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Cutaneous melanoma histopathologic features and laboratory findings as predictors of sentinel lymph node status and progression-free survival: a single-center experience

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Abstract

Introduction: Cutaneous melanoma (CM) is the most aggressive cutaneous malignancy. The aim of the study was to determine the predictive value of primary tumor histopathologic features and laboratory findings used in routine CM follow-up for sentinel lymph node (SLN) biopsy results and progression-free survival (PFS).
Methods: This retrospective study included 157 patients. Planar images were acquired after an intradermal injection of 18 to 30 MBq of 99mTc-nanocolloid in 0.3 ml at two to eight sites 5 to 10 mm from the surgical scar. SLN excision was performed a day after lymphoscintigraphy.
Results: In a logistic regression analysis, Breslow thickness, ulceration status, and mitotic rate showed possible predictive significance for SLN biopsy results, with serum lactate dehydrogenase (LDH) being the only independent predictor ($p = 0.042$). The difference in survival distributions reached statistical significance for Breslow thickness, mitotic rate, and LDH ($p < 0.05$, Kaplan–Meier, log-rank test). In a Cox regression analysis, Breslow thickness was a possible predictor of PFS and mitotic rate was an independent predictor ($p = 0.025$).
Conclusions: LDH is an independent predictor of SLN histopathology findings, and mitotic rate is an independent predictor of PFS.

Keywords: ^{99m}Tc-nanocolloid, Breslow thickness, melanoma, mitotic rate, sentinel lymph node

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Introduction

Cutaneous melanoma (CM) is the most aggressive cutaneous malignancy, causing up to three in every four skin cancer–related deaths, despite accounting for only 4% of all skin cancer cases (1). Studies suggest that time to definitive treatment is an important factor in the overall survival of these patients (2).

Melanoma spreads from the site of occurrence to the draining lymph node through a process involving the migration of cells into local lymphatics (3). This lymph node is considered a sentinel lymph node (SLN). Sentinel lymph node biopsy (SLNB) is a procedure of utmost importance in the staging, prognosis, and survival of patients with CM (4). It is performed after lymphatic mapping, using 99mTc-labeled colloids injected intradermally around the primary location of CM, with possible utilization of single-photon emission computed tomography (SPECT/CT) and intraoperative application of a gamma probe (4–5).

Serum lactate dehydrogenase (LDH) is a well-known biomarker for metastatic melanoma patients, and acidic cytoplasmic protein S100B is considered a specific and reliable immunohistochemical marker in these patients. Elevated blood levels of either one of these are associated with poor response and poor overall survival (6). Melanoma characteristics, including type, Breslow thickness, Clark level, mitotic rate, and ulceration status, are correlated with the prognosis in patients with CM, with Breslow tumor thickness being the strongest prognostic factor (7). We evaluated the predictive significance of these histopathologic and laboratory parameters, as well as demographic characteristics and imaging findings for histopathology findings of SLN and progression-free survival (PFS).

Methods

Patients

The study included stage 1 and stage 2 cutaneous melanoma patients that underwent lymphoscintigraphy with 99mTc-nanocolloid at the Center for Nuclear Medicine with Positron Emission Tomography (PET) of the University Clinical Center of Serbia between March 2017 and March 2025 for detection of SLN. A total of 157 patients were enrolled in the first part of the study, which analyzed the predictive value of prognostic factors for SLN positivity. Patients that lacked data related to specific laboratory parameters and histopathology findings were excluded from the study. The second part of the study, in which the predictive value of melanoma histopathologic features and laboratory findings for PFS was evaluated, included 115 and 68 patients, respectively, due to some patients being lost in the follow-up. Patients with concomitant malignancy and patients that died due to a cause other than CM were also excluded from this part of the study.

Data collection

Data were obtained retrospectively using institutional medical records. Demographic variables (age and sex), the location of the surgical scar, the number of dosages applied around the scar, the

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presence or absence of lesions suspected of a metastatic disease observed with other imaging methods, CM characteristics (type, Breslow thickness, Clark level, mitotic rate, and ulceration status), laboratory findings (S100 protein, LDH), the location of a detected SLN, the appearance of a second lymph node in another lymph node group and its location when appropriate, the absence or presence of SLN on dynamic and early static acquisitions, and the histopathology findings of SLN were recorded. Progression of the disease proven either by biopsy or imaging (ultrasound, multidetector computed tomography [MDCT], magnetic resonance, or PET-CT) as well as the time from the procedure to progression or loss to follow-up were also recorded.

Protocol and image analysis

All the examinations were performed at the University Clinical Center of Serbia and reported by two nuclear medicine specialists. Planar images were acquired on a Symbia S gamma camera (Siemens Medical Solutions USA Inc., Malvern, PA, USA) in all patients after an intradermal injection of 18 to 30 MBq of ^{99m}Tc-nanocolloid in 0.3 ml at two to eight sites 5 to 10 mm from the surgical scar. The number of injections was determined based on the size of the scar. The injection sites were marked with a marker pen. The acquisitions were carried out using a low-energy high-resolution (LEHR) collimator with a zoom of ×1 and maintaining the energy window at 140 keV. A dynamic anteroposterior acquisition was performed immediately after the radiopharmaceutical injection with a 128 × 128 matrix for a total of 10 min. Dynamic acquisition was immediately followed by a 10-minute early static anteroposterior image acquisition. After a post-injection interval of 1 hour, a 10-minute late static acquisition was performed. SPECT/CT was performed on Symbia Intevo 6 (Siemens Medical Solutions USA Inc., Malvern, PA, USA) when necessary. A CT scan was carried out without the application of an intravenous contrast agent, covering an identical field of view to planar imaging. The SPECT acquisition followed immediately after that. After the acquisition, the data obtained were analyzed on a SYNGO workstation (Siemens Medical Systems, Forchheim, Germany). The obtained images were interpreted visually. The first focal zone of radiopharmaceutical accumulation above the background, detected outside of the area of application, was considered SLN. SLN was marked with a marker pen while the patient was lying in the same position as he would be lying on the operating table the next day. The appearance of other focal zones was also noted. When two focal zones were detected at the same moment, both were marked.

Surgical approach

The excision of SLN was performed a day after lymphoscintigraphy. The surgical technique involved an incision of the skin and subcutaneous tissue at the location of the previously marked SLN. The lymph basin was palpated, after which the area with the highest radioactivity was detected using nuclear surgical cadmium telluride (CdTe) SOE11 and cesium iodide (CsI) SOE16 gamma probes (Eurorad, Eckbolsheim, France). Careful tissue preparation and dissection of the SLN with an ex vivo verification of the radioactivity level in the removed lymph node with a probe were performed, followed by reconstruction, drainage, and closure of the operative wound. The specimen was sent for standard histopathological verification.

Follow-up

Follow-up was conducted through clinical reports. PFS was defined as the time from SLNB, which was obtained from medical records, to the detection of metastatic disease proven by either biopsy or imaging, or until the end of the follow-up.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 31.0, Armonk, NY, USA) and the R statistical package. The Kolmogorov–Smirnov test was performed to test continuous variables for normal distribution. Minimum, median, and maximum values were calculated for continuous variables due to their non-normal distribution, and proportions were calculated for categorical variables. Univariable binary logistic regression analysis was performed to determine the significance of demographic factors, CM histopathologic features, and laboratory and imaging findings in predicting the histopathology findings of SLN. Multivariable logistic regression analysis was used to assess the independent predictive significance of the variables that previously showed statistical significance in predicting the histopathology results in univariable logistic regression analysis. The Kaplan–Meier method was used to estimate the probability of survival past the given time points, with the survival distributions of groups of patients being compared for equality with a log-rank test. In addition, the predictive value of demographic factors, CM histopathologic features, SLN histopathology results, and laboratory findings for PFS were assessed using Cox regression univariate and multivariate analysis. A p-value < 0.05 was considered statistically significant for all the tests. For the numerical variables, cutoff values for differentiating between benign and malignant SLNB findings and progression of the disease during follow-up or a lack thereof were determined using receiver operating characteristic (ROC) curve analysis for quantitative tests when feasible.

Results

Patient characteristics

The study included 157 patients. The median age was 64 years (range: 23–88), and 80 (50.96%) patients were men. The most common locations of a surgical scar around which the radiotracer was applied were the interscapular region (15/157), the left lower leg (13/157), and the left upper arm (13/157). The number of dosages applied around the scar varied between two and eight (median: four). Five patients had suspected metastasis previously detected with other imaging methods in the region where SLN was found, and only three patients had positive imaging findings in terms of a suspected metastatic disease outside the region of SLN. The most common locations of a detected SLN (Figs. 1a–c) were the left axillary region (42/157), the right axillary region (29/157), the left inguinal region (24/157), and the right inguinal region (24/157). The most common location of the second lymph node, observed in the lymph node group other than that of the SLN, was the right axillary region (15/46). Histopathology findings in SLN were positive for CM in 28 patients (17.83%) and negative in 129 patients (82.17%). CM characteristics and laboratory findings are presented in Table 1.

Logistic regression analysis

Neither age (odds ratio [OR] 1.00; 95% confidence interval [CI], 0.97–1.03; p = 0.93, univariable logistic regression analysis) nor sex (OR 0.51; 95% CI, 0.22–1.20; p = 0.12, univariable logistic regression analysis) showed statistical significance in predicting the histopathology results of SLN in univariable logistic regression analysis. In addition, the predictive significance of SLN appearance on dynamic acquisition (OR 0.29; 95% CI, 0.39–2.10; p = 0.22, univariable logistic regression analysis) and early static acquisition (OR 0.56; 95% CI, 0.14–2.24; p = 0.41; univariable logistic regression analysis) for malignant findings in SLN could not be confirmed. In univariable logistic regression analysis of CM characteristics, Breslow thickness, ulceration status, and mitotic rate showed possible predictive significance for positive histopathological findings in SLN, whereas Clark level and CM type were not significant predictors. On the other hand, LDH blood level was a significant predictor of malignant findings in SLN in univariable logistic regression analysis, whereas S100 was not (Table 2). However, among four explanatory variables that showed predictive significance in univariable logistic regression analysis, only LDH blood level > 280 U/l was an independent predictor of malignant findings in SLN in multivariable logistic regression analysis (OR 3.99; 95% CI, 1.05–15.10; p = 0.042, multivariable logistic regression analysis; Table 3). In the receiver operating characteristic (ROC) curve analysis for SLN histopathology results, a cutoff value of 3.3 mm for Breslow thickness (area under the curve [AUC] 0.69; 95% CI, 0.58–0.79; ROC curve analysis for the quantitative test) with a sensitivity of 64.3% and specificity of 67.5% (Fig. 2a) and a cutoff value of 281 U/l for LDH (AUC 0.65; 95% CI, 0.47–0.82; ROC curve analysis for the quantitative test) with a sensitivity of 66.7% and specificity of 71.7% (Fig. 2b) were found.

Table 1 | **Histopathologic features and laboratory findings (157 patients).**

Variable	Values
Type	
Superficial, *n* (%)	98 (71.53)
Nodular, *n* (%)	32 (23.36)
Lentigo maligna, *n* (%)	3 (2.19)
Acral lentiginous, *n* (%)	1 (0.73)
Spitz, *n* (%)	3 (2.19)
Breslow thickness (mm), median (range)	2.50 (0.08–22.50)
Clark level	
2, *n* (%)	4 (2.61)
3, *n* (%)	33 (21.57)
4, *n* (%)	110 (71.90)
5, *n* (%)	6 (3.92)
Number of mitoses/mm², median (range)	3 (0–32)
Ulceration status	
Absent, *n* (%)	87 (62.14)
Present, *n* (%)	53 (37.86)
S100 protein (µg/l)	
≤ 0.1, *n* (%)	78 (87.64)
› 0.1, *n* (%)	11 (12.36)
LDH (U/l)	
≤ 280, *n* (%)	45 (64.30)
› 280, *n* (%)	25 (35.70)

LDH = serum lactate dehydrogenase.

The Kaplan–Meier method and Cox regression analysis

A total of 115 patients were included in the second part of the study, in which the probability of survival past the given time points and the predictive value of demographic characteristics, CM features, and SLN histopathology findings for PFS were estimated, and 68 patients were included when the predictive value of laboratory findings for PFS was evaluated. Progression of the disease in the follow-up period was observed in 23 patients (20.0%).

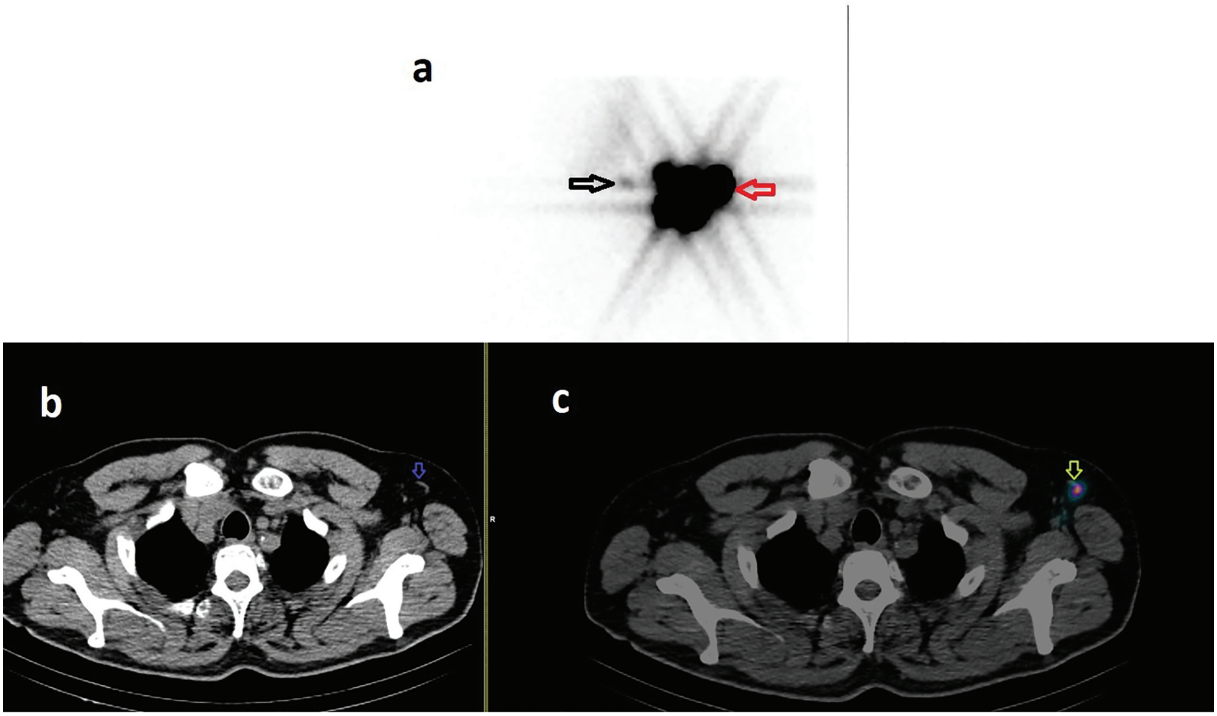

Figure 1 | Lymphoscintigraphy and single-photon emission computed tomography (SPECT/CT) with 99mTc-nanocolloid of two patients with cutaneous melanoma: (a) lymphoscintigraphy image (anterior view) of a 66-year-old male patient with cutaneous melanoma of the left clavicular region (superficial type, Breslow thickness 1.4 mm, Clark IV, 1 mitosis/mm², ulceration status negative, serum lactate dehydrogenase (LDH) 202 U/l, S100 0.06 µg/l) showing a focal zone of an increased uptake of the radiopharmaceutical (black arrow) cranially and medially to the applied dosages (red arrow). Histopathology of the sentinel lymph node (SLN) was negative for metastatic disease, without progression of the disease in the 9-month follow-up period; (b, c) SPECT/CT of a 62-year-old male patient with cutaneous melanoma of the left upper arm (Breslow thickness 4.2 mm, Clark IV, 32 mitoses/mm², ulceration status positive, LDH 371 U/l, S100 0.08 µg/l) showing an increased uptake of the radiopharmaceutical (yellow arrow) in the left axillary lymph node (blue arrow). Histopathology was negative for metastatic disease, but three in-transit melanoma metastases were detected in the left lower arm subcutaneous tissue 9 months after SLN biopsy.

In the ROC curve analysis for progression of the disease after the procedure, cutoff values of 3.8 mm for Breslow thickness (AUC 0.69; 95% CI, 0.55–0.83; ROC curve analysis for the quantitative test), with a sensitivity of 59.1% and specificity of 82.4% (Fig. 2c), and a mitotic rate of 4 mitoses/mm² (AUC 0.65; 95% CI, 0.52–0.77; ROC curve analysis for the quantitative test), with a sensitivity of 66.7% and specificity of 59.3% (Fig. 2d), were found. The median follow-up was 14 weeks (Fig. 3a). A log-rank test was performed to determine whether there were differences in the survival distribution for groups of patients with different demographic characteristics, CM features, SLN histopathology findings, and laboratory findings. The mean PFS was shorter in men (45.23 ± 5.05 months) than in women (52.35 ± 4.56 months), but the survival distributions for the two sexes were not statistically significantly different (x^2 (1) = 3.820; p = 0.051, Kaplan–Meier, log-rank test). The mean PFS was shorter in patients with positive SLN histopathology results (42.09 ± 7.33 months) than in patients with negative SLNB results (52.12 ± 3.73 months), but the survival distributions were not significantly different (x^2 (1) = 0.517; p = 0.472, Kaplan–Meier, log-rank test; Fig. 3b). Statistical significance was also not reached for the type of melanoma, Clark level, ulceration status (Fig. 3c), or S100 protein blood level (p > 0.05, Kaplan–Meier, log-rank test). However, the difference in survival distributions reached statistical significance for LDH > 280 U/l compared to values < 280 U/l (x^2(1) = 4.524; p = 0.033, Kaplan–Meier, log-rank test; Fig. 3d), Breslow thickness > 3.8 mm (x^2(1) = 18.444; p < 0.001, Kaplan–Meier, log-rank test; Fig. 3e), and mitotic rate > 4 (x^2(1) = 5.562; p = 0.018, Kaplan–Meier, log-rank test; Fig. 3f, Table 4). In univariate Cox regression analysis, neither age (OR 1.01; 95% CI, 0.98–1.05; p = 0.389, univariate Cox regression analysis) nor sex (OR 2.30; 95% CI, 0.97–5.48; p = 0.059, univariate Cox regression analysis) were statistically significant predictors of PFS. Furthermore, among CM histopathologic features, SLN histopathology results, and laboratory findings, only the number of mitoses/mm² (OR 1.08; 95% CI, 1.03–1.14; p = 0.002, univariate Cox regression analysis) and Breslow thickness (OR 1.16; 95% CI, 1.02–1.31; p = 0.021, univariate Cox regression analysis) were possible predictors of PFS in univariate Cox regression analysis, whereas the num-

Table 2 | **Univariable logistic regression analysis for histopathologic features and laboratory findings (157 patients).**

Parameter	OR	95.0% CI		$p^\dagger$
		Lower	Upper	
Superficial type	0.64	0.28	1.46	0.288
Nodular type	1.75	0.69	4.45	0.239
Breslow thickness	1.18	1.05	1.32	**0.005**
Clark level	2.02	0.85	4.82	0.114
Number of mitoses/mm²	1.07	1.01	1.13	**0.031**
Ulceration status	2.73	1.14	6.51	**0.024**
S100 protein	1.11	0.22	5.75	0.900
LDH	5.33	1.56	18.20	**0.007**

†statistically significant p-values (< 0.05) are in bold.
OR = odds ratio, CI = confidence interval, LDH = serum lactate dehydrogenase.

Table 3 | **Multivariable logistic regression analysis for histopathologic features and laboratory findings (157 patients).**

Parameter	OR	95.0% CI		$p^\dagger$
		Lower	Upper	
Breslow thickness	1.13	0.92	1.39	0.225
Ulceration status	1.33	0.28	6.36	0.723
Number of mitoses/mm²	1.00	0.89	1.11	0.937
LDH	3.99	1.05	15.10	**0.042**

†statistically significant p-values (< 0.05) are in bold.
OR = odds ratio, CI = confidence interval, LDH = serum lactate dehydrogenase.

Table 5 | **Univariate and multivariate Cox regression analysis for histopathologic features and laboratory findings.**

Parameter	OR	95.0% CI		$p^\dagger$
		Lower	Upper	
Univariate Cox regression analysis for histopathologic features (115 patients)				
Superficial type	0.91	0.40	2.07	0.815
Nodular type	0.28	0.07	1.20	0.086
Breslow thickness	1.16	1.02	1.31	**0.021**
Clark level	1.21	0.46	3.14	0.699
Number of mitoses/mm²	1.08	1.03	1.14	**0.002**
Ulceration status	2.14	0.89	5.16	0.091
SLN histopathology	1.43	0.53	3.87	0.480
Multivariate Cox regression analysis for histopathologic features (115 patients)				
Number of mitoses/mm²	1.07	1.01	1.13	**0.025**
Breslow thickness	1.12	0.97	1.30	0.128
Univariate Cox regression analysis for laboratory findings (68 patients)				
S100 protein	2.61	0.30	22.68	0.385
LDH	5.21	0.95	28.59	**0.057**

†statistically significant p-values (< 0.05) are in bold.
OR = odds ratio, CI = confidence interval, LDH = serum lactate dehydrogenase, SLN = sentinel lymph node.

Table 4 | **Log-rank test results and mean progression-free survival in months by groups.**

Parameter	Value, mean ± standard error (months)		Chi-square (x^2)	Log-rank test p-value†
Histopathologic features (115 patients)				
Type	Superficial	Other		
	52.32 ± 4.49	46.78 ± 4.71	0.056	0.813
Type	Nodular	Other		
	59.49 ± 4.34	46.59 ± 4.01	3.422	0.064
Breslow thickness (mm)	≤ 3.8	> 3.8		
	57.50 ± 3.73	30.17 ± 5.06	18.444	**< 0.001**
Clark level	II and III	IV and V		
	53.39 ± 5.14	49.67 ± 4.41	0.152	0.697
Number of mitoses/mm²	≤ 4	> 4		
	55.22 ± 3.92	40.87 ± 5.23	5.562	**0.018**
Ulceration status	Yes	No		
	41.25 ± 5.65	52.23 ± 4.37	3.040	0.081
SLN histopathology	Malignant	Benign		
	42.09 ± 7.33	52.12 ± 3.73	0.517	0.472
Laboratory findings (68 patients)				
S100 protein (μg/l)	> 0.1	≤ 0.1		
	20.60 ± 3.04	51.51 +/- 4.81	0.819	0.365
LDH (U/l)	> 280	≤ 280		
	26.85 ± 3.44	60.50 +/- 4.00	4.524	**0.033**

†statistically significant p-values (< 0.05) are in bold.
OR = odds ratio, CI = confidence interval, LDH = serum lactate dehydrogenase, SLN = sentinel lymph node.

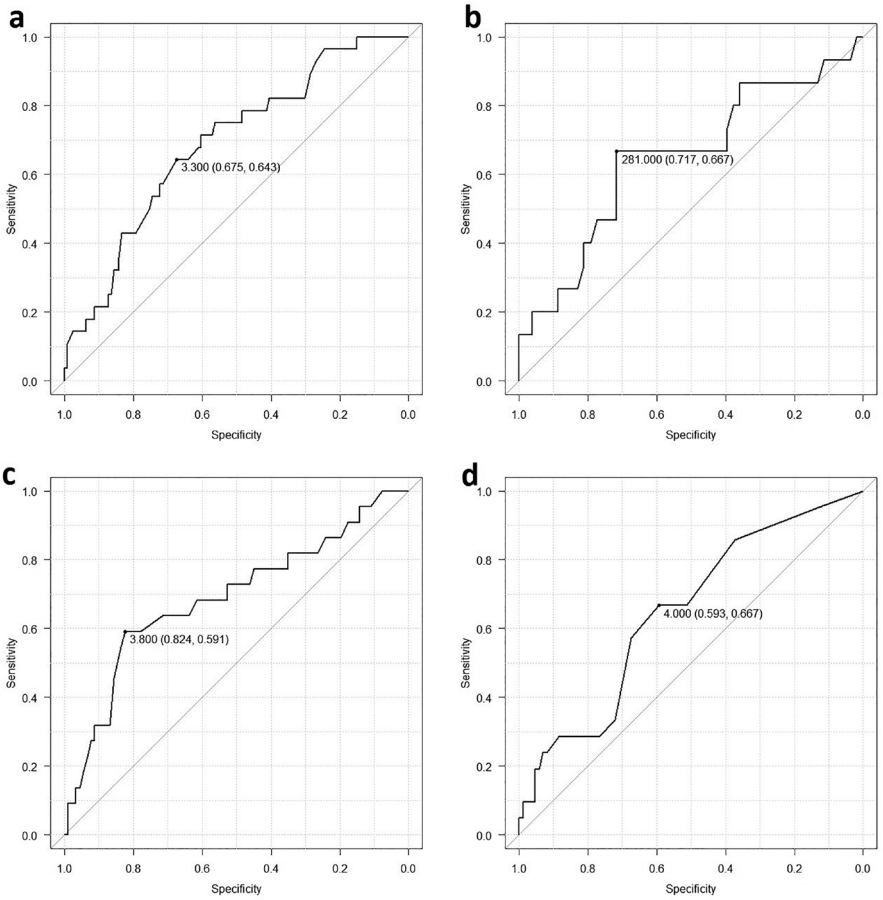

Figure 2 | Receiver operating characteristic (ROC) curve analysis for quantitative tests: curves showing sensitivity and specificity for different cutoff values of (a) Breslow thickness and (b) serum lactate dehydrogenase for the sentinel lymph node (SLN) histopathology results, and (c) Breslow thickness and (d) mitotic rate for progression of the disease in the follow-up period.

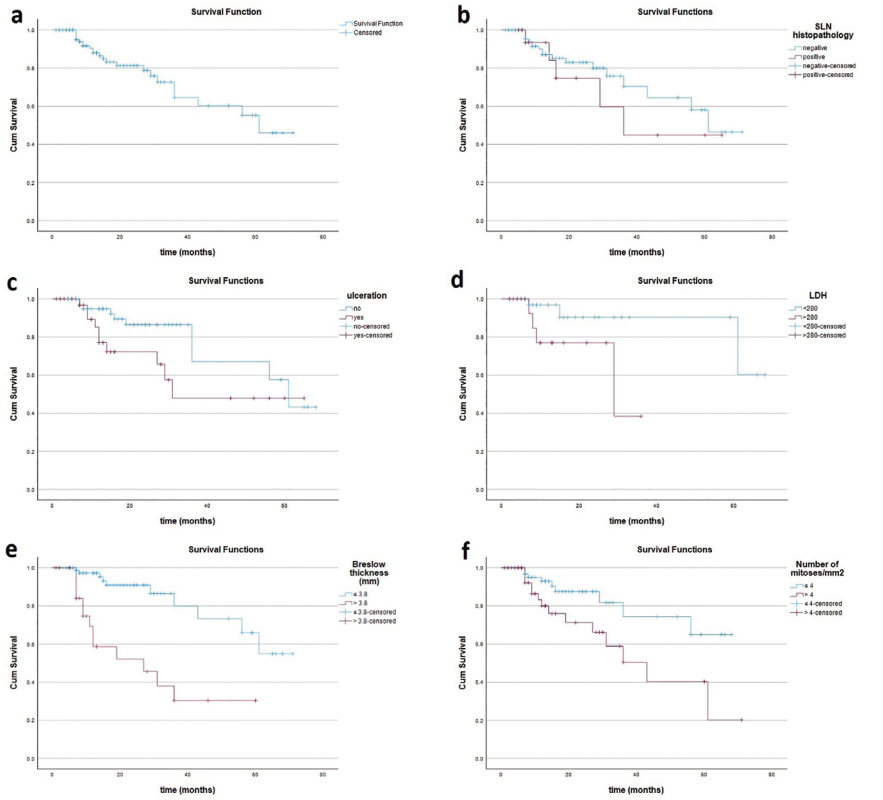

Figure 3 | Kaplan–Meier survival analysis of progression-free survival (PFS) for (a) all patients and according to (b) sentinel lymph node histopathology, (c) ulceration status, (d) lactate dehydrogenase, (e) Breslow thickness, and (f) mitotic rate.

ber of mitoses/mm² remained an independent predictor of PFS in multivariate Cox regression analysis (OR 1.07; 95% CI, 1.01–1.13; $p = 0.025$, multivariate Cox regression analysis; Table 5).

Discussion

Although debates have been initiated on whether SLNB is a necessity in the era of gene therapy and immunotherapy (8) and the possible use of diagnostic tools such as gene expression profiling in detecting high-risk patients (9), SLNB retains its significance in managing patients with CM (10). Recently, attempts have been made to better select patients to undergo SLNB based on clinical and histopathological predictors, and to therefore improve cost-effectiveness (11). This study demonstrated that only LDH blood level was an independent predictor of SLN histopathology findings. In addition, the results showed that Breslow thickness, ulceration status, and mitotic rate are possible predictors of SLN histopathology findings.

According to some studies, age may be associated with SLNB results (12, 13). Some of these studies suggest that elderly patients have a higher SLN positivity rate (14), whereas others suggest that younger patients have a higher risk of SLN being positive for malignancy in CM patients (15). On the other hand, there are studies that indicate a lack of predictive significance of age for histopathology results (16), which is consistent with our results. Jeremić et al. were also unable to prove the predictive value of sex for SLN positivity (16). Santos et al. reached similar conclusions (17). These results are consistent with ours as well. However, a study by Berghe et al. suggested that men might be at higher risk of SLN positivity (18). We also evaluated the predictive significance of SLN appearance on dynamic and early static acquisitions, with the idea that the rapidity of lymph drainage from the CM primary location could be a predictor of SLN positivity. However, the predictive value of early SLN appearance on lymphoscintigraphy could not be proven.

The most recent systematic review and meta-analysis showed predictive significance of Breslow thickness ≥ 0.8 mm, the presence of ulceration, and a higher mitotic rate for SLN positivity, with ulceration status being the most predictive factor, whereas the Clark level was not a significant predictor (19). The results of our study suggest that the same features are possible predictors of positive SLN histopathology results, but no independent predictive value could be confirmed for any of these features. The results of the aforementioned meta-analysis by Huang et al. are partially consistent with earlier meta-analyses on this topic from 7 years ago (20) and 9 years ago (21), which also suggested the predictive value of Breslow thickness, ulceration status, and mitotic rate, but also of a Clark level either higher than IV (20) or III (21), which is not consistent with our results. Different SLN positivity rates are reported for different types of melanoma, ranging from below 10%, such as in lentigo maligna melanoma (22), to 25% in acral lentiginous melanoma (23), which was associated with a higher risk of SLN positivity compared to that of superficial spreading melanoma in the study by Cheraghlou et al. (24). However, the type of melanoma was not a significant predictor of SLN positivity in our study, which is consistent with the findings of Jeremić et al. (16).

Although LDH and S100 protein have been proven to be important prognostic factors with high specificity for disease progression in patients with melanoma (25), earlier studies suggested a lack of predictive value of either biomarker in determining SLN status (26, 27). These results are only partially consistent with our findings, which showed a predictive significance of LDH blood level and a lack thereof for the S100 protein for SLN histopathology results. No recent study has evaluated the predictive value of these biomarkers for SLN positivity. In the future, more extensive studies using artificial intelligence models as tools for predicting SLN positivity should be considered, as has been the practice in some recent ones (28, 29).

In this study, mitotic rate was an independent predictor of PFS, and Breslow thickness was a possible predictor. In the last three editions of the American Joint Committee on Cancer (AJCC) staging system, ulceration status and Breslow thickness were used to categorize CM patients into thickness (T) stages (30). Mitotic rate was not recommended as a staging criterion in the most recent edition because Breslow thickness and ulceration status proved to have higher predictive value for the outcome (31). Nevertheless, multiple studies suggest the importance of mitotic rate as a prognostic factor in CM. A multicentric Italian study confirmed that the mitotic rate was an independent predictor of disease-free survival in CM with Breslow thickness > 1 mm (32). In a 2025 study, a higher mitotic rate proved to be a negative predictor of recurrence-free survival, along with Breslow thickness, both in patients with anti-programmed cell death protein 1 treatment and in patients with no treatment (33). In a large 2018 cohort study that included 71,235 patients, the mitotic rate was an independent predictor of survival in CM patients (34). This is consistent with our findings. Despite the Breslow measurement having a few limitations, such as a possible underestimation of thickness in the case of positive ulceration status (35), it remains one of the strongest prognostic factors for PFS, as suggested by multiple studies (33, 36), and it has been the most commonly used variable in the prediction tools for melanoma survival (37). This is also consistent with our results, which showed Breslow thickness to be a possible predictor of PFS. Karakousis et al. found that patients with positive ulceration status had a shorter melanoma-specific survival (hazard ratio 1.9) (38). Another study also suggested lower survival in thin melanoma patients with positive ulceration status, with an increase in the survival difference with time (39). This disagrees with our results, which may be due to the limited number of ulcerated cases in our group of patients. In our study, patients with positive ulceration status had shorter PFS, but statistical significance was not reached. The sixth AJCC melanoma staging guidelines used the Clark level of invasion and ulceration in addition to the tumor Breslow thickness to subdivide thin melanoma as T1a or T1b. In the seventh edition (2009) of the AJCC classification, Clark level, which was recommended along with Breslow thickness and ulceration status as a variable for discriminating between T1a and T1b stages in the previous edition, was no longer recommended for these purposes (7). In our study, CM patients with Clark level IV–V had shorter survival compared to those with Clark level II–III, but the difference in survival distributions did not reach statistical significance. In a 2009 multi-center study, SLN histopathology was the most important prognostic factor for melanoma-specific survival (40). Jafari et al. reported similar findings (41). In our study, the survival difference between groups was not statistically significant, although patients with benign SLN histopathology findings did have a longer mean PFS. SLN histopathology results could not be proven to be a predictor for PFS either. However, both studies mentioned above had a larger number of patients than this study. Therefore, the reason for the discrepancy with our results can most likely be attributed to the limited num-

ber of patients with malignant SLN findings in the second part of our study, in which PFS was analyzed.

Fan et al. suggested a shorter survival of patients with higher LDH and S100 protein levels (42). Another study from 2024 found that S100 is an independent predictor of metastatic disease in melanoma patients, and LDH is a possible predictor (25). Both LDH alone (43) and together with S100 protein (6) were also found to be predictors for survival in melanoma patients treated with immune checkpoint inhibitors targeting programmed death receptor-1 in separate studies. In this study, the survival difference between patients with high and normal LDH was statistically significant, which was not the case for S100 protein.

We should acknowledge the main limitations of the study. This was a single-center retrospective study with a limited number of cases with ulcerations, positive SLNs, and availability of laboratory parameters, especially in the part of the study in which PFS was estimated. The sample was reduced to 115 and 68 patients in this part of the study due to loss of follow-up of some, which led to some predictors not reaching statistical significance, even though a difference in the mean PFS was observed.

Conclusions

In this study, LDH blood level was the only independent predictor of SLN histopathology results, and Breslow thickness, ulceration status, and mitotic rate were possible predictors of SLN status. Age, sex, imaging findings, Clark level, type of melanoma, and S100 protein blood level did not show predictive significance for SLNB results. Furthermore, the difference in survival distributions reached statistical significance for Breslow thickness, mitotic rate, and LDH. Mitotic rate was an independent predictor of PFS, and Breslow thickness was a possible predictor.

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