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ORIGINAL ARTICLE

Predictors of sentinel lymph node status of cutaneous melanoma in Serbian patients

Stevan Jokic[1], Ivan Markovic[1,2], Zoran Bukumiric[2,5], Vladimir Jokic[1], Marija Rakovic[1], Jovana Tripkovic[1], Dejan Stojiljkovic[1], Igor Spurnic[1], Marko Jevric[1], Marija Matic[3,5], Danijela Dobrosavljevic[4,5]

[1]Institute of Oncology and Radiology of Serbia, Department of Surgical Oncology, Belgrade; [2]Institute of Medical Statistics and Informatics, Belgrade; [3]Institute of Medical and Clinical Biochemistry, Belgrade; [4]Clinic of Dermatology and Venereology, Clinical Center of Serbia, Belgrade; [5]Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Summary

Purpose: *Presence of metastasis in sentinel lymph node (SLN) is considered to be the most important factor in terms of patient survival. The main aim of this study was to identify predictors of positive SLN in Serbian patients with melanoma.*

Methods: *This retrospective study was conducted on 147 patients. Univariate chi-square and univariate logistic regression analyses were used to identify the association between prognostic factors and positive SLN. Receiver Operating Characteristics (ROC) was conducted to find the Breslow thickness cutoff point at which to perform SLN biopsy (SLNB). Kaplan-Meier analysis was used to evaluate disease-free survival (DFS), and log rank test was applied to compare differences between groups.*

Results: *Breslow thickness and Clark level ($p \leq 0.05$), presence of ulceration and a high mitotic rate (>6 mitoses/mm^2) ($p<0.001$) were significant independent predictors of SLN metastasis. ROC curve showed that Breslow thickness of 2.8 mm was the most suitable cutoff point for SLN positivity (sensitivity 86%, specificity 67%). Furthermore, Breslow thickness and presence of ulceration were found to be associated with DFS ($p<0.05$).*

Conclusions: *Patients with Breslow thickness ≥2.8 mm, ulceration, and high mitotic rate are at higher risk for SLN metastasis. In addition, high Breslow thickness and presence of ulceration are associated with decreased DFS. These results indicate that multiple selection criteria should be used when performing and predicting SLN metastasis and disease recurrence.*

Key words: *cutaneous melanoma, predictors, sentinel lymph node*

Introduction

The most common human cancers are malignant skin tumors. The incidence of cutaneous melanoma is not only rising rapidly worldwide, but it also has a very high mortality rate [1,2]. Caucasians have about 20-fold higher risk of developing cutaneous melanoma when compared to darker-skinned populations [3]. According to the American Joint Committee of Cancer (AJCC) staging system, Breslow thickness, ulceration and SLN metastasis are the strongest prognostic factors in melanoma patients without clinical evidence of metastasis [4]. However, among these factors, SLN metastasis is considered as the most important in terms of survival prediction and melanoma treatment [5,6].

SLNB is a technique validated by the Multicenter Selective Lymphadenectomy Trial (MSLT-1). The importance of SLNB lies in both detecting occult nodal metastasis and in overall treatment

Correspondence to: Marija Matic, MD, PhD. Institute of Medical and Clinical Biochemistry, Faculty of Medicine, University of Belgrade, Doctor Subotica 8, 11000 Belgrade, Serbia.
Tel: +381 11 3643272, E-mail: marija_opacic@yahoo.com
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prognosis as well. In 1992, Morton and colleagues proposed SLNB using a single blue dye technique which was revised by Gershenwald et al. [7]. Guidelines for the SLNB use in melanoma were recently published by the Society of Surgical Oncology and the American Society of Clinical Oncology (SSO/ASCO) and the National Comprehensive Cancer Network (NCCN) [5]. The biopsy of "sentinel" avoids the elective dissection of regional lymph nodes from the drainage area of the primary skin melanoma, but still reflecting the exact histological status of this area [8].

However, selection SLNB criteria are still under discussion. Namely, usually a surgical oncologist performs SLNB in T1b patients (thin melanoma, Breslow thickness ≤1mm with ulceration and/or Clark level >III) or in the presence of regression [9]. On the other hand, other researchers have proposed that age, sex, mitotic rate and vertical growth phase (VGP) are also significant predictors of SLN positivity [10,11]. In recent years, however, mitotic rate has attracted a lot of attention as both a prognostic and predictive factor for SLN metastasis [12]. Nevertheless, in the latest version of the Cancer Staging Manual of the AJCC, mitotic rate is recognized only as a prognostic factor in thin melanomas [13].

The main aim of this study was to identify predictors of SLN metastasis in Serbian patients with cutaneous melanoma. A secondary goal was to evaluate the role of these factors in predicting DFS.

Methods

This study was designed as a retrospective observational study.

Study population

The database of the National Cancer Referral Center (Institute of Oncology and Radiology of Serbia, Belgrade, Serbia) was used in a 5-year period (January 1st, 2012 - December 31st, 2016). Out of 902 melanoma patients, the study included 147 melanoma patients (male 49%, female 51%) with stage I and II who underwent SLNB. Their mean age was 53.9±15.1 years and all were diagnosed with a single lesion of cutaneous melanoma.

Patients with non identifiable SLN during the SLNB were excluded from the study. Patients younger than 18 years or with previously diagnosed malignancy were also excluded. All of the patients provided written informed consent and the study protocol was approved by the Ethics Committee of the Medical Faculty, University of Belgrade and Ethics Committee of the Institute of Oncology and Radiology of Serbia, Belgrade. This research was carried out in compliance with the Helsinki Declaration. SLN status was categorized as positive in the presence of melanoma cells in the SLNB, and negative if there were no malignant cells.

SLN status

SLNB was performed as previously described [14]. Surgeons used either vital blue dye and radioactive colloid or combination of these two methods for locating SLNs. Positive SLNB after technetium 99 administrations was identified by gamma camera. According to the protocol, all blue nodes and all nodes containing >10% of the radioactive colloid were the hottest nodes marked as SLN and have been removed. All SLN were further histologically analyzed with hematoxylin & eosin, as well as with immunohistochemical S100 protein staining. Immunohistochemical staining for HMB-45 or MART-1 was also performed. Lymphatic drainage to the following nodal basins was classified as axillary, cervical (including supraclavicular and parotid) and inguinal.

In this study the dependent variable was SLN status. Independent clinical variables were patient gender and age (40 *vs* >40 years) and anatomic location of the primary melanoma (head and neck, trunk and extremities). Independent histological characteristics were as follows: Breslow thickness (0.8, 0.81-2, 2.01-4, and >4 mm, according to the latest TNM version) [15], Clark level, ulceration (present *vs* absent), regression (present *vs* absent), mitotic rate (0, 1-5, or 6 mitoses/mm^2), and tumor infiltrating lymphocytes (TILs) (absent, nonbrisk and brisk).

According to the new classification pT1 tumor 1 mm or less in thickness is categorized as pT1a with 0.8 mm or less in thickness without ulceration and pT1b with 0.8 mm in thickness with ulceration or more than 0.8 mm but no more than 1mm in thickness, with or without ulceration [15].

Statistics

Statistical analyses were performed using the IBM SPSS 22 software (IBM Corporation, Armonk, NY, USA). The results were presented as frequencies (%) and means. For numeric data with non normal distribution and ordinal data the Mann-Whitney U test was used. Chi square test and Fisher's exact test were used to test differences between nominal data (frequencies). Association between the dependent variables and potential predictors was analyzed by univariate analysis. Kaplan-Meier analysis was used to evaluate DFS, and the log rank test was applied to compare differences between groups. A p value of less than 0.05 was considered statistically significant.

Results

Table 1 summarizes the clinical and histological patient characteristics stratified by SLN status. SLN metastasis was significantly associated with increased Breslow thickness (p=0.001) and Clark level (p=0.048), presence of ulceration and a high mitotic rate (p<0.001). SLN status was not associated with sex, age, anatomic location, regression or tumor infiltration (p>0.05).

Univariate analysis of SLN positivity as dependent variable was further performed (Table 2).

Patients with each higher Breslow thickness had 2.6-fold higher risk for SLN metastasis (OR=2.62, 95% CI=1.38-4.99, p=0.003). Similarly, patients with each higher Clark level were at 2.21-fold higher risk for SLN metastasis development (OR=2.21, 95% CI=1.01-4.86, p=0.048). The presence of ulceration increased the risk of SLN metastasis about 9 times (OR=8.97, 95% CI=2.39-33.70, p=0.001), while higher mitotic rate increased the risk of SLNB positivity about 8 times (OR=7.93, 95% CI=2.33-26.96, p=0.001).

The ROC curve for the Breslow thickness in SNL positivity is shown on Figure 1. The cut off values with >2.8 mm were 86% for sensitivity and 67% for specificity, while the ROC curve presented an area under the curve of 0.79 (95% CI=0.72-0.85).

Kaplan-Meier analysis showed that the mean survival rate for all patients was 41.3 months (95% CI=39.3–43.3), while the mean time to first recurrence was 32 months (95% CI=28.1–35.9).

Log rank test analysis of the first melanoma recurrence according to gender, anatomic localization, Breslow thickness, ulceration and mitotic rate in patients with localized cutaneous melanoma is shown on Table 3. The mean time to relapse was decreasing with higher Breslow thickness (p=0.001). Namely, patients with Breslow thickness 0.81-2.00 mm had 33.9 months mean time to relapse, while those with Breslow thickness 2.01-4.00 mm and >4.00 mm had mean time to relapse

Table 1. Clinical and histological characteristics of patients according to SLNB results

Characteristics	*Patients with negative SNB, n (%)*	*Patients with positive SNB, n (%)*	*p value*
Age (years)			0.525
≤ 40	30 (23)	2 (13)	
> 40	103 (77)	13 (87)	
Sex			0.353
Female	70 (53)	6 (40)	
Male	63 (47)	9 (60)	
Anatomic localization			0.580
Head and neck	8 (6)	1 (7)	
Extremities	62 (47)	9 (60)	
Trunk	63 (47)	5 (33)	
Breslow thickness, mm			0.001
≤ 0.8	29 (22)	0 (0)	
0.81-2	49 (37)	2 (14)	
2.01-4	27 (20)	5 (36)	
> 4.00	27 (21)	7 (50)	
Clark level			0.048
1	3 (2)	0 (0)	
2	22 (17)	0 (0)	
3	52 (39)	5 (36)	
4	52 (39)	8 (57)	
5	4 (3)	1 (7)	
Ulceration			<0.001
Present	37 (31)	12 (80)	
Absent	83 (69)	3 (20)	
Regression			1.000
Present	6 (30)	1 (25)	
Absent	14 (70)	3 (75)	
Mitotic rate			<0.001
0	5 (4)	0 (0)	
1-5	84 (73)	4 (29)	
> 6	27 (23)	10 (72)	
Tumor infiltration			0.270
Present	71 (91)	8 (80)	
Absent	7 (9)	2 (20)	

of 26.4 and 17.8 months, respectively. Also there was a statistically significant difference in time to relapse between patients with or without ulceration (p=0.016). DFS was decreased if melanoma was located in the head and neck (mean time 23.2 months), as well as on the trunk (mean time 23.5 months), compared to extremities' melanoma localization (mean time 33.8 months), but this difference did not reach statistical significance (p=0.413). Melanoma patients with high mitotic rate lower mean time of first recurrence but this difference was not statistically significant (p=0.156).

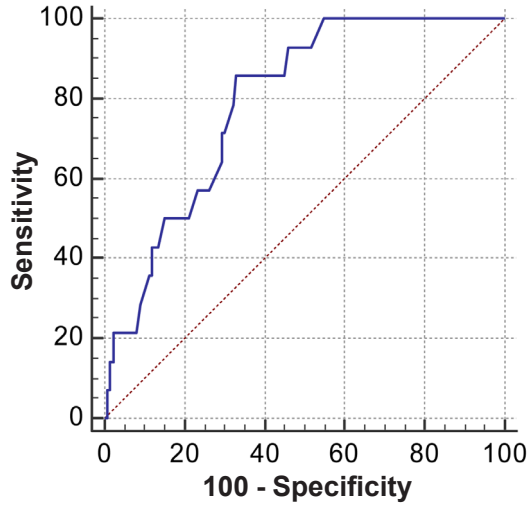

Figure 1. Receiver operating characteristic (ROC) curve for Breslow thickness in SNLB positivity with an area under the curve of 0.79 (95% CI 0.72–0.85).

Table 2. Univariate logistic regression of predictors of positive SLNB result in patients with cutaneous melanoma

Variables	*Odds ratio (95% CI)*	*p value*
Age (years)		0.418
≤40	1.00	
>40	1.89 (0.40-8.86)	
Sex		0.357
Male	1.00	
Female	0.60 (0.20-1.78)	
Anatomic localization		
Head and neck	1.00	
Trunk	0.64 (0.07-6.14)	0.695
Extremities	1.16 (0.13-10.41)	0.894
Breslow thickness	2.62 (1.38-4.99)	0.003
Clark level	2.21 (1.01-4.86)	0.048
Ulceration		0.001
Absent	1.00	
Present	8.97 (2.39-33.70)	
Regression		0.841
Absent	1.00	
Present	0.78 (0.07-9.08)	
Mitotic rate	7.93 (2.33-26.96)	0.001
Tumor infiltration		0.293
Absent	1.00	
Present	2.54 (0.45-14.35)	

Table 3. Log rank test of the first recurrence according to sex, anatomic localization, Breslow thickness, ulceration and mitotic rate in patients with cutaneous melanoma

Variables	*Time to first recurrence (months)*	*95% CI*	*p value*
Sex			0.046
Male	27.9	22.3 – 33.5	
Female	34.6	29.7 – 39.5	
Anatomic localization			0.413
Head and neck	23.2	11.9 – 34.5	
Trunk	23.5	18.3 – 28.7	
Extremities	33.8	29.1 – 38.6	
Breslow thickness, mm			0.001
≤ 0.8	*		
0.81-2.00	33.9	28.3-39.5	
2.01-4.00	26.4	22.7-30.1	
> 4.00	17.8	14.2-21.3	
Ulceration			0.016
Absent	34.2	29.5 – 38.8	
Present	21.0	17.4 – 24.6	
Mitotic rate			0.159
0	*		
1-5	32.2	27.5-36.9	
> 6	21.1	16.6-25.5	

Discussion

This study has shown in a cohort of 147 Serbian patients with cutaneous melanoma stage I and II that Breslow thickness and Clark level, mitotic rate and presence of ulceration are significant independent predictors of SLN metastasis. Furthermore, Breslow high thickness and presence of ulceration were found to be associated with poor DFS.

A recent meta-analysis of Gyorki et al. summarized the results of 10 studies, and reported outcomes for SLNB in melanomas >4 mm thick, proving that SLNB status is an independent prognostic factor for patients with T4 melanoma [16]. According to the AJCC staging system, Breslow thickness, ulceration and SLN metastasis are considered as the most important prognostic factors in melanoma patients [17], while mitotic rate is recognized only as a prognostic factor in the subgroup of thin melanomas [11]. We have found an overall SLNB positivity rate of 10%, which is a bit lower in comparison to a reported rate of 15-25% [19]. Our success rate for SLNB (proportion of patients successfully mapped, PSM) was 98%, which compares favorably to the results of similar series and an overall reported PSM of 98% (87-100%) [5]. The results of the present study have shown that SLN metastasis was significantly associated with increased Breslow thickness and Clark level, presence of ulceration and high mitotic rate, while no association was found for sex, age, anatomic location, regression and tumor infiltration. Our results are in concordance with the study of Tejera-Vaquerizo et al. who also found that ulceration, tumor thickness and high mitotic rate were independently associated with SLN metastasis in the multivariate regression analysis [20]. Statistical results were obtained from Konofaos et al. who showed that positive SLNB is related with high mitotic rate, Breslow thickness and lymph vessels' infiltration [21].

Moreover, the results of our study demonstrated that Breslow thickness and Clark level, together with the presence of ulceration and high mitotic rate have important roles in predicting SNL metastasis. Namely, the presence of ulceration increases the risk of SLN metastasis about 9-fold, while high mitotic rate increases the risk almost 8 times. In addition, patients with Breslow thickness >0.8mm and Clark level 5 had about 2-fold higher risk of SLN metastasis. Similarly to our findings, ulceration has been highlighted as an important independent predictor of SLN involvement in several studies [9,20]. Although the mitotic rate has not been consistently observed to predict SLN metastasis in melanoma, we found significant predictive role of this parameter, which is in concordance with the studies of Nagore et al. and Azzola et al. [12,22]. Interestingly, in the subgroup of melanomas with Breslow thickness ≤0.8 mm, the frequency of SLN involvement was 0%, probably as result of relatively small number of patients. Since Breslow thickness is traditionally considered as the most important predictor of SLN metastasis, after ROC analysis we obtained a cutoff of 2.8 mm as the best value for differentiating risk of lymph node metastasis. Results of point studies reported lower cutoff values (between 1.50-2.0 mm) [23,24] although any cutoff point must be taken very carefully into consideration and it should be reasonable to use multiple selection factors in patients with cutaneous melanoma.

Furthermore, we analyzed the association of these prognostic factors with the recurrence of melanoma. The overall time to first recurrence was 32 months, but significant shorter DFS was observed in males. Indeed, it is well established that male gender represents an adverse prognostic factor in melanoma [25,26]. In our study, log rank test showed that only Breslow thickness and ulceration significantly affected melanoma recurrence. Namely, time to relapse was decreasing with higher Breslow thickness (p=0.001). Similarly, in the presence of ulceration time to relapse was only 21 months in contrast to 34.2 months in patients without ulceration (p=0.016). Thus, Breslow thickness and presence of ulceration are considered the most important predictors of disease recurrence.

Patient age and anatomic location were not statistically significant associated with both SLN metastasis and recurrence. Nevertheless, the shortest time to recurrence in our study, which was observed in the case of head & neck and trunk anatomic location, has also been confirmed by other authors [27,28]. Indeed, these findings are biologically plausible since worse DFS and overall survival in the case of axial location of melanoma might be a consequence of the increased vascularization of axial tumors. In addition, the complexity of the lymph system at the level of head & neck seems to result in an increased likelihood of hematogenous metastasis [29].

A recent prospective study of Lee et al. [30] with 2986 patients has shown that male gender, local/in-transit recurrence and head & neck primary melanomas are associated with false negative SLNB. Furthermore, a predictive role of false negative SLNB in melanoma-specific survival with disease thickness <4 mm was found, with higher rate of distant metastasis and regional recurrences.

Based on our results we can conclude that patients with higher Breslow thickness, ulceration and high mitotic rate are at higher risk for SLN metastasis. In addition, Breslow thickness >0.8 mm and presence of ulceration are associated with decreased DFS. These results indicate that multiple selection criteria should be used when performing and predicting SLN metastasis and disease recurrence.

Conflict of interests

The authors declare no conflict of interests.

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