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ORIGINAL ARTICLE

Should we report Breslow density, a new concept in cutaneous melanoma?

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Abstract

Introduction: Breslow density is a newly defined biomarker, independent of Breslow thickness. We aimed to investigate the relationship of Breslow density with other clinicopathological prognostic factors and its effect on the overall survival and disease-free survival in patients with cutaneous melanomas. *Materials & Methods:* This was a single-centre retrospective study of patients (n = 19) diagnosed with cutaneous malignant melanomas in our hospital between 2011 and 2019 were included in the study. The exclusion criteria were *in situ* melanomas, punch or incisional biopsies and metastasis at the time of the diagnosis. Breslow density was determined by re-evaluating slides obtained at the time of the initial diagnoses. The effect of Breslow density on survival was determined using univariate and multivariate Cox proportional risk analyses. *Results:* In terms of the overall survival, mortality risk increased as Breslow density increased (p = 0.044). Breslow density was not significantly associated with the overall survival in the multivariate model (p = 0.078). In terms of disease-free survival, the risk of metastasis or recurrence increased 1.229-fold in accordance with an increase in Breslow thickness (CI: 1.057-1.428), whereas increased Breslow density increased the metastasis or recurrence risk 1.059-fold (CI: 1.008-1.112). In the multivariate model, only Breslow density was statistically significant (p = 0.046). *Conclusions:* As a semi-quantitative and subjective measurement, Breslow density is not a completely accurate representation of the invasive tumour load. However, the measurement is practical and low cost and requires no additional equipment. Therefore, Breslow density can be measured in every laboratory. Considering the value of Breslow density in predicting the prognosis in patients with cutaneous melanomas and strong inter-observer compliance observed in the present study, we believe that it would be useful to include this measurement in pathology reports.

Keywords: Breslow density, histopathology, melanoma, prognosis, survey

INTRODUCTION

Malignant melanomas (MMs) are the result of transformation of melanocytes from melanin-producing neural crest cells into melanocytic neoplasms.[1] MMs account for 2% of all skin cancers and are the leading cause of skin cancer-related deaths.[2] The incidence rates of skin cancer continue to increase rapidly worldwide, with 160,000 new cutaneous MM cases diagnosed each year.[3] Although previous melanoma research suggested that patients diagnosed with early-stage disease have a better prognosis than those diagnosed with late-stage disease, more recent studies reported a significant difference between the mortality rates of patients with early-stage disease.[4-6]

The inclusion of immune checkpoint inhibitors and targeted drugs in the treatment of melanoma patients with advanced-stage disease has become routine in recent years, with the selection of the most appropriate treatment methods based on risk-associated grouping.[7] An accurate estimate of the risk of metastasis and mortality is important, both in terms of informing patients and treatment management. Some studies based on tumour biology demonstrated that gene expression profiling may provide individualised prognostic information.[4,8,9] However, gene expression profiling is time consuming and involves high-cost procedures. Furthermore, it cannot be carried out in all laboratories because of the need for technical expertise and specific

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equipment. Therefore, the identification of new prognostic bioindicators is important to improve risk classification of melanomas. At present, TNM staging remains the gold standard for risk classification.[7] This staging system is accepted in the 8th edition of the American Joint Committee on Cancer (AJCC), updated in 2020.[10] In the TNM system, the T stage is determined by ulceration and tumour thickness, currently known as Breslow thickness (BT).

Breslow first pointed out the prognostic importance of tumour thickness in his study on 98 cases of cutaneous melanomas in 1970 and suggested in the same study that tumour volume may be a better prognostic indicator than tumour thickness.[11] Since then, many studies have demonstrated the prognostic importance of tumour volume in malignant melanomas.[12-14] However, the methods for calculating tumour volume recommended in most of these studies involved obtaining numerous tumour blocks and performing tumour sectioning, thereby markedly increasing both laboratory costs and workloads. Rashed *et al*.[7] and Saldanha *et al*.[15,16] have proposed a new parameter named Breslow density (BD), which includes both BT and tumour volume, and reported that this parameter is prognostically stronger than BT alone. BD can be evaluated using only haematoxylin-eosin-stained slides.

The availability of parameters, such as BD, that can be evaluated using only haematoxylin-eosin-stained slides is important, as many people, especially those in developing countries, cannot afford high-cost tests based on molecular studies. Furthermore, many laboratories do not have the equipment required to conduct such tests. Thus far, there are three promising published studies related to BD in the literature.[7,15,16] The same research team conducted all three studies. The repeatability of these studies needs to be demonstrated prior to the inclusion of BD measurements in routine protocols. Thus, the aim of the present study was to investigate the relationship of BD with other clinicopathological prognostic factors and to determine its impact on overall survival (OS) and disease-free survival (DFS) in patients diagnosed with cutaneous MMs in our institute.

MATERIALS AND METHODS

This was a single-centre retrospective study and approved by the ethics committee of our institution (Date: 01.12.2020, Approval No: 14/14).

Patient Selection

The archives of the pathology department of our hospital were screened, and files and slides from patients diagnosed with cutaneous MMs between 2011 and 2019 were reviewed. *In situ* melanomas, punch and incisional biopsies in which the tumour thickness could not be accurately assessed and cases with metastasis at the time of diagnosis were excluded from the study. The latter group was excluded because DFS and metastasis-free survival (MFS) could not be evaluated. From an initial patient population of 25 cases, six cases were excluded for the following reasons: metastasis at the time of diagnosis ($n = 2$), *in situ* melanomas ($n = 2$) and punch biopsies ($n = 2$). Thus, 19 cases were included in the study.

Histopathological Analysis

The haematoxylin-eosin-stained slides of the 19 patients were re-evaluated. The prognostic parameters recommended by the cancer reporting protocol of the College of American Pathologists (i.e. histological subtype, BT, Clark stage, pathological stage [pT], ulceration, lymphovascular invasion [LVI], microsatellites, neurotropism, regression and tumour-infiltrating lymphocytes [TILs]) were reviewed.[17] The pathological stage was determined according to the 8th edition of the AJCC.[10] Two observers (IES and DG) evaluated BD in all the slides in a blinded fashion.

Measurement of Breslow Density

BD was measured in the invasive area where BT had been measured in the formalin-fixed paraffin-embedded sections. BD was measured according to the five-step method described by Rashed *et al*.,[7] as follows:

Step 1: The location containing the deepest melanoma cells was determined;

Step 2: Created a low-power virtual window centred on the deepest melanoma cell horizontally bounded by ×10 field (2.2 mm with Olympus BX53 microscope using a Plan ×10/0.22 objective) width. The horizontal section passing through the deepest melanoma cell was accepted as the lower border of the examination window. The epidermal basement membrane in the horizontal plane was determined as the upper border of the same frame.

Step 3: At low power, the predicted window was shifted horizontally to the area

containing the highest density of dermal melanoma cells including the deepest melanoma cells.

Step 4: Centerfield on the preceding window from the previous step, the view was switched from low power to a ×10 objective. Histological landmarks that defined the two horizontal edges of the ×10 field (e.g. a specific rete tip, hair follicles, tumour/inflammatory cell clusters or sweat ducts) were identified. At low power, a precise window was reconstructed using horizontal landmarks of the basal layers and the deepest melanoma cells. In very thick melanomas, where the area could not be seen, even at low power, vertical scanning of the entire rectangular area was performed.

Step 5: The percentage area of dermal stromal tissue occupied by the melanoma cells was recorded as the BD to the nearest 5% but with precision to the nearest 1% for scores below 5% and above 95%.

As clear from the above protocol, each window focused on a fixed position (the location containing the deepest melanoma cells), the horizontal dimension was the same in all cases (×10 field width, 2.2 mm for our microscope). The vertical size varied between cases and it was the same as BT.[7] The tumour burden score (TBS) was calculated as BT × BD/100.[15]

Statistical Analysis

All data were analysed using the Statistical Package for the Social Sciences V.21.0 software package (SPSS Inc., Armonk, NY, US). Quantitative variables were expressed as the median and interquartile range, and categorical variables were expressed as numbers (*n*) and percentages (%). As shown by the Shapiro–Wilk test, the data were not normally distributed. The difference in quantitative data between the two groups was analysed using the Mann–Whitney *U* test. Differences in quantitative data in three or more groups were determined using the Kruskal–Wallis test. Spearman's correlation test was used for correlation analysis. Univariate and multivariate hazard ratios were determined using the Cox proportional hazards method. Inter-observer (IES and DG) consistency was analysed using Krippendorff's test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

In the study, 11 (57.9%) of the patients were females, and 8 (42.1%) were males. All the cases were nodular variant. Ulceration, microsatellites, LVI, neurotropism, regression, mild-moderate TILs and severe TILs were detected in 9 (47.4%), 3 (15.8%), 4 (21.1%), 3 (15.8%), 2 (10.5%), 11 (55%) and 6 (30%) of the 19 patients, respectively. Six (31.6%) cases were p53, and 13 (68.4%) cases were pT4, respectively. Recurrence and metastasis developed in 5 (26.3%) and 7 (36.8%) of the patients during the follow-up period, respectively, 9 (47.4%) of whom died.

The median age of the patients was 63 years. The median number of mitotic count was 4.2. The median BT, BD and TBS were 5 mm, 70% and 3.75, respectively. The median metastasis-free survival was 37 months, and the median OS and median DFS were 42 and 28 months, respectively. The descriptive statistics for the quantitative variables are presented in Table 1.

Table 1: Descriptive statistics for quantitative variables

Variables	Median (Interquartile range)	Minimum-Maximum
Age	63.00 (50.00-79.00)	21.00-88.00
Mitotic count\mm^2	4.20 (1.70-6.30)	0.80-13.90
Breslow thickness(mm)	5.00 (3.13-7.00)	2.10-18.00
Breslow density%	70.00 (60.00-85.00)	10.00-95.00
Total Breslow score	3.75 (1.75-4.80)	0.42-17.10
Metastasis-free survival (month)	37.00 (8.00-67.00)	1.00-106.00
Overall survival (month)	42.00 (22.00-67.00)	4.00-106.00
Disease-free survival (month)	28.00 (8.00-67.00)	3.00-106.00

The consistency between the observers (IES and DG) in the evaluation of BD was very high (α: 0.988, $p < 0.001$).

BD showed a correlation with BT (Rho: 0.590, $p = 0.008$), age (Rho: 0.494, $p = 0.031$) and the mitotic count (Rho: 0.575, $p = 0.010$). BD was significantly higher in those who developed metastasis or died ($p = 0.012$ and $p = 0.010$, respectively). BD was significantly associated with LVI and TILs ($p = 0.035$ and $p = 0.031$, respectively). The median BD in the patient group with LVI was 87.5% versus 70% in the group without LVI. The median BD was 40% in the group without TILs and 77.5% and 67.5% in the groups with mild-moderate and severe TILs, respectively. The relationships of BD and other prognostic factors with recurrence, metastasis and death are summarised in Table 2.

Table 2: Relationship of Breslow density with prognostic factors, recurrence, metastasis and death

Variables	Categories	N (%)	Median BD (Interquartile range)	p
Gender	*Female*	11(57.9)	65.00 (45.00-75.00)	**0.031**
	Male	8(42.1)	82.00 (71.25-93.75)	
Tumour site	*Central*	4(21.1)	60.00 (21.25-72.50)	0.100
	Periferal	15(78.9)	75.00 (65.00-90.00)	
pT	*3a*	2(10.5)	57.50 (55.00-60.00)	0.354
	3b	4(21.1)	67.50 (50.00-85.00)	
	4a	5(26.3)	75.00 (40.00-82.50)	
	4b	8(42.1)	77.50 (66.25-95.00)	
CLARK	*IV*	15(78.9)	75.00 (60.00-85.00)	0.596
	V	4(21.1)	67.50 (23.75-88.75)	
Ulcer	*Absent*	10(52.6)	67.50 (52.50-76.25)	0.065
	Present	9(47.4)	80.00 (67.50-95.00)	
Microsatellite	*Absent*	16(84.2)	70.00 (57.50-82.50)	0.254
	Present	3(15.8)	80.00 (75.00-87.50)	
LVI	*Absent*	15(78.9)	70.00 (55.00-80.00)	**0.035**
	Present	4(21.1)	87.50 (75.00-87.50)	
Neurotropism	*Absent*	16(84.2)	72.50 (62.50-87.50)	0.359
	Present	3(15.8)	70.00 (40.00-72.50)	
Regression	*Absent*	17(89.5)	70.00 (60.00-80.00)	0.142
	Present	2(10.5)	87.50 (85.00-90.00)	
TILs	*None*	3(15)	40.00 (25.00-50.00)	**0.031**
	Mild/moderate	10(55)	77.50 (70.00-85.00)	
	Severe	6(30)	67.50 (55.00-95.00)	
Relapse	*Absent*	14(73.7)	67.50 (52.50-82.50)	0.130
	Present	5(26.3)	80.00 (72.50-90.00)	
Metastasis	*Absent*	11(57.9)	65.00 (45.00-75.00)	**0.012**
	Present	8(42.1)	82.50 (71.25-93.75)	
Death	*Absent*	10(52.6)	65.00 (52.50-71.25)	**0.010**
	Present	9(47.4)	85.00 (72.50-95.00)	

BD: Breslow density, p<0.05 is statistically significant.

Table 3: Investigation of risk factors affecting overall survival by Cox regression analysis

	Univariate		Multivariate	
	HR (%95 Cl)	p	HR (%95 Cl)	p
Breslow thickness	1.117 (0.994-1.256)	0.064	1.043 (0.894-1.217)	0.590
Breslow density	1.049 (1.001-1.098)	**0.044**	1.066 (0.993-1.144)	0.078
TBS	1.116 (0.998-1.248)	0.055	*	*
Ulcer	1.206 (0.323-4.506	0.780	0.300 (0.052-1.737)	0.179

HR: Hazard ratio, (%95 confidence interval). TBS: Total Breslow score. p<0.05 is statistically significant. *TBS is not included in multivariate analysis as it depends on Breslow thickness and Breslow density.

The independent risk factors that affected OS were analysed using univariate and multivariate Cox proportional hazards methods. In terms of the mortality risk, OS increased as BD increased. The parameters BT, TBS and presence of ulceration had no significant impact on OS ($p = 0.064$, $p = 0.055$ and $p = 0.780$, respectively). BD was a significant predictor of OS in the multivariate model ($p = 0.078$) (Table 3).

The independent risk factors that affected DFS were analysed using univariate and multivariate Cox proportional hazards methods. According to the results of the univariate analysis, in terms of DFS, increased BT elevated the metastasis or recurrence risk 1.229-fold (Cl: 1.057-1.428). Increased BD and an increase in the TBS elevated the metastasis or recurrence risk 1.059-fold (CI: 1.008-1.112) and 1.226-fold (CI: 1.062-1.416), respectively. In the multivariate model, only BD was statistically significant as independent risk factors that affected DFS ($p = 0.046$) (Table 4). Figure 1 shows the association of the parameters BT, BD and TBS with OS.

DISCUSSION

The estimation of metastasis and mortality risk by clinicians in any cancer type is difficult but important in terms of both informing patients and treatment management. Recent studies showed that targeted treatments and immune therapies, when administered in an appropriate adjuvant setting, led to a 50% increase in recurrence-free survival in patients with stage III melanomas.[18,19] This finding demonstrates the importance of accurate predictive prognostic factors in the selection of appropriate treatment options. Although novel molecular tests are promising in this regard, they are not practical due to a lack of accessibility and high cost. Therefore, novel, lower cost prognostic bioindicators that are more easily applicable in the clinical setting are needed.

Rashed *et al*.[7] first described the use of BD, which provides information on the tumour load, in their series of 100 melanoma cases and reported that BD was a prognostic bioindicator. Various studies have investigated the effect of tumour volume on the prognosis in cutaneous MM.[12-14,20] Voss *et al*.[20] calculated the tumour volume based on a mathematical calculation of ellipsoid volume in their series of 122 cases. They calculated the tumour volume using the formula $\pi/12 \times$ diameter a $\times$ diameter b $\times$ Breslow tumour thickness, where the diameters of the tumours were measured macroscopically. They reported that melanomas with a tumour volume exceeding a threshold value of 140 mm^3 have a significantly worse prognosis compared with tumours below this threshold value. Unlike BD measurements, most methods are not suitable for use in routine pathology practice because of their high costs and difficulty in application. The measurement of BD incurs no additional costs or technical load, and BD can be measured on

Table 4: Investigation of risk factors affecting disease-free survival by Cox regression analysis

	Univariate		Multivariate	
	HR (%95 Cl)	p	HR (%95 Cl)	p
Breslow thickness	1.229 (1.057 –1.428)	**0.007**	1.178 (0.972-1.428)	0.094
Breslow density	1.059 (1.008 –1.112)	**0.022**	1.100 (1.002-1.207)	**0.046**
Total Breslow score	1.226 (1.062-1.416)	**0.005**	*	*
Ulcer	1.224 (0.282-4.592)	0.764	0.112 (0.011-1.109)	0.061

HR: Hazard ratio, (%95 confidence interval). p<0.05 is statistically significant.

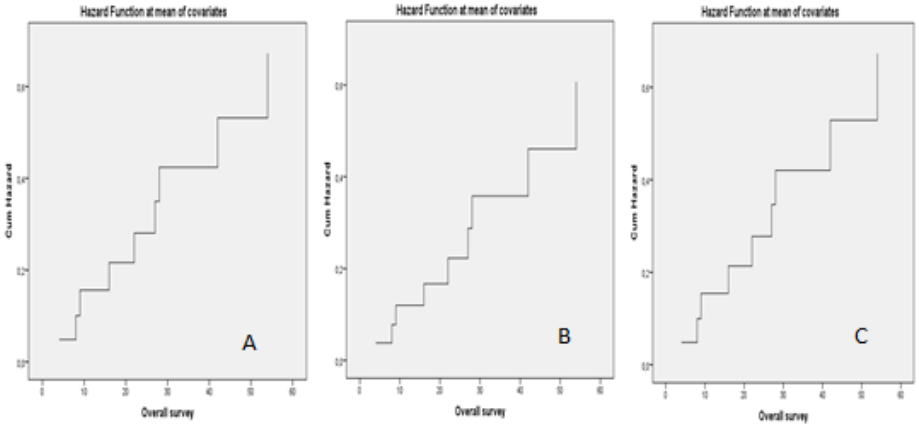

FIG. 1: Risk function graphs for overall survival time are shown for parameters of Breslow thickness (A), Breslow density (B) and Total Breslow score (C).

the same sample section as BT. Rashed *et al*.[7] and Saldanha *et al*.[15] noted that the measurement of BD is understandable and easily applicable and that consistency between observers is high. We also found that the measurement of BD was easy and quick. Likewise, consistency between the observers was high in our study.

In a continuation of the study by Rashed *et al*.,[7] Saldanha *et al*.[15] reported that BD is an independent prognostic factor and that BD higher than 65% indicates a worse prognosis. Their study was based on 1,329 cases with cutaneous MM. In another study, Saldanha *et al*.[16] reported that a parameter named the calculated tumour area, which includes tumour density, was a stronger prognostic indicator than BT.

The primary purpose of the present study was to determine the impact of BD on the prognosis of patients with cutaneous MMs. With this aim in mind, we investigated the relationship between BD and known prognostic parameters and the potential correlation of BD with BT. BT, ulceration, mitotic count, LVI, neurotropism and TILs are known prognostic parameters in melanomas.[21,22] Age, localisation and sex are also prognostic criteria.[23] Both Rashed *et al*.[7] and Saldanha *et al*.[15] reported that BD is associated with BT. Saldanha *et al*.[15] also found that BD is associated with age and the mitotic count. In our study, consistent with the literature, BD was correlated with BT (Rho: 0.590, $p = 0.008$), age (Rho: 0.494, $p = 0.031$) and mitotic count (Rho: 0.575, $p = 0.010$). BD was similar in males and females in the study by Saldanha *et al*.[15] In contrast, the median BD was significantly higher in males than females (82% and 65%, respectively) in the present study. This finding raises the question as to whether hormonal factors affect BD in females.

Rashed *et al*.[7] developed Cox regression models to investigate the effect of BD on the prognosis in patients with cutaneous melanomas but did not mention its relationship with known prognostic parameters. Saldanha *et al*.[15] evaluated the relationship of BD with age, tumour localisations, histological subtypes, microsatellites, ulceration, mitotic counts, tumour stages (AJCC-7th ed) and BT. However, they did not discuss the relationship of BD with regression, neurotropism, LVI and TILs. They attributed the exclusion of these parameters to a lack of consensus in the literature on their prognostic importance. However, regression, neurotropism, LVI and TILs are accepted as prognostic criteria in the cancer reporting protocols of the College of American Pathologists, and it recommends that these parameters should be included in pathology reports.[17] Furthermore, these parameters are used by pathologists in many countries worldwide to guide treatment decision making in patients with cutaneous melanomas. Hence, in contrast to Saldanha *et al*.,[15] we included these parameters in our study.

Saldanha *et al*.[15] found that BD was higher in the presence of microsatellites and ulceration. In contrast, we detected no statistically significant difference between the cases with and without microsatellites and ulceration in our study. However, BD was associated with LVI and TILs in our study ($p = 0.035$ and $p = 0.031$, respectively). In our study, the median BD was 87.5% in the patients with LVI and 70% in those without LVI. Previous studies reported that an

increased level of TILs is a favourable prognostic criterion in melanomas and that it indicates the immune system response to the tumour invasion [22, 24]. In our study, the median BD was 40% in the group without TILs ($n = 3$) and 77.5% and 67.5% in the groups with mild/moderate ($n = 10$ cases) and severe ($n = 6$) TILs, respectively. Taking into consideration that an increase in BD is a poor prognostic indicator and that TILs are a favourable prognostic factor, the absence of TILs would be expected to be associated with a worse prognosis.

The aforementioned may explain the outcome (i.e. mortality) in the three patients in the present study in which TILs were not detected. However, a high BD also means increased proximity between cells. The latter may have caused lymphocyte chemotaxis by increasing interactions between the cells. More comprehensive cohort studies are needed to investigate the relationship between TILs and BD and to clarify the nature of this relationship.

Rashed *et al*.[7] and Saldanha *et al*.[15] used the 7th edition of the AJCC TNM staging system in their studies. They noted that 85.1% of the patients with low BD (< 25%) were Stage 1A. In our study, as BD was correlated with BT, we expected BD to be associated with the T stage. However, we found no significant relationship between BD and pathological T stage. The latter is essentially based on BT. In our study, the absence of a statistically significant association might be due to the high T stage (pT3 and pT4) of the patients. In addition, the 8th AJCC TNM staging system includes some changes.[10] Although the mitotic index is included in the T category of the 7th edition of the AJCC TNM staging system, it is excluded from this category in the 8th edition.[10] In addition, the pT1 category in the 8th edition of the AJCC TNM staging system was changed, where pT1a denotes BT < 0.8 mm with or without ulceration and pT1b denotes BT < 0.8 mm plus ulceration or 0.8–1 mm plus the presence or absence of ulceration.[10] We used the 8th edition of the AJCC TNM staging system in our study. Thus, we included only BT, BD and ulceration in our multivariate Cox regression model and not the mitotic count.

Rashed *et al*.[7] stated that BD represents a novel morphological prognostic bioindicator, independent of BT. Saldanha *et al*.[15] noted that BD is a very significant predictor of OS, melanoma-specific survival and metastasis-free survival and that BD explains melanoma-specific survival better than does BT. However, they emphasised that the best explanatory model involves the combined use of BD and BT.[7,15] In our study, in the univariate Cox regression analysis, only BD had a statistically significant effect on OS ($p = 0.044$), with BT, TBS and presence of ulceration not affecting OS ($p > 0.050$). In the multivariate Cox regression model, BD was not an independent risk factor for OS ($p = 0.078$). In the univariate analyses, BT, BD and TBS were significant predictors of DFS ($p = 0.007$, $p = 0.022$ and $p = 0.005$, respectively), whereas only BD was an independent risk factor for DFS in the multivariate Cox regression analysis ($p = 0.046$). Furthermore, BD was significantly higher in the cases that developed metastasis or died in the follow-up period compared with those without metastasis or those who survived ($p = 0.012$ and $p = 0.010$, respectively).

The present study has some limitations. These include the single-canter design, small sample size and absence of pT1 and pT2 stage tumours.

In conclusion, BD is a predictive factor for OS and DFS. Its predictive value in the estimation of DFS is higher than that in the estimation of OS. BD is an independent prognostic indicator of DFS. BD is a semi-quantitative and subjective measurement. Thus, it is not a completely accurate representation of the invasive tumour load. However, it a practical measurement that can be performed in every laboratory due to its low cost and lack of requirement for additional equipment. Considering the value of BD in predicting the prognosis in patients with cutaneous melanomas and strong inter-observer compliance, we believe that the inclusion of BD in pathology reports would be useful.

Authors' contribution: Ilke Evrim Secinti: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing -Original Draft, Visualization. Didar Gursoy: Conceptualization, Methodology, Writing -Review & Editing. Tugce Erturk, Tumay Ozgur, Isa Dede and Esin Dogan: Investigation, Resources, Data curation.

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