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SENTINEL LYMPH NODE INVOLVEMENT AND A HIGH BRESLOW INDEX ARE INDEPENDENT FACTORS OF RISK FOR EARLY RELAPSE OF MELANOMA

THESE

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Rapport de synthèse

Le ganglion sentinelle (GS) se défini comme le premier ganglion de la chaîne ganglionnaire qui draine le territoire anatomique où siège une tumeur et, par conséquent, celui ayant le plus de possibilités de recevoir des métastases. La combinaison des deux techniques de détection du GS existantes, lymphoscintigraphie et coloration, permettent de déceler le GS dans 95-100% des cas. Le taux d'attente métastatique du GS varie entre 16 et 21% des patients. Dans 50 à 87% des cas, le GS est le seul site de métastase et la probabilité de trouver des micro-métastases dans des ganglions appartenant aux relais supérieurs sans atteinte du GS est estimée à moins de 2%. Ces chiffres relèvent l'importance de la détection du GS. L'emploi de cette technique offre de nombreux avantages par rapport à la lymphadénéctomie élective que nous décrirons. Selon Rousseau et al., il existe une probable association entre le statut du GS et la survie de la maladie. Cette interprétation et celles d'autres auteurs soulignent la pertinence clinique du statut du GS dans le mélanome. En ce qui concerne la survie sans maladie (DFS) et la survie globale (OS), aucune différence significative n'a été observée entre les patients ayant subi une résection complète immédiate des ganglions lymphatiques et ceux qui d'abord ont subi une résection chirurgicale et analyse du GS secondaire, suivies par une dissection élective en cas de positivité. L'objectif de cette étude prospective était d'évaluer la pertinence de la positivité tumorale du GS dans l'évaluation des risques de rechute du mélanome.

Cette étude a confirmé l'intérêt de la scintigraphie des ganglions lymphatiques (associée à la technique de coloration par bleu et celle de détection par sonde portable) dans l'identification du GS comme approche thérapeutique au stade précoce du mélanome. Elle a montré, en autre, que le statut du GS et l'indice de Breslow sont des facteurs de risque indépendants importants de rechute chez des patients atteints d'un mélanome au stade précoce. La combinaison de ces deux paramètres a permis de créer des groupes de patients à risque de rechute différents qui pourraient conduire à l'adaptation des protocoles de thérapie en fonction de ces risques.

Sentinel lymph node involvement and a high Breslow index are independent factors of risk for early relapse of melanoma

SLN-Befall und hoher Breslow-Index sind unabhängige Risikofaktoren eines Frührezidivs beim Melanom

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Keywords
Sentinel lymph node, melanoma, Breslow index, factor of risk, relapse

Summary
Aim: The clinical relevance of sentinel lymph node (SLN) analysis was evaluated prospectively and compared with other known risk factors of relapse in early stage melanoma. Methods: Surgery was guided by lymphoscintigraphy, blue dye and gamma probe detection. SLN were analysed by haematoxylin eosin (HE) histochemistry and multimarker immunohistochemistry (IHC). Disease free survival (DFS) was evaluated with Kaplan-Meier plots according to different parameters and Cox analyses of variance. Results: From 210 patients a total of 381 SLN were excised. Lymphoscintigraphy identified all excised SLN with only 2 false positive lymphatic lakes. Fifty patients (24%) had tumour positive SLN. With a mean follow-up of 31.3 months, 29 tumour recurrences were observed, 19 (38%) in 50 SLN positive and 10 (6%) in 160 SLN negative patients. Strong predictive factors for early relapse ($p < 0.0005$) were SLN positivity and a high Breslow index. Conclusion: SLN tumour positivity is an independent factor of high risk for early relapse with a higher power of discrimination than the Breslow index.

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Schlüsselwörter
Wächterlymphknoten, Melanom, Breslow-Index, Risikofaktor, Rezidiv

Zusammenfassung
Ziel: Die klinische Bedeutung des Wächterlymphknoten(SLN)-Befalls wurde prospektiv in Bezug auf das Frühauftreten eines Rezidivs analysiert und mit anderen bekannten Risikofaktoren beim Melanom im Frühstadium verglichen. Methode: Nach der Resektion, gestützt auf Szintigraphie, Patentblau V und intraoperative Gamma-Sondenmessung, wurden die SLN mit Hämatoxylin-Eosin(HE)-Färbung und Multimarker-Immunohistochemie (ICH) untersucht. Tumorfreie Überlebenszeit wurde für verschiedene Parameter nach der Kaplan-Meier-Methode und der Cox-Varianzanalyse analysiert. Ergebnisse: Bei 210 Patienten wurden 381 SLN untersucht. Die Szintigraphie identifizierte alle Lymphknoten, mit zwei falsch positiven Ergebnissen. Fünfzig Patienten (24%) wiesen einen Tumorbefall des SLN auf. Bei einer mittleren Beobachtungszeit von 31,3 Monaten wurden 29 Rezidive festgestellt, davon 19 (38%) bei 50 SLN-positiven und 10 (6%) bei 160 SLN-negativen Patienten. Den höchsten prädiktiven Wert ($p < 0,0005$) für ein frühzeitiges Rezidiv wiesen SLN-Befall und Breslow-Index auf. Schlussfolgerung: SLN-Befall ist ein unabhängiger Risikofaktor für ein früh rezidivierendes Melanom mit besserer Diskriminanz als der Breslow-Index.

The application of the sentinel lymph node (SLN) technique to melanoma has been introduced in 1992 by Morton et al. (7) and extended with scintigraphy by Alex et al. in 1993 (1). The combination of lymphoscintigraphy with the blue dye technique allows identifying the SLN in 95 to 100% of the patients. Metastatic SLN have been found in 16 to 21% of patients (5, 7, 9, 10, 12-14). In case of SLN positivity in melanoma, SLN was the unique site of metastasis in 50 to 87% of patients submitted to elective lymphadenectomy. Furthermore, metastases that bypassed the SLN were found in less than 2% of patients (5, 9, 10, 13). These data show the importance of SLN analysis in patients with melanoma. The selective excision of sentinel lymph node(s) offers several advantages compared to elective lymph node dissection: First, the large majority of patients without lymph node disease will not undergo unnecessary lymph node dissection, a procedure connected with a high incidence of complications such as wound infection, seroma formation and lymphoedema. Secondly, the sentinel node can be analysed most carefully using multiple slicing combined with detailed histologic and immunhistologic analysis and reverse transcription-polymerase chain reaction (RT-PCR). Such an extended analysis cannot be applied routinely to the multiple nodes from elective lymph node dissections.

Rousseau et al. (12) showed that stratification of melanoma patients according to the American Joint Committee on Cancer (AJCC) staging criteria revealed an increasing risk of SLN metastases with successive stage groups. According to these authors, the ability of the AJCC staging system to predict survival was likely due to its ability to predict the risk of occult nodal, notably SLN disease, given the significant association of SLN status and survival. This interpretation again highlights the clinical relevance of the SLN status in melanoma.

With regard to disease free survival (DFS) and overall survival (OS), no significant difference was observed between pa-

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tients having undergone immediate complete resection of lymph nodes of the primary basin and patients who first had surgical resection and analysis of the SLN followed by secondary elective lymph node dissection in case of positivity (4).

Only a few studies focus on long-term results of selective sentinel node surgery due to the recent development of the method and the fact that randomised studies are ongoing. The goal of this prospective study was to evaluate the relevance of SLN tumour positivity in relapse risk assessment of melanoma.

Patients, materials, methods

The study was performed in the frame of a SLN protocol conducted by the Groupe Mélanome Lémanique. The study protocol was approved by the ethical commission and patients gave their written, informed consent to participate. Two hundred and ten successive patients entered the study. Patients' characteristics are shown in Table 1. None of the patients presented clinical or radiological evidence of metastatic lymph node disease at the moment of SLN excision. Breslow thickness was comprised between 0.45 and 10.0 mm, four patients could not be classified by this parameter. Histology typing was: superficial spreading in 89 patients among which 15 ulcerative, nodular in 69 patients including 23 ulcerative, acral-lentiginous in 23 patients including six ulcerative. Six patients presented other different histologies and 23 lesions were not classified. Clark indices ranged from II to V, six patients being not classified.

SLN triple detection technique

The SLN triple technique, including preoperative lymphoscintigraphy, blue coloration and per-operative detection with a handheld gamma probe, was applied to all patients. All lymphatic nodes identified by the triple technique were considered SLN and excised.

Lymphoscintigraphy was realised the day before surgery using intradermic injection of ^{99m}Tc-microcolloid (Lymphoscint Solco®, n = 54) or ^{99m}Tc-nanocolloid (Nanocoll®, n = 150) and Nanocis®, n = 6). An activity of 10 MBq per site was injected at two to six points, depending on lesion size. Static imaging of 5 minutes using a matrix of 256 × 256 and further static imaging of 2 minutes (matrix 256 × 256) combined with an external transmission source was realised. The location of the SLN(s) was/were marked in ink on the skin.

The SLN(s) was/were localised peroperatively by radioactivity detection at the labelled site(s) using a portable probe „Neoprobe 1000" (Neoprobe Corp., Dublin, Ohio, USA) equipped with a detector of 11 mm diameter or the „pol.hi.tech. ScintiProbe MR-100" equipped with two detectors of 11 and 18 mm diameter.

One or several slow (2 to 3 minutes) intradermic injections of patent blue (Patent V®) or Evans blue (Evans®) were performed around the melanoma site or the surgical scar 10 to 15 minutes before surgery. The SLN surgery was performed at the site defined the day before using lymphoscintigraphy and the precise localisation determined with the hand held gamma probe and visual detection of the dye.

Tab. 1 Patient's characteristics

		number of patients
total		210
sex	men	107
	women	103
age	mean	53.7
	range	15–79
melanoma localization	upper extremity	39
	lower extremity	74
	trunc	81
	head and neck	16
Breslow thickness (mm)	≤1.49	72
	1.5–2.38	66
	≥2.39	68
	unknown	4
	range	0.45–10
melanoma histology	superficial spreading	89
	nodular	69
	acra-lentiginous	23
	others	6
	unclassable	16
	unknown	7
ulceration	yes	53
	no	157

Histopathology, further treatment and follow up

All excised SLN were immediately sent for histopathological examination without fixation. Alternate slices of 2 mm thickness throughout the SLN were subjected to histology and immunohistology analysis. Slices were paraffin embedded and 3 to 5 sections per slice subjected to classical histology using haematoxyline-eosin (HE) staining. Immunohistochemistry (IHC) analysis was performed for protein S-100, MART-1/Melan-A, Tyrosinase and HMB 45, except for nine patients for whom histology alone was performed. MART-1/Melan-A, Tyrosinase and HMB 45 are proteins specifically expressed by melanocytes and most melanoma while protein S-100 is expressed by different neuroendocrine tissues and tumours.

Disease free survival was defined as interval from surgery to first observation of relapse. All patients presenting tumour-negative SLN by HE and IHC criteria were followed every 3 to 12 months by clinical examination and whole body CT without further treatment. Elective node dissection was proposed to patients presenting tumour-positive SLN by HE and/or IHC criteria, independently from the Breslow index.

Statistics

Different clinical and pathological factors including age, sex, location and histological classification of melanoma, Breslow index, ulceration, number and tumour positivity/negativity of SLN have been analysed as predictive elements of disease evolution. Analysis of DFS was performed using the Kaplan-Meier method. Univariate and multivariate analysis on the basis of Cox's proportional hazards regression

localisation	1 site	2 sites homolateral	2 sites bilateral	3 sites	total
upper extremity	36	3 (8%)	0	0	39
lower extremity	71	2 (3%)	0	1 (1%)	74
trunc	49	9 (11%)	21 (25%)	2 (2%)	81
head & neck	11	4 (27%)	1 (7%)	0	16
total	167	18	22	3	210

Tab. 2 Lymphatic draining sites

model were used to associate covariates to DFS. Breslow thickness was treated as a continuous variable for both univariate and multivariate analysis. P-values <0.05 were considered significant. All analyses were performed using the BMDP statistical Software.

Results

In 210 patients, 385 SLN have been identified by lymphoscintigraphy. Two additional sites (0.5%) identified by lymphoscintigrapy as SLN were false positive and corresponded to lymphatic lakes. Blue coloration detected 375/385 SLN (97%) and gamma probe 377/385 (98%). Combined blue coloration and/or the hand held probe allowed the peroperative localization of 381 SLN (99%, 1.81 SLN per patient) that were excised and analysed. For the other four sites identified by lymphoscintigraphy (1%), complementary nodal surgery did not allow to find a radioactive lymph node.

Draining sites, post-surgical complications

Single SLNs were detected in 102 patients, two SLNs in 72 patients and more thand three SLNs in 36 patients. A large number of multiples draining sites were observed in trunk and head & neck melanomas (Tab. 2). Draining to three sites, all homolateral was observed in three patients. 97% of the upper and lower extremities drained to the axillary and inguinal nodes, respectively, while trunc melanomas drained preferentially to axillary nodes (83%) and head and neck melanomas to cervical nodes (44%).

Concerning morbidity after SLN resection, six cases of lymphocele (3%), four cases of local infection (2%), one case of allergy (grade I) against blue dye (0.5%), one case of pulmonary embolism (0.5%) and one case of scar pain (0.5%) were observed.

Histopatholgy, resection of sites

A total of 68 (18%) tumour positive SLN were observed by HE staining and/or IHC. Fourty-three SLN were positive by HE staining as well as IHC, 16 were positive by IHC alone and nine were positive by HE staining while immunohistology had not been performed. Furthermore, 161 control lymph nodes were excised and analysed from 61 patients (2.6 control nodes per patient). All control nodes were negative by HE and IHC. On a per patient basis, 50 patients out of 210 (24%) had positive nodes by HE and/or IHC.

40/50 patients presenting metastases of the SLN by HE/IHC criteria had complementary elective resection of all nodes in the sites identified for SLN by lymphoscintigraphy. All complementary resections were performed within three months of SLN surgery. From three up to 47 nodes were excised at secondary node surgery. One or more further positive nodes were found in 10 of these 40 patients (25%).

Relapse, SLN status, survival

Mean follow-up after SLN resection was 31.3 months (range: 6–66 months). Four patients were lost to follow up 4, 9, 29 and 32 months respectively after SLN surgery without evidence of relapse and were entered considered in live as such in the survival analysis. Out of 210 patients 29 relapsed with local and/or distant disease one to 55 months after SLN surgery (Tab. 3).

We observed 19 relapses which occurred in 50 SLN positive patients and 10 in 160 SLN negative patients. Fourty patients out of 50 SLN positive patients accepted elective node resection. Ten of them were positive in the histopathological examination of the complementary nodes. We observed eight recurrences in the group of 10 positive elective node resections (80%) and only 3/30 (10%) in the group of negative elective node resections.

At evaluation, 196 of 210 patients were alive. Thirteen patients had died from disease, 10 of 19 patients with relapse and positive SLN, three among the 10 patients who relapsed despite negative SLN. One patient, who was SLN negative and without evidence of relapse, had died from an unrelated cause.

According to Kaplan-Meier analysis the overall survival probability in our patient population was 92% at three years (Fig. 1a). SLN status proved to be the most significant predictive factor of relapse: Relapse probability was 12% in SLN negative and 54% in SLN positive patients ($p < 0.0001$). Already at one year relapse probability in SLN positive patients was high (25%), compared with only 3% in SLN negative patients (Fig. 1b). The mean Breslow index of all patients was 2.32 + SD 1.53 mm. It was 2.06 + SD 1.40 mm for patients with negative SLN, 3.15 + SD 1.69 mm in SLN positive (HE and/or IHC) patients and 3.4 + SD 1.86 mm in patients whose tumour recurred.

DFS probability according to Breslow index as a contiuous variable was highly significant ($p < 0.0001$). When evaluated for three similar sized subgroups that presented Breslow indices of ≤1.49 (n = 72), 1.5 to 2.38 (n = 66) and ≥2.39 (n = 68) the overall probability of relapse was 5, 25 and 37%, respectively. Relapse probability at one year after surgery in these subgroups was 0, 5 and 18%, respectively (Fig. 1c). DFS according to sex was close to significant ($p = 0.06$), relapse probability being 18 and 28% for women and men, respectively (Fig. 1d).

Histology showed a small trend towards significance ($p = 0.21$), relapse probability

Tab. 3 Major disease and survival parameters of 29 relapsed patients

patient	sex	melanoma				SLN		elective resect.	relapse (month*)		last control in life (month*)	died from disease (month*)
		locali.	Breslow	ulcer	histology	number	HE/IHC		local	distant		
1	F	LE	1.47	no	superficial s.	1	P	P	13	20		34
2	F	LE	2,27	no	acra-lent	1	P	ND	49			63
3	M	trunc	5.1	no	nodular	2	P	ND		34	59	
4	F	trunc	3.8	no	superficial s.	1	P	ND		4	51	
5	M	trunc	3.2	yes	nodular	2	P	ND	6	1		18
6	F	LE	2.5	no	unknow	1	P	P	6	6		24
7	M	UE	2.32	yes	superficial s.	1	P	P		7		8
8	F	LE	4	yes	nodular	1	P	P	13	17	34	
9	F	H&N	2.7	no	other	4	P	P	5	13	22	
10	M	LE	2.5	yes	acra-lent	2	P	P		11		15
11	M	trunc	4	no	nodular	1	P	P		1		3
12	F	LE	6.5	yes	nodular	2	P	P	12		18	
13	M	LE	2.4	no	nodular	1	P	ND	27	27		41
14	M	LE	2.9	no	nodular	3	P	ND	5		46	
15	M	UE	5.5	no	nodular	2	P	ND		33	33	
16	M	LE	9	no	acra-lent	1	P	ND	2	2		19
17	M	LE	4.4	no	nodular	1	P	N	4	25	43	
18	M	trunc	3	no	superficial s.	2	P	N	8	15		21
19	M	trunc	2.2	yes	superficial s.	3	P	N	16	16	20	
20	M	trunc	4	no	nodular	2	N	ND	8		47	
21	M	UE	2.3	no	nodular	1	N	ND	10	19		21
22	M	H&N	1.8	no	superficial s.	1	N	ND	34	42	42	
23	F	LE	2.3	yes	superficial s.	1	N	ND	18		39	
24	M	H&N	1.2	no	unclassable	3	N	ND	26	26	34	
25	M	H&N	2.2	no	superficial s.	2	N	ND		34	34	
26	M	LE	2.3	no	nodular	2	N	ND	23		31	
27	M	trunc	1.95	no	superficial s.	3	N	ND	8			12
28	F	LE	8	no	nodular	1	N	ND	36	55	55	
29	M	UE	2.8	no	nodular	1	N	ND	26	27		32

P: positive; N: negative; ND: not done; UE: upper extremity, LE: lower extremity; H&N: head and neck; *months after excision of SLN

being highest for nodular histology. Survival prediction showed no clear difference in trend among the other histological subgroups. Primary melanoma localization showed a similar trend of difference ($p = 0.21$) with the highest probability for relapse for melanoma of the head and neck, followed by lower and upper extremities melanoma. The other parameters evaluated, notably ulceration, age and number of SLN, were far from reaching statistical significance concerning probability of relapse.

In the multivariated analysis, the Cox analysis indicated that the most important risk factor was SLN positivity ($p = 0.0001$) followed by the Breslow index ($p = 0.0004$) and patient's sex ($p = 0.055$).

When analysing the relapse probability for the combination of Breslow index and SLN positivity again in three similar sized subgroups defined as

- SLN negative and Breslow index <1.5,
- SLN negative and Breslow index between 1.5 to 3.5,
- SLN positive and/or Breslow >3.5, relapse probabilities were 3, 16 and 48%, respectively (Fig. 1e).

Finally, SLN positivity in combination with a Breslow index of ≥2.39 was observed in 28 patients, and 15 of these patients relapsed. The Kaplan-Meyer analysis gave a very high relapse probability of 72% for these patients, the probability in the first year being 42% with a 95% confidence interval of between 25 and 63%.

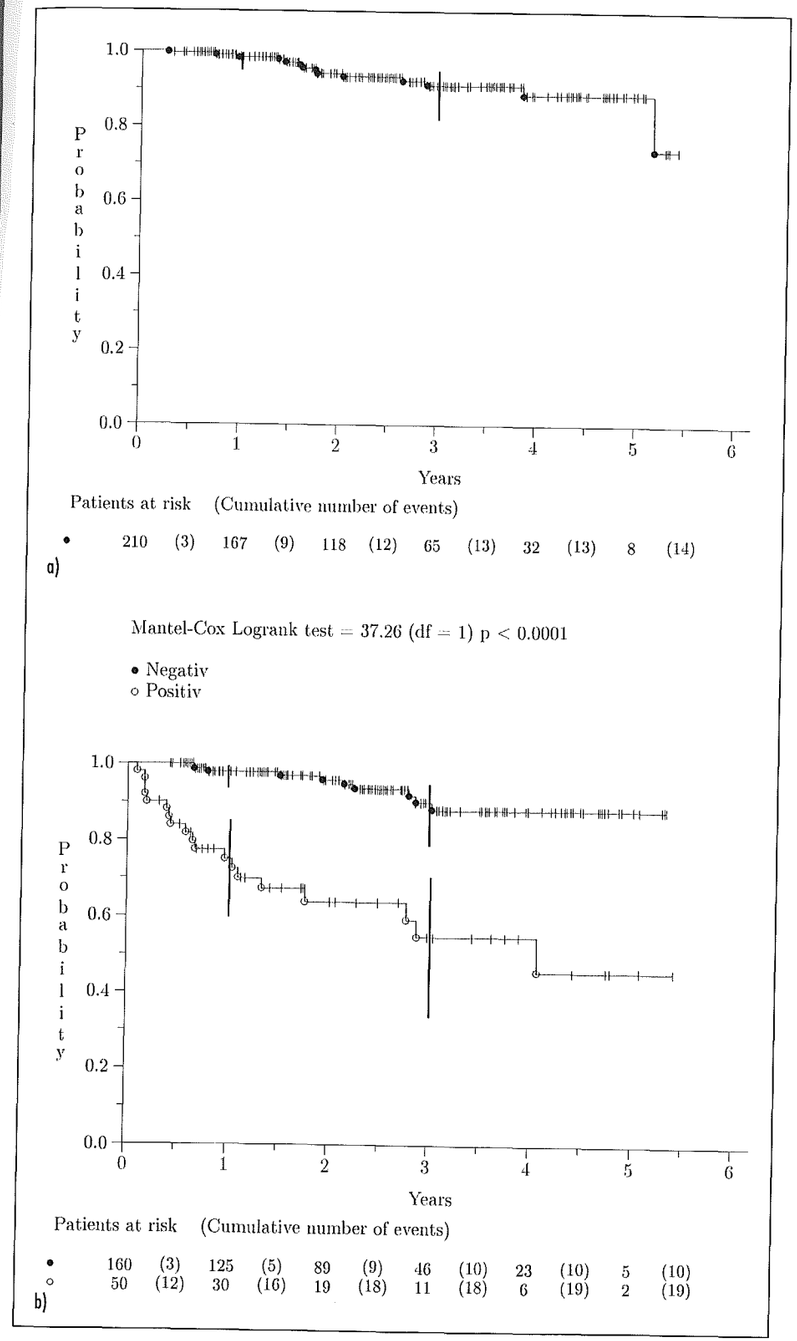

Fig. 1 Overall survival (a) and disease free survival (b-e); vertical bars: 95% confidence interval
a) overall survival probability: 92% at three years
b) histology between negative and positive SLN patients; overall relapse probability (3 years): 10% and 45%

Discussion

This study aimed at evaluating SLN scintigraphy in early stage melanoma. A second aim was the evaluation of the prevalence of tumour recurrence in SLN positive patients. Performed as multicenter study, scintigraphy was analysed as part of the triple SLN detection method. Immunohistochemical analysis was performed for four parameters.

RT-PCR evaluation was performed in a subgroup of these patients. These results have been presented previously (11). Overall, we did not observe any particular difference of relapse probability between RT-PCR positive and negative patients. None of the RT-PCR positive patients with negative HE/IHC evaluation presented a relapse in this evaluation. In fact, the debate on the relevance of PCR as a predictive factor for relapse in melanoma patients remains open (2, 3). RT-PCR positive and HE/IHC negative patients might represent a subgroup of patients with very minor disease. The survival probability of these patients appears to be better in the first years than that of HE/IHC SLN positive patients, but they might be prone to relapse at a later stage.

The lymphatic imaging of SLN in melanoma patients allowed visualising all 381 SLN in this cohort of 210 patients in the sense that no further SLN were detected by the blue coloration method and the gamma probe. However, while these latter methods revealed essential and complementary information for surgery, a few SLN were missed (1%) even when using the combination of both of them. In the absence of the peroperative SLN identification, selective surgery of the relevant lymph node basin had to be performed and the histological analysis of the multiple lymph nodes was therefore suboptimal.

In view of the high detection rate of >97% with each of the preoperative methods, one or the other method might be chosen by the surgeon without major change in the selective SLN resection rate. However, the combination of blue coloration and gamma probe technique allowed to resect and analyse the highest percentage of SLN. Scintigraphy had a false positive detection

rate of 2/385 (0.5%), both false positives being due to lymphatic lakes. This already low rate could possibly be further reduced by using late imaging, however the gain might be judged insufficient in this pathology.

In view of the favourable detection rate of SLN by scintigraphy, it is confirmed here that this method is essential in SLN surgery of melanoma. It notably allowed in many patients to become aware of multiple draining sites of melanoma, especially when situated on the trunk and close to the median line. However, also infrequent localisation of SLN such as popliteal or humeral sites could be identified and allowed the surgeon to program the intervention adequately.

When considering the histopathological examination, we found a clear difference between the classical HE coloration and immunohistochemistry in favour of the latter one. While all HE positive SLN that were also examined by immunohistochemistry were confirmed, the latter method detected a significantly higher number of metastases. We used the overall detection rate of these two methods to correlate it with the clinical outcome. The combination of both techniques was necessary, since immunohistochemistry could not be performed in a few occasions.

In this series, 24% of patients presented tumour positive SLN by HE/IHC criteria. When analysing SLN positivity according to histological criteria, nodular melanoma showed the highest incidence of SLN positivity (35%), followed by acral-lentiginal histology (26%) and superficial spreading melanoma (16%). These results differ partially from those described by Vuylsteke et al. (14) who found similarly that nodular melanoma had the highest probability for SLN positivity, followed, however, by superficial spreading in his series. Acral lentiginal melanoma, which presented only 1% in the patient population of Vuylsteke compared with 11% in our group, had no particular tendency for SLN positivity.

In this study, primary melanoma located on the legs as compared to other localisations showed a higher incidence of SLN positivity. A correlation of SLN positivity with primary tumours on the appendices has been described previously by Rousseau et al. (12).

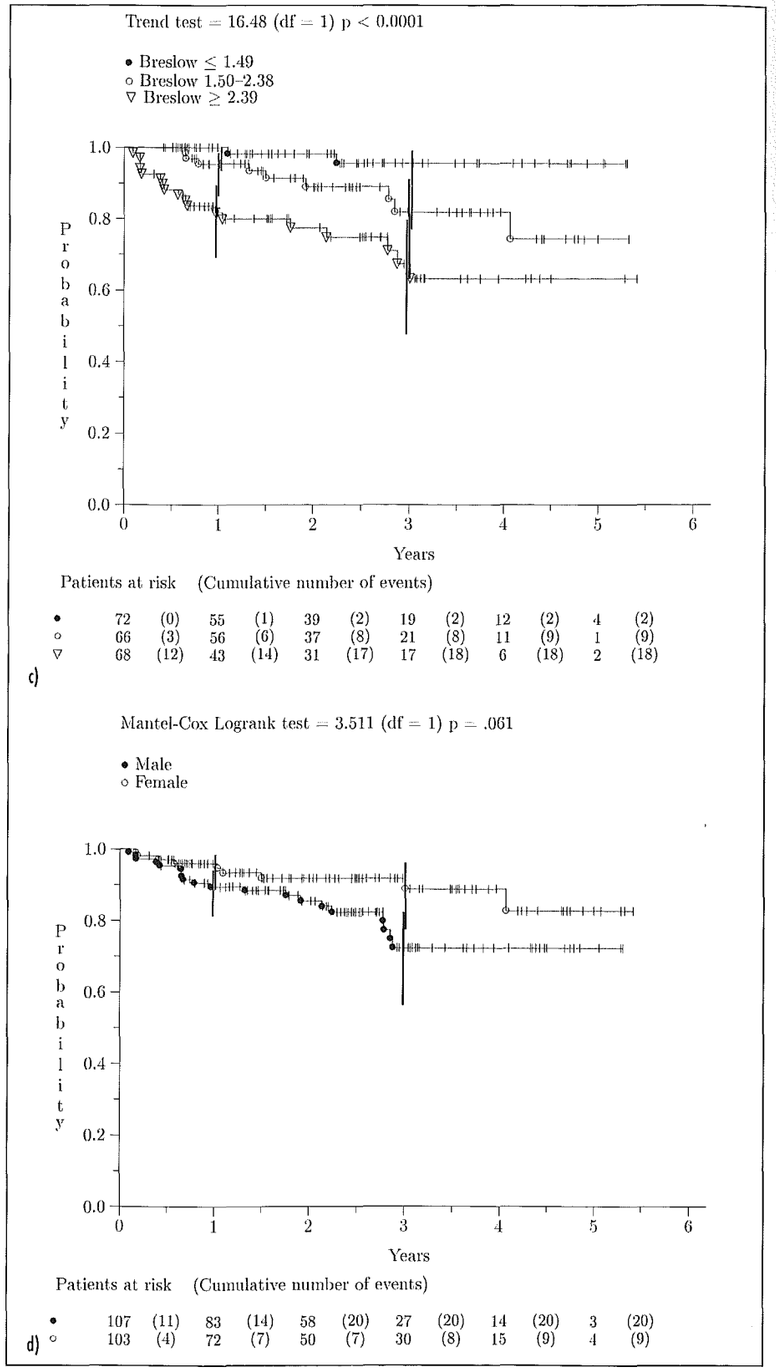

Fig. 1 c) relapse probability (3 years) of 4, 18, and 37% in the subgroups with Breslow indices of ≤1.49 (n = 72), 1.5 to 2.38 (n = 66) and ≥2.39 (n = 68)
d) relapse probability according to sex (3 years): 12 and 28% for women and men

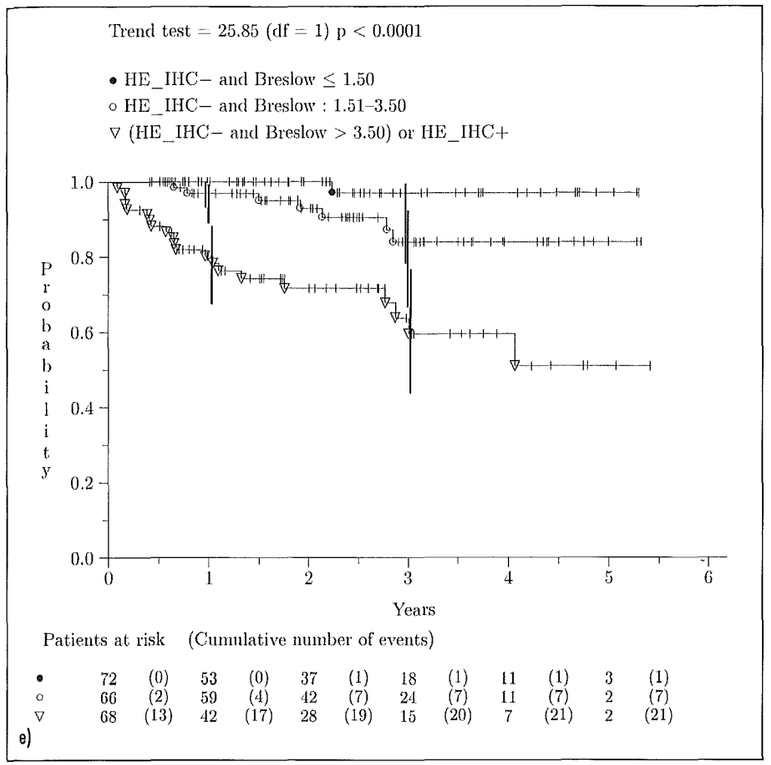

Fig. 1 e) histology and Breslow index: relapse probability (3 years): 3, 16, and 41% for subgroups with HE/IHC and Breslow index ≤1.50, HE/IHC negative and Breslow index 1.51–3.50 and HE/IHC negative and Breslow index >3.50 or HE/IHC positive, respectively.

We observed 29 tumour relapses one to 55 months post surgery of primary melanoma. The univariate and multivariate analysis showed that the highest risk factors for relapse were those of SLN positivity and a high Breslow index. SLN positivity and a Breslow index above 2.38 turned out as strong predictive factors of early relapse in agreement with the observations of Vuylsteke et al. (14), Mozzillo et al. (8), Rousseau et al. (12) and Morton et al. (6). Ulceration has been described as another predictive factor of relapse by two of the three mentioned authors (8, 14). However, this was not the case in the patient population presented here. This study included 53 patients with ulcerated melanoma. Their risk of relapse appeared only marginally higher ($p = 0.58$) than that of patients without ulceration.

Relapse probability according to patient's sex was close to significant ($p = 0.06$) with more than 50% increased relapse probability for men compared with women. Concerning tumour histology, some tendency was observed ($p = 0.21$), nodular melanoma showing a highest probability of tumour recurrence. However, the number of patients per subgroup was small, the observation period short and thus do not permit a conclusive statement. Similarly, localization of primary melanoma showed only some tendency of discrimination for relapse. However, age and number of SLN, were far from showing any tendency as predictive factors for recurrence.

Our statistical analysis has shown the very similar contribution of SLN positivity and a high Breslow index for defining patients with high risk of early relapse. This observation prompted the evaluation of different combination of these two parameters. Patients presenting simultaneously the two risk factors have a very high relapse probability. The association between Breslow index and SLN status furthermore allowed to grouping the patients according to their relapse probabilities. These groups in turn could profit from therapies of differential aggressiveness, depending on the individual patients relapse probability.

Conclusion

This study confirmed the particular relevance of lymph node scintigraphy in the context of carefully performed blue colour and gamma probe detection technique in the SLN approach of early stage melanoma. Furthermore, we showed that the SLN status and the Breslow index are independent, strong predictive factors for early relapse in patients with early stage melanoma. The combination of these two parameters allowed the subdivision of patients in groups with very high, intermediate and low risk of relapse and might lead consequently to the adaptation of therapy protocols according to the individual risk.

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References

1. Alex JC, Weaver DL, Fairbank JT et al. Gamma-probe-guided lymph node localization in malignant melanoma. Surg Onc 1993; 2: 303–308.
2. Carlson GW, Murray DR, Lyles RH et al. The amount of metastatic melanoma in a sentinel lymph node: does it have prognostic significance? Ann Surg Oncol 2003; 10: 578–581.
3. Davids V, Kidson SH, Hanekom GS. Melanoma patient staging: histopathological versus molecular evaluation of the sentinel node. Melanoma Research 2003; 13: 313–324.
4. Essner R, Conforti A, Kelley MC et al. Efficacy of lymphatic mapping, sentinel lymphadenectomy, and selective complete lymph node dissection as a therapeutic procedure for early-stage melanoma. Ann Surg Oncol 1999; 6: 442–449.
5. Miliotes G, Albertini J, Berman C et al. The tumor biology of melanoma nodal metastasis. Am Surg 1996; 62: 81–88.

6. Morton DL, Thompson JF, Cochran AJ et al. Sentinel-node biopsy or nodal observation in melanoma. N Engl J Med 2006; 35: 13.
7. Morton DL, Wen DR, Wong JH et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg 1992; 127: 392–399.
8. Mozzillo N, Caracò C, Chiofalo MG et al. Sentinel lymph node biopsy in patients with cutaneous melanoma: outcome after 3-years follow-up. J Cancer Surg 2004; 30: 440–443.
9. Pijpers R, Collet JG, Meijer S et al. The impact of dynamic lymphoscintigraphy and gamma probe guidance on sentinel node biopsy in melanoma. Eur J Nucl Med 1995; 22: 1238–1241.
10. Reintgen D, Cruse CW, Wells K et al. The orderly progression of melanoma nodal metastases. Ann Surg 1994; 220: 759–767.
11. Rimoldi D, Lemoine R, Kurt A-M et al. Detection of micrometastases in sentinel lymph nodes from melanoma patients: direct comparison of multimarker molecular and immunopathological methods. Melanoma Research 2003, 13: 511–520.
12. Rousseau DL, Ross MI, Johnson MM et al. Revised american joint committee on cancer staging criteria accurately predict sentinel lymph node positivity in clinically node-negative melanoma patients. Ann Surg Oncol 2003; 10: 569–574.
13. Thompson JF, McCarthy WH, Bosch CMJ et al. Sentinel lymph node status as indicator of the presence of metastatic melanoma in regional lymph nodes. Melanoma Res 1995; 5: 255–260.
14. Vuylsteke RJCLM, van Leeuwen PAM, Statius MG et al. Clinical outcome of stage I/II melanoma patients after selective sentinel lymph node dissection: long-term follow-up results. J Clin Oncol 2003; 6: 1057–1065.

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