disease on the basis of a positive sentinel node biopsy were then excluded (n=758), as well as patients who underwent an elective lymph node dissection and were found to have one or more positive nodes (n=204). Finally, patients were excluded if they had local recurrence or in transit metastasis simultaneously with macroscopic nodal disease (n = 456). The data retrieved for each patient included patient demographics, primary tumor characteristics, nodal metastatic tumor details, and surgery, follow-up and survival information.

Patients

After excluding patients according to the criteria described above, 551 melanoma patients who underwent TLND at MIA after presenting with macroscopic disease in regional lymph nodes between 1971 and 2010 were identified. Eighty-nine patients (16%) underwent TLND before 1990 and the remainder after this time. A single primary melanoma had been diagnosed in 264 patients (47.8%), while 287 patients (52.5%) underwent TLND for nodal melanoma from an unknown primary site (MUP). Patients were diagnosed with MUP when the palpable nodal melanoma was histologically confirmed and there was no history of a synchronous or metachronous primary tumor, and after thorough examination of the skin and potential non-cutaneous primary melanoma sites.

Diagnosis and surgery

All MKP patients underwent wide local excision and regional LN dissection at MIA at the time of presentation with clinical lymph node involvement (n=264). The diagnosis of metastatic melanoma in regional lymph nodes was usually confirmed preoperatively by fine needle biopsy[25,26], and the absence of distant disease was demonstrated radiographically using a variety of imaging techniques including ultrasonography of lymph node fields and/or the liver, chest X-ray, computed tomography, positron emission tomography, and/or magnetic resonance imaging.

Six co-authors performed the majority of the lymph node dissections included in this study (A.J.S., M.J.Q., R.P.M.S., K.F.S., J.R.S. and J.F.T.). The quality of their TLND surgery, as reflected by lymph node retrieval parameters, has been documented in a prior publication.[27] MIA-affiliated pathologists at the Royal Prince Alfred Hospital, Sydney undertook the histopathological assessment of all surgical specimens.

Statistics

Statistical analyses were performed with SPSS 17.0 software (SPSS PASW Inc. Chicago, IL). Variables were coded for statistical analysis as mentioned in table 1.

Table 1 Patient, tumor and lymph node dissection data for MUP and MKP patients

	MUP patients n (%) 287 (52.2)	**MKP patients** n (%) 264 (47.8)	p*
Gender			
Female	83 (28.9)	79 (29.9)	
Male	204 (71.1)	185 (70.1)	0.852
Age (yr)			
Mean±SD	53.6±0.97	57.3±1.02	0.008
≤ 56	153 (53.3)	117 (44.3)	
> 56	134 (46.7)	147 (55.7)	0.041
Primary site			
Extremity	-	111 (42.9)	
Trunk	-	82 (31.7)	
Head & Neck	-	66 (25.5)	
Missing	-	5	-
Thickness (mm)			
Median (IQR)	-	3.95 (2.22-6.08)	-
≤ 1.00 / T1	-	20 (8.1)	
> 1.00 – ≤ 2.00 / T2	-	32 (12.9)	
> 2.00 – ≤ 4.00 / T3	-	81 (32.7)	
> 4.00 / T4	-	115 (46.4)	-
Missing	-	16	
Tumour mitotic rate (/mm^2)			
Median (IQR)	-	6 (3-13)	-
Clark level			
II	-	12 (5.3)	
III	-	37 (16.3)	
IV	-	125 (55.1)	
V	-	53 (23.3)	-
Missing	-	37	
Histologic subtype			
SSM	-	37 (18.9)	
NM	-	117 (59.7)	
Other	-	42 (21.4)	-
Missing	-	68	
Ulceration			
Absent	-	92 (41.3)	
Present	-	131 (58.7)	-
Missing		41	

Table 1 (continued)

	MUP patients n (%) 287 (52.2)	**MKP patients** n (%) 264 (47.8)	p*
Regression			
Absent	-	79 (48.2)	
Present	-	85 (52.2)	-
Missing	-	100	
Site of LND			
Neck	74 (25.9)	71 (27.1)	
Axilla	133 (46.5)	96 (36.6)	
Groin	70 (24.5)	81 (30.9)	
Other	9 (3.1)	14 (5.3)	0.075
Missing	1	2	
Nr. of harvested nodes			
Median (IQR)	21 (12-31)	18 (12-28)	0.210
Nr. of positive nodes			
Median (IQR)	1 (1-3)	2 (1-3)	0.047
1	159 (57.0)	127 (48.8)	
2-3	69 (24.7)	76 (29.2)	
>3	51 (18.3)	57 (21.9)	0.166
Missing	8	4	
Lymph node ratio (%)			
Median (IQR)	8 (5-19)	11 (5-26)	0.021
≤ 10	157 (57.7)	125 (49.6)	
10 - ≤ 25	67 (24.6)	63 (25.0)	
> 25	48 (17.6)	64 (25.4)	0.071
Missing	15	12	
Extracapsular extension			
Absent	221 (77.0)	199 (75.4)	
Present	66 (23.0)	65 (24.6)	0.689
Adjuvant Radiotherapy			
No	208 (72.5)	195 (73.9)	
Yes	79 (27.5)	69 (26.1)	0.773

MUP = Melanoma with Unknown Primary; MKP = Melanoma with Known Primary; SSM = Superficial Spreading Melanoma; NM = Nodular Melanoma; IQR = Inter Quartile Range

* P-values were calculated using the Fisher exact test, Chi-square test or Mann-Whitney U test.

Fisher's exact, Chi-square and Mann-Whitney U tests were used to calculate p-values for differences between MUP and MKP patients. For univariate survival analysis, Kaplan-Meier curves and log rank tests were assessed. A Cox proportional hazards model using backward stepwise selection was performed for multivariate analyses.

Disease-free survival (DFS) was measured from the date of LN dissection to the date of any first recurrence. Distant metastasis-free survival (DMFS) was measured from the date of LN dissection to the date of first distant recurrence. Melanoma-specific survival (MSS) was measured from the date of LN dissection to the date of death from melanoma. Patients without such an event were censored at the time of last follow-up.

RESULTS

Of the 551 patients who presented with macroscopic nodal disease, 26% had a neck dissection, 42% had an axillary dissection, 28% had a groin dissection and 4% had a dissection of another LN field (e.g. epitrochlear or popliteal). Mean and median follow-up times for MUP patients were 5.1 and 3.3 (interquartile range (IQR) 1.1 – 7.6) years, respectively. Mean and median follow-up times for MKP patients were 3.8 and 1.4 (IQR 0.7 – 4.6) years, respectively. There was no change in the incidence of the diagnosis of MUP over the study period.

Differences between patient populations

Significant differences were present between MUP and MKP patients (Table 1). The mean ages were 53.6 (standard deviation (SD) ±0.97) years for MUP patients and 57.3 (SD ±1.02) years for MKP patients (p=0.008). The median number of positive nodes was significantly higher in MKP patients (p=0.047) as well as the positive LN ratio (p=0.021). The administration of adjuvant radiotherapy was not significantly different between the two groups, with 27.5% of MUP patients and 26.1% of MKP patients receiving radiotherapy postoperatively (p=0.773).

Survival analyses

In univariate analyses for all patients, the following were significant prognostic factors for reduced DFS: known primary melanoma, greater number of positive LNs, higher positive LN ratio, extracapsular tumor extension (ECE) and administration of adjuvant post-operative radiotherapy (all $p<0.05$) (Table 2). After multivariate analyses for DFS, significant adverse prognostic factors for survival were: known primary melanoma ($p<0.001$), ECE (p=0.010), administration of adjuvant radiotherapy ($p<0.001$) and the presence of >3 positive nodes ($p<0.001$) (Table 3). MKP was a significant adverse prognostic factor for DMFS in univariate analysis ($p<0.001$).

In univariate analyses significant adverse prognostic factors for MSS were: MKP, older age, increased number of positive LNs, increased positive LN ratio, ECE, and administration of adjuvant radiotherapy (Table 2). After multivariate analyses, the

Table 2 – Univariate analysis of prognostic factors in all patients

Variable	Disease-Free Survival			Melanoma-Specific Survival		
	HR	95% CI	P*	HR	95% CI	P*
Known primary						
Absent	Ref			Ref		
Present	1.88	1.46 – 2.42	< 0.001*	2.25	1.70 – 2.98	< 0.001*
Gender						
Female	Ref			Ref		
Male	0.90	0.68 – 1.18	NS	1.06	0.78 – 1.43	NS
Age (yr)						
Mean±SD	1.00	1.00 – 1.01	NS	1.01	1.01 – 1.02	0.002
Site of LND						
Neck	Ref			Ref		
Axilla	0.77	0.56 – 1.05	NS	1.01	0.71 – 1.44	NS
Groin	1.06	0.77 – 1.47	NS	1.06	0.73 – 1.54	NS
Nr. of harvested nodes						
Median (IQR)	1.00	0.99 – 1.01	NS	1.01	1.00 – 1.02	NS
Nr. of positive nodes						
Median (IQR)	1.05	1.03 – 1.07	< 0.001	1.06	1.03 – 1.08	< 0.001
1	Ref			Ref		
2-3	1.34	0.99 – 1.82	NS	1.43	1.02 – 1.99	0.036
> 3	2.40	1.76 – 3.26	< 0.001	2.39	1.71 – 3.35	< 0.001
Lymph node ratio (%)						
Median (IQR)	1.93	1.17 – 3.19	0.010	2.02	1.19 – 3.43	0.009
≤ 10	Ref			Ref		
10 - ≤ 25	1.52	1.12 – 2.06	0.007	1.30	0.93 – 1.84	NS
> 25	1.88	1.37 – 2.60	< 0.001	2.10	1.50 – 2.95	< 0.001
Extracapsular extension						
Absent	Ref			Ref		
Present	1.44	1.07 – 1.92	0.015	1.93	1.42 – 2.63	< 0.001
Adjuvant Radiotherapy						
No	Ref			Ref		
Yes	2.10	1.60 – 2.76	< 0.001	2.28	1.72 – 3.02	< 0.001

HR = Hazard Ratio, CI = Confidence Interval, IQR = Interquartile range, NS = Non significant, Ref = Reference

**P-values are calculated using the log rank test*

following factors remained significant: MKP (p<0.001), older age (p=0.023), ECE (p<0.001), administration of adjuvant radiotherapy (p<0.001) and > 3 positive nodes (p<0.001) (Table 3).

For DFS and MSS, positive LN ratio was not assessed in the multivariate model due to correlation with the number of positive nodes. When positive LN ratio was

Table 3 – Multivariate Cox's hazard regression analysis of prognostic factors in all patients

Variable	Disease-Free Survival			Melanoma-Specific Survival		
	HR	95% CI	P*	HR	95% CI	P*
Known primary						
Absent	Ref			Ref		
Present	1.91	1.48 – 2.47	< 0.001*	2.35	1.77 – 3.13	< 0.001*
Age (per yr)			NS**	1.01	1.00 – 1.02	0.023
Nr. of positive nodes						
1	Ref			Ref		
2-3	1.22	0.90 – 1.66	NS	1.26	0.90 – 1.76	NS
> 3	1.84	1.34 – 2.54	< 0.001	1.90	1.34 – 2.71	< 0.001
Extracapsular extension						
Absent	Ref			Ref		
Present	1.44	1.07 – 1.92	0.015	1.56	1.13 – 2.15	0.007
Adjuvant Radiotherapy						
No	Ref			Ref		
Yes	2.10	1.60 – 2.76	< 0.001	1.89	1.40 – 2.55	< 0.001

HR = Hazard Ratio, CI = Confidence Interval, NS = not significant, Ref = Reference
**P-values are calculated using the log rank test*
***Not significant in univariate analysis*

included in the multivariate model (in place of the number of positive nodes) it was not a significant predictor of DFS (p=0.920) or OS (p=0.753).

Five-year cumulative DFS in the absence and presence of ECE was 51.4% and 36.2% respectively (p=0.015), while 5-year cumulative MSS in the absence and presence of ECE were 62.4% and 37.3% respectively (p<0.001) (Figure 1A). Five-year MSS according to the number of positive nodes were 64.8% for 1 positive node, 54.9% for 2 or 3 positive nodes and 38.7% for > 3 positive nodes (p<0.001) (Figure 1B).

Figure 1 – Melanoma-specific survival of melanoma patients for A) extracapsular extension and B) the number of positive nodes

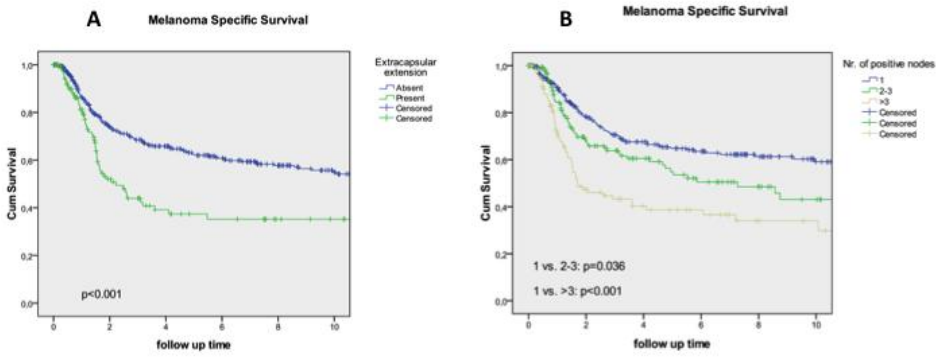
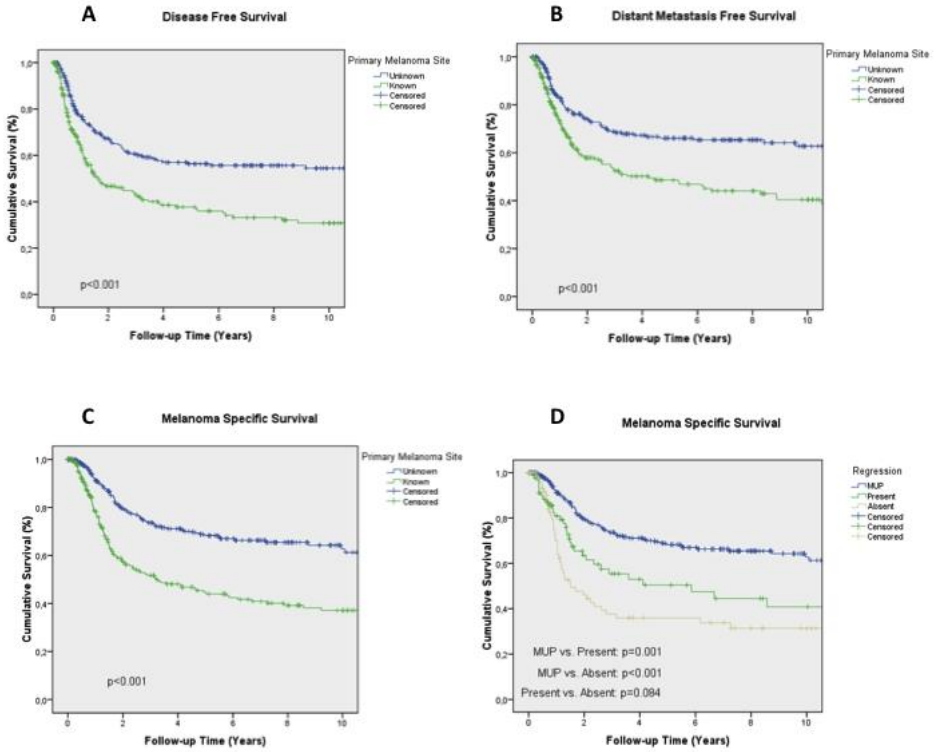


Figure 2 – A) Disease-free survival, B) Distant metastasis-free survival and C) Melanoma-specific survival for unknown versus known primary melanoma, and D) Melanoma-specific survival for unknown primary versus known primary with regression versus known primary without regression



The 5-year cumulative DFS for MUP patients was 56.3% and for MKP patients it was 37.7% (p<0.001) (Figure 2A). The 5-year cumulative DMFS was 66.1% and 48.6% for MUP and MKP patients, respectively (p<0.001) (Figure 2B). For MSS, 5-year cumulative survival was 68.3% for MUP patients and 44.7% for MKP patients (p<0.001) (Figure 2C). When MKP patients were stratified for regression of the primary melanoma, the 5-year MSS was 50.4% for patients with regression and 35.9% for patients with no regression of the primary (p=0.083) (Figure 2D).

DISCUSSION

This retrospective, single institution study reports what is, to the best of our knowledge, the largest series of MUP patients who have undergone TLND for macroscopic nodal disease. It demonstrated that after TLND, patients with a MUP have better DFS, DMFS and MSS than patients with a MKP (Table 3, Figure 2A-C). The presence of

ECE, >3 positive LNs and the administration of adjuvant radiotherapy were all independent prognostic factors for reduced DFS and MSS (Table 3, Figure 1A-B). MUP patients also had a better prognosis than MKP patients with a primary melanoma that showed regression. MKP patients with a primary melanoma associated with regression had a clear trend towards better MSS than MKP patients with a primary without regression but this was not statistically significant (Figure 2D).

There were some differences between the MUP and the MKP patients. MKP patients were significantly older (p=0.008) and had a higher positive LN ratio (p=0.021) and a greater number of involved nodes (p=0.047). A higher positive LN ratio was correlated with worse survival (Table 2).[28,29] Even when these differences were accounted for in multivariate survival analysis, MUP patients continued to have a significantly better outcome (Table 3).

Administration of adjuvant radiotherapy was a significant independent prognostic factor for adverse DFS and MSS (Table 2-3). This is counter-intuitive, but probably reflects selection bias. Adjuvant radiotherapy was administered to 42.7% of patients with ECE compared to 21.9% of patients with no ECE (p<0.001). Thus patients with more advanced disease were selected for adjuvant radiotherapy, which is the most likely explanation of their worse outcome.

The significantly better overall outcome after TLND for stage III MUP patients compared to MKP patients in this study is in line with some other studies reported in recent years.[14-16] In Table 4, results of the largest and most recent studies addressing the outcome for MUP patients are presented. All studies demonstrated higher survival rates for MUP patients compared to MKP patients, although this was not always statistically significant. The study performed by Lee et al., containing the largest number of MUP patients reported prior to the present study, produced results that were similar to ours, with MUP patients having a better outcome than MKP patients.[15]

Several theories have been proposed to explain the occurrence of MUP.[2,14,15,30,31] Firstly, it is possible that a primary melanoma could exist but remain unidentified. Whenever a MUP is suspected, a thorough physical examination is recommended. However, a detailed search for a non-cutaneous primary melanoma, e.g. involving colonoscopy, ophthalmic and nasopharyngeal examination, is not routinely performed at our institution since the incidence of non-cutaneous melanomas is extremely low.[32,33] A second possibility is that an excised melanoma could have been misdiagnosed as a benign naevus. These two possibilities can be excluded by performing thorough and careful physical and pathological examinations and reviewing the histopathology of any previously biopsied pigmented lesions.

A third possible explanation is that a melanoma may arise de novo within a LN from ectopic nodal naevus cells. In a report by Shenoy et al, a single axillary LN containing melanoma and naevus cells was described, with an apparent transition

Table 4 – Published series reporting the outcome after lymph node dissection for stage III melanoma patients with an unknown primary and a known primary

Reference (year)	Groups	Nr. of patients	5-year DFS	p††	5-year OS	p††
Present study* (2011)	MUP	287	56%		68%	
	MKP	264	38%	<0.001	45%	<0.001
Prens[16] (2011)	MUP	47	25%		43%	
	MKP	292	21%	0.619	27%	0.06**
Rutkowski[17] (2010)	MUP	59	44%		41%	
	MKP	400	28%	0.09	36%	0.45
	MUP†	59	44%		41%	
	MKP†	57	29%	0.29	39%	0.14
Lee[15] (2008)	MUP	262	NR		55%	
	MKP	1309	NR	N/A	44%	0.0021**
	MUP†	221	47%		58%	
	MKP†	221	35%	0.066	40%	0.0006
Cormier[14] (2006)	MUP	71	43%		55%	
	MKP	466	30%	0.14**	42%	0.04**
Schlagenhauff[6] (1997)	MUP	37	NR		50%	
	MKP	NR	NR	N/A	36%	0.14
Anbari[9] (1997)	MUP	20	NR		57%‡ 19%‡	
	MKP	46	NR	N/A		0.008
Norman[18] (1992)	MUP	18	NR		NR	
	MKP	38	NR	0.15	NR	0.96
Wong[4] (1987)	MUP	188	NR		46%	
	MKP	387	NR	N/A	37%	0.10

DFS = Disease Free Survival; OS = Overall Survival; MUP = Melanoma with Unknown Primary; MKP = Melanoma with Known Primary; NR = Not Reported; N/A = Not Applicable
** Melanoma Specific Survival has been reported*
† Matched paired analysis
†† log rank tests were used to calculate p-values
‡ 4-year overall survival rates have been reported
***After multivariate analysis, Prens et al. reached p=0.03 (hazard ratio 0.62 for having MUP in OS), Lee et al. reached p=0.0001 (hazard ratio of 1.51 for having MKP in OS) and Cormier et al. demonstrated p=0.006 (hazard ratio of 0.61 for having MUP in OS) and p=0.057 (hazard ratio of 0.73 for having MUP in DFS).*

from benign naevus cells to dysplastic naevus cells to melanoma cells. It was suggested that this provided evidence that the melanoma had arisen primarily in the LN.[30] Our finding of fewer tumor-positive LNs in MUP patients gives some support to this hypothesis, i.e., a primary nodal melanoma may have a lesser ability to spread to other LNs because it is at an earlier phase in its acquisition of a metastatic phenotype

at the time it is detected clinically than is a nodal metastatic tumor from a cutaneous primary.

A fourth theory for the occurrence of MUP, probably the most frequently discussed in the literature, hypothesises that an endogenous immune response results in complete spontaneous regression of the primary tumor, which is therefore unrecognised.[3,15,31,35] Spontaneous tumor regression has been noted amongst others cancers, but is most well-described in melanoma.[36] Regression of the primary tumor does not seem to be associated with a more favorable prognosis, although this remains controversial.[31,37,38] In the present study, 52.2% of MKP patients had partial regression of their primary. The outcome for MKP patients in whom regression of the primary was identified was better than the outcome for MKP patients with no primary melanoma regression, although this did not reach statistical significance ($p=0.084$). Both groups had a significantly worse outcome than MUP patients ($p<0.05$) (Figure 2D). This finding indicates that primary tumor regression might be correlated with a better prognosis. Immune-induced regression of a primary melanoma (after it has given rise to a nodal metastasis) could be the cause of a better outcome for MUP patients as a consequence of the immune response also being directed against any metastatic tumour.[39]

The rate of MUP in patients presenting with clinically palpable nodal disease and treated with TLND in our institution was 52.5% (247/551). This is substantially higher than in previous studies, which have reported MUP rates of 12.8 – 16.7% (Table 4).[14-17] However, in contrast to many prior reports, the selection criteria were much more restrictive in the current study, and only patients whose initial presentation was with palpable LN disease were included. The MKP patients were only included when they had a single primary melanoma, and underwent both their wide excision and TLND for palpable disease at the time of their initial presentation to Melanoma Institute Australia. This might also be a factor contributing to the higher five-year survival rates for MKP patients compared to those reported in the literature (Table 4). However, when all melanoma patients who underwent TLND at MIA between 1970 and 2010 were considered, the MUP rate was similar to the rates reported in the literature (i.e. 13.4%).

In conclusion, this large retrospective study demonstrated that MUP patients with macroscopic disease in LNs had a better outcome after TLND than MKP patients with equivalent disease at the time of presentation. Possible factors that might contribute to the improved survival in MUP patients include a more active tumor-directed immune response against the tumor (manifest by complete regression of the primary tumor) in some cases, and de novo origin of the melanoma within a LN in other cases. ECE, >3 positive LNs and adjuvant radiotherapy were factors associated with a worse prognosis. Our results suggest that all patients presenting with macroscopic

melanoma in a LN and no evidence of metastatic melanoma elsewhere should be treated as patients with stage III disease, with curative intent, even when there is no known primary melanoma.

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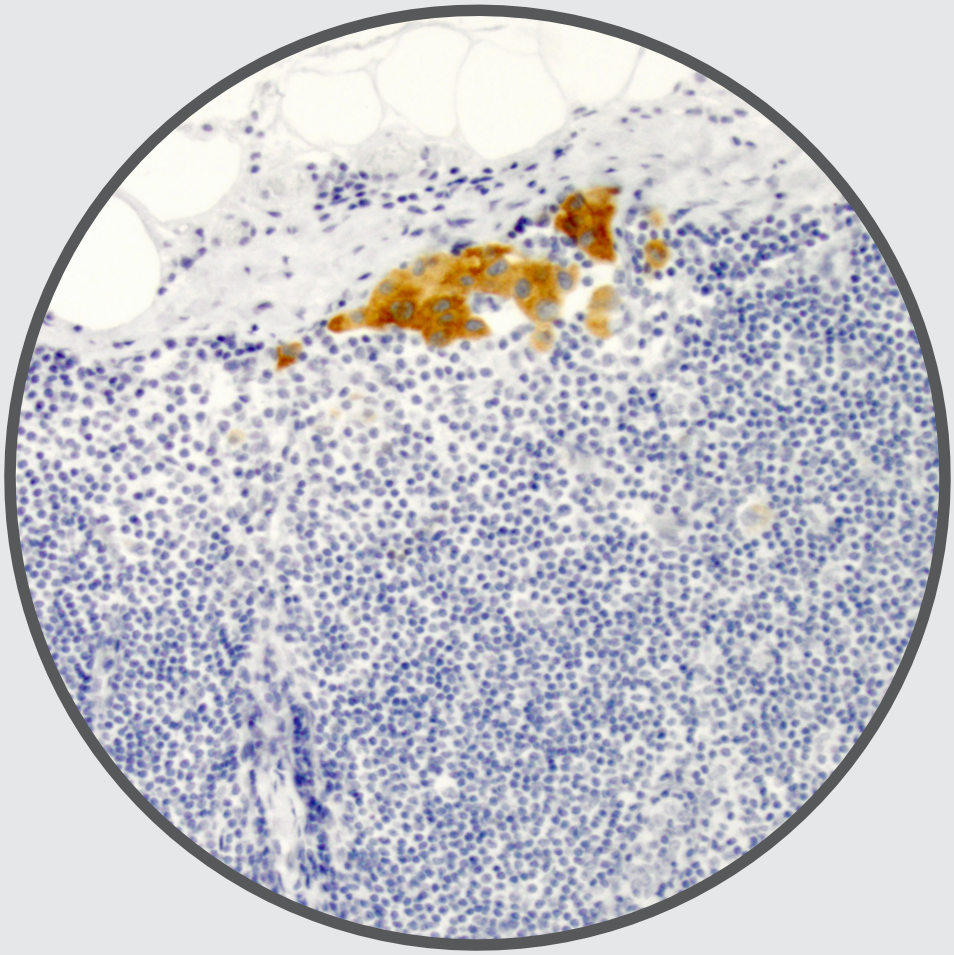
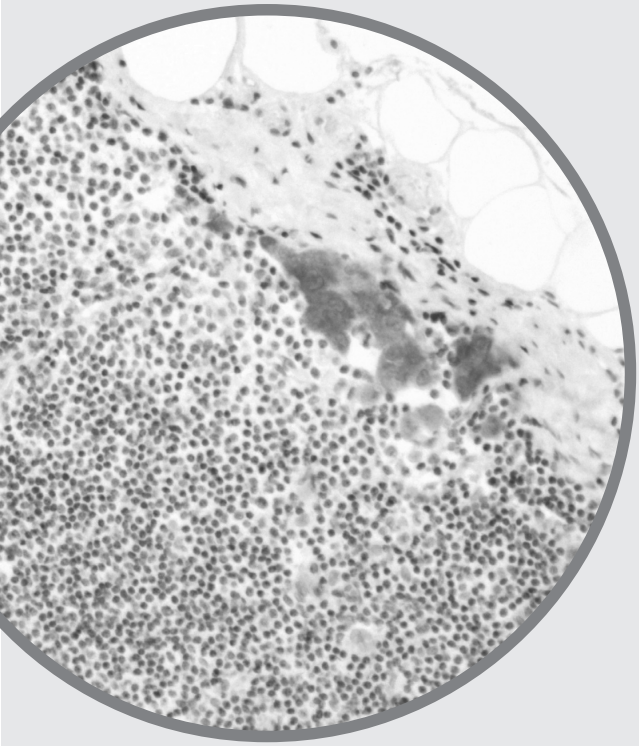


Part IV

Summary, general discussion and future perspectives

Chapter 12

Summary



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This thesis is divided in three parts. **Part I** describes the management and prognosis of stage I – II melanoma patients. **Part II** and **III** describes the management and prognosis of stage III patients separated in, respectively, patients with micrometastases and patients with macrometastases.

In **chapter 2** of **part I** the management and outcome are evaluated of nearly 6000 patients with a single primary melanoma treated with wide local excision at the Melanoma Institute Australia (MIA), Sydney, Australia. Of these patients treated between 1992 and 2008, approximately 50% underwent sentinel node biopsy (SNB) and 50% did not undergo SNB, but were observed (OBS). After univariate analysis, patients with intermediate thickness melanoma (T2 – T3; > 1.0 – 4.0 mm) had significantly better outcome when undergoing SNB. After correcting for baseline differences that occurred between both groups in multivariate analyses, SNB patients had improved disease free survival (DFS), but no better distant metastasis free (DMFS) and melanoma specific survival (MSS) in patients with any primary thickness.

In **chapter 3**, the lymphatic drainage pattern of melanoma is addressed. All patients treated at the MIA between 1993 and 2010 with melanoma on the ear were included in this study. The specific location of 111 primary tumors, classified according to the embryologically-derived anatomical subunits of the ear, were correlated with their mapped 281 sentinel nodes (SNs) identified by lymphoscintigraphy (LS). The helical rim was the location with highest prevalence (55.0%), followed by the lobule (24.3%). In conclusion, lymphatic drainage of the ear has no predictive pattern and there is no drainage to the contralateral neck. Most commonly drainage is to cervical level II (36.4%) and the preauricular and postauricular LN fields. LS can accurately identify the lymphatic drainage pattern and is essential for accurate SN identification and reliable SNB.

Part II of this thesis starts with **chapter 4** reporting on a study regarding 421 SNB patients treated at the Erasmus University Medical Centre – Daniel den Hoed Cancer Centre. The 121 (28.7%) SN positive patients were stratified for SN tumor burden according to the Rotterdam criteria (largest diameter of the largest lesion < 0.1 mm, 0.1 – 1.0 mm and > 1.0 mm). Besides the high identification rate of SN metastases by the EORTC MG SN pathology work-up protocol, the protocol identified a high rate of minimal tumor burden, i.e. 18% (22/121), as well. These patients with Rotterdam criteria < 0.1 mm had an estimated 5-year overall survival (OS) rate of 91% and a non-sentinel node (NSN) positivity rate in the specimen of the completion lymph node dissection (CLND) of 0%. This was not significantly different from the outcome of SN negative patients.

In **chapter 5**, the outcome of SN positive patients is analyzed in a multicenter setting. Nine EORTC MG centers combined their data into the largest number of

SN positive patients in the world. Between 1993 and 2008, 1080 melanoma patients were diagnosed with SN tumor burden. The Rotterdam criteria and Dewar criteria (subcapsular vs. non-subcapsular metastases) were determined. Patients with Rotterdam criteria < 0.1 mm had an estimated 5-year OS rate of 91% and NSN positivity of 9%. The so-called Rotterdam Dewar Combined (RDC) criteria identified patients with metastases < 0.1 mm located subcapsularly having an estimated 5- and 10-year MSS rate of 95% and NSN positivity of 2%. Long-term follow-up is necessary, since median follow-up was 3 years. However, in absence of results of prospective trials, the latter group might be classified as SN negative patients, therefore possibly not indicated for an immediate CLND.

The aim of the study presented in **chapter 6** was to validate the results of chapter 5 by adding data of an Australian center and a European center. Moreover, tumor penetrative depth (TPD) was added as a SN tumor burden parameter. After classifying the added SN positive patients for the different SN tumor burden parameters in an equivalent matter, outcome was analyzed for the total cohort (n=1539) and for each of the eleven centers individually. The maximum tumor size, the intranodal location and the TPD provided prognostic and predictive information in the total cohort, but their prognostic significance varied considerably between the different centers. Differences could have been occurred due to differences in SN detection, removal and examination techniques such as the number and location of SN slides to be cut, but also due to sample size limitations or patient characteristic differences.

Until now, all SN positive patients analyzed in **part II** underwent immediate CLND. Patients with minimal SN tumor burden (< 0.1 mm, especially when located subcapsularly), however, have very good prognosis and might not be indicated for CLND. Thus, the question remains: what if these patients do not undergo CLND? In **chapter 7**, 61 SN positive patients without CLND with a median follow-up of 4 years are analyzed. This group is compared to 1113 SN positive patients with CLND, all from ten EORTC MG centers. Performing CLND did not significantly influence MSS, however, patients without CLND had more favorable prognostic factors. After performing matched pair analysis, CLND did not demonstrate a therapeutic benefit as well. In patients with minimal SN tumor burden, outcome was significantly better in patients with TPD ≤ 0.3 mm with CLND compared to patients without CLND. Patients with micrometastases < 0.1 mm and/or located subcapsularly with CLND had no significantly better MSS than the same group without CLND. These latter two results should be considered with great caution due to the small sample sizes of the subgroups and due to the retrospective setting with probable selection bias.

Part III describes the management and outcome of melanoma patients with macrometastases in the lymph nodes. When palpable lymph nodes are present a therapeutic lymph node dissection (TLND) is indicated. Despite proper management,

survival can vary substantially due to the presence or absence of prognostic risk factors. In **chapter 8**, a mathematical model, i.e. nomogram, has been developed to predict recurrence within 2 years after TLND in a cohort of 504 patients from Brisbane, Australia. The nomogram included the following independent predictors of 2-year recurrence: number of positive lymph nodes, extracapsular extension, nodular histopathological subtype and post-operative seroma. The predictive value of the nomogram was successfully validated in a cohort of 331 patients from Rotterdam, the Netherlands.

Chapter 9 of this thesis evaluates the therapeutic surgical management of patients with clinically detectable nodal metastases to the groin. At the Erasmus University Medical Centre – Daniel den Hoed Cancer Centre, 121 patients underwent an ilioinguinal or combined superficial and deep groin lymph node dissection (CGD) and 48 patients underwent an inguinal or superficial groin lymph node dissection (SGD). Survival and local control did not differ for patients with palpable groin metastases treated by CGD or SGD. Morbidity rates were not significantly different between both groups as well, although CGD patients had a trend towards more chronic lymphedema. Preoperative computed tomography (CT) could accurately identify a negative deep lymph node field with 91%. Patients without pathological iliac nodes on CT might safely undergo SGD, while patients with multiple positive nodes or positive deep nodes on CT might be indicated for CGD.

Chapter 10 and 11 evaluate the outcome of patients with melanoma of unknown primary (MUP). In **chapter 10**, an entire cohort analysis of a single center is described, while in **chapter 11** restrictive inclusion criteria were assessed to include MUP and MKP patients from a single center for analyses. In the study described in **chapter 10**, patients who underwent a therapeutic lymph node dissection (TLND) for clinically detectable disease at the Erasmus University Medical Centre – Daniel den Hoed Cancer Centre were included. Between 1982 and 2009, 47 (13.7%) MUP patients and 295 patients with a known primary melanoma (MKP) were treated with TLND. After multivariate analysis, patients with MUP demonstrated a significantly better OS than MKP patients. Extracapsular extension and an increased number of positive nodes are significantly associated with worse prognosis.

In **chapter 11**, the outcome of 287 MUP patients who were treated between 1971 and 2010 was analyzed. The control group consisted of 264 MKP patients who were treated in the same time frame with wide local excision for a single primary melanoma and TLND for nodal metastases and had their follow-up at the MIA. MUP patients had improved DFS, DMFS and MSS over MKP patients. Extracapsular extension, an increased number of positive nodes and adjuvant radiotherapy were independent prognostic factors for MSS. MKP patients whose primary was in regression had a trend towards improved survival compared to those without regression of

the primary. Explanations of the significantly improved outcome of MUP patients in **chapter 10 and 11** may be an endogenous immune response resulting in regression of the primary or that melanoma cells arise de novo within a lymph node.

CONCLUSIONS

Chapter 2: Melanoma patients who underwent wide local excision and sentinel node biopsy had improved disease free survival, but no improved distant metastasis free and melanoma specific survival over patients who underwent wide local excision only.

Chapter 3: Lymphatic drainage of the ear has no predictive pattern. Drainage to the contralateral neck did not occur. Lymphoscintigraphy is essential to accurately identify the sentinel node.

Chapter 4: SN negative patients had significantly improved survival over sentinel node positive patients. The Rotterdam criteria significantly stratify patients for overall survival. Patients with < 0.1 mm SN metastases (18% of sentinel node positive patients) had five-year overall survival of 90% which is comparable to the survival of sentinel node negative patients and significantly improved survival over patients with 0.1-1.0 mm and > 1.0 mm SN metastases.

Chapter 5: Patients with metastases < 0.1 mm located subcapsularly in the sentinel node had 5- and 10-year melanoma specific survival of 95% and non-sentinel node positivity of 2%. These patients might be spared the additional completion lymph node dissection.

Chapter 6: The maximum sentinel node tumor size, the intranodal location and tumor penetrative depth provided prognostic information for melanoma specific survival and predictive information for non-sentinel node status after completion lymph node dissection.

Chapter 7: Completion lymph node dissection did not demonstrate to have a therapeutic benefit, especially in patients with minimal sentinel node tumor burden.

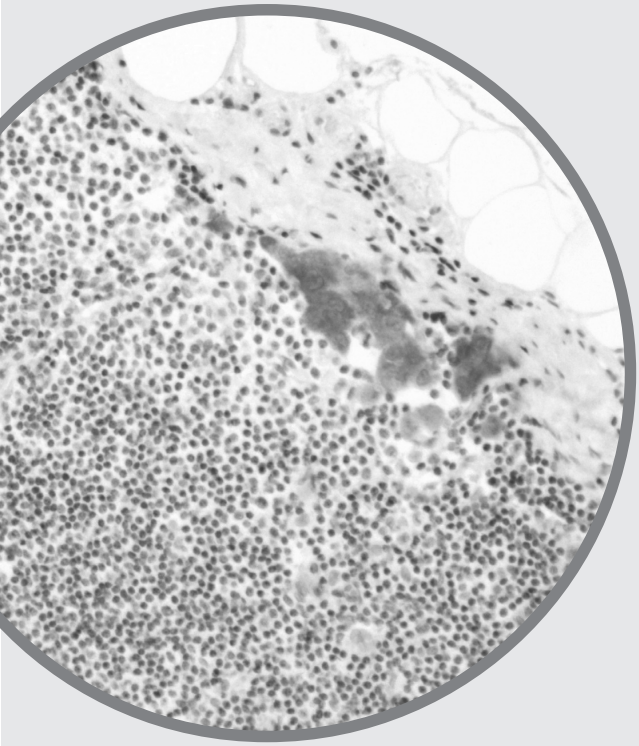
Chapter 8: A nomogram with independent predictive factors of 2-years recurrence, i.e. number of positive lymph nodes, extracapsular extension, nodular histopathological subtype and post-operative seroma, was developed and validated for patients with palpable metastases who underwent therapeutic lymph node dissection.

Chapter 9: Survival and local control did not differ for patients with palpable groin metastases treated by combined superficial and deep groin dissection or superficial only groin dissection. Patients with a single superficial positive node and preoperative negative deep nodes on CT scan should undergo superficial groin dissection only.

Chapter 10: Patients with melanoma of unknown primary had improved survival over patients with a known primary melanoma. Extracapsular extension and an increased number of positive nodes are significantly associated with worse prognosis.
Chapter 11: Patients with melanoma of unknown primary had improved disease free, distant metastasis free and melanoma specific survival over patients with a known primary. Regression of a primary demonstrated a trend towards improved survival.

Chapter 13

Nederlandse samenvatting



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Dit proefschrift is verdeeld in drie delen. **Deel I** beschrijft het beleid en de prognose van stadium I – II melanoompatiënten. **Deel II** en **III** beschrijven het beleid en de prognose van stadium III patiënten in, respectievelijk, patiënten met micrometastasen (niet-palpabel en gedetecteerd door middel van schildwachtklier (SWK) procedure) en patiënten met macrometastasen (palpabele ziekte).

In **hoofdstuk 2** van **deel I** zijn het beleid en de prognose beschreven van bijna 6000 patiënten waarbij het primair melanoom is verwijderd door middel van chirurgische excisie in het Melanoma Institute Australia (MIA) te Sydney, Australië. Van deze patiënten die behandeld zijn tussen 1992 en 2008 heeft ongeveer 50% een SWK procedure ondergaan. De andere 50% is geobserveerd gedurende follow-up (OBS). Na univariate analyse was de overleving voor patiënten met een gemiddelde tumordikte (T2 - T3; > 1.0 – 4.0 mm) significant beter voor diegene die een SWK procedure hebben ondergaan. Nadat in mulitvariate analyse gecorrigeerd was voor de significante verschillen tussen de SWK en de OBS groep, hadden patiënten in de SWK groep een verbeterde ziektevrije overleving, maar geen betere afstandsmetastase-vrije overleving of melanoom-specifieke overleving.

In **hoofdstuk 3** wordt het lymfedrainage patroon van het melanoom beschreven. Alle patiënten behandeld in het MIA tussen 1993 en 2010 met een melanoom op het oor werden in deze studie geïncludeerd. De specifieke locaties van 111 primaire tumoren, geclassificeerd volgens de embryologisch afgeleide anatomische localisaties van het oor, werden gecorreleerd met de 281 SWK-en geïdentificeerd door middel van lymfoscintigrafie (LS). De helix was de locatie met de hoogste prevalentie (55.0%), gevolgd door de oorlel (24.3%). Het patroon van lymfedrainage van het oor was niet te voorspellen. Er werd geen drainage naar de contralaterale hals gezien. Metastasering vindt meestal plaats naar het cervicale niveau II (36.4%) en naar de preauriculaire en postauriculaire lymfeklierregio's. LS kan nauwkeurig het drainagepatroon identificeren en is essentieel voor nauwkeurige SWK identificatie en een betrouwbare SWK procedure.

Deel II van dit proefschrift begint met **hoofdstuk 4**. In deze studie zijn 421 SWK patiënten geïncludeerd die behandeld zijn in de Erasmus Medisch Centrum – Daniel den Hoed kliniek te Rotterdam. Het percentage SWK positieve patienten was hoog (28.7%). De tumorgrootte van de 121 (28.7%) SWK-positieve patiënten is geanalyseerd en de patiënten zijn ingedeeld volgens de Rotterdam criteria (grootste diameter van de grootste laesie <0.1 mm, 0.1 – 1.0 mm en > 1.0 mm). Binnen de SWK-positieve groep heeft het SWK pathologie protocol van de EORTC melanoomgroep een hoog percentage patiënten met minimale tumorgrootte (Rotterdam criteria <0.1 mm) geïdentificeerd van 18% (22/121). Deze patiënten hebben een geschatte 5-jaars overleving van 91% en een percentage niet-schildwachtklier (NSWK) positieve patienten na completerende lymfeklierdis-

sectie (CLKD) van 0%. Deze prognose is niet significant verschillend van die van SWK-negatieve patiënten.

In **hoofdstuk 5** wordt de prognose van SWK-positieve patiënten geanalyseerd in een multicentrische setting. Negen centra binnen de EORTC melanoomgroep voegden hun gegevens samen. Tussen 1993 en 2008 werden 1080 melanoompatiënten gediagnosticeerd met tumor in de SWK. De Rotterdam criteria en Dewar criteria (subcapsulair versus niet-subcapsulair gelegen metastasen) werden bepaald voor elke SWK metastase. Patiënten met Rotterdam criteria < 0.1 mm hadden een geschatte 5-jaars overleving van 91% en NSWK-positiviteit na CLKD van 9%. De zogenaamde Rotterdam-Dewar gecombineerde (RDC) criteria identificeerden bij patiënten met < 0.1 mm subcapsulair gelegen metastase een geschatte 5- en 10-jaars melanoomspecifieke overleving van 95% en NSWK-positiviteit van 2%. Lange-termijn follow-up is noodzakelijk omdat de mediane follow-up 3 jaar was. Deze laatste groep met minimale tumorgrootte heeft een overleving die niet lijkt te verschillen van die van SWK-negatieve patiënten. Een aanvullende operatie, een CLKD, met hoge morbiditeit zoals chronisch lymfoedeem zou deze patiënten bespaard kunnen blijven.

Het doel van de studie die beschreven is in **hoofdstuk 6** was het valideren van de studie in hoofdstuk 5. Patiënten die behandeld zijn in twee andere centra en een nieuwe variabele werden meegenomen in de analyse. De penetratiediepte van de tumor in de SWK (TPD) werd toegevoegd als een SWK tumor karakteristiek. De totale cohort (n=1593) werd geanalyseerd alsmede ieder van de elf centra apart. De maximale grootte van de tumor, de locatie en de TPD hadden allen een prognostische waarde voor overleving en een voorspellende waarde voor additionele lymfekliermetastasen na CLKD in de totale cohort. Hun prognostische betekenis verschilde echter aanzienlijk tussen de verschillende centra. Verschillen in de prognose zouden het gevolg kunnen zijn van verschillen in de SWK detectie, verwijdering en onderzoekstechnieken zoals het aantal en de locatie van de gesneden SWK coupes. Het zou ook een gevolg kunnen zijn van verschillen in de samenstelling van de groepen met betrekking tot aantallen en patient kenmerken.

In alle voorgaande hoofdstukken in **deel II** zijn alle SWK positieve patiënten geanalyseerd die een CLKD hebben ondergaan. Patiënten met minimale SWK metastase (< 0.1 mm, vooral wanneer subcapsulair gelegen) hebben een zeer goede prognose. Gezien het feit dat deze patiënten een additionele CLKD hebben ondergaan blijft de vraag: wat als deze patiënten geen CLKD hadden ondergaan? In **hoofdstuk 7** zijn 61 SWK positieve patiënten die geen CLKD hebben ondergaan, geanalyseerd. De mediane follow-up was 4 jaar. Deze groep werd vergeleken met 1113 SWK positieve patiënten met aanvullende CLKD. Alle patiënten waren behandeld in tien EORTC MG centra. Na analyse bleek er geen significant betere overleving te zijn na het uitvoeren van een CLKD. Patiënten zonder additionele chirurgie hadden echter

meer gunstige prognostische factoren zoals kleinere metastasen. Na het uitvoeren van een matched pair analyse van twee groepen met 61 patiënten met vergelijkbare prognostische factoren werd een therapeutisch voordeel van een CLKD ook niet aangetoond.

Deel III beschrijft het management en de prognose van melanoompatiënten met (palpabele, klinisch detecteerbare) macrometastases in de lymfeklieren. In **hoofdstuk 8** zijn nomogrammen gecreëerd en gevalideerd. De cohort groep bestond uit 504 patiënten uit Brisbane, Australie met palpabele (klinisch detecteerbare) macrometastasen die een therapeutische lymfeklierdissectie (TLKD) hebben ondergaan. De primaire uitkomstmaten waren de recidiefkans en de overleving binnen 2 jaar na TLKD. Het aantal positieve lymfeklieren en extranodale groei waren onafhankelijk significante factoren voor een recidief binnen 2 jaar en voor de 2-jaars overleving. Histologisch nodulair type melanoom en postoperatieve seroomvorming waren additionele onafhankelijke significante factoren voor een recidief binnen 2 jaar en leeftijd was dat voor de 2-jaars overleving. Het nomogram werd succesvol gevalideerd in een vergelijkbare groep van 331 patiënten uit Rotterdam, Nederland.

Hoofdstuk 9 van dit proefschrift heeft de chirurgische behandeling van patiënten met klinisch aantoonbaar nodale metastasen naar de lies geevalueerd. In het Erasmus Universitair Medisch Centrum / de Daniel den Hoed kliniek ondergingen 121 patiënten een ilioinguinale lymfeklierdissectie (LKD) en ondergingen 48 patiënten een inguinale LKD. Overleving en lokale controle verschilden niet in beide groepen. De postoperatieve morbiditeit was niet significant verschillend tussen beide groepen, hoewel ilioinguinale LKD patiënten een trend naar meer chronische lymfoedeem lieten zien. Preoperatieve computertomografie (CT) kon een negatieve iliacale lymfeklier nauwkeurig identificeren in 91% van de patiënten met een ilioinguinale LKD. Patiënten zonder pathologische iliacale lymfeklieren op CT zouden veilig een inguinale LKD kunnen ondergaan, terwijl patiënten met meerdere positieve klieren of positieve iliacale klieren op CT een ilioinguinale LKD kunnen ondergaan.

In hoofdstuk 10 en 11 zijn studies behandeld die patiënten met melanoom met een onbekende primaire tumor (MUP) hebben onderzocht. De overleving tussen deze groep patiënten en patiënten met een bekend primair melanom (MKP) werd vergeleken. In **hoofdstuk 10** werd een totale cohort analyse van een enkel centrum beschreven, terwijl in **hoofdstuk 11** strikte inclusiecriteria werden gebruikt om MUP en MKP patiënten te selecteren voor een nauwkeurigere analyse. In de studie beschreven in **hoofdstuk 10** waren alle patiënten die een TLKD voor klinisch detecteerbare ziekte in de Erasmus Universitair Medisch Centrum – Daniel den Hoed kliniek inbegrepen. Tussen 1982 en 2009 werden 47 (13.7%) MUP patiënten en 295 MKP patiënten behandeld met een TLKD. Na multivariate analyse bleek dat patiënten met MUP een significant betere overleving hadden dan MKP patiënten.

Extranodale groei en positieve klieren waren significant geassocieerd met een slechtere prognose.

In **hoofdstuk 11** is de studie besproken waar 287 MUP patiënten zijn geanalyseerd die tussen 1971 en 2010 met een TLKD zijn behandeld in het Melanoma Institute Australia (MIA) in Sydney, Australië. De controlegroep bestond uit 264 MKP patiënten die behandeld werden in hetzelfde tijdsbestek met radicale excisie van een enkel primair melanoom, TLKD voor macrometastasen en follow-up in het MIA hadden. MUP patiënten hadden een significant verlengde ziekte-vrije en afstandsmetastase-vrije periode en een significant verbeterde algehele overleving vergeleken met MKP patiënten. Extranodale groei, het aantal positieve klieren en adjuvante radiotherapie waren onafhankelijke prognostische factoren voor slechtere prognose. MKP patiënten bij wie de primaire tumor in regressie was hadden een niet significante, maar verbeterde overleving in vergelijking met mensen zonder regressie van de primaire tumor. Een verklaring voor de aanzienlijk verbeterde overleving voor MUP patiënten in **hoofdstuk 10 en 11** zou een endogene immuunrespons kunnen zijn welke resulteert in regressie van het primaire melanoom. Tevens zou het kunnen zijn dat de melanoomcellen de novo ontstaan binnen een lymfeklier.

CONCLUSIES

Hoofdstuk 2: Melanoompatiënten die een schildwachtklier procedure hebben ondergaan hadden een verbeterde ziektevrije overleving, maar geen verlengde periode tot afstandsmetastasen en geen verbeterde ziektespecifieke overleving.
Hoofdstuk 3: Lymfedrainage van melanoom van het oor heeft geen voorspellend patroon van metastasering. Metastasering naar de contralaterale hals trad niet op. Lymfoscintigrafie is essentieel om de schildwachtklier nauwkeurig te identificeren.
Hoofdstuk 4: Schildwachtklier negatieve patiënten hadden significant betere overleving dan schildwachtklier positieve patiënten. De Rotterdam criteria stratificeren patiënten significant voor algemene overleving. Patiënten met <0.1 mm schildwachtklier metastasen (18% van de schildwachtklier positieve patiënten) hadden een vijfjaars overleving van 90% wat vergelijkbaar is met de prognose van schildwachtklier negatieve patiënten en significant beter dan patiënten met 0.1-1.0 mm en > 1.0 mm metastasen.
Hoofdstuk 5: Patiënten met < 0.1 mm metastase subcapsulair gelegen in de schildwachtklier hadden 5- en 10-jaar ziekte-specifieke overleving van 95% en een niet-schildwachtklier positiviteit in het completerende lymfeklierdissectie preparaat van 2%. Deze patiënten zouden een aanvullende completerende lymfeklierdissectie bespaard kunnen blijven.

Hoofdstuk 6: De maximale grootte van de schildwachtklier, de intranodale locatie en de tumor penetratiediepte zijn prognostisch onafhankelijke factoren voor ziekte specifieke overleving en hebben een voorspellende waarde voor niet-schildwachtklier metastasen.

Hoofdstuk 7: Completerende lymfeklierdissectie bij schildwachtklier positieve patiënten had geen therapeutisch effect, vooral bij patiënten met minimale schildwachtklier metastase.

Hoofdstuk 8: Nomogrammen met risicofactoren voor het voorspellen van een recidief binnen 2 jaar en de 2-jaars overleving na therapeutische lymfeklierdissectie in verband met palpabele lymfeklieren werden verwezenlijkt. De nomogrammen bestonden uit de volgende factoren: Het aantal positieve lymfeklieren, extranodale groei, een nodulaire melanoom, postoperatieve seroomvorming en leeftijd.

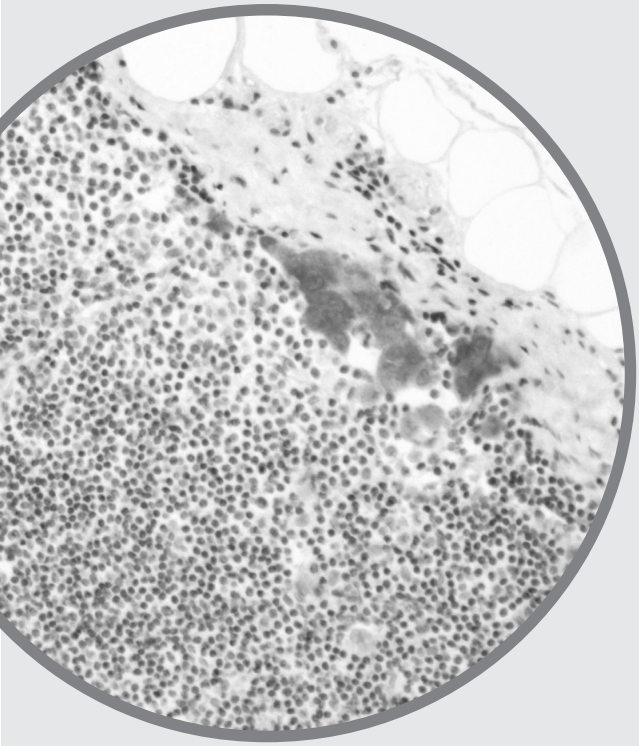
Hoofdstuk 9: Overleving en lokale controle waren niet verschillend voor patiënten met palpabele inguinale lymfeklieren behandeld met ilioinguinale lymfeklierdissectie of alleen inguinale lymfeklierdissectie. Patiënten met een enkele inguinale metastase in de lymfeklier en preoperatieve negatieve iliacale klieren op CT-scan zouden een inguinale lymfeklierdissectie kunnen ondergaan.

Hoofdstuk 10: Patiënten met melanoom van een onbekende primaire tumor hadden een significante betere overleving dan patiënten met een bekend primair melanoom. Extranodale groei en het aantal positieve lymfeklieren was significant geassocieerd met een slechtere prognose.

Hoofdstuk 11: Patiënten met melanoom van onbekende primaire origine hadden een verlengde ziektevrije en afstandsmetastase vrije periode en een verbeterde ziektespecifieke overleving. Regressie van de primaire tumor toonde een niet significant verbeterde overleving.

Chapter 14

Discussion and future perspectives



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SENTINEL NODE BIOPSY

Twenty years after its introduction, SNB is standardized in cancer centers worldwide for staging in melanoma patients. According to analyses from the Surveillance, Epidemiology and End-Result (SEER) database, only 53% of eligible patients had undergone SNB between 2004 and 2008 in the United States.[1] SNB is highly recommended for patients with intermediate-thickness melanoma (T2 and T3; Breslow thickness of 1 to 4 mm) at any anatomic site.[2] (Chapter 2) For patients with thick melanomas (T4; Breslow thickness > 4 mm), the use of SNB is accepted as staging purpose and to facilitate regional control, although the risk of occult distant metastases is present.[2, 3] For patients with thin melanomas (T1; Breslow thickness < 1 mm), SNB is more controversial. Most claim that SNB should only be offered to those with risk factors such as ulceration or mitotic rate ≥ 1/mm and melanoma > 0.75 mm thick.[2, 4]

The SN status is the most prognostic factor for melanoma specific survival.[5, 6] In approximately 20%, patients are diagnosed with a positive SN which implies that their prognosis decreases significantly. SN negative patients have 5-year MSS rates of approximately 90%.[5, 6] Heterogeneous groups of SN positive patients have 5-year MSS rates ranging from over 50% to around 75%.[7, 8] In most guidelines, patients with proven melanoma metastases in one or more SNs should undergo an "early" completion lymph node dissection (CLND), sparing the patients without SN metastases the additional surgery.[2] According to analyses from the National Cancer Data Base, only 50% of 2942 SN positive patients treated in 2004 and 2005 in the United States underwent CLND.[9]

Multicenter Selective Lymphadenectomy Trial – 1 (MSLT-1)

The MSLT-1 is the only randomized controlled trial (RCT) to date, which randomized primary melanoma patients (60:40) for SNB, followed by early CLND when SN positive, or for nodal observation (OBS), followed by therapeutic lymph node dissection (TLND) when regional nodal relapse. Between January 1994 and March 2002, 2001 patients with primary melanoma ≥ 1.00 mm or having Clark level IV or V were randomized. The study completion date was June 2012, 10 years after the last patient accrual. Interim-results on the primary outcome measure are published in 2006, on morbidity in the nodal metastases group in 2010 and on surgery in stage IV patients in 2012. Final results and results on the total cohort have not been published or presented (yet). An overview and minor update of both MSLTs has been published in 2012, but did not report on the primary end-point (melanoma-specific survival) for the original cohort.[10]

Only patients with melanomas 1.2 – 3.5 mm in thickness were selected for the interim-analysis in 2006, because "statistical modeling indicated that the timing of

CLND probably affects survival among patients within this range". The primary endpoint, i.e. prolonged melanoma-specific survival (MSS) for SNB patients compared to nodal observation, has not been reached. In the nodal metastases subgroups, SN positive patients who underwent CLND had improved survival over OBS patients with nodal relapse who underwent TLND (p=0.004). A survival benefit is only seen in a subgroup of a subgroup of the primary aim group. The SN hypothesis that SNB will detect occult metastases, which will eventually all progress to aggressive regional or distant disease, is tested in the interim-analyses. After 5 years the rate of nodal metastases between the SNB group (15.6% + 3.8% = 19.4%) and the OBS group (16.1%) has a difference of 3.3%. A similar percentage of 2.8% has been detected in chapter 2 of this thesis (follow-up of 3.5 years). Longer follow-up will identify more patients with nodal metastases or these patients have very low volume metastases that never will progress to clinically detectable disease. Several reports in this thesis have identified this possible SN "false-positive" group. In contrast, the recent update of MSLT-1 provided by Dr. Morton describes an equivalent incidence of nodal metastases in both groups after 8 until 10 years of follow-up. For patients with intermediate thickness melanomas, the incidence of nodal metastasis was 19.8% in the SNB group compared to 20.5% in the OBS group.[10] An additional 3.9% of the OBS group in MSLT-1 had recurrence in the regional nodal field between follow-up years 5 to 8. It will remain unclear if these percentages also apply for the total study cohort, since these data are not (yet) presented. At the same time, some SN positive patients might not have developed clinically relevant lymph node metastases, but might have succumbed to distant metastases prior to this.

Moreover, pathology work-up and analysis play a very important role in determining these percentages and can skew these results. A more extensive pathology protocol and different analysis of SN tumor burden might detect more clinically insignificant SN metastases.[11] A higher SN positivity rate will be detected which has been demonstrated in chapter 4.[6, 12] Follow-up of studies in the present thesis should be expanded by several years to support these results.

In the two decades MSLT-1 was running, the field of melanoma has changed significantly. Imaging techniques, e.g. PET-CT scans and ultrasound(US)-guided fine-needle aspiration cytology (FNAC), have improved. New therapeutic agents have become available. SN melanoma experts gained more experiences, especially on the field of pathology. This all will make the final results of MSLT-1 not easy to interpret into the new reality of everyday practice.

SENTINEL NODE PATHOLOGY PROTOCOL

After the surgical procedure of removing SNs, the SNs are sent to the pathology department for pathological examination. Pathology work-up protocols are different worldwide and no standard approach for the SNs has been adopted. Substantial differences in identifying, removing and analyzing SNs exist between different cancer centers. Agreement on the SN pathology work-up protocol is an issue highly discussed in recent years.[6, 13, 14] In a very recent survey of 142 academic institutions in the United States on the histopathological evaluation of SNs, 32 (28%) institutions responded to the questionnaire. Twenty-six (81%) institutions had a protocol for SN examination. Nine (28%) centers cut the SN in half (bivalve), whereas 59% cut the SN at even intervals without specifically commenting about any orientation to the hilum. The number of levels cut varied from 1 to 8. Histologic protocols varied vastly among institutions.[15] In general, three protocols are being assessed according to literature. Cochran et al. of the John Wayne Cancer Institute developed a protocol.[16] The European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group uses the protocol developed by Cook et al.[17] The Melanoma Institute Australia handles a protocol formed by Scolyer et al, which is adapted by the Cochran protocol.[18]

There are differences between the protocols, i.e. where the sections in the SN are cut, how many sections are cut and how large the interval between sections is. It is unclear what the influence is of the different approaches, but they evidently will lead to differences in decision making during daily clinical practice. Some bivalve through the hilum, some through the longest axis and others cut sections at random. Some suggest that most metastases are located near the hilum, while others state that evenly sized melanoma metastases are located throughout the nodes, so sectioning either in the central area or in the peripheral area does not matter.[13] Another issue is the number of sections cut from each halve or part of the SN. In general, according to the EORTC MG protocol by Cook, 12 sections should be cut from each SN, which is more sections than other protocols recommend.[17, 18] More extensive sectioning and examination of SNs increases detection rate of SN metastases from 14% up to almost 30%.[6, 12, 17, 19-22] Moreover, it increases the detection rate of submicrometastases.[6] Sectioning more than 50 µm could miss submicrometastases, since sometimes submicrometastases are smaller than 50 µm.[17] Experience tells us that every extra section cut from an SN may reveal that a very small deposit is in fact much larger.[13, 20] Patients with minimal SN tumor burden will in some cases show to have more advanced disease than was previously shown. At the same time, negative patients will become upstaged to minimal SN tumor burden patients. When cutting more, more clinically irrelevant metastases will appear.[11] Complete sectioning would lead to high

detection rates, but at the same time this will cost much more time for sectioning and analyses of these sections, which in turn is a financially costly matter.[14] In conclusion, pathology work-up is not performed equivalent over different centers in the world, which could be an important cause in the differences in prognostic significance of the SN tumor burden parameters.

PATHOLOGY SLIDE ANALYSIS

In the last decade, numerous studies identified several different factors in melanoma patients with SN tumor burden predicting the risk of death and/or the risk of additional non-sentinel node (NSN) involvement.[6, 7, 23-46] Most often assessed are the microanatomic location,[7, 25, 26, 43, 44], the tumor penetrative depth (TPD)[31, 40, 43] and tumor size as maximum diameter of the largest lesion[6, 24, 26-28, 33, 35, 40-43, 47]. All were predictors for survival, NSN involvement or both.

Van Akkooi et al. on behalf of the EORTC MG pointed out how to specifically report the anatomic location and the maximum size of SN metastasis.[48] Murali et al. made guidelines for assessment of the SN and its metastasis.[49] Both parties recommend a practical methodology. The tumor penetrative depth is defined as the maximum distance of melanoma cells from the interior margin of the respective SN capsule.[31, 50] A subcapsular metastasis is defined as the location of all lesions in the SN strictly confined to the subcapsular sinus or the paratrabecular and not tattered or irregularly shaped.[25, 48] The maximum size of the metastasis is defined as the measurement of the largest diameter of only the largest deposit with no interruption by lymphocytes.[32, 48]

When clinical decision making, for instance to undergo a CLND or observation, is based on the assessment of the above mentioned micromorphometric parameters, there is an absolute need for agreement between observers. Murali et al. calculated the interobserver agreement on measurement of quantitative and qualitative SN tumor burden parameters.[49] Quantitative parameters as the maximum size of the largest deposit and TPD had an excellent degree of agreement, while a qualitative parameter as the location of deposits in the SN had a moderate degree of agreement.[49] TPD, classified according to the S-classification, provides important prognostic and predictive information and assesses both size and location of the SN metastases, as well as the RDC criteria does[31, 38, 43] There are, however, differences present. For example, a patient with a large subcapsular lesion which is 1.4 mm in diameter could have a minimal TPD, i.e. < 0.3 mm (S1), and thus be addressed to the group of patients with the best prognosis. At the same time a very small lesion, which is located somewhere far from a capsule, could be seen as an S3 classification (most

aggressive disease), when this might be far less.[48] These two examples clarify that the TPD classified according to Starz et al. does not always correctly reflect the location of the SN metastases, while the RDC-criteria does reflect both factors on all lesions. TPD and the S-classification should be included in addition to the Dewar criteria, Rotterdam criteria and Rotterdam-Dewar Combined (RDC) criteria in forthcoming analyses of SN positive patients.

PROGNOSTIC HETEROGENEITY OF SENTINEL NODE POSITIVE PATIENTS

Outcome of SN positive patients is determined by the predictive value for non-sentinel node (NSN) status in the CLND specimen and the prognostic value for survival. The group of SN positive patients is a heterogeneous group of patients with survival rates ranging from approximately 30% to over 90% in various subgroups.[51] Patients with high volume SN tumor burden have significant worse outcome than patients with low volume tumor burden.[5, 25, 31, 32, 42, 43, 51] A meta-analysis performed by Nagaraja et al. demonstrated the heterogeneity of SN positive patients in the likelihood of NSN metastases with 12 significantly different predictive factors.[52] SNB has implemented in the TNM staging system since a decade.[53] The American Joint Committee on Cancer (AJCC) Melanoma Staging Committee anticipated that microscopic tumor burden will be included in the analysis for the eight edition of the Cancer Staging Manual.[54, 55]

Subgroups are defined by micromorphometric parameters and their classification. The anatomic location is most efficiently divided into subcapsular located versus non-subcapsular located metastases according to the Dewar criteria.[25] Subcapsular metastases represent approximately 20-30% of SN positive patients and have better outcome than non-subcapsular metastases.[25, 26, 35, 39, 43, 56, 57] TPD beneath the SN capsule is classified according to Starz et al. in S1 (≤ 0.3 mm), S2 (> 0.3 – ≤ 1.0 mm) and S3 (> 1.0 mm) metastases.[31] Patients with S1 metastases are about 30% of SN positive patients and have improved survival over patients with S2 and, subsequently, S3 metastases.[31, 38, 39, 43, 50, 56-59] The maximum size is classified according to the Rotterdam criteria, i.e. < 0.1 mm, 0.1 – 1.0 mm and > 1.0 mm metastases.[32] Patients with < 0.1 mm represent around 10-15% of SN positive patients and have better outcome than patients with 0.1 – 1.0 mm and > 1.0 mm metastases.[6, 12, 26, 32, 38, 41-43] Several studies using other cut-off points (0.5, 1.0 and/or 2.0 mm) for subgroups of maximum size demonstrated a significant influence on survival as well.[35, 39, 56, 60, 61] In the only meta-analysis performed in the field of SN tumor burden, patients with < 0.1 mm metastases had the lowest odds ratio for having NSN metastases. The parameter was devoid for heterogeneity.[52] In chapter six the location and the maximum size

in the SN were combined into the RDC (Rotterdam Dewar combined) criteria. The S-classification also represents the size and location, however, not the maximum size in any direction. Patients with < 0.1 mm metastases located subcapsularly only represented 6% of SN positive patients and had excellent survival, i.e. 5- and 10-year MSS rates of 95%, compared to the other subgroups.[26] (Chapter 5) A maximum SN tumor size > 1mm separated a cohort of SN positive patients into two equivalent sized groups and was the most consistent independent predictor of NSN positivity and poorer DFS and MSS in individual centers, and in the combined cohort. (Chapter 6)

Several studies demonstrated that patients with minimal SN tumor burden had excellent survival equivalent to the survival of SN negative patients.[6, 26, 32, 38, 42, 58] Few studies statistically compared the outcomes of SN negative patients and patients with minimal SN tumor burden (Table 1).[6, 38, 62, 63] When comparing SN negative patients to SN positive patients with < 0.1 mm metastases, no significant differences were identified for DFS and MSS. Meier et al. performed analyses on 697 melanoma patients in their institute with a median follow-up of 5 years.[38] There was no significant difference in disease free (DFS) (p=0.183) and overall survival (OS) (p=0.400) between SN negative patients (n=480) and SN positive patients with Rotterdam criteria < 0.1 mm (n=85). Meier et al. also reported that the additional parameters of tumor penetrative depth (TPD) and capsular involvement had no impact on prognosis in patients with Rotterdam criteria < 0.1 mm. The proposed group by Meier et al. with the best

Author (year)	Group of patients	No. of patients	Median follow-up (mo)	Median Breslow Thickness (mm)	Ulceration (%)	Disease Free survival			Melanoma Specific Survival		
						5-yr DFS rates (%)	P	HR	5-yr MSS rate (%)	P	HR
Van der Ploeg [6] (2010) †	SN negative	300	55	2.00	21.7	82%	0.902	N/A	90%	0.762	N/A
	< 0.1 mm	22*	66	1.90	31.8	80%			91%		
Meier [38] (2010)	SN negative	480	45.5**	1.70	19.4	± 85%			± 90%		
	< 0.1 mm	85*		N/A	N/A	± 78%	0.183	N/A	± 86%	0.400	N/A
Scheri [62] (2007)	SN negative	1168	57**	1.20	15.5	89%			94%		
	< 0.2 mm	57*		1.70	14	74%	0.0008	N/A	89%	0.02	N/A
Ollila [63] (2009)	SN negative	488	26**	1.1	16	83%			N/A		
	< 0.1 mm	33*		2.1	24	78%	0.048	N/A	N/A	0.40	N/A

CLND = early Completion Lymphadenectomy after a positive SNB, N/A = Not Available
** Scheri et al: 5 of 57 patients did not undergo CLND, Ollila et al: 3 of 22 patients did not undergo CLND, Meier et al: 42 of 217 SN positive patients did not undergo CLND, 22 due to minimal SN tumor burden, van der Ploeg et al: 7 of 22 patients did not undergo CLND*
*** The median follow-up of the total number of patients included in the study has only been provided*
† Additional analyses after publication have been performed by authors

outcome was the Hannover scoring system group 1. This group consisted only of patients with Rotterdam criteria < 0.1 mm.[38]

The above mentioned results are confirmed by a study performed with the Rotterdam cohort.[6] (Chapter 4) Some additional analyses have been performed for a reply to Satzger et al.[64] SN negative patients from the Rotterdam cohort (n=298) had a median follow-up of 4.6 years and median Breslow thickness of 2.0 mm, while patients with Rotterdam criteria < 0.1 mm (n=23) had 5.5 years follow-up and 1.9 mm thick melanoma, respectively. SN negative patients did not have a significantly different DFS (p=0.902) and OS (p=0.762) compared to patients with < 0.1 mm SN metastases.[6]

Others feel that patients with isolated tumor cells (ITC) cannot be compared to SN negative patients.[62, 63, 65] Scheri et al. revealed that < 0.2 mm metastases cannot be considered as SN negative.[62] Scheri et al., compared outcome of patients with SN metastases ≤ 0.2 mm (n=57) to SN negative patients (n=1168).[62] With a median follow-up time of 4.8 years, improved DFS (p=0.0008) and OS (p=0.02) were demonstrated for SN negative patients. However, the SN negative group had a median Breslow thickness of 1.20 mm, which is less than a normal SN negative population, e.g. the MSLT-I trial (1.80 mm) patients, and significantly less than patients with SN tumor burden < 0.2 mm (1.70 mm) in their study. Thus, it does not seem that this is a true reflection of the outcome of SN negative patients, from most cohorts and has lead to a survival rate of 94%, which is much higher than reported by other studies in the literature.[62] Furthermore, When changing the minimal cut-off from 0.1 to 0.2 mm, five-year MSS rates decrease significantly from over 90% to approximately 80%.[26] Murali et al. demonstrated that 20 patients with < 0.1 mm metastases cannot be safely regarded as SN negative. When extra sections were cut from the SNs, patients were upstaged and had worse survival.[65] Murali et al, however, introduced a new bias, as SN negative patients were not upstaged. In theory, when extra sections are also cut from the group of SN negative patients, the "true" patients with < 0.1 mm metastases might be identified. With a non-extensive SN pathology work-up protocol, patients with ITC's or < 0.1 mm metastases might not be safely regarded as clinically irrelevant and/or SN negative. With an extensive SN pathology work-up protocol, < 0.1 mm metastases might be clinically irrelevant.

As well as differences in pathology work-up and protocols, differences in any part of the SNB technique, e.g. nuclear medicine and surgery, can affect the accuracy of SNB.[66] Chapter 6 demonstrated that several parameters of SN tumor burden provided important prognostic information, but had prognostic variability between different centers. It will be difficult to overcome all differences, but consensus on pathology work-up and analyses might have great impact on treatment of patients who underwent SNB.

MANAGEMENT OF SENTINEL NODE POSITIVE PATIENTS

In most cancer centers, standard treatment for all SN positive patients is CLND. However, Bilimoria and colleagues demonstrated, surprisingly, that only 50% of patients with melanoma diagnosed with a positive SN in 2004 and 2005 in the USA underwent CLND. 24 After conducting a worldwide web-based survey on opinions about CLND of melanoma surgeons, Pasquali et al demonstrated that 91.8% of 193 surgeons recommend CLND in patients with a positive SN. Decision-making is based on patient comorbidities and SN tumor burden.[67] Henderson already stated that it is a logical and necessary progression to explore whether it is possible to define the subgroups of SN positive patients who will and who will not benefit from a potentially morbid procedure as CLND.[68] McMasters believes it is also appropriate to suggest that CLND sometimes should be avoided. However, he feels we should wait for randomized controlled trials, although he argues we would cure the same number of patients if we did not perform CLND.[69]

The therapeutic benefit of CLND is unknown and it attends with high morbidity.[70-73] Some have hypothesized that not all SN positive patients might be indicated for immediate "early" CLND.[27, 32, 42, 58, 70] Others have reported that all SN positive patients should undergo CLND.[39, 62, 69, 74, 75] No additional metastases are detected in approximately 80 per cent of patients undergoing CLND. Furthermore, CLND is associated with considerable greater morbidity than SNB alone[71, 72, 76-79]. Complication rates from 23% to 61% are reported after CLND with lymphedema, wound infection, haematoma/seroma formation and sensory nerve injury as most common complications.[79, 80] Results from the Sunbelt Melanoma Trial demonstrated complication rates of 23 per cent after CLND and 5 per cent for patients who underwent SNB alone.[79] In a study by de Vries et al investigating the quality of life, patients undergoing CLND had more problems than patients who underwent SNB alone.[81]

Some retrospective studies have reported on the outcomes of patients who did not undergo CLND (Table 2).[58, 70, 73, 82] SN positive patients who did not undergo CLND had similar DFS and MSS as those with CLND. However, selection bias was evident in these nonrandomized retrospective studies, a problem highlighted by Henderson.[68] The study mentioned in chapter seven performed matched-pair analysis to overcome the selection bias. MSS was not significantly different between both groups consisting of 61 patients with minimal SN tumor burden.[82] Patients with high volume SN tumor burden seem to have more therapeutic benefit from undergoing CLND than patients with minimal SN tumor burden.[82] Patients with low SN tumor burden could probably be spared the morbidity associated with CLND.[52] The possible survival benefit of patients undergoing CLND could not only be limited to patients with high volume SN tumor burden. Patients with intermediate primary thickness melanoma

Author (year)	Group of patients	No. of patients	Median follow-up (mo)	Median Breslow Thickness (mm)	Ulceration (%)	Disease Free survival			Melanoma Specific Survival		
						5-yr DFS rates (%)	P	HR	5-yr MSS rate (%)	P	HR
Van der Ploeg IM* [58] (2009)	No CLND	20	32	1.8	N/A	83% (3-yr)			100% (3-yr)		
	CLND	50	34	3.8	N/A	60% (3-yr)	0.40	N/A	80% (3-yr)	0.04	N/A
Kingham [70] (2010)	No CLND	42	32	3.5	62	± 45%			± 68%		
	CLND	271	43	2.8	44	± 40%	0.63	N/A	± 58%	0.26	N/A
Wong [73] (2006)	No CLND	134	20	2.60	32.8	80% (3-yr)			80% (3-yr)		
	CLND	164	36	2.85	50	88% (3-yr)	0.07	N/A	74% (3-yr)	0.65	N/A
Van der Ploeg AP** [82] (2012)	No CLND	61	48	2.50	45				66.0		
	CLND	1113	34	3.00	49.4	N/A	N/A	N/A	66.9	0.60	0.89
	No CLND	61	48	2.50	45				66.0		
	CLND†	61	44	2.50	45	N/A	N/A	N/A	69.0	0.64	0.86

CLND = early Completion Lymphadenectomy after a positive SNB, N/A = Not Available
** Patients without CLND consist of patients with tumor penetrative depth (TPD) ≤ 1.0 mm (SI and SII), while patients with CLND consist of patients with TPD > 1.0 mm (SIII).*
*** Patients without CLND had, amongst others, a median maximum SN tumor size of 0.40 mm, while patients with CLND had a median maximum SN tumor size of 1.40 mm.*
† After matched-pair analysis, both groups had a median maximum SN tumor size of 0.40 mm.

(> 1.00 – 4.00 mm) are most likely to have therapeutic benefit from undergoing CLND.[83] Other significant predictive factors such as primary tumor thickness should be considered in the decision to perform CLND.[60, 84]

MULTICENTER SELECTIVE LYMPHADENECTOMY TRIAL – 2 (MSLT-2) AND EORTC 1208 STUDY (MINITUB)

Prospective trials are evidently the only studies that can overcome the drawbacks of above-mentioned nonrandomized retrospective studies. A randomized controlled trial, i.e. the Multicenter Selective Lymphadenectomy Trial-II (MSLT-II), is currently ongoing to investigate if all SN-positive patients have therapeutic benefit when CLND has been performed. Unfortunately, SN tumor burden is not taken into consideration. Chapters 4, 5, 6 and 7 of this thesis underline the strong prognostic impact of SN tumor burden on melanoma specific survival. Obvious benefit of the MSLT-2 is the fact that it is a randomized controlled trial. MSLT-II opened in 2005, the estimated

completion date is in 2022. A prospective single arm study by the EORTC Melanoma Group, the EORTC 1208 or "Minitub" study, is in process to examine if patients with minimal SN tumor burden are safely to undergo observation instead of CLND. Patients with minimal SN tumor burden are defined as patients with metastases ≤ 0.1 mm anywhere or ≤ 0.4 mm subcapsularly located. We have to wait for preliminary results from both these studies to make evidence based clinical decisions regarding the management of patients. Based on the results of studies performed in a large multicenter international retrospective setting it might be safe to withhold CLND in SN positive patients with ≤ 0.1 mm metastases, especially when located subcapsularly, when an extensive SN pathology work-up protocol is assessed. All other SN positive patients should undergo CLND.

MANAGEMENT OF PATIENTS WITH MACROSCOPIC (CLINICALLY DETECTABLE) DISEASE

Compared to the more heterogeneous range in 5-year survival of approximately 30 to 90% in patients with nodal microscopic disease, patients with nodal macrometastases have a narrower range of 5-year survival of around 30 to 50%.[51] Chapter 10 demonstrated that patients who underwent a neck dissection had a survival benefit over patients with inguinal dissection. This is counter-intuitive to head and neck melanomas, which are associated with worse prognosis compared to truncal or extremity melanomas. A less predictive drainage pattern of the head and neck area might be one of the causes. (Chapter 3) At the same time, it might also reflect more rigorous patient selection in case a neck dissection is considered compared to other dissection types. To our best knowledge, only one study analyzed the differences between prognoses of different lymph node dissection sites. Results stated in chapter 10 were confirmed by Wevers et al; performing a neck dissection was an independent prognostic factor for improved MSS over other dissection sites.[85] Earlier detection of palpable disease in the head and neck region might have an impact of the improved outcome.

In part two of the present thesis, it has been demonstrated that the number of positive nodes was the most important prognostic factor in patients with macrometastases. Extracapsular extension, postoperative seroma, adjuvant radiotherapy and nodular histological subtype were other independent prognostic factors for survival. Analysis of the AJCC staging system committee demonstrated that outcome in patients with macrometastases (n=440) was not predicted by primary melanoma characteristics.[51] In the most recent years, the number of positive nodes divided by the number of excised nodes, i.e. the lymph node (LN) ratio, has gained in popularity. LN ratio is an independent prognostic factor for survival.[86-88] Furthermore, it can be

used as a quality parameter. It has been suggested that removal of at least 15, 8 and 6 lymph nodes per positive lymph node in, respectively, the neck, axilla and groin lymph node fields should maintain good surgical quality.[89] More research needs to be performed to detect if LN ratio is a stronger predictor for survival than the number of positive nodes. In other types of cancer such as gastric cancer and colorectal cancer, LN ratio was an independent prognostic factor for survival as well.[90, 91] The number of positive nodes was the most prognostic factor in a predictive tool, i.e. a nomogram, which has been created in chapter 8 to predict 2-year recurrence in patients with macroscopic disease who underwent TLND. A web-based predictive tool has been created for clinicians to predict overall outcome for early-stage melanoma patients (www.melanomaprognosis.org). Multiple independent predictors of survival for patients with stage III melanoma were assessed to calculate estimated 1-, 2-, 5- and 10-year survival rates. The number of positive nodes remained the most important independent predictor of survival.

Before the era of SNB, most melanoma patients presented with macroscopic (clinically detectable) disease.[92] Primary management of patients presenting with macroscopic disease and no presence of distant metastases has not changed. A therapeutic lymph node dissection (TLND) should be performed. Controversial is which level of dissection should be assessed, especially in the groin area. For micrometastases detected by SN, performing combined superficial (inguinal) and deep (iliac) lymph node dissection (CGD) is more controversial than for palpable macrometastatic disease. Deep lymph node involvement occurs in 15% of SN positive patients and in approximately 30% of patients with palpable disease.[93] Spillane et al. state that all patients should be considered for CGD, especially when having palpable disease, due to high rates of pelvic disease.[94] Of patients who underwent CGD, 39% had involved deep LNs (of which only 9.3% had surgery for a positive SN). Pelvic recurrence in patients who underwent superficial groin dissection (SGD) was only 6.7% (7 of 105). In chapter 9, this rate was 10%. Deep lymph node involvement was present in 25% of CGD patients. As mentioned in chapter nine and according to most literature, DFS and OS were not significantly different between performing SGD or CGD.[95-97] No melanoma patients with one superficial positive node had positive deep lymph nodes and preoperative CT had a high negative predictive value for deep lymph nodal involvement. Patients with one positive palpable superficial groin lymph node can be advised to undergo superficial groin dissection. Thus, we hypothesize that the "watch and wait" approach for pelvic involvement is safe. There has never been a randomized controlled trial which compared CGD vs SGD for palpable disease. Prospective trials will provide more definite conclusions whether deep iliac nodal removal can be avoided in a specific subgroup of patients. Currently two studies are being set-up worldwide to examine this issue.

Chapter 10 and 11 of the present study have similar results. Patients with macrometastases who underwent TLND having an unknown primary melanoma (MUP) had improved survival over those with a known primary (MKP). This confirms the results of the largest study performed to date by Lee et al.[28] Management of MUP patients continues to be similar to management of MKP patients. The improved DFS, DMFS and MSS and similar management underline the insignificance of rigorous searching for a primary melanoma when palpable disease is present.[98] The cause of the improved outcome is unknown, however, regression of the primary or the de novo origin of melanoma cells in lymph nodes might play a role.

FUTURE PERSPECTIVES

Final results of the MSLT-1 are tanxiously o be awaited within the next year(s). A therapeutic benefit for all SNB patients will most probably not be identified. Patients with intermediate thickness melanoma (between 1 and 4 mm in Breslow thickness) might have therapeutic benefit from undergoing SNB. The therapeutic benefit of CLND for SN-positive patients is unknown. It will take 10 to 15 years for MSLT-2 and EORTC 1208 study to come up with final results and definitive answers to whether CLND has a therapeutic benefit and to whether some SN positive patients could safely be spared CLND. In the meantime the discussion will carry on and interim-results are already highly awaited.

Compliance and agreement on a routine pathology work-up protocol is essential in this discussion. For example, an extensive pathology protocol will identify more clinically irrelevant micrometastases than a limited protocol. In future pathology slide analyses, we recommend that at least the maximum SN tumor size, the intranodal location and tumor penetrative depth will be analyzed, though correctly measured, and will be classified according to the Rotterdam criteria, Dewar criteria and S-classification. SN tumor burden will become important for clinical decision-making regarding future adjuvant medical (e.g. interferon-alpha, molecular-targeted strategies (BRAFi/MEKi) and/or immunotherapy (anti-CTLA-4 / anti-PD-1)) and/or surgical (e.g. CLND) therapy. Molecular profiling of primary melanomas might add new promising risk factors as lymphangiogenic biomarkers to currently used clinico-pathological features. Imaging techniques as PET-CT scans and ultrasound-guided fine needle aspiration cytology (US-FNAC) have already and will shine new lights on SN management in the forthcoming years. Regarding macroscopic (clinically detectable) disease in the groin area, prospective trials are currently being set up to provide more definite conclusions on the necessity of iliac lymph node dissection.

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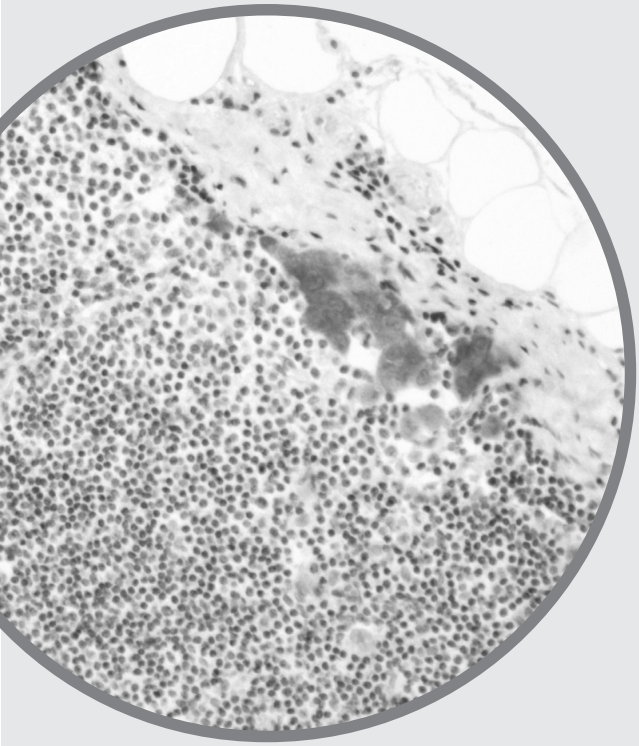
Chapter 15

Acknowledgements



De afgelopen jaren hebben velen in meer of mindere mate een bijdrage geleverd aan de totstandkoming van dit proefschrift. Hieronder is getracht om hen met gebruik van de juiste superlatieven te bedanken.

Mijn hooggeleerde promotor, prof.dr. A.M.M. Eggermont. Beste Lex, ook al verliet je vrij snel Rotterdam voor het grotere doel in Parijs (en de rest van de wereld…) waarmee je tijdschema nog drukker werd dan het al was, nooit heb ik het idee gehad dat het ten koste ging van mij. Als een onuitputtelijke bron van energie, kennis en humor was je tijdens onze contacten van onschatbare waarde. Bedankt voor alle ervaringen die je mij geschonken hebt.

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My supervisors and colleagues at the Melanoma Institute Australia in Sydney, Australia; dear prof.dr. J.F. Thompson, Prof, dear prof.dr. R.E. Scolyer, Richard, dear Dr. Spillane, Andrew, dear Lauren, dear Kaye and all the others that made my stay at the institute so easy; Thank you for your support and time that you have spent on me during (and after) my stay at your beautiful Poche Centre. I had the time of my life in Sydney and hope to return to Melanoma Institute in the near future to pay you all a visit. A special thanks goes to Sandro, my Italian colleague from Padova, thank you for all the laughter and interesting discussions during coffee and lunch breaks.

Good luck with your clinical, scientific and social career in the UK. I will never forget our time at the MIA.

The EORTC Melanoma Group; thank you for your endless support in the last four years. Dear members of the Melanoma Group, thank you for inspiring moments during conferences and meetings. Dear Stefan (Suciu), thank you for your effortless help with statistics. Beste Senada (Koljenovic), jij was er vanaf het begin tot het einde bij; van pathologie slides in het Josephine Nefkens tot Milaan, Mallorca en uiteindelijk met de fotograaf weer terug naar de slides. Dank voor je gezelligheid en geduldige hulp met het maken van de cover tussen je eigen drukke werkzaamheden door. Jij ook succes met jouw verdere succesvolle carrière.

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Lieve familie, Pap en Mam, Frederique, Robbert, Bas en Julia, Iris, Jeroen en Claas, Hans en Marlieke, hoe fijn zijn de momenten dat wij samen zijn, zeker in het Zuiden. Ik heb zo'n idee dat de familie uitbreiding nog niet ten einde is.. En gelukkig maar, want het wordt er alleen maar gezelliger op. Het is heerlijk om met je naasten over je vak te kunnen praten, maar gelukkig hebben wij er genoeg niet-medico's bij gehaald voor een gezond evenwicht.

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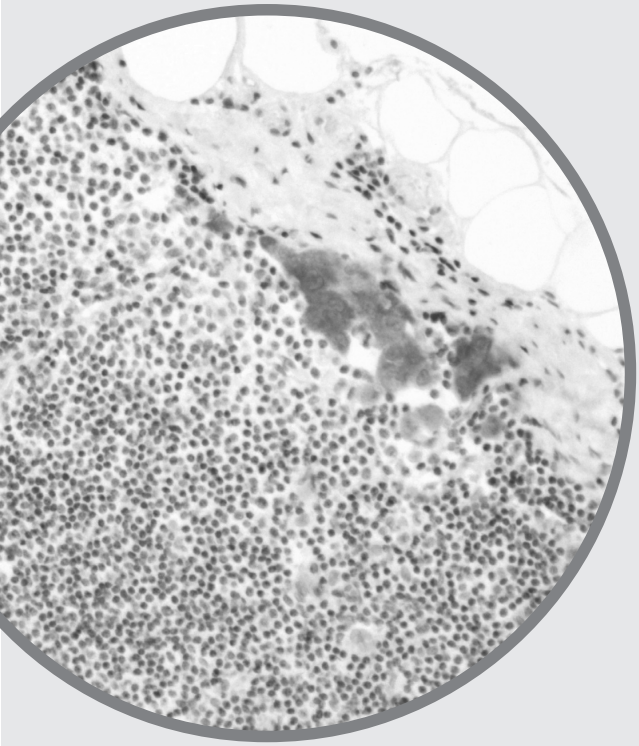
Chapter 16

List of publications



- van der Ploeg AP, van Akkooi AC, Schmitz PI, Koljenovic S, Verhoef C, Eggermont AM.
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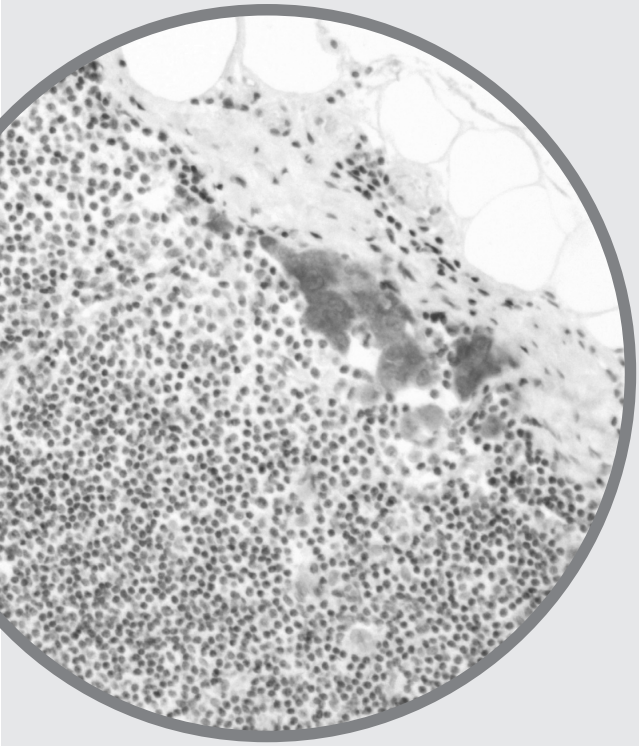
  Outcome following sentinel node biopsy plus wide local excision versus wide local excision only for primary cutaneous melanoma: analysis of 5840 patients treated at a single institution
  Ann Surg, in press

Chapter 17

Curriculum vitae

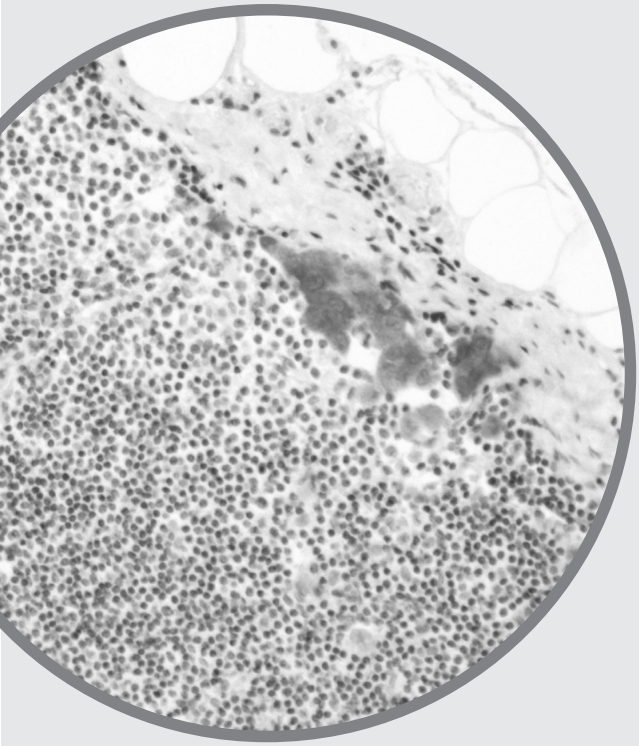


Augustinus Paulus Theodorus van der Ploeg, the author of this thesis, was born on the 6th of July 1987 in Heerlen, the Netherlands. Raised in Valkenburg, the Netherlands, Stijn finished high school (gymnasium) in 2005 at the Bernardinuscollege in Heerlen. In the same year, he continued to study Medicine at the Erasmus University Rotterdam. He performed research in 2009 regarding the value of sentinel node biopsy in melanoma patients at the department of Surgical Oncology at the Erasmus Medical Center – Daniel den Hoed Cancer Center in Rotterdam, the Netherlands, under supervision of Dr. A.C.J. van Akkooi and Prof.dr. A.M.M. Eggermont. After finishing the first four years of Medicine and receiving his doctorate title in January 2010, he interrupted his studies temporarily to continue to work at the department of Surgical Oncology as a PhD student under supervision of the above mentioned and Prof.dr. C. Verhoef. In 2010, he spent eight months in Sydney, Australia working on his PhD thesis at the Melanoma Institute Australia under supervision of Prof. dr. J.F. Thompson and Prof.dr. R.A. Scolyer. During his time as a research fellow in Rotterdam and Sydney, he has become a young investigator of the European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group and worked on several projects regarding the prognosis and management of early stage melanoma patients. This resulted in the present PhD thesis. He was awarded with the Young Investigator Award during the seventh ISNS meeting in Yokohama, Japan in November 2010, the Stichting Hippocrates Studiefonds award in July 2011 and the Outstanding Abstract Award during the Paris Melanoma Conference in Paris, France in April 2012. He continued his medical studies in August 2011. After finishing his electives of trauma surgery at the Groote Schuur Hospital in Cape Town, South Africa and general surgery at the Ikazia hospital in Rotterdam, he obtained his medical degree on 23 August 2013. He started working as a resident of surgery at the general surgery department of the Ikazia hospital in Rotterdam, the Netherlands on 1 September 2013.

Augustinus Paulus Theodorus van der Ploeg, de auteur van dit proefschrift, is geboren op 6 juli 1987 te Heerlen. Stijn groeit op in Valkenburg, Zuid-Limburg, en behaalt zijn gymnasium diploma aan het Bernardinuscollege te Heerlen in het jaar 2005. In september 2005 start hij de studie geneeskunde aan de Erasmus Universiteit Rotterdam. Eind 2009 verricht hij zijn afstudeeronderzoek over de waarde van sentinel node biopsie bij melanoompatiënten op de afdeling Chirurgische Oncologie van de Daniel den Hoed kliniek te Rotterdam, verbonden aan het Erasmus Medisch Centrum, onder leiding van Dr. A.C.J. van Akkooi en Prof.dr. A.M.M. Eggermont. In januari 2010 behaalt hij zijn doctoraal waarna hij zijn studie tijdelijk onderbreekt om te promoveren op dezelfde afdeling in de Daniel den Hoed kliniek onder leiding van bovengenoemde en Prof.dr. C. Verhoef. Tijdens zijn promotie verblijft hij in 2010 voor een periode van 9 maanden in Sydney, Australië, waar hij werkzaam is in het Poche Centre, Melanoma Institute Australia, onder leiding van Prof.dr. J.F. Thompson en Prof.dr. R.A. Scolyer. Tijdens zijn periode als onderzoeker in Rotterdam en Sydney heeft hij aan verschillende projecten gewerkt betreffende melanoompatiënten met lymfekliermetastasen. Dit heeft geleidt tot dit proefschrift. In november 2010 verkrijgt hij de Young Investigator Award op het zevende ISNS congres in Yokohama, Japan, in juni 2011 de Stichting Hippocrates Studiefonds prijs en in april 2012 de Outstanding Abstract Award op de Paris Melanoma Conference in Parijs, Frankrijk. In augustus 2011 is hij begonnen aan zijn coschappen om zijn studie geneeskunde af te ronden. Op 23 augustus 2013 heeft hij zijn artsexamen behaald nadat hij zijn keuze coschap trauma chirurgie in het Groote Schuur ziekenhuis te Kaapstad, Zuid-Afrika en zijn oudste coschap algemene chirurgie in het Ikazia ziekenhuis te Rotterdam succesvol heeft afgerond. Vanaf 1 September 2013 is hij begonnen als arts-assistent chirurgie in het Ikazia ziekenhuis te Rotterdam.

Chapter 18

PhD portfolio



SUMMARY OF PhD TRAINING AND TEACHING ACTIVITIES

Name PhD student:
Augustinus Paulus Theodorus van der Ploeg
Erasmus MC Department:
Surgical Oncology
PhD period:
January 2010 – February 2014

Promotors:
Prof. A.M.M. Eggermont, MD PhD
Prof. C. Verhoef, MD PhD
Supervisor:
Dr. A.C.J. van Akkooi, MD PhD

1. PhD training		
	Year	**Workload (ECTS)**
General courses		
- Introduction to clinical research	2011	1
- Biostatistics for clinicians	2011	1
- Good Clinical Practice	2013	0.5
Research skills		
- Statistics (SPSS and STATA)	2010	2
Presentations – National conferences		
- NVvH Chirurgendagen 2011	2011	0.5
- NVvH Chirurgendagen 2012	2012	0.5
Presentations – International conferences		
- 7th SMR meeting, Sydney, Australia	2010	0.5
- 7th ISNS meeting, Yokohama, Japan	2010	0.5
- Annual SSO meeting, San Antonio, Texas, USA	2011	0.5
- 2011 EMCC, Stockholm, Sweden	2011	1
- 2011 EORTC MG fall meeting, Barcelona, Spain	2011	1
- Annual Paris Melanoma Conference, Paris, France	2012	0.5
- 2012 EORTC MG fall meeting, Milan, Italy	2012	1
- 2013 EMCC, Amsterdam, the Netherlands	2013	1
- 2013 EORTC MG fall meeting, Mallorca, Spain	2013	0.5
2. Teaching activities		
	Year	**Workload (ECTS)**
Supervising		
- Graduate students	2010-2013	5