Sentinel Node Tumor Load Assessment in Melanoma

Dilemmas and Clinical Management

Alexander Christopher Jonathan van Akkooi

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Dilemmas and Clinical Management

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Dilemmas en Klinische Consequenties

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Rien sans Dieu (nothing without God), the van Akkooi family motto.

Contents

Chapter 1

General Introduction to the Thesis

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INTRODUCTION

Malignant Melanoma is the most aggressive form of skin cancer. Worldwide, the incidence of melanoma has risen sharply of the past three decades[1-5]. On the 1st of January 2007, there were nearly 800,000 people alive in the USA alone, who were diagnosed with a melanoma[6]. This increase is characterized largely by an increase in thin melanomas (1 mm or less; T1 tumors). Prognosis of American Joint Committee on Cancer (AJCC) stage I / II ranges between 95% for T1 and 45% for T4 melanomas[7].

DISSEMINATION

There are two general hypotheses on the metastatic spread of melanoma: the first is that melanoma will undergo a simultaneous lymphatic and hematogenous spread. Lymph node metastases are considered indicators, not governors, of metastatic disease. The regional lymph nodes may be the first site where metastatic disease is diagnosed as a result of sentinel node staging or as the first clinical appearance of metastases by ultrasound or by physical examination. In the absence of the utilization of early diagnostic tools however, regional nodal involvement will become apparent only when involved nodes become palpable.

Evidence for this hypothesis can be found in a study by Meier et al., where 50.2% of 466 melanoma patients presented with regional lymph node metastases as first metastatic site and 28.1% presented with a distant metastasis as first site, yet in both groups the time to distant metastases was nearly identical (both after a median time of approximately 25 months)[8]. Another study by Slingluff et al. in almost 5000 patients demonstrated virtually identical rates of lymph node metastases, distant metastases and mortality for melanoma patients per primary tumor thickness category[9].

Moreover, 4 randomized controlled trials (RCT) on elective lymph node dissections (ELND) where unable to demonstrate a survival benefit for early lymphadenectomy[10-13]. Thus, there seems to be a simultaneous lymphatic and hematogenous spread of metastatic disease.

The second hypothesis is that melanoma spreads through a cascade of orderly progression, where the regional lymph nodes serve as governors of disease, rather than indicators of metastatic spread. The disease will spread from the primary tumor to the regional lymph nodes, before eventually disseminating to distant organs, which finally becomes the cause of death for melanoma patients[14].

This hypothesis led to the propagation of elective (immediate) lymph node dissections and to a series of RCTs to investigate the potential improvement on survival due to this procedure. When it was found that elective lymph node dissections could not establish

a significant survival benefit for the entire population, it was suggested that there might be a subgroup of patients that could benefit from ELND. However, the effect of ELND was diluted, due to the majority (80%) of patients, who are node negative. Therefore, it was suggested that specific targeting of node positive patients by CLND after positive SN procedure, would potentially lead to a survival benefit[15].

ELECTIVE LYMPH NODE DISSECTION (ELND)

The excellent meta-analysis by Hochwald and Coit[16] has analyzed numerous retrospective series of ELND versus Wide Local Excision (WLE) only, followed by a Therapeutic Lymph Node Dissection (TLND), only in those cases with regional lymph node recurrences. These retrospective studies have demonstrated conflicting results, some in favor and some opposed to the routine use of ELND[9,16-30].

More importantly, the 4 randomized controlled trials (RCT) to address this subject; the WHO-1[10], the Mayo Clinic Trial[11], the Intergroup trial[31] and the WHO-14[12], could not demonstrate a clear survival benefit for populations, where about 20% had occult nodal metastases.

However, subgroup analyses have suggested a potential survival benefit in a subset of patients < 60 years of age with intermediate thickness (1 – 2mm) melanomas, especially the non-ulcerated melanomas[31]. It has also been suggested that node positive patients in the ELND arm had an improved survival compared to the node positive WLE only patients, although this did not translate into any overall survival benefit for all patients[12]. Some have suggested this effect to be diluted by the majority of node negative patients and claimed that only specific targeting of node positive patients could demonstrate a survival benefit[15]. This has been one of the basis to investigate the therapeutic value of the sentinel node biopsy followed by a CLND.

SENTINEL NODE HYPOTHESIS

Donald Morton, introduced the SLNB in melanoma in the early 1990s[15]. The SN has been defined as the first draining lymph node from a tumor. This node is the node at greatest risk to harbor (occult) metastases, as it is the first station in the cascade. The selective biopsy, through a minimally invasive procedure, and extensive pathological examination of this SN should be able to accurately predict both survival and further non-SN (NSN) lymph node metastases in the same lymph node basin for melanoma patients.

Although the SN procedure is primarily a staging procedure, it was suggested that the use of this procedure would identify a subgroup of patients, only those with clinically

occult, microscopic metastases, who might benefit from early completion lymph node dissection (CLND), whilst sparing the majority of melanoma patients an unnecessary and morbid ELND.

SNLB TECHNIQUE AND INDICATION FOR SLNB

The sentinel node is usually detected by the use of the triple technique, which includes a pre-operative lymphoscintigraphy, the intraoperative use of patent blue and the intra-operative use of a Geiger counter[32].

SN positivity rates vary considerably in the literature, usually rates around 15% to 20% are reported, but rates may vary from 10% to 30% in selected populations, depending on the mean/median Breslow thickness, percentage of ulcerated primary tumors of the population and the extent of the pathological work-up used to examine the SN[32-35]. A study by Doubrovsky et al. demonstrated that SN staging identified micrometastases more accurately than bivalving did with ELND[36].

HISTO-PATHOLOGICAL ASSESSMENT OF THE SN

Basically, three 'standard' pathology protocols are currently being used worldwide for the work-up of SNs; the John Wayne Cancer Institute (JWCI) protocol developed by Cochran et al.[37], the Sydney Melanoma Unit (SMU) protocol by Scolyer et al.[38] and the EORTC Melanoma Group protocol by Cook et al.[39] Although some individual centers' protocols may vary slightly from these 'standard' approaches.

Most authors recommend to bisect the SN through the hilum and its longest dimension, because it has been shown that most SN metastases are located close to the central meridian[40], although it has also been suggested to slice into 1mm slices from a random point[41]. This should be followed by the examination of multiple slides from each half of the node by haematoxylin-eosin (HE) and by Immunohistochemistry (IHC).

Thereafter, there is no consensus on how many sections should be examined, how big the step-interval should be, and which immunostains should be used. Although, the most commonly used immunostains include: S-100, HMB-45, Melan-A / MART-1 and / or tyrosinase[37,39,42-44].

Cook et al. demonstrated that progressively more detailed pathology work-up protocols lead to an increase in SN positivity, which was only marginally lower than RT-PCR identified positivity rates, but virtually free of false positive results[39].

AIM OF THIS THESIS

The subject of **Part I** of this thesis is sentinel node tumor burden in melanoma. In **Chapter 2** we report on the rate of sentinel node positivity of 262 melanoma patients from the Erasmus University Medical Center – Daniel den Hoed Cancer Center, who underwent a sentinel node procedure. Moreover, outcome of these patients was analyzed. **Chapter 3** describes the European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group methods for the measurement of SN tumor burden and the microanatomic location of metastases within the SN. In **Chapter 4** we have first analyzed our own experience and outcome related to SN tumor burden in 77 SN positive patients from the Erasmus University Medical Center – Daniel den Hoed Cancer Center. This lead us to analyze the impact of minimal SN tumor burden on the possible survival benefit for the SN procedure and early completion lymph node dissection versus patients, who did not undergo a SN, but developed lymph node metastases, which required a lymph node dissection during follow-up. This is described in **Chapter 5**. We validated the results from our single center experience on SN tumor burden in a multicenter fashion in **Chapter 6**. Finally, the results of SN tumor burden and the influence of the microanatomic location were analyzed and validated in 1080 SN positive patients from 10 EORTC MG centers, which is shown in **Chapter 7**.

In **Part II** an alternative staging procedure, ultrasound (US) guided fine needle aspiration cytology (FNAC) was analyzed. In **Chapter 8** we report on the results of US-guided-FNAC in 400 stage I / II melanoma patients. **Chapter 9** analyzes to a deeper extent the new set of morphology criteria, which were used to increase the accuracy of US-guided-FNAC as alternative staging procedure. **Chapter 10** reports on the use of Reverse Transcription Polymerase Chain Reaction (RT-PCR) to possibly further increase SN positivity rates.

Finally, **Chapter 11** summarizes the entire thesis, but also a general discussion will be presented here, together with the conclusions of this work.

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Part I

Sentinel Node Positivity and SN Tumor Burden Heterogeinty, Assessment and Prognosis

Chapter 2

High positive sentinel node identification rate by EORTC melanoma group protocol Prognostic indicators of metastatic patterns after sentinel node biopsy in melanoma

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ABSTRACT

Methods to work-up sentinel nodes (SN) vary considerably between institutes. This single institution study evaluated the positive SN-identification rate of the EORTC Melanoma Group (MG) protocol and investigated the prognostic value of the SN status regarding disease-free survival (DFS) and overall survival (OS) and evaluated the locoregional control after the SN procedure. Multivariate and univariate analyses using Cox's proportional hazard regression model was employed to assess the prognostic value of covariates regarding DFS and OS.

The positive SN-identification rate was 29% at a median Breslow thickness of 2.00 mm and the false-negative rate was 9.4%. Breslow thickness and ulceration of the primary correlated with SN status. SN status, ulceration and site of the primary tumour correlated with DFS. SN status and ulceration of the primary correlated with OS. The in-transit metastasis rate correlated with SN-positivity, Breslow thickness and ulceration. Projected 3-year OS was 95% in SN-negative and 74% in SN-positive patients. Transhilar bivalving of the SN with step sections from the central planes is simple and had a high SN-positive detection rate of about 30%. The SN status is the most important predictive value for DFS and OS. In-transit metastasis rates correlated with SN-positivity, Breslow thickness and ulceration of the primary.

INTRODUCTION

Of all the different types of cancer, melanoma has a share of 1% of all cases. Metastatic behavior and survival correlate with risk factors such as tumour thickness and the presence of ulceration of the primary, the presence and number of metastatic regional lymph nodes and non-visceral or visceral metastases[1].

A number of underpowered randomized trials have evaluated the impact of the adjuvant surgical procedure the elective lymph node dissection (ELND) in melanoma and have failed to demonstrate a survival advantage by ELND[2-5]. The most recent randomized trial, the WHO 14 demon-strated a potential benefit in patients with micrometastatic disease in the ELND specimen[5] and suggested that the Sentinel Node procedure might therefore be of benefit to patients in the management of primaries >1.5 mm.

Also the long-term follow-up results of the USA Intergroup trial showed some potential benefit in patients with melanomas of intermediate thickness[4], as did a database matched paired analysis in patients with primary melanomas between 1.2mm and 3.5 mm, by Morton and co-workers[6].

At the basis of these developments is the work of Mortonin the late 1980's and early 1990's, who formulated the sentinel node (SN) procedure, which is based on the concept that a tumour will undergo an orderly progression of dissemination with the local lymphatic system as primary route of metastasis[7]. Whether this SN procedure, followed by complete lymph node dissection in case of a positive SN, results in survival benefit has been investigated in the Multicenter Selective Lymphadenectomy Trial (MSLT-I), which has not yet reached full maturity for final analysis.

Identification rates of positive SN in patients with primary melanomas thicker than 1.0 mm vary considerably in the literature. Usually rates of 15–20% are reported. Vulysteke[8] found 19% SN-positive patients in a total of 209 patients with a median Breslow thickness of 1.41 mm. Doubrovsky[9] and Gerschenwald[10] found 18% and 15% SN-positive patients in a total of 672 and 580 patients with amedian Breslow thickness of 2.30mm and 1.80mm respectively. Balch[1] and Morton[11] found 13.9% and 19% SN-positive patients in a total of 3126 and 1159 patients respectively.

Methods to work-up SN vary considerably between institutes. This single institution study evaluates the positive SN-identification rate of the EORTC Melanoma Group (MG) protocol. This study also investigates the prognostic value of the SN status regarding disease-free survival and overall survival and it evaluates the locoregional control, specifically on recurrence patterns in the SN investigated lymph node basin(s) and on rates of in-transit metastasis after the SN procedure.

PATIENTS AND METHODS

Patients

From October 1997 to May 2004, 262 patients with malignant melanomas, with a Breslow thickness of at least 1.00mm and/or at least a Clark level IV or if ulceration was present, underwent a sentinel lymph node biopsy (SLNB) at our institute (Erasmus Medical Center, Daniel den Hoed Cancer Center, Rotterdam, the Netherlands). Patient characteristics, operation notes and follow-up were all entered in a prospective database. The average age was 48 years (range 16–83 years). The mean Breslow thickness was 2.76mm (range 0.60–15.00 mm). The baseline characteristics of these 262 patients are described in Table 1.

Triple technique

To identify and retrieve the correct and all SN, the triple technique was used[12,13]. Firstly, a preoperative lymphoscintigraphy (LS) after four intradermal injections of 99m-labeled Tcalbumin nanocolloid (Nanocoll, Amersham Health, Gipharma, Saluggia, Italy) around

Table 1 Characteristics for all 262 patients

	N	%
Gender		
Male	116	44%
Female	146	56%
Primary Tumor Location		
Arm	40	15
Leg	113	43
Trunk	91	35
Head/Neck	18	7
Histology		
SSM	126	48
NM	90	34
ALM	4	2
Other	3	1
Unclassified	39	15
Clark Level		
II	5	2
III	110	42
IV	121	46
V	10	4
Undeterminable	16	6
Ulceration		
Present	73	28
Absent	189	72

SSM = Superficial Spreading Melanoma, NM = Nodular Melanoma, ALM = Acrolentiginous Melanoma.

the excision site of the primary tumour on the day of the surgery was performed. Scanning was carried out immediately after the injection and again after 2 h. Secondly, intraoperative use of handheld gamma detection probe (Europrobe, PI Medical Diagnostic Equipment B.V., Sneek, the Netherlands) was used to verify the location of SNs. And finally, shortly before surgery, patent blue dye (Laboratoire Guerbet, Aulnay-sous-Bois, France) was injected intradermally next to the initial site of the melanoma, to help localize the SN visually during the operation.

A lymph node was considered to be a SN if it was stained blue, 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[14,15].

Surgical procedure

Most of the patients had already undergone (diagnostic) excision of the primary tumour elsewhere. They were treated by a local wide re-excision (with margins according to the guidelines of the Dutch Melanoma Workgroup), unless the diagnostic excision was considered wide enough or if the primary tumour was located in regions of the body where re-excision could not be performed with primary closure. During the same operation, the SN(s) were surgically removed with the help of the triple technique as described previously. In 10 patients re-excision had already taken place in another hospital and only removal of the SN(s) was performed.

Pathological analysis

All sentinel nodes after June 2002 (n = 112) were sent for pathological assessment according to the protocol by Cook[16], which is the EORTC MG guideline for pathological examination of SN. In brief, lymph nodes were fixed for 24 h in buffered formalin. After fixation they were cut in half through the hilum and its longest dimension and embedded in paraffin. In rare cases, exceptionally large nodes were sectioned parallel to the first cut in order to fit in the blocks. Five serial step sections of 4 lm each were cut from each face of the lymph node, and staining with H&E, S100 and HMB-45 was performed. There was a slight difference between the protocol by Cook[16] and that used before June 2002 (n = 150), in that serials sections were made with 50 lm intervals, which were 250 lm intervals before that time.

Follow-up

Patients were all followed at the outpatient clinic. Recurrences were scored as local recurrence, in-transit metastasis, regional lymph node recurrence, distant lymph node metastasis, subcutaneous metastasis or visceral metastasis. Patients with a negative SN who developed recurrence in the sentinel lymph node basin were further analyzed as false negative SN biopsy patients.

Statistical analysis

Categorical variables were tabulated by SN status and the imbalance of those groups was tested using the Fisher's exact test. Imbalances in continuous variables were tested using the Kruskal–Wallis test. Disease-free and overall survival was defined as time from SN biopsy till recurrence or death respectively. In-transit metastasis was defined as time from SN biopsy till in-transit recurrence. Patients without such an event on their last contact were censored at that time. Analysis of those endpoints was performed using the Kaplan–Meier approach. The log-rank test was used to evaluate a difference in survival between groups. Univariate and multivariate analyses using the Cox's proportional hazard regression model were performed to assess the prognostic value of covariates with respect to disease-free survival and overall survival. Few values for Clark and Breslow were missing. A single imputation algorithm was used in order to include those patients in the multivariate analysis. P-values of less than 0.05 were considered as significant. All statistical analyses were performed with Stata version 8.2 (Stata Corporation, College Station, Texas, USA).

RESULTS

SN identification and status

In 262 patients, 256 underwent preoperative lymphoscintigraphy (LS), 6 did not have the preoperative lymphoscintigraphy due to logistical problems. In these 256 patients, a total of 334 lymph node basins were recognized through LS, with a total of 601 lymph nodes recognized. This resulted in an average of 1.80 lymph node per lymph node basin. During all the operations a total of 510 lymph nodes were harvested (they were either stained blue and/or radioactive, see Patients and Methods), with an average of 1.95 lymph node per patient. It also yielded a rate of 85% of all the nodes found in the LS. However, at least one SN was found in all patients and therefore the procedure was considered to have a success rate of 100%.

In the 262 patients, 77 patients (29.4%) were considered to have a positive SN after the pathological examination of their nodes. There were no differences in SN-positivity between gender and age. Median Breslow thickness was 1.90mm for SN-negative patients and 2.95mm for SN-positive patients. The distribution pattern of tumour characteristics for SN-positive patients is summarized in Table 2.

Not shown in Table 2 is the analysis of the two different patient cohorts, for which two slightly different pathology protocols, as mentioned previously, were used. These two cohorts (250 lm versus 50 lm intervals) did not significantly differ from each other for mean Breslow thickness, 2.94mm versus 2.54 mm, or ulceration, 27% versus 29%. Both cohorts also did not significantly differ from each other for positive SN identification rate, 30.7% versus 27.7%.

Table 2 Characteristics for SN positive patients

	N	%	P
Primary Tumor Location			
Arm	8	20	
Leg	35	31	
Trunk	32	34	
Head/Neck	3	17	n.s.
Histology			
SSM	38	30	
NM	28	31	
ALM	1	25	
Other	1	33	
Unclassified	9	23	n.s.
Breslow Thickness			
< 1.00 mm	2	17	
1.01 – 2.00 mm	25	21	
2.01 – 4.00 mm	27	34	
> 4.00 mm	20	48	0.005
Clark Level			
II	2	40	
III	33	30	
IV	33	27	
V	4	40	
Undeterminable	5	31	n.s.
Ulceration			
Present	30	41	
Absent	47	25	0.015

SSM = Superficial Spreading Melanoma, NM = Nodular Melanoma, ALM = Acrolentiginous Melanoma.

A total of 76 completion lymphadenectomies were performed in 77 SN-positive patients. One patient refused completion lymphadenectomy. Of the 76 patients who underwent completion lymphadenectomies, 61 did not reveal any further positive nodes (80%). Five patients (7%) had one additional metastatic node and ten patients (13%) had two or more additional metastatic nodes.

Recurrences

The median follow-up was 23.3 months (range: 0–82 months). The estimated 3-year overall recurrence-free survival for SN negative patients was 88% and 52% for SN-positive patients ($P < 0.001$). The estimated 5-year overall recurrence-free survival for SN-negative patients was 87% and 51% for SN-positive patients ($P < 0.001$) (Fig. 1b). Table 3 shows the distribution of the first recurrence sites. SN positive patients had a significantly increased risk of developing any form of recurrence compared to SN-negative patients.

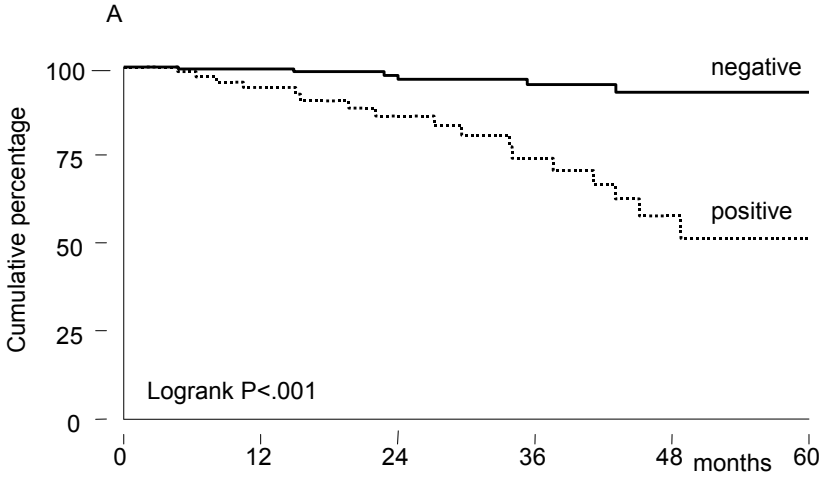

Figure 1A Kaplan-Meier estimated 5-year survival curves for overall survival

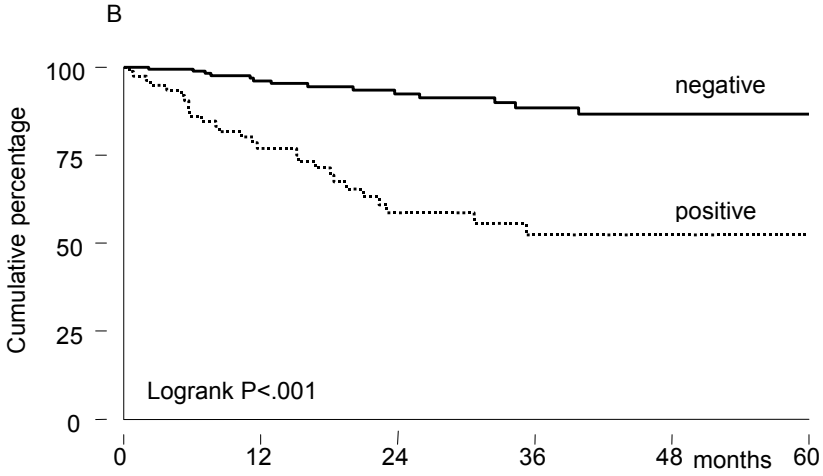

Figure 1B Kaplan-Meier estimated 5-year survival curves for disease-free survival

In-transit metastases

The estimated 5-year in-transit metastasis rate was 8% for SN-negative patients versus 20% for SN-positive patients ($P < 0.001$) (Fig. 2a). The estimated 5 year in-transit metastasis rate was also significantly dependent upon the Breslow thickness ($P < 0.001$) (Fig. 2b) and ulceration status ($P = 0.03$) (Fig. 2c).

False negative results

Thus far, 8 false negative patients were seen (9.4% false negative rate). All these SN were retrospectively reviewed by a staff pathologist (M.K.) and remained node-negative. In three patients, fewer nodes were retrieved than seen on the lymphoscintigraphy. These

Table 3 The distribution of all first recurrence sites

Type of first recurrence	SN neg	%		SN pos	%	
Locoregional failure						
Local recurrence	2	1.1		4	5.2	
In-transit metastasis	4	2.2	6.5	7	9.1	22.1
Regional lymph node	6	3.2		6	7.8	
Distant failure						
Distant lymph node	1	0.5		3	3.9	
Subcutaneous metastasis	2	1.1	3.8	3	3.9	27.3
Visceral metastasis	4	2.2		15	19.5	
Total	19	10.3		38	49.4	

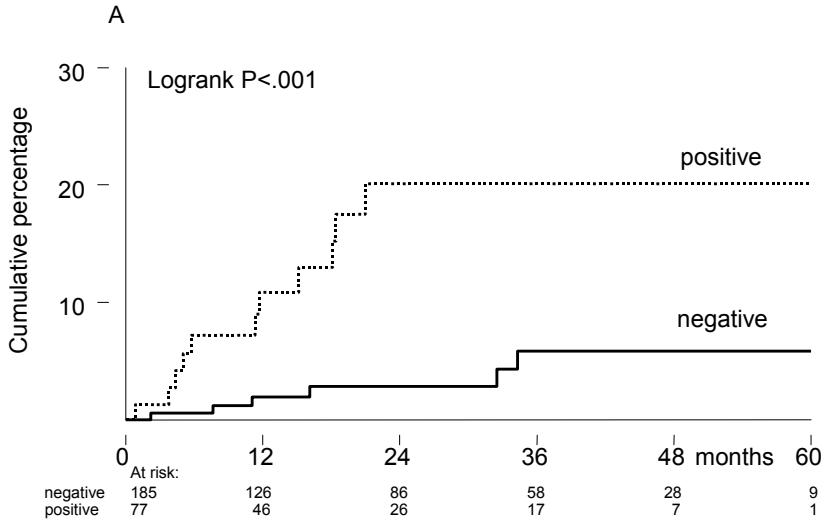

Figure 2A In-transit metastasis rate according to SN positivity

nodes were possibly missed during the operation. These patients developed a nodal recurrence after 21, 24 and 8 months, respectively. Three patients developed distant (n = 1) or in-transit (n = 2) metastases and subsequently a positive node in the regional basin after 13, 11 and 9 months, respectively. Two patients were not treated according to the protocol. In one patient only blue dye was used and another patient SN biopsy was performed 5 months after reexcision. These were considered technical failures.

Survival

The estimated 3-year overall survival (OS) rate according to the SN status was 95% for SN-negative and 74% for SN-positive patients ($P < 0.001$). The estimated 5-year OS rate according to the SN status was 93% for SN-negative and 51% for SN positive patients,

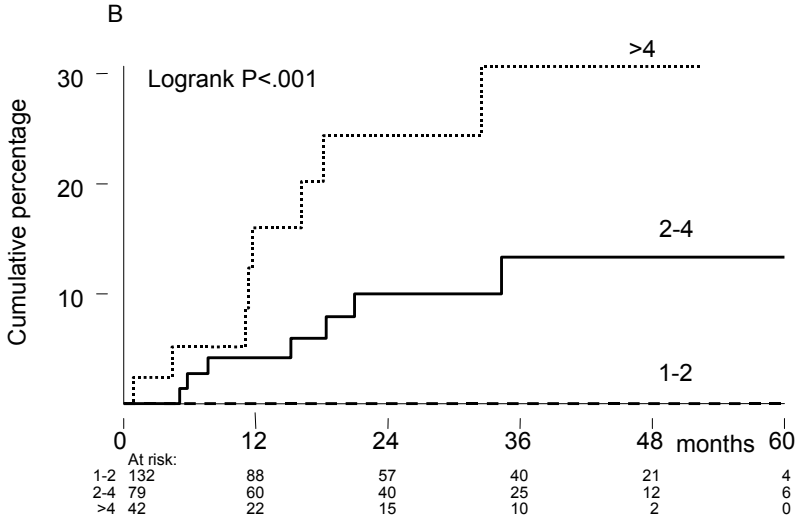

Figure 2B In-transit metastasis rate according to Breslow thickness

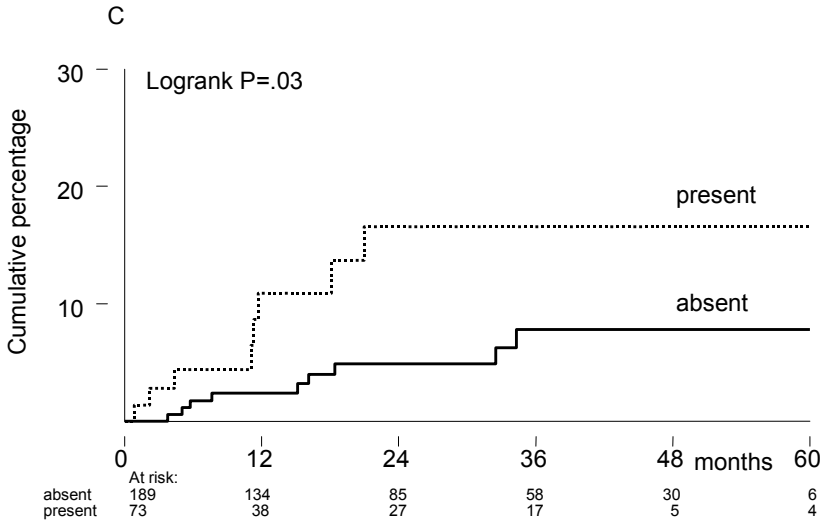

Figure 2C In-transit metastasis rate according to ulceration status

respectively (P < 0.001) (Fig. 1a). The estimated 5-year survival rates for four different categories of Breslow thickness, namely, <1.00 mm, 1.01–2.00 mm, 2.01–4.00mm and >4.00 mm, were 100%, 86%, 77% and 65% respectively (P = 0.11) (Fig. 3a).

The estimated 5-year survival rates in the presence or absence of ulceration of the primary tumour, was 86% in the absence and 60% in the presence of ulceration, respec-

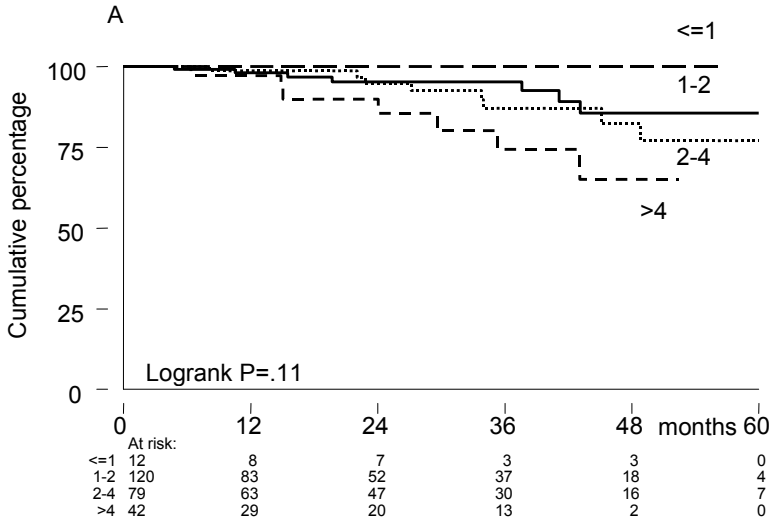

Figure 3A Kaplan-Meier estimated 5-year overall survival according to Breslow thickness

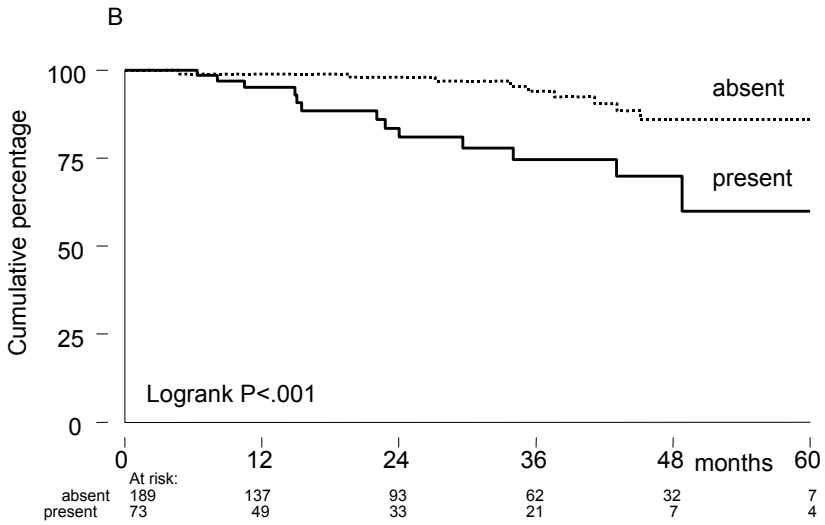

Figure 3B Kaplan-Meier estimated 5-year overall survival according to ulceration status

tively (P < 0.001) (Fig. 3b). The estimated 5-year survival rates according to the number of involved sentinel lymph nodes was 93% for no metastatic sentinel lymph nodes, 54% for one metastatic sentinel lymph node and 47% for multiple (two or more) metastatic sentinel lymph nodes (P < 0.001) (Fig. 4).

Table 4 shows an overview of Cox's univariate regression analyses for disease-free and overall survival. Also a Cox's proportional hazard regression model was used to

Table 4 Cox univariate regression analyses of disease-free and overall survival

	DFS			OS		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Age						
≤ 50	1			1		
> 50	1.09	0.59 – 2.02	0.77	0.76	0.33 – 1.77	0.53
SN status						
Negative	1			1		
Positive	5.51	2.89 – 10.53	< 0.001	7.25	2.86 – 18.40	< 0.001
Gender						
Female	1			1		
Male	1.14	0.62 – 2.10	0.68	0.91	0.40 – 2.08	0.82
Location						
Extremity	1			1		
Central	2.29	1.23 – 4.28	0.009	1.59	0.70 – 3.61	0.27
Ulceration						
Absent	1			1		
Present	2.72	1.47 – 5.02	0.001	3.76	1.65 – 8.59	0.002
Breslow						
≤ 2.00 mm	1			1		
2.01 – 4.00 mm	3.23	1.46 – 7.14	0.004	1.69	0.63 – 4.53	0.30
> 4.00 mm	5.28	2.26 – 12.36	< 0.001	3.27	1.14 – 9.34	0.027
Clark						
II, III	1			1		
IV	1.27	0.65 – 2.50	0.49	0.57	0.23 – 1.40	0.22
V	5.00	1.92 – 13.03	0.001	2.44	0.69 – 8.67	0.17

DFS = Disease-Free Survival, OS = Overall Survival, HR = Hazard Ratio, CI = Confidence Interval.

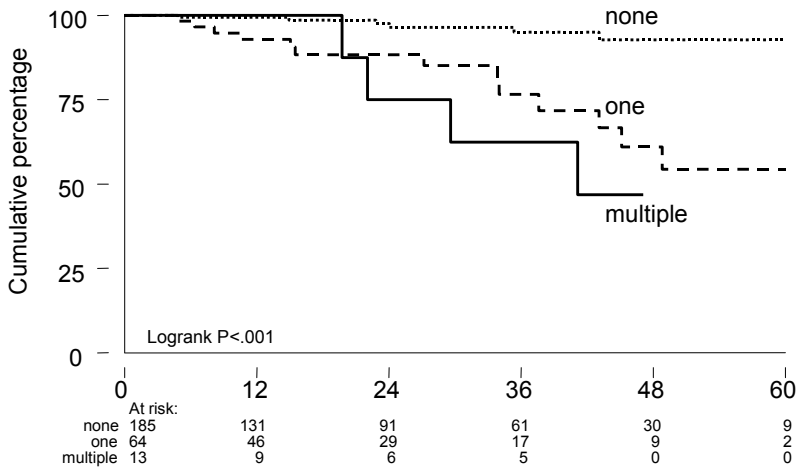

Figure 4 Overall survival according to the number of metastatic sentinel lymph nodes

Table 5 Cox proportional hazard regression analyses of disease-free and overall survival

	DFS			OS		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Age						
≤ 50	1			1		
> 50	1.17	0.61 – 2.22	0.64	1.02	0.42 – 2.48	0.97
SN status						
Negative	1			1		
Positive	5.71	2.81 – 11.60	< 0.001	7.29	2.65 – 20.10	< 0.001
Gender						
Female	1			1		
Male	0.75	0.39 – 1.44	0.38	0.77	0.32 – 1.82	0.54
Location						
Extremity	1			1		
Central	3.31	1.66 – 6.60	0.001	2.13	0.89 – 5.10	0.09
Ulceration						
Absent	1			1		
Present	3.33	1.58 – 7.03	0.002	4.66	1.76 – 12.34	0.002
Breslow						
≤ 2.00 mm	1			1		
2.01 – 4.00 mm	1.94	0.82 – 4.56	0.13	0.94	0.32 – 2.74	0.91
> 4.00 mm	1.15	0.38 – 3.52	0.81	0.58	0.13 – 2.57	0.47
Clark						
II, III	1			1		
IV	1.38	0.67 – 2.87	0.39	0.71	0.26 – 1.92	0.28
V	11.63	2.96 – 45.76	< 0.001	5.43	0.94 – 31.27	0.06

DFS = Disease-Free Survival, OS = Overall Survival, HR = Hazard Ratio, CI = Confidence Interval.

determine the influence of different covariates on the disease-free and overall survival rates (Table 5).

As seen in Table 5, the SN status, location, ulceration and a high Clark level (V) had a significant influence on the disease free survival. SN status and ulceration had a significant influence on the overall survival. Breslow thickness was not a significant factor in this model, however Breslow thickness as a factor for disease-free survival approached significance (P = 0.13). The reason that Breslow thickness was not a significant factor might be due to the small patient population.

DISCUSSION

In this single institution study we confirm the high detection rate of the EORTC MG protocol. In the present study at least one SN was found in 100% of the patients during the surgical biopsy, this is comparable with other studies, which reported success rates between 98.5% and 100%[8,10,17,18]. The false negative rate found in the present study was 9.4%, this is also comparable with other studies, in which rates between 7% and 18% are reported[17-20].

The Cook protocol[16] for the histopathologic work-up and examination of SN reported a higher positive SN identification rate (29.4%) than most other large studies[1,8-11,17,21,22], which all had detection rates between 14% and 20%. Our patient population did not differ from those studies with respect to the crucial prognostic factors (Table 6). The median Breslow thickness reported by Gerschenwald[10], Vuylsteke[8] and Doubrovsky[9] were 1.80 mm, 1.41mm and 2.30mm respectively.

In this current study there was a median Breslow thickness of 2.00mm and a SN-positive rate of 29.4% is the highest ever reported. The essential difference in the pathological work-up of the SN between the present study and other studies[1,8-11,17,21,22] is transhilar bivalving and taking most step-sections fromthe central hilar planes of the lymph node. Despite the higher rate of SN positivity, survival rates were similar to other studies (Table 6). The increase in SN positivity may reflect an increase in diagnosis of minimal and perhaps biologically less aggressive disease and therefore further research needs to be done on the clinical relevance of the increase in SN-positive detection rates.

The SN technique is based on the now well-supported hypothesis that melanoma lymphatic metastases follow an orderly progression through afferent lymphatic channels to SNs before spreading into other regional, non-SN[10,23]. The current study supports this hypothesis, as 80% of the patients, who had at least one positive SN and received

Table 6 Review of other SN studies

Name	%SN+	Pt.	Mean	Median	Ulc.(%)	SN+DFS	SN+OS	SN-DFS	SN-OS
Morton[11]	19.8%	1159			65%		70.6%		88.4%
Gerschenwald[10]	15%	580	2.40	1.80	23.7%	56%(3yr)	70%(3yr)	89%(3yr)	97%(3yr)
Vuylsteke[8]	19%	209	1.78	1.41	17%	50%	67%	88%	92%
Doubrovsky[9]	18%	672	2.90	2.30	31.8%		59%		87.5%
Balch[1]	13.9%	3126					58%		
Carlson[22]	17.7%	592			13%	59%(3yr)	77%(3yr)	86%(3yr)	92%(3yr)
Estourgie[17]	24%	250	2.70		31.6%	53%	64%	80%	89%
Kretschmer[21]	29.1%	244		2.30	34.8%	38.6%	54.4%	77.7%	90.1%
DDHCC	29.4%	262	2.76	2.00	28%	51%	51%	87%	93%

DDHCC = Daniel den Hoed Cancer Center, DFS = Disease free survival, OS = Overall Survival

a subsequent lymphadenectomy, did not reveal any other positive nodes in the nodes resected during the lymphadenectomy.

The 5-year disease-free survival rates were 87% and 52% for SN-negative and positive patients, respectively in our study. These rates are comparable with several other studies, which report 5-year disease-free survival rates of between 78% and 88% and between 39% and 53% for SN-negative and positive patients, respectively[8-11,17,21,22]. Also the 5-year overall survival rates of the present study, 93% and 51% for SN-negative and SN-positive patients, respectively, are comparable with several other studies, which report similar overall survival rates of between 92% and 88% for SN-negative patients, and overall survival rates of between 54% and 67% for SN-positive patients[8-11,17,21,22].

Whether SN staging will have an impact on overall survival remains to be seen: a recent study by Doubrovsky[9] shows that the reason SLNB is superior to ELND is due to the difference in histopathological protocols used to examine the lymph nodes, however SLNB patients had no survival advantage compared to ELND patients in this retrospectively matched control study. More importantly the interim analysis of the MSLT-I trial does not suggest any survival benefit for the overall population with high-risk primary melanomas. Survival rates at 5 years are virtually identical at 87% and 86% irrespective of whether or not a SN procedure has been performed[11]. Whether a complete lymph node dissection at the time of the identification of a positive SN has an impact on survival is unclear at the moment as well. But, survival rates at 5 years are reported significantly higher in the SN-positive patient population than in patients that did not undergo an SN-staging and underwent a delayed lymph node dissection at a later stage, because of positive nodal disease. However, this is not a strictly randomized comparison and it may well be that patients who develop clinically positive disease represent a biologically unfavorable selection amongst the patients, as compared to the complete set of SN-positive patients[11]. Since the overall outcome in the overall population is not different between patients that have or have not undergone a SN procedure it is clear that the data thus far presented are incomplete, as they have not provided insight in the curves of SN-negative patients (including the SN false-negative patients), which may well be significantly worse than the Observation patients that never developed nodal disease, just has been observed in the WHO-1 trial[2]. The present study can not address this dilemma, as there is no group of patients that did not undergo a SN procedure, but only had a local wide excision or only had an ELND.

Another important issue is the alleged increased rate of in-transit metastasis after the SN procedure. Thomas[24] reported a higher rate of in-transit metastasis after SN biopsy plus lymphadenectomy (20.9%). Another recent study by Estourgie[25] reported a rate of 23% in-transit metastasis in SN-positive patients. The present study shows an estimated 5-year in-transit metastasis rate of 20% in SN-positive patients (who subsequently underwent a lymph node dissection). However, the theory that the SN procedure itself

leads to more in-transit metastases can be refuted. The study by Estourgie[25] shows a major unbalance in prognostic tumour characteristics Breslow thickness and ulceration of the primary between the two groups that were compared. These were 3.8 mm versus 2.9mm and 48% versus 22% ulceration present for the SN and palpable lymph node groups, respectively.

The present study shows that both in the SN-negative and in the SN-positive patient groups the ulceration status and Breslow thickness of the primary tumour influences the in-transit metastasis rate. This correlation is significant for the Breslow thickness ($P < 0.001$) and ulceration ($P = 0.03$). Recent publications with large patient populations concur with this observation[26,27]. SN-positive patients have a significantly increased risk of developing any form of recurrence compared to SN-negative patients. Studies with much larger case numbers seem to demonstrate that the increase in the in-transit metastasis rate is not real, but due to a prolonged recurrence-free interval, since the SN procedure avoids nodal recurrences, thereby increasing the chance of in-transit metastases to manifest as a first recurrence site. The overall in-transit probability however remains unchanged; independent of whether early or delayed excision of nodal metastases is performed[21,28]. Many comments[28-32] by international authors point out that the presumption by Thomas[24] and by Estourgie[25] that sentinel lymph node biopsy would lead to an increased rate of in-transit metastasis is not true. Therefore, the suggestion that SN biopsy should be abandoned, because of the supposed risk, is unjustified.

In spite of the absence of proof of a survival benefit associated with SN staging, the procedure is quite useful for stratifying patients in randomized phase III systemic adjuvant therapy trials, to create more homogeneous patient populations to determine whether adjuvant systemic trials are of benefit[33]. Moreover SN-staging may well improve long term locoregional control in the lymph node basin compared to the patients who underwent a delayed lymph node dissection[11].

At the same time it is clear that ultrasound of the regional lymph nodes may also be able to achieve this by detecting very small non-palpable lymph node metastases, thus offering an alternative to a SN procedure[34,35].

In conclusion, this study confirms that the EORTC MG protocol performs well in detecting a high rate of nearly 30% of positive SN in patients with cutaneous melanomas >1 mm. Essential is transhilar bivalving and step-sectioning from the central hilar planes of each face of the lymph node. SN status is the strongest predictive factor for disease-free and overall survival. Breslow thickness and ulceration influence SN status. The SN status is the most important predictive value for disease-free and overall survival. Ulceration is the single most predictive factor for survival. In-transit metastasis rates correlate with SN-positivity, Breslow thickness and ulceration of the primary. The SN status is currently the most powerful prognostic tool available and is a mandatory stratification tool for every prospective adjuvant systemic therapy trial.

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Chapter 3

Expert opinion in melanoma: The sentinel node; EORTC Melanoma Group recommendations on practical methodology of the measurement of the microanatomic location of metastases and metastatic tumour burden

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SUMMARY

The Sentinel Node (SN) status has been recognized to be the most important prognostic factor in melanoma. Many studies have investigated additional factors to further predict survival / lymph node involvement. The EORTC Melanoma Group (MG) has formulated the following question: How should we report the microanatomic location and SN tumor burden?

The EORTC MG recommends the following: The EORTC MG SN pathology protocol or a similarly extensive protocol, which has also been proven to be accurate, should be used. Only measure what you can see not what you presume. Cumulative measurements decrease the accuracy and reproducibility of measuring. The most reproducible measure is a single measurement of the maximum diameter of the largest lesion in any direction (1-D). If there is any infiltration into the parenchyma, this lesion can no longer be considered solely subcapsular. Reporting of the microanatomic location of metastases should be an assessment of the entire sentinel node, not only of the largest lesion. Multifocality reflects a scattered metastatic pattern, not to be confused with multiple cohesive foci, which fall under the regular location system. A subcapsular metastasis should have a smooth usually curved outline not ragged or irregular.

We recommend all pathologist report the following items per positive SN for melanoma patients: The Microanatomic Location of the metastases according to Dewar et al. for the entire node. The SN Tumor Burden according to the Rotterdam Criteria for the maximum diameter of the largest metastasis expressed as an absolute number and SN Tumor Burden stratified per category; < 0.1 mm or 0.1 – 1.0 mm or > 1.0 mm.

INTRODUCTION

The Sentinel Node (SN) biopsy has become a routine staging procedure for primary melanoma patients without any clinical evidence of regional or distant metastases. Depending on the extent of the pathology protocol used and the Breslow thickness of the population, SN positivity rates range from 15% to 33%[1-6]. It has been demonstrated that SN positive patients (approximately 50 – 70% survival at 5-years) have a significantly worse prognosis compared to patients, who are SN negative (approximately 90% survival at 5-years)[3,4,6,7].

Since its introduction in the 1990s increasingly more centers worldwide have been performing, and more patients have been undergoing, SN procedures for melanoma, each year. Together with this increase in the number of performed procedures, came an increase in scientific projects to research and evaluate the efficacy and results of the SN procedure to a deeper extent. Although these studies have answered some questions, they have perhaps raised more new questions at the same time.

One of these issues is the importance of SN tumor burden and possible clinical implications. Many studies have assessed this issue, but agreement has not been achieved[8-22]. One conclusion can be drawn: the prognosis of patients decreases with increasing SN tumor burden, no matter how you measure this, even if only by approximate measurements[8-22].

Since all these studies have used different methods and most often have not elaborated on how they practically measured SN tumor burden or established the microanatomic location of a metastasis, the following questions present themselves in the everyday clinical practice of pathologists in the reporting of SN tumor burden:

How should we report the microanatomic location of metastases within the SN and how should we measure the amount of tumor burden within the SN?

The following comments are based on the experience of the authors following reporting of several thousands of sentinel node biopsies. They are practical responses to frequently arising questions. They are still subject to further evaluation and may be shown to be suboptimal in the light of further studies, but seem the most appropriate in the current state of understanding of sentinel lymph nodes and melanoma.

LITERATURE OVERVIEW

From the early 2000s onwards, a number of studies have identified certain factors, which predicted survival and / or additional non-SN positivity in the Completion Lymph Node Dissection (CLND) specimen. Table 1 summarizes the main results from these studies.

Table 1 Overview of SN tumor burden studies in the literature

Author	# of pos SNs	Characteristics	Groups	Survival	CLND positive
Ranieri et al. [8]	90	Maximum Diameter	≤ 3 mm	86% (3-yrs)	
			> 3 mm	27% (3-yrs)	
Carlson et al. [9]	104	Maximum Diameter	Isolated or cluster of melanoma cells	86% (3-yrs)	
			≤ 2 mm	90% (3-yrs)	
			> 2 mm	57% (3-yrs)	
Reeves et al. [10]	98	Maximum Diameter (≤ 2 mm or > 2 mm) and Ulceration Status of the Primary	0		0%
			1		16%
			2		31%
Starz et al. [11]	70	Infiltration from the capsule	≤ 0.3 mm	±80% (5-yrs)	
			> 0.3 ≤ 1.0 mm	±90% (5-yrs)	
			> 1 mm	±60% (5-yrs)	
Dewar et al. [12]	146	Microanatomic Location	Subcapsular		0%
			Combined		11%
			Parenchymal		19%
			Multifocal		37%
			Extensive		42%
Vuylsteke et al. [13]	80	Maximum Diameter (< 0.3 mm and ≥ 0.3mm), Breslow Thickness (< 2.5 mm and ≥ 2.5 mm) and non-SN status	0	94% (5-yrs)	
			1	56% (5-yrs)	
			2	30% (5-yrs)	
Sabel et al. [14]	232	Extracapsular Extension (ECE) and ≥ 3 positive SNs	ECE		OR 3.2
			≥ 3 positive SNs		OR 65.8
Pearlman et al. [15]	90	Maximum Diameter	≤ 2 mm	85% (5-yrs)	6%
			> 2 mm	47% (5-yrs)	45%
van Akkooi et al. [16]	74	Maximum Diameter	< 0.1 mm	100% (5-yrs)	0%
			0.1 – 1.0 mm	63% (5-yrs)	19%
			> 1.0 mm	35% (5-yrs)	
Govindarajan et al. [17]	127	Maximum Diameter	≤ 0.2 mm		0%
			0.2 – 2.0 mm		10.5%
			> 2.0 mm		26.1%
Satzger et al. [18]	101	Capsule Invasion, Tumor Infiltrative Depth (< 2 mm or ≥ 2 mm) and size of largest tumor deposit (< 30 cells or ≥ 30 cells)	0	±100% (5-yrs)	
			1	±90% (5-yrs)	
			2	±55% (5-yrs)	
			3	±20% (5-yrs)	
Debarbieux et al. [19]	98	Maximum Diameter	≤ 2mm	±80% (5-yrs)	
			> 2 mm	±35% (5-yrs)	

Scheri et al. [20]	214	Maximum Diameter	≤ 0.2 mm	87% (5-yrs)	12%
Gershenwald et al. [21]	309	Maximum Diameter and Tumor Square Area	≤ 0.5 mm		5.3%
			≤ 0.1 mm^2		3.7%
EORTC Melanoma Group by van Akkooi et al. [22]	388	Maximum Diameter and Microanatomic Location	< 0.1 mm	91% (5-yrs)	3%
			0.1 – 1.0 mm	61% (5-yrs)	21%
			> 1.0 mm	51% (5-yrs)	32%
			Subcapsular		8%
			Combined		32%
			Parenchymal		19%
			Multifocal		15%
			Extensive		40%

As can be observed in Table 1, a number of different characteristics and combinations of these characteristics have been tested with similar and/or different cut-off values. These characteristics include most often the maximum diameter of the metastases[8-10,15-17,19-22], but also the tumor infiltration from the capsule inwards and the microanatomic location[11-13,16,22,23]. Other factors have also been investigated, such as Breslow thickness, ulceration of the primary, extracapsular extension (ECE) or capsule invasion, the square area of the metastases and the number of positive sentinel nodes[10,13,14,18,21,24].

Although, sometimes very elaborate, very detailed and time consuming and, sometimes very rough measurements, have been predictive of survival and/or CLND positivity, none of these studies has been able to answer the following crucial question:

Since only approximately 20% of all SN positive patients is CLND positive and the CLND has the risk of considerable morbidity; can we identify a group of SN positive patients, which we can safely (with regard to regional control and survival) spare a CLND?

Supporters of this idea have argued that these tiny lesions within the SN are clinically non-relevant and should therefore be considered prognostically false positive[25,26]. Patients with minimal SN tumor burden have excellent survival rates, which are identical to SN negative patients[16,22,27,28]. Moreover, these patients have similar primary tumor characteristics to SN negative patients and they rarely, if ever have additional lymph node metastases in their CLND specimen[16,22,27,28]. Finally, the Multicenter Selective Lymphadenectomy Trial-1 (MSLT-1) did not demonstrate any survival benefit for patients undergoing a SN procedure followed by a CLND when positive, compared to patients who only received wide local excision (WLE) followed by a Delayed Lymph Node Dissection (DLND) when metastases became clinically apparent[4].

Opposition to this idea has argued that all these retrospective studies could only be performed on excised sentinel nodes and that the excision of the SN might have already been beneficial for these patients[29-31]. Similarly, CLND has been performed in all these patients with minimal SN tumor burden and although metastases were rarely seen in the CLND specimen, the CLND specimen was usually analyzed only by bivalving and

H&E staining[29-31]. Micrometastases could therefore easily have been missed. Moreover, the MSLT-1 subgroup analysis suggested a survival benefit for SN positive patients compared with WLE node positive patients[4].

Two prospective studies, the MSLT-2 and the EORTC Melanoma Group MINITUB trial, currently both being conducted with different viewpoints, are addressing the issue of performing or omitting a CLND in all SN positive patients or in minimal SN tumor burden patients only, respectively.

MEASURING QUESTIONS

Tumors are three-dimensional (3-D), not one-dimensional (1-D), would a 3-D calculation of the metastasis be the most accurate measure?

This would seem logical, but unfortunately this does not seem to work, for a number of reasons: we only have access to a two dimensional (2-D) slice of the SN, therefore any addition of a third dimension would be, either through a complicated computer calculation, or a rough estimate of the researcher, which has a considerable inter-observer spread.

Moreover, metastases do not grown in nice square or cubic forms, but most often spread along the curve of the capsule or trabeculae, which is difficult to measure in 2-D or 3-D and thus leads to a tremendous spread in reporting and therefore to inaccuracy. At the same time, this also shows us, why 2-D calculations (tumor square area) are also less accurate.

Most often there is not one single lesion within the SN, there are a number of lesions visible, would measuring all and adding these to a total be the most accurate measure?

This does seem logical, but unfortunately this would require a considerable amount of work for a pathologist to report per sentinel node, which is practically not feasible. Moreover, if one measurement has inter-observer spread, multiple measurements are certainly increasingly inaccurate: Cumulative measurements are time consuming, decrease the accuracy and reproducibility of measurements and are therefore not recommended.

Through the use of an adequate SN protocol (such as the recommended EORTC Melanoma Group protocol[1,5]), two lesions may be visible on one slide, but perhaps these would become one if, deeper sections were available; how can this be assessed?

The most important credo for this issue is: only measure what you can see. This means that if two lesions are interrupted by lymphocytes (or other cells or structures), these are to be considered as two separate lesions. Although it is plausible that these two lesions could be one connective lesion on a deeper level, if this cannot be observed, these lesions should still be considered as two separate lesions, unless or until evidence has been presented that this assumption is untrue.

It is sometimes difficult to measure the 1-D maximum diameter of a metastasis, when it spreads along the curve of the capsule (and thus is not a straight lesion), especially when the metastasis is large, how should this be measured?

Although 1-D measurements of the maximum diameter of metastases seems quite simple, it can be difficult, certainly in cases where there are multiple metastases, metastases are large and/or metastases have a curved shape along the capsule or trabeculae. The answer to this is very simple and pragmatic: Do not waste time on very accurately measuring the very large metastases, because they will already belong to the group of tumor burden with a bad prognosis (i.e. > 1, > 2 or > 3 mm, depending on your classification system). You can 'eyeball' this, recognize this without measurement, since it is not important to differentiate between say 7.8mm and 8.3mm in maximum diameter, as both would have a bad prognosis. More time should be given to measuring smaller lesions and lesions close to a threshold. Thresholds are currently only for the prognosis of patients, but certainly if and when they have clinical management implications, it would be very important to thoroughly measure metastases close to a threshold.

Smaller curved lesions, i.e. lesions up-to 1mm in maximum diameter, even if showing a slight curve, can be measured sufficiently accurately by a straight line between the furthest points. Larger lesions will already be assigned bad prognosis and thus not complicate matters with difficult curve measurements. Therefore, it is appropriate to measure in a straight line, in any direction; the maximum diameter and it will be sufficiently accurate (within 0.1 mm difference) between different observers.

There are a number of metastases, how do I know which one has the largest maximum diameter?

You may not know initially, but you can usually differentiate most by simply screening these lesions. Often there is one lesion, which is clearly largest, which saves you the time of measuring many smaller lesions. Sometimes, especially when lesions are small, they might be in the same order of size. In such cases measurement of all these to differentiate which one is the largest would be necessary to accurately reflect the maximum diameter of the largest deposit.

Sometimes the metastasis is mostly in the subcapsular space, but some cells, such as a few loose cells or a small cluster, seem dissociated from the main metastasis and no longer solely confined to the subcapsular space. What type of microanatomic involvement is this?

Subcapsular involvement is only observed, when there is a well-defined and cohesive lesion, solely confined to the subcapsular space or paratrabecular. When there is any lesion inside the parenchyma, either connective to the main lesion as infiltrative satellite or tentacle, or as a separate cluster within the parenchyma, interrupted by lymphocytes between the two, the metastases can no longer be solely regarded as subcapsular and this lesion should be considered as combined involvement (subcapsular and parenchymal).

Quite often there are multiple metastases. The majority of the metastases are confined to the subcapsular space, including the largest lesion. However, there is another smaller lesion with parenchymal infiltration. Should we report the microanatomic location of the largest lesion or of the entire node?

Microanatomic location is a reflection of the biologic behavior of the metastases within the entire SN, not just of one single lesion. Although the size of the largest lesion gives good prognostic information, the microanatomic information of this single lesion does not reflect the biology of the disease in the entire node. Therefore, any parenchymal involvement, anywhere within the SN should be judged as such. Only when all metastases, in case of multiple lesions, are confined to the subcapsular space, can the metastases be considered as solely subcapsular.

The histopathological examination of a SN often reveals multiple deposits of tumor. Is this considered multifocality within the microanatomic location classification or should there be a minimum number of foci to be considered as such?

Literally multifocality means more than one focus. However, in the microanatomic classification system, this reflects the pattern of, several to many, usually tiny lesions, but most importantly these groups are scattered throughout the greater part of the node.

There is also another, very different pattern of multiple metastases, which can be numerous metastases, but entails a pattern of clearly cohesive lesions and certainly not one of widely scattered loose cells (this is a combined pattern). Therefore, a cut-off threshold for the number of lesions to be considered for either of these patterns cannot be specified, but the decision is based on the overall morphological distribution of the lesions.

EORTC MELANOMA GROUP RECOMMENDATIONS:

- The EORTC Melanoma Group SN pathology protocol or a similarly extensive protocol, which has also been proven to be accurate, should be used.
- Only measure what you can see, not what you presume.
- Cumulative measurements decrease the accuracy and reproducibility of measuring. This includes tumor square area (2-D) and 3-D reconstructions.
- The most reproducible measure is a single measurement of the maximum diameter of the largest lesion in any direction (1-D).
- If there is any infiltration into the parenchyma, this lesion can no longer be considered solely subcapsular.
- Reporting of the microanatomic location of metastases should be an assessment of the entire sentinel node, not only of the largest lesion.
- Multifocality reflects a scattered metastatic pattern, not to be confused with multiple cohesive foci, which fall under the regular location system (subcapsular, combined, parenchymal or extensive).
- A subcapsular metastasis should have a smooth usually curved outline not ragged or irregular.
- We recommend all pathologist report the following items per positive SN for melanoma patients:
    1) The Microanatomic Location of the metastases according to Dewar et al. for the entire node.
    2) The SN Tumor Burden according to the Rotterdam Criteria for the maximum diameter of the largest metastasis expressed as an absolute number (e.g. 0.6 mm).
    3) SN Tumor Burden stratified per category; < 0.1 mm or 0.1 – 1.0 mm or > 1.0 mm.

TEACHING EXAMPLES

Figure 1

Shows a completely subcapsular metastasis, which has a smooth outline, not irregular or ragged (like figure 3). Due to the curved shape, it might be somewhat of a challenge to measure, but our recommendation is to measure in a straight line from one end to the other of the largest cohesive cluster (as shown in figure 1).

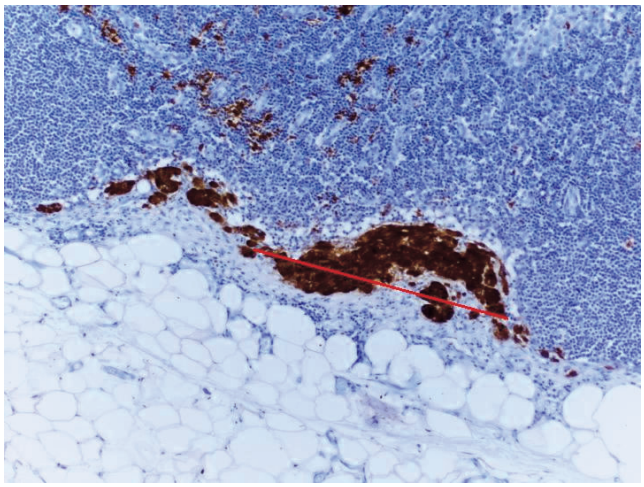

Figure 1 Subcapsular Metastasis

Figure 2

Although this metastasis is for the largest part confined to the subcapsular space, beginning infiltration into the parenchyma is visible. Therefore this metastasis is to be considered a combined (subcapsular and parenchymal) lesion.

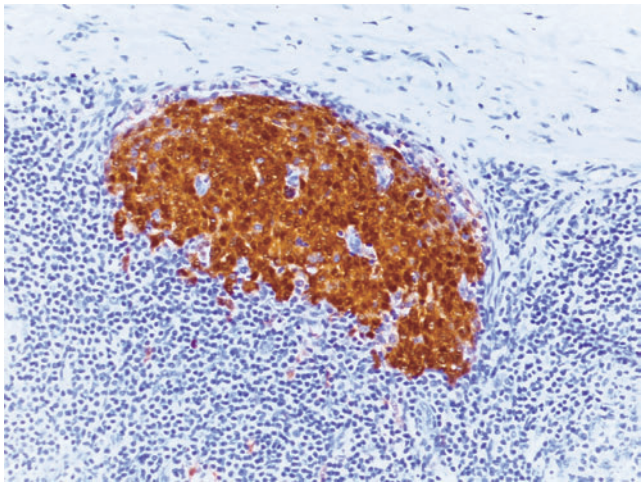

Figure 2 Combined Metastasis

Figure 3

This metastasis is located for the most in the subcapsular space, however, compared to Figure 1, this metastasis does clearly not have a smooth outline, but is very irregular and ragged. Therefore this lesion is to be considered combined (subcapsular and parenchymal) involvement.

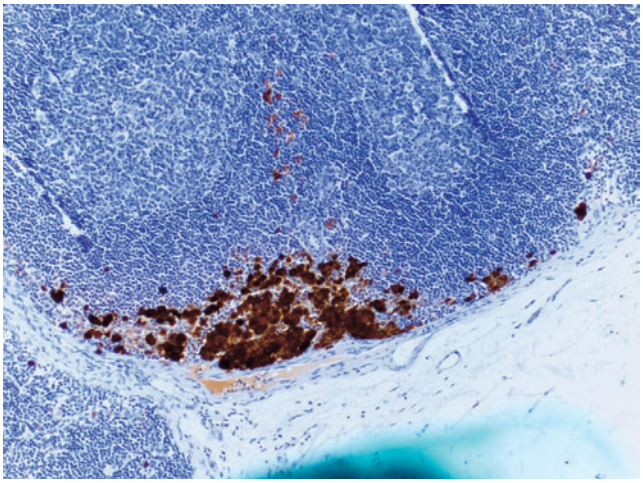

Figure 3 Combined Metastasis

Figure 4

This slide shows us 3 clear separate lesions, one located solely in the subcapsular space, which is smooth and regularly shaped. But there are also two others, which are located solely in the parenchyma. Although there are multiple foci, these lesions are very cohesive and not at all scattered. Therefore this is not to be considered a multifocal involvement, but it is also a combined (subcapsular and parenchymal) type of involvement.

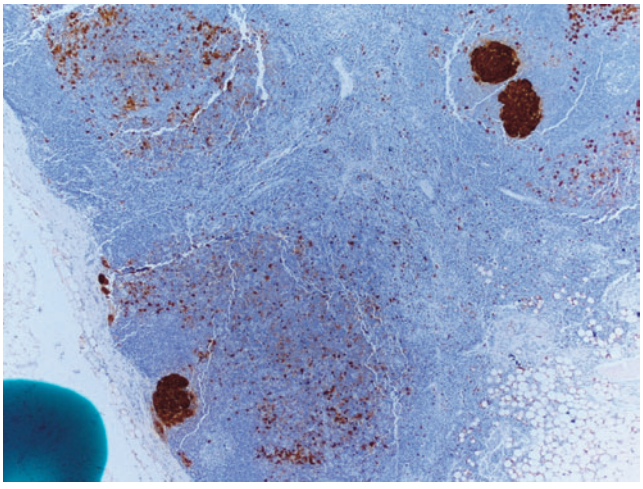

Figure 4 Combined Metastasis

Figure 5

This slide demonstrates a multifocal pattern of metastases. The cells and small clusters of cells are scattered throughout the greater part of this lymph node. It is clearly different from the pattern shown in Figure 4, where there are three well-defined lesions.

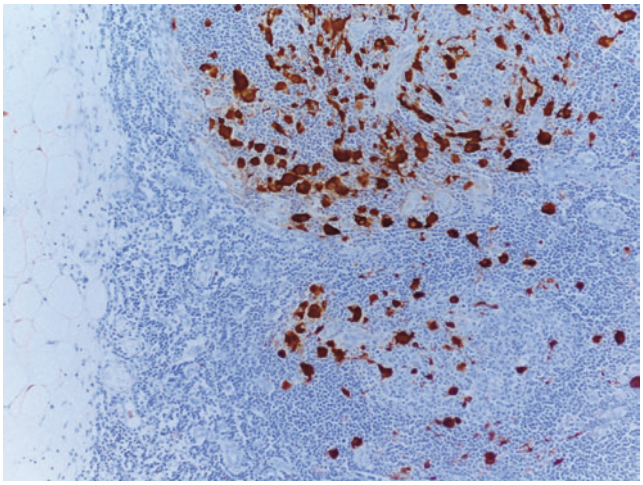

Figure 5 Multifocal Metastasis

Figure 6

This lymph node is almost solely taken in with a metastasis. The metastasis is disrupting the normal anatomic structure of the lymph node. This pattern is called the extensive involvement pattern. There is no size limitation (cervical nodes can be small, yet still almost completely displaced by tumor) to this type of involvement.

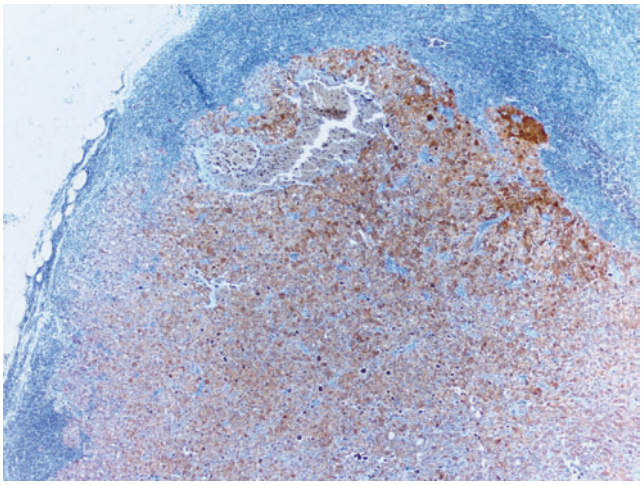

Figure 6 Extensive Metastasis

Figure 7

This metastasis is the largest within the SN of this patient. It is confined to the subcapsular space (smooth and regularly shaped) and the size is < 0.1 mm in maximum diameter.

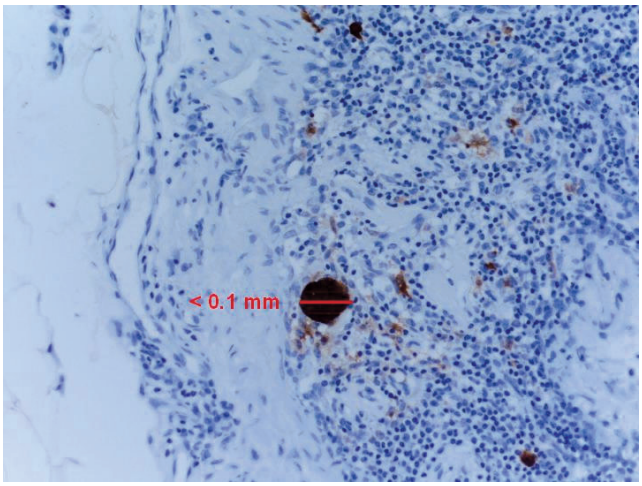

Figure 7 Subcapsular < 0.1 mm Sub-micrometastasis

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Chapter 4

Clinical relevance of melanoma micrometastases (<0.1mm) in sentinel nodes; are these nodes to be considered negative?

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ABSTRACT

As only about 20% of sentinel node (SN) positive melanoma patients have additional non-SN lymph node involvement in the Completion Lymph Node Dissection (CLND) specimen, we tried to identify a SN positive patient group, which can be spared CLND. Micro anatomic analyses of metastatic SNs were performed to identify patient/tumor and/or SN factors predicting additional non-SN positivity as well as disease free and overall survival.

SN positivity was found in 77 of 262 stage I/II patients, included into a prospective database (10/97-5/04). Of 74 patients pathology material was available for re-evaluation. Micro anatomic analyses categorized topography of SN-metastases, Starz classification and amount of SN tumor burden. Additional non-SN positivity, DFS, OS and was calculated for all analyses.

Mean Breslow thickness was 3.5mm (0.8 - 12.0); mean FU was 35 (6 – 81) months. There was no additional non-SN positivity for SN-micrometastases <0.1mm. Topography of SN involvement had no impact on OS. Estimated 5-yr OS rates for the different groups of <0.1mm, 0.1-1.0mm and >1.0mm SN tumor burden were 100%, 63% and 35% respectively. Distant metastases were exceedingly rare (1/16 = 6.3%) in <0.1mm SN-positive patients. On multivariate analysis the SN tumor burden was the most important prognostic factor for DFS (P=0.005) and OS (P=0.03).

Distant metastasis-free survival was identical (91%) to the 5-yr OS of SN negative patients, the estimated 5-yr OS was 100% for these patients and additional non-SN positivity was not observed. Therefore, our data suggest that patients with sub-micrometastases (<0.1mm) in the SN may be judged as SN negative, as non-stage III, and are highly unlikely to benefit from CLND, which we no longer recommend.

INTRODUCTION

The Sentinel Node (SN) procedure is a staging procedure for primary melanoma patients without clinically detectable nodal metastases[1,2]. Depending on known prognostic factors of the primary tumor, such as the Breslow thickness and ulceration, 15 – 30% of the clinical stage I or II patients have histopathologically identifiable metastases in their SNs[2-4]. Most patients with a positive SN undergo completion lymph node dissection (CLND) with approximately 10% to 33% of the non-SNs in the specimen containing further metastases[2,4-7]. The SN status has been demonstrated to be the most powerful prognostic value for disease-free (DFS) and overall survival (OS) of patients with primary melanoma. SN negative patients can achieve excellent long-term survival, whereas approximately 35 – 50% of SN positive patients die of their disease within 5 years[2-4,8-10]. A number of studies have tried to identify patient, tumor and SN characteristics that predict additional non-SN positivity[6,7,11-18]. Breslow thickness and ulceration of the primary tumor have been identified as prognostic factors for additional non-SN positivity[13-15]. More often SN tumor burden has been described as prognostic factor for additional non-SN positivity and overall survival[7,13-17]. The location of the metastasis in the SN has also been described by Dewar et al. as prognostic factor for additional non-SN positivity[18]. Other classifications have been developed to divide SN positive patients in different risk groups for additional non-SN positivity and survival[11,12,15]. On the other hand, McMasters et al. found no significant characteristics in their group of melanoma patients studied for additional non-SN positivity or for survival[6].

Methods for the histopathological work-up of SNs varies considerably between institutes and new pathology protocols[19,20] may have lead to an increase in SN positivity. This increase in SN positivity may reflect an increase in diagnosis of minimal and perhaps biologically less aggressive disease[2]. Because only a small proportion of SN positive patients has additional nodal involvement at the time of SN, it has become increasingly important to identify factors that may predict non-SN positivity, which might spare SN positive patients the unnecessary morbidity of a completion lymph node dissection.

This study was performed to analyze if any patient and/or tumor characteristics, SN tumor burden or the location of the metastasis in the SN, might be prognostic for additional non-SN positivity, DFS and OS. As a result, an attempt was made to identify patients who could be spared the morbidity of a CLND, without compromising their survival chances.

METHODS

Patients with tumor positive SNs were identified from the SN database at the Erasmus University Medical Center – Daniel den Hoed Cancer Center (Rotterdam, the Netherlands). This database consists of 262 patients, who were operated on between October 1997 and May 2004, of whom 77 were SN positive (29%). The median and mean Breslow thickness of all 262 patients was 2.0mm and 2.8mm respectively. Briefly, inclusion criteria for patients to undergo a SN biopsy consisted of a tumor with at least 1.00 mm Breslow thickness or Clark IV/V or ulceration of the primary tumor. Exclusion criteria were palpable lymph node metastases or signs of distant metastases.

SNs were identified by the standard triple technique of preoperative lymphoscintigraphy with the use of a radioactive nanocolloid, intraoperative use of patent blue dye and the intraoperative use of a hand-held gamma probe. The SNs were pathologically analyzed according to the EORTC Melanoma Group protocol[19], which required transhilar bivalving and stepsectioning from both faces of the lymph node. Staining was performed with H&E, S100 and HMB-45.

SN slides of 3 (out of 77) patients could not be retrieved from the archives and therefore 74 patient's slides were re-evaluated for this study. During re-evaluation location of the metastasis in the SN was determined according to Dewar et al.[18] in the various categories; only subcapsular involvement, only parenchymal involvement, combined subcapsular and parenchymal involvement, multiple discrete deposits (multifocal involvement), or extensive involvement of a large proportion of the SN.

Patients were divided by SN characteristics into different categories according the S classification (old and new versions) by Starz et al.[11,12]. The old S classification consisted of three categories; S1, S2 and S3 and these categories were based on the number of positive sections (n) and the maximum distance from the interior margin to the capsule of the SN (d). The criteria for these respective categories was $n \leq 1$ and $d \leq 1$mm for S1, $n > 2$ and $d \leq 1$mm for S2 and $n > 2$ and $d > 1$mm for S3.[11] The new S classification had different criteria for the three different categories SI, SII and SIII, these were $d \leq 0.3$mm for SI, $d > 0.3$mm and ≤ 1mm for SII and $d > 1$mm for SIII[12]. The size of the SN tumor burden was also recorded, three different tumor burden size groups were defined, submicrometastases (clusters of more than 10 cells, but < 0.1mm), tumor burden 0.1mm – 1mm and tumor burden > 1mm. If multiple lesions were present within a SN, the largest lesion was recorded.

Statistical analyses were all performed with Stata version 8.2 (Stata Corporation, College Station, Texas, USA). Disease-free and overall survival were calculated from time of SN until recurrence of the disease or death respectively. Patients without such an event at their last follow-up were censored at that time. Univariate analyses of end-points was performed using the Kaplan-Meier method and the logrank test. Multivariate analyses to

determine the prognostic value of covariates regarding disease-free and overall survival were performed using the Cox's proportional hazard model. P values of less than 0.05 were considered as significant.

RESULTS

Baseline characteristics of all reviewed patients (n=74) are depicted in Table 1. The average age was 47 years (range 16 – 76 years). The mean and median Breslow thickness were 3.5mm and 3.0mm, respectively (range 0.8 – 12.00mm). The distribution of SN characteristics, the S classification (old and new versions) according to Starz et al.[11,12] and the location according to Dewar et al.[18] are shown in Table 2. The mean and median follow-up time were 35 and 30 (6 – 81) months respectively. In patients with sub-micrometastases (< 0.1mm) SN tumor burden, the mean and median Breslow thickness were 2.4mm and 1.7mm, respectively and 37% of these patients had ulcerated primary tumors. This was compared to the SN-negative population in the whole Rotterdam series and found to be very similar as in the SN-negative population the mean and median Breslow thickness were 2.5 mm and 1.9 mm, respectively and 23% of these patients had ulcerated primary tumors[2].

CLND characteristics were missing for 7 patients, because either they refused CLND (n=2) or preferred follow-up of with ultrasound (n=5). Therefore, only 67 patients were

Table 1 Baseline patient and tumor characteristics of all 74 patients

	N	%
Gender		
Male	40	54%
Female	34	46%
Histology		
SSM	37	50%
NM	28	38%
Unclassified	9	12%
Clark		
II	2	3%
III	32	43%
IV	32	43%
V	4	5%
Unclassified	4	5%
Ulceration		
Present	29	39%
Absent	45	61%

Table 2 Sentinel Node characteristics

	N	%
SN Basin		
Inguinal	41	55%
Axillary	27	37%
Neck	3	4%
Other	3	4%
Number of positive SNs		
One	59	80%
Two or more	15	20%
Location		
Subcapsular	31	42%
Parenchymal	8	11%
Combined	12	16%
Multifocal	12	16%
Extensive	11	15%
SN Tumor Burden		
< 0.1mm (sub-micro)	16	22%
0.1 – 1.0mm	41	55%
> 1.0mm	17	23%
S classification (old)		
S1	16	22%
S2	40	54%
S3	18	24%
S classification (new)		
SI	27	36%
SII	30	41%
SIII	17	23%

analyzed for additional non-SN positivity. Table 3 shows an analysis of additional non-SN positivity by patient, tumor and SN characteristics. The number of positive SNs was a significant predictor of additional non-SN positivity (P=0.02) as well as the absence of ulceration (P=0.05). Borderline not significant (P=0.07) was SN tumor burden (<0.1mm vs. ≥ 0.1mm). The estimated 3-year DFS for all SN positive patients was 57%, 3-year and 5-year OS were 80% and 63% respectively.

The estimated OS for the three different categories of the old S classification was not statistically significant (Fig.1A) (P=0.16), however, the new S classification did approach significance with an estimated 3-year OS of the respective categories of 96%, 80% and 57% (Fig.1B) (P=0.055). The OS according to the distribution of the different location categories by Dewar was significantly different per category and is shown in Figure 2A (P=0.05). Figure 2B shows the OS of a simplified classification of Extensive versus

Table 3 Additional non-SN positivity by patients, tumor and SN characteristics

	Number of patients	Additional non-SN involvement	P
Sex			
Male	37	14%	
Female	30	17%	0.72
Age			
≤ 50	35	14%	
> 50	32	16%	0.88
Breslow			
≤ 2.00mm	23	22%	
2.01 – 4.00mm	24	17%	
> 4.00mm	18	6%	0.35
Clark			
2 / 3	31	16%	
4 / 5	32	16%	0.96
Ulceration			
Absent	42	21%	
Present	25	4%	0.05
Number of pos. sections			
1	7	29%	
2	9	0%	
3	51	16%	0.27
Starz (old)			
1	16	13%	
2	34	18%	
3	17	12%	0.82
Starz (new)			
I	26	23%	
II	25	8%	
III	16	13%	0.30
Number of pos SNs			
One	52	10%	
Multiple	15	33%	0.02
Dewar Location			
Subcapsular	30	13%	
Parenchymal	7	29%	
Combined	11	9%	
Multifocal	9	22%	
Extensive	10	10%	0.75
SN Tumor Burden			
< 0.1mm (sub-micro)	15	0%	
0.1 – 1.00mm	36	22%	
> 1.00mm	16	13%	0.12
SN Tumor Burden			
< 0.1mm (sub-micro)	15	0%	
≥ 0.1mm	52	19%	0.07*

** Fisher's test P = 0.10*

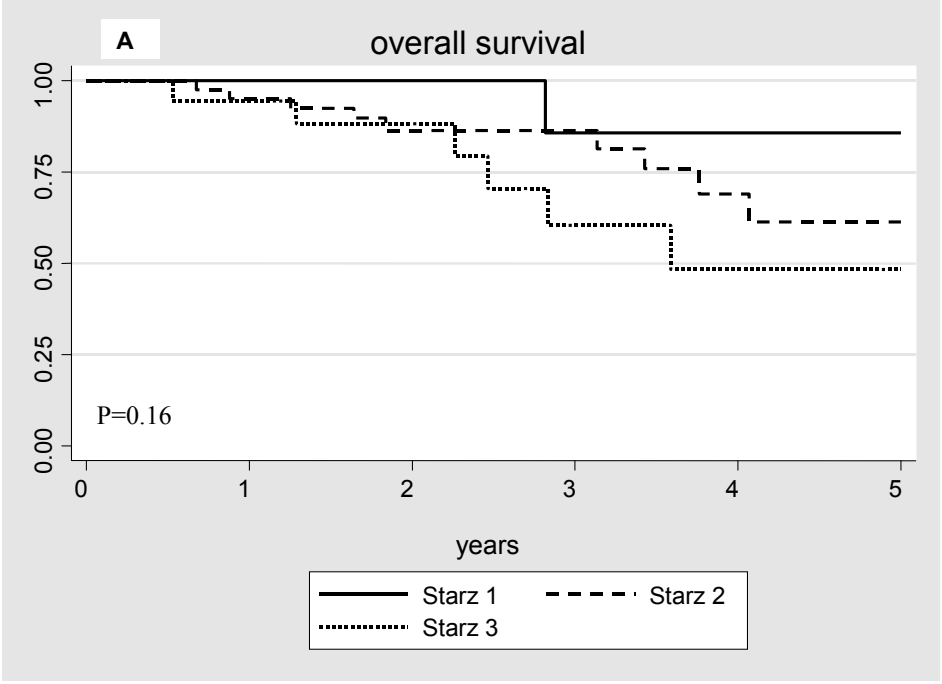

Figure 1A Kaplan-Meier estimated 5-year overall survival according to the original S classification by Starz

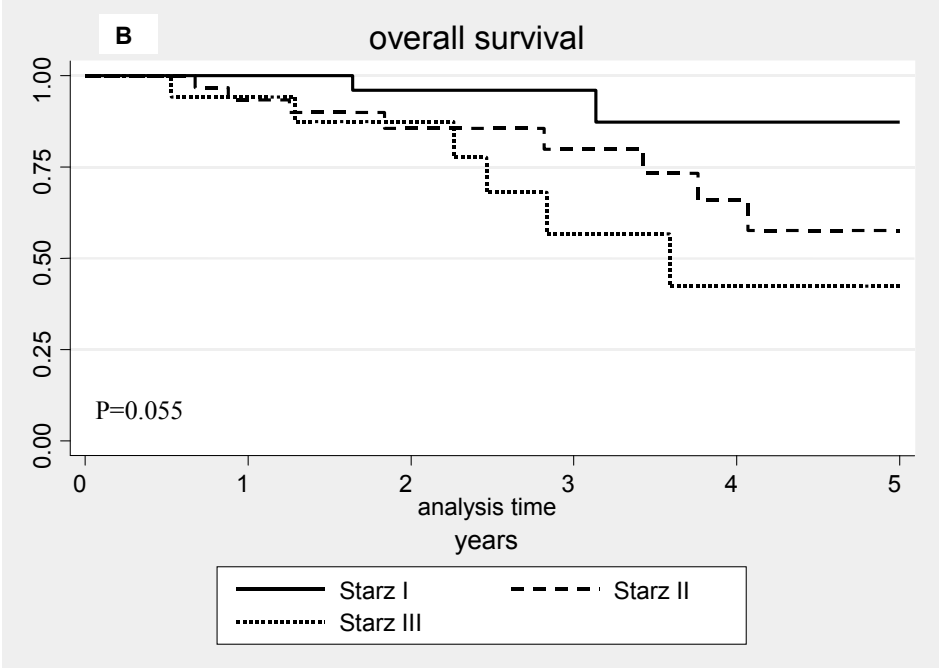

Figure 1B Kaplan-Meier estimated 5-year overall survival according to the simplified S classification by Starz

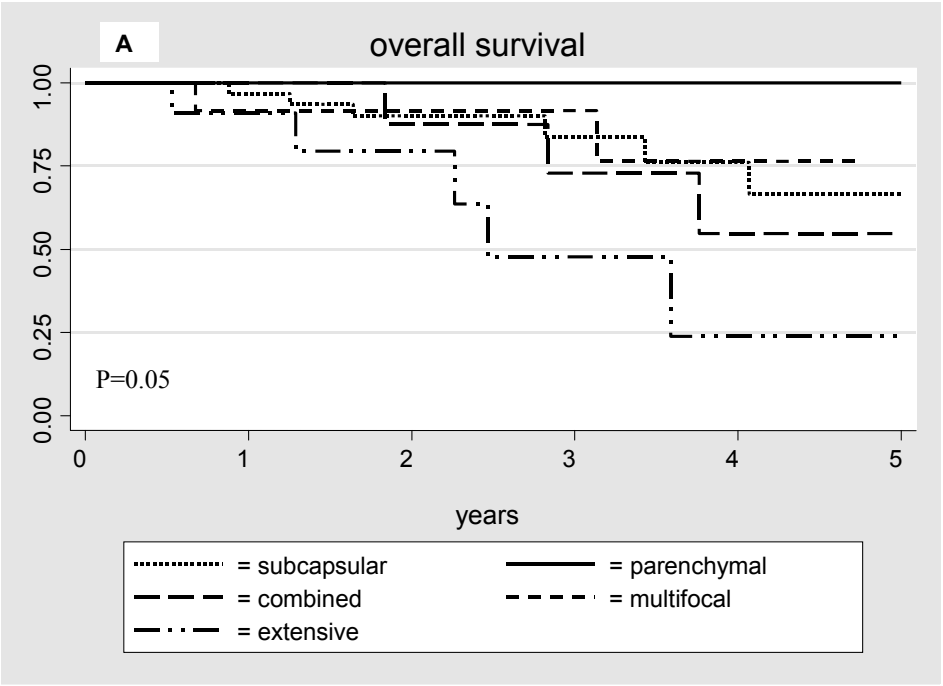

Figure 2A Kaplan-Meier estimated 5-year overall survival according to the location categories by Dewar

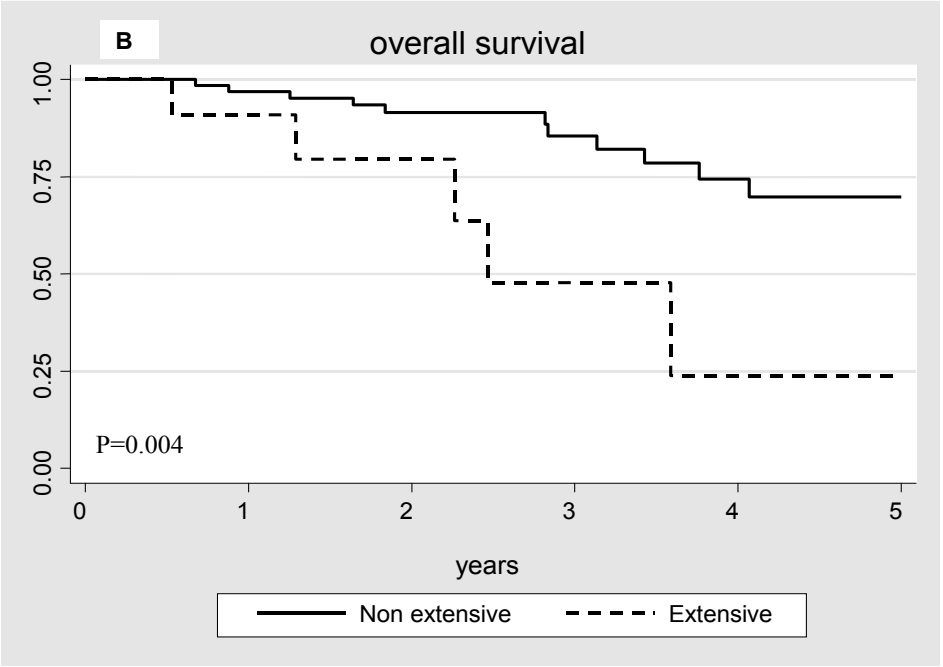

Figure 2B Kaplan-Meier estimated 5-year overall survival according to the location categories by extensive versus non-extensive SN involvement

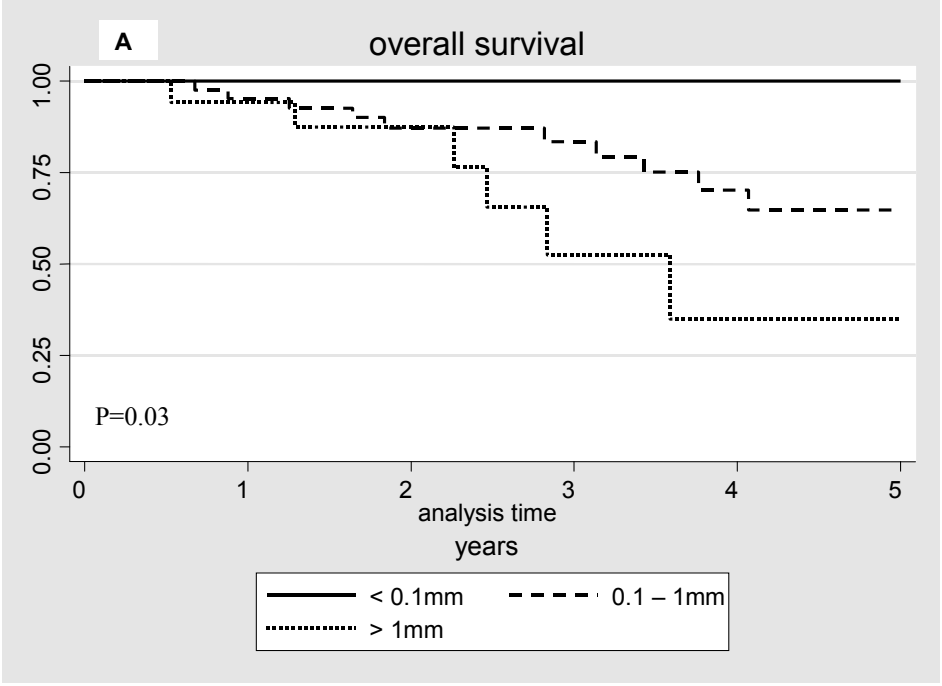

Figure 3A Kaplan-Meier estimated 5-year overall survival according to SN tumor burden

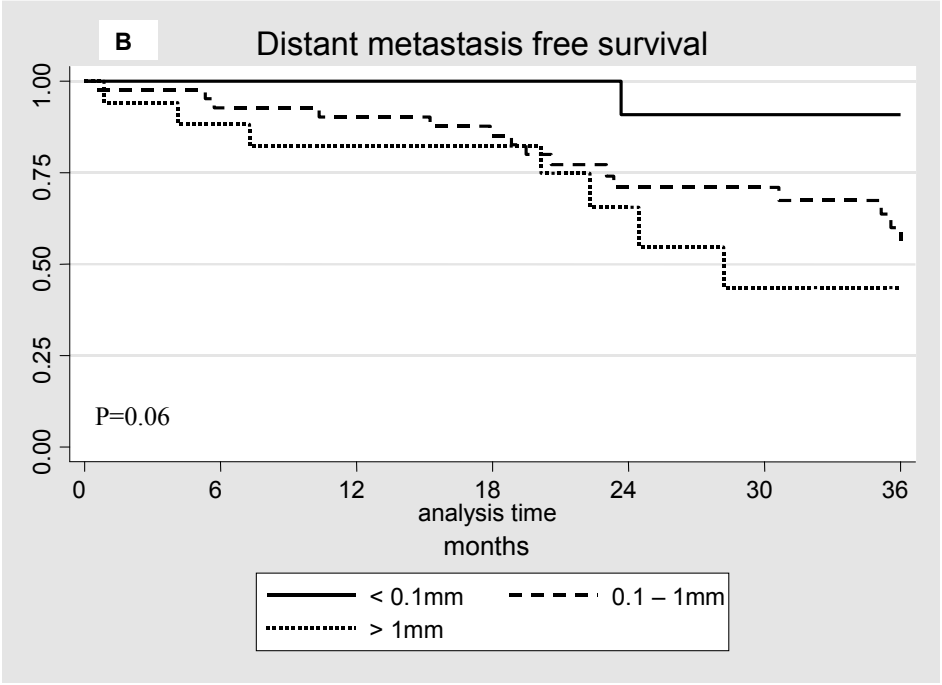

Figure 3B Kaplan-Meier estimated 3-year distant metastasis free survival according to SN tumor burden

Non-Extensive metastatic involvement of the SNs according to the Dewar classification, which is also significantly different for the two groups (P=0.004).

The estimated 5-years OS rates were significantly different between the different SN tumor burden groups and were 100%, 63% and 35% respectively (Fig.3A) (P=0.03). Two patients with sub-micrometastasis (<0.1mm) involvement developed a recurrence of the disease during follow-up. One patient had a local recurrence at the site of the primary and subsequently developed an in-transit metastasis. During follow-up the patient also developed multiple lung metastases, but is still alive with disease after 30 months. The other patient developed an in-transit metastasis, which was removed surgically and is alive and without evidence of disease after 41 months follow-up. Figure 3B shows the distant metastasis free survival (DMFS) according to the three different groups of SN tumor burden (P=0.06). The estimated 3-year DMFS for the respective groups was 91%, 56% and 44%.

The estimated 5-year OS rates for ulcerated and not ulcerated primary tumors were 45% and 75% respectively (Fig.4) (P=0.01). Gender, age, the number of positive sections per SN, the number of positive SNs, Breslow thickness and Clark level all had no significant effect on estimated survival rates in the univariate analysis.

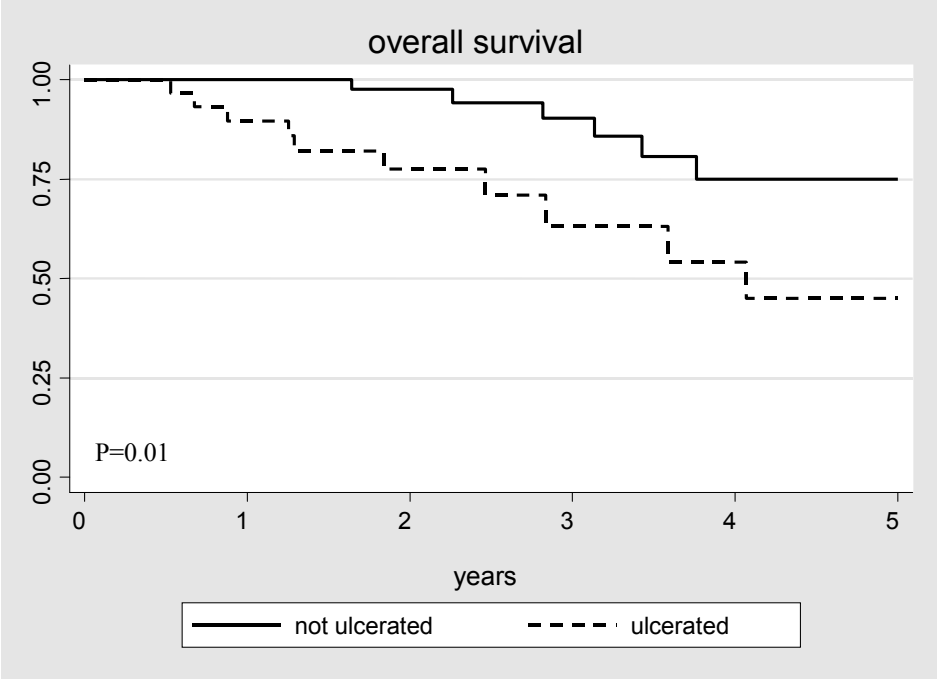

Figure 4 Kaplan-Meier estimated 5-year overall survival according to ulceration status of the primary tumor

Table 4 Univariate Cox proportional hazard regression analyses of disease-free and overall survival

	DFS			OS		
Covariate	**HR**	**95% CI**	**P**	**HR**	**95% CI**	**P**
Sex						
Female	1			1		
Male	0.99	0.48 – 2.06	0.98	0.46	0.17 – 1.28	0.14
Age						
≤ 50	1			1		
> 50	0.58	0.28 – 1.25	0.16	0.52	0.18 – 1.49	0.21
Breslow						
≤ 2.00mm	1			1		
2.01 – 4.00mm	2.57	0.96 – 6.88		1.68	0.53 – 5.30	
> 4.00mm	2.80	1.02 – 7.71	0.069	1.08	0.29 – 4.04	0.64
Clark						
2 / 3	1			1		
4 / 5	1.59	0.74 – 3.43	0.24	0.65	0.24 – 1.80	0.41
Ulceration						
Absent	1			1		
Present	2.25	1.08 – 4.69	0.03	3.43	1.24 – 9.47	0.02
Number of pos sections						
1	1			1		
2	1.95	0.18 – 21.50		3.49	0.63 – 19.19	
3	4.33	0.59 – 31.95	0.17	12.18	2.21 – 66.98	0.15
Starz (old)						
S1/2	1			1		
S3	2.77	1.30 – 5.90	0.008	2.14	0.78 – 5.89	0.14
Starz (new)						
SI	1			1		
SII	1.84	1.35 - 2.33		2.25	1.56 – 2.94	
SIII	3.39	2.90 - 3.88	0.01	5.05	4.36 – 5.74	0.02
Multiple SNs						
One	1			1		
Multiple	1.74	0.77 – 3.94	0.18	1.68	0.54 – 5.25	0.37
Dewar Location						
Not extensive	1			1		
Extensive	3.48	1.53 – 7.93	0.003	4.18	1.44 – 12.17	0.009
SN Tumor Burden						
< 0.1mm (single cells)	1			1		
0.1 – 1.00mm	3.70	0.85 – 16.01		3.12	1.32 – 7.35	
> 1.00mm	7.37	1.61 – 33.72	0.01	9.78	7.11 – 12.45	0.01

Table 5 Multivariate Cox proportional hazard regression analysis of disease-free and overall survival

	Univariate		Multivariate	
Covariate	**DFS**	**OS**	**DFS**	**OS**
Primary tumor factors				
Breslow thickness	0.069	n.s.	n.s.	n.s.
Ulceration	0.03	0.02	0.05	n.s.
SN factors				
Starz (new)	0.01	0.02	n.s.	n.s.
Dewar location	0.003	0.009	n.s.	n.s.
Tumor load	0.01	0.01	0.005	0.03

Table 6 The distribution of different characteristics according to the Dewar classification

	Total	**Subcapsular**	**Parenchymal**	**Combined**	**Multifocal**	**Extensive**	**P**
N	74	31	12	8	12	11	
%		42%	16%	11%	16%	15%	
Mean Breslow	3.5	3.1	2.35	3.4	3.4	5.4	0.14
Mean depth of the metastasis	1.08	0.27	0.64	0.93	0.29	4.67	<0.001
Add. Non-SN	15%	13%	29%	9%	22%	10%	0.74

Table 4 shows the univariate analysis for disease free survival (DFS). SN tumor burden (P=0.005) and ulceration (P=0.05) were significant independent prognostic factors for DFS on multivariate analysis (Table 5). Table 4 also shows the univariate analysis for overall survival. Only SN tumor burden (P=0.03) was a significant independent prognostic factor for overall survival on multivariate analysis (Table 5).

DISCUSSION

The SN procedure is the most accurate staging procedure for regional lymph node metastases in melanoma patients, without the substantial morbidity associated with an elective lymph node dissection. The SN status has been recognized as the most important prognostic factor for disease-free and overall survival of melanoma patients[1-4]. Patients with negative SN status generally have an excellent long-term survival with 90 – 95% 5-years OS, whereas a positive SN status is associated with a 5-years OS rate of 50 – 65%[2-4,8-10].

Approximately 67% to 90% of SN positive patients do not have further non-SNs that contain tumor deposits in the completion lymph node dissection specimen[2,4,5,7]. As a consequence, the majority of SN positive patients undergo unnecessary surgery with its associated morbidity. It has been proposed by other authors to identify patient, tumor

and SN characteristics, which could be predictive of non-SN positivity, in order to spare SN positive patients needless surgery[12-18], similar to the widely accepted situation in breast cancer. Breast cancer patients with sub-micrometastases (<0.2mm) in the SN do not undergo a completion axillary lymph node dissection, because these patients will not recur regionally, and therefore additional surgery can be safely omitted[21,22].

In melanoma, the tumor and SN characteristics; Breslow thickness and ulceration of the primary tumor, the number of positive SNs, SN metastatic tumor burden and tumor penetrative depth within the SN have all been identified as indicative factors for additional non-SN positivity[13-16]. These factors are all merely indicative and to this date no factor has been identified, which can absolutely predict non-SN positivity. Reeves et al.[15] proposed a size/ulceration classification of SN positive patients by the size of the metastasis in the SN and the ulceration status of the primary. This SU score was a significant prognostic indicator for additional non-SN positivity, but Gietema et al.[23] did not demonstrate the predictive value of this classification in their study. In the present study the *absence* of ulceration was predictive of additional non-SN positivity (P=0.05), this contradicting outcome cannot be explained and might be due to the relatively small number of patients in this study. On the other hand, the *presence* of ulceration was an independent prognostic factor for DFS on multivariate analysis (P=0.05) as was previously demonstrated by us and other authors[2,24,25]. The number of positive SNs was a significant predictive factor for further additional non-SN positivity (P=0.02), both in the present study and in a previous study of Salti et al[26].

The micro anatomic classification by Dewar et al.[18] seemed to be a promising prognostic tool for SN positive patients. However, the size (or the depth) of the SN tumor burden is considerably different for the different locations of metastases. In the present study the size of SN tumor burden seems to be the essential factor and not so much the location of the metastases. This is supported by the study of Dewar et al., because the locations with very small SN tumor burden have an excellent prognosis in contrast to the locations with larger sizes of SN tumor burden. In the study by Scolyer et al.[17] neither subcapsular nor parenchymal deposits of the metastases were a significant predictive factor for additional non-SN positivity. They did demonstrate, however, that the group of patients with extensive involvement had a higher rate of additional non-SN metastases compared to the patients with non-extensive involvement. SN tumor burden was an independent prognostic factor for additional non-SN positivity in the study of Vuylsteke et al.[14], no additional non-SN involvement was seen in patients with SN metastases of <0.03mm. However, Carlson et al.[7] did not find SN tumor burden to be a significant predictor of non-SN involvement in their patient population. In the present study no additional non-SN positivity in the group of patients with minimal SN tumor

burden (<0.1mm) was demonstrated, but this was borderline statistically not significant (p=0.07).

Breslow thickness was identified by studies by Lee et al.[13] and Sabel et al.[16] as an indicative for additional non-SN positivity, but this was not a predictive factor for additional non-SN positivity in the present study. Nor were gender, age, Clark level, number of positive sections and Starz classifications (old and new) predictive factors for additional non-SN positivity in the present study.

In the present study, SN tumor burden was a significant prognostic factor for overall survival (P=0.03). More importantly there was an estimated 5-year overall survival rate of 100% in the group of patients with single cells metastatic involvement of the SN. Despite two local failures, distant metastases are exceedingly rare (1/16 = 6.3%) and the estimated 5-year distant metastasis-free survival of 91% was identical to the SN negative patient group, previously reported[2]. Mean and median Breslow thickness and ulceration of the primary tumors of SN negative patients was also comparable with minimal SN tumor burden (< 0.1mm). The median Breslow thickness was 1.9mm vs. 1.7mm, mean Breslow thickness was 2.5mm vs. 2.4mm, ulceration was 23% vs. 37% for SN negative vs. minimal SN tumor burden (<0.1mm), respectively[2]. Furthermore, no additional non-SN positivity was seen and, although this was not significant, it seems likely that it would be significant in a larger patient population. Considering all this, the amount of SN tumor burden seems to be an excellent prognostic factor for overall survival.

In the study of Vuylsteke et al.[14] survival was only reported to be significantly important for SN tumor burden in combination with the Breslow thickness and the presence/absence of additional non-SN metastases. This classification does not seem be a very useful classification for clinical use. Carlson et al.[7] found the SN tumor burden to be a significant indicative for survival, however three groups (isolated melanoma cells, clusters of cells and ≤ 2mm) had very similar 3-year overall survival rates in contrary to the group of patients with SN tumor burden larger than 2mm, which had a significantly worse estimated overall survival. In a review study on the different applications of the TNM classification for all tumor types, Hermanek et al.[27] studied the significance of isolated tumor cells (sub-micrometastases, defined as deposits ≤ 0.2mm) and concluded that isolated clusters of tumor cells should be distinguished from micrometastases. Also, because the prognostic significance of isolated clusters of tumor cells is unknown, it should not be considered in the TNM classification for any tumor type, but should be documented none-the-less[27].

The location classification according to Dewar et al.[18] was not a significant prognostic factor for disease-free or overall survival on uni- and multivariate analyses (results not shown). However, when simplified to extensive versus non-extensive 'the location' was a significant prognostic factor for DFS and OS on univariate analysis, but did not remain

as an independent prognostic factor on multivariate analysis. The location of the metastasis seems to be a less significant prognostic factor for DFS and OS than the size of the SN tumor burden. Ulceration of the primary tumor and the new Starz classification were both significant factors on univariate analysis for overall survival (both P=0.02). But, neither remained as an independent prognostic value for OS after multivariate analysis.

The present study shows an important observation, that sub-micrometastasis (<0.1mm) involvement of the SN, or what others call isolated clusters of melanoma cells (more than 10 cells) is a biologically very different from 'larger' micrometastatic disease. As a consequence of this different, less aggressive disease, which has an identical estimated 5-year distant metastasis free survival (91%) comparable to the OS rate for SN negative patients, and a 0% non-SN positivity rate these patients could possibly be spared the morbidity of a CLND, without compromising their survival chances.

The interim results of the MSLT-I trial[9] shows in the ITT analysis a 13% survival benefit for the SN positive patients over patients which underwent a delayed or therapeutic lymph node dissection (TLND). However, patients with sub-micrometastases were considered SN positive and stratified as such into this trial[28,29]. Our data, in 22% (16 / 74) of the SN positive patients the tumor burden was < 0.1mm and projected 5-yr survival was 100%. This indicates that these patients (possibly up to 22% of the population) might be considered as "biologically" false positive and will probably incorrectly improve the outcome of patients who underwent a SN procedure versus the patients that underwent a TLND in the observation arm of the MSLT-1 trial.

Sub-micrometastases (clusters of more than 10 cells, but <0.1mm) may not be considered as metastatic melanoma and as a consequence these patients are highly unlikely to benefit from CLND. Therefore we explain this situation to the patient and do not recommend CLND for melanoma sub-micrometastases. Importantly these patients have such excellent prognosis that they should not be stratified as stage III patients, when entered into adjuvant therapy trials. As a consequence randomizing patients with RT-PCR positive sentinel nodes for additional lymph node dissection seems highly unlikely to be of any benefit to patients and thus it's added value in clinical trials is very doubtful.

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Chapter 5

Multivariable analysis comparing outcome after sentinel node biopsy or therapeutic lymph node dissection in patients with melanoma

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ABSTRACT

Sentinel Node (SN) status is the most important prognostic factor for overall survival (OS) in stage I/II melanoma patients. However, its therapeutic value remains unclear. We recently reported that SN-positive patients with submicroscopic involvement of the SN (clusters of cells < 0.1 mm) had a distant recurrence rate of only 9% at 5 years, which is as good as SN-negative patients. We thus hypothesized that these SN patients may be considered SN-negative.

Here we compare outcome of CLND in SN+ patients with outcome in TLND patients with palpable nodes, treated in a tertiary referral centre. Survival rates were calculated from date of primary excision. All patients with primary melanomas on extremities or trunk were included. We identified 188 patients; 124 TLND patients ('82 – '05) and 64 CLND patients ('97 – '05). Median follow-up was 56 and 37 months, respectively. There were no significant differences between both groups regarding Breslow thickness, ulceration, gender, and site of the primary.

On univariate analysis site of the primary tumor (extremity versus trunk) ($P<0.001$), Breslow thickness ($P=0.005$), ulceration ($P<0.001$) were prognostic for OS. There was a non-significant 13% difference in OS for the CLND compared to the TLND group ($P=0.12$). Excluding SN patients with submicrometastases (n=15) reduced the difference in OS to 6% ($P=0.42$).

The present study did not show a significant survival benefit for SN+CLND compared to TLND, especially not when patients with submicrometastases were excluded.

INTRODUCTION

Lymph node involvement is the most significant prognostic factor for survival and recurrence in malignant melanoma. The presence of lymph node metastases decreases the 5-year survival by 40% to 50% compared to patients without nodal metastases. The management of the regional lymph node basin either by immediate or delayed lymph node dissection, is an ongoing area of debate. Elective lymph node dissection (ELND) did not significantly improve survival in several randomized trials[1-4]. The sentinel lymph node biopsy (SLNB), developed by Morton and colleagues[5], identifies patients with clinically occult lymph node metastases who may benefit from completion lymph node dissection (CLND). Primarily though the SLNB is to be considered a diagnostic staging procedure rather than a therapeutic procedure. The sentinel node (SN) status has been shown to be the most important prognostic factor for disease-free and overall survival of stage I / II melanoma patients[6-9]. The therapeutic value of SLNB followed by early CLND has not yet been demonstrated.

However, not all SN-positive patients may not necessarily be all "biologically positive" patients, in the sense that a fraction of the SN-positive patients may have had a tumor load that does not necessarily represent truly metastatic disease that will progress locally or systemically. We reported in an analysis of our own data on the concept of "biologically false positive" SN patients[10]. Patients with submicrometastases, which were defined as clusters of more than 10 cells, but < 0.1mm, had identical patient and tumor characteristics as SN negative patients. More importantly, none of these patients had additional lymph node metastases in the CLND specimen and the survival rates were also identical for submicrometastases patients as for SN negative patients. Therefore, these patients might be considered biologically false positive and we concluded that these patients should not be included into the SN positive patient population.

The aim of the present study was to analyze our own series of melanoma patients with nodal disease in order to determine if those who were subjected to SLNB followed by CLND fared better than those who presented with palpable lymph node metastases and subsequently underwent TLND. Disease-free (DFS) and overall survival (OS) were evaluated and factors that influence prognosis were assessed.

PATIENTS AND METHODS

Patients

From 1982 through 2005, 303 consecutive patients were treated for regional lymph node metastases at our institution (Erasmus Medical Centre, Daniel den Hoed Cancer Centre, Rotterdam, The Netherlands) (Figure 1). 236 patients underwent TLND for

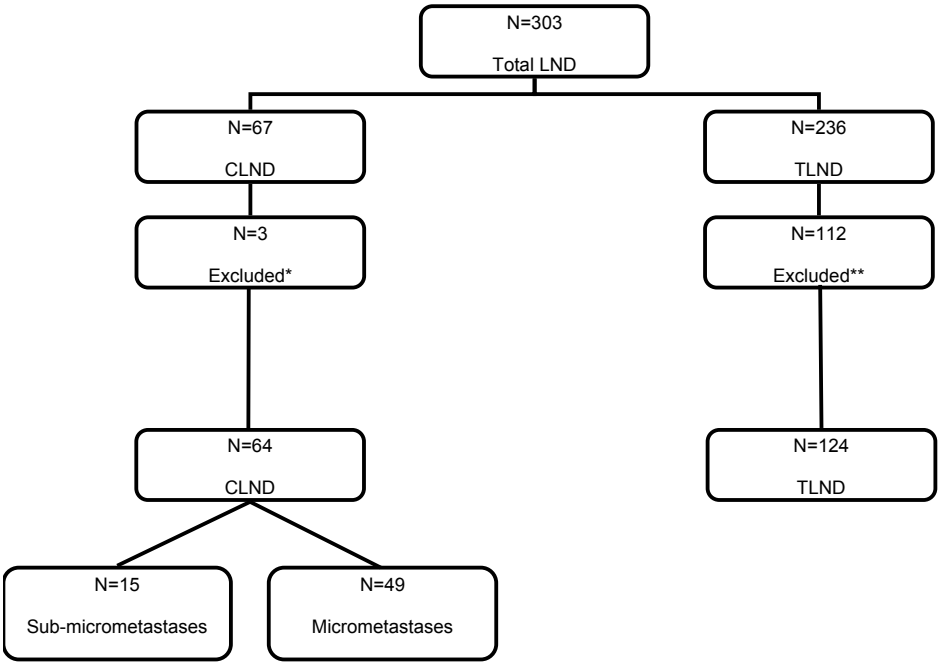

Figure 1 Patient population selection flowchart

palpable lymph node metastases with curative intent from 1982 through 2005[11]. None of the included patients had clinical evidence of systemic disease at the time of the lymph node dissection. SLNB followed by CLND was performed in 67 patients between 1997 and 2005. SLNB was indicated for malignant melanoma with a minimum Breslow thickness of 1.00 mm and/or Clark level IV/V or if ulceration was present. Patients with unknown Breslow thickness were not considered for further analysis. Furthermore, all patients with head and neck primary tumors were excluded from the analysis; this was 2 for the CLND group and 37 for the TLND group, respectively. This resulted in the analysis of 124 patients who underwent TLND and 64 patients who underwent CLND.

Patient, tumor and dissection characteristics were obtained from hospital records. Primary tumor details: Breslow thickness, Clark level, tumor location and tumor ulceration were established. The interval between diagnosis of the primary tumor and lymph node dissection was calculated. The different types of dissections were divided into ilio-inguinal and axillary dissections. Number of tumor positive lymph nodes, defined as N1 (one positive lymph node), N2 (two or three positive lymph nodes) and N3 (more than three positive lymph nodes) and the presence of extra capsular extension (ECE) was recorded.

All patients were routinely monitored at the outpatient clinic. Recurrences were scored as locoregional, regional lymph node, distant lymph node, distant subcutaneous or visceral metastasis.

Lymphatic mapping and surgical procedures

The triple technique was used for sentinel node identification, as described previously[12]. In most patients, treatment of the primary tumor was performed in referring hospitals. Standard treatment of the primary melanoma was local excision with adequate safety margins according to the guidelines of the Dutch Melanoma Workgroup. Patients underwent CLND within six weeks from sentinel lymphadenectomy. In the case of axillary metastases, levels I - III were excised and when indicated, the minor pectoral muscle was resected. Ilio-inguinal 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.

Pathological analysis

Primary tumors and the specimens from the lymph node dissections were examined using routine techniques of haematoxylin and eosin (H&E) staining. Additional staining to S100 or MART-1 was applied when the presence of melanoma cells in the dissection specimen was uncertain. Total number of harvested lymph nodes, number of tumor positive lymph nodes, the presence of ECE and the presence of non-radical resection margins were reported.

Sentinel nodes underwent a different pathology protocol. After 24-hour fixation in buffered formalin the sentinel nodes were cut in half through the hilum and its longest dimension and embedded in paraffin. In rare cases, exceptionally large nodes were sectioned parallel to the first cut in order to fit in the blocks. Five serial step sections of 4 µm each were cut from both faces of the sentinel lymph node and subsequently stained with H&E, S100 and HMB-45. Until June 2002 the intervals were 250 µm and after this time they were reduced to 50 µm intervals, as described by the EORTC melanoma group protocol of Cook et al.[7,13].

Adjuvant therapy

In the later years of this study, several patients were accrued into the European Organization for Research and Treatment of Cancer (EORTC) 18952 trial or the EORTC 18991 trial. The EORTC 18952 trial has evaluated the effects of adjuvant therapy with intermediate doses of interferon alpha 2b and did not show a significant survival benefit for patients in the treatment group[14]. The EORTC 18991 trial will evaluate the role of long-term treatment with pegylated interferon[15]. Patients were considered for local radiotherapy in case of narrow or irradical resection margins, excessive nodal involvement (4 or more positive lymph nodes), ECE or simultaneous in-transit, subcutaneous or skin metastases in the operation area.

Statistical analyses

Statistical analyses were all performed with Stata version 9.1 (Stata Corporation, College Station, Texas, USA). Baseline values were compared with the chi-square test for proportions or the Mann-Whitney-U test for ordered data. Disease-free survival (DFS) and overall survival (OS) were calculated from the time of primary tumor excision to the time of recurrence (DFS) or to the time of death (OS) respectively and were censored at the last contact date if there were no events. Univariate analyses of end-points were performed using the Kaplan-Meier method and the logrank test. Multivariate analyses to determine the prognostic value of covariates regarding disease-free and overall survival were performed using the Cox's proportional hazard model. P values of less than 0.05 were considered significant.

RESULTS

This study included 188 patients (99 males and 89 females), who either underwent a completion lymph node dissection (CLND) after a positive sentinel node (SN) (n=64), or a therapeutic lymph node dissection (TLND) for a palpable lymph node metastasis (n=124). The median age of the entire group was 52 years (range: 15 – 82 years); the median age per group was 47 years (range: 15 – 76 years) for CLND patients and 53 years (range: 23 – 82 years) for TLND patients. The mean and median Breslow thicknesses of the primary tumors of the entire patient population were 3.8mm and 2.3mm (range 0.43 – 52.00mm), respectively. The mean and median Breslow thickness was 3.4mm and 2.8mm for the CLND group; these were 4.1mm and 2.0mm for the TLND group. The distribution of patient and tumor characteristics for the different groups is depicted in Table 1. The median follow-up of all patients was 47 months; this was 37 months for the CLND group and 56 months for the TLND group.

Univariate analyses

On univariate analysis of the total patient population (CLND and TLND together) the Breslow thickness (P=0.004), ulceration status (P=0.002) and the site (P<0.001) of the primary tumor were significant prognostic factors for disease-free survival. Gender, age, Clark level, number of positive lymph nodes and the different treatment modalities were no significant prognostic factors for DFS on univariate analysis.

On univariate analysis for overall survival, the Breslow thickness (P=0.005), ulceration (P<0.001) and the site (P<0.001) of the primary tumor were significant prognostic factors. Gender, age, Clark level, number of positive lymph nodes and the different treatment modalities were no significant prognostic factors for OS on univariate analysis. There was a 13% (non-significant, P=0.12) difference in OS between the total CLND and

Table 1 Patient and tumor characteristics

	TLND (N = 124)	CLND (N = 64)	P †
Gender			
Male	65 (52.4%)	34 (53%)	
Female	59 (47.6%)	30 (47%)	0.93
Age			
< 60 years	76 (61.3%)	51 (80%)	
> 60 years	48 (38.7%)	13 (20%)	0.01
Site			
Extremity	74 (59.7%)	36 (56%)	
Trunk	50 (40.3%)	28 (44%)	0.65
Breslow			
T1 / 2 (≤ 2.00)	64 (51.6%)	24 (38%)	
T3 (2.01 – 4.00)	29 (23.4%)	22 (34%)	
T4 (> 4.00)	31 (25.0%)	18 (28%)	0.14
Clark*			
II	8 (7.1%)	2 (3%)	
III	28 (25.0%)	30 (48%)	
IV	66 (58.9%)	28 (45%)	
V	10 (8.9%)	2 (3%)	0.02
Ulceration			
Present	45 (36.3%)	25 (39%)	
Absent	79 (63.7%)	39 (61%)	0.71
Number of nodes			
N1	53 (42.7%)	47 (73%)	
N2 (2 / 3)	35 (28.2%)	11 (17%)	
N3 (> 3)	36 (29.0%)	6 (9%)	<0.0001

*Values in parentheses are percentages. *Number of patients was 112 for total lymph node dissection (TLND) and 62 for completion lymph node dissection (CLND); 14 patients had an unknown Clark invasion level. †Log rank test.*

TLND groups (Fig.2A). When the patients with submicrometastases (cluster of more than 10 cells, <0.1mm) were excluded from the SN/CLND group, the difference in OS was reduced to 6% (NS; P=0.42) (Fig.2B).

Multivariate analysis

A multivariate analysis was performed for disease-free and overall survival; the results are depicted in Table 2 and Table 3 respectively. On multivariate analysis, site (extremity versus trunk) and ulceration status of the primary tumor were independent prognostic factors for both DFS and OS. The different treatment types (SN/CLND vs. WLE and late TLND) were not an independent prognostic factor for DFS or OS.

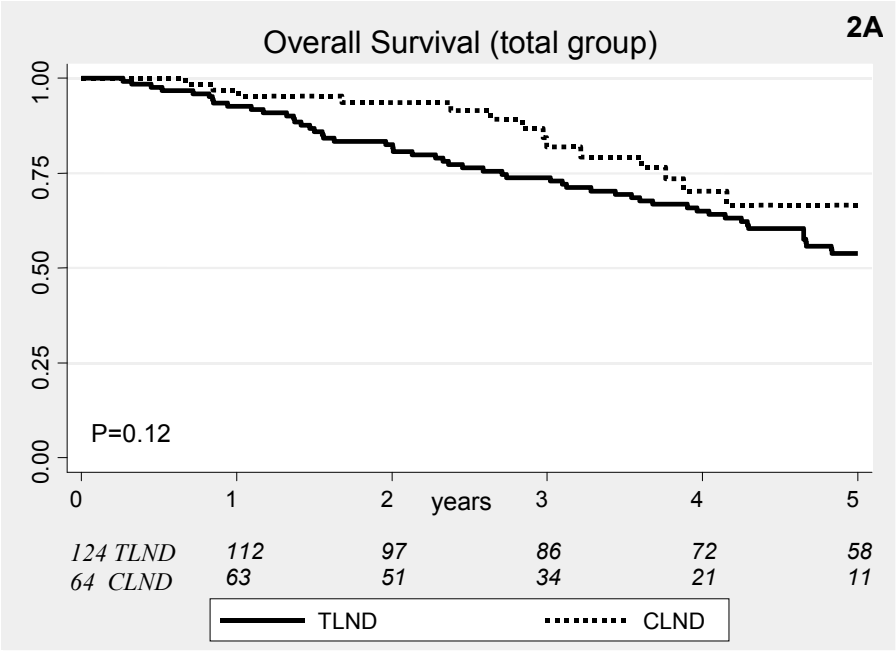

Figure 2A Kaplan-Meier estimated overall survival according to the different treatment modalities for the total group

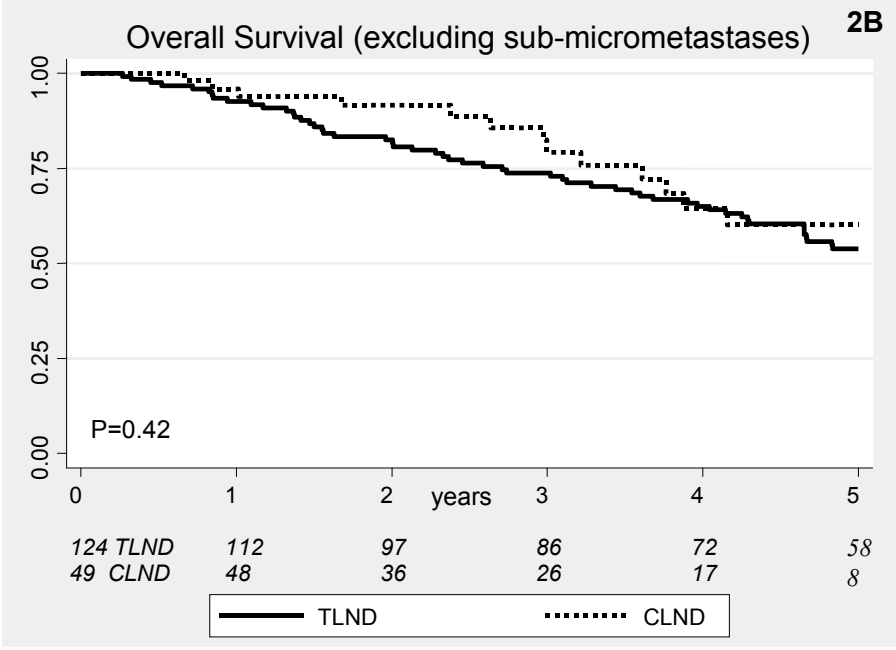

Figure 2B Kaplan-Meier estimated overall survival according to the different treatment modalities, excluding the submicrometastases

Table 2 Multivariable analysis for disease-free survival

Co-variate	HR	95 % CI	P Value Univariable*	P Value Multivariable†
Age				
< 60 years	1			
≥ 60 years	1.00	0.99 – 1.02	0.52	n.s.
Gender				
Female	1			
Male	1.35	0.88 – 2.08	0.17	n.s.
Primary location				
Extremity	1			
Trunk	2.66	1.72 – 4.10	<0.001	< 0.001
Breslow thickness				
T1/2 (≤ 2.00mm)	1			
T3 (2.01 – 4.00mm)	2.06	1.23 – 3.45		
T4 (> 4.00mm)	2.11	1.26 – 3.55	0.0038	n.s.
Clark				
II	1			
III	0.75	0.28 – 1.99		
IV	1.12	0.44 – 2.81		
V	1.20	0.37 – 3.93	0.48	n.s.
Ulceration				
Absent	1			
Present	1.93	1.25 – 2.97	0.0023	< 0.001
Number of positive nodes				
N1 (1 node)	1			
N2 (2/3 nodes)	1.34	0.79 – 2.27		
N3 (> 3 nodes)	1.63	0.99 – 2.69	0.15	n.s.
Treatment modality				
CLND	1			
TLND	1.28	0.78 – 2.08	0.33	n.s.

*HR = Hazard Ratio, CLND = Completion Lymph Node Dissection, TLND = Therapeutic Lymph Node Dissection, *Kaplan–Meier analysis (log rank test); †Cox's proportional hazard model.*

DISCUSSION

Over the last decade increasingly more physicians worldwide have started to perform sentinel node procedures on stage I/II melanoma patients. The SN-status has been shown to be the most important prognostic factor for disease-free and overall survival[6-9,16]. For future adjuvant trials and translational research the SN procedure is therefore an important modality in the treatment of melanoma patients. However, the SN procedure does not seem to lead to a survival benefit.

Table 3 Multivariable analysis for overall survival

Co-variate	HR	95 % CI	P Value Univariable*	P Value Multivariable†
Age				
< 60 years	1			
≥ 60 years	1.01	0.99 – 1.02	0.43	n.s.
Gender				
Female	1			
Male	1.22	0.75 – 1.98	0.42	n.s.
Primary location				
Extremity	1			
Trunk	2.40	1.48 – 3.89	0.0003	< 0.001
Breslow thickness				
T1/2 (≤ 2.00mm)	1			
T3 (2.01 – 4.00mm)	2.26	1.26 – 4.07		
T4 (> 4.00mm)	2.30	1.28 – 4.14	0.0046	n.s.
Clark				
II	1			
III	0.73	0.27 – 1.97		
IV	0.72	0.28 – 1.86		
V	1.23	0.38 – 4.05	0.60	n.s.
Ulceration				
Absent	1			
Present	2.39	1.48 – 3.88	0.0002	< 0.001
Number of positive nodes				
N1 (1 node)	1			
N2 (2/3 nodes)	1.47	0.82 – 2.65		
N3 (> 3 nodes)	1.68	0.95 – 2.97	0.16	n.s.
Treatment modality				
CLND	1			
TLND	1.60	0.89 – 2.90	0.12	n.s.

*HR = Hazard Ratio, CLND = Completion Lymph Node Dissection, TLND = Therapeutic Lymph Node Dissection, *Kaplan–Meier analysis (log rank test); †Cox's proportional hazard model.*

Some retrospective reports suggested that early completion lymph node dissections (CLND) in SN positive patients, may lead to a survival benefit for these patients[17,18]. But, these studies have encountered several statistical and methodological difficulties, such as the problem of stage migration. Essner et al. suggest a 19% survival benefit for SN positive patients compared to ELND positive patients[19]. The number as well as the site of sections in the work up of a SN, and the introduction of immunohistochemistry (IHC) by SN staging, has led to the identification of more patients as stage III patients, compared to one or two sections after the standard bivalving of lymph nodes in the evaluation

of elective lymph node dissection specimens in the old days. This increase in staging accuracy favors SN identified patients compared to ELND patients. Doubrovsky et al. have also reported this phenomenon, but this did not influence survival in the Sydney Melanoma Unit series[20].

A study by Kretschmer et al.[21] showed a significant survival benefit of 13% at 5 years for SN positive patients treated with a CLND compared to TLND patients. In the recently published MSLT-1 data of the 1.2mm-3.5mm subgroup, Morton et al. reported that SN positive patients had a survival benefit of 20% at 5 years compared to patients who underwent WLE only and developed clinically palpable nodal metastases[22]. Although these results seem promising for a potential therapeutic effect of SN biopsy followed by CLND, some issues need to be clarified. For one, not just the 1.2mm-3.5mm subgroup interim analysis[22], but also the overall final results of the MSLT-1 will need to be reported[23,24]. Moreover, in the reported subgroup analysis, the OS was statistically not different between the SN group and the control group (87.1% vs. 86.6%, respectively)[22]. This is surprisingly, because the impact on overall survival for the SN positive group should have made a difference of 3-4% for overall survival in all patients.

Another important issue is raised by a recent study from our institute[10] and a similar one from the M.D. Anderson Cancer Centre[25]. Both studies showed that not all positive sentinel nodes are to be considered the same and it is questionable if all microscopically detected disease will develop into clinically relevant disease[10,26]. The data from our centre shows that there is a significant group of SN positive patients, those with submicrometastases (cluster of more than 10 cells, <0.1mm), have similar primary tumor characteristics, but also survival rates, as SN negative patients[10].

We therefore conducted the present study to analyze differences in survival between "biologically positive" SN patients and patients treated for palpable metastatic disease. Overall there was a non-significant difference of 13% in OS at 5 years between both treatment modalities when including all SN detected disease. However, once patients with sub-microscopic disease were excluded from the analysis, the non-significant difference in OS became even less significant and was reduced to only 6%.

Because SN staging was not standard hospital policy for head and neck primary tumors, there was a major imbalance in this factor between both therapy groups. Since it has been reported that patients with head and neck primary melanomas have a worse prognosis this imbalance was not acceptable and therefore these patients were excluded from the analysis. The patient groups were well balanced for the most important prognostic factors, Breslow thickness and ulceration. The ulceration status and the site of the primary tumor (extremity versus trunk) were independent prognostic factors for DFS and OS in this population of stage III melanoma patients.

Without analyzing rate of submicrometastatic SNs as well as the rate of false negative SN assessments, which will both benefit the survival rate of the SN positive patient, the

thus far reported survival benefit is overestimated. The present study did not demonstrate a survival benefit for SN positive patients, who subsequently underwent CLND compared to patients that underwent WLE only and subsequently developed palpable nodal disease treated by TLND. Excluding submicrometastatic (<0.1mm) positive patients from the analysis is important to increase the validity of the analysis.

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Chapter 6

Minimal Sentinel Node (SN) Tumor Burden According to the Rotterdam Criteria is the Most Important Prognostic Factor for Survival in Melanoma Patients. A Multicenter Study in 388 SN Positive Patients

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ABSTRACT

Background

The more intensive sentinel node (SN) pathologic workup, the higher the SN-positivity rate. This is characterized by an increased detection of cases with minimal tumor burden (SUB-micrometastasis < 0.1 mm), which represents different biology.

Methods

The slides of positive SN from three major centers within the EORTC Melanoma Group were reviewed and classified according to the Rotterdam Classification of SN Tumor Burden (<0.1mm; 0.1-1mm; >1mm) maximum diameter of the largest metastasis. The predictive value for additional nodal metastases in the completion lymph node dissection (CLND) and disease outcome as disease free (DFS) and overall survival (OS) were calculated.

Results

In 388 SN positive patients, with primary melanoma, median Breslow thickness was 4.00mm; ulceration was present in 56%. 40 patients (10%) had metastases <0.1mm. Additional nodal positivity was found in only 1 of 40 patients (3%). At a mean follow-up of 41 months, estimated OS at 5 years was 91% for metastasis < 0.1 mm, 61% for 0.1 – 1.0 mm, and 51% for >1.0mm ($p<0.001$). SN tumor burden increased significantly with tumor thickness. When the cut-off value for SUB-micrometastases was taken at <0.2mm (such as in breast cancer), the survival was 89% and 10% had additional non-SN nodal positivity.

Conclusion

This large multicenter dataset establishes that patients with SUB-micrometastases < 0.1 mm have the same prognosis as SN negative patients and can be spared a CLND. A <0.2mm cut-off for SUB-micrometastases does not seem correct for melanoma, as 10% additional nodal positivity is found.

INTRODUCTION

After a number of underpowered trials failed to demonstrate a survival benefit for the elective lymph node dissection (ELND)[1-4], the introduction by Morton and co-workers of a new technique, sentinel node (SN) biopsy, gathered great popularity[5,6]. The hypothesis was proposed that SN biopsy would identify those patients that could possibly benefit from the removal of all regional lymph nodes and this could lead to a survival benefit. The Multicenter Selective Lymphadenectomy Trial (MLST-I) was performed to evaluate if wide local excision (WLE) combined with a SN procedure, followed by a completion lymph node dissection (CLND) in case of a positive SN, would result in a survival benefit over WLE only, followed by a therapeutic lymph node dissection (TLND) in case of a nodal recurrence during follow-up.

The MSLT-I failed to demonstrate a survival benefit in favor of the SN procedure[7]. Subgroup analyses were presented, which suggested that the outcome in SN positive patients who underwent a CLND was better than WLE patients who underwent a TLND during follow-up[7]. However, SN positive patients represent a mix of patients with good and bad biology, reflected by SN tumor burden, tumor location within the SN and the number of nodes involved. Thus sentinel node positive patients may well behave biologically different from patients with clinical nodal recurrence after WLE only and therefore the interpretation of the results of such a post-randomization comparison should be done with great caution and considered exploratory only.

A number of studies have classified SN metastases by different criteria and have discussed the differences in biologic behavior that they may represent[8-14]. The observation of extremely favorable outcome in patients with < 0.1 mm SUB-micrometastases according to the Rotterdam Criteria needed to be validated by reclassifying positive sentinel nodes from other centers.

The aims of the present study were to increase the study power compared to our previous single center experience[8]. Moreover to analyze the occurrence rate of minimal SN tumor burden in different centers and to correlate this to Breslow thickness and the extent of the pathological work-up of the SN. And finally, to evaluate the survival rate of minimal SN tumor burden in this multicenter study.

PATIENTS AND METHODS

Data from the prospective melanoma databases from three major cooperating centers within the network of the EORTC Melanoma Group (MG), were combined for the purpose of this study. Participating centers were; Erasmus University Medical Center – Daniel den Hoed Cancer Center, Rotterdam, the Netherlands, M.Sklodowska-Curie Memorial Cancer

Center and Institute of Oncology, Warsaw, Poland and the Charité, Humboldt University of Berlin, Germany. Between 1993 and 2007, a total of 2200 melanoma patients underwent a SN procedure in the three cooperating centers. In total 388 SN positive patients (17.6%), whose slides were available for reviewing, had sufficient patient, primary tumor information and follow-up to be included into this study. 86 Patients (22%) were included from Berlin, 95 patients (25%) from Rotterdam and 207 patients (53%) from Warsaw, respectively. Primary tumor, patient and follow-up characteristics were available and all pathology slides of the SNs of these patients were re-evaluated for the purpose of this study. Baseline characteristics are summarized in Table 1.

Table 1 Patient, primary tumor characteristics of all patients

Center	
Berlin	86 (22%)
Rotterdam	95 (25%)
Warsaw	207 (53%)
Gender	
Male	205 (53%)
Female	183 (47%)
Age	
< 60	263 (68%)
≥ 60	125 (32%)
Location primary tumor	
Extremity	192 (49%)
Trunk	185 (48%)
Head & Neck	11 (3%)
Breslow Thickness	
≤ 1.00 mm	20 (5%)
1.01 – 2.00 mm	68 (18%)
2.01 – 4.00 mm	133 (34%)
> 4.00 mm	157 (40%)
Unknown	10 (3%)
Clark level	
II	13 (3%)
III	127 (33%)
IV	183 (47%)
V	54 (14%)
Unknown	11 (3%)
Ulceration	
Present	216 (56%)
Absent	136 (35%)
Unknown	36 (9%)

All patients had a previously diagnosed melanoma with a minimum Breslow thickness of 1.00 mm or Clark IV / V or with an ulcerated primary tumor. Patients subsequently underwent a SN procedure, which was identified with the use of the triple technique, which is described in detail elsewhere[15-17]. In short, the triple technique consists of a preoperative lymphoscintigraphy, peroperative use of patent blue and a handheld gamma detection probe. A lymph node was considered a SN if it stained blue, 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[18,19]. Patients from Berlin were subjected to a preoperative ultrasound examination of the regional lymph nodes for the purpose of another study described elsewhere[20]. Nonetheless, these patients proceeded to undergo a SN procedure according to the protocol described above.

Pathology Work-up and Review

The pathological work-up of the SNs was the same for all three participating centers. It was performed according to the EORTC Melanoma Group pathology protocol developed by Cook et al.[21] The sentinel lymph nodes were fixed for 24 h in buffered Formalin. After fixation they were bivalved through the hilum in its longest dimension and embedded in paraffin. In rare cases, exceptionally large nodes were sectioned parallel to the first cut in order to fit in the blocks. Five serial step sections of 4 µm each were cut from each face of the lymph node, and staining with H&E, S100 and HMB-45 was performed. Initially the serial sections were made with 250 µm intervals, a change in policy in 2002 decreased the interval to 50 µm. Warsaw had a slightly different approach to the pathological work-up than the other centers, because of budgetary issues. In the pathological examination of Warsaw patients, only H&E staining was performed first. If this was negative other slides (which were already cut at the described intervals) were stained with S100 and HMB-45.

All slides of the included patients were reviewed again for the purpose of this study. SN tumor burden was measured according to the Rotterdam Criteria[8], which consist of the following: Measure the maximum diameter (in any direction) of the largest lesion on a slide. All positive slides are examined and this process of measuring the largest lesion in repeated. The largest value overall (which is the largest diameter measured anywhere on one slide in one patient) has been defined is the amount of SN tumor burden (in mm). If a patient had multiple positive SNs, the largest maximum diameter of any of the SNs is the largest overall and thus the amount of SN tumor burden for this patient. Categories were made for SN tumor burden. SUB-micrometastases have been defined as a maximum diameter of < 0.1 mm[8]. Other categories are 0.1 – 1.0 mm and > 1.0 mm.

The location of the metastases was also recorded, according to the Dewar criteria for the micro-anatomic location of the metastasis[11]. This was either; subcapsular, parenchymal, combined, multifocal or extensive.

Follow-up

Follow-up was gathered at the outpatient clinic according to a follow-up schedule of the specific countries. Basically this involves regular palpation of the lymph node basins at the outpatient clinic at 3 – 4 month intervals for the first two to three years. And at 6 month intervals for years 3 – 5. Routine chest X-rays were performed once a year. In Berlin only, patients were subjected to routine ultrasound of lymph node basins. Other imaging techniques, such as CT, MRI or PET-scans were not routinely performed, but only on indication.

Statistical Analysis

Statistical analyses were all performed with Stata®, version 10.0 (Stata Corporation, College Station, Texas, USA). Disease-free (DFS) and Overall Survival (OS) were calculated from the date of SN biopsy until the date of first recurrence or death, respectively and were censored at the last contact date if there were no events. Univariate analyses of endpoints were performed using the Kaplan-Meier method and the log rank test. Multivariate analyses to determine the prognostic value of covariates regarding disease-free and overall survival were performed using the Cox's proportional hazard model. P values of less than 0.05 were considered as significant.

RESULTS

Baseline characteristics of all 388 included SN positive patients are summarized in Table 1. The median Breslow thickness of all patients was 4.00 mm. Median Breslow thickness of the respective centers was Rotterdam 2.9 mm, Berlin 3.4 mm and Warsaw 4.0 mm. The mean / median duration of follow-up was 41 / 36 months (range 1 – 139 months). The mean / median follow-up per center was; 44 / 40 months for Rotterdam, 36 / 32 months for Berlin and 41 / 37 for Warsaw patients. 28 Patients refused to undergo a completion lymph node dissection (CLND) after a positive SN; therefore CLND was available of 360 patients (93%). Of these 92 patients (25%) had additional non-sentinel nodes involved in their CLND specimen.

Table 2 shows the distribution of SN tumor burden according to the Rotterdam criteria for all patients and by center. Overall rate of SUB-micrometastases was 10% of all patients, this rate was higher for Berlin (22%) and Rotterdam (19%), but lower for Warsaw (2%). Table 2 also shows the distribution of SN tumor burden according to

Table 2 The distribution of SN tumor burden according to the Rotterdam criteria for all patients and by center and by AJCC Breslow thickness category of the primary tumor.

	< 0.1 mm	**0.1 – 1.0 mm**	**> 1.0 mm**
Center			
Berlin	18 (22%)	30 (37%)	34 (41%)
Rotterdam	18 (19%)	46 (50%)	29 (31%)
Warsaw	4 (2%)	57 (28%)	145 (70%)
Overall	40 (10%)	133 (35%)	208 (55%)
			P<0.001
Breslow Thickness of the Primary Tumor			
T2 (Breslow 1.01 – 2.00 mm)	13 (19%)	33 (49%)	22 (32%)
T3 (Breslow thickness 2.01 – 4.00 mm)	16 (12%)	44 (34%)	70 (54%)
T4 (Breslow thickness > 4.00 mm)	7 (5%)	46 (30%)	100 (65%)
			P<0.001

Table 3 The rate of additional non-SN positivity according to Rotterdam criteria for SN tumor burden and for every category of micro-anatomic location of the metastases according to Dewar.

	Additional non-SN negative	**Additional non-SN positive**
SN Tumor Burden		
< 0.1 mm	35 (97%)	1 (3%)
0.1 – 1.0 mm	98 (79%)	26 (21%)
> 1.0 mm	136 (68%)	64 (32%)
		P=0.001
Micro-Anatomic Location		
Subcapsular	57 (92%)	5 (8%)
Parenchymal	54 (81%)	13 (19%)
Combined	95 (68%)	44 (32%)
Multifocal	23 (85%)	4 (15%)
Extensive	39 (60%)	26 (40%)
		n.s.

Breslow thickness of the primary tumor. Patients with primary tumors ≤ 1.00 mm were seen only infrequently and therefore not included into this table. Table 3 shows the rate of additional non-SN positivity according to Rotterdam criteria for SN tumor burden, which significantly increased from 3% in SUB-micrometastases (< 0.1 mm) to 32% in SN metastases > 1.0 mm (P=0.001). Additionally, the rate of non-SN positivity for SN tumor burden ≤ 0.2 mm was 10% (n=4).

The distribution of the micro-anatomic location of the metastases according to Dewar was; 20% subcapsular, 17% parenchymal, 38% combined, 8% multifocal and 17% extensive. Table 3 also shows the amount of additional non-SN positivity for every category of micro-anatomic location of the metastases according to Dewar. Subcapsular metastases showed the lowest rate of non-SN positivity at 8%.

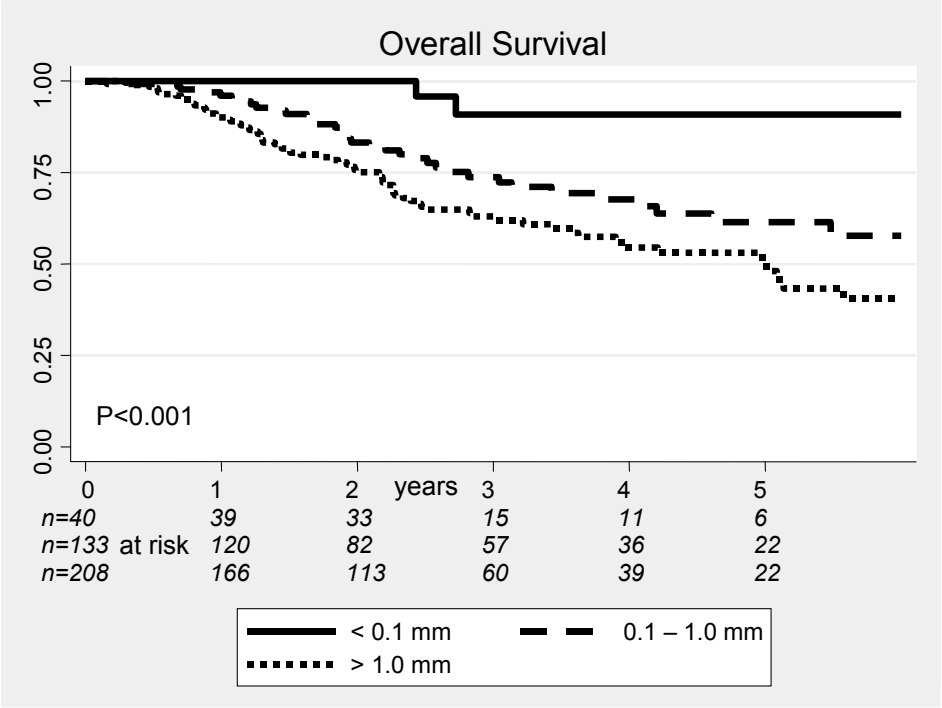

Figure 1 Kaplan-Meier estimated 5-year overall survival according to the Rotterdam criteria for SN tumor burden

Kaplan-Meier estimated 5-year overall survival for the different categories of SN tumor burden according to the Rotterdam criteria were; 91% for SUB-micrometastases (< 0.1 mm SN tumor burden), 61% for 0.1 – 1.0 mm SN tumor burden and 51% for SN tumor burden > 1.0 mm, respectively (Figure 1) (P<0.001). Estimated 5-year OS for SN tumor burden ≤ 0.2 mm was 89%.

Finally, univariate and multivariate analyses for overall survival were performed. Univariate prognostic factors for OS were; Breslow thickness (P<0.001), Clark level (P=0.05), ulceration status (P<0.001), center (P=0.02), subcapsular vs. other location of involvement (P<0.001) and SN tumor burden (P<0.001). Gender, age and primary tumor site were not significant on univariate analysis. Table 4 shows the multivariate analysis for OS. SN tumor burden and T4 primary tumors (> 4.00 mm Breslow thickness) were independent prognostic factors for OS.

DISCUSSION

In this large multicenter study, conducted within the network of the EORTC Melanoma Group, we were able to look at the importance of SN tumor burden with authorative

Table 4 Multivariate analysis for overall survival.

Covariate	P-Value	Covariate	HR	P-Value
Gender	n.s.	Breslow thickness		
Age	n.s.	T1	1	
Clark	n.s.	T2	1.43	0.59
Ulceration	n.s.	T3	2.45	0.14
		T4	4.85	*0.008*
		SN tumor burden		
		< 0.1 mm	1	
Center	n.s.	0.1 – 1.0 mm	4.56	*0.038*
Dewar Location	n.s.	> 1.0 mm	5.51	*0.02*

validation power. This study evaluated the outcome of 388 SN positive patients, which is almost twice the amount of nodal positive patients included into the MSLT-1[7]. The present study confirms that patients with SUB-micrometastases (maximum diameter < 0.1 mm) had an excellent 5-year overall survival rate of 91% (Figure 1). This survival rate is the same as for SN negative patients (90 – 94%) as reported in the MSLT-1 and most other SN procedure studies[7,12,17,22-25].

Due to the infrequency of recurrences in the SUB-micrometastatic patient group, we could not analyze recurrence patterns between the different groups of SN tumor burden. However, the 4 patients in the SUB-micrometastases group who developed a recurrence had typical melanoma recurrence patterns. Three patients developed in-transit metastases as the first site of recurrence, one developed a regional lymph node recurrence as first site. Subsequently three patients died of disease after 30, 33 and 34 months of follow-up and one patient, who only had an in-transit recurrence remains free from disease after 47 months of follow-up after resection of the recurrence. All other SUB-micrometastases patients remained free from disease during the course of follow-up.

In line with the differences in estimated survival rates (Figure 1), the difference in prognosis between SUB-micrometastasis and extensive tumor burden is visible to the eye, when examining the pathology slides under a microscope (Figures 2A and 2B). The observed survival together with the multivariate analysis (Table 4) demonstrates that the method of measuring the amount of SN tumor burden according the Rotterdam criteria was the most important prognostic factor for survival.

Moreover additional non-SN positivity in the CLND specimen was observed only in 1 patient (3%) for SUB-micrometastases patients. It is identical to the reported false negative rate of the MSLT-1 trial and other SN studies[7,26].

All together, the excellent survival, the very low rate of additional non-SN positivity and the similarity to SN negative patients, leads us to believe that not all SN positive patients progress to palpable nodal disease if the SN was never excised. This is supported by evidence from a recent case-control study by Koskivuo et al. were the rate of

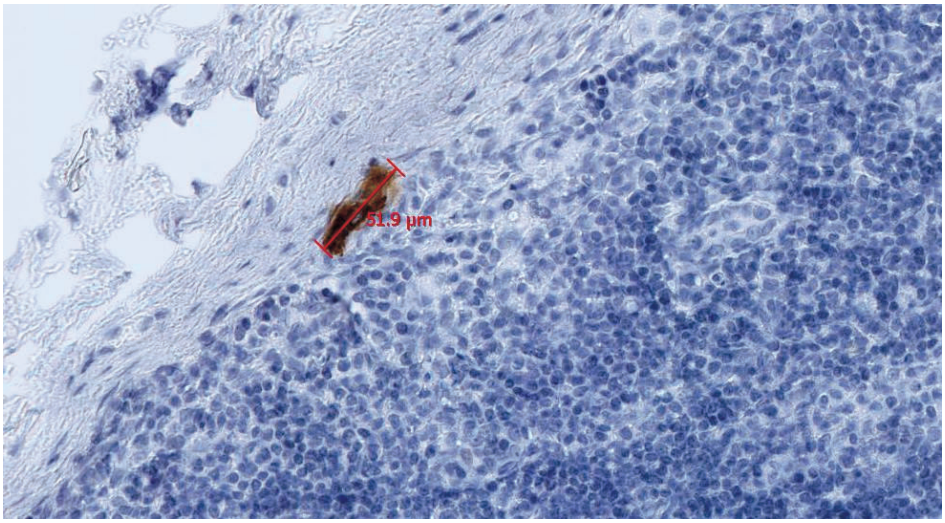

Figure 2A Photograph of a SUB-micrometastasis (<0.1 mm)

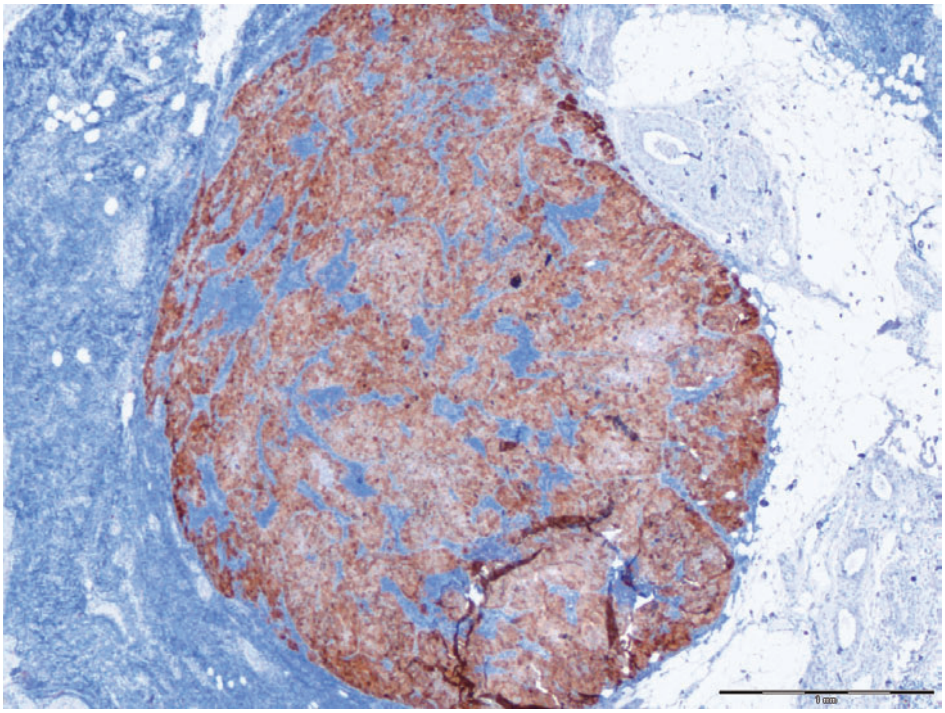

Figure 2B Photograph of an extensive metastasis (> 2.0 mm)

SN positivity was 16.4%, but only 11.7% of WLE only patients developed lymph node metastases during (longer) follow-up, even without taking into consideration the false negative SN patients[27].

There was an interesting difference in the occurrence rate of SUB-micrometastases between the different participating centers, approximately 20% in both Berlin and Rotterdam, but only 2% in Warsaw (P<0.001) (Table 2). This could possibly be the result of a less extensive pathology protocol used in Warsaw, which could have missed smaller SN tumor burden. However, the explanation for this observation is also shown in Table 2 and by the median Breslow thickness reported per center. As Warsaw's patients had the thickest median Breslow thickness by far (4.0 mm vs. 3.4 and 2.9 mm), the amount of SN tumor burden increased significantly with the increase in primary tumor stage (Table 2). The larger amount of SN tumor burden in Warsaw was likely to be the result of the higher primary tumor stages. This observation is supported by the evidence from the multivariate analysis (Table 4), in which center, previously a significant prognostic factor for OS on univariate analysis, was no longer a prognostic factor for survival in the multivariate analysis, but tumor burden and T4 primary tumor stage remained as independent prognostic factors for OS. Thus, the amount of SN tumor burden seems to be a reflection on how advanced the primary tumor is.

Some authors have suggested that 0.2 mm should be taken as a cut-off value for SUB-microscopic SN tumor burden in melanoma patients, similar to the cut-off value in breast cancer SNs[10,12,28,29]. We have considered this scenario too and the overall survival rate for < 0.2 mm SUB-micrometastases seems very promising with an estimated 5-year overall survival rate of 89%, which is very similar to SN negative patients. However, the additional non-SN positivity rate in < 0.2 mm metastases was considerably higher than in < 0.1mm metastases (10% vs. 3%). This is exactly in line with the observation from a recent study by Scheri et al., were patients with SN tumor burden < 0.2 mm (median Breslow thickness 1.7 mm) had a very good prognosis of 89%. However, the study of Scheri et al. did demonstrate that patients with < 0.2 mm metastases had a worse prognosis than SN negative patients. An explanation for this observed difference in survival may lay in the very low mean Breslow thickness of the SN negative patient population (1.2 mm) and therefore the exceptionally good survival of 94%[12,30]. The cut-off value for melanoma patients should therefore be different from the current situation in breast cancer.

Whilst the SN procedure is the best predictor of survival so far and the amount of SN tumor burden is an even greater predictor of survival, the question raised with the data from this large multicenter study is whether patients with SUB-micrometastases (defined as < 0.1 mm in maximum diameter) could safely be spared a CLND and avoids its morbidity, such as wound infections and limb edema[31-34]. The data from this large multicenter study suggests that patients with SN SUB-micrometastases according to the Rotterdam criteria (< 0.1 mm) have an identical survival rate as SN negative patients and they are at very low risk to develop nodal recurrence. Therefore they should be

considered as 'biologically false positive' and can be spared the need and morbidity of a CLND. Patients with SUB-microscopic disease should be considered as SN negative patients and only included as such into adjuvant therapy trials. It is our recommendation that patients are stratified into new adjuvant therapy trials according to the Rotterdam criteria for SN tumor burden.

Thomas has recently revealed that a reason for the survival benefit in the MSLT-1 trial subgroup analyses could be due to a proportion of false positive results taken into consideration in this analysis[7,35]. In his calculations Thomas has demonstrated that as much as 24% could be considered false positive SN patients in the MSLT-1 trial, which is very similar to the rate of SUB-microscopic disease as seen in Berlin (22%) and Rotterdam (19%) in the present study[35]. If patients with SUB-microscopic disease in the present study have such a good 5-year overall survival, because of, or in spite of undergoing a SN and/or CLND remains unclear. Possibly the MSLT-2 will answer this question, but it is also the subject of another study conducting within the EORTC Melanoma Group network, the MINITUB study, which might have a better chance to demonstrate this, since patients will be selected according to the amount of SN tumor burden.

In conclusion, SN tumor burden according to the Rotterdam criteria is the most important prognostic factor for overall survival in this large multicenter study. This study validates the previous observation that patients with SUB-micrometastases (defined as < 0.1 mm in maximum diameter) have an excellent estimated 5-year overall survival rate and seldom have additional non-SN positivity, which does not seem to differ from SN negative patients (90 – 94%). The correct cut-off value for melanoma SUB-micrometastases seems to be < 0.1 mm rather than < 0.2 mm (as in breast cancer). Patients with SUB-micrometastases (< 0.1 mm) may be considered as 'biologically false positive', identical to SN negative patients and therefore be spared a CLND and only included into adjuvant therapy trials as SN negative.

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Chapter 7

Prognosis in sentinel node-positive melanoma patients is accurately defined by the Combined Rotterdam Tumor Load and Dewar Topography Criteria

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ABSTRACT

In patients where a Completion Lymph Node Dissection (CLND) has been performed after a positive Sentinel Node (SN) Biopsy (SNB), only one in five show additional non-SN (NSN) metastases. In the last decade, many specialists in the melanoma field have tried to identify criteria that determine the group of patients which have good prognosis and do not develop positive NSNs. The aim of this large multicenter study was to evaluate SN tumor load as a predictive and prognostic factor for NSN involvement and survival.

Between 1993 and 2008, 1080 patients (509 women and 571 men) were diagnosed with tumor burden in the SN in 9 EORTC Melanoma Group (MG) centers of which 1009 patients (93%) underwent CLND. Median Breslow thickness was 3.00 mm. The median follow-up time was 37 months. Patients with submicrometastases (Rotterdam Criteria <0.1 mm) reconfirmed to have an estimated 5-year OS rate of 91% and a low NSN positivity rate of 9%. The most predictive and prognostic parameter in our study was the RDC (Rotterdam-Dewar Combined) Criteria. Patients with submicrometastases located subcapsular only (RDC Criteria) had a NSN positivity rate of 2% and an estimated 5- and 10-year MSS of 95%.

Until the time that currently running randomized prospective trials, such as the EORTC MINITUB study and the MSLT-II, have evidence based conclusions, the EORTC Melanoma Group proposes that patients with tumor burden <0.1 mm according to the Rotterdam Criteria, especially when found in the subcapsular area only, seem to be indicated for observation instead of CLND. The RDC Criteria has the most prognostic value and the best predictivity for NSN status, implying the importance of the Rotterdam Criteria and the Dewar Criteria to be applied as micromorphometric parameter in each patient with tumor burden in the SN.

INTRODUCTION

Since the introduction of the sentinel lymph node (SN) biopsy (SNB) by Morton *et al* in the early 90s[1,2], SNB has been broadly accepted as a highly accurate diagnostic method of identifying early lymph node micrometastasis in melanoma patients. Although a survival benefit of undergoing a SNB followed by early completion lymph node dissection (CLND) compared to nodal observation has not yet been shown in the Multicenter Selective Lymphadenectomy Trail – I (MSLT-I), SN tumor burden has been shown to be the most important prognostic factor for melanoma patients with early-stage disease[3].

Approximately one in five patients who had a SNB has evidence of tumor burden in the SN and undergoes a completion lymph node dissection (CLND). In patients where a CLND has been performed, again only one in five patients shows additional non-SN (NSN) metastases. Many specialists in the melanoma field have tried to identify the correct patient group to undergo a CLND and, possibly even more importantly, to identify the correct group, which can safely be spared unnecessary surgery (CLND) and its possible great morbidity, such as chronic lymph edema[4-37]. Ongoing prospective multicenter studies are aiming to accurately identify the group of patients that can be considered for observation instead of CLND. The two most prominent studies are the MSLT-II and the EORTC MINITUB study[38,39].

Diverse morphometrical parameters of SN tumor burden have been suggested to provide prognostic information. In this study, two important parameters are assessed, i.e. the microanatomical location (Dewar Criteria)[26] and the maximum diameter of the largest tumor lesion (Rotterdam Criteria)[9,18]. The EORTC Melanoma Group (MG) recommends all pathologists to report these criteria for each positive SN patients[40].

The aim of this large multicenter study was to consider the amount of SN tumor load as a predictive and prognostic factor for NSN involvement and survival. Moreover, the aim was to evaluate the outcome of our previous multi center experience[18]. Outcome in this study might suggest a certain group of patients that in the future might be indicated for observation instead of CLND.

PATIENTS AND METHODS

Patients

Patients with a positive SNB after wide local excision (WLE) of a malignant melanoma in 9 major collaborating EORTC Melanoma Group (MG) centers were included in this retrospective study. Participating EORTC MG Centers are shown in Table 1. Between 1993 and 2008, 1080 patients were diagnosed with tumor burden in the SN. These patients were all collected into a database with personal information, information on previous medical

history, information on disease and follow-up information. Baseline characteristics are summarized in Table 1.

Table 1 Baseline Characteristics of 1080 SN Positive Patients

Gender	n		**Clark**	n	
Male	571	53%	I	2	0%
Female	509	47%	II	33	3%
Center			III	266	25%
DDHCC	115	11%	IV	614	57%
CHUB	86	8%	V	117	11%
MMCCIO	245	23%	Unknown	48	4%
RSCH	214	20%	**Ulceration**		
AVL	116	11%	Absent	603	56%
IGR	68	6%	Present	477	44%
VU	107	10%	**Rotterdam Criteria**		
UMCG	56	5%	< 0.1 mm	113	10%
EIO	73	7%	0.1 – 1.0 mm	457	42%
Age			> 1.0 mm	510	47%
≤ 50	523	48%	**Dewar Criteria**		
> 50	557	52%	Subcapsular	181	17%
Location			Combined	423	39%
Extremity	643	60%	Parenchymal	154	14%
Trunk	405	37%	Multifocal	41	4%
Head & Neck	32	3%	Extensive	152	14%
Histology			Unknown	129	12%
SSM	401	37%	**NSN status**		
NM	347	32%	Negative	797	74%
Other	332	31%	Positive	212	20%
Breslow			Unknown	71	7%
T1 (≤ 1.00mm)	53	5%			
T2 (1.01 – 2.00 mm)	270	25%			
T3 (2.01 – 4.00 mm)	434	40%			
T4 (> 4.00 mm)	323	30%			

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 = Vrij Universiteit, Amsterdam, the Netherlands
UMCG = University Medical Center Groningen, Groningen, the Netherlands
EIO = European Insitute of Oncology, Milan, Italy

All patients underwent therapeutic re-excision of the melanoma before the SN procedure. Tumor free margins of 1 cm surrounding the lesion were excised with melanoma smaller than or equal to 2 mm Breslow. Margins of 2 cm surrounding the lesion were excised with melanoma larger than 2 mm Breslow. Melanoma in face, head and neck and distal extremities always were excised with tumor free margins of at least 2 mm. Finally, the defected areas were closed via primary closure or split skin graft.

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

Completion Lymph Node Dissection (CLND) was not performed in all SN positive patients. In 71 patients (6.6%) CLND was not executed due to diverse reasons. Rejection of further treatment, the occurrence of distant metastasis between SNB and CLND and the presence of minimal tumor burden were reasons of not undergoing a CLND.

The triple technique

After WLE of the malignant melanoma the SN procedure followed, with the use of the triple technique. The triple technique is described in detail elsewhere.[41-43] In short, the triple technique consists of (1) pre-operative lymphoscintigraphy (LS), undertaken within 24 hours of the operation being performed, (2) peroperative use of patent blue and (3) a handheld gamma detection probe to detect the SN or SNs. A lymph node is identified as a SN if stained blue, 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.

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

Pathology

In the nine EORTC centers, all SNs were basically 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 bivalved 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 in-between different numbers of sections. Finally, all sections were stained with H&E and S100 and/or MelanA. There were slight local differences to 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 (AVL and UMCG), other experienced melanoma specialists re-classified

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.[9,18] Dewar Criteria could be examined for 951 patients (88%).

The Dewar Criteria defines the micro anatomic location of the melanoma lesion.[26] Micro anatomic locations are: subcapsular, parenchymal, combined, multifocal or extensive. Because Dewar identified that the subcapsular group had a better prognosis than any other, we have also grouped the locations into two groups: subcapsular and non-subcapsular, 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.[4-8,10-14,16,17,19] With this reason, we attended other cut-off points in our analyses instead of <0.1 mm, i.e. <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, i.e. RDC (Rotterdam-Dewar Combination) Criteria (<0.1 subcapsular, <0.1 non-subcapsular), combining the two most predictive and prognostic subgroups of the parameters.

Cases with any difficulty in determine the different micromorphometric parameters were discussed during EORTC MG meetings, which takes place every 6 months.

Statistics

Univariate analyses for NSN positivity were performed using a chi square test. Univariate analyses of endpoints for survival were performed 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 hazard 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: gender (male, female), centers (9 EORTC centers), age (≤50, >50 years), location of the melanoma (extremities, trunk, head & neck), histology of the melanoma (SSM, NM, other), Breslow thickness (T1, T2, T3, T4), Clark level (II, III, IV, V), ulceration (absent/unknown and present), Rotterdam Criteria (<0.1 mm, 0.1-1.0 mm or >1.0 mm), Rotterdam Criteria II (<0.2 mm, 0.2-1.0 mm or >1.0 mm), Rotterdam Criteria III (<0.3 mm, 0.3-1.0 mm or >1.0 mm), Rotterdam Criteria IV (<0.4 mm, 0.4-1.0 mm or >1.0 mm), Dewar Criteria (subcapsular, parenchymal, combined, multifocal, extensive, unknown), Dewar Criteria II (subcapsular, non-subcapsular), RDC Criteria (<0.1 subcapsular, <0.1 non-subcapsular) and, for survival analysis only, NSN status (negative, positive, unknown).

Statistics were performed with STATA version 11.1 (StataCorp LP, College Station, TX, USA).

RESULTS

Characteristics

Baseline characteristics are summarized in Table 1. This study included 1080 melanoma patients (509 women and 571 men) with a positive SN procedure in a period over 16 years. Average age was 51 (range 6 – 88) years. Mean and median Breslow thicknesses were 4.00 mm and 3.00 (range 0.1 – 90) mm respectively. The mean/median follow-up time for the entire group was 3.8 / 3.0 years (46 / 37 (range 1 – 172) months). The mean/median time to first recurrence was 3.2 / 2.3 years (38 / 27 months). At last follow-up 336 of 1080 patients (31%) were deceased.

In Table 2, the characteristics Breslow thickness, ulceration rate and subgroups of the Rotterdam Criteria and the Dewar Criteria are compared between the nine EORTC MG Centers. With a median Breslow thickness of 4.00 mm and an ulceration percentage of

Table 2 Characteristics per EORTC Center

Center		DDH- CC	CHUB	MM- CCIO	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%
	<0.1	17%	26%	3%	11%	4%	9%	11%	23%	4%
Rotterdam Criteria	***0.1 – 1.0***	48%	35%	33%	46%	32%	50%	49%	57%	52%
(mm)	***>1.0***	35%	40%	64%	43%	64%	41%	40%	20%	44%
	Subcapsular	30	40	4	18	34	15	15	NA	NA
	Combined	30	29	47	50	41	50	56	NA	NA
Dewar Criteria	***Parenchymal***	13	12	22	17	0	26	19	NA	NA
	Multifocal	12	8	4	1	5	3	0	NA	NA
	Extensive	15	12	24	13	21	6	10	NA	NA

NA = Not Applicable
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 = Vrij Universiteit, Amsterdam, the Netherlands
UMCG = University Medical Center Groningen, Groningen, the Netherlands
EIO = European Insitute of Oncology, Milan, Italy

64%, the MMCIO (Warsaw, Poland) is the center with the worst prognostic group of SN positive patients. 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, the histology and the location of the primary, Clark level, Breslow thickness, the Rotterdam Criteria, the 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 (Rotterdam Criteria <0.1mm) was 9%, while 16% of patients with Rotterdam Criteria 0.1 – 1.0 mm had positive NSNs and 25% with >1.0 mm of SN tumor burden. NSN positivity rates in 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 in patients with subcapsular metastases was 7% and 22% in patients with non-subcapsular metastases. The subgroup of patients with the best predictivity for NSN status was the group with subcapsular metastases smaller than 0.1 mm, which showed positive NSNs in only 2% of patients.

Survival

Outcome of both univariate and multivariate analyses are demonstrated in Table 4. Because of multicollinearity in multivariate analyses due to the covariates Rotterdam Criteria (with different cut-off values) and RDC Criteria, separate multivariate analyses were performed. On multivariable analyses of the covariates regarding melanoma specific survival (MSS), gender, Breslow thickness (T3 and T4), ulceration, the Rotterdam Criteria (with different hazard ratio's for different cut-off values), RDC Criteria and NSN status were independent prognostic factors. Dewar or Dewar II Criteria were not significant on multivariate analyses.

The Kaplan-Meier 5- and 10-year OS rates of patients with Rotterdam Criteria <0.1 mm were 91% and 81%, followed by 71% and 54% in the group with 0.1 – 1.0 mm and 57% and 46% in the >1.0 mm group. The Kaplan-Meier 5- and 10-year DFS rates of patients with Rotterdam Criteria <0.1 mm were 83% and 83%, followed by 61% and 49% in the group with 0.1 – 1.0 mm and 40% and 32% in the >1.0 mm group. The Kaplan-Meier 5- and 10-year MSS rates of patients with Rotterdam Criteria <0.1 mm were 92% and 87%, followed by 74% and 57% in the group with 0.1 – 1.0 mm and 59% and 48% in the >1.0 mm group. (Figure 1)

Table 3 Association between Clinicopathological Factors and the Detection of Metastases in Non-Sentinel Nodes (NSN)

Predictive factor		NSN Positive (%)	NSN Negative (%)	NSN unknown (%)	P Value
Gender					
	Female	111 (22)	360 (71)	38 (7)	
	Male	101 (18)	437 (77)	33 (6)	0.094
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)	<0.0005
Histology					
	SSM	76 (19)	297 (74)	28 (7)	
	NM	88 (25)	244 (70)	15 (4)	
	Other	48 (14)	256 (77)	28 (8)	0.003
Location					
	Extremity	123 (19)	466 (72)	54 (8)	
	Trunk	82 (20)	310 (77)	13 (3)	
	Head & Neck	7 (22)	21 (66)	4 (13)	0.011
Age					
	≤ 50	101 (19)	398 (76)	24 (5)	
	> 50	111 (20)	399 (72)	47 (8)	0.032
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)	0.011
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)	<0.0005
Ulceration					
	Absent	103 (17)	457 (76)	43 (7)	
	Present	109 (23)	340 (71)	28 (6)	0.052

Table 3 (continued)

Predictive factor	NSN Positive (%)	NSN Negative (%)	NSN unknown (%)	P Value
Rotterdam Criteria				
< 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)	<0.0005
Rotterdam Criteria II				
<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)	<0.0005
Rotterdam Criteria III				
<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)	<0.0005
Rotterdam Criteria IV				
<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)	<0.0005
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)	<0.0005
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)	<0.0005
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)	<0.0005

Centers:
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
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VU = Vrij 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; NSN= Non-Sentinel Node

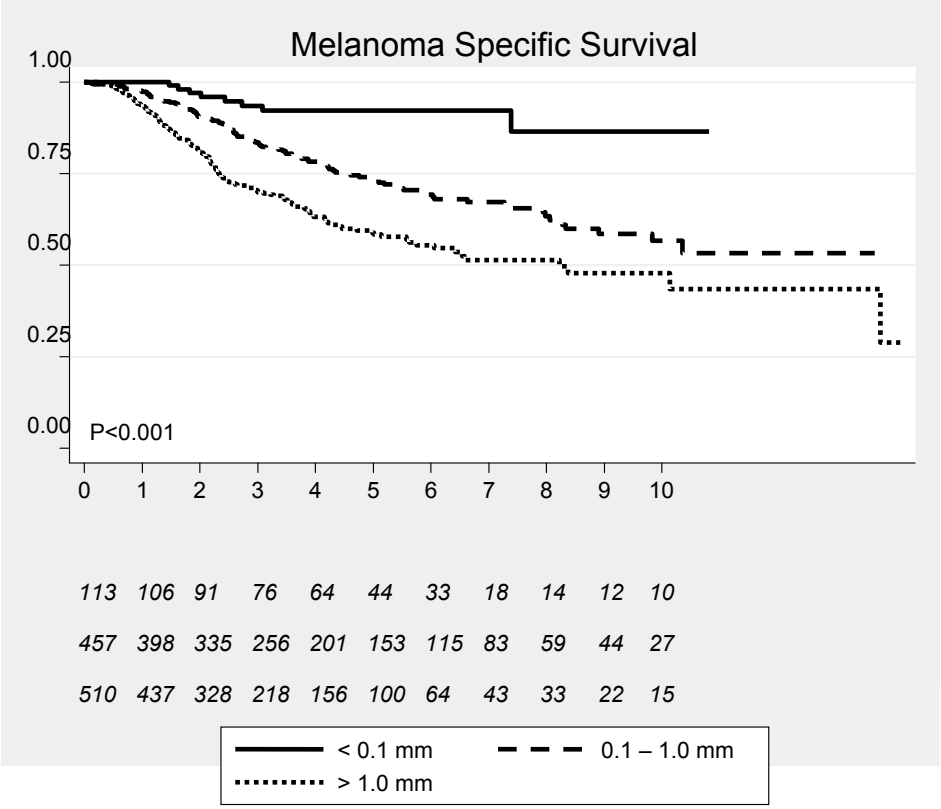

Figure 1 Melanoma Specific Survival for SN Tumor Burden according to the Rotterdam Criteria

The Kaplan-Meier 5- and 10-year MSS rates of patients with other cut-off points then the Rotterdam Criteria, i.e. <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 of patients with subcapsular metastases were 81% and 71% and the Kaplan-Meier 5- and 10-year MSS rates of patients with non-subcapsular metastases were 66% and 52%. (Figure 2)

The Kaplan-Meier 5- and 10-year MSS rates of patients with RDC Criteria <0.1 mm subcapsular were both 95%, while the 5- and 10-year OS rates of patients with RDC Criteria <0.1 mm non-subcapsular were 88% and 80%. (Figure 3)

DISCUSSION

This is the largest study ever performed in this field, evaluating almost three times more patients with tumor burden in the SN than a previous report of the EORTC Melanoma Group (MG)[18], reports of two studies performed in the USA[6,17] and a report from the

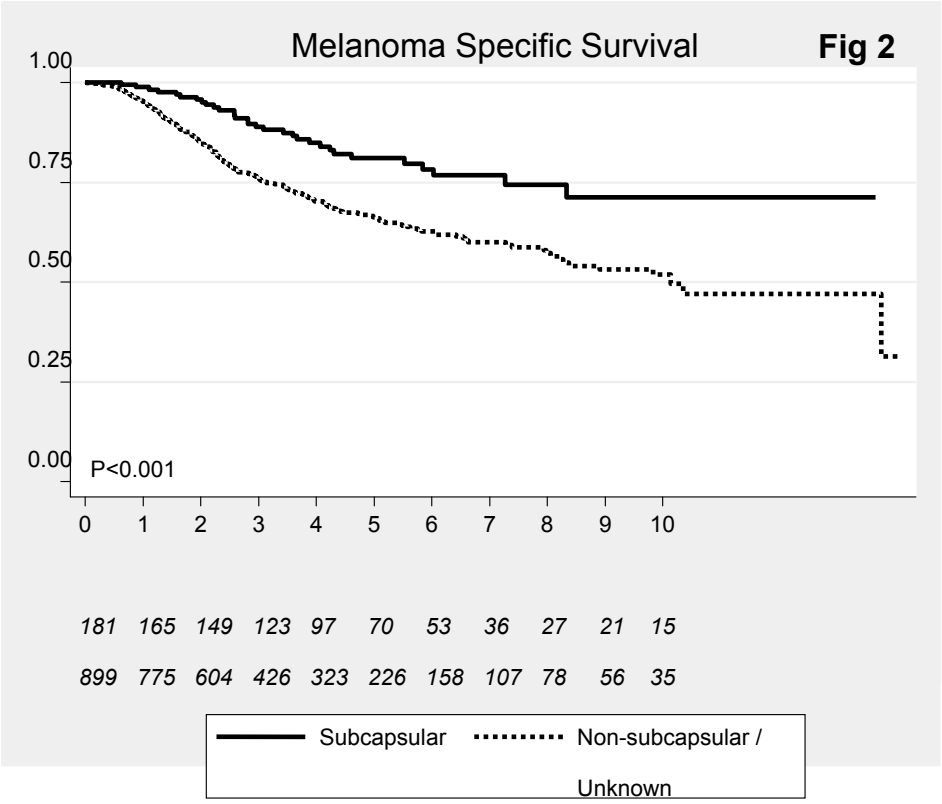

Figure 2 Melanoma Specific Survival for SN Tumor Burden according to the Subcapsular Location

Melanoma Institute Australia in Sydney[20]. This study investigated prognostic factors for survival and predictive factors for non-Sentinel Node (NSN) status by addressing two different histological parameters of classifying SN tumor load.

Compared to our earlier results, we confirm that patients with submicrometastases (Rotterdam Criteria <0.1 mm) had an estimated 5-year OS rate of 91% comparable with SN negative patients.[18] The NSN positivity rate in this larger cohort of patients is low with a NSN positivity rate of 9%. The most predictive and prognostic parameter in our study was the RDC (Rotterdam-Dewar Combined) Criteria. Patients with submicrometastases located subcapsular solely had a NSN positivity rate of 2% and an estimated 5- and 10-year MSS of 95%. These patients have very good prognostic outcome and an identical survival as SN negative patients. Although a CLND was performed in 93.4% of these patients, it seems that these patients very likely do not benefit from a CLND, as they have a clinical course that is indistinguishable from SN negative patients. Therefore we propose that these patients are spared a CLND.

SN tumor load is an excellent prognostic factor for survival. 5-year estimated disease free survival (DFS) rates for SN positive patients reported are 38-65% compared with 77-

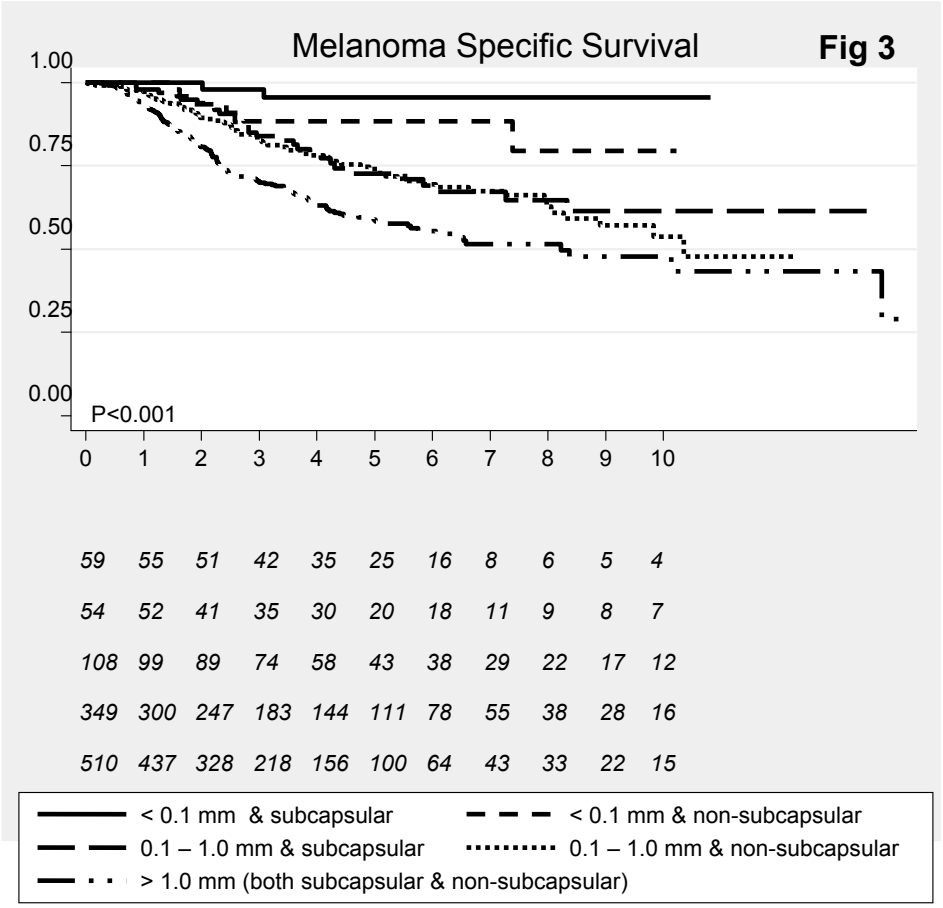

Figure 3 Melanoma Specific Survival for SN Tumor Burden according to the Rotterdam Dewar Combined (RDC) Criteria

89% for SN negative patients.[16,45] 5-year estimated overall survival (OS) rates for SN positive patients reported are 54-75% compared with 88-94% for SN negative patients.[12,45-47]

Different micromorphometric parameters for tumor load in the SN have been addressed in many previous studies to identify the most predictive measurement. Other more or less frequently observed measurements of SN tumor burden are the tumor penetrative depth, the square area, the percentage area, the number of metastatic foci, the number of positive SNs, the extracapsular spread and the capsular invasion.[11,14,15,17,21-24,30,31,34,37] Others combined primary melanoma and/or SN characteristics into working models for predicting survival and/or NSN status[13,14,17,20,29,30,34,37]

An important fact regarding the assessment of micromorphometric parameters is their reproducibility and accuracy which has been mentioned in recent reports discussing the pathological work-up and measurement of SN tumor deposits.[40,48] Murali *et al* observed

the agreement of assessment of histological parameters between 7 different pathologists. Quantitative 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.[48] In conclusion, besides containing predictive and prognostic value, a measurement of SN tumor load must not be time-consuming, inaccurate or difficult to reproduce. This supports the vision of the EORTC MG to maintain the measurement of SN tumor burden as simple as possible.

Many studies addressed other cut-off points then the 0.1 mm and 1.0 mm used by the Rotterdam Criteria measuring the largest diameter of the largest lesion.[4-19] (Table 5) With this reason, we scrutinized the cut-off point of 0.1 mm in this study and addressed other cut-off points, that is 0.2 mm as in SN positive breast cancer patients[49,50] and addressed in other studies[10,12], 0.3 mm as in the S-Classification[22] and 0.4 mm as suggested by van der Ploeg *et al*.[19] NSN positivity increased rapidly and in consent in patients with these other cut-off points having positive NSNs in 13-14% of positive SN patients. (Table 3) Survival rates in these groups showed similar worse outcome. 5-year MSS survival rates were 80-81% and 10-year MSS survival rates were 70-74%. Of all four different cut-off points, < 0.1 mm according to the Rotterdam Criteria had the best prognostic outcome and predictive value. (Table 3, 4) The other three cut-off points addressed had worse survival than SN negative patients (80-81% compared to 88-94%), while patients with submicrometastases according to the Rotterdam Criteria had similar survival (91%).

The most important question regarding predictive and prognostic parameters for NSN status and survival is; do parameters identify a group of patients that would be indicated for observation instead of CLND? We suggest that they do – the group of patients with submicrometastases especially when located subcapsular solely – but it remains unclear if it is because of, or in spite of undergoing the CLND, and even the primary SN excision. The question remains if these patients would have had the same outcome when they did not undergo a CLND. The outcome of a good prognostic group of patients without CLND has never been published, although two recent studies described the difference between a group of SN positive patients who did not and a group who did undergo CLND after a positive SN.[51,52] There was no significant difference in locoregional control and disease specific survival between both groups indicating it seems CLND possibly does not have a survival benefit.

Obviously all retrospective studies, including this one, have the traditional downside that can only be overcome by a prospective randomized controlled trial. Several prospective trials are running to further investigate the possibility to reduce the 80% unnecessary CLND operations. The two most prominent studies are the MSLT-II and the EORTC MINITUB study.[38,39] Until the time comes that these studies will have final outcome and hard conclusions can be made, suggestions of other options than CLND can be discussed and proposed.

Table 4 Univariate and Multivariate Analyses of Covariates regarding Melanoma Specific Survival

	Univariate			Multivariate		
	HR	95% CI	P Value	HR	95% CI	P Value
Gender						
Female	1			1		
Male	1.38	1.10 – 1.73	0.006	1.31	1.04 – 1.64	0.022
Center						
DDHCC	1					
CHUB	1.75	1.02 – 3.01	0.042			
MMCCIO	1.93	1.23 – 3.04	0.004			
RSCH	1.53	0.95 – 2.46	0.081			
AVL	1.09	0.65 – 1.84	0.74			
IGR	1.07	0.51 – 2.22	0.87			
VU	1.54	0.93 – 2.55	0.091			
UMCG	0.83	0.43 – 1.60	0.58			
EIO	1.78	1.02 – 3.10	0.041			n.s.
Histology						
SSM	1					
NM	1.44	1.10 – 1.88	0.009			
Other	1.51	1.13 – 2.01	0.005			n.s.
Location						
Extremity	1					
Trunk	1.07	0.85 – 1.36	0.55			
Head & Neck	1.18	0.66 – 2.13	0.57			n.s.
Age						
≤ 50	1					
> 50	1.24	0.99 – 1.55	0.063			n.s.
Clark						
II	1					
III	1.45	0.63 – 3.36	0.39			
IV	2.07	0.92 – 4.66	0.081			
V	3.43	1.48 – 7.99	0.004			
Unknown	2.23	0.84 – 5.96	0.108			n.s.
Breslow						
T1	1			1		
T2	1.07	0.51 – 2.27	0.85	-	-	n.s.
T3	1.92	0.94 – 3.93	0.075	1.53	1.10 – 2.13	0.012
T4	3.74	1.83 – 7.64	< 0.001	2.45	1.73 – 3.45	<0.001
Ulceration						
Absent	1			1		
Present	2.11	1.68 – 2.64	< 0.001	1.50	1.18 – 1.92	0.001
Rotterdam Criteria						
< 0.1	1			1		
0.1 – 1.0	3.28	1.72 – 6.25	< 0.001	2.65	1.38 – 5.06	0.003
> 1.0	5.36	2.83 – 10.13	< 0.001	3.30	1.73 – 6.31	<0.001

Table 4 (continued)

	Univariate			Multivariate		
	HR	95% CI	P Value	HR	95% CI	P Value
Rotterdam Criteria II						
<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	< 0.001	1.83	1.23 – 2.72	0.003
Rotterdam Criteria III						
<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	< 0.001	1.77	1.26 – 2.50	0.001
Rotterdam Criteria IV						
<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	< 0.001	1.59	1.17 – 2.17	0.003
Dewar Criteria						
Subcapsular	1					
Combined	1.88	1.29 – 2.76	0.001			
Parenchymal	1.94	1.23 – 3.05	0.004			
Multifocal	1.46	0.72 – 2.95	0.297			
Extensive	3.62	2.38 – 5.51	< 0.001			
Unknown	1.61	1.02 – 2.56	0.042			n.s.
Dewar Criteria II						
Subcapsular	1					
Non-subcapsular	2.04	1.43 – 2.92	< 0.001			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	0.006	4.53	1.37 – 14.91	0.013
0.1 – 1.0 non-subcapsular	5.92	1.87 – 18.69	0.002	5.01	1.58 – 15.88	0.006
> 1.0 non and subcapsular	9.36	2.99 – 29.32	<0.001	6.17	1.95 – 19.45	0.002
NSN status						
Negative	1			1		
Positive	2.46	1.89 – 3.22	< 0.001	2.12	1.62 – 2.79	< 0.001
Unknown	1.45	1.09 – 1.93	0.011	1.68	1.26 – 2.25	< 0.001

HR = Hazard Ratio; CI = Cumulative Index

Centers:

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 = Vrij 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; NSN= Non-Sentinel Node

Table 5 Overview of Literature for Predictive Factors for Non-Sentinel Node (NSN) Involvement and 5-year estimated Overall Survival (OS) rates

Parameters for tumor burden in the Sentinel Node	*Studies where parameter was assessed for NSN status*	*Analyzed number of NSN positive (% of total) patients*	*Most prognostic subgroup(s) of variable for NSN status*	*NSN Positivity rate of subgroup (%)*	*5-year estimated OS rate (%)*
Size as largest diameter of largest lesion	Ranieri 2002	13 (14)	≤3 mm	-	86 (3y)
	Carlson 2003	15 (16)	Isolated tumor cells	-	86 (3y)
			≤2 mm	-	90 (3y)
e.g.	Lee 2004	46 (24)	<2 mm	16	-
Rotterdam	Sabel 2005	34 (15)	Micrometastasis	2	-
Criteria	Pearlman 2006	17 (21)	≤2 mm	6	85
	van Akkooi 2006	10 (15)	<0.1 mm	0	100
	Govindarajan 2007	20 (16)	≤0.2 mm	0	-
	Debarbieux 2007	22 (22)	≤2 mm	18	±80
			≤1 mm (smallest diameter)	13	-
	Scheri 2007	NA †	≤0.2 mm	12	87
	Roka 2008	18 (21)	≥2 mm	8	-
	Rossi 2008	20 (21)	≤2 mm	16	-
	Satzger 2008	28 (16)	<0.1 mm	0	-
			<1 mm	9	-
			<2 mm	11	-
	Guggenheim 2008	22 (22)	≤2 mm	16	-
	Gershenwald 2008	48 (14)	≤0.5 mm	5	-
			≤2 mm	8	-
	van Akkooi 2008	91 (23)	<0.1 mm	3	91
	van der Ploeg IM 2009	15 (13)	<0.1 mm	0	100
	Present study	184 (17)	<0.1 mm	9	91
Microanatomic Location	Dewar 2004	24 (16)	Subcapsular	0	-
	van Akkooi 2006	10 (15)	Combined	9	-
e.i. Dewar	Govindarajan 2007	20 (16)	Sinusoidal	0	-
Criteria	Roka 2008	18 (21)	Non-extensive	13	-
	Rossi 2008	20 (21)	Subcapsular	0	-
	Gershenwald 2008	48 (14)	Subcapsular	10	-
	Frankel 2008	29 (21)	Subcapsular	10	-
	van Akkooi 2008	91 (23)	Subcapsular	8	-
	van der Ploeg IM 2009	15 (13)	Subcapsular	3	83
	Present study	184 (17)	Subcapsular	7	81

NSN = Non-Sentinel Node; OS = Overall Survival; NA = Not Applicable
† Only the group of patients with isolated tumor cells and known NSN status were included. 6 of 52 (12%) patients with ≤0.2 mm had NSN positivity in this study.

In conclusion, this study of the EORTC Melanoma Group, proposes that patients with tumor burden <0.1 mm according to the Rotterdam Criteria are indicated for observation instead of CLND, especially when found in the subcapsular area only. The measurement of SN tumor burden as the largest diameter of the largest lesion and as the tumor penetrative depth is straightforward, reproducible and not time-consuming. The RDC Criteria has the most prognostic value and the best predictivity for NSN status, implying the importance of the Rotterdam Criteria and Dewar Criteria to be applied as micromorphometric parameter in each patient with tumor burden in the SN.

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Part II

Ultrasound guided fine needle aspiration cytology (FNAC) as alternative to surgical SN staging

Chapter 8

Rotterdam Criteria for SN Tumor Burden and the Accuracy of Ultrasound (US) guided Fine Needle Aspiration Cytology (FNAC): Can US guided FNAC replace SN staging in Melanoma Patients?

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ABSTRACT

Background:

SN status is the most important prognostic factor for overall survival (OS) for stage I/II melanoma patients and the role of the SN procedure as a staging procedure has long been established. However a less invasive procedure as Ultrasound (US) guided FNAC would be preferred. Aim of this study was to evaluate the accuracy of US guided FNAC and compare the results with histology after SN surgery was performed in all patients.

Methods:

400 consecutive patients, who underwent lymphoscintigraphy, subsequently underwent an US-exam prior to the SN procedure. When the US-exam showed a suspicious or malignant pattern, patients underwent a FNAC. Median Breslow thickness was 1.8 mm., follow-up was 42 months (mean, range 4 – 82). We considered the US-FNAC positive if either US and/or FNAC were positive. If US was suspicious, but FNAC negative, it was considered negative.

Results:

US guided FNAC identified 51/79 (65%) of SN metastases. Specificity was 99% (317/321) with a PPV of 93% and NPV of 92%. SN positive identification rate by US guided FNAC increased from 40% in stage pT1a/b to 79% in stage pT4a/b. US guided FNAC detected SN tumors > 1.0mm in 86% of cases, SN tumors of 0.1 – 1.0mm in 46% and SN tumors < 0.1mm in 23%. Estimated 5-year OS rates were 92% for US guided FNAC negative, 51% for positive patients.

Conclusions:

US-guided FNAC of SNs is highly accurate. Up to 65% of the SN positive patients in our institution could have been spared a SN procedure.

INTRODUCTION

Epidemiologists report that not only melanoma incidence, but also melanoma mortality has been rising over the past decades[1,2]. Breslow thickness and ulceration of the primary tumor are important prognostic factors[3,4]. However, the nodal status of melanoma patients has been demonstrated to be the overriding factor predicting disease outcome[3-5]. Identifying patients with nodal metastases as early as possible, so they might benefit from the early removal of these metastatic nodes, before the disease could spread any further, is the goal of the sentinel node (SN) staging procedure. This is based on the concept of an orderly progression of lymphatic dissemination to the regional draining SN as first station, which occurs in a majority of approximately 90% of all melanoma patients[6]. The big advantage of the SN procedure over elective lymph node dissection (ELND) is that only those patients with metastases in their SN will undergo a Completion Lymph Node Dissection (CLND). In the past the role of ELND was investigated, but a number of underpowered studies failed to demonstrate a true benefit[7-10]. The Multicenter Selective Lymphadenectomy Trial (MSLT-I) was developed to answer the question if SN followed by early CLND would have an overall survival benefit in intermediate thickness melanoma patients[11]. Although the published interim results of the MSLT-I study showed a significant impact on disease-free survival (DFS), and a subgroup analysis suggestive of improved survival for node positive patients, this did not translate into an overall survival benefit in the ITT (Intention To Treat) population[11,12]. However, the status of the SN procedure as a staging procedure has been established widely for a number of years. Whilst the SN procedure is the best predictor of survival so far, it is still an invasive procedure, usually carried out under general anesthesia.

The current state of results entitles us to enter new research fields, such as the role of ultrasound (US) in the staging of stage I/II melanoma patients. US has been increasingly incorporated and accepted as a follow-up tool for melanoma patients in Europe and Australia[13-15]. It is also used for follow-up in the MSLT-II trial, currently recruiting patients[16]. A previous study from our institute by Voit et al. revealed that US can accurately identify which lymph node is the SN prior to the excision by the surgeon[17].

The aim of the present study was to evaluate the accuracy of US guided FNAC in the detection of melanoma metastases to the SN, prior to patients undergoing the SN procedure. The golden standard for this study was the final histological analysis of the SN excised during surgery. Survival analyses for different patient groups have been performed.

PATIENTS AND METHODS

Patients

We report on the analysis of a prospectively defined database of 400 consecutive melanoma patients with a primary melanoma (AJCC Stage I/II) scheduled to undergo a SN procedure at department of Dermatology of the Charité, Humboldt University of Berlin, Germany. Primary tumors had at least a Breslow thickness of ≥ 1mm, or regardless of Breslow thickness, tumors were Clark IV / V, ulcerated or showed signs of regression.

Patients' primary tumor data were not known in all cases prior to the US examination of the regional lymph node basin(s). The institutional ethical review board approved the study and informed consent was obtained from all patients enrolled. Recruitment into this study started in 2001 and we now report the first 400 consecutive patients.

Methods

All patients were scheduled for SN and were examined by US in B-Mode and Power Doppler after lymphoscintigraphy, since lymphoscintigraphy proved to be helpful prior to the US examination. In case of a suspicious or malignant result during the US examination, at least 3 FNACs of the lesion were performed. Afterwards the patients proceeded to undergo the SN surgery later the same day or the next day. During the study there was a shift in the hospital policy, allowing the surgeon to proceed directly to performing a therapeutic lymph node dissection (TLND), in cases were there was a positive FNAC (n=14).

Ultrasound (US)

Pre-operatively we performed a high-resolution US examination of the lymphatic basin and the lymphatic drainage of the tumor. All US examinations were performed using the high-end device Technos (ESAOTE, Italy) equipped with 3 transducers between 3.5 and 14 MHz (B-mode, 30 pictures per second, color Doppler, Power Mode). The lymph node was measured, classified as benign [b], suspicious [s] or malignant [m].

Table 1 summarizes the morphology criteria used for this ultrasound classification. To be considered for either ultrasound category (suspicious or malignant), at least one of the morphology criteria summarized in Table 1 had to be present. In cases of malignant ultrasound exams, the presence of a balloon shaped lymph node, with or without peripheral perfusion had to be observed. Peripheral perfusion is an early sign of involvement, whereas balloon shaped lymph nodes and the loss of central echoes are late signs that correspond to advanced microscopic involvement. When none of the criteria was present or if echopoor islands were present, the node was considered benign.

The region was always examined in comparison to the contra lateral side. All examinations were performed by experienced sonographers (CV & GS).

Table 1 Morphological Criteria for Suspicion on Ultrasound

	Malignant	Suspicious	Benign
Balloon Shaped Lymph Node*	X		
Loss of Central Echoes#		X	
Peripheral Perfusion$		X	
Hump Structure**		X	
Cap Structure##			X
Loss of Central Perfusion$$		X	
Echopoor Islands***		X	X

** Balloon Shaped Lymph Node = Echopoor round enlarged lymph node, usually without any central echoes*
 # Loss of Central Echoes = Observation that a lymph node has lost central echoes or has still some residual central echoes, but these are wandering towards the rim, giving an asymmetrical central aspect
$ Peripheral Perfusion = Perfusion at the rim of a space occupation lesion in ultrasound depicted by PowerMode
*** Hump Structure = Asymmetrical broadening of the parenchyma like a camel hump*
Cap structure = Cap like structure as broadening of the parenchyma to the smaller end of an ovally shaped lymphnode as described by Kahle et al.[40]
$$ Loss of Central Perfusion = Cental perfusion of a space occupying lesion in ultrasound measured by PowerMode
**** Echopoor Islands = Echofree areas like islands within an elsewhere normally appearing lymph node with central echoes and echopoor parenchyma; interrupting the normal architecture of this lymph node.*

FNAC

FNAC was performed with a hand-held "Binder"-valve, which provides an especially short distance between the button for initiation of aspiration and the region of interest. This makes it possible to aspirate even very small targets without losing contact with the lesion in the process. US-guided FNAC uses an alcoholic fluid as a conductor medium, thus minimizing the danger of infection. The fine needle for superficial lymph nodes has a diameter of approximately 0.4 mm (26G). For deeper lymph nodes (depth > 25 mm) a 22 G lumbar puncture needle is used. The negative pressure for aspiration is performed with a 20 ml syringe by fixing the plunger at the 10 ml position, creating an approximate negative pressure of about -300 cmH_2O. We performed at least 3 aspirations under sonographic guidance to receive multiple smears for representative cytodiagnostic evaluation. A smear was considered to be technically efficient if it contained approximately 100 cells. FNAC procedures performed in small targets such as intranodal areas within a sentinel node with a needle diameter of only 0.4 mm often achieve a smaller number of cells and thus tend to give 'unrepresentative' results, these cases were considered negative. In order to deliver representative results, at least three FNAC procedures must be performed, in those cases where the cytologist deemed the aspirated material macroscopically insufficient, a possible extra (fourth) FNAC could be performed.

Pathological Review

SNs were identified by the triple technique, which consists of the preoperative lymphoscintigraphy with the use of radioactive nanocolloid, intraoperative use of patent blue dye and the intraoperative use of a handheld gamma probe. The SNs were histologically worked-up by the EORTC Melanoma Group protocol for pathological examination[18]. This requires transhilar bivalving of the nodes and stepsections from both faces of the lymph node. Staining was performed with H&E, S-100 and Melan-A. The SN metastases were micro-anatomically analyzed for location according to Dewar et al[19]. and for SN tumor burden by the Rotterdam Criteria; maximum diameter of the largest lesion < 0.1 mm, 0.1 – 1.0 mm or > 1.0 mm[20,21]. Due to a change in hospital policy during the course of this study, following preliminary results, some patients with a positive FNAC proceeded immediately to undergo a TLND (n=14), these nodes were examined by routine bivalving and H&E staining, not by an advanced SN protocol.

Statistics

To assess diagnostic value of US guided FNAC, sensitivity, specificity, positive and negative predictive values were calculated using the Pearson's Square test. The combination of US guided FNAC was only counted as a positive test if, either the US and/or FNAC was positive. If US was suspicious, but FNAC was negative, it was considered as a negative result. Disease-free and overall survival were calculated from time of US until recurrence of the disease or death respectively. Patients without such an event at their last follow-up were censored at that time. Univariate analyses of end-points was performed using the Kaplan-Meier method and the log-rank test. P values of less than 0.05 were considered as significant. The statistical analyses were performed with Stata version 8.2 (Stata Corporation, College Station, Texas, USA).

RESULTS

Baseline characteristics of all 400 patients are summarized in Table 2. Mean and median Breslow thickness was 1.5 mm and 1.8 mm, respectively. Mean age at the time of US was 58 years. Mean and median follow-up of all patients were both 42 months (range 4 - 82 months). A total of 79 patients (20%) had metastases in their SN on histology. For the different AJCC categories of Breslow thickness this was 4%, 9%, 29%, 56%, respectively. Ultrasound was considered malignant in 45 (11%), suspicious in 112 (28%) patients and benign in 243 (61%). FNAC was performed in a total of 134 patients (34%). Unfortunately, in 4 cases there was not sufficient time to perform a FNAC, 19 cases yielded an unrepresentative FNAC result, due to inadequate smears (< 100 cells). All 23 patients have been analyzed as FNAC negative.

Table 2 Characteristics of all 400 patients and their Primary Tumors

	N (%)		N (%)
Gender		Clark	
Male	219 (55%)	II	9 (2%)
Female	181 (45%)	III	152 (38%)
Histology		IV	215 (54%)
SSM	275 (69%)	V	21 (5%)
NM	81 (20%)	Unknown	3 (1%)
LMM	17 (4%)	Ulceration	
ALM	17 (4%)	Present	130 (33%)
Unknown	10 (3%)	Absent	252 (63%)
Breslow		Unknown	18 (4%)
T1 (≤1.00 mm)	121 (30%)	Location	
T2 (1.01 – 2.00 mm)	126 (32%)	Extremity	185 (46%)
T3 (2.01 – 4.00 mm)	85 (21%)	Trunk	171 (43%)
T4 (> 4.00 mm)	68 (17%)	Head & Neck	44 (11%)

Of the 400 patients, 331 patients (83%) had a single draining basin, 61 patients (15%) had two draining basins and 8 patients (2%) had three draining basins, all of which were examined. 4 patients with multiple draining basins had one positive FNAC, none had multiple positive FNACs from different basins. These 4 patients subsequently underwent surgical SN procedure of all draining basins. 1 of these 4 patients had two positive SN basins; the others had only one positive SN basin.

Table 3 demonstrates the value of US-FNAC, it shows an overview of the sensitivity, specificity, positive and negative predictive value of the combination of US guided FNAC in total and also per T stage. Table 3 also summarizes the results for the three most important ultrasound morphology criteria.

Table 3 Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of the combination Ultrasound-guided-FNAC for all 400 patients, per T-stage and according to Separate Ultrasound Morphology Criteria

	Sens.	**Spec.**	**PPV**	**NPV**
Balloon Shaped Lymph Node	30%	100%	96%	85%
Loss of Central Echoes	60%	92%	65%	90%
Peripheral Perfusion	77%	82%	52%	93%
T1	2/5 (40%)	116/116 (100%)	2/2 (100%)	116/119 (97%)
T2	6/11 (55%)	113/115 (98%)	6/8 (75%)	113/118 (96%)
T3	13/25 (52%)	59/60 (98%)	13/14 (93%)	59/71 (83%)
T4	30/38 (79%)	29/30 (97%)	30/31 (97%)	29/37 (78%)
All Patients	51/79 (65%)	317/321 (99%)	51/55 (93%)	317/345 (92%)

Sens. = Sensitivity, Spec. = Specificity, PPV = Positive Predictive Value, NPV = Negative Predictive Value, US = Ultrasound, FNAC = Fine Needle Aspiration Cytology

Table 4 The Distribution of US-FNAC Positivity according to SN Tumor Burden

	SN Neg	< 0.1 mm	0.1 – 1.0 mm	> 1.0 mm	Total
US-FNAC *Negative*	317/321 (99%)	10/13 (77%)	13/24 (54%)	4/28 (14%)	345
US-FNAC *Positive*	4/321 (1%)	3/13 (23%)	11/24 (46%)	24/28 (86%)	55
Total	321	13	24	28	400

NOTE. Fourteen patients underwent direct complete lymph node dissection, for a total of 79 patients with node-positive disease. Abbreviations: US, ultrasound; FNAC, fine-needle aspiration cytology; SN, sentinel node.

There was one case were the FNAC was 'false positive', because histological examination of the SN was negative. However, this patient soon developed a regional nodal recurrence in the same nodal basin where the FNAC was performed, so most likely this node was the ultrasound-identified node and the SN retrieved by surgery was not the nodal metastasis found with FNAC.

For all patients the technique demonstrated a 65% sensitivity rate, a 99% specificity rate, a 93% positive predictive value, and a 92% negative predictive value. Of the 79 SN positive patients 28 patients (35%) were false negative on US-FNAC. For the entire population this translated into 7% and 8% of all patients, which were incorrectly identified as SN positive and negative, respectively.

Because 14 patients underwent a TLND after positive FNAC, without a previous SN, only 65 (of the 79) positive SNs could be micro-anatomically analyzed for SN metastasis location and tumor burden. 13 Patients (20%) had metastases < 0.1 mm. 37% had 0.1 – 1.0 mm SN tumor burden and 43% had a SN tumor burden > 1.0 mm. (Table 4). All 14 patients with a positive FNAC, followed by a TLND, demonstrated at least 1 positive lymph node on routine bivalving and H&E staining.

Survival

The Kaplan-Meier estimated 5-years overall survival rate was 92% for US-FNAC negative versus 51% for US-FNAC positive patients, respectively (Figure 1A). Figure 1B shows the Kaplan-Meier estimated 5-year overall survival for the groups; US-FNAC and histology negative (true negative patients) 93%, US-FNAC and histology positive (true positive patients) 53% and US-FNAC negative, but histology positive (false negative patients) 71%. The distant metastasis-free survival (DMFS) was calculated for the same three patient groups. The Kaplan-Meier estimated 5-years DMFS was 92% for the US-FNAC & histology negative patients, 35% for the US-FNAC and histology positive patients and 82% for the US-FNAC negative, but histology positive patients (Figure 2).

The Kaplan-Meier estimated 5-year overall survival rates according to the Rotterdam Criteria for SN tumor burden were 93% for SN negative patients, 92% for metastases < 0.1mm, 46% for 0.1 – 1.0 mm and 51% for > 1.0 mm (Figure 1C).

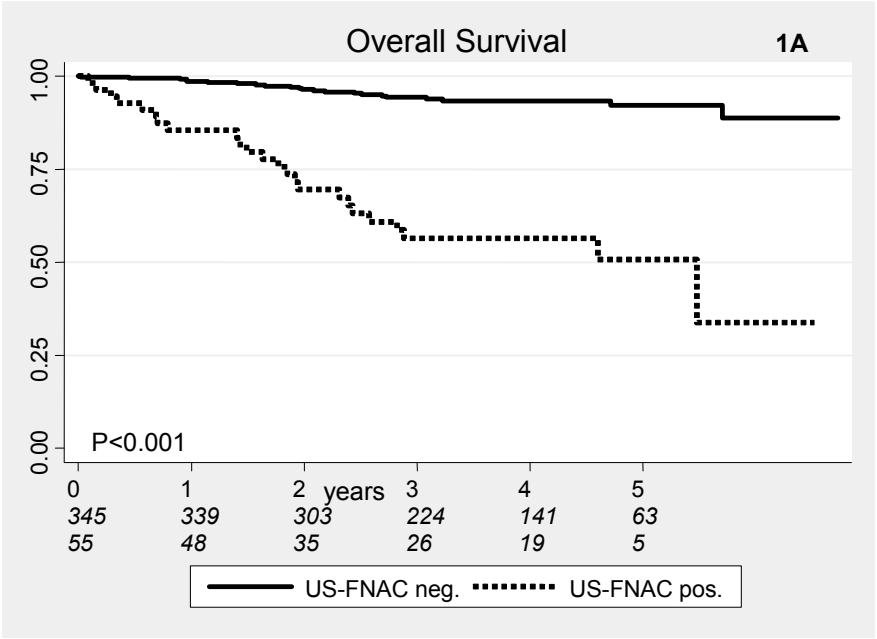

Figure 1A The Kaplan-Meier Estimated 5-years Overall Survival of all 400 patients according to US-FNAC status

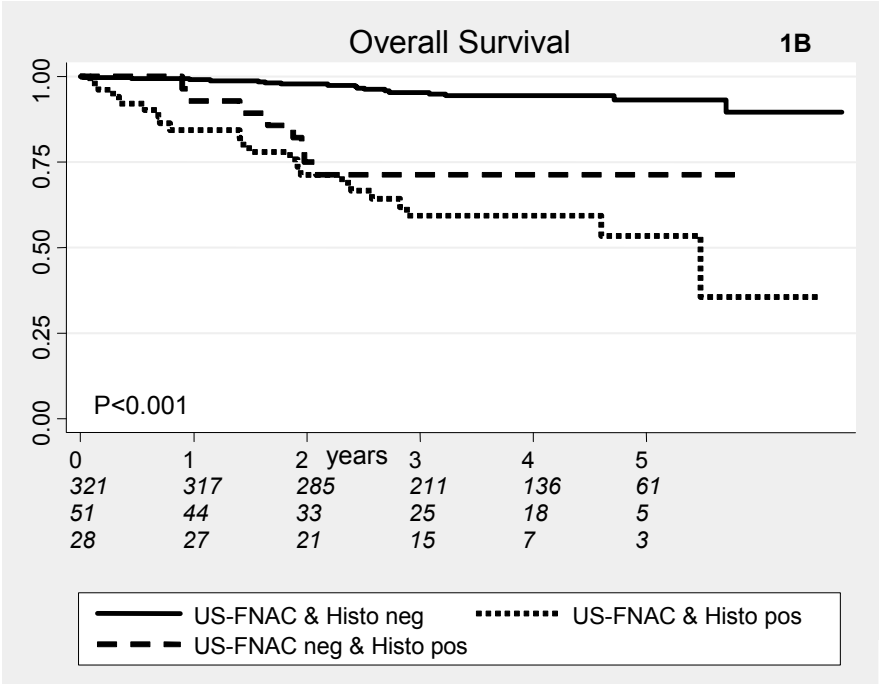

Figure 1B The Kaplan-Meier Estimated 5-years Overall Survival of all 400 patients according to US-FNAC and histology status

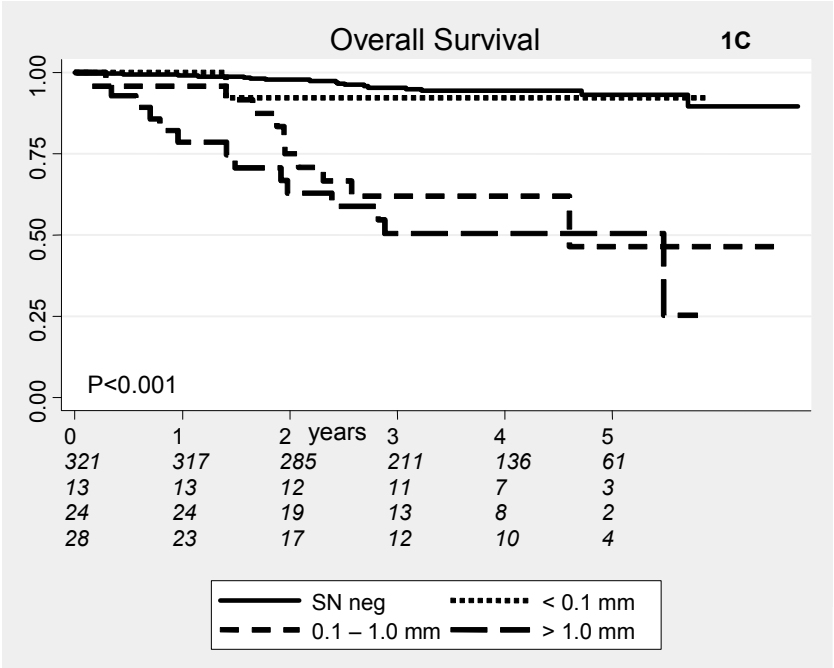

Figure 1C The Kaplan-Meier Estimated 5-years Overall Survival of all 400 patients according to the amount of SN Tumor Burden

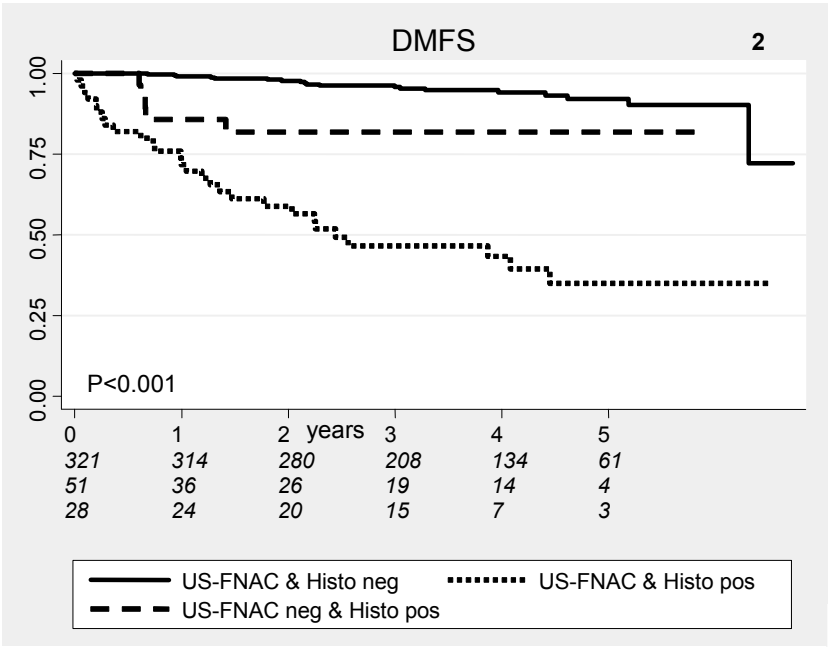

Figure 2 The Kaplan-Meier Estimated 5-years Distant Metastasis-Free Survival (DMFS) according to US-FNAC and histology status

DISCUSSION

The present study demonstrates, in the largest US guided FNAC melanoma patient cohort ever to be reported, that US in combination with FNAC is a highly accurate pre-surgical SN staging procedure for stage I/II melanoma patients. In our hands this technique has an overall sensitivity of 65%, which is the highest rate ever reported. Rossi et al. reported a rate of 12/31 (39%), Starritt et al. reported a rate of 7/33 (21%) and van Rijk et al. reported a rate of 12/37 (34%)[22-24]. A possible clarification for this large difference in sensitivity compared to previous studies could be the introduction and recognition of peripheral perfusion as sign of early involvement.

Another possible reason for the increase in sensitivity in our study is the easy access to quick cytology reports and the more frequent use of FNAC. Other centers do not have access to an overnight FNAC report and will therefore not perform a FNAC. At our center patients undergo subsequently a lymphoscintigraphy, a US exam with or without FNAC and a SN procedure the next day. The definitive FNAC report will be available for the surgeon, prior to the scheduled operation.

In other centers, such as the Sydney Melanoma Unit, FNAC is only performed in those cases, where already a large disruption of the US image has been observed. Most stage I/II melanoma patients, scheduled to undergo a SN, will not yet have such advanced SN disease and therefore the yield and sensitivity is lower than reported in the present study. In contrast, at our center, FNAC is performed quite often, when there is a small, early disruption of the US image. However, due to the single institution nature of the present study, we stress that the results of this study need to validated in a multicenter prospective study.

Importantly, in the present study, the frequency of node positivity varies from 4% to 9% in pT1 and pT2 stages, i.e. an uncommon event. In stages pT3 and pT4 node positivity occurs with a higher frequency of 29% to 56% respectively. The sensitivity increased significantly from 40% in pT1 to 80% in pT4. This is analogous to the situation in breast cancer, were US guided FNAC detects a large proportion of the nodal metastases preoperatively, especially in the higher T stages, thereby reducing the number of SN operations[25-27]. The survival rates of 92% for US-FNAC negative versus 51% for US-FNAC positive patients in our study are identical to the survival rates from numerous large studies in the literature.[3-5,11,28].

Arguments against the preoperative use of US guided FNAC in melanoma patients are that, although US guided FNAC can detect about two-thirds of SN metastases preoperatively, it will still miss one-third. Therefore US guided FNAC will not be able to replace SN staging in melanoma patients. However, the question we want to address is: which metastases is US-FNAC missing and what are the consequences?

The present study has demonstrated that there is a close correlation between the sensitivity of US guided FNAC detection of SN metastases and the size of SN metastases. Whereas only a few SNs with metastases < 0.1 mm were detected by a suspicious US (n=3; none were positive on FNAC), up to 86% of metastases > 1.0 mm were detected by US guided FNAC. A number of studies have demonstrated that minimal, most often subcapsular, SN tumor burden has an excellent prognosis that does not differ from SN-negative patients[19-21,29,30], although these results were not confirmed by some other studies on this subject[31,32].

The 7% false negative-rate of US-FNAC of the total population is in the same range as reported for the SN-procedure, which ranges between 7% and 25%, and thus this argument cannot be used against US-FNAC[4,11,33]. Moreover US-FNAC can be repeated in an outpatient follow-up setting[14,34-36]. So, even if a patient does not have a US guided FNAC detectable SN metastases at first, it could possibly be detected at a very early phase during follow-up. Therefore this could be considered as an acceptable alternative to current SN staging. The ongoing MSLT-II trial is also addressing the value of US guided FNAC as tool in detecting early relapses and might give more insight into the role for US guided FNAC[16].

US-FNAC has the obvious benefit to reduce the number of surgical SN procedures and thereby the costs of the surgery, most often performed under general anesthesia and its associated short- and long-term morbidity. Morbidity rates of 4.6% to 13.4% have been reported, in most cases this entails wound infections, hematomas/ seromas and in some cases lymphedema, also for the SN negative patients[11,37-39]. We argue that US-FNAC can avoid these costs in most, if not all, of the 80% (SN-negative) of all stage I/II melanoma patients.

With a positive predictive value of 93% and a negative predictive value of 92% the US guided FNAC identified 65% of the SN positive patients pre-operatively in our single institution experience. We hope that this report will initiate further multicenter studies to determine the reproducibility of these excellent results in daily practice in multiple institutions. Such prospective studies could also evaluate the learning curve in institutions not familiar with this technique.

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Chapter 9

Ultrasound morphology criteria predict metastatic disease of the sentinel nodes in patients with melanoma

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ABSTRACT

Background:

We have shown that Ultrasound (US) guided Fine Needle Aspiration Cytology (FNAC) can accurately identify the Sentinel Node (SN). Moreover, US-guided-FNAC prior to the surgical SN procedure could identify up to 65% of all SN metastases. Here we analyzed in detail the different US morphologic patterns of SN metastases.

Method:

From 2001 to 12/2007 a total of 650 melanoma patients scheduled for SLND have been examined. We present the first 400 with sufficient follow-up (mean 40, median 39 mo.). Several morphologic characteristics were scored. In case of suspicious/ clearly malignant US patterns a FNAC was performed. The final histology was considered the golden standard.

Results:

Median Breslow was 1.8 mm. The sensitivity and PPV of the most important factors were: peripheral perfusion (PP) present (77%, 52%), loss of central echoes (LCE) (60%, 65%) and balloon shape (BS) (30%, 96%). Together these factors has a sensitivity of 82% and PPV of 52% ($P<0.001$). PP identified more patients with lower volume disease. PP and, combined BS and LCE were independent prognostic factors for survival, HR 2.19 ($P<0.015$) and 5.50 (<0.001).

Conclusion:

Pre-operative US and FNAC can identify 65% of SN metastases and thus reduce the need for surgical SN procedures. Peripheral perfusion is an early sign of involvement and of crucial importance to achieve a high identification rate. Balloon shape and loss of central echoes are late signs of metastases. We recommend US evaluation to identify those patients, who can directly proceed to a CLND after a positive US-guided FNAC of the SN.

INTRODUCTION

The involvement of regional lymph nodes is the most important prognostic factor for overall survival of AJCC stage I/II melanoma patients[1,2]. A sentinel node (SN) procedure provides early identification of metastases in the excised lymph node[3,4]. The SN technique achieves a detection rate of approximately 97% by combining technetium-99m lymphoscintigraphy, intraoperative blue-dye-mapping and gamma-probe identification[5-7]. In case of a tumor positive SN, usually a complete lymph node dissection (CLND) is performed.

Ultrasound (US) combined with fine needle aspiration cytology (FNAC) has been shown to identify regional metastases earlier than the physical examination with high sensitivity and specificity during the follow-up of melanoma patients[8,9]. The question remains if US-FNAC can also play a role in the early lymph node staging of AJCC stage I/II melanoma patients.

We have previously shown[10] the value of ultrasound in the detection of SNs before surgery. The morphology of these lymph nodes detected as a SN on ultrasound and the operatively excised SNs corresponded very accurately (Sensitivity 79%)[10]. We also demonstrated the high accuracy and high identification rate of 65% of US-FNAC in the detection of SN metastases, prior to surgery[11]. However, tyrosinase RT-PCR applied to the FNAC specimen did not further improve detection rates[12]. The study showed a possible 65% reduction in SN procedures for tumor involved SNs, with a spread of 40% - 79% depending on tumor stage and of 23% - 86% depending on the SN tumor burden classified according to the Rotterdam Criteria[11,13,14]. Overall this translated into a possible 13% reduction in SN procedures for all stage I / II melanoma patients. Importantly US-FNAC failed to identify only 7% with micrometastases[11].

The aim of the present study was to evaluate a number of specifically defined morphology US patterns and to correlate this with tumor involvement of the SN. Correlations between morphology patterns were analyzed, as was the correlation to SN tumor burden. Possible combinations of patterns and the amount of positive patterns were analyzed. Survival of different patient groups was calculated and a multivariate analysis was performed.

PATIENTS AND METHODS

Patients

Our prospective database included 650 consecutive melanoma patients who were scheduled for SN (July 2001 - December 2007). This study consists of the first 400 patients with sufficient follow-up. The institutional ethical review board approved the study and

Table 1 Summary of the clinical data

	N (%)		N (%)
Gender		Clark	
Male	219 (55%)	II	9 (2%)
Female	181 (45%)	III	152 (38%)
Histology		IV	215 (54%)
SSM	275 (69%)	V	21 (5%)
NM	81 (20%)	Unknown	3 (1%)
LMM	17 (4%)	Ulceration	
ALM	17 (4%)	Present	130 (33%)
Unknown	10 (3%)	Absent	252 (63%)
Breslow		Unknown	18 (4%)
T1 (≤1.00 mm)	121 (30%)	Location	
T2 (1.01 – 2.00 mm)	126 (32%)	Extremity	185 (46%)
T3 (2.01 – 4.00 mm)	85 (21%)	Trunk	171 (43%)
T4 (> 4.00 mm)	68 (17%)	Head & Neck	44 (11%)

Abbreviations: SSM, superficial spreading melanoma; NM, nodular melanoma; LMM, lentigo maligna melanoma; ALM, acrolentiginous melanoma.

informed consent was obtained from all patients enrolled. All melanomas included into this study had at least a 1.0 Breslow thickness, or were Clark IV / V, ulcerated or regressed (Table 1).

Methods

All patients were scheduled to undergo a SN procedure. Following the lymphoscintigraphy they were examined by US in B-Mode and Power Doppler. US was aimed at clearly depicting the location of the suspected SN prior to surgery and stating whether it seemed involved or not. Other lymph node basins were secondarily also evaluated by US. If US depicted a suspicious SN, FNAC followed for verification of the lesion. US patterns were collected into the prospective database prior to the gathering of the cytology/histopathology results. Of the 400 patients, 373 patients' pictures have been saved as jpg.data. Aspirated material was cytologically evaluated within hours and the results were available prior to the scheduled SN biopsy. The cytological findings were reported to the surgeon, it was left to his discretion how to proceed with surgery, either SN or LND. If the US did not show any suspicious nodes or if the cytology was negative, the patients proceeded to undergo the scheduled SN in every case (see flow-chart in Figure 1). Histopathological patterns according to Dewar et al(15) and measurements of SN tumor burden according to the Rotterdam Criteria by van Akkooi et al.[13,14] were correlated with ultrasound morphology data.

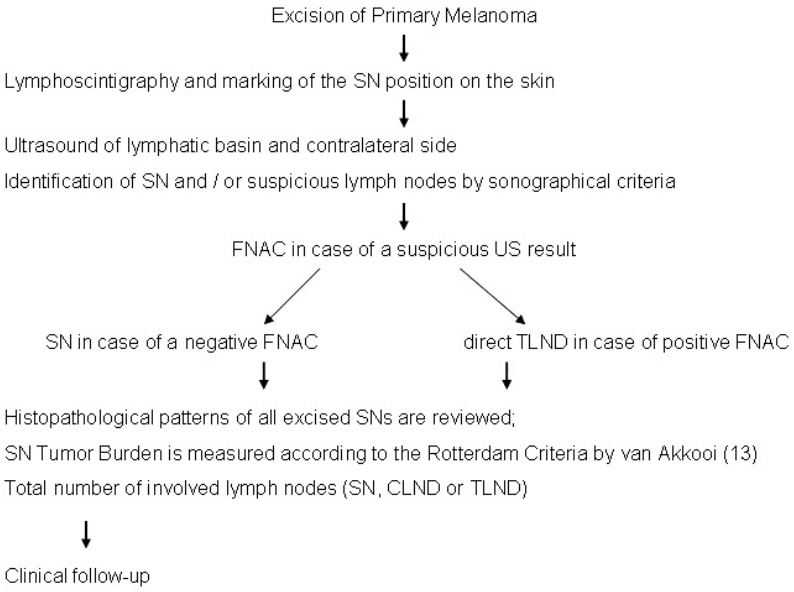

Figure 1 Flow-chart

Ultrasound Technique and Image Analysis

All US examinations were performed using the high-end device Technos (ESAOTE, Genova, Italy) equipped with 3 transducers between 3.5 and 14 MHz (B-mode, 30 pictures per second, color Doppler, Power Mode). The lymph node was measured, the pattern was described and it was classified in real-time as either benign [b], suspicious [s] or malignant [m] by a trained expert ultrasonographist (C.V.).

All images were analyzed by 2 experts with a computerized workstation (ESAOTE, Genova, Italy), but a hard copy of the images was also made on film for the purpose of annotation. Nodal size was calculated as: The maximum and minimum bi-dimensional length in the orthogonal plane of each SN was measured to the nearest millimeter on the transverse images with the caliper tool.

The following 7 pre-defined morphological criteria were assessed: 1) Hump Structure, 2) Echo-poor Islands, 3) Cap Structure, 4) Loss of Central Perfusion, 5) Presence of Peripheral Perfusion, 6) Loss of Central Echoes (including displacement of the central echo to the periphery) and 7) Balloon Shaped Lymph Node (Figure 4; Pictures I – VIII).

Fine needle aspiration in detail

FNAC was performed with a hand-held "Binder"-valve as described elsewhere[16]. The special design of the valve enables aspiration of even very small targets. The fine needle for superficial lymph nodes has a diameter of approximately 0.4 mm (26G). A smear was considered to be technically efficient if it contained approximately 100 cells. FNAC procedures performed in small targets such as intranodal areas within a sentinel node

with a needle diameter of only 0.4 mm often achieve a smaller amount of cells and thus tend to give limited 'unrepresentative' results. In order to deliver representative results, multiple and repeated FNAC procedures must be performed.

Histopathological evaluation of excised SN

In brief, lymph nodes were fixed for 24 h in buffered formalin. After fixation they were cut in half through the hilum and its longest dimension and embedded in paraffin. In rare cases, exceptionally large nodes were sectioned parallel to the first cut in order to fit in the blocks. Five serial step sections of 4 µm each were cut from each face of the lymph node, and staining with H&E, S100 and HMB-45 was performed.

Microanatomic location of the metastases and SN tumor burden were assessed according to the criteria by Dewar[15] and according to the Rotterdam Criteria for SN tumor burden[13,14].

Statistics

To assess the diagnostic value of individual and combinations of US patterns for involved sentinel nodes, sensitivity and specificity of those patterns as well as positive or negative predictive values were calculated. Correlations were tested with Pearson's square test. Overall Survival (OS) was calculated from SN date until death or censored at date of last follow-up, if no events had taken place. Univariate analyses of OS was performed using the Kaplan-Meier method and the log-rank test. Multivariate analyses to determine the prognostic value of covariates regarding OS were performed using the Cox's proportional hazard model. Statistical analyses were all performed with Stata®, version 10.1 (Stata Corporation, College Station, Texas, USA). P values of less than 0.05 were considered as significant.

RESULTS

Median Breslow was 1.8 mm. 54% showed a Clark IV primary. 32.5% of patients presented with an ulcerated primary tumor. Baseline patient and tumor characteristics are shown in Table 1. Table 2 summarizes the occurrence of individual morphological criteria and combinations of specific criteria and shows the correlations between peripheral perfusion, loss of central echoes and balloon shaped lymph nodes. Peripheral perfusion is often seen in cases with loss of central echoes (71.4%) or presence of a balloon shaped lymph node (96%), whilst loss of central echoes (17.7%) and presence of a balloon shaped lymph node (20.4%) are only seen in a minority of cases where peripheral perfusion is present. With 83.3% and 71.4% respectively, loss of central echoes and balloon shaped lymph nodes are closely correlated to each other.

Table 2 Occurrence of different individual and combinations of morphological criteria and their correlations

Criterion	N	%
None	166	44.5%
Hump Structure	125	33.5%
Echo-poor Island	27	7.2%
Cap Structure	44	11.8%
Loss of Central Perfusion	86	23.1%
Presence of Peripheral Perfusion	113	30.3%
Loss of Central Echoes	28	7.5%
Balloon Shaped Lymph Node	24	6.4%
Loss of Central Echoes and/or Balloon Shaped	32	8.6%
Presence of Peripheral Perfusion and/or Loss of Central Echoes / Balloon Shaped	121	32.4%
Metastasis	**77**	**20.6%**

Table 3 Correlations between criteria

Correlations	Peripheral Perfusion	Loss of Central Echoes	Balloon Shaped Lymph Node
Presence of Peripheral Perfusion	X	17.7 % (12 / 113)	20.4% (23 / 113)
Loss of Central Echoes	71.4% (20 / 28)	X	71.4% (20 / 28)
Balloon Shaped Lymph Node	96% (23 / 24)	83.3% (20 / 24)	X

The sensitivity, specificity, PPV, NPV and P-values of all individual morphology patterns and specific combinations of patterns are shown in Table 3. The presence of peripheral perfusion, loss of central echoes and balloon shaped lymph nodes demonstrated the highest sensitivity and PPV rates. The combination of these 3 criteria showed 82% sensitivity and a 52% PPV, with a specificity of 80% and a NPV of 94% (P<0.001). To illustrate the significance of these results a comparison with sensitivity results reported by other studies is shown in Table 4. Subsequently these 3 criteria and combinations of these 3 criteria were correlated to SN tumor burden (Table 5).

Kaplan-Meier estimated 5-year overall survival according to peripheral perfusion was 81% and 92% for present and absent, respectively (Figure 2A) (P<0.001). The Kaplan-Meier estimated 5-year OS for the loss of central echoes was 49% vs. 92% when echoes were still present (Figure 2B) (P<0.001). The respective Kaplan-Meier estimated 5-year Os rates for the presence and absence of a balloon shaped lymph node was 48% and 92% (Figure 2C) (P<0.001). Figure 3A shows the Kaplan-Meier estimated OS curve for the presence of a balloon shaped lymph node and/or loss of central echoes (56%) vs. peripheral perfusion only (87%) vs. neither of these criteria (93%) (P <0.001). Figure 3B

Table 4 Sensitivity, Specificity, PPV, NPV and P-Value of the individual morphological criteria and for combinations of criteria.

Morphology Criteria	**Sens.**	**Spec.**	**PPV**	**NPV**	P-Value
Hump Structure	21%	72%	55%	86%	P < 0.001
Echo-poor Islands	21%	96%	59%	82%	P < 0.001
Cap Structure	8%	87%	14%	78%	n.s. (P = 0.221)
Central Perfusion Absent	25%	77%	22%	80%	n.s. (P = 0.705)
Presence of Peripheral Perfusion	**77%**	**82%**	**52%**	**93%**	**P < 0.001**
Loss of Central Echoes	**60%**	**92%**	**65%**	**90%**	**P < 0.001**
Balloon Shaped Lymph Node	**30%**	**100%**	**96%**	**85%**	**P < 0.001**
Balloon Shaped and/or Loss of Echoes	**34%**	**98%**	**81%**	**85%**	**P<0.001**
Peripheral Perfusion Present and/or Balloon / Loss of Echoes	**82%**	**80%**	**52%**	**94%**	**P<0.001**

NOTE. Bold font indicates statistical significance. Abbreviations: Sens. = Sensitivity, Spec. = Specificity, PPV = positive predictive; NPV = negative predictive value; NS = not significant.

Table 5 Comparison of sensitivity with other studies.

	Sensitivity
Starritt et al. [32]	21%
van Rijk et al. [33]	34%
Rossi et al. [31]	39%
Current Study: Loss of Echoes / Balloon Shaped	*34%*
Include: Peripheral Perfusion	**82%**

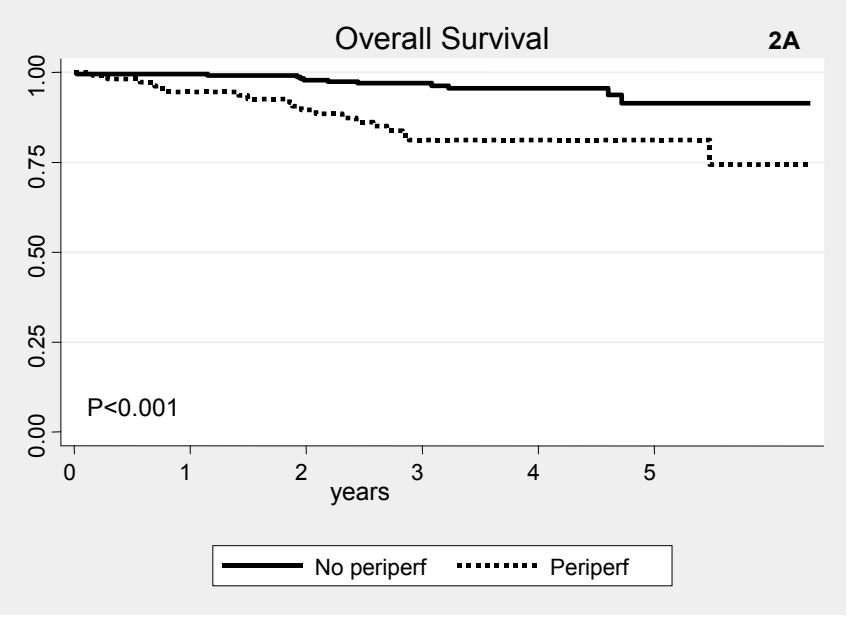

Figure 2A Overall Survival According to Peripheral Perfusion

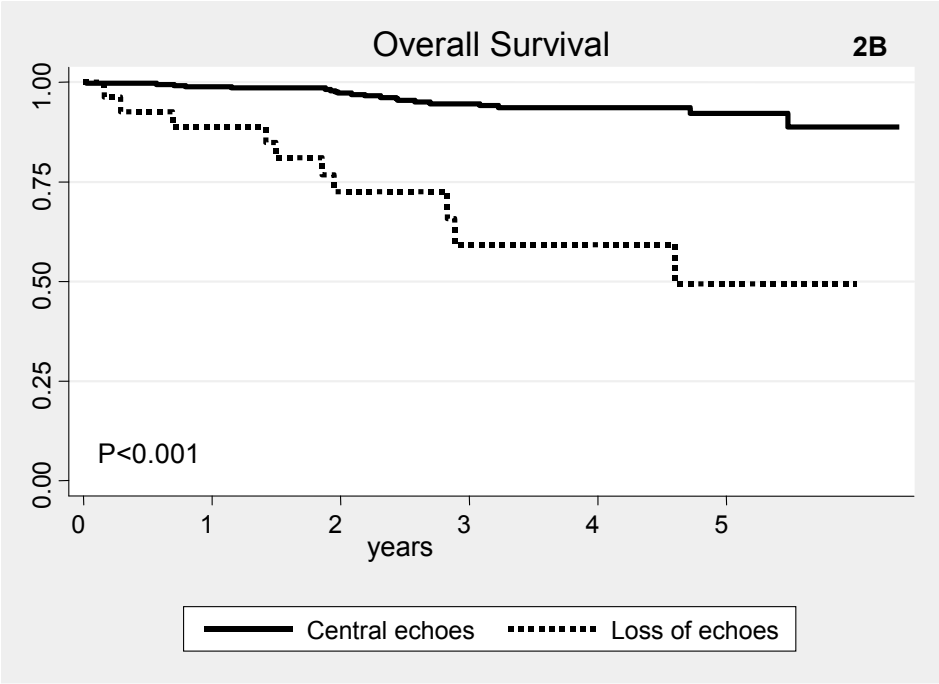

Figure 2B Overall Survival According to Loss of Central Echoes

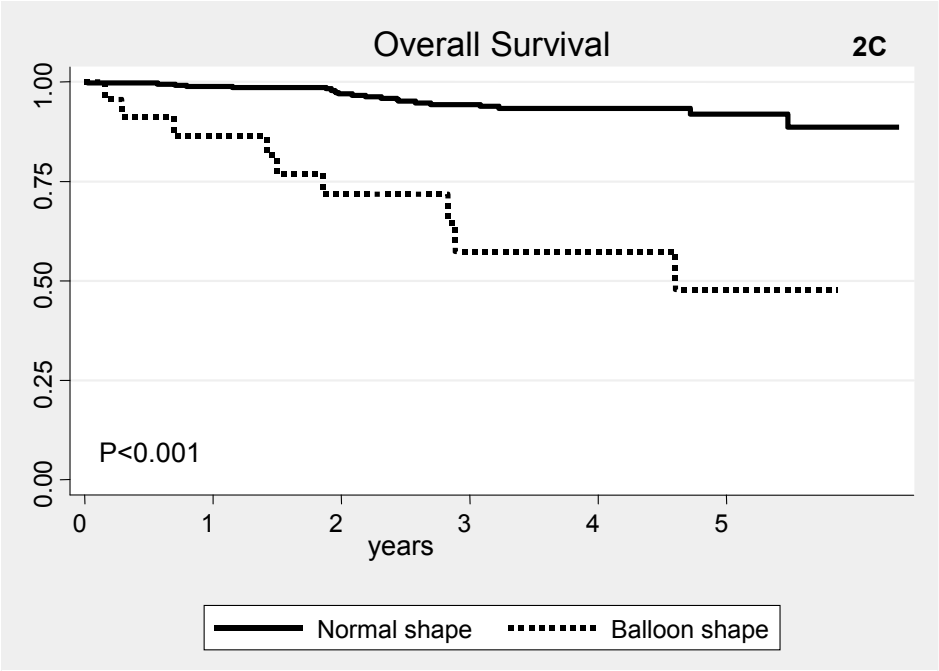

Figure 2C Overall Survival According to Loss Balloon Shaped Lymph Node

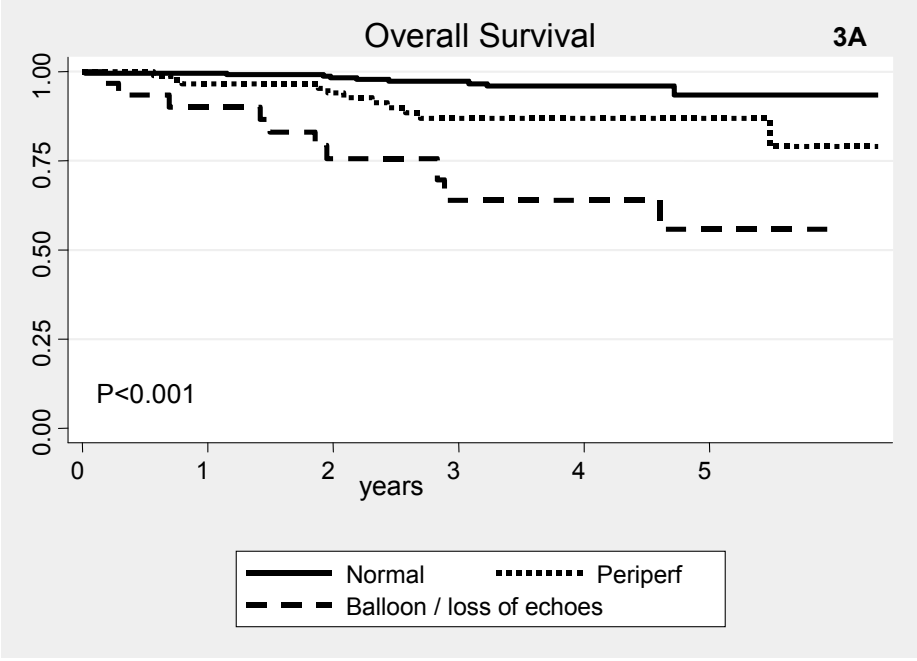

Figure 3A Overall Survival for Balloon Shaped Lymph Node/Loss of Central Echoes vs. Peripheral Perfusion vs. Neither.

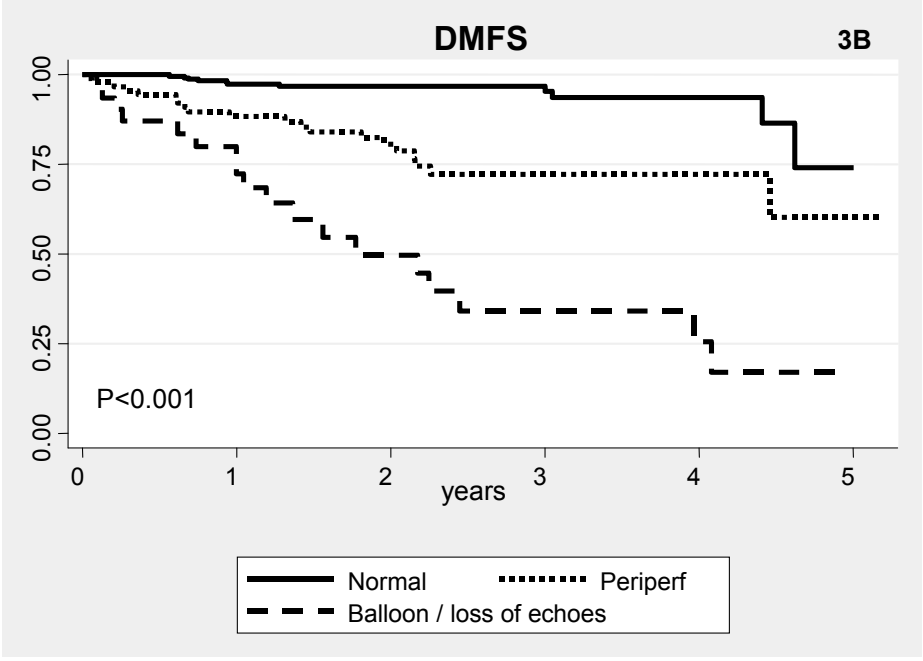

Figure 3B Distant Metastasis-Free Survival for Balloon Shaped Lymph Node/Loss of Central Echoes vs. Peripheral Perfusion vs. Neither.

Table 6 SN tumor burden according and multivariate analyses according to different morphological criteria.

	< 0.1 mm	0.1 – 1.0 mm	> 1.0 mm	P-Value	HR	95% CI	P
Peripheral Perfusion	*75%*	*54%*	*90%*	*P<0.001*			
Loss of central Echoes	8%	20.8%	42.5%	P<0.001			
Balloon Shaped	8%	12.5%	47.5%	P<0.001			
Loss of echoes and/or Balloon Shaped	8%	25%	47.5%	P<0.001			
Peripheral Perfusion and/or Loss of Echoes/Balloon Shaped	*75%*	*71%*	*90%*	*P<0.001*			
Hump					1.22	0.57 – 2.60	0.616
Island					1.49	0.67 – 3.29	0.328
Cap					0.88	0.30 – 2.56	0.811
Central Perfusion					0.99	0.49 – 2.03	0.986
Balloon / loss of echoes					*5.50*	*2.85 – 10.62*	*<0.001*
Peripheral Perfusion					*2.19*	*1.16 – 4.14*	*0.015*

shows the respective Kaplan-Meier estimated 5-year DMFS, which was 26% vs. 60% vs. 74%, respectively (P<0.001).

Table 6 summarizes the multivariate analysis for overall survival. The loss of central echoes and/or presence of a balloon shaped lymph node was an independent prognostic factor for survival with the highest hazard ratio (5.5, P<0.001), whereas the presence of peripheral perfusion was also an independent prognostic factor for OS, but with a lower hazard ratio (2.19, P=0.015).

The Vasallo/Solbiati-index, which should be >2 in most cases of benign lymph nodes, was checked[17,18] and was not a statistically significant predictor of involvement, nor was it a predictor of non-involvement (*data not shown*). Alike, size of lymph nodes was not a useful characteristic for involvement; i.e. increasing size was not translated into an increasing number of detected malignant SNs (*data not shown*).

DISCUSSION

Malignant melanoma primarily metastasizes to the regional lymph nodes[3]. Once a lymph node is involved, the 5- or 10-year survival rate drops dramatically[1,2]. It has been shown that the status of the SN after SLNB is the most important single prognostic factor, although a benefit of a SLNB on survival could not be shown[4,19]. Moreover, it was calculated that a positive sentinel lymph node might have no adverse prognostic relevance in up to one-third of patients[19]. Furthermore, in the same patients, progression to palpable nodal disease might not have occurred even if the positive sentinel

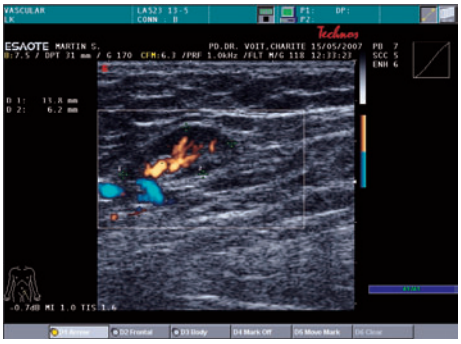

Fig 4, Pic I Reactively enlarged lymph node; Central Echoes and Central Perfusion Present

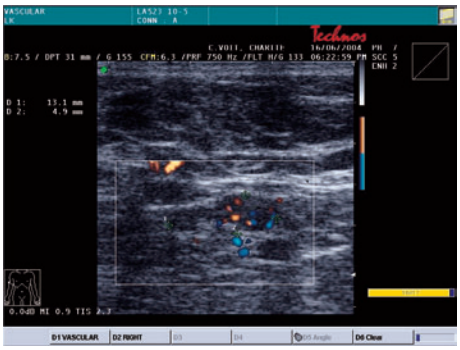

Fig 4, Pic II Hump Structure

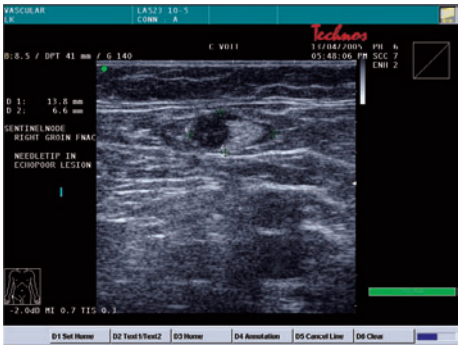

Fig 4, Pic III Echo-poor Island

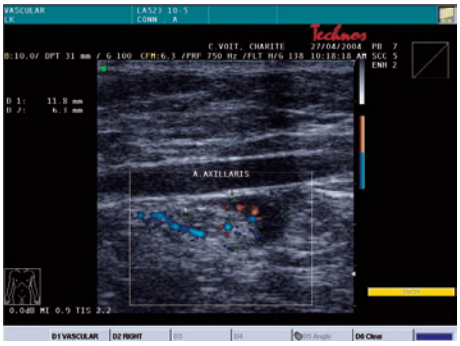

Fig 4, Pic IV Cap Structure

node had not been removed[14,19,20]. Thus US-guided FNAC can be considered as another valid staging method, in the absence of a survival benefit for the surgical SN procedure. This might have the advantage that approximately 80% of stage I/II melanoma patients without SN metastases will not have to undergo a surgical staging procedure.

For physicians unfamiliar with this in vivo high resolution ultrasound technique of the SN, it may be difficult to imagine that a tumor involved SN could show a typical US morphologic pattern[21]. During immunologic processes like infection or peripheral tumor growth, a lymph node loses its basic structure and proceeds to a reactive state with characteristic structural changes. This transformation tends to be reversible as long as no neo-vascularization arises or (micro-) metastases exist within the lymph node. The shape of the SN without tumor involvement (like any other lymph node) tends to be oval and builds an echo-poor peripheral band, like the pulp around the stone of a cherry. The hilum reveals increased echoes and there is central perfusion. Hypervascularization within the periphery, signals the location of a beginning process (=involvement) in the parenchyma that is suspicious for malignancy. A balloon-shaped parenchyma is always highly suspicious for malignancy, especially if it contains the above-mentioned local hyper-perfusion.

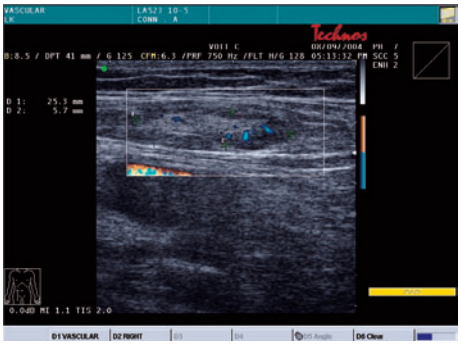

Fig 4, Pic V Loss of Central Perfusion

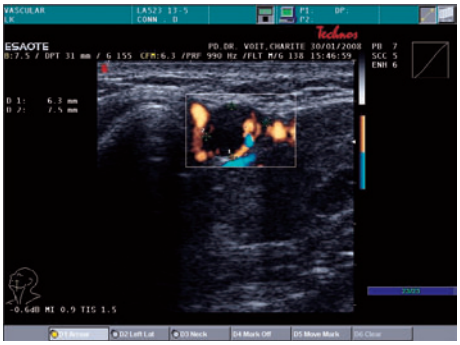

Fig 4, Pic VI Presence of Peripheral Perfusion

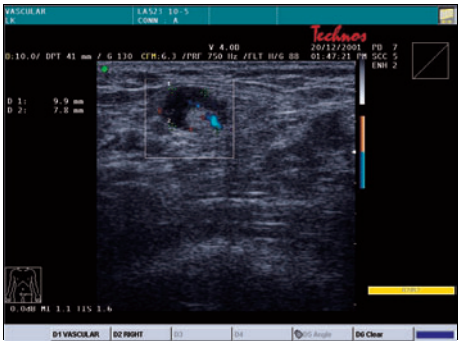

Fig 4, Pic VII Displacement of central echo to the periphery / Loss of central echoes

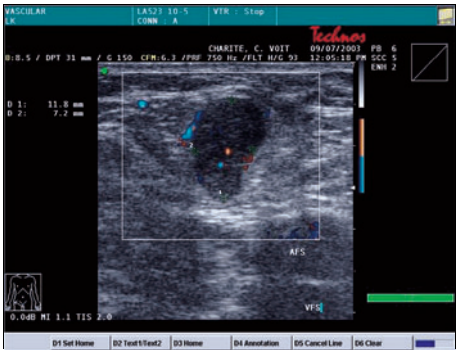

Fig 4, Pic VIII Balloon Shaped Lymph Node

In most cases, melanoma cells are lying free/loose, disconnected from the normal lymph node structures within the lymph node. These cells are well suited for the aspiration process, which has demonstrated tremendously high specificity of 100%, meaning that the probability of producing false positive findings is nearly zero[8,22].

The relatively low sensitivity of FNAC in the SN can be explained by the sometimes small size (< 1 mm) of our targets. The US-FNAC technique is therefore not only limited to the US recognition of a suspicious pattern, but also to the sometimes small area within the lymph node, which has to be aspirated. The histological pattern of a subcapsular metastasis is often a small lesion, spread along the width of the capsule, which delivers a great challenge to the physician performing the FNAC, because it is easy to puncture through the lesion into the parenchyma. These are the cases where it is very likely *not* to achieve a positive FNAC, although the US pattern is highly suspicious.

A number of older studies have identified some guideline characteristics for the morphologic changes of a lymph node involved by a malignant tumor. First Vassallo et al. observed marked differences between benign and malignant nodes in terms of the longitudinal-transverse diameter ratio, the hilus and cortical widening; nodal size

was not significantly different for benign and malignant nodes[17]. Later, the experienced group around Uren, described ultrasound features diagnostic of the presence of palpable nodal metastases. The involved lymph node thickness was more than two-thirds of the lymph node length and could show the presence of low-level echoes[23]. Further, more recent studies recommend the application of high resolution ultrasound for lymph node in the follow-up[24] as well as for in-transit metastases[25], however they do not give more detailed descriptions on specific ultrasound patterns suspicious for malignancy. Recently, the preliminary data of the value of ultrasound in the Multicenter Selective Lymphadenectomy Trial-2 (MSLT-2) were presented. Ultrasound only identified 8 out of 193 metastases (4.2%) in the MSLT-2[26].

For a different type of cancer, thyreoid cancer, there is more experience and a study by Leboulleux et al. describes morphological patterns in neck lymph nodes[27]. In thyreoid cancer, a cystic appearance, hyperechoic punctuations, loss of hilum, and peripheral vascularization could be considered as major ultrasound criteria for lymph node involvement[27]. Lymph nodes with a hyperechoic hilum should be considered as benign. Peripheral vascularization had the best sensitivity-specificity compromise. Round shape, hypoechogenicity and the loss of hilum taken as single criteria were not specific enough to suspect malignancy[27]. Although this is a very useful system for thyreoid cancer and the study did recognize similar morphological criteria, it is unsure if this can simply be extrapolated to melanoma.

Very recently an **in vitro** study was conducted by breast cancer specialists describing cortical morphologic features of axillary lymph nodes as a predictor of metastasis in breast cancer. Similarly, lymph nodes with central echoes were considered benign, whereas hypoechoic nodes were considered suspicious for malignancy[28]. Maximum asymmetrical cortex thickness, as shown in another recent breast cancer study[28] combined with irregular hypervascularization turned out to be the most important feature for predicting SN involvement. There was no correlation between size and probability of malignancy of a lymph node[9,28] which could be confirmed in the present study on melanoma.

In the present study we have shown improved results of US-guided FNAC in melanoma as compared to other series, including the large prospective randomized trial, the MSLT-2[26,29,30]. Therefore, there must be a difference in the US morphology criteria applied. Importantly, many studies, in which ultrasound is performed do not describe the criteria used for these examinations. It is illustrative that the sensitivity reported by Rossi et al., van Rijk et al. and Starritt et al.[29-31], all range between 21% and 39%, which is virtually identical to the 30% sensitivity rate reported by us for balloon shaped lymph nodes or the 34% for both balloon shaped and loss of central echoes.

To our knowledge the present study is the first prospective study to evaluate and categorize ultrasound morphology patterns of in vivo examinations of SN in melanoma patients and correlate these results with histopathological findings and survival.

The most pathognomonic criteria for an involved lymph node were the loss of central echoes and/or a balloon shaped lymph node, this is reflected in the high positive predictive values of 65 and 96%, respectively. This is also shown in the correlation to larger SN tumor burden (42.5% and 47.5% of metastases >1 mm, respectively), the worse survival outcome (56% at 5-years) and the high HR of 5.5 for OS. When compared to peripheral perfusion, which identified more melanoma metastases than loss of echoes and balloon shaped lymph node, which is reflected in a higher sensitivity of 77%. Correlated to SN tumor burden, peripheral perfusion alone detects more patients with smaller volume disease. It seems that peripheral perfusion is an early sign of lymph node involvement by a metastasis and that this remains in later stages of metastasis development. In these later stages of metastasis development the central core echo (and thus the anatomy) of the lymph node is displaced to the periphery and as the lymph node expands under pressure of large volume of fast growing melanoma cells it finally becomes balloon shaped. Our recommendation is that specifically these 3 factors; peripheral perfusion, loss of central echoes (including displacement to the periphery) and balloon shaped lymph node, should be used in any future ultrasound study for melanoma patients.

Moreover, US can be very useful in treatment planning, providing the surgeon with additional information about surrounding structures, blood vessels and suspicious findings in additional (non-sentinel) lymph nodes. In our opinion routine US-FNAC should be planned, after lymphoscintigraphy and prior to operative SN staging. If the US-FNAC of the SN reveals a positive cytology, the operative SN can be replaced by an immediate lymph node dissection, thus saving both the surgeon and the patient a second surgical procedure.

In our hands, US-FNAC can identify up to 65% of all SN metastases in the subgroup of all SN positive patients. At our center, peripheral perfusion, loss of central echoes and a balloon shaped lymph node were the most important factors for tumor involvement of the SN and survival. Peripheral perfusion is an early sign of involvement, whereas loss of central echoes (including displacement to the periphery) and balloon shaped lymph nodes are late signs of already larger volume metastases.

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Chapter 10

Impact of Molecular Staging Methods in Primary Melanoma: RT-PCR of Ultrasound Guided Aspirate of the Sentinel Node does not Improve Diagnostic Accuracy, but RT-PCR of Peripheral Blood does predict Survival

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ABSTRACT

Purpose

This study analyzes (1) the value of tyrosinase reverse-transcriptase polymerase chain reaction (RT-PCR) of aspirates obtained by ultrasound-guided fine-needle aspiration cytology (US-FNAC) of sentinel nodes (SNs) in patients with melanoma before sentinel lymph node biopsy (SLNB) and (2) the value of RT-PCR of blood samples of all SLNB patients.

Patients and Methods

Between 2001 and 2003, 127 patients with melanoma (median Breslow depth, 2.1 mm) underwent SLNB. FNAC was performed in all SNs of all patients pre- and post-SLNB. The aspirates were partly shock-frozen for RT-PCR and were partly used for standard cytology. Peripheral blood was collected at the time of SLNB and at every outpatient visit thereafter.

Results

Thirty-four (23%) of 120 SNs were positive for melanoma. SN involvement was predicted by US-FNAC with a sensitivity of 82% and a specificity of 72%. Additional tyrosinase RT-PCR revealed the same sensitivity of 82% and a specificity of 72%. At a median follow-up time of 40 months from first blood sample, peripheral-blood RT-PCR was a significant independent predictor of disease-free survival (DFS) and overall survival (OS; $P < 0.001$).

Conclusion

US-FNAC is highly accurate and eliminates the need for SLNB in 16% of all SLNB patients. RT-PCR of the aspirate or excised SN does not improve sensitivity or specificity. RT-PCR of blood samples predicts DFS and OS.

INTRODUCTION

The technique of sentinel lymph node biopsy (SLNB) was originally defined as selective removal of the first draining node (or nodes) from a tumor, called the sentinel node (SN). After the surgical removal of the SN, the SN is thoroughly examined by means of an extensive pathology protocol. This technique identifies early metastatic involvement in the regional lymph node(s)[1].

Ultrasound (US), in combination with fine needle aspiration cytology (FNAC) of regional lymph nodes, is able to detect lymph node metastasis both at the time of presentation of a primary melanoma and for surveillance at follow-up[2-5]. US guided FNAC of sentinel nodes is not the standard of care yet, but it is enjoying rapid implementation in many clinics, because it was shown to eliminate the need for unnecessary surgical SN procedures in approximately 30% of all patients with breast cancer[6,7]. We have previously shown that US-guided FNAC is highly accurate and eliminates the need for SLNB in 16% of all examined cases.8 SLNB has been reported to have a false-negative rate that ranges from 6% to 13%[9]. Approximately one third of these initially false negative SNs can be proven to be positive by increasing the number of step sections of the sentinel nodes[10]. There is some evidence that undetected SN metastases will not necessarily become clinically apparent and that these small, most frequently subcapsular metastases are not necessarily relevant to overall survival[11,12].

The aims of the present study are to evaluate the accuracy of (1) additional reverse-transcriptase polymerase chain reaction (RT-PCR) of aspirates retrieved through preoperative US-guided FNAC, (2) RT-PCR of the excised SN, and (3) RT-PCR of peripheral blood.

PATIENTS AND METHODS

Patients

In this prospective study, 127 consecutive patients scheduled for SLNB after the excision of a melanoma were enrolled after providing written informed consent. All patients had a melanoma of at least 1.00mmin thickness or, in patients with melanomas less than 1.00mm,they were Clark grade IV or V, ulcerated, or showed regression. The study was approved by the local ethical committee and conducted in accordance with the Declaration of Helsinki.

Methods

Evaluation of patients was performed according to the following schedule. First, a lymphoscintigraphy was performed, followed by US-guided FNAC of the sentinel node.

Subsequently, patients underwent SLNB within 24 hours of the lymphoscintigraphy. The SN was retrieved through the use of the triple technique of lymphatic mapping, which includes the preoperative lymphoscintigraphy (already made), intraoperative use of patent blue dye, and the intraoperative use of a handheld gamma probe. Finally, a second FNAC was performed of the SN after it was excised operatively. All FNAC material was used in part for cytology and another part was shock-frozen for molecular biology with tyrosinase RT-PCR. Furthermore, small pieces of the hilum region and of the afferent draining lymphatic vessels were cut out of the excised SN, which were also examined for the presence of melanoma cells by tyrosinase RT-PCR.

The SNs then proceeded to the pathologist for regular work-up. All excised SNs and the corresponding afferent lymphatic drainage are examined in step sections in hematoxylin and eosin staining and staining against HMB-45 and Melan-A according to the European Organization for Research and Treatment of Cancer Melanoma Group protocol for the work-up of SNs[13].

A blood sample was taken at time of SLNB for all patients. Further blood samples were collected when patients presented for clinical examination during their regular scheduled follow-up at 3- or 6-month intervals depending on the American Joint Committee on Cancer stage, which is in accordance with the guidelines of the German Dermatology Society.

Classification by US. Our classification by US and FNAC were previously reported[4,5,14]. Preoperatively, we performed a high-resolution US examination of the lymphatic basin and the lymphatic drainage of the tumor by using the high-end device Technos (ESAOTE, Genoa, Italy) equipped with three transducers between 3.5 and 13 MHz.

FNAC. Methods and results of FNAC were previously reported[3,15]. The fine needle only has a diameter of 0.4 mm (26G). Multiple aspirates were obtained for cytology and for tyrosinase RT-PCR. The smears were dehumidified before staining. To get a representative result, a number of 100 cells per smear are expected.

Tyrosinase RT-PCR of Fine Needle Aspirates (FNA-PCR)

RNA isolation: FNAC material. Approximately 0.3 µL of aspirate was shock-frozen in liquid nitrogen and stored at -80°C for subsequent molecular biologic evaluation. Total RNA was extracted from the mini-cell pellet by means of RNeasy total RNA kit (Qiagen, Hilden, Germany). RNA was quantified by ultraviolet spectrophotometry at 260 nm and 280 nm and stored at -80°C. A total of 1.5 µg of total RNA was transcribed.

cDNA synthesis. Reverse transcription was performed with the Super-Script First-Strand System for RT-PCR (Invitrogen, Carlsbad, CA) according to instructor's protocol. The cDNA synthesis was carried out with 1.5 µg of RNA, 500 ng of Oligo (dT)12-18 primer, 10 nmol/L of dNTP-Mix, 200 U of SuperScript II (Invitrogen, Carlsbad, CA), followed by an *Escherichia coli* RNase H treatment (2 U). *Nested tyrosinasePCR*. Primers for a nested

tyrosinase PCR were used as described[14,16]. Thirty cycles were run using a schedule described elsewhere[14,17]. To amplify the β2m housekeeping gene, PCR was performed under the same condition and concentration as for tyrosinase. β2m PCR product size is 165 base pairs (for sequences of primers for β2m and porphobilinogen deaminase, refer to Max et al[18,19]).

Quality control experiments. After an inter-laboratory trial of the European Organization for Research and Treatment of Cancer for inter-laboratory quality assurance[20], several steps were taken to detect any cross-over contamination and were performed after the protocol as published[21]. If results of patient samples were discordant, the procedure starting from RNA preparation was rerun twice until two concordant results could be seen. The specificity of PCR products was examined by sequencing all positive samples.

Quantitative real-time PCR. Real-time PCR was conducted on a Light-Cycler instrument (Roche, Mannheim, Germany) using primer specific for PBGD and tyrosinase (HTYR3, HTYR4) in a single-round PCR. Probes for LightCycler PCR (PBDD-3FL, PBGD-5LC, Tyr-3FL, Tyr-5LC) were purchased from Metabion, Martinsried, Germany. The sequences as used are described by Keilholz et al[22].

Statistics

To evaluate the diagnostic value of RT-PCR of the US-guided FNAC before patients underwent SLNB and of the second FNAC of the surgically excised SN, sensitivity, specificity, and positive and negative predictive values were calculated separately for each of these methods. These results were compared with the sensitivity and specificity of the cytology of US-guided FNAC alone (no RT-PCR) and the final histologic evaluation by the pathologist.

Disease-free survival (DFS) time and overall survival (OS) time were calculated from the date of the SLNB (first blood sampling) until first recurrence or death, respectively. DFS and OS were analyzed with the method of Kaplan and Meier. Furthermore, because serial tyrosinase blood RT-PCR measurements per patient were performed, a proportional hazards model with RT-PCR as a time dependent covariate was fitted and a hazard ratio with 95% CIs and *P* values was calculated. RT-PCR result was considered as a timedependent prognostic factor because several RT-PCR results per patient were available, and RT-PCR results changed over time[23]. Statistical analyses were performed with SAS version 8.2 (SAS Institute, Cary, NC).

RESULTS

In total, we evaluated 141 SNs in 127 patients. No US- or FNAC related morbidity or complications occurred during this study. Detailed patients and tumor characteristics

Table 1 Summary of the clinical data

Characteristic			No. of Patients	%
Gender:	Female		66	52
	Male		61	48
Age in years: median (min-max)			60 (20-88)	
Breslow tumor depth in mm: median (min-max)			2.1 (0.4-18.0)	
Breslow tumor depth in mm:		≤1mm	30	24
		> 1 to ≤ 2 mm	31	24
		> 2 to ≤ 4 mm	35	28
		> 4 mm	29	23
		Missing	2	1
Primary Tumor Site:		head, neck	14	11
		limbs	61	48
		trunk	51	40
		unknown	1	1
Type of Histology:		SSM	82	65
		NM	30	24
		LMM	2	2
		ALM	5	4
		Others	8	6
Ulceration:	no		74	58
	yes		53	42
Regression:	no		77	61
	yes		50	39
Clark Level:	II		1	1
	III		49	39
	IV		65	51
	V		10	8
	Missing		2	1
AJCC staging:	Ia		26	20
	Ib		20	16
	IIa		18	14
	IIb		21	17
	IIc		6	5
	IIIa		12	9
	IIIb		11	9
	IIIc		13	10

Abbreviations: SSM, superficial spreading melanoma; NM, nodular melanoma; LMM, lentigo maligna melanoma; ALM, acral lentiginous melanoma; AJCC, American Joint Committee on Cancer.

are listed in Table 1. Of the 120 SNs recognized by US-guided FNAC before the SLNB, 34 were histologically malignant. The combined technique of the preoperative US-guided FNAC of the SN correctly classified 28 of 34 histologically malignant cases as positive, thus achieving a sensitivity of 82%. This combination of US and FNAC also correctly identified 62 of 86 histologically proven benign SNs as not involved, reflecting a specificity of 72% (Table 2)[8].

Table 2 also lists the sensitivity and specificity results of the tyrosinase RT-PCR analyses of all the different times and locations, as retrieved from the patients. Location of

Table 2

	Sensitivity	Specificity	PPV	NPV
US	79%	72%	53%	90%
FNAC	59%	100%	100%	85%
Pre-SLNB US-guided RT-PCR	50%	99%		
Pre-SLNB US-guided FNAC & RT-PCR	82%	72%		
Post-SLNB RT-PCR	76%	92%		
Post-SLNB-RT-PCR from afferent vessel	50%	92%		
Post-SLNB-RT-PCR from hilum	85%	72%		
US and FNAC combined	82%	72%	54%	91%

Abbreviations: US, ultrasound; FNAC, fine-needle aspiration cytology; RTPCR, reverse-transcriptase polymerase chain reaction; PPV, positive predictive value; NPV, negative predictive value; SLNB, sentinel lymph node biopsy.

Table 3

		Histology			
		H&E	*Immunostains*	**FNAC**	**RT-PCR**
Negative	n = 86	86 (100 %)	86 (100%)	86 (100%)	85 (99%)
Positive	n = 34	31 (91%)	34 (100%)	19 (56%)	17 (50%)

Abbreviations: SN, sentinel node; FNAC, fine-needle aspiration cytology; RT-PCR, reverse-transcriptase polymerase chain reaction; H&E, hematoxylin and eosin.

positive RT-PCR in the lymphatics (hilum of SN and lymphatic vessels) were checked and analyzed; however, these analyses did not identify new metastases, were considered nonrelevant, and are therefore not further presented.

Table 3 shows an overview of the results with regard to histology (hematoxylin and eosin and/or immunostains), FNAC, and RT-PCR. For patients with discordant results by these techniques (ie, cases where all three results were not all positive or all negative), discrepancies and final survival outcome are listed in Table 4.

Serial Blood Tyrosinase RT-PCR

The median follow-up time from first blood sampling is 40 months (range, 0 to 60.0 months). Eighteen of the 127 patients have had a positive blood RT-PCR at least once or repeatedly. DFS for patients with blood RT-PCR positive at least once versus patients for whom blood RT-PCR was always negative is shown in Figure 1A. OS for patients with blood RT-PCR positive at least once versus patients for whom blood RT-PCR was always negative is shown in Figure 1B.

The Cox proportional hazards model with RT-PCR results as time-dependent variables (ie, a test result was carried forward as long as it was replaced by the following result) showed a hazard ratio for DFS of 11.7(95%CI, 4.6 to 29.7;P<0.001). Another Cox proportional hazards model for OS showed a hazard ratio of 25.4 (95% CI, 10.9 to 59.3; P<0.001).

Table 4

Patient #	Histology	FNAC	RT-PCR	Outcome	FU (months)
11	Positive	Non Diagnostic	Negative	DOD 24/03/06	57
29	Positive	Negative	Negative	NED 02/05/07	67
33	Positive	Negative	Negative	DOD21/03/04	29
48	Positive	Negative	Negative	DOD 21/01/04	23
55	Positive (IHC)	Negative	Negative	NED 22/07/02	4*
56	Positive (IHC)	Negative	Negative	DOD 22/05/05	38
60	Positive	Not Done	Negative	DOD 28/04/04	25
69	Positive	Positive	Negative	DOD 14/01/05	32
72	Positive	Negative	Negative	NED 02/07/07	61
86	Positive	Negative	Negative	DOD 01/09/04	24
89	Positive	Positive	Negative	DOD 01/09/04	24
90	Positive	Negative	Negative	NED 02/05/07	56
94	Positive	Negative	Positive	DOD 28/03/04	18
102	Positive	Negative	Negative	NED 13/11/07	61
111	Positive	Positive	Negative	DOD 08/06/07	54
115	Positive	Positive	Negative	NED 15/11/07	59
117	Positive	Negative	Negative	DOD 28/01/05	24
131	Positive	Negative	Positive	NED 20/12/07	55
133	Negative	Negative	Positive	NED 21/02/07	44

Abbreviations: FNAC, fine-needle aspiration cytology; RT-PCR, reverse-transcriptase polymerase chain reaction; DOD, died of disease; NED, no evidence of disease; IHC, immunohistochemistry.
** Lost to follow-up.*

In an additional real-time RT-PCR assay for quantization of tyrosinase according to Keilholz et al[22] and for porphobilinogen deaminase, housekeeping gene was used. Melanoma aspirates from SNs and their paired peripheral blood samples were analyzed only if they tested positive in qualitative RT-PCR. Tyrosinase-mRNA was not detected in healthy donor blood samples. Patients with stage III disease expressed this marker more frequently and at higher levels in peripheral blood as compared with those with earlier stage disease. The diagnostic sensitivity was optimal in blood samples containing more than 0.1 pg/μL of porphobilinogen deaminase.

DISCUSSION

In this study, we show that US-guided FNAC is reliable in detecting metastatic involvement of the SN in patients with melanoma, but that the results are not improved by RT-PCR of the aspirate. The 16% pre-SNLB identification of a positive SN by US-guided FNAC alone and, more importantly, the 82% identification rate of an involved SN by

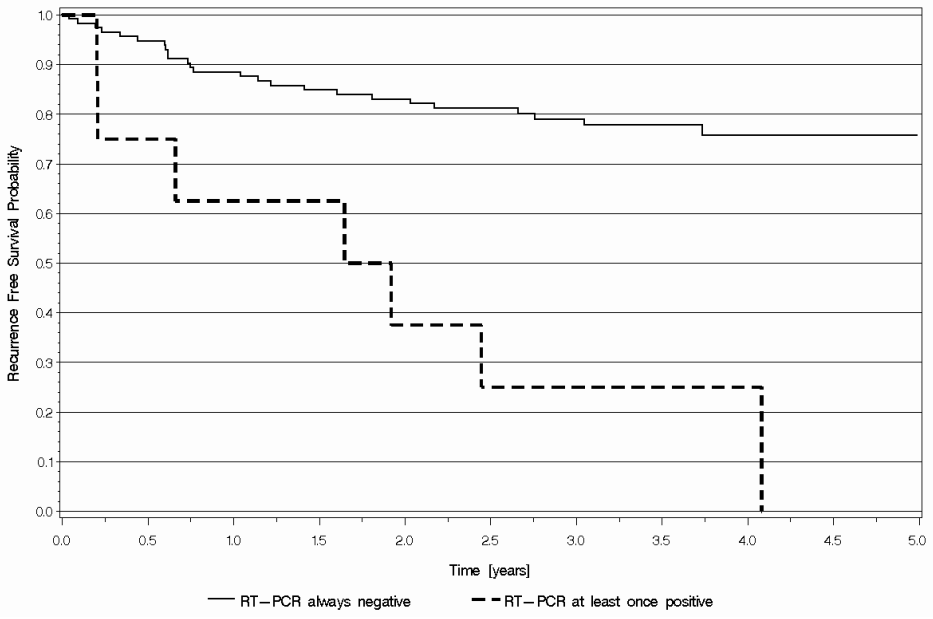

Figure 1A Disease-free survival from the time of first blood sampling stratified for Reverse transcriptase polymerase chain reaction (RT-PCR) that is always negative and RT-PCR that is positive at least once.

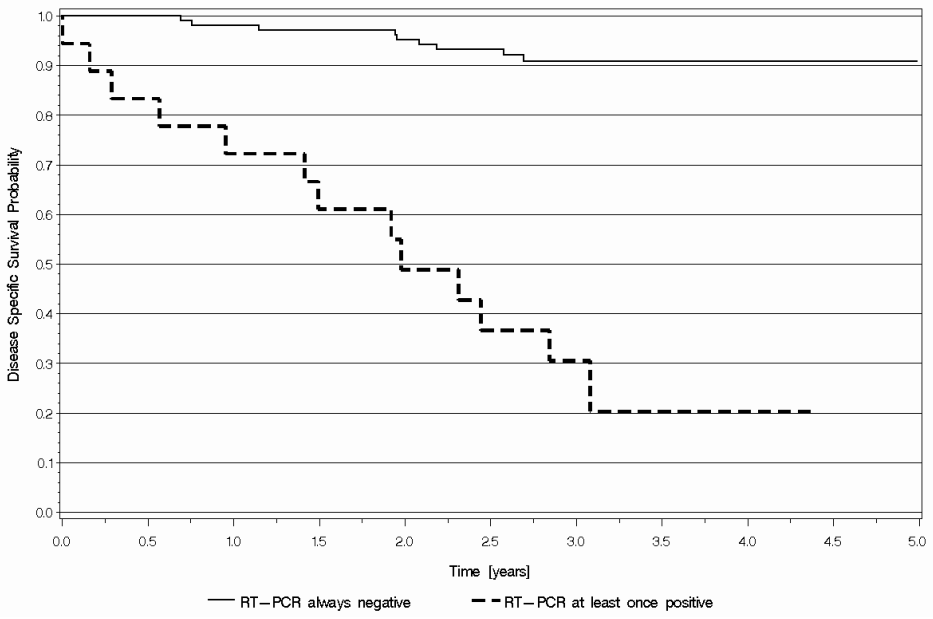

Figure 1B Overall survival from the time of first blood sampling stratified for reverse transcriptase polymerase chain reaction (RT-PCR) that is always negative and RT-PCR that is positive at least once.

US-guided FNAC is virtually identical to that which has been reported for US-guided FNAC in the Rotterdam breast cancer study[24].

However, this is not improved by additional RT-PCR of the aspirate nor of the excised SN. In fact, RT-PCR of aspirated pre-SLNB material shows virtually identical sensitivity and specificity compared with the FNAC results of the same node. Whereas RT-PCR of post-SLNB material does not improve the sensitivity compared with the final histology results, the use of both FNAC and RT-PCR of US guided material of one and the same SN does increase the sensitivity of this pre-SLNB technique to 82%. So in approximately 16% of all patients, an SLNB procedure can be avoided by performing US guided FNAC of the lymph nodes up front[8].

Regarding the prognostic value of micrometastases in the SN, their predictive role for the involvement of the non-SNs and the clinical consequences of a false-negative staging as a result of undetected micrometastases are still under debate[25]. The Multicenter Selective Lymphadenectomy Trial-1 trial has demonstrated that implementation of SNLB in patients with primary melanomas does not seem to improve survival[9]. In this trial, SLNB did not seem to improve survival in the overall population of 2001 patients, nor in the 1,327 patients with intermediate-thickness melanomas (1.2 to 3.5 mm). At the same time, the trial report suggests that SNLB is beneficial for SN-positive patients with 1.2- to 3.5-mm melanomas. This conclusion concerns a subgroup analysis, which shows a much smaller difference in survival when corrected for false negatives.

Recently, the largest experience by far of RT-PCR evaluation of SNs indicated that this procedure did not further enhance prognostic value of SN staging[26]. This Sunbelt trial report, which represents the largest experience (more than 1,400 patients) could not prove any benefit of this examination for the additional work-up of the SN[26]. A total of 1,446 patients with histologically negative SNs underwent RT-PCR analysis and showed no difference in DFS, distant metastasis–free survival (DMFS), or OS between the RTPCR–positive and RT-PCR–negative patients.

PCR-based detection of melanoma cells in SNs of patients was reviewed in a recent meta-analysis covering 22 studies with 4,019 patients who underwent SLNB for clinical stage I to II cutaneous melanoma. PCR status of SN was shown to have a clinically valuable prognostic power in patients with melanoma, but caution is warranted to avoid overestimating of results[27].

This is accordance with the conclusions of the present study and of another observation regarding the lack of prognostic value of the presence of submicrometastasis (<0.1mm) in the SN, because these cases had the same DFS, DMFS, and OS prognosis as SN-negative patients[12]. These observations would indicate that clinically relevant disease and disease volume are related and that the detection of tumor cells below a certain threshold (<0.1mmand/or by RT-PCR alone in the absence of hematoxylin and

eosin/immunohistochemical histopathologic evidence) does not represent clinically relevant metastatic disease.

With even more sophisticated histopathologic protocols[28], the detection rate of submicroscopic disease will increase and lead to the reporting of higher SN-positive rates, which may not be clinically relevant. The struggle to find earlier, prognostically better, and more reliable tumor markers is not new[29]. However, to date, the conventional staging methods by SN histology and US-guided FNAC are the best we have.

RT-PCR testing of more than one marker in peripheral blood was associated with shorter DFS and DMFS but no change in OS in the Sunbelt trial[26]. Here, a single-marker RT-PCR for the detection of tyrosinase was used, although in the literature, the use of multimarker assays in peripheral blood was reported to increase sensitivity (sometimes, however, at the expense of specificity)[30,31]. Apart from the often reported inter-laboratory differences between use of quantitative versus qualitative RT-PCR schedules, peripheral blood testing was positive in two smaller studies[21,32], but more importantly, it was negative in this large study (Sunbelt trial; n=1,446)[26].

However, our own recent study had an extremely long median follow-up duration (from the first blood sample to the last follow-up examination or death) of 6.3 years (range, 0.9 to 8.6 years)[21]. Second, here the study population comprised exclusively patients with stage II and III melanoma (ie, patients who have a higher probability to have recurrence) as compared with the study population in the Sunbelt trial. Third, the tyrosinase result was modeled as a time-dependent variable, because results changed over time. This study had shown a strong association between PCR detected in peripheral blood and OS. In a proportional hazards regression analysis, PCR positivity was an important predictor (hazard ratio = 12.6; 95% CI, 3.4 to 46.3;

$P<0.001$)[21]. A recent letter by Qualgino et al[33] stressing the role of the time-dependent calculations of the serial tyrosinase measurements was directed to the authors of the Sunbelt trial, and the answer was that after recalculations in the proposed manner, the blood tyrosinase turned out to be predictive of outcome. Even a single positive test result in tyrosinase RT-PCR from peripheral blood seemed to be a warning for metastases, and several positive test results might be taken as reliable hint for disease progression[32].

In conclusion, US-guided FNAC had previously been proven to be an accurate staging method for patients before they undergo an SN procedure. Additional RT-PCR of fine-needle aspirates or of the SN did not further improve results. We recommend US-guided FNAC pre-SNLB as a simple method to reduce the number of operative SN procedures. RT-PCR could be valuable when taken from peripheral blood of patients with high-risk melanoma to predict recurrence and/or survival.

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Chapter 11

Summary, General Discussion and Conclusions

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SUMMARY

The subject of **Part I** of this thesis is sentinel node tumor burden in melanoma. In **Chapter 2** we reported on the high rate of sentinel node positivity (29%) of the EORTC Melanoma Group pathology protocol for the work-up of 262 melanoma patients from the Erasmus University Medical Center – Daniel den Hoed Cancer Center, who underwent a sentinel node procedure. The SN status was the most important factor for disease-free and overall survival. Importantly, in-transit metastases rates were correlated to SN status, Breslow thickness and ulceration status of the primary. There was no evidence for an increased rate of in-transit metastases by performing a SN procedure.

Chapter 3 describes the European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group methods for the measurement of SN tumor burden and the microanatomic location of metastases within the SN. The EORTC MG recommends the following: The EORTC MG SN pathology protocol or a similarly extensive protocol, which has also been proven to be accurate, should be used. Only measure what you can see not what you presume. Cumulative measurements decrease the accuracy and reproducibility of measuring. The most reproducible measure is a single measurement of the maximum diameter of the largest lesion in any direction (1-D). If there is any infiltration into the parenchyma, this lesion can no longer be considered solely subcapsular. Reporting of the microanatomic location of metastases should be an assessment of the entire sentinel node, not only of the largest lesion. Multifocality reflects a scattered metastatic pattern, not to be confused with multiple cohesive foci, which fall under the regular location system. A subcapsular metastasis should have a smooth usually curved outline not ragged or irregular.

In **Chapter 4** we have first analyzed our own experience and outcome related to SN tumor burden in 77 SN positive patients from the Erasmus University Medical Center – Daniel den Hoed Cancer Center. Sub-micrometastases (<0.1 mm) were found in 16 patients (22%) of the 74 patients, who had slides available for re-evaluation. Estimated 5 years overall survival rates for the different groups of <0.1mm, 0.1-1.0mm and >1.0mm SN tumor burden were 100%, 63% and 35% respectively. Distant metastases were exceedingly rare (1/16 = 6.3%) in <0.1mm SN-positive patients. On multivariate analysis the SN tumor burden was the most important prognostic factor for disease-free survival (P=0.005) and overall survival (P=0.03). Therefore we proposed that patients with metastases <0.1 mm have a clinical course that is indistinguishable from SN negative patients and might not benefit from CLND.

This lead us to analyze the impact of minimal SN tumor burden on the possible survival benefit for the SN procedure and early completion lymph node dissection versus patients, who did not undergo a SN, but developed lymph node metastases, which required a lymph node dissection (TLND) during follow-up. This is described in

Chapter 5. Survival rates were calculated from date of primary excision. All patients with primary melanomas on extremities or trunk were included. We identified 188 patients; 124 TLND patients ('82 – '05) and 64 CLND patients ('97 – '05). Median follow-up was 56 and 37 months, respectively. There were no significant differences between both groups regarding Breslow thickness, ulceration, gender, and site of the primary. On univariate analysis site of the primary tumor (extremity versus trunk) (P<0.001), Breslow thickness (P=0.005), ulceration (P<0.001) were prognostic for OS. There was a non-significant 13% difference in OS for the CLND compared to the TLND group (P=0.12). Excluding SN patients with submicrometastases (n=15) reduced the difference in OS to 6% (P=0.42). Thus we concluded that there was no significant survival benefit for SN+CLND compared to TLND, especially not when patients with submicrometastases were excluded.

We validated the results from our single center experience on SN tumor burden in a multicenter fashion in **Chapter 6**. In 388 SN positive patients from 3 EORTC MG centers, the median Breslow thickness was 4.00mm and ulceration was present in 56%. 40 patients (10%) had metastases <0.1mm. Additional nodal positivity was found in only 1 of 40 patients (3%). At a mean follow-up of 41 months, estimated overall survival at 5 years was 91% for metastasis < 0.1 mm, 61% for 0.1 – 1.0 mm, and 51% for >1.0mm (p<0.001). SN tumor burden increased significantly with Breslow tumor thickness. When the cut-off value for SUB-micrometastases was taken at <0.2mm (such as in breast cancer), the survival was 89% and 10% had additional non-SN nodal positivity.

Finally, the results of SN tumor burden and the influence of the microanatomic location were analyzed and validated in 1080 SN positive patients from 10 EORTC MG centers, which is shown in **Chapter 7**. Between 1993 and 2008, 1080 patients (509 women and 571 men) were diagnosed with tumor burden in the SN in 9 EORTC Melanoma Group (MG) centers of which 1009 patients (93%) underwent CLND. Median Breslow thickness was 3.00 mm. The median follow-up time was 37 months. Patients with submicrometastases (Rotterdam Criteria <0.1 mm) reconfirmed to have an estimated 5-year OS rate of 91% and a low non-SN positivity rate of 9%. The most predictive and prognostic parameter in our study was the RDC (Rotterdam-Dewar Combined) Criteria. Patients with submicrometastases located subcapsular only (RDC Criteria) had a NSN positivity rate of 2% and an estimated 5- and 10-year MSS of 95%.

In **Part II** an alternative staging procedure, ultrasound (US) guided fine needle aspiration cytology (FNAC) was analyzed. In **Chapter 8** we report on the results of US-guided-FNAC in 400 stage I / II melanoma patients. All 400 patients underwent lymphoscintigraphy, subsequently underwent an US-exam prior to the SN procedure. When the US-exam showed a suspicious or malignant pattern, patients underwent a FNAC. Median Breslow thickness was 1.8 mm., follow-up was 42 months (mean, range 4 – 82). We considered the US-FNAC positive if either US and/or FNAC were positive. If US was suspicious, but FNAC negative, it was considered negative. US guided FNAC

identified 51/79 (65%) of SN metastases. Specificity was 99% (317/321) with a Positive Predictive Value (PPV) of 93% and Negative Predictive Value (NPV) of 92%. SN positive identification rate by US guided FNAC increased from 40% in stage pT1a/b to 79% in stage pT4a/b. US guided FNAC detected SN tumors > 1.0mm in 86% of cases, SN tumors of 0.1 – 1.0mm in 46% and SN tumors < 0.1mm in 23%. Estimated 5-year OS rates were 92% for US guided FNAC negative, 51% for positive patients.

Chapter 9 analyzes to a deeper extent the new set of morphology criteria, which were used to increase the accuracy of US-guided-FNAC as alternative staging procedure. Several morphologic characteristics were scored. In case of suspicious/ clearly malignant US patterns a FNAC was performed. The final histology was considered the golden standard. The sensitivity and PPV of the most important factors were: peripheral perfusion (PP) present (77%, 52%), loss of central echoes (LCE) (60%, 65%) and balloon shape (BS) (30%, 96%). Together these factors has a sensitivity of 82% and PPV of 52% (P<0.001). PP identified more patients with lower volume disease. PP and, combined BS and LCE were independent prognostic factors for survival, HR 2.19 (P<0.015) and 5.50 (<0.001). Pre-operative US and FNAC can identify 65% of SN metastases and thus reduce the need for surgical SN procedures. Peripheral perfusion is an early sign of involvement and of crucial importance to achieve a high identification rate. Balloon shape and loss of central echoes are late signs of metastases. We recommend US evaluation to identify those patients, who can directly proceed to a CLND after a positive US-guided FNAC of the SN.

Chapter 10 reports on the use of Reverse Transcription Polymerase Chain Reaction (RT-PCR) to possibly further increase SN positivity rate. Between 2001 and 2003, 127 patients with melanoma (median Breslow depth, 2.1 mm) underwent SLNB. FNAC was performed in all SNs of all patients pre- and post-SLNB. The aspirates were partly shock-frozen for RT-PCR and were partly used for standard cytology. Peripheral blood was collected at the time of SLNB and at every outpatient visit thereafter. Thirty-four (23%) of 120 SNs were positive for melanoma. SN involvement was predicted by US-FNAC with a sensitivity of 82% and a specificity of 72%. Additional tyrosinase RT-PCR revealed the same sensitivity of 82% and a specificity of 72%. At a median follow-up time of 40 months from first blood sample, peripheral-blood RT-PCR was a significant independent predictor of disease-free survival (DFS) and overall survival (OS; $P < 0.001$). RT-PCR of the aspirate or excised SN does not improve sensitivity or specificity. RT-PCR of blood samples predicts DFS and OS.

GENERAL DISCUSSION

SN status and prognosis

Numerous studies have demonstrated that sentinel-node status is an independent prognostic factor for survival. For patients with negative sentinel nodes, the reported 5-year disease-free survival (DFS) rates are 77–89% compared with 38–65% for patients with positive sentinel nodes.[1-7] Moreover, 5-year overall survival rates are generally reported to be 83–94% for sentinel-node-negative patients compared with 54–75% for patients with positive nodes.[7-11] Table 1 shows an overview of the DFS and overall survival rates in the literature.

False-negative rate

Some patients with negative sentinel nodes do have regional nodal relapses in the sentinel node sampled basin. These patients are considered sentinel node false negative. Many investigators have reported very low false negative rates of between 1.5% and 4.1% for the sentinel node procedure.[11-13] Some researchers, however, have rightfully argued that this seems to be an underestimation of the true rate of false negativity, because the false negative rate should not be calculated as a percentage of the entire population, but rather as the amount of false negative results divided by the amount of false negatives plus the true positive rates. In other words, false negatives / false negatives + true positives x 100% = false negative rate.[14] False negative rates calculated by this method have yielded very different rates to others documented in the literature, and vary between 9–21%.[13,15] Table 1 summarizes true false negative rates for a number of pivotal studies. False negativity may be the consequence of a failure in the surgical procedure or the result of a sampling failure in the histopathology protocol.[4] Another hypothesis for false negativity is that an in-transit metastasis has not yet reached the regional lymph node basin, but does so later on, causing a regional nodal relapse.[13]

Cook *et al*.[21] demonstrated that increased sectioning and staining leads to a significant increase in sentinel node positivity rates from 17.4% to 34%. This is due to the recognition of micrometastases in other areas of the lymph node, which were not yet visible on previous sections. Whilst other studies by Karim *et al*.[38] and Nowecki *et al*.[39] demonstrated that 33% and 24.6%, respectively of the false-negative sentinel nodes actually contained occult metastases that were not identified either on the original slides, but also in the deeper recut sections.

The prognosis of false-negative patients is uncertain. Their survival might be poor, due to an advanced metastatic lesion, which might completely block lymph flow to the first draining sentinel node from the tumor, and thus also the flow of patent blue dye and nanocolloid to the true sentinel node and thereby lead to a false-negative result. This poor prognosis could also be the result of a less-intensive follow-up scheme, because these

Table 1 Sentinel Node positivity, false negative, DFS and OS rates

Study	Number of patients	Mean Breslow thickness	Median Breslow thickness	Ulc (%)	SN+ (%)	FN rate (%)	DFS SN- (%)	DFS SN+ (%)	OS SN- (%)	OS SN+ (%)
MSLT-1 Morton *et al.* (2006)[12]	769	1.98	1.8	26	15.9	17.5% (26/148)	83.1%	53.4%	90.2%	72.3%
Gershenwald *et al.* (2008)[55]	2,203				16.3					
Guggenheim *et al.* (2008)[14]	392	2.5			27.3	10.1% (12/119)	89.1%	65%		
Sassen *et al.* (2008)[80]	2,303	2.5	2.0	26.8	16.8					
Koskivuo *et al.* (2007)[46]	305	2.0	1.1	24.5	16.4	9% (5/55)			≈90%	≈75%
Scheri *et al.* (2007)[68]	1,382				15		89%		94%	
Doubrovsky *et al.* (2004)[16]	672	2.9	2.3	31.8	18				87.5%	59%
Balch *et al.* (2000)[13]	3,126				13.9					58%
Nowecki *et al.* (2006)[39]	1207		2.4	42	18.9	20% (57/285)			87.9%	56.8%
Cascinelli *et al.* (2006)[81]	1,108			33	15.9	21% (47/223)			90.6%	75.4%
Yee *et al.* (2005)[82]	1,169				14.6	13.2% (22/167)			90%	56%
Carlson *et al.* (2008)[31]	1,287	1.88		22.2	17.6				83.3%	57.6%
van Akkooi *et al.* (2006)[15]	262	2.76	2.0	28	29.4	9.4% (8/85)	88% (3 years)	52% (3 years)	95% (3 years)	74% (3 years)

Abbreviations: DFS, disease-free survival; FN, false negative; NR, not reported; OS, overall survival; SN, sentinel node; Ulc, ulceration; y, year.

patients were wrongly considered node negative. By contrast, the prognosis of false-negative patients might be the same or better than true positives, as they might have had only a very low amount of metastatic disease, which was missed on serial sectioning.

In the MSLT-1 trial the 3-year overall survival rate of patients who were false-negative was 68.4%, compared with the 5-year overall survival rate of 72.3% for sentinel node patients with a true positive node.[4] A study by Nowecki *et al.*,[39] however, did not confirm these findings; the 5-year survival rates for false-negative patients were similar to true sentinel node-positive patients (53.7% versus 56.8%, respectively; P=0.9). Despite these varied false-negative rates and the uncertain prognosis of false-negative patients, the sentinel node procedure currently remains the most accurate staging tool for melanoma patients, and is associated with a high sensitivity and specificity.

Discussion of the MSLT-1 trial

The only prospective randomized clinical trial to examine the efficacy of lymphadenectomy is the Multicenter Selective Lymphadenectomy Trial-1 (MSLT-1). This trial was designed in the 1990s to address the following question: "To determine whether wide excision of the primary with intraoperative lymphatic mapping followed by selective lymphadenectomy will effectively prolong overall survival compared to wide excision of the primary melanoma alone."[4] In this trial, 2,001 patients were randomized to undergo WLE only followed by a total lymph-node dissection in case of a clinical nodal relapse or WLE plus SLNB followed by CLND in cases of positive sentinel nodes.

Although the final analysis of the trial data has not yet taken place, the data monitoring committee recommended publication of data after the third (of five) planned interim analyses in 2006.[4] The paper by Morton and coauthors reported on 1,269 patients with intermediate thickness melanomas (1.2–3.5 mm) of the total 2,001 melanoma patients that were randomized.[4] There was a small, but significant, DFS benefit for the SLNB group (78.3 versus 73.1% P=0.009);[4,16-20] however, this did not translate into melanoma-specific-survival benefit. With a median follow-up of 59.8 months, the 5-year melanoma-specific-survival rates were 87.1% for the SLNB-arm compared with 86.6% for the WLE-only arm (P=0.58).[21,22] The authors of the study concluded that SLNB identifies patients with nodal metastases whose survival can be prolonged by immediate lymphadenectomy.[23-27] This statement was based on a subgroup analysis and has lead to a widespread and vivid debate.

Supporters of this conclusion have argued that patients with sentinel-node metastases had a significantly improved survival compared with patients who underwent CLND in the WLE-only group (72.3% versus 52.4%, respectively; P = 0.004).[4] This is supported by a similar outcome in two retrospective case-control studies.[26-29] Opponents to this conclusion have argued that this conclusion it is not valid for the following reasons. First, the analysis only takes into account a pre-specified group of the 1,269 patients with intermediate thickness (1.2 – 3.5 mm) tumors of the total 2,001 patients randomized. Second, they argue that this conclusion does not take into account the SLNB patients who were false negative (that is, patients who were negative after the SLNB procedure and who have regional nodal relapse in the sentinel node basis).[27-31] Third, the rate of nodal positivity in the SLNB group was considerably higher than in the WLE-only group (19.2% versus 15.6% at 5-years).[4,26] Therefore it has been suggested by some investigators[50] that a certain proportion of patients with positive sentinel nodes are to be considered "prognostically false positive", because the positive nodes contain single cell involvement or "submicrometastatic lesions" only. These type of metastases may not represent established metastases, but may represent a type of dormant metastases, which does not seem to develop into clinically relevant disease.

Prognostic false positivity

False positivity is a well recognized phenomenon in many diagnostic tests. The analysis by Thomas, published in 2008, was the first to support the hypothesis of prognostic false positivity for the sentinel node procedure in patients with melanoma.[26] Thomas suggested that the concept of prognostic false positivity could play an important part in the analysis of the MSLT-1 trial. Moreover, this concept is based on a mathematical model, which used the interim results of the MSLT-1 trial, as published in 2006. As the regional relapse rate at 5 years was not identical in the two MSLT-1 arms (19.3% (15.9% (SN true positive) +3.4% (false negative SN) for sentinel node patients versus 16.1% for WLE only patients), Thomas calculated that 24% of sentinel-node positive patients was prognostically false positive.

We found further evidence that submicrometastatic tumor load may fall into this phenomenon, as the rate of minimal sentinel node tumor burden according to the Rotterdam criteria is approximately 20% in a multicenter study in 388 patients with a median Breslow thickness of 4.00 mm, which was very similar to the rate as calculated by Thomas.[26,32] Moreover, the patient and tumor characteristics of patients with minimal sentinel node tumor burden do not differ from patients with a negative sentinel node, and importantly the survival rates do not differ.[32]

The proposal of "prognostically false positivity" has been met with much criticism. The most important critique is that these "prognostically false positive" sentinel node metastases might be very slowly progressing tumors, which could potentially lead to late disease recurrences (5–10 years after SLNB) and therefore the regional metastases rates will in time become equal for the both MSLT-1 arms.[28,32-36] Moreover, the excision of these small lesions could have the potential to be curative in this subset of patients.[30,36-42]

An opposing argument is that prognostic false positivity is a known phenomenon with each and every prognostic test. This would be in line with the prognostically false positive rates of 15–20% of bone marrow aspirations in breast, gastric and colon cancer patients, which do not later progress to overt distant clinical metastases, as noted in a study by Lindemann and coauthors.[43]

Role of micrometastatic heterogeneity

A number of researchers have devised different methods of measuring sentinel node tumor burden as a further prognostic factor for survival or non-sentinel node positivity. Thus we may identify the patient populations with non-sentinel node lymph-node metastases in the sentinel node basin, who might benefit from CLND or are unlikely to benefit from such a procedure. As all patients with positive sentinel nodes undergo routine CLND, but only approximately 20% of all sentinel-node positive patients have non-sentinel node metastases, the correct identification of this group could possibly spare approximately 80% of sentinel node-positive patients an unnecessary and morbid CLND.[26]

A number of different factors regarding tumor characteristics and tumor have been tested, with similar or different cut-off values for assessment of these parameters. Table 2 summarizes the literature identified by our review search on this subject. As can be

Table 2 Methods to analyze SN tumor burden and outcomes

Reference	Number of positive SNs	Characteristics	Groups	Survival (%)	CLND positive (%)
Ranieri *et al.*(2002)[83]	90	Maximum diameter	≤3 mm	86 (3 y)	
			>3 mm	27 (3 y)	
Carlson *et al.* (2003)[84]	104	Maximum diameter	Isolated or cluster of melanoma cells	86 (3 y)	
			≤2 mm	90 (3 y)	
			>2 mm	57 (3 y)	
Reeves *et al.* (2003)[62]	98	Maximum diameter (≤ 2 mm or > 2 mm) and ulceration status of the primary	0		0
			1		16
			2		31
Starz *et al.* (2004)[59]	70	Infiltration from the capsule	≤0.3 mm	±80 (5 y)	
			>0.3 ≤ 1.0 mm	±90 (5 y)	
			>1 mm	±60 (5 y)	
Cochran *et al.* (2004)[61]	90	Relative tumor area (% of node involved)	<1%	NA	0
			1–4%		16.7
			≥4%		65.4
Dewar *et al.* (2004)[58]	146	Microanatomic location	Subcapsular	NA	0
			Combined		11
			Parenchymal		19
			Multifocal		37
			Extensive		42
Vuylsteke *et al.* (2005)[60]	80	Maximum diameter (<0.3 mm and ≥ 0.3 mm), Breslow thickness (<2.5 mm and ≥2.5 mm) and non-SN status	0	94 (5 y)	
			1	56 (5 y)	
			2	30 (5 y)	
Sabel *et al.*[63] (2005)	232	ECE and ≥3 positive SNs	ECE	NA	OR 3.2
			≥ 3 positive SNs		OR 65.8
Pearlman *et al.* (2006)[85]	90	Maximum diameter	≤ 2 mm	85 (5 y)	6
			> 2 mm	47 (5 y)	45
van Akkooi *et al.* (2006)[53]	74	Maximum diameter	< 0.1 mm	100 (5 y)	0
			0.1 – 1.0 mm	63 (5 y)	19
			> 1.0 mm	35 (5 y)	
Govindarajan *et al.* (2007)[56]	127	Maximum diameter	≤ 0.2 mm	NA	0
			0.2 – 2.0 mm		10.5
			> 2.0 mm		26.1

Table 2 (continued)

Reference	Number of positive SNs	Characteristics	Groups	Survival (%)	CLND positive (%)
Satzger *et al.* (2007)[64]	101	Capsule invasion, tumor infiltrative depth (<2 mm or ≥2 mm); size of largest tumor deposit (<30 cells or ≥30 cells)	0	±100 (5 y)	NA
			1	±90 (5 y)	
			2	±55 (5 y)	
			3	±20 (5 y)	
Debarbieux *et al.* (2007)[86]	98	Maximum diameter	≤ 2mm	±80 (5 y)	
			> 2 mm	±35 (5 y)	
Roka *et al.* (2008)[65]	85	Maximum diameter (≤2 mm or >2 mm) and ulceration status of the primary	0	NA	12
			1		28
			2		36
Guggenheim *et al.* (2008)[14]	114	Maximum diameter	< 2 mm	NA	16.4
			≥ 2 mm		30.8
Frankel *et al.* (2008)[66]	136	Relative tumor area (% of node involved), ECE and number of positive SNs	≤1 %	NA	9.4
			>1%		32.8
			ECE present		19.2
			ECE absent		66.7
			1 pos SN		16.8
			2 pos SNs		28.6
			3 pos SNs		57.1
Scheri *et al.* (2007)[68]	214	Maximum diameter	≤0.2 mm	87 (5 y)	12
Gershenwald *et al.* (2008)[55]	309	Maximum diameter and tumor square area	≤0.5 mm	NA	5.3
			≤0.1 mm^2		3.7
van Akkooi *et al.* (2008)[54]	388	Maximum diameter and microanatomic location	<0.1 mm	91 (5 y)	3
			0.1–1.0 mm	61 (5 y)	21
			>1.0 mm	51 (5 y)	32
			Subcapsular	NA	8
			Combined		32
			Parenchymal		19
			Multifocal		15
			Extensive		40
van Akkooi *et al.* (2009)[69,70]	663	Maximum diameter	<0.1 mm	93 (5 y)/ 93 (10 y)	6
			0.1–1.0 mm	71 (5 y)/ 58 (10 y)	16
			>1.0 mm	57 (5 y)/ 40 (10 y)	28

Abbreviations: CLND, completion lymph node dissection; ECE, extracapsular extension; NA, not applicable; OR, overall rate; SN, sentinel node; y, year.

observed from Table 2, the number of different characteristics and cut-off values tested has led to a large volume of heterogeneous evidence, which is not easy to interpret.

The most often used characteristic in all these studies has been the maximum diameter of the metastases.[34] Tumor infiltration from the capsule inwards and the microanatomic location are also frequently used characteristics in the documented studies.[35] Other factors have also been investigated, such as Breslow thickness, ulceration of the primary, extracapsular extension or capsule invasion, the square area of the metastases, the number of metastatic foci, the relative area of the metastases and the number of positive sentinel nodes.[30,36,37,40] The main conclusion from these heterogeneous studies is that, either measured with very accurate, sometimes even computer assisted reconstructions, or measured with inaccurate, sometimes very rough, measures, tumor burden is predictive for survival and/or non-sentinel node positivity.

One of the first and most frequently used staging systems for sentinel node tumor burden was developed by Starz and colleagues[37] and was updated a few years later.[37] This system evaluates the infiltration from the capsule inwards. In this system, three subgroups of infiltration were defined (≤0.3 mm, 0.3–1.0 mm and >1.0 mm), and a study using this system showed that only patients with capsule infiltration >1 mm had a significantly worse survival (60%) compared with the other subgroups (80% and 90%, respectively).[33] Limitations of this study were the short median follow-up period and the limited number of patients (70) who were sentinel node positive. Moreover, small parenchymal lesions are difficult to measure using this system, as it can be unclear where the closest capsule is in relation to the metastatic border.

In the same year a study by Cochran *et al*.[37] expressed SN tumor burden as the percentage of the lymph-node area involved by metastases. Patients with <1% of the lymph node involved by metastases did not have any further non-sentinel node involvement, whilst 16.4% of the patients with 1–4% of their sentinel node involved by metastases had non-sentinel node involvement and 65.4% of patients with >4% involved lymph node had further non-sentinel node metastases.[28] The basic rationale of this system is logical. However, the practical everyday use seems doubtful, as the researchers in this study required a complicated computer system to accurately assess this percentage of volume. Without such an expensive system, which most reporting pathologists lack, it could easily deteriorate into a rough estimation of relative involved square area. Moreover, if a single measurement has inter-observer variability, square-area measurements have an even larger inter-observer variability, as this measurement requires two dimensions (X and Y). Moreover, it is unclear how best to measure curved lesions and how to measure multiple lesions, which is also time-consuming and inaccurate.

Dewar *et al*.[57] were the first to analyze the microanatomic location of the metastases and concluded that patients with subcapsular metastases, which accounted for 26% of all metastases did not have any non-sentinel node metastases. However, the study was

scrutinized for the difficulty in establishing the microanatomic location. Moreover, the mean infiltration of the subcapsular group was merely 0.18 mm, which reflects the small metastases that form this subcapsular group.

A study from our group has evaluated three staging systems; the Starz classification, the microanatomic location according to Dewar and our own Rotterdam Criteria for sentinel node tumor burden, which simply reports the maximum diameter of the largest lesion. In 77 patients, 16 patients had limited metastases of <0.1 mm in maximum diameter.[28] These patients have similar patient and primary tumor factors as sentinel node-negative patients.[28] Moreover, none of these patients with <0.1 mm metastases had additional non-sentinel node positivity and the survival rate was virtually identical to sentinel-node-negative patients[28] (Figure 1). The Rotterdam Criteria proved to be a more accurate prognostic factor than the Starz classification or the microanatomic classification in our series.[44] The study was, however, scrutinized for the limited sample size and short follow-up (mean of 35 months).

Govindarajan and coauthors[55] also assessed the maximum diameter as a measure for sentinel node tumor burden, only with a different cut-off value of <0.2 mm compared to <0.1 mm used in our study.[31] None of the 13 patients (of a total of 127) with metastases <0.2 mm had non-sentinel node positivity in the CLND specimen.[31] This study was also scrutinized for the limited sample size and short follow-up (median of 31.2 months).

The findings from the study by Scheri and coauthors, however, did not confirm these results. In this study patients with minimal sentinel node tumor burden (defined as <0.2 mm in maximum diameter) were compared to a cohort of patients with negative

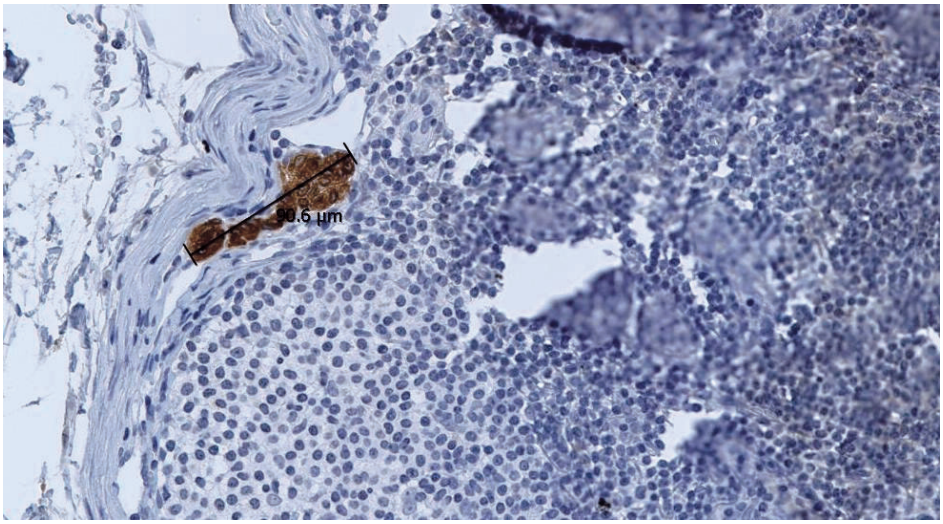

Figure 1 Histopathology picture of a sub-micrometastasis (< 0.1 mm) as defined by the Rotterdam Criteria for SN tumor burden

sentinel nodes.[44] Patients with metastases <0.2 mm had a significantly worse 5-year overall survival compared with patients with negative sentinel nodes (87% versus 94%, *P*=0.02).[44]Patients with metastases <0.2 mm also were at considerable risk for further non-sentinel node metastases with a CLND positivity rate of 12% reported for these patients.[44] There was also debate on the results of this study, as patients from the early sentinel node era (that is, 1990s whereby protocols were less exact and might have led to an underestimation of SN tumor burden) were also included, which raised questions on the adequacy of the extent of the pathology protocol used for these nodes.[32] Moreover, the patients with a negative sentinel node had an extremely good survival of 94%, which is much higher than usually observed for such patients.[32] This result could be related to the very low median Breslow thickness of the sentinel-node-negative patient population, which was merely 1.2 mm compared with a 1.8 mm Breslow thickness, which was more common for node negative patients from the MSLT-1 trial.[32]

Finally, in the largest series to date of 388 sentinel-node positive patients, the EORTC Melanoma Group cooperation study demonstrated that sentinel node tumor burden—according to the Rotterdam Criteria—is the most important prognostic factor for survival.[45,46] This study independently validated the results from a previous single center experience in a large multicenter fashion. Patients with metastases <0.1 mm had similar prognosis to patients with negative sentinel nodes; moreover, the CLND positivity rate of the patients with metastases was merely 3%, which is identical to false-negative rates reported for the sentinel node procedure.[45,46] The study also evaluated the <0.2 mm cut-off value and it was concluded that although the survival of patients with <0.2 mm did not differ significantly from patients with negative sentinel nodes (89%), the CLND positivity rate was significantly higher at 10%.[45,46]

Recently, a study by Murali *et al*.[47] evaluated the inter-observer agreement of different sentinel node tumor burden staging systems. For this study, seven experienced pathologists reviewed the slides of 44 patients. The study concluded that quantitative parameters were highly reproducible between observers. Location and extracapsular spread were less reproducible. At the same time a study by the EORTC Melanoma Group has extensively dealt with practical difficulties in the measurement of sentinel node tumor burden.[48] Based on the results of this study The EORTC Melanoma Group has recommended the use of the Rotterdam Criteria for the measurement of sentinel tumor burden as the maximum diameter of the largest lesion. It is our experience that the measurement of the maximum diameter of the largest lesion is the easiest and best reproducible characteristic to measure SN tumor burden. This has lead us to propagate the Rotterdam Criteria for SN tumor burden as the simplest prognostic factor[49].

All these aforementioned retrospective studies have demonstrated the prognostic value of sentinel node tumor burden. These studies, however, have not answered some crucial questions and perhaps have even raised some new questions. Outstanding

questions that need to be addressed include: Would these tiny micrometastases have progressed to palpable clinical disease? Since the CLND was performed in all patients, and in those with small-volume disease, was the CLND curative or unnecessary for these patients? All these studies are retrospective studies, most of them with limited follow-up periods, what will happen if follow-up matures? If the sentinel node was excised to determine the minimal sentinel node tumor burden status, could the sentinel node procedure have been curative in these patients?

A recent study by de Boer *et al*.[50] in patients with breast cancer has demonstrated that the prognosis of patients with isolated tumor cells in the sentinel node is significantly worse compared to sentinel-node-negative patients. Moreover, patients with isolated tumor cells in the sentinel node benefited from adjuvant therapy.[50] Therefore, it seems we have not yet identified the target patient population that might benefit from CLND in melanoma. Prospective trials are necessary to address this issue.

Currently, the MSLT-2 trial is investigating this issue. In this study, SN positive patients will be randomized to undergo a CLND or to observation. This study is currently accruing patients. The EORTC Melanoma Group is currently conducting its own registration study (MINITUB) to further address this issue. Patients with positive sentinel nodes and minimal tumor burden will be offered to not undergo a CLND, but be followed-up through regular ultrasound examinations.

ULTRASOUND AS AN EMERGING STAGING TOOL

Alternative staging procedures to the sentinel node procedure are also being investigated. These new avenues include RT-PCR, PET-CT, MRI scanning amongst others. Ultrasound (US)-guided fine-needle aspiration cytology (FNAC) is a commonly used staging tool for other types of cancer, such as breast cancer or thyroid cancer. Yet initial studies of US-guided-FNAC were disappointing, as sensitivity rates of around 30% were reported in patients with melanoma.[51-53] A cut-off value for detection of metastases using US was approximately 4.5 mm in the maximum diameter. However, most sentinel node metastases are smaller than 4.5 mm and therefore the authors concluded that this technique was unlikely to be cost-effective in the pre-sentinel node setting.

However, recently studies from our group introduced a new set of morphology criteria for the detection of sentinel node metastases in melanoma detected by US, which differ from the patterns used in breast or thyroid cancer. Peripheral perfusion is the single new criterion, which seems to be responsible for this staggering increase in early metastatic detection by US-guided FNAC. Moreover, the more frequent use of FNAC and overnight reporting of the cytology has made this an attractive procedure[54,55].

Table 3 Nodal metastasis rate and rate of increase according to time for sentinel node (SN) and observation (OBS) arms of the Multicenter Selective Lymphadenectomy Trial-1 (MSLT-1)

	SN-arm	Increase	OBS-arm	Increase	Difference
0 years	15.6%	15.6%	0%		100%
3 years	18.9% (± 1.4%)	3.3% 1.1%/yr	13.6% (± 1.6%)	13.6% 4.4% / yr	5.3%
5 years	19.4% (± 1.4%)	0.5% 0.25%/yr	16.1% (± 1.7%)	2.5% 1.25% / yr	3.3%
7 years	19.6% (± 1.5%)	0.2% 0.1%/yr	17.0% (± 1.7%)	0.9% 0.45%/yr	2.6%
10 years	20.8%	1.2% 0.4%/yr	20.5%	3.5% 1.2%/yr	0.3%

From: Morton, 6th Biannual International Sentinel Node Society meeting, Sydney 2008
Increase in SN arm, years 5–10: 1.4% = 0.3%/year
Increase in OBS arm, years 5–10: 2.9% = 0.6%/year

Ultrasound-guided-FNAC might thus be a very promising tool, as was demonstrated in these studies whereby 65% of all sentinel-node positive patients could have been identified prior to the surgical excision of the sentinel node. This translated into 13% for the entire stage I–II melanoma patient population, which had a 20% sentinel-node positivity rate.[55] Therefore only 7% of all patients were incorrectly not identified by US-FNAC and the possible implications of this false-negative rate are currently under debate, but it might be a cost-effective alternative to the current sentinel-node staging approach.[56]

The possibility of US-guided FNAC as alternative became more current in light of a recent discussion in the Annals of Surgical Onolcogy[57,58], the discussion focuses on a possible survival benefit for patients treated by sentinel lymph node biopsy (SNLB) procedure compared with observation (OBS) and the potential cost-effectiveness of SNLB in the light of such a supposed survival benefit, based on the third interim results of the prospective Multicenter Selective Lymphadenectomy Trial-1 (MSLT-1)[4].

Interestingly, the discussion also focuses on the nodal relapse rates for both arms of the MSLT-1 trail (Table 3). Strikingly, there seems to be an increase in late relapses in both arms, which might either be the result of selection bias, as follow-up has not yet matured to 10 years in the entire MSLT-1 population and thus might lead to an overestimation of the data, or these continuous late relapses in both arms may indicate a failure rate of completion lymph node dissection (CLND) completeness. Interestingly we did not see any late relapses in our submicrometastases (<0.1 mm) patients[28,32].

In light of the lack of survival benefit for the sentinel node (SN) procedure from the point of randomization in the MSLT-1 trial, the cost-effectiveness of the SN procedure as a staging procedure is debatable. Recently a study by Voit et al. demonstrated that presurgical ultrasound (US)-guided fine-needle aspiration cytology (FNAC) has a sensitivity of 65% compared with surgical SN procedure[55]. Moreover, the sensitivity of US-guided FNAC increases significantly with increasing SN tumor burden[55].

Considering that only 15–30% of all stage I/II melanoma patients are SN positive, 70–85% are negative but still undergo a SN procedure[4]. US-guided FNAC has the potential to save 65–80% of SN-positive patients a SN staging procedure and to save an estimated 61–92% of SN-negative patients a surgical procedure, and the accompanying costs. Here we would like to submit the argument for ultrasound-guided FNAC as a cost-effective alternative scenario to the surgical SN procedure.

For the purpose of these calculations, based on the data by Voit et al., we considered that 40% will undergo an US with FNAC whereas 60% will have a benign US and will not undergo a FNAC[55]. Moreover, 50% will be FNAC positive and 50% will be FNAC negative. Finally, the negative patients will undergo routine US follow-up (four times a year), with an average of one FNAC.

At our centers in The Netherlands and Germany, an SN procedure and 1 day of hospital stay would cost an average of €1254.83. Thus, for a scenario of 100 stage I/II melanoma patients, the total cost would be €125,483. Moreover, these calculations do not take into account the time spent in the operating theatre, which could be used for other patients, and the strain on the waiting list for operations.

For the US-guided FNAC scenario, an average ultrasound exam would cost €58.99 (without FNAC) and a US-guided FNAC would cost an average of €168.61 at our centers. Figure 2 shows the flowchart of US-guided-FNAC and the sum of the costs. Total costs of US-guided FNAC as an alternative would be €10,283.80 + €45,831.40 = €56,115.20. This

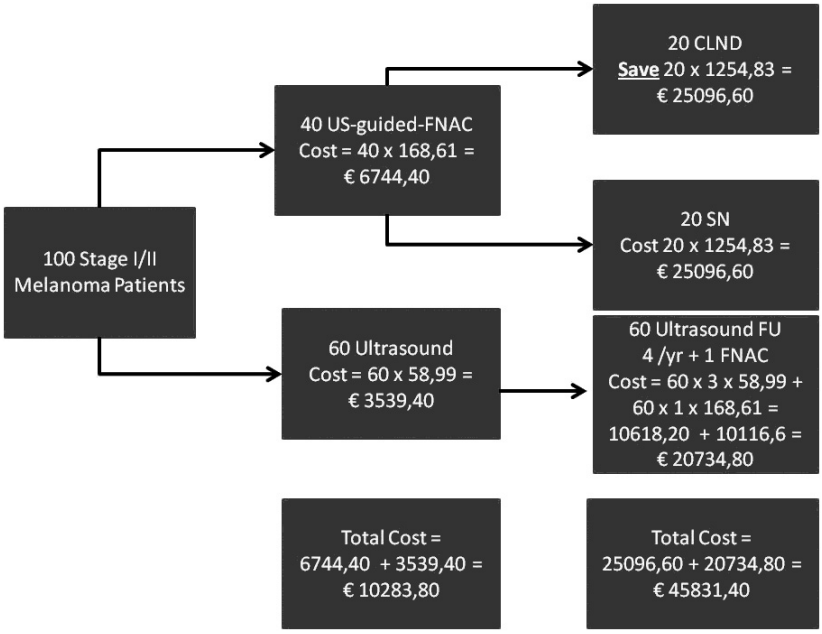

Figure 2

is considerably lower than the €125,483 for 100 SN procedures, corresponding to a cost reduction of 55%.

In reality the savings will be higher as ultrasound is increasingly used for the follow-up of melanoma patients, even after a negative SN or after a CLND for a positive SN. At present, these patients undergo in an increasing number of centers an ultrasound exam twice a year for 5–10 years. This would lead to costs up to 2 x 5 x 100 x €58.99 = €58,990 (without any FNAC over the entire follow-up period). Thus the potential saving might even be much higher, approximately 70%.

Thus, US-guided FNAC emerges as an alternative and cost-effective staging procedure compared with surgical SN, with the potential to save up to €69,367.80 (>50%) at our centers, plus the obvious benefits such as saving patients unnecessary surgery, morbidity, operation theatre time, and reducing the strain on the waiting list for operations.

Therefore there is a need to reproduce the results from the study by Voit et al. in a prospective multicenter fashion, to establish the value of US-guided FNAC, before this staging approach can be recommended.

CONCLUSIONS

The SLNB is a very accurate staging procedure for stage I–II melanoma patients. In spite of reasonable false-negative rates (9–21%), the sensitivity and specificity is good. The sentinel node status is an important prognostic factor for CLND positivity, DFS and overall survival. The sentinel node procedure followed by immediate CLND does not seem to improve survival, although further studies are needed to confirm or refute that it might be beneficial for a subset of patients. The final results of MSLT-1 are still pending. Sentinel node tumor burden determines survival and the need for CLND. It is not yet certain what the clinical implications are of sentinel node tumor burden, specifically minimal sentinel node tumor burden. It seems that patients with sentinel node micrometastases <0.1mm have a clinical course that is indistinguishable from sentinel-node-negative patients and that routine CLND may not be indicated in these patients. The EORTC Melanoma Group recommends the use of the Rotterdam Criteria for the measurement of sentinel node tumor burden. The MSLT-2 and the EORTC Melanoma Group MINITUB studies are currently evaluating the significance of sentinel node tumor burden and the need for CLND. Sentinel node staging is the most accurate staging in melanoma patients, but future staging approaches might include a role for US-guided FNAC.

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Samenvatting

Hoofdstuk 1 is een inleiding tot dit proefschrift. In **Deel I** van dit proefschrift wordt het belang van de hoeveelheid tumor in de schildwachtklier (SWK) van melanoom patiënten onderzocht. In **Hoofdstuk 2** rapporteerden wij het hoge percentage SWK positieve patiënten (29%) met behulp van het European Organization for Research and Treatment of Cancer (EORTC) Melanoom Groep (MG) protocol voor de pathologische bewerking van een SWK in 262 melanoom patiënten, die werden behandeld in het Erasmus MC – Daniel den Hoed. De SWK status is de belangrijkste prognostische factor voor (ziekte-vrije) overleving. Het percentage in-transit metastasen was gecorreleerd aan SWK status, Breslow dikte en ulceratie status van de primaire tumor. Het verrichten van een SWK procedure leidde niet tot het optreden van meer in-transit metastasen.

Hoofdstuk 3 beschrijft de EORTC MG methoden voor het opmeten van tumor hoeveelheid en microanatomische locatie van de metastasen in de SWK. De EORTC MG beveelt de volgende zaken aan: gebruik van het EORTC MG protocol voor de pathologie van de SWK of een gebruik van een even extensief protocol, die ook bewezen accuraat is. Alleen opmeten wat je ziet, niet wat je vermoed. Cumulatieve metingen zijn minder accuraat en slechter reproduceerbaar dan een enkele meting. De maximale diameter van de grootste laesie (1-D), ongeacht welke richting, is het beste reproduceerbaar. Als het parenchym betrokken is bij een laesie, dan kan de laesie niet meer als subcapsulair beschouwd worden. Rapporteren van de microanatomische locatie van een metastase dient een reflectie te zijn van de gehele lymfklier en niet alleen van de grootste laesie. Een multifocaal patroon is een verstrooid patroon, niet te verwarren met multipele gebonden laesies, die onder het normale systeem voor microanatomische locatie vallen. Een subcapsulaire laesie dient een gladde rand te hebben, niet irregulair of gekarteld.

In **Hoofdstuk 4** hebben we allereerst onze eigen ervaring en uitkomsten van SWK tumor hoeveelheid onderzocht in 77 SWK positieve patiënten uit het Erasmus MC – Daniel den Hoed. Sub-micrometastasen (< 0.1 mm) werden gezien in 16 uit de 74 patiënten (22%), waarvan het materiaal beschikbaar was voor herbeoordeling. 5 jaar overleving voor de groepen tumor hoeveelheid <0.1 mm, 0.1 – 1.0 mm en > 1.0 mm was 100%, 63% en 35%, respectievelijk. Afstandsmetastasen werden zeer zeldzaam gezien in de groep patiënten met metastases < 0.1 mm (1/16 = 6.3%). Multivariaat analyse toonde aan dat de SWK tumor grootte de belangrijkste prognostische factor was voor ziektevrije (P=0.005) en totale overleving (P=0.03). Dit leidde ertoe dat wij stelden dat patiënten met metastases <0.1 mm een klinisch beloop hebben, die niet te onderscheiden is van SWK negatieve patiënten.

Het heeft ertoe geleid dat we de invloed van minimale SWK tumor grootte op een potentieel overlevingswinst voor de SWK procedure gevolgd door een vroege com-

pleterende lymfklier dissectie (CLKD) versus patiënten, die geen SWK ondergingen, maar lymfkliermetastasen ontwikkelde gedurende follow-up, die een therapeutische lymfklier dissectie (TLKD) nodig hadden. Dit wordt beschreven in **Hoofdstuk 5**. Overleving werd in beide groepen berekend vanaf de datum van de excisie van de primaire tumor. Alle patiënten hadden een melanoom op de ledematen of de romp. Er waren 188 patiënten, 124 TLKD ('82 – '05) and 64 CLKD patiënten ('97 – '05). Mediane follow-up was respectievelijk 56 en 37 maanden. Er waren geen significante verschillen tussen beide groepen met betrekking tot de Breslow dikte, percentage geulcereerde primaire tumoren, geslacht of locatie van de primaire tumor. Univariate analyse toonde aan dat locatie (ledemaat versus romp) (P<0.001), Breslow dikte (P=0.005) en ulceratie (P<0.001) significante prognostische factoren waren voor overleving. Er was een 13% verschil in overleving, die niet statistische significant was, tussen de CLKD en TLKD groepen (P=0.12). Als patiënten met sub-micrometastases (n=15) werden geëxcludeerd van de analyse, verdween de overlevingswinst, het verschil werd slechts 6% (P=0.42). Derhalve concludeerden wij dat er geen overlevingswinst was voor het verrichten van een SWK procedure, gevolgd door CLKD in geval van een positieve SWK in vergelijking met TLKD, zeker wanneer patiënten met sub-micrometastasen buiten beschouwing werden gelaten.

We hebben onze resultaten gevalideerd in een multicenter studie in **Hoofdstuk 6**. De mediane Breslow dikte in 388 SWK positieve patiënten uit 3 EORTC MG centra was 4.00 mm, ulceratie werd gezien in 56% van de patiënten. 40 patiënten (10%) hadden sub-micrometastasen < 0.1 mm. Slechts 1 patiënt had een positieve CLKD uit deze 40 patiënten (3%). Met een gemiddelde follow-up van 41 maanden was de 5-jaars overleving respectievelijk 91%, 61% en 51% voor patiënten met metastasen <0.1 mm, 0.1 – 1.0 mm en > 1.0 mm (P<0.001). SWK tumor grootte nam significant toe met de toename van de Breslow dikte. Als we < 0.2 mm als afkapwaarde voor een sub-micrometastase namen (gelijk aan de situatie bij borstkanker), was de overleving 89%, maar had 10% een positieve CLKD.

Tot slot warden deze resultaten van SWK tumor grootte, inclusief de microanatomische locatie geanalyseerd en gevalideerd in 1080 SWK positieve patiënten uit 10 EORTC MG centra, in **Hoofdstuk 7**. Tussen 1993 en 2008 werden er 1080 patiënten (509 vrouwen, 571 mannen) gediagnosticeerd met een positieve SWK. 1009 patiënten (93%) onderging een CLKD. Mediane Breslow dikte was 3.00 mm en de mediane follow-up was 37 maanden. We bevestigden dat patiënten met sub-micrometastasen (Rotterdam Criteria < 0.1 mm) een uitstekende 5-jaars overleving hadden van 91%. Tevens hadden deze patiënten een laag percentage positieve CLKD's van 9%. De belangrijkste prognostische factor was de RDC (Rotterdam-Dewar Combined) Criteria. Patiënten met sub-micrometastasen, die subcapsulair waren hadden 5- en 10-jaars melanoom specifieke overleving van 95% en slechts in 2% van de patiënten was er sprake van een positieve CLKD.

In **Deel II** wordt een alternatieve stagerings methode onderzocht, namelijk de echo-geleide cytologische punctie. In **Hoofdstuk 8** rapporteerden wij de resultaten van 400 stadium I/II melanoom patiënten, die een echo-geleide cytologische punctie ondergingen. Alle 400 patiënten ondergingen eerst een lymfoscintigrafie. Vervolgens ondergingen zij allemaal een echo onderzoek. Indien het echo onderzoek verdachte of maligne patronen toonde, werd er een cytologische punctie verricht. Mediane Breslow dikte was 1.8 mm, gemiddelde follow-up was 42 maanden (4 – 82 maanden). De techniek van echo-geleide cytologische punctie werd positief beschouwd, indien de echo maligniteit toonde en/of de cytologische punctie positief was. Indien de echo verdachte kenmerken toonde, maar de cytologische punctie negatief was, werd de test als negatief beschouwd. Echo-geleide cytologische punctie identificeerde 51/79 (65%) van SWK metastasen. Specificiteit was 99% (317/321) met een Positief Voorspellende Waarde (PVW) van 93% en een Negatief Voorspellende Waarde (NVW) van 92%. De identificatie steeg van 40% in pT1 tot 79% pT4 melanomen. De techniek identificeerde SWK tumoren groter dan 1 mm in maximum diameter in 86% van de gevallen. Tumoren van 0.1 – 1.0 mm werden in 46% correct geïdentificeerd en tumoren kleiner dan 0.1 mm werden slechts correct geïdentificeerd in 23% van de gevallen. 5-jaars overleving was 92% en 51% voor respectievelijk echo-geleide cytologische punctie negatieve en positieve patiënten.

In **Hoofdstuk 9** analyseren wij de nieuwe morfologische criteria, die gebruikt zijn in het echo onderzoek, die leidden tot deze stijging in diagnostische waarde van de echo-geleide cytologische punctie als alternatieve stagerings procedure. Sensitiviteit en PVW van de belangrijkste factoren was: perifere perfusie (PP) aanwezig 77%, 52%. Verlies van centrale echo (LCE) 60%, 65%. Ballon vormige lymfklier (BS) 30%, 96%. Gezamenlijk hadden deze factoren een sensitiviteit van 82% en PVW van 52% ($P<0.001$). Perifere perfusie identificeert meer patiënten met beperkte hoeveelheid tumor in de SWK. PP en LCE gecombineerd met BS waren onafhankelijke prognostische factoren voor overleving met een relatief risico van 2.19 ($P<0.015$) en 5.50 ($P<0.001$), respectievelijk. Voorafgaand aan de chirurgische schildwachtklier procedure kan echo-geleide cytologische punctie 65% van patiënten met een SWK metastase identificeren en daarmee de noodzaak van een chirurgische SWK procedure verminderen. Perifere perfusie is een vroeg teken van metastasering in de lymfklier bij melanoom patiënten en is daarmee cruciaal voor een hoog detectie percentage. Ballon vormige lymfklieren en verlies van centrale echo's zijn late tekenen van metastasering. We bevelen het gebruik van echo-geleide cytologische puncties aan om daarmee patiënten te identificeren, die een SWK procedure bespaard kan worden, door meteen een CLKD te ondergaan.

Hoofdstuk 10 beschrijft het gebruik van Reverse Transcription Polymerase Chain Reaction (RT-PCR) om daarmee het identificatie percentage van een positieve SWK te verhogen. Tussen 2001 en 2003 ondergingen 127 melanoom patiënten (mediane

Breslow dikte 2.1 mm) een schildwachtklier procedure. Een echo-geleide cytologische punctie werd verricht in alle patiënten voor en na de SWK procedure. Het materiaal van de puncties werden deels bevroren en deels gebruikt voor standaard cytologisch onderzoek. Perifeer bloed werd afgenomen ten tijde van de SWK procedure en tijdens ieder polikliniek bezoek gedurende de follow-up. 34 van de 120 schildwachtklieren (23%) was positief voor melanoom. Een SWK metastase werd door echo-geleide cytologische punctie met een sensitief van 82% en specificiteit van 72% beoordeeld. Aanvullende tyrosinase RT-PCR toonde dezelfde sensitiviteit (82%) en specificiteit (72%). Er was een mediane follow-up van 40 maanden. RT-PCR van perifeer bloed was een significante prognostische voorspeller van (ziekte-vrije) overleving ($P<0.001$). RT-PCR van cytologische puncties of de geëxcideerde SWK verhoogt de sensitiviteit en specificiteit niet. RT-PCR van perifeer bloed was wel voorspellend voor (ziekte-vrije) overleving.

List of Publications

- *van der Ploeg APT,* ***van Akkooi ACJ,*** *Rutkowski P, Nowecki ZI, Michej W, Mitra A, Newton-Bishop JA, Cook M, van der Ploeg IM, Nieweg OE, van den Hout MF, van Leeuwen PA, Voit CA, Cataldo F, Testori A, Robert C, Hoekstra HJ, Verhoef C, Spatz A, and Eggermont AMM.*
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Curriculum Vitae

CURRICULUM VITAE

Alexander Christopher Jonathan van Akkooi, the author of this thesis, was born on the 16th of April 1981 in Bridgeport, Connecticut, USA. He attended high school at the Erasmiaans Gymnasium in Rotterdam, the Netherlands, finishing in 1999. He continued to study Medicine at the Erasmus University Rotterdam the same year. During this period he started to work as a ward assistant at the Department of Surgical Oncology of the Erasmus University Medical Center – Daniel den Hoed Cancer Center. This was also the location, where he commenced with his first scientific work, when he started work on his Master thesis. After completing his Master thesis in 2005, he stayed at the department of surgical oncology of the Erasmus University Medical Center – Daniel den Hoed Cancer Center to continue work on this PhD thesis, under supervision of Prof. Dr. A.M.M. Eggermont and Dr. J.H.W. de Wilt. He continued this work, whilst completing his internships and achieving the title of MD (Medical Doctor). On the 1st of January 2009 he started work as a surgical resident in training for surgeon at the Maasstad Hospital, Rotterdam (Education Head Dr. E. van der Harst). In the period of working on this PhD thesis he has become a member of the Steering Committee of the European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group (MG) and is the Prinicipal Investigator of the EORTC MG prospective study, MINITUB.

CURRICULUM VITAE

Alexander Christopher Jonathan van Akkooi, de auteur van dit proefschrift, werd geboren op de 16de van April 1981, in Bridgeport, Connecticut, Verenigde Staten van Amerika. In 1999 behaalde hij zijn VWO diploma aan het Erasmiaans Gymnasium in Rotterdam. Het zelfde jaar begon hij zijn studie geneeskunde aan de Erasmus Universiteit Rotterdam. Tijdens deze studie begon hij, bij wijze van bijbaan, te werken als afdelings assistent op de afdeling chirurgische oncologie van het Erasmus MC – Daniel den Hoed. Hier zette hij ook zijn eerste stappen in een academische carrière door zijn afstudeeronderzoek alhier te verrichten. Na zijn afstudeeronderzoek voltooid te hebben, bleef hij werkzaam op deze afdeling om verder te gaan met het eerste werk in het kader van dit proefschrift, hetgeen gesuperviseerd werd door Prof. Dr. A.M.M. Eggermont en Dr. J.H.W. de Wilt. Hij ging door met het werken aan zijn proefschrift, terwijl hij zijn co-schappen voltooide om zodoende de graad van doctorandus (drs.) in de geneeskunde en derhalve arts te worden. Op 1 Januari 2009 startte hij met zijn opleiding tot chirurg, als assistent chirurgie in het Maasstad Ziekenhuis, Rotterdam (opleider Dr. E. van der Harst). Gedurende de periode, die hij besteedde aan het werken aan dit proefschrift, is hij lid geworden van het Bestuur van de European Organization for Research and Treatment of Cancer (EORTC) Melanoom Groep (MG) en is hij de hoofd verantwoordelijke voor de prospectieve EORTC MG studie, de MINITUB.