Factors Predicting Recurrence and Survival in Sentinel Lymph Node-Positive Melanoma Patients

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Background: Studies which have found that histologic features of metastatic melanoma in sentinel lymph nodes (SNs) were predictive of survival have differed considerably in their design and results. We investigated in detail the influence of SN tumor characteristics and clinical and primary tumor parameters on clinical outcomes in a large cohort of patients treated in a single center.

Methods: In SN-positive melanoma patients, the association of clinical, primary tumor, and SN tumor features [maximum size (MaxSize), % cross-sectional area of SN occupied by tumor (%CS), tumor-penetrative depth (TPD), intranodal location of tumor, extranodal spread (ENS), and perinodal lymphatic invasion (PLI)] with disease-free (DFS), distant metastasis-free (DMFS), and melanoma-specific (MSS) survival was analyzed.

Results: In 409 SN-positive patients, independent predictors of poorer DFS were primary tumor features [anatomic site: head/neck (HR = 3.65, 95% CI 1.65–8.08) and limbs (HR = 2.46, 95% CI 1.21–4.98) compared with trunk; ulceration (HR = 1.70, 95% CI 1.15–2.51); satellites (HR = 2.85, 95% CI 1.49–5.44)], SN tumor features [MaxSize (HR = 1.53, 95% CI 1.04–2.26); ENS in SN (HR = 2.05, 95% CI 1.06–4.00); PLI (HR = 1.85, 95% CI 1.11–3.07)], and positive CLND (HR = 1.92, 95% CI 1.26–2.91). Factors independently predictive of poorer MSS were age ≥50 years (HR 1.64, 95% CI 1.01–2.67), primary tumor features [ulceration (HR = 2.55, 95% CI 1.44–4.52); satellites (HR = 3.95, 95% CI 1.83–8.49)], and ENS in SN (HR = 2.34, 95% CI 1.06–5.13).

Conclusions: The use of clinical, primary tumor, and SN tumor characteristics shown to be independent predictors of clinical outcomes in melanoma patients will assist in accurate prediction of prognosis and optimize clinical management.

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A sentinel lymph node (SN) is any node that receives direct lymphatic drainage from the site of a primary tumor. SN biopsy (SNB) has been shown to be an accurate means of assessing the tumor-harboring status of the regional lymph node field in patients with clinically localized melanoma, and SN status is an important prognostic factor.[1–9] SNB is associated with much less morbidity than elective complete regional lymph node field dissection,[10–12] which was previously the principal means of staging regional lymph nodes in patients with clinically node-negative disease.[13]

The results of the first Multicenter Sentinel Lymphadenectomy Trial (MSLT-I) showed that SNB was an accurate method of staging patients with melanoma.[10,14] However, completion lymph node dissection (CLND) in patients with positive SNs reveals tumor in regional nonsentinel lymph nodes (NSNs) in only approximately 20% of cases. Previous studies have investigated features of patients, their primary tumors, and the tumor deposits in SNs in attempts to identify factors predictive of NSN involvement.[15–22] Many predictive factors have been identified, although the results vary between individual studies depending on the methods used and the parameters considered. We have recently analyzed an extensive range of clinical, primary tumor, and SN tumor characteristics, and proposed a weighted score (Non-Sentinel Node risk score, N-SNORE) for accurate stratification of the risk of NSN involvement in SN-positive patients.

The results of the fourth interim analysis of MSLT-I suggest that there was a substantial survival benefit for SN-positive patients undergoing CLND, when compared with those who were subjected to wide excision of the primary tumor and subsequent regional lymph node dissection only if nodal disease became clinically apparent.[14,23] A survival benefit for patients undergoing SN biopsy has also been suggested by other studies (including a meta-analysis of 6 studies).[7,24–26]

Identification of parameters independently predictive of clinical outcomes in SN-positive patients may assist clinical management. Some previous studies have investigated the influence of SN tumor characteristics on clinical outcomes and patient survival. They found that SN tumor parameters such as size of tumor deposit(s),[21,27–31] relative area of SN tumor,[31,32] tumor penetrative depth,[16,33,34] volume of tumor,[35] intranodal location of tumor,[31] extranodal spread of tumor (ENS),[21,36] density of dendritic leukocytes in the paracortex of involved SNs,[32] and CXCL4 expression in SN metastases[37] were predictive of survival, and size of tumor deposit(s) correlated with disease recurrence.[22,38] However, these studies differed in the patient populations, histologic protocols, and methods of parameter assessment, which makes it difficult to compare their results.

In this large study of SN-positive melanoma patients diagnosed and managed at a single specialist center, we investigated in detail the influence of SN tumor characteristics and clinical and primary tumor parameters on regional lymph node recurrence, distant metastasis, and survival.

METHODS

Patients with primary cutaneous melanoma who underwent SNB between 1991 and 2008 were identified from the database of Melanoma Institute Australia (MIA). Patients with more than one primary melanoma and those for whom histologic slides of their SNs were not available for review were excluded. The primary melanoma was reported (or reviewed in cases diagnosed in external laboratories) by pathologists at the Royal Prince Alfred Hospital (RPAH), Sydney, Australia. Clinical and pathologic information on the primary melanomas was retrieved from the databases of MIA and RPAH.

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At MIA, SNB is offered to patients with melanoma ≥1.00 mm in thickness, and to those with thin melanomas (<1.00 mm) if they are considered at high risk (presence of ulceration, high mitotic rate, or Clark level IV/V invasion). Patients found to have melanoma involving SNs are offered CLND, which is usually performed within 2 months from the date of the SNB procedure, and within 3 months from the date of diagnosis of the primary melanoma. Patients were considered to have developed a lymph node recurrence (LNR) if metastatic melanoma was identified in a regional lymph node dissection (RLND) specimen >3 months after diagnosis of the primary melanoma. Recurrences beyond the regional node field were recorded as distant metastases (DM).

During the study period, SNs were processed as follows: The SNs were bisected longitudinally in a paramedian plane and embedded in paraffin blocks with the cut surfaces facing upward. Four consecutive sections were cut, the first and fourth were stained with hematoxylin–eosin, and the second and third sections were stained immunohistochemically with S-100 and HMB-45.

Clinical features (age at diagnosis of primary melanoma, sex) and primary tumor characteristics [histologic subtype, Breslow thickness, Clark level, ulceration, mitotic rate (MR), desmoplasia, tumor-infiltrating lymphocytes (TILs), regression (defined as partial or complete replacement of invasive melanoma by angiofibroplasia, with/without associated inflammation and melanophages), satellites, lymphovascular invasion (LVI), and neurotropism] were recorded. Histologic sections of all SNs were reviewed by a single pathologist (RM) with expertise in their examination. SN features that were assessed were: number of SNs harvested (NoSN); number (NoPosSN) and proportion (%PosSN) of SNs containing metastatic melanoma; number of tumor deposits; maximum size of largest melanoma deposit (MaxSize), estimated percentage of SN cross-sectional area involved by melanoma (%CS), tumor penetrative depth (TPD, defined as the maximum depth of invasion into the SN of tumor measured from the nodal capsule,[18] also known as S-score[16,17]), intranodal location of tumor deposits (confined to the subcapsular zone only, involving the nodal parenchyma, or multifocal, regardless of the nodal zone involved), and the presence of ENS and perinodal lymphatic invasion (PLI). If more than 1 SN contained melanoma, the highest/worst score for each parameter was recorded. MaxSize was categorized in 3 ways: (1) ≤2 mm and >2 mm; (2) using the so-called "Rotterdam" criteria[28]: <0.1 mm, 0.1 to 2.0 mm, 2.0 to 10.0 mm, and >10 mm; and (3) criteria used by Gershenwald et al[39]: ≤0.5 mm, 0.5 to 2.0 mm, 2.0 to 10.0 mm, and >10.0 mm. MaxSize, %CS, TPD, and intranodal location were considered to be SN tumor burden indices.

The relationship of categorical clinico-pathologic parameters with LNR and DM was initially assessed using cross-tabulations and Pearson χ^2 tests. Univariate logistic regression analysis was used to investigate the association of categorical and continuous clinico-pathologic variables with LNR and DM; variables which yielded a P value of <0.1 were then included in multivariate logistic regression models. Because SN tumor characteristics, sex, and regression were associated with CLND status (data not shown), multivariate logistic regression models were initially studied including interaction terms including CLND status and SN tumor parameters. Survival indices were defined as duration from diagnosis of primary melanoma to the first occurrence of distant metastasis (distant metastasis-free survival, DMFS), first melanoma recurrence (disease-free survival, DFS), or melanoma-related death (melanoma-specific survival, MSS). Survival analysis was carried out using the Kaplan-Meier method with log-rank tests, and univariate and multivariate Cox proportional hazards models (including analysis of interactions between CLND status and SN tumor parameters). The criterion for entry into the multivariate analysis was a P value <0.1 in the univariate analysis. Cases in which the endpoints did not occur during the follow-up period were censored. P values <0.05 were considered to be statistically significant.

The study conforms to the guidelines set forth by the Sydney South West Area Health Service Ethics Committee.

RESULTS

Four hundred nine patients (254 male patients and 155 female patients) had a single primary melanoma and 1 or more positive SNs. The median age at diagnosis of primary melanoma was 55.6 years (range 6.0–93.5 years). A total of 1029 SNs (median 2, range 1–12) were removed, of which 528 (51.3%) (median 1, range 1–6) were positive for metastatic melanoma. Three hundred fifteen (77.0%) patients had 1 positive SN, and 94 (23.0%) patients had >1 positive SN. CLND was performed in 309 (75.6%) patients (189 male patients and 120 female patients), 53 (17.2%) of whom had NSNs positive for metastatic melanoma. The mean and median follow-up durations were 39.1 and 30.8 months, respectively (range 1.2–248.2 months).

Time to LNR ranged from 2.7 to 237.6 months (mean 23.3, median 12.3). Factors significantly associated with the occurrence of LNR were: age >50 years, primary melanoma features (presence of regression and LVI), SN tumor characteristics (parenchymal vs. subcapsular location and TPD >1.0 mm), and CLND positivity (Table 1). Multivariate analysis showed that age >50 years, the presence of LVI in the primary tumor, and CLND status were independent predictors of LNR (Table 1). SN tumor characteristics (%CS, TPD, and parenchymal location) were the only significant predictors of shorter time to LNR, whereas CLND status and ENS showed a trend toward a shorter time to LNR (Table 2). Multivariate analysis of parameters predictive of time to LN recurrence was not performed due to the close correlation and multicollinearity of the SN tumor indices.

Primary tumor features (presence of ulceration and satellites) and presence of ENS in SNs were independent predictors of DFS, DMFS, and MSS. In addition, poorer DFS was independently associated with primary tumor site (head/neck and limbs vs. trunk), SN tumor features (MaxSize >2 mm, presence of PLI) and positive NSN in CLND (Table 3); other factors independently predictive of DMFS were male sex, primary tumor features (absence of TILs), and SN tumor MaxSize >10 mm (Table 4); and age ≥50 years was an additional independent predictor of MSS (Table 5). CLND status was not an independent predictor of DMFS or MSS.

MaxSize criteria were compared with respect to their predictive value (Fig. 1). DFS, DMFS, and MSS differed significantly using the 2 mm cutoff. The Gershenwald criteria provided significant stratification of DFS, DMFS, and MSS. However, this was largely due to the fact that survival times in only the >10 mm group (and the >2 mm group for DFS) differed considerably from those of the other groups. Similarly, the difference in survival using the "Rotterdam" criteria was only significant for DFS and DMFS (but not MSS), and was largely due to the difference between the >2 mm and the ≤2 mm groups.

DISCUSSION

In previous studies, older age,[36,40] Breslow thickness,[36,40] ENS,[21,36] and NSN positivity in CLND[8,36,40–42] have been shown to be an independent predictors of survival in SNB-positive melanoma patients. Number of positive SNs was not found to be a significant predictor of DFS or overall survival.[41] NSN positivity has also been shown to be a significant predictor of poor DMFS.[36] DMFS is a useful measure because serious, life-threatening disease progression is regarded as the endpoint, in contrast to DFS, which also includes

TABLE 1. Association of Clinico-Pathologic Factors with Development of Lymph Node Recurrence During Follow-Up

				Univariate Analysis			Multivariate Analysis		
Parameter	**Level**	**N**	**LNR%**	***P***	**OR**	**95% CI**	***P***	**OR**	**95% CI**
Clinical									
Age	<50 years	161	6.2		1.00			1.00	
	≥50 years	248	15.3	0.007	2.73	1.32–5.66	0.26	1.76	0.66–4.74
Sex	Female	155	9.7		1.00				
	Male	254	13.0	0.31	1.39	0.73–2.66			
Primary tumor									
Thickness	0.01–1.00 mm (ref)	23	13.0		1.00				
	1.01–2.00 mm	107	10.3	0.70	0.76	0.20–2.99			
	2.01–4.00 mm	173	13.9	0.91	1.07	0.30–3.89			
	>4.00 mm	104	8.7	0.52	0.63	0.16–2.54			
MR*	0 (ref)	17	17.6		1.00			1.00	
	1–2	82	4.9	0.08	0.24	0.05–1.19	0.38	0.38	0.05–3.24
	3–5	135	9.6	0.32	0.50	0.13–1.96	0.28	0.34	
	6–10	90	14.4	0.74	0.79	0.20–3.13	0.99	0.99	0.05–2.39
	>10	73	16.4	0.90	0.92	0.23–3.69	0.81	0.79	
									0.14–6.83
									0.12–5.40
Ulceration	Absent	260	11.2		1.00				
	Present	147	12.2	0.74	1.11	0.59–2.08			
TILs	Absent	265	12.8		1.00				
	Present	142	9.2	0.27	0.69	0.35–1.34			
Regression	Absent	356	10.1		1.00			1.00	
	Present	51	21.6	0.02	2.44	1.15–5.18	0.07	2.62	0.92–7.47
Satellites	Absent	387	11.1		1.00				
	Present	20	20.0	0.23	2.00	0.64–6.26			
LVI	Absent	373	10.5		1.00			1.00	
	Present	34	23.5	0.03	2.64	1.12–6.22	0.20	2.30	0.65–8.19
Neurotropism	Absent	398	11.6		1.00				
	Present	9	11.1	0.97	0.96	0.12–7.82			
No. of SNs									
NoSN	1 (ref)	106	12.3		1.00				
	2	124	10.5	0.67	0.84	0.37–1.90			
	≥3	179	12.3	1.00	1.00				
						0.48–2.08			
NoPosSN	1 (ref)	315	12.1		1.00				
	2	79	11.4	0.87	0.94	0.43–2.03			
	≥3	15	6.7	0.53	0.52				
						0.07–4.07			
%PosSN	≤50%	127	13.4		1.00				
	>50%	282	11.1	0.49	0.80	0.43–1.50			
SN tumor features									
MaxSize	≤2 mm	253	9.9		1.00				
	>2 mm	156	14.7	0.14	1.58	0.86–2.89			
%CS	≤1% (ref)	184	10.3		1.00				
	>1 and ≤10%	125	9.6	0.84	0.92	0.43–1.98			
	>10%	100	17.0	0.11	1.78				
						0.88–3.60			
TPD	≤0.3 mm (ref)	145	6.9		1.00			1.00	
	>0.3 and ≤1.0 mm	98	13.3	0.10	2.07	0.87–4.92	0.02	12.42	1.47–104.8
	>1.0 mm	162	15.4	0.02	2.46	1.14–5.33	0.03	9.88	1.24–78.42
Intranodal location	Subcapsular	194	8.2		1.00		†		
	Parenchymal	211	15.2	0.03	1.99	1.05–3.75			
ENS	Absent	387	11.4		1.00				
	Present	22	18.2	0.34	1.73	0.56–5.35			
PLI	Absent	357	12.0		1.00				
	Present	52	9.6	0.61	0.78	0.29–2.06			
CLND status	Negative	256	6.6		1.00			1.00	
	Positive	53	24.5	<0.001	4.57	2.06–10.13	0.004	3.92	1.55–9.91

*MR stratified as 0 vs. ≥1 was not significant; nor was Clark level or primary site or desmoplasia; nor was MaxSize using 1 mm cutoff, Rotterdam criteria,[28] or Gershenwald criteria.[39]

†Model with TPD was stronger than that including intranodal location.

LNR%, proportion of patients suffering lymph node recurrence during follow-up; N, number of patients; NoSN, number of SNs harvested; OR, odds ratio; P, significance level; %PosSN, proportion of SNs containing metastatic melanoma; 95% CI, 95% confidence interval for OR.

TABLE 2. Association of Clinico-Pathologic Parameters with Time to Lymph Node Recurrence (Only Significant Parameters are Shown)

		Months to LNR		Univariate Cox Regression Analysis		
Parameter	**Level**	**Mean***	**Median***	***P***	**HR**	**95% CI**
MaxSize†	<0.1 mm (ref)				1.00	
	0.1–1.0 mm			0.03	0.24	0.07–0.85
	>1.0 mm			0.45	0.67	0.23–1.92
%CS	≤1%	37	14		1.00	
	>1 and ≤10%	17	10	0.27	1.54	0.72–3.29
	>10%	13	11	0.03	2.21	1.08–4.54
TPD	≤0.3 mm	44	19		1.00	
	>0.3 mm and ≤1.0 mm	24	10	0.11	2.00	0.85–4.73
	>1.0 mm	14	11	0.02	2.53	1.13–5.66
Location	Subcapsular	41	18		1.00	
	Parenchymal	14	10	0.03	2.13	1.08–4.20
ENS	Absent	25	13		1.00	
	Present	9	6	0.09	2.52	0.87–7.26
NSN status	Negative	37	11		1.00	
	Positive	12	12	0.08	2.11	0.93–4.80

*Time to LNR estimates using Kaplan-Meier method.
†MaxSize using cutoffs of 1 mm, 2 mm, and Gershenwald criteria[39] were not significant.
HR, hazard ratio; LNR, regional lymph node recurrence; *P*, significance level; 95% CI, 95% confidence interval for HR.

local, in transit and nodal recurrences as endpoints. The results of the present large study are similar to those of previous studies, but differ from them in the greater number of clinicopathologic parameters assessed. We identified clinical, primary tumor features, and SN tumor characteristics as being independent predictors of survival. Features of the primary tumor (location in trunk vs. limbs, presence of ulceration and satellites) and SN tumor (MaxSize >2 mm and presence of ENS and PLI) and positive NSN in CLND specimens were predictive of DFS. Clinical (male sex), primary tumor (presence of ulceration and satellites, absence of TILs) and SN tumor (MaxSize >10 mm, presence of ENS) features were independent predictors of DMFS, when controlled for CLND status. Clinical features (age >50 years), primary tumor features (presence of ulceration and satellites) and SN tumor features (presence of ENS and PLI) were independent predictors of MSS, when controlled for CLND status.

The association of older age with poorer MSS and male sex with DMFS suggests that immunological and hormonal factors may play a role in disease progression in SN-positive melanoma patients. Male sex was also associated with an increased risk (albeit falling just short of statistical significance in multivariate analysis) of NSN positivity.

Number of positive SNs was not an independent predictor of survival. SN tumor burden indices such as MaxSize, TPD, and %CS have been shown to be associated with clinical outcome. Increasing TPD (particularly >1 mm) has been shown to be predictive of poorer survival,[16,17,33,43] and in a comparative study, the predictive ability of TPD was slightly better than that of MaxSize.[33] Increasing cross-sectional area of tumor in SNs measured by computerized morphometry has been shown to be independently predictive of poorer survival.[44] We found that TPD, %CS, and intranodal location of tumor deposits were not independent predictors of survival when controlled for other SN tumor parameters, and therefore, based on our results, it seems that MaxSize is the best measure of tumor burden in terms of predicting clinical outcome.

The optimal MaxSize cutoffs for prognostic stratification, however, are not established. MaxSize cutoffs differ between studies and include ≤2 mm/>2 mm,[29,45–47] ≤3 mm/>3 mm,[48] <0.1 mm/0.1 to 0.1 mm/>1 mm,[28] and ≤0.5 mm/0.5 to 2.0 mm/2.0 to 10.0 mm/>10.0 mm.[39] Increasing MaxSize has been shown to be associated with recurrence and poorer survival in different studies.[22,29,45–48] Comparison of results of these studies is difficult because different MaxSize cutoffs and different survival indices have been measured. The issue is further complicated by the fact that the histologic protocols used for sampling SNs vary between studies; more extensive sectioning protocols are known to result in detection of more tumor deposits,[49,50] potentially altering the MaxSize. Based on findings in the SN-positive breast cancer population, MaxSize cutoffs of <0.2 mm, 0.2 to 2 mm, and >2 mm were initially assessed in melanoma patients. It has since been established that poor clinical outcomes (which are rare in breast cancer patients) do occur when MaxSize in melanoma SNs is <0.2 mm. The Rotterdam group[28,51] and others[33] showed that MaxSize categorized into <0.1 mm, 0.1 to 0.1 mm, and >1 mm groups was predictive of survival. They found that patients with MaxSize <0.1 mm rarely suffered adverse clinical outcomes,[28,51] and proposed that these patients should be considered to be SN-negative.[51] However, other authors have demonstrated adverse outcomes (albeit rare) in patients with MaxSize <0.1 mm.[27] Based on their findings, the EORTC melanoma group[52,53] recommended that pathologists assess and report MaxSize as an absolute number and according to the Rotterdam criteria, along with intranodal location of tumor in SN (using the Dewar classification[19]).

Our results showed that although MaxSize was a significant predictor of DFS and DMFS, the 2 mm cutoff and the Gershenwald criteria were better predictors than the Rotterdam criteria. Based on our results (Tables 3–5, Fig. 1), a simple MaxSize classification of ≤2 mm versus >2 mm (or strata of ≤2 mm, 2–10 mm and >10 mm) would appear to be the best predictor of clinical outcomes. This simple scheme is likely to be more reproducible and will potentially be less affected by variations in sectioning protocols, particularly in the case of very small (0.1–0.2 mm) metastases, which may be prove to be larger in deeper sections. Furthermore, in the 24 patients with MaxSize <0.1 mm, after a median follow up period

TABLE 3. Association of Clinico-Pathologic Parameters with Disease-Free Survival

				Univariate Analysis			Multivariate Analysis		
Parameter	**Level**	**5YS**	**10YS**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**
Clinical									
Age	<50 years (ref)	59.1	56.7		1.00			1.00	
	≥50 years	37.0	29.9	<0.001	1.92	1.40–2.64	0.07	1.42	0.97–2.08
Sex	Female (ref)	54.0	40.5		1.00			1.00	
	Male	40.5	39.1	0.02	1.44	1.05–1.97	0.08	1.41	0.96–2.07
Primary tumor									
Primary site	Trunk (ref)	65.0	65.0		1.00			1.00	
	Head & neck	44.7	33.5	0.02	2.20	1.17–4.14	0.001	3.65	1.65–8.08
	Limbs	42.7	35.6	0.02	1.96	1.13–3.41	0.01	2.46	1.21–4.98
Thickness	0.01–1.00 mm (ref)	78.0	78.0		1.00			1.00	
	1.01–2.00 mm	55.4	55.4	0.21	1.83	0.72–4.67	0.13	2.59	0.77–8.76
	2.01–4.00 mm	43.4	37.5	0.02	2.86	1.16–7.07	0.11	2.70	0.81–9.00
	>4.00 mm	33.9	25.4	0.006	3.64	1.45–9.15	0.21	2.22	0.64–7.66
Ulceration	Absent	54.3	47.8		1.00			1.00	
	Present	31.0	28.8	<0.001	1.93	1.44–2.59	0.008	1.70	1.15–2.51
Satellites	Absent	47.5	41.4		1.00			1.00	
	Present	15.0	15.0	<0.001	3.48	2.00–6.03	0.002	2.85	1.49–5.44
LVI	Absent	47.3	40.9		1.00			1.00	
	Present	30.8	30.8	0.007	1.89	1.19–2.98	0.25	1.42	0.78–2.57
No. of SNs									
NoPosSN	1 (ref)	46.9	43.6		1.00			1.00	
	2	44.2	22.1	0.20	1.27	0.88–1.82	0.53	1.16	0.74–1.81
	≥3	19.7	19.7	0.003	2.83	1.43–5.58	0.07	2.01	0.94–4.30
SN tumor features									
Maximum size*†	≤2 mm (ref)	52.8	42.6		1.00			1.00	
	>2 mm	34.4	34.4	<0.001	1.78	1.33–2.39	0.03	1.53	1.04–2.26
%CS†	≤1% (ref)	51.6	40.7		1.00				
	>1 and ≤10%	49.7	49.7	0.52	1.13	0.79–1.61			
	>10%	28.7	28.7	<0.001	2.09	1.58–2.96			
TPD†	≤0.3 mm (ref)	52.8	46.2		1.00				
	>0.3 and ≤1.0 mm	46.7	31.8	0.11	1.40	0.93–2.10			
	>1.0 mm	38.5	38.5	<0.001	1.87	1.33–2.64			
Intranodal location†	Subcapsular	54.9	48.0		1.00				
	Parenchymal	36.8	31.5	<0.001	1.82	1.34–2.46			
ENS	Absent	47.0	40.9		1.00			1.00	
	Present	21.9	21.9	0.001	2.47	1.46–4.20	0.03	2.05	1.06–4.00
PLI	Absent	48.4	45.2		1.00			1.00	
	Present	26.6	13.3	0.003	1.78	1.21–2.60	0.02	1.85	1.11–3.07
CLND status‡	Negative	53.9	48.5		1.00			1.00	
	Positive	21.0	10.5	<0.001	2.70	1.85–3.94	0.002	1.92	1.26–2.91

*Other MaxSize cutoffs were not significant in multivariate models: 1 mm, Rotterdam criteria,[28] Gershenwald criteria.[39]

†All SN tumor burden indices were significant predictors when included in multivariate models, but MaxSize model was slightly stronger, and the results of this model are shown.

‡Interactions between CLND status and SN tumor features were not significant.

HR, hazard ratio; *P*, significance level; 5YS and 10YS, proportion of patients surviving at 5 and 10 years after diagnosis of primary melanoma; 95% CI, 95% confidence interval for HR.

Not significant in univariate analysis: Clark level, desmoplasia, tumor-infiltrating lymphocytes, regression, neurotropism, mitotic rate, number of SN harvested, % of SNs positive.

of 40.1 months (range 7.5–83.7 months), 5 (20.8%) patients died of melanoma, 2 patients died of unknown causes, and 1 patient remained alive with residual melanoma. These findings argue against the use of MaxSize as a sole criterion for estimating prognosis in SN-positive melanoma patients. Further studies are clearly needed to determine the clinical significance of very small melanoma deposits in SNs.

It could be argued that the differences in our findings compared with other studies using the Rotterdam criteria are due to the differences in sectioning protocol used in our study and the EORTC protocol. Many authors have demonstrated that more extensive protocols for sectioning SNs will result in a larger number of metastases being detected.[49,50,54,55] It is therefore possible that protocols involving more extensive sectioning will result in higher values for SN tumor indices than less extensive protocols, by virtue of examining a greater cross-sectional area of the SN. In addition, it has recently been demonstrated that melanoma metastases do not preferentially localize to a particular region of the lymph node, and that they may be found anywhere within the node.[56] Therefore, protocols in which a greater extent of the SN is examined may yield more representative values for SN tumor burden indices. However, as we have stated previously,[57,58] the extent of the protocol used should be based

TABLE 4. Association of Clinico-Pathologic Parameters with Distant Metastasis-Free Survival

				Univariate Analysis			Multivariate Analysis		
Parameter	**Level**	**5YS**	**10YS**	***P***	**HR**	**Parameter**	**Level**	**5YS**	**10YS**
Clinical									
Age	<50 years (ref)	67.9	65.6		1.00				
	≥50 years	60.0	39.7	0.10	1.38	0.94–2.04			
Sex	Female (ref)	72.9	53.5		1.00			1.00	
	Male	57.3	51.0	0.02	1.63	1.08–2.46	0.005	2.16	1.26–3.71
Primary tumor									
Thickness	0.01–1.00 mm (ref)	88.2	88.2		1.00			1.00	
	1.01–2.00 mm	67.2	50.4	0.18	2.67	0.63–11.33	0.38	2.52	0.33–19.28
	2.01–4.00 mm	65.9	48.3	0.07	3.70	0.90–15.28	0.63	1.66	0.21–13.02
	>4.00 mm	49.0	49.0	0.02	5.66	1.36–23.62	0.60	1.75	0.22–14.13
MR*	0 (ref)	78.3	39.2		1.00			1.00	
	1–2	76.4	47.8	0.78	0.85	0.27–2.68	0.71	1.30	0.34–5.02
	3–5	71.1	62.2	0.81	1.14	0.39–3.29	0.84	0.87	0.23–3.25
	6–10	51.9	51.9	0.11	2.38	0.82–6.91	0.77	1.22	0.32–4.69
	>10	34.2	34.2	0.04	3.16	1.08–9.26	0.45	1.68	0.43–6.52
Ulceration	Absent	72.0	58.3		1.00			1.00	
	Present	47.7	45.0	<0.001	2.15	1.47–3.14	0.03	1.85	1.06–3.24
TILs	Absent	60.3	47.7		1.00			1.00	
	Present	75.9	60.7	0.06	0.66	0.43–1.01	0.003	0.44	0.26–0.76
Satellites	Absent	64.8	52.4		1.00			1.00	
	Present	19.9	19.9	0.001	3.07	1.54–6.10	0.03	2.75	1.10–6.89
LVI	Absent	66.1	52.8		1.00			1.00	
	Present	34.8	34.8	0.001	2.49	1.48–4.18	0.06	1.91	0.97–3.77
No. of SNs									
NoPosSN	1 (ref)	65.6	52.5		1.00			1.00	
	2	54.7	54.7	0.07	1.50	0.96–2.34	0.83	1.06	0.61–1.87
	≥3	55.9	55.9	0.15	2.11	0.77–5.79	0.10	2.75	0.84–9.05
SN tumor features									
Maximum size†	<0.50	74.6	49.8		1.00			1.00	
	0.51–2.00	65.0	50.3	0.40	1.28	0.72–2.28	0.41	1.35	0.66–2.79
	2.01–10.00	60.1	54.1	0.04	1.85	1.04–3.28	0.18	1.67	0.79–3.54
	>10.00	16.9	16.9	<0.001	5.24	2.37–11.59	0.03	3.15	1.13–8.78
%CS‡	≤1% (ref)	69.3	49.9		1.00				
	>1 and ≤10%	67.9	56.6	0.81	1.06	0.67–1.69			
	>10%	45.9	45.9	0.002	1.99	1.28–3.08			
TPD‡	≤0.3 mm (ref)	67.8	62.1		1.00				
	>0.3 and ≤1.0 mm	67.6	37.4	0.53	1.18	0.70–2.01			
	>1.0 mm	57.9	50.6	0.07	1.50	0.97–2.31			
Intranodal location‡	Subcapsular	69.4	56.8		1.00				
	Parenchymal	57.3	46.3	0.049	1.47	1.00–2.15			
ENS	Absent	65.0	52.8		1.00			1.00	
	Present	31.9	31.9	0.001	3.01	1.61–5.63	0.002	3.70	1.64–8.36
PLI	Absent	66.6	53.7		1.00			1.00	
	Present	43.0	43.0	0.006	1.93	1.21–3.08	0.08	1.75	0.94–3.23
CLND status§	Negative	67.4	57.5		1.00			1.00	
	Positive	43.0	22.9	0.001	2.19	1.37–3.51	0.36	1.31	0.74–2.31

*MR stratified as 0 vs. ≥1 was not significant.

†Other MaxSize cutoffs were not significant in multivariate models: 1 mm, 2 mm, Rotterdam criteria.[28]

‡Not significant in separate multivariate models.

§Interactions between CLND status and SN tumor features were not significant.

HR, hazard ratio; *P*, significance level; 5YS and 10YS, proportion of patients surviving at 5 and 10 years after diagnosis of primary melanoma; 95% CI, 95% confidence interval for HR.

Not significant in univariate analysis: primary site, Clark level, desmoplasia, regression, neurotropism, mitotic rate, number of SN harvested, % of SNs positive.

on the balance between the benefits derived from the chosen protocol (in terms of accurate assessment of SN status and prediction of prognosis), and the financial, temporal, and labor demands of the protocol. Unfortunately, comparison of different sectioning protocols and determination of their relative benefits and shortcomings in our patient cohort was beyond the scope of this study. Such a comparison (preferably in a large patient cohort) is urgently needed to establish the optimal sectioning protocol for SNs to enable standardization of clinical practice and comparability of the results of research studies in this field.

TABLE 5. Association of Clinico-Pathologic Parameters with Melanoma-Specific Survival

				Univariate Analysis			Multivariate Analysis		
Parameter	**Level**	**5YS**	**10YS**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**
Clinical									
Age	<50 years (ref)	68.5	64.1		1.00			1.00	
	≥50 years	56.0	30.0	0.008	1.70	1.15–2.53	0.04	1.64	1.01–2.67
Sex	Female (ref)	68.9	40.3		1.00				
	Male	56.3	48.6	0.12	1.36	0.92–2.02			
Primary tumor									
Thickness	0.01–1.00 mm (ref)	84.7	84.7		1.00			1.00	
	1.01–2.00 mm	67.4	47.4	0.24	2.40	0.56–10.18	0.41	2.36	0.31–18.32
	2.01–4.00 mm	64.8	39.4	0.08	3.49	0.85–14.37	0.58	1.80	0.23–14.22
	>4.00 mm	43.3	40.6	0.01	5.89	1.42–24.41	0.43	2.33	0.29–18.76
MR*	0 (ref)	83.0	41.5		1.00			1.00	
	1–2	80.0	45.0	0.69	1.30	0.36–4.65	0.15	3.35	0.64–17.70
	3–5	63.6	48.4	0.33	1.81	0.55–6.04	0.29	2.36	0.48–11.66
	6–10	50.4	43.4	0.03	3.76	1.12–12.59	0.23	2.69	0.53–13.53
	>10	36.3	27.2	0.009	5.09	1.51–17.18	0.08	4.24	0.85–21.16
Ulceration	Absent (ref)	73.3	54.1		1.00			1.00	
	Present	41.9	31.5	<0.001	2.80	1.92–4.08	0.001	2.55	1.44–4.52
Satellites	Absent (ref)	63.7	44.3					1.00	
	Present	8.5	8.5	<0.001	5.02	2.78–9.04	<0.001	3.95	1.83–8.49
LVI	Absent (ref)	64.0	44.2		1.00			1.00	
	Present	34.7	26.1	0.001	2.38	1.43–3.95	0.50	1.27	0.64–2.52
No. of SNs									
NoPosSN	1 (ref)	64.6	44.2		1.00			1.00	
	2	49.3	49.3	0.03	1.61	1.04–2.49	0.36	1.29	0.75–2.21
	≥3	59.5	59.5	0.10	2.35	0.86–6.44	0.16	2.33	0.73–7.45
SN tumor features									
Maximum size†,‡	≤2 mm (ref)	63.9	41.7		1.00				
	>2 mm	56.2	43.9	0.05	1.45	1.00–2.10			
%CS	≤1% (ref)	59.9	40.4		1.00			1.00	
	>1 and ≤10%	76.3	47.2	0.52	0.86	0.53–1.37	0.61	0.86	0.47–1.55
	>10%	44.9	38.2	0.004	1.86	1.22–2.84	0.24	1.38	0.81–2.37
TPD‡	≤0.3 mm (ref)	65.4	50.3		1.00				
	>0.3 and ≤1.0 mm	60.6	33.8	0.22	1.38	0.83–2.29			
	>1.0 mm	59.6	44.9	0.09	1.46	0.95–2.25			
Intranodal location‡	Subcapsular	67.1	51.0		1.00				
	Parenchymal	56.3	36.4	0.02	1.58	1.08–2.30			
ENS	Absent (ref)	62.9	44.3	0.001	2.95			1.00	
	Present	30.0	30.0			1.58–5.51	0.03	2.34	1.06–5.13
PLI	Absent (ref)	65.1	47.3		1.00			1.00	
	Present	39.1	24.4	0.001	2.14	1.37–3.33	0.10	1.67	0.92–3.04
CLND status§	Negative	66.7	48.3		1.00			1.00	
	Positive	44.4	26.7	0.004	1.98	1.243.17	0.48	1.24	0.69–2.23

*MR stratified as 0 vs. ≥1 was not significant.

†Other MaxSize cutoffs were not significant in multivariate models: 1 mm, Rotterdam criteria,[28] Gershenwald criteria.[39]

‡All were nonsignificant predictors when included in separate MVA models (all of which were roughly equally predictive); results of %CS model (highest test statistic) are shown.

§Interactions between CLND status and SN tumor features were not significant.

HR, hazard ratio; *P*, significance level; 5YS and 10YS, proportion of patients surviving at 5 and 10 years after diagnosis of primary melanoma; 95% CI, 95% confidence interval for HR.

Not significant in univariate analysis: primary site, Clark level, desmoplasia, tumor-infiltrating lymphocytes, regression, neurotropism, number of SN harvested, % of SNs positive.

A major strength of this study is the range of clinicopathologic parameters (including ENS and PLI) analyzed in a large cohort of uniformly treated patients. Furthermore, multivariate survival analyses controlled for CLND status. This was done because the CLND status in SN-positive patients is usually known soon after the diagnosis and excision of primary melanoma, and we wished to determine parameters that were predictive of clinical outcomes, regardless of the CLND status.

The results show that clinical examination and thorough histologic evaluation of primary tumor and SN features in melanoma patients provide important prognostic information with regard to disease recurrence, distant metastasis, and survival. This information will be useful in providing patients with an accurate estimate of prognosis, will aid management decisions, and will assist in selecting patients for entry into clinical trials.

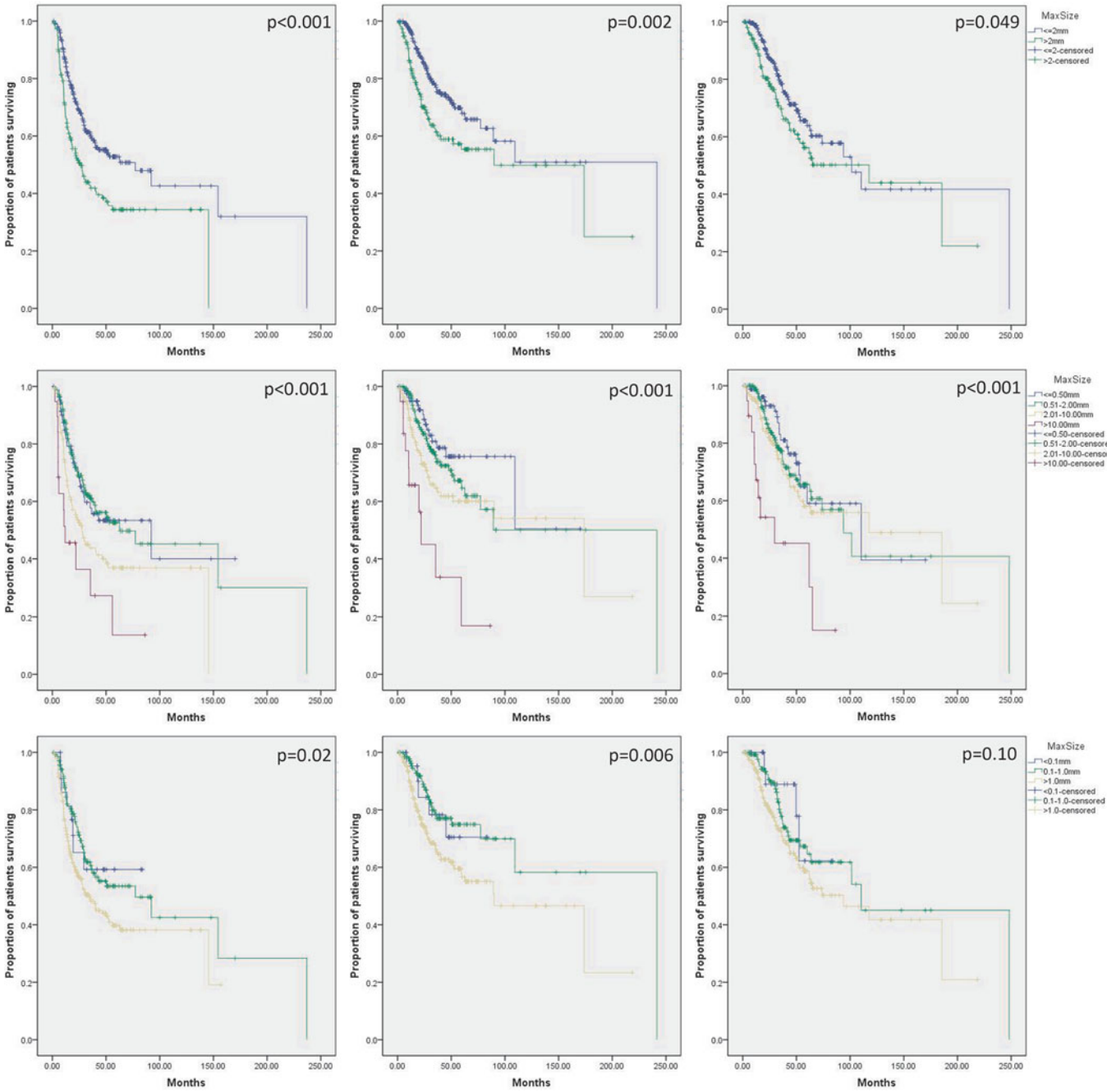

FIGURE 1. Association of patient survival with maximum size of largest SN tumor deposit (using various criteria): top row–≤2 mm vs. >2 mm; middle row (criteria used by Gershenwald et al)–≤0.5 mm, 0.5–2.0 mm, 2.0–10.0 mm, and >10.0 mm; bottom row (criteria used by Rotterdam group)–<0.1 mm, 0.1–1.0 mm, and >1.0 mm. Survival estimates using Kaplan-Meier method; significance levels (*P* values) calculated using log-rank tests.

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