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

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ABSTRACT

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

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

1. Introduction

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

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diagnostic procedure for patients with clinically negative lymph nodes.

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

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

2. Patients and methods

2.1. Patients

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

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

All patients underwent therapeutic re-excision of the melanoma before the SN procedure according to National Guidelines. Tumour-free margins of at least 1 cm were achieved in melanomas smaller than or equal to 2 mm Breslow thickness. Melanomas larger than 2 mm in Breslow thickness were excised with a tumour-free margin of at least 2 cm or less for distal acral and head and neck primaries for feasibility limits or cosmetic reasons. Finally, the defected areas were closed via primary closure or split skin graft. At the same time as wide local excision of the malignant melanoma the SN procedure was performed.

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

Table 1 – Patient characteristics for all 421 patients.

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

SSM, superficial spreading melanoma; NM, nodular melanoma; ALM, acrolentiginous melanoma; SN, sentinel node, NSN, non-sentinel node.

2.2. Sentinel lymph node procedure

At our centre the SN is identified by the use of the triple technique, described in detail elsewhere.[4] Basically, patients are first seen at the nuclear medicine department for a pre-operative lymphoscintigraphy (LS). The LS should be undertaken within 24 h of the operation being performed, by four intradermal injections of radioactive nanocolloid around the primary tumour or the scar of the primary tumour excision. Scanning should be carried out immediately after the injection for approximately 10–15 min and again (= delayed) after 2 h.

Secondly, intraoperative use of a handheld gamma detection probe should be used to verify the location of the SNs.

Thirdly, patent blue should be injected pre-operatively in the operating theatre, again through four intradermal injections around the primary tumour or the scar of the primary tumour excision (this does not have to be the same 4 locations). The blue is also used to verify the identity of the SNs. A lymph node was considered to be a SN if it was stained blue, or if it had an in situ radioactivity count of at least three times that of the background count, or if it had an *ex vivo* radioactivity count of at least ten times greater than that of the background count.

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

Multivariate	DFS			OS		
	HR	95% CI	*P*	HR	95% CI	*P*
Gender						
Female	1			1		
Male	1.31	0.90–1.90	0.16	1.73	1.09–2.76	0.02
Age						
⩽50 years	1			1		
>50 years	1.31	0.90–1.91	0.15	1.32	0.83–2.09	0.24
Location						
Extremities	1			1		
Head and neck/trunk	1.53	1.05–2.23	0.03	1.9	1.19–3.03	0.006
Histology						
SSM	1			1		
NM	1.7	1.13–2.55	0.01	2.03	1.24–3.32	0.004
Breslow						
⩽2.00 mm	1			1		
2.01 to ⩽4.00 mm	1.91	1.20–3.05		1.87	1.05–3.34	
>4.00 mm	3.53	2.18–5.70	<0.00005	3.59	2.00–6.46	<0.00005
Clark						
2, 3	1			1		
4	1.13	0.74–1.72		0.85	0.51–1.41	
5	2.8	1.49–5.28	0.003	2.23	1.02–4.91	0.044
Ulceration						
Absent	1			1		
Present	2.49	1.71–3.64	<0.00005	3.4	2.14–5.39	<0.00005
SN status						
Negative	1			1		
Positive	3.75	2.57–5.47	<0.00005	3.64	2.29–5.77	<0.00005
NSN status						
Negative	1			1		
Positive	6.43	3.90–10.60	<0.00005	3.92	2.11–7.30	<0.00005
Rotterdam Criteria						
Negative	1			1		
<0.1	1.06	0.38–2.93		0.82	0.20–3.42	
0.1–1.0	3.9	2.45–6.22	<0.00005	3.47	2.00–6.01	
>1.0	6.86	3.99–11.82		6.62	3.69–11.87	<0.00005
Rotterdam Criteria						
Negative, <0.1	1			1		
0.1–1.0	3.83	2.44–5.99		3.58	2.08–6.18	
>1.0	7.21	4.45–11.68	<0.00005	7.29	4.08–13.02	<0.00005

SSM, superficial spreading melanoma; NM, nodular melanoma; SN, sentinel node; NSN, non-sentinel node.

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

2.3. *Pathological features*

All SNs were worked up according to the EORTC MG protocol.[5] Within 24 h lymph nodes are placed in formalin. After fixation the SN will be bivalved through the hilum. Each half sentinel node will be examined in six serial step sections cut at 50 μm intervals. All sections are stained with H&E and S100 and/or Melan A. Spare sections are made at each level for a number of difficult cases where additional immunochemistry is needed. Two individual specialised pathologists at our institute worked up all SNs. Tumour burden was scored according to the Rotterdam Criteria (<0.1 mm, 0.1–1.0 mm and >1.0 mm largest diameter of the largest metastasis in the SN).[6]

2.4. *Follow-up*

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

2.5. Statistics

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

Analyses were executed with the following variables: sex (female or male), age (⩽50 or >50 years), location of the melanoma (extremities or trunk/head and neck), histology of the melanoma (superficial spreading melanoma (SSM) or nodular melanoma (NM)), Breslow thickness (⩽2.0 mm, 2.01 to ⩽4.0 mm or >4.0 mm), Clark level (II and III, IV or V), ulceration (absent or present), SN status (positive or negative), NSN status (no additional nodes or additional nodes) and Rotterdam Criteria (negative and <0.1 mm, 0.1–1.0 mm or >1.0 mm).

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

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

3. Results

3.1. Patient characteristics and SN status

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

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

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

Multivariate	DFS			OS		
	HR	95% CI	P	HR	95% CI	P
Location						
Extremities				1		
Head and neck/trunk				2.95	1.73–5.03	<0.00005
Histology						
SSM				1		
NM				1.86	1.07–3.26	0.03
Ulceration						
Absent	1			1		
Present	2.23	1.49–3.33	<0.0005	2.76	1.55–4.90	0.001
NSN status						
Negative	1			1		
Positive	4.8	2.75–8.35	<0.0005	4.29	2.13–8.65	<0.0005
Rotterdam Criteria						
Negative, <0.1	1			1		
0.1–1.0	2.63	1.63–4.24		2.2	1.18–4.11	
>1.0	5	2.75–8.35	<0.0005	4.27	2.27–8.02	<0.0005

SSM, superficial spreading melanoma; NM, nodular melanoma; NSN, non-sentinel node.

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

	SN positivity rate	# Patients SN positive	NSN positivity rate	# Patients with additional NSN tumour after positive SN(s)
Our institute	28.70%	121 (0.287 * 421)	11.20%	14 (0.112 * 121)
Other literature	20% (13.9%–29.4%)	84 (0.2 * 421) (58.5–123.8) (0.139 * 421–0.294 * 421)	20% (14–28%)	17 (0.2 * 84) (8.2–34.7) (0.14 * 58.5–0.28 * 123.8)

SN, sentinel node; NSN, non-sentinel node.

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

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

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

3.2. *Survival*

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

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

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

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

The 5 year estimated OS rates for patients with Rotterdam Criteria divided into three categories, namely, <0.1 mm, 0.1–1.0 mm and >1.0 mm, were, respectively, 91%, 65% and 36% ($p = 0.002$). The 5-year estimated OS rate was 90% for SN-negative patients (Fig. 2).

3.3. *Prognostic factors*

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

Table 4 shows the result of the multivariate proportional hazard regression analysis. The Rotterdam Criteria, ulceration and NSN status had an independent significant influence on both DFS and OS. The location and histology of the primary melanoma had a significant influence on OS.

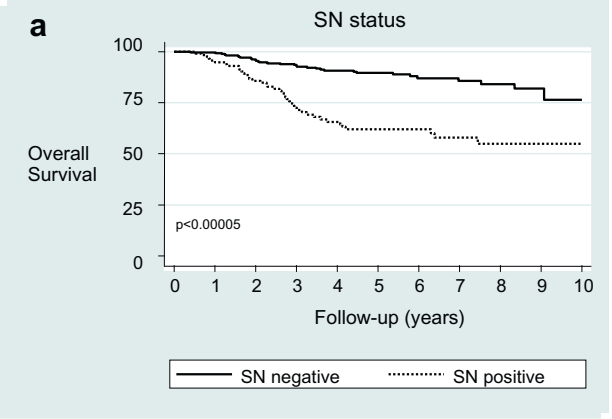

Fig. 1 – Kaplan–Meier estimated 10-year overall (a) and disease-free (b) survival curves for SN status.

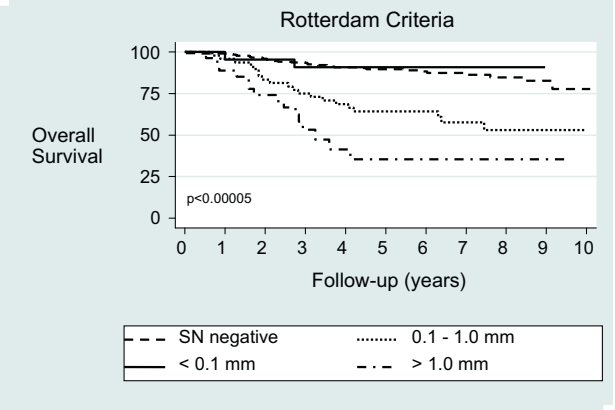

Fig. 2 – Kaplan–Meier estimated 10-year overall survival curves for the Rotterdam Criteria.

For the purpose of multivariate analysis, SN negative and tumour burden <0.1 mm were grouped as one, because in the univariate analysis they had a virtually identical outcome.

4. Discussion

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

This study identified a high SN positivity rate of 28.7% (121/421). SN positivity rates in the literature differ from 13.9% to 29.4% with median Breslow thicknesses from 1.1 to 3.0 mm.[2–4,7–28] A previous report from our centre identified the highest rate of SN-positive patients.[4] The factors ulceration rate, mean and median Breslow thicknesses, false negative rates, survival rates and number of patients included do not seem to be correlated with the SN-positive rates. For example, the current study has a lower ulceration rate, lower mean and median tumour thicknesses than several other studies, yet our SN positivity rate is higher.[11,20,22]

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

The sensitivity of the EORTC MG SN pathology protocol is apparent, because the current study demonstrated that 65% of SN-positive patients have metastases <1.0 mm and 18% has metastases <0.1 mm in maximum diameter according to the Rotterdam Criteria.[30] (Table 1) 7 patients with minimal SN tumour burden have not undergone a CLND, yet none of these has developed a recurrence or died due to melanoma. Furthermore, none of the 15 patients with submicrometastases who underwent a CLND showed non-SN positive lymph nodes. This indicates that in this group of patients CLND may be forfeited. Although our pathological work-up of the SN is very sensitive, the question remains if all detected tumour cells are of clinical importance. These are merely preliminary results; further prospective studies on SN tumour burden are currently ongoing to examine the clinical relevance of minimal SN tumour burden, such as the EORTC MG MINITUB registry study or the randomised phase III MSLT-2 trial.[31,32]

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

The 5-year overall survival (OS) rates of the present study are 90% and 62% for SN-negative and SN-positive patients, respectively. These rates are comparable with many other studies showing OS rates from 87.5% to 94% for SN-negative patients and rates from 42.9% to 75.4% for SN-positive patients.[2–4,10–12,15,17–20,23,26,27] The 5-year disease-free survival (DFS) rates of this study are 82% among those with a negative SN procedure and 51% among those with a positive SN procedure. Both survival rates are comparable with several other studies showing rates from 75.9% to 89.1% for SN-negative patients and rates from 35.2% to 65% for SN-positive patients.[2,4,9,12,15,18,19,23,25,26] Compared to our previous report, the OS and DFS rates of SN-negative and SN-positive patients have only changed marginally, due to the increased follow-up with more events. Interestingly, the high SN positivity rate with the detection of increasingly more patients with early metastatic disease has not improved the survival rates for SN-positive or for SN-negative patients, when compared to other studies. This might suggest a lack of therapeutic benefit of undergoing a SN followed by early CLND.

A benefit of the SN is that it is a minimally invasive procedure with a low complication rate compared to the morbidity and expense of a lymph node dissection.[34–37] It spares SN-negative patients an unnecessary CLND. However, only 14–28% of the SN-positive patients undergoing a CLND of the regional nodes have positive non-SNs.[38] Thus, approximately 80% of the SN-positive patients will have undergone an unnecessary operation with the possible risks of known complications and morbidity, such as wound infections and lymph oedema.[34,39] The current study shows an additional positive nodal rate in the CLND specimen of 11.2% (12/107), which is quite low compared to the literature and our previous report, which demonstrated a CLND positivity rate of 14.7% (10/68). The clinical procedure regarding a CLND has not changed in recent years. Our hypothesis is that our high sensitive pathology model could explain our low CLND positivity rate. Higher detection rate of SN minimal tumour burden correlates with more negative nodes in the CLND specimen. Moreover, our SN positivity rate is higher than others in the literature; therefore our CLND positivity rate is relatively lower than others in the literature. Yet, in absolute numbers the amount is equal (Table 4).

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

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

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

Conflict of interest statement

None declared.

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