Prognostic Significance of Microscopic Tumor Burden in Sentinel Lymph Node in Patients with Cutaneous Melanoma

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Abstract. *Background/Aim: Sentinel lymph node (SLN) biopsy provides useful prognostic information for patients with melanoma. The present study sought to determine the prognostic value of SLN tumor burden on overall survival (OS) and disease-free survival (DFS). We also assessed its association with non-sentinel lympth node (NSLN) involvement. Patients and Methods: We conducted a retrospective review of 138 patients with cutaneous melanoma, who were found to have positive SLNs from 2000 to 2011. SLN tumor burden was measured in the maximum diameter of the largest tumor focus. OS and DFS were assessed by the Kaplan-Meier method and Cox proportional hazard regression model. A logistic regression model was used to evaluate the association between SLN tumor burden and NSLN positivity. Results: On multivariable analysis, SLN tumor burden was significantly associated with OS (hazard ratio (HR)$_{>1\ vs.\ \leq 1mm}$=5.15; 95% confidence interval (CI)=2.32-11.44; p<0.0001) and DFS rate (HR>1 vs. ≤1mm=3.02; 95% CI=1.37-6.67; p=0.0064). On univariate analysis, SLN tumor burden was significantly associated with NSLN positivity (OR>1 vs. ≤1mm=3.41; 95% CI=1.03-11.27; p=0.04). Conclusion: SLN tumor burden, by measuring the maximum diameter of the largest tumor focus, is significantly associated with OS, DFS and NSLN involvement.*

Sentinel lymph node (SLN) status is one of the strongest prognostic factors of survival in patients with melanoma (1, 2). The 5-year survival rate was 72.3% for patients with tumor-positive SLNs and 90.2% for patients with tumor-negative SLNs in the MSLT-1 study (3). The usual but controversial recommendation for patients with cutaneous melanoma and a positive SLN is a complete lymph node dissection (CLND) in order to achieve regional disease control. Whether undergoing CLND improves survival is being investigated by the ongoing Multicenter Selective Lymphadenectomy Trial II (4).

The frequency of finding additional metastases in non-sentinel lymph node (NSLN) in patients with positive SLN is variable with a reported range of 6-29% (5-17). It is generally thought that only patients with positive NSLN would derive benefit from CLND. Therefore, there have been significant efforts to identify a group of patients who were not likely to have a positive NSLN in an attempt to spare them from CLND, which is commonly associated with considerable morbidity (6-8, 10-28). One of the pathologic parameters that have been evaluated in this regard is SLN tumor burden. There is mounting evidence that SLN tumor burden is prognostic of overall survival, disease recurrence and NSLN metastases but the microscopic classifications used in different study groups were variable and so were the reported results (6-8, 10, 15, 18-20, 29, 30).

In the present study, we examined the prognostic role of tumor burden in SLN on overall survival (OS) and disease-free survival (DFS) in patients with cutaneous melanoma using different microscopic classifications. We also sought to assess clinical and histological predictors of additional NSLN metastases.

Patients and Methods

Patients. The database of a plastic surgery clinic in Minnesota was searched for patients with a single primary cutaneous melanoma who had one or more positive SLNs from 2000 to 2011. The primary melanomas and SLN were reported (or reviewed in cases diagnosed in external laboratory) by pathologists at Abbott

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Northwestern Hospital, Minnesota. Patients were offered SLN biopsy if they had a melanoma of >1.0 mm in Breslow depth or <1.0 mm in Breslow depth with high-risk histological features, such as primary tumor ulceration and high Clark level, especially if Breslow thickness was greater than 0.75 mm. Patients had a wide local excision of a melanoma at the time of SLN biopsy. SLN biopsy was performed using the triple technique (31). Patients with more than one primary melanoma and those whose histologic slides were not available for review were excluded from the study. CLND was not performed in all SLN positive patients due to several reasons including refusal of further treatment and presence of minimal tumor burden in the SLN. SLN biopsies were performed by a single plastic surgeon. After SLN biopsy, patients were followed at the plastic surgery clinic and their medical oncology clinic. Due to close collaboration between the plastic surgery clinic and medical oncology clinic, most of the oncology follow-up information was available for review. Approval of this study was granted by the University of Minnesota Institutional Review Board.

Pathological work-up and review. Harvested SLNs were fixed in formalin, embedded in paraffin and underwent multistep sectioning. A pathologist or pathology assistant examined the specimen grossly and identified the SLNs. SLNs were cut at 2-mm intervals parallel to the long axis. Each processed paraffin block from the sentinel nodes was cut at five levels separated by about 40 micrometers depending on the size and thickness of the node. Levels 1, 3 and 5 were for routine hematoxylin (H) and eosin (E) staining. Levels 2 and 4 were to be placed on immunohistochemical staining (IHC) slides. Melan A and Melanoma cocktail (Tyrosinase and HMB45; supplier, address) were used for IHC staining. The slides that did not have information on SLN tumor burden in the primary pathology report were re-examined by a dedicated dermatopahtologist. Tumor burden was obtained by measuring the maximum diameter of the largest tumor focus. When there were two or more positive SLNs, the one with the largest maximum diameter in any of the SLNs was used. CLND specimens were analyzed by routine H and E analysis only.

Statistical analysis. The study end-points were OS and DFS after diagnosis of stage III disease. OS and DFS were calculated from time of SLN biopsy until death and first melanoma recurrence. Patients without such events were censored at their last follow-up. Outcomes were ascertained using a combination of clinical records and the Social Security Death Index (SSDI). We assessed six different cutoff points of SLN tumor burden based on a review of recently published literature (7, 10, 12, 15, 16, 18, 20); SLN tumor burden I (≤1, >1 mm), SLN tumor burden II (≤0.1, 0.1 to 1, or >1 mm), SLN tumor burden III (≤0.2, 0.2 to 1, or >1 mm), SLN tumor burden IV (≤2, >2 mm), SLN tumor burden V (≤3, >3 mm), SLN tumor burden VI (≤4, >4 mm). Because of multicollinearity between different criteria for SLN tumor burden, separate multivariate analyses were performed. SLN tumor burden was also assessed as a continuous variable on a linear scale. To determine the best cutoff values for SLN tumor burden, the Wald chi-square of each microscopic classification obtained by multivariable analysis was compared. Survival estimates were evaluated by the Kaplan-Meier method and the log-rank test. Multivariate analyses, to determine the prognostic values of covariates in regard to OS and DFS, were performed using the Cox's proportional-hazard model with the entry criterion of a p-value <0.10 in the univariate analyses. Breslow approximation was used to handle ties. Univariate logistic regression model was used to evaluate the association of various clinicopathologic parameters with NSLN positivity. Variables with a p-value <0.10 were included in the multivariate logistic regression model. To assess whether undergoing CLND affects OS and DFS, additional analyses were performed. Statistical analyses were performed by the SAS software, version 9.3. p-Values <0.05 were considered to be statistically significant in all analyses.

Results

Between 2000 and 2011, there were 1,157 patients who underwent sentinel lymph node biopsy. One hundred and fifty-six (13.4%) patients of those enrolled were found to have positive SLN status. After excluding 8 patients who underwent SLN biopsy for recurrent melanoma, 5 patients with no available pathology report or slide, 4 patients with no follow-up and 1 patient with two primary cutaneous melanomas, 138 patients were included in the study. Clinicopathological features of the 138 patients with positive SLNs are listed in Table I. The average age was 53.9 years (interquartile range, 43-64 years). The primary disease sites were the head and neck (n=31, 22.5%), extremities (n=47, 34.1%) and trunk (n=60, 43.4%). The mean and median Breslow thicknesses were 2.7 and 2.0 mm, respectively. Seventy-five percent of patients had one positive SLN and 25% of patients had more than one SLN. Among the 138 patients, 111 patients opted to undergo CLND. After excluding one patient whose NSLN status was unavailable, we found that 13 patients (11.8%) had additional metastases in NSLN. The median and mean follow-up time were 22 and 36 months, respectively (range=2-133).

Overall survival and disease-free survival. Results of univariate and multivariate analyses of risk factors for OS and DFS are summarized in Tables II and III. The Kaplan-Meier analysis of OS and DFS stratified by different microscopic classifications are shown in Figure 1.

On univariate analysis of OS, age on a continuous scale (hazard ratio (HR)=1.04; 95% confidence interval (CI)=1.02-1.07; p=0.0002), Breslow thickness ($HR_{T4\ vs.\ T1/T2}$=6.26; 95% CI=2.68-14.61; p<0.0001) and SLN tumor burden I-VI ($HR_{>1\ vs.\ \leq 1mm}$=5.63; 95% CI=2.72-11.65; p<0.0001) were significantly associated with worse OS (see Table II for results of SLN tumor burden II-VI). On multivariate analysis of OS, which included variables with a p-value <0.1 on univariate analysis, age on a continuous scale (HR=1.04; 95% CI=1.02-1.07; p=0.0003), Breslow thickness ($HR_{T4\ vs.\ T1/T2}$=3.71; 95% CI=1.43-9.63; p=0.007) and SLN tumor burden I-VI ($HR_{>1\ vs.\ \leq 1mm}$=5.15; 95% CI=2.32-11.44; p<0.0001) remained statistically significant. When SLN tumor burden was assessed as a continuous variable, it was significantly associated with OS (HR=1.076; 95% CI=1.035-1.117; p=0.0002).

On univariate analysis of DFS (Table III), Breslow thickness ($HR_{T4\ vs.\ T1/T2}$=6.77; 95% CI=2.95-15.49; p<0.0001), presence of ulceration (HR=2.51; 95% CI=1.35-4.65; p=0.004), angiolymphatic invasion (HR=4.27; 95% CI=2.15-8.47; p<0.0001), Clark level ($HR_{5\ vs.\ 2/3}$=9.61; 95% CI=1.20-77.04; p=0.03) and SLN tumor burden I-VI were significantly associated with a shorter DFS. Multivariate analyses with inclusion of variables with a p-value <0.10 on univariate analysis revealed that only Breslow thickness ($HR_{T3\ vs.\ T1/T2}$=2.78; 95% CI=1.10-7.00; p=0.03) and SLN tumor burden I-VI were significant prognostic factors for DFS.

Among 6 microscopic classifications, the cutoff value of 1 mm was identified as the most significant cutoff point for both OS (p<0.0001) and DFS (p=0.0064) as the Wald chi-square statistic was maximized with this cutoff point (data not shown). As shown in Figure 1a, the estimated OS at 5 years was 75.6% in patients with SLN tumor burden ≤1mm and 34.6% in patients with SLN tumor burden >1mm. The curves of patient with SLN tumor burden ≤0.1mm and patients with SLN tumor burden of 0.1-1.0 mm tended to collapse, which indicates similar OS between two groups (Figure 1b). A similar pattern was observed in regards to DFS (Figure 1c-d).

Our data indicate that patients with positive SLNs show a pattern of recurrence and mortality, which is related to the size of the SLN deposits. Patients with node metastases of ≥1 mm showed a recurrence risk of 74% by 48 months. In contrast, patients with nodal metastases of <1 mm showed a recurrence risk of 35% at 48 months. After 48 months, no recurrences were seen in patients with <1 mm SLN deposit (Figure 1c).

Of note, CLND status was not associated with OS ($HR_{CLND\ vs.\ no\ CLND}$=0.68; 95% CI=0.31-1.36; p=0.68). On univariate analysis of DFS, CLND status was not associated with DFS ($HR_{CLND\ vs.\ no\ CLND}$=1.14; 95% CI=0.57-2.26; p=0.72). The Kaplan-Meier curves show no significant difference in OS and DFS (data not shown).

Predictors of metastatic disease in NSLNs. Table IV shows the results of univariate and multivariate analyses of clinicopathologic factors regarding NSLN status. By univariate analysis, Breslow thickness (≤2 *vs*. >4mm; OR 5.10; 95% CI=1.33-19.54; p=0.01), angiolymphatic invasion (OR=4.89; 95% CI=1.36-17.61; p=0.02), number of positive SLN (cutoff point: 1; OR=4.87; 95% CI=1.45-16.30; p=0.01) and SLN tumor burden I-VI were significantly associated with positive NSLN status. By multivariate analysis including significant variables, only the number of positive SLN (OR=4.27; 95% CI=1.15-15.82; p=0.03) remained statistically significant, whereas Breslow thickness, angiolymphatic invasion and SLN tumor burden did not. Patients with SLN tumor burden equal to or less than 0.1 mm and 0.2 mm were found to have a low NSLN positivity rate of 3.9% and 3.2%, respectively, as opposed to 20.5% in patients with SLN tumor burden greater than 1.0 mm.

Table I. *Clinical and pathological characteristic of 138 melanoma patients with positive sentinel lymph nodes*.

Characteristics	No.	%
Age at SLN dissection (years)		
Mean	53.9	
Median	54	
IQR	43-64	
Gender		
Male	84	60.9%
Female	54	39.1%
Location		
Head and neck	31	22.5%
Upper extremity	16	11.6%
Lower extremity	31	22.5%
Trunk	60	43.4%
Breslow thickness		
Mean	2.7	
Median	2.0	
0-1.0 mm	17	12.4%
1.01-2.0 mm	54	39.4%
2.01-4.0 mm	43	31.4%
>4.0 mm	23	16.8%
Clark level		
II	1	0.7%
III	12	8.8%
IV	113	82.5%
V	11	8.0%
Ulceration		
Present	48	34.8%
Absent	82	59.4%
Unknown	8	5.8%
Angiolymphatic invasion		
Present	21	15.2%
Absent	107	77.5%
Indeterminate/Unknown	10	7.3%
Perineural invasion		
Present	5	3.6%
Absent	125	90.6%
Indeterminate/Unknown	8	5.8%
Regression		
Present	9	6.5%
Absent	89	64.5%
Unknown	40	29.0%
Satellite		
Present	5	3.6%
Absent	101	73.2%
Indeterminate/Unknown	32	23.2%
Type of melanoma		
Superficial spreading	36	26.0%
Nodular	32	23.2%
Acral-lentiginous	2	1.5%
Superficial spreading/Nodular	2	1.5%
Desmoplastic	1	0.7%
Unknown	65	47.1%
Number of Positive SLN		
1	103	74.6%
≥2	35	25.4%
NSLN status		
Positive	13	11.8%
Negative	97	88.2%
Vital status		
Alive	101	73.2%
Dead	34	24.6%
Unknown	3	2.2%

SLN, Sentinel lymph node; IQR, interquartile range.

Table II. *Univariate and multivariate analyses of clinicopathological parameters regarding overall survival.*

		Univariate			Multivariate**		
Variable	No.	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age, years		1.04	1.02-1.07	0.0002	1.04	1.02-1.07	0.0003
Gender							
Female	54	1.00			1.00		
Male	84	1.57	0.77-3.24	0.21	1.23	0.53-2.37	0.76
Location							
Axial*	91	1.00			1.00		
Extremity	47	0.48	0.21-1.10	0.08	0.48	0.20-1.15	0.10
Breslow thickness							
T1/T2	71	1.00			1.00		
T3	43	2.47	1.02-5.97	0.045	2.56	0.98-6.68	0.055
T4	23	6.26	2.68-14.61	<0.0001	3.71	1.43-9.63	0.007
Ulceration							
Absent/unknown	90	1.00					
Present	48	1.60	0.81-3.14	0.17			
Angiolymphatic invasion							
Absent/unknown	117	1.00					
Present	21	1.67	0.75-3.69	0.21			
Perineural invasion							
Absent/unknown	133	1.00					
Present	5	2.10	0.64-6.92	0.22			
Number of positive SLN							
1	103	1.00					
>1	35	1.10	0.51-2.37	0.81			
NSLN status							
Negative	97	1.00					
Positive	13	2.05	0.76-5.55	0.16			
Total number of positive LN		1.09	0.88-1.34	0.43			
SLN tumor burden I (mm)							
≤1	92	1.00			1.00		
>1	46	5.63	2.72-11.65	<0.0001	5.15	2.32-11.44	<0.0001
SLN tumor burden II (mm)							
≤0.1	41	1.00			1.00		
0.1-1.0	47	0.87	0.26-2.92	0.82	0.89	0.25-3.24	0.86
>1.0	46	4.74	1.79-12.56	0.002	4.47	1.61-12.39	0.004
SLN tumor burden III (mm)							
≤0.2	48	1.00			1.00		
0.2-1.0	40	0.95	0.29-3.17	0.96	1.03	0.29-3.70	0.96
>1.0	46	5.06	2.05-12.50	0.0004	4.43	1.68-11.66	0.003
SLN tumor burden IV (mm)							
≤2	105	1.00			1.00		
>2	32	4.71	2.36-9.41	<0.0001	4.82	2.18-10.69	0.0001
SLN tumor burden V (mm)							
≤3	111	1.00			1.00		
>3	26	4.63	2.29-9.37	<0.0001	3.70	1.66-8.25	0.001
SLN tumor burden VI (mm)							
≤4	116	1.00			1.00		
>4	21	4.20	2.05-8.60	<0.0001	3.03	1.32-6.94	0.009

HR, Hazard ratio; SLN, sentinel lymph node; NSLN, nonsentinel lymph node; LN, lymph node; CI, confidence interval. *Axial: head and neck, trunk. **Because of multicollinearity between different criteria for SLN tumor burden, separate multivariate analyses were performed. The displayed results of other variables (age, sex, location, Breslow) were obtained using cut-off points of SLN tumor burden I.

Discussion

Various microscopic classifications to measure SLN tumor burden including maximum diameter of the largest tumor focus, invasion depth (32), and metastatic area (26) have been assessed for their association with prognosis in patients with melanoma. Measurement of maximum diameter of the largest tumor focus is an easy and best reproducible prognostic factor

Table III. *Univariate and multivariate analyses of clinicopathological parameters regarding disease-free survival.*

	Univariate			Multivariate**		
Variable	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age, years	1.02	1.00-1.04	0.04	1.02	1.00-1.04	0.12
Gender						
Female	1.00			1.00		
Male	1.06	0.57-1.97	0.87	0.86	0.40-1.81	0.68
Location						
Axial*	1.00					
Extremity	1.34	0.70-2.55	0.38			
Breslow thickness						
T1/T2	1.00			1.00		
T3	4.43	1.99-9.86	0.0003	2.78	1.10-7.00	0.03
T4	6.77	2.95-15.49	<0.0001	2.48	0.81-7.57	0.11
Ulceration						
Absent/unknown	1.00			1.00		
Present	2.51	1.35-4.65	0.004	1.12	0.64-1.94	0.70
Angiolymphatic invasion						
Absent/unknown	1.00			1.00		
Present	4.27	2.15-8.47	<0.0001	1.26	0.69-2.29	0.46
Perineural invasion						
Absent/unknown	1.00					
Present	1.77	0.43-7.35	0.43			
Clark level						
2/3	1.00			1.00		
4	4.45	0.61-32.56	0.14	4.67	0.51-43.08	0.17
5	9.61	1.20-77.04	0.03	2.68	0.22-32.49	0.44
Number of positive SLN						
1	1.00			1.00		
>1	1.86	0.99-3.50	0.056	2.01	0.78-5.12	0.15
NSLN status						
Negative	1.00			1.00		
Positive	2.19	0.95-5.05	0.07	1.00	0.30-3.32	0.99
Total number of positive LN	1.10	0.95-1.27	0.21			
SLN tumor burden I (mm)						
≤1	1.00			1.00		
>1	3.45	1.84-6.45	0.0001	3.02	1.37-6.67	0.0064
SLN tumor burden II (mm)						
≤0.1	1.00			1.00		
0.1-1.0	1.55	0.66-3.64	0.32	1.64	0.41-6.58	0.48
>1.0	3.79	1.73-8.31	0.0009	4.43	1.22-16.13	0.02
SLN tumor burden III (mm)						
≤0.2	1.00			1.00		
0.2-1.0	1.42	0.64-3.19	0.39	1.23	0.34-4.40	0.75
>1.0	3.51	1.75-7.04	0.0004	3.92	1.24-12.45	0.02
SLN tumor burden IV (mm)						
≤2	1.00			1.00		
>2	2.89	1.68-4.97	0.0001	2.44	1.08-5.49	0.03
SLN tumor burden V (mm)						
≤3	1.00			1.00		
>3	2.72	1.53-4.86	0.0007	1.96	0.78-4.87	0.15
SLN tumor burden VI (mm)						
≤4	1.00			1.00		
>4	2.21	1.18-4.13	0.01	1.38	0.46-4.16	0.57

HR, Hazard ratio; SLN, sentinel lymph node; NSLN, non-sentinel lymph node; LN, lymph node; CI, confidence interval. *Axial: head and neck, trunk. **Because of multicollinearity between different criteria for SLN tumor burden, separate multivariate analyses were performed. The displayed results of other variables (age, sex, Breslow, ulceration, angiolymphatic invasion, number of positive SLN, NSLN status) were obtained using cut-off points of SLN tumor burden I.

Table IV. *Univariate and multivariate analyses of covariates regarding nonsentinel lymph node positivity.*

Variable	No.	No. of NSLN positive patients (%)	Univariate		Multivariate**	
			Odds ratio (95% CI)	*p*-Value	Odds ratio (95% CI)	*p*-Value
Age, years						
≤50	47	5 (10.6)	1.00		1.00	
>50	63	8 (12.7)	1.22 (0.37-4.00)	0.74	2.05 (0.55-10.15)	0.36
Gender						
Male	67	9 (13.4)	1.00			
Female	43	4 (9.3)	0.66 (0.19-2.30)	0.52		
Location of Tumor						
Axial	75	11 (14.7)	1.00			
Extremity	35	2 (5.7)	0.35 (0.07-1.69)	0.19		
Breslow thickness (mm)						
≤2	56	5 (8.9)	1.00		1.00	
2-4	35	2 (5.7)	0.62 (0.11-3.38)	0.11	0.79 (0.13-4.66)	0.35
>4	18	6 (33.3)	5.10 (1.33-19.54)	0.01	2.98 (0.54-16.35)	0.14
Ulceration						
Absent	69	6 (8.7)	1.00			
Present	41	7 (17.1)	2.16 (0.67-6.95)	0.20		
Angiolymphatic invasion						
Absent/Unknown	94	8 (8.5)	1.00		1.00	
Present	16	5 (31.3)	4.89 (1.36-17.61)	0.02	1.11 (0.39-3.16)	0.84
Perineural invasion*						
Absent/Unknown	106	13 (11.8)	1.00			
Present	4	0 (0)	0.77 (0.03-21.21)	0.88		
Clark level						
II/III	11	1 (9.1)	1.00			
IV	89	9 (10.1)	1.13 (0.13-9.83)	0.40		
V	10	3 (30.0)	4.29 (0.37-50.21)	0.11		
No. of positive SLN						
1	78	5 (6.4)	1.00		1.00	
≥2	32	8 (25.0)	4.87 (1.45-16.30)	0.01	4.27 (1.15-15.82)	0.03
SLN tumor burden I (mm)						
≤1	71	5 (7.0)	1.00		1.00	
>1	39	8 (20.5)	3.41 (1.03-11.27)	0.04	2.05 (0.44-9.56)	0.36
SLN tumor burden II (mm)						
≤0.1	26	1 (3.9)	1.00		1.00	
0.1-1.0	42	4 (9.5)	2.63 (0.28-24.94)	0.96	2.44 (0.23-25.99)	0.78
>1.0	39	8 (20.5)	6.45 (0.76-55.09)	0.05	3.74 (0.34-40.80)	0.31
SLN tumor burden III (mm)						
≤0.2	31	1 (3.2)	1.00		1.00	
0.2-1.0	37	4 (10.5)	3.53 (0.37-33.34)	0.59	3.28 (0.31-34.30)	0.61
>1.0	39	8 (21.6)	8.00 (0.94-67.96)	0.04	4.61 (0.43-49.78)	0.27
SLN tumor burden IV (mm)						
≤2	83	7 (8.4)	1.00		1.00	
>2	26	6 (23.1)	3.26 (0.99-10.78)	0.05	1.58 (0.33-7.73)	0.57
SLN tumor burden V (mm)						
≤3	89	7 (7.9)	1.00		1.00	
>3	20	6 (30.0)	5.02 (1.47-17.16)	0.01	2.68 (0.60-12.02)	0.20
SLN tumor burden VI (mm)						
≤4	93	8 (8.6)	1.00		1.00	
>4	16	5 (31.3)	4.83 (1.34-17.40)	0.02	1.98 (0.36-10.83)	0.43

SLN, Sentinel lymph node; NSLN, nonsentinel lymph node; CI, confidence interval. *Used Firth estimation to deal with the issue of quasi or complete separation of data points. **Because of multicollinearity between different criteria for SLN tumor burden, separate multivariate analyses were performed. The displayed results of other variables (age, Breslow, angiolymphatic invasion, number of positive SLN) were obtained using cut-off points of SLN tumor burden I.

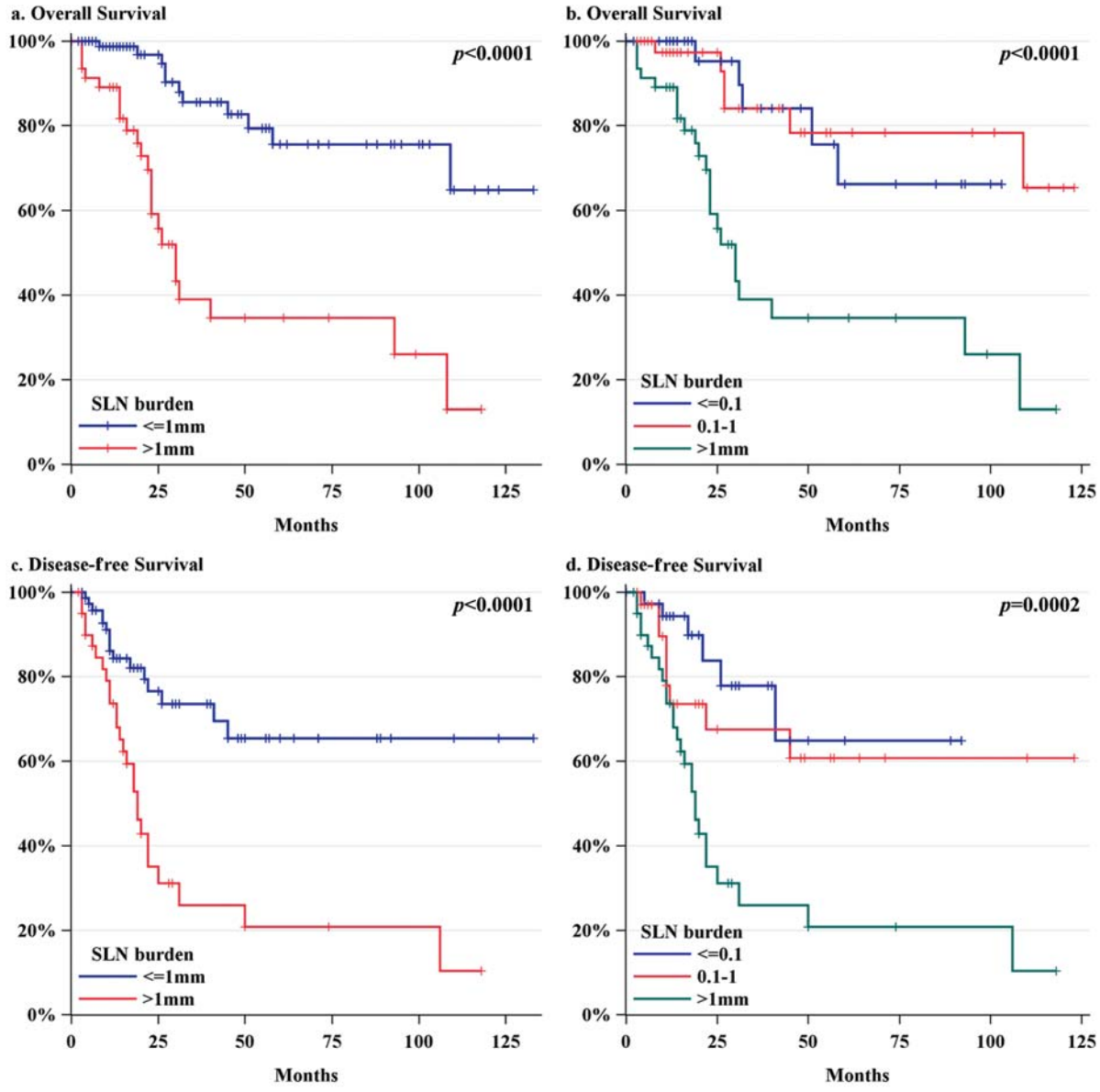

Figure 1. *a. Kaplan-Meier estimated overall survival for melanoma patients with maximum metastasis size ≤1 mm and >1 mm. b. Kaplan-Meier estimated recurrence for melanoma patients with maximum metastasis size ≤0.1 mm, 0.1-1.0 mm, or >1 mm. c. Kaplan-Meier estimated recurrence for melanoma patients with maximum metastasis size ≤1 mm and >1 mm. d. Kaplan-Meier estimated recurrence for melanoma patients with maximum metastasis size ≤0.1 mm, 0.1-1.0 mm, or >1 mm.*

to measure SLN tumor burden (24, 33). In this case series of 138 patients with a single cutaneous melanoma and one or more positive SLNs, we found that SLN tumor burden provided important prognostic information regarding OS and DFS in line with the results of previous studies (6, 7, 10, 12, 13, 15, 16, 18-20, 24, 26). In addition, our data identified the maximum diameter of 1 mm as the most significant cut-off point in terms of OS and DFS. The five-year survival rate was markedly different for SLN tumor burden greater or less than 1 mm (75.6% *vs*. 34.6%).

We assessed cut-off points of 0.1 mm and 0.2 mm to evaluate whether they represent different biological behavior-as suggested by previous studies (7, 15). In contrast to the results of previous studies, which observed better prognosis in patients with SLN tumor deposits ≤0.1 mm or ≤0.2 mm, compared to those with SLN tumor deposits greater than the aforementioned cut-off values, our study showed similar OS, DFS and hazard rate between patients with SLN tumor burden ≤0.1 mm and those with SLN tumor burden 0.1-1.0 mm. Similarly, there were no differences in OS, DFS and

hazard rate between patients with SLN tumor burden ≤0.2 mm and those with SLN tumor burden 0.2-1.0 mm suggesting similar biologic behavior between the two groups. Van der Ploeg *et al*. (16) observed that patients who had SLN tumor size <0.1 mm in the subscapular area had a similar melanoma-specific survival compared to SLN negative patients. In the present study, because many pathology reports missed information on the location of metastasis within the node, we were not able to assess this finding.

On the basis of our data, SLN tumor burden, Breslow thickness, angiolymphatic invasion and number of positive SLNs (1 *vs*. ≥2) may be useful in predicting NSLN involvement. Gershenwald *et al*. (8) reported similar clinicopathological prognostic factors that predicted the presence of positive NSLN (with the exception of angiolymphatic invasion that was not included in their analysis), which supports our findings. A number of clinical scoring systems to stratify patients with melanoma with positive SLNs according to risk have been developed (8, 12). However, to our knowledge, none of them have been validated prospectively. The clinicopathological factors, which were found to be predictive of NSLN involvement in our study, will need to be incorporated and assessed in future studies with a larger number of patients.

A few studies observed no additional involvement of NSLN in patients with SLN tumor burden less than 0.1 mm or 0.2 mm (7, 23, 34) but those studies were limited by a relatively small number of patients in that group. Our study showed a low prevalence of NSLN involvement in patients with SLN tumor burden ≤0.1 mm or 0.2 mm (3.9% and 3.2% respectively) but the odds ratio between patients with SLN tumor burden ≤0.1mm and those with SLN tumor burden 0.1-1.0 mm did not reach statistical significance. Until more data are available, we should be cautious in excluding patients based on SLN tumor burden alone.

One of the strengths of our study is that this is a single-Institution study and SLN biopsies were performed by a single plastic surgeon during the whole study period, which ensures a consistency in practice. One of the weaknesses of our study is that we did not have data on patients whose SLN biopsy was negative. Comparing patients with no SLN involvement to those with minimal SLN tumor burden would have shed more light to differences in OS and DFS in these two groups of patients. Another weakness is that the pathological protocols used to examine SLN have changed during the study period, which might have affected the sensitivity of detecting SLN metastases in the study population. A standardized protocol for detection of metastases needs to be developed to produce more reliable pathologic information (35).

In conclusion, SLN tumor burden by measuring the maximum diameter of the largest tumor focus is one of the strongest prognostic factors for OS and DFS. To determine the best cut-off points for SLN tumor burden, various microscopic classifications will need to be assessed in future studies with larger cohorts. Incorporation of SLN tumor burden in the American Joint Committee on Cancer (AJCC) staging system or prognosis scoring models should also be considered to improve prognostic accuracy for patients with cutaneous melanoma.

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