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ORIGINAL ARTICLE – MELANOMAS

Influence of Sentinel Lymph Node Tumor Burden on Survival in Melanoma

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ABSTRACT

Background. Completion lymph node dissection (CLND) is the standard procedure for patients with positive sentinel lymph nodes (SLN). With extensive pathological workup, increased numbers of small metastatic deposits are detected in SLN. This study evaluated the prognostic significance of SLN metastatic deposits $\leq$ 0.2 mm in patients treated in a referral cancer center in Brazil.

Methods. Patients with stage I/II melanoma, consecutively submitted to a SLN procedure by the same surgeon from 2000 to 2006, were evaluated. All positive SLN and randomly selected negative cases were reviewed by two pathologists. Different prognostic factors and SLN tumor burden were recorded. Additional positive non-SLN after CLND, and disease outcome were evaluated.

Results. Of 381 patients who underwent SLN biopsy, 103 (27%) were positive. The mean/median Breslow tumor thickness in the overall group was 3.4/2.0 mm and in the SLN positive patients was 5.72/4.0 mm. Among these patients, 48 (47%) had metastatic deposits >2 mm (macrometastasis), 49 (47%) had metastatic deposits $\leq$2 mm but >0.2 mm (micrometastasis), and 6 (6%) had metastatic deposits $\leq$0.2 mm (submicrometastasis). Additional positive non-SLN were detected in 29% of patients with macrometastasis, in 25% of patients with micrometastasis, and in 0% of patients with submicrometastases. At median follow-up of 35 months, the estimated 3-year overall survival was 92% for negative SLN, 64% for micrometastases, 53% for macrometastases, and 100% for submicrometastases ($P < 0.001$).

Conclusion. In the present study, patients with SLN metastatic deposits $\leq$0.2 mm had no additional positive non-SLNs, and no recurrences or deaths were recorded, suggesting that their prognosis is equivalent to that of patients with negative SLN.

Cutaneous melanoma frequently disseminates to regional lymph nodes. Sentinel lymph node biopsy (SLN) was introduced in 1992 as a minimally invasive procedure to identify patients with lymph node metastases who could benefit from completion lymph node dissection (CLND).[1] The procedure has been improved with use of preoperative lymphoscintigraphy and intraoperative use of gamma probe with blue dye injection, and several studies have validated the effectiveness of lymph node mapping in accurately identifying the first lymph node to receive drainage from the tumor.[2–5]

However, the therapeutic value of this procedure remains under dispute, since controlled studies demonstrating prolonged survival in patients undergoing this procedure are still lacking.[6] The Multicenter Selective Lymphadenectomy Trial (MLST-I) compared wide local excision (WLE) plus SLN biopsy with WLE, and failed to demonstrate a survival benefit in favor of the SLN procedure.[6] Moreover, in approximately 80% of SLN positive nodes, no additional metastases are found in the dissection specimen, and those patients are at risk of associated morbidity.[7,8] Nevertheless, subgroup analyses suggested that the outcome in SLN-positive patients who underwent CLND was better than in patients who had WLE and

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underwent therapeutic lymph node dissection (TLND) during follow-up.[6]

Histopathological involvement in SLNs can range from isolated melanoma cells to complete effacement of lymph node structures by tumor infiltration.[3,9–13] The great majority of patients with SLN metastases will have only a single tumor-positive lymph node, and surgeons have attempted to determine if these patients, or particular subgroups of them, could safely be spared CLND. Different micromorphometric classifications have been proposed based on invasion depth from the capsule (Starz), maximum diameter (Rotterdam criteria) or location within the node (Dewar).[14–16] According to the American Joint Committee on Cancer (AJCC) staging system for cutaneous melanoma, all patients with microscopic lymph node involvement (micrometastases), but without lymph node enlargement, are staged as N1a.[17] However, several groups have suggested that patients with "submicrometastases," defined by some as lesions smaller than 0.2 mm and by others as lesions smaller than 0.1 mm, present a better prognosis, similar or even identical to that of patients without SLN metastases.[15,18–20]

The purpose of the present study was to evaluate the influence of SLN tumor burden, in particular the significance of SLN metastatic deposits smaller than 0.2 mm, on the prognosis of a homogenous population treated by the same team in a reference cancer center.

METHODS

Study Design and Population

A retrospective cohort of 381 patients consecutively submitted to SLN biopsy and lymphatic mapping at a single institution (Brazil's Instituto Nacional de Câncer, INCA) from May 2000 to December 2006 was analyzed. The inclusion criteria were patients with confirmed histological diagnosis of cutaneous melanoma, absence of lymph node enlargement and Breslow greater than 1 mm or presence of ulceration, Clark IV or V. There were no limits regarding gender, age or skin color. Melanomas located in the head and neck were excluded.

SLN Biopsy and Lymphatic Mapping

SLN biopsy and lymphatic mapping were performed by a single surgeon (J.F.N.R.). Dynamic lymphoscintigraphy was performed on the morning before surgery with intradermal injection of 1 mCi 99Tcm-phytate. Vital blue dye was injected subdermally around the primary melanoma or biopsy site at time of surgery. SLNs were localized with a handheld gamma probe intraoperatively, and by visual inspection for blue dye. In vivo and ex vivo counts of the radiolabeled lymph nodes were obtained and compared with nodal bed counts before and after removal. All "hot" and "stained" lymph nodes were considered as SLNs and excised.

Pathological Assessment and Treatment Choices

SLNs were bivalved, and imprints were prepared for peroperative evaluation. If positive, CLND was immediately performed. Otherwise, the SLNs were processed in the pathology laboratory, stained with hematoxylin and eosin (H&E) and with the immunohistochemical markers HMB 45, Melan A, and S100 without a standard protocol. CLND was scheduled in all positive cases. The micromorphometric classification adopted was based on maximum diameter. Invasion depth from the capsule was not evaluated. Metastatic deposits identified in the SLNs had their larger diameter measured using an optical microscope with an ocular micrometer. The largest value defined the SLN's tumor burden. If multiple positive SLNs were present, the largest maximum diameter in any of the SLNs was considered.[15,20] The number of metastatic deposits in each SLN was also evaluated. All positive cases and 35 randomly selected negative cases were reviewed independently by two pathologists (S.de O.R. and M.de A.A.). Discordant cases were reviewed jointly by the same pathologists to reach consensus. Two positive SLN cases were reclassified as negative after review (1.9%), while the 35 negative cases remained unaltered upon review (100%). Since the smallest diameter observed was 0.12 mm, making the 0.1 mm cutoff infeasible, we adopted the 0.2 mm cutoff, and results were stratified into four categories: negative SLN, positive SLN with macrometastases (>2 mm), positive SLN with micrometastases ($\leq$2 mm but >0.2 mm), and positive SLN with submicrometastases ($\leq$0.2 mm).

Data Collection

Data were retrospectively collected from the Institute's medical records and electronic databases. Postoperative follow-up was performed in the melanoma outpatient clinic and consisted of clinical examination every 3 months and chest radiography every 6 months. Other examinations were performed according to the patient's need. Patient current status was classified as alive without evidence of disease, alive with disease, dead from disease or dead from other cause.

Statistical Analysis

Data were analyzed by using SPSS version 13.0. The chi-square test was used to evaluate relationships between

categorical variables. Disease-free survival (DFS) and overall survival (OS) were calculated from date of SLN biopsy to date of first recurrence or death, respectively, censored at date of last contact if there were no events. Univariate estimations of survival were performed by Kaplan–Meier method and compared by using the log-rank test. Multivariate analysis was performed with the Cox proportional-hazards regression model including variables with statistical significance on univariate analysis. $P < 0.05$ was considered statistically significant. The study was approved by the institutional ethics committee.

RESULTS

The clinicopathologic features of the patients are listed in Table 1. There were 208 women (55%) and 173 men (45%), with a median age of 53 years (range 16–89 years). The primary disease sites were the extremities ($n = 229$, 60%) and trunk ($n = 152$, 40%). Most patients ($n = 330$, 87%) were White. Median follow-up was 35 months.

Four patients with negative SLN were lost to follow-up but were alive without disease at last contact. Two patients had sentinel node drainage to two different sites, and both sites were positive. Both patients underwent CLND and are alive without disease.

SLN Biopsy Results

After pathologic review, 103 patients (27%) were classified as positive SLN and 278 (73%) as negative SLN. Among patients with positive SLNs, 48 (47%) had metastatic deposits >2 mm and were classified as macrometastases, 49 (47%) had metastatic deposits ≤2 mm but >0.2 mm and were classified as micrometastases, and 6 (6%) had metastatic deposits ≤0.2 mm and were classified as submicrometastases. A median of 1 SLN was removed from each patient (range 1–6), and a median of 12 lymph nodes were retrieved during CLND (range 1–44). Among patients with micrometastases, 77% had only one positive SLN; this included 69% with macrometastases, and all patients with submicrometastases.

The proportion of patients with at least one positive non-SLN on CLND specimens was 26%. In individuals with macrometastases, 14/48 (29%) had at least one positive

TABLE 1 Clinicopathologic features of the 381 patients submitted to SLN biopsy

Number of patients and %	Overall	Negative SLN	Positive SLN		
	381 (100%)	278 (73%)	103 (27%)		
			Submicrometastasis 6 (6%)	Micrometastasis 49 (47%)	Macrometastasis 48 (47%)
Age, years (median)	53	55	46	58	53
Sex (%)					
Male	45	45	33	41	50
Female	55	55	67	59	50
Color, White (%)	87	87	83	82	88
Primary disease site (%)					
Trunk	40	37	83	43	46
Extremities	60	63	17	57	54
Breslow, mm (median)	2.00	1.71	1.8	3.2	5.0
Ulceration, yes (%)	28	25	17	35	44
Regression, yes (%)	30	34	50	25	13
Clark (median)	IV	III	III	IV	IV
Median no. SLN	1.0	1.0	2.0	2.0	1.0
Median no. of positive SLN	1.0	–	1.0	1.0	1.0
Median no. of metastatic deposits in SLN	3.0	–	1.5	2.0	5.0
Positive non-SLN (%)	26	–	0	25	29
No. of metastatic deposits in SLN (%)					
Five or more	45	–	0	35	60
Fewer than five	55	–	100	65	40
Death rate (%)	17	10	0	33	48
Recurrence rate (%)	25	15	0	47	63

SLN sentinel lymph node

non-SLN on CLND; in individuals with micrometastases, 12/49 (25%) had at least one positive non-SLN on CLND; however, no further metastases were found in patients with submicrometastases.

Among patients with positive SLN, 45% had five or more metastatic deposits in SLN: 0% in submicrometastases, 35% in micrometastases, and 60% in macrometastases patients. The median number of metastatic deposits in SLN was 3 in positive SLN group: 5 in the macrometastases group, 2 in the micrometastases group, and 1.5 in the submicrometastases group.

Clinical Outcomes

On univariate analysis (Table 2), the following variables were correlated with worse DFS and OS: Breslow higher than 2 mm, ulceration, lack of regression, acral lesions, age greater than 50 years, male sex, five or more metastatic deposits, and positive sentinel lymph nodes.

Out of the 381 patients, there were 94 relapses (25%) and 66 deaths (17%) due to disease. The recurrence rate was 51% in patients with positive SLNs and 15% in patients with negative SLNs. The recurrence rate was 63% in patients with macrometastases, 47% in those with micrometastases, and 0% in those with submicrometastases. Accordingly, the death rate was 38% in patients with positive SLNs and 10% in patients with negative SLNs. The death rate was 48% in patients with macrometastases, 33% in patients with micrometastases, and 0% in those with submicrometastases (Table 1).

The estimated OS at 3 years was 92% in patients with negative SLN, 100% in patients with submicrometastases, 64% in patients with micrometastases, and 53% in those with macrometastases (Fig. 1). However, there were no statistical differences in OS between patients with submicrometastases and those with negative SLN ($P = 0.44$), nor between patients with micrometastases and those with macrometastases ($P = 0.25$), and also no differences when comparing OS in patients with metastatic deposits of 0.2–1 mm with those with metastatic deposits of 1–2 mm ($P = 0.39$). The same findings also were observed regarding disease-free survival (Fig. 2). However, patients with submicrometastases and those with negative SLN had better OS and DFS than did patients with micrometastases.

When patients with five or more metastatic deposits in the positive SLN were compared with those with fewer than five metastatic deposits in the SLN, OS at 3 years was 47 vs. 70% ($P = 0.009$) and DFS at 3 years was 35 vs. 63% ($P = 0.011$). Also, when patients with micrometastases and fewer than five metastatic points were compared

TABLE 2 Univariate analyses of 381 patients submitted to SLN biopsy

Variables	Patients, *N* (%)	5-Year overall survival (%)	*P*-value	5-Year relapse-free survival (%)	*P*-value
No. of metastatic deposits in SLN (%)					
≥5	46 (45%)	34	0.009	29	0.011
<5	57 (55%)	66		60	
Breslow					
<2 mm	180 (50%)	92	<0.001	87	<0.001
≥2 mm	181 (50%)	60		57	
Ulceration					
Yes	107 (28%)	58	0.006	58	<0.001
No	273 (72%)	78		76	
Acral lesions	80 (21%)	61	0.034	57	0.009
Others	301 (79%)	78		75	
Age					
≥50 years	226 (59%)	70	0.028	64	0.002
<50 years	155 (41%)	82		81	
Gender					
Male	173 (45%)	67	0.026	62	0.009
Female	208 (55%)	80		78	
Regression					
Yes	115 (30%)	97	<0.001	92	<0.001
No	265 (70%)	70		64	
SLN status					
Positive	103 (27%)	50	<0.001	45	<0.001
Negative	278 (73%)	84		80	

SLN sentinel lymph node

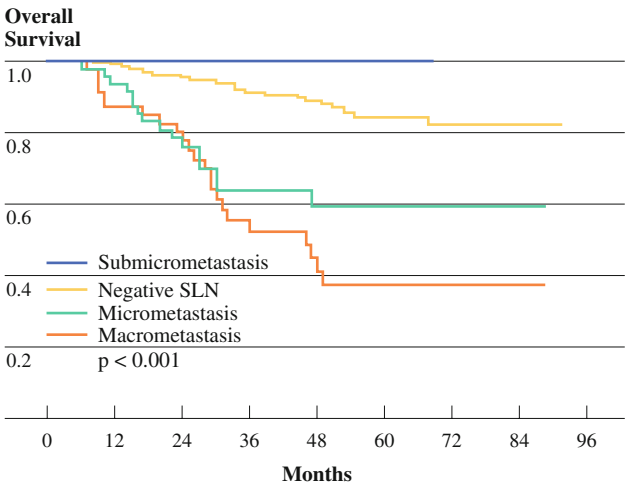

FIG. 1 Kaplan–Meier analysis of overall survival of patients with negative SLNs, micrometastases, submicrometastases, and macrometastases ($P < 0.001$)

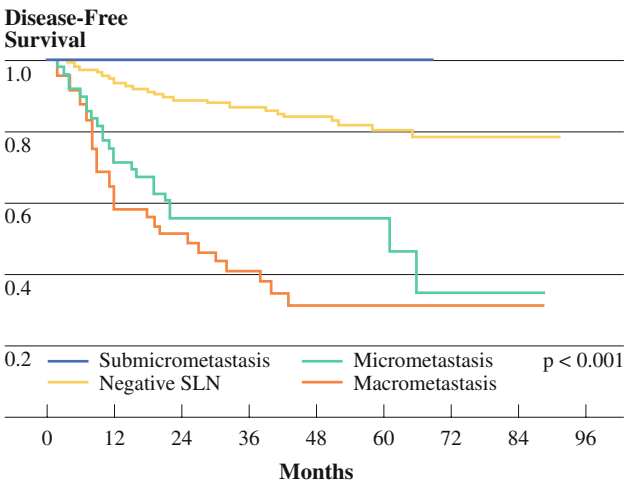

FIG. 2 Kaplan–Meier analysis of disease-free survival of patients with negative SLNs, micrometastases, submicrometastases, and macrometastases ($P < 0.001$)

with those with negative SLN, OS at 3 years was 76 vs. 92% ($P = 0.032$) and DFS at 3 years was 68 vs. 87% ($P = 0.002$). The number of positive SLN did not correlate with worse OS or DFS in the micrometastases and macrometastases group.

Multivariate Analysis

On multivariate analysis, presence of positive SLNs, five or more metastatic deposits, Breslow >2 mm, absence of regression, and age >50 years were all independently related with worse OS. Likewise, presence of positive SLN, five or more metastatic deposits, ulceration, absence of regression, age >50 years, and male sex were independently related with worse DFS (Table 3).

DISCUSSION

There is ongoing controversy regarding the benefits provided by the strategy of sentinel lymph node biopsy followed by subsequent CLND in positive patients. Recent evidence suggests that patients with very small lymph node involvement might have outcomes similar to those without lymph node involvement.[8,15,18–20]

In line with this, the present study evaluates the outcome of 381 patients with melanoma submitted to SLN biopsy and lymphatic mapping in a reference cancer center in Brazil. Among the 103 patients with lymph node involvement, only 6 (6%) had submicroscopic disease, defined as metastatic lymph node lesions smaller than 0.2 mm. Of note, none of these patients had positive lymph nodes after the CLND. Their failure-free and overall survival were both 100%. In a recent paper, van der Ploeg et al. also did not observe relapses in the submicroscopic subgroup.[8] They put forward the idea that patients with tumor invasion diameter up to 0.4 mm should not undergo completion dissection.

TABLE 3 Multivariate analysis of 381 patients submitted to SLN biopsy

Variables	Overall survival					Disease-free survival				
	β	Exp (*β*)	CI 95% Exp (*β*)		*P*	*β*	Exp (*β*)	CI 95% Exp (*β*)		*P*
Positive SLN vs. negative SLN	−1.736	0.176	0.094	0.329	0.000	−1.686	0.185	0.109	0.314	0.000
Breslow ≥2 vs. <2 mm	−.829	0.436	0.216	0.883	0.021	−0.475	0.622	0.365	1.059	0.080
Regression yes vs. no	1.246	3.476	1.056	11.443	0.040	1.260	3.526	1.593	7.804	0.002
Ulceration yes vs. no	−0.480	0.619	0.345	1.108	0.106	−0.630	0.533	0.335	0.847	0.008
Acral lesions vs. others	−0.261	0.770	0.433	1.370	0.374	−0.205	0.815	0.503	1.319	0.404
Age ≥50 vs. <50 years	−0.789	0.454	0.244	0.846	0.013	−0.787	0.455	0.274	0.756	0.002
Gender male vs. female	−0.494	0.610	0.363	10.026	0.062	−0.504	0.604	0.390	0.936	0.024
Metastatic deposits ≥5 vs. <5	−0.778	0.459	0.229	0.923	0.029	−0.692	0.501	0.279	0.898	0.020

β coefficient, *Exp (β)* relative hazard, *CI 95% Exp (β)* relative hazard 95% confidence interval, *SLN* sentinel lymph node

In their cohort, compared with our series, a similar proportion of patients were considered submicroscopic (5%), although they followed the Rotterdam criteria of a 0.1 mm cutoff. In contrast, previous studies describe a higher proportion of patients with submicroscopic disease, ranging from 4 to 26%.[15,18–20] This discrepancy might reflect not only tumor burden at diagnosis but also how systematically the lesions were looked for in the pathological examination. In a recent multicenter study involving patients from three different centers (Berlin, Rotterdam, and Warsaw), submicroscopic disease was observed in 20% of patients in the first two centers, while in the latter it was observed in only 4%.[20] The authors point out that the median Breslow thickness was substantially larger in Warsaw patients: 4 vs. 2.9 and 3.4 mm in Berlin and Rotterdam, respectively. Moreover a less extensive pathology protocol was used in Warsaw. In our series, Breslow thickness among patients with SLN involvement was also 4 mm, and due to the retrospective nature of our study, a standardized pathology protocol could not be used. The findings of large tumor burden and advanced disease at diagnosis are typical features of cancer in developing countries, and reflect complex factors related to the health system and the sociocultural background of the population.[21]

Discrepancies in the proportion of patients with submicroscopic metastases across studies may explain differences in outcome observed in some series. The 0.2 mm cutoff point was tested by other authors with better results. In a recent study, there was no relapse or death, and no additional positive lymph nodes after CLND with metastatic deposits smaller than 0.2 mm.[18] In two other recent studies, however, while the estimated 5-year overall survival was excellent, the number of positive non-SLNs ranged from 10 to 12% with metastatic deposits smaller than 0.2 mm.[19,20] Besides, in the first study, patients with minimal residual disease in SLN also had a significantly worse outcome compared with negative patients and CLND could be necessary to correctly treat and possibly curing these patients.[19] However, in the second study, the 0.1 mm cutoff point was considered ideal, since patients in this category had only 3% positivity in non-SLN, with an estimated 5-year overall survival of 91%.[20] In our series, the smallest tumor deposit observed was 0.12 mm, making the 0.1 mm cutoff point impractical. However, patients with metastases of less than 0.2 mm had 0% positivity in non-SLN, with an estimated 5-year overall survival of 100%.

It should be highlighted that higher cutoff points (2 and 1 mm) in SLN have been previously examined, in order to identify patients that could be considered as false positives and spared CLND.[15,18–20,22–26] Corroborating data from previous studies, we also identified an elevated number of positive non-SLN after CLND in macro- and micrometastases subgroups (29 and 25%, respectively).[13,15,18–20,22–26] Furthermore according to data published elsewhere, in our hands, patients with metastasis smaller the 2 and 1 mm cutoff points had significantly higher relapse and death rates than did patients with negative SLN (Figs. 1 and 2).[15,18–20,22–26] Analyzed together the existing data suggest that those higher cutoff points are not ideal, and we should pursue more refined ones such as a standardized submicroscopic cutoff.

The number of metastatic deposits in SLNs was also evaluated in the present study. The mean number of metastatic deposits in SLNs was 3.2, similar to other studies, where it ranged from 3.1 to 3.9.[18,27] The median number of metastatic deposits correlated with tumor burden, and patients with five or more metastatic deposits had significantly worse survival compared with those with fewer than five metastatic deposits. Also, patients with micrometastases and fewer than five metastatic deposits had significantly worse survival compared with negative SLN group (OS 76 vs. 84%; $P = 0.032$; DFS 68 vs. 80%; $P = 0.002$) and an elevated number of additional positive nodes after CLND (16%). Few studies have evaluated this parameter, and none of them suggested an impact on OS.[18,25,27] Although our findings suggest that this information might help to define patients who would not benefit from CLND, validation on prospective cohorts is required. Alternatively, other studies have attempted to measure histological tumor burden assisted by software, either by bidimensional or tridimensional measurements.[28–30] These methods are time consuming, and the results so far are not convincing.

In sum, by identifying that patients with metastatic deposits smaller than 0.2 mm in SLNs had excellent outcome and could have been spared CLND, we believe that our data derived from a large number of patients homogeneously treated in a reference cancer center add to the current literature. Furthermore, the well-characterized prognostic factors obtained from uni- and multivariate analyses (Tables 2, 3) define our population as homogeneous and strengthen our data. We are aware, though, of some caveats to our study. Its retrospective format that precluded the use of a standardized pathology protocol, the use of a 0.2 instead of 0.1 mm cutoff for submicroscopic disease, and the inclusion of a population with Breslow of 4 mm may limit comparison with other series using different criteria.

Large, randomized trials such as the Multicenter Selective Lymphadenectomy Trial II (MSLT-II) and the EORTC melanoma group MINITUB coupled to the definition of the best micromorphometric parameter to analyze SLN are eagerly awaited and will help to better settle the matter.

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