Mitotic rate is a more reliable unfavorable prognosticator than ulceration for early cutaneous melanoma: A 5-year survival analysis

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Abstract. The presence of ulceration has been considered as one of the most important primary tumor characteristics of cutaneous malignant melanoma (CMM) for predicting patient outcome. Yet recently, scientific attention has been drawn towards another microscopic feature of primary tumors, the mitotic rate (MR). The present study aimed to examine the relationship between the presence of ulceration and the mitotic rate and clinicopathological characteristics and melanoma patient survival, and to discuss the results in the context of AJCC melanoma staging recommendations. Tissue samples were obtained from 104 patients treated for CMM. In classical H&E staining, the mitotic rate and the presence of ulceration were evaluated. Non-mitogenic tumors were defined as having 0 mitoses/mm^2, low mitogenic potential, 1-2 mitoses/mm^2 and highly mitogenic tumors, ≥3 mitoses/mm^2. In the entire group of 104 patients, a high mitotic rate (hMR) and ulceration were highly negative prognostic factors, and indicated considerably shorter overall survival, cancer-specific overall survival and disease-free survival. Notably, hMR appeared to have a statistically significant negative impact on survival in early melanomas in both the pT1 (P=0.001) and pT2 subgroups (P=0.006). Kaplan-Meier analysis of the remaining subsets (pT3 and pT4) did not reveal any important differences in the 5-year survival with regard to MR values. The presence of ulceration also had a prognostic significance for early melanomas, but only for pT1 tumors (P=0.05). Multivariate analysis confirmed that hMR was strongly associated with an unfavorable prognosis. Ulceration had no prognostic significance in the Cox proportional hazards model. Considering the biology of melanoma, hMR seems to be a more reliable parameter than the presence of ulceration. The value of MR categorizes melanomas into tumors with low or high proliferative potential, thus giving direct information concerning their capacity to infiltrate deeper layers of the dermis and, potentially, to generate regional lymph node and distant metastases.

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Introduction

Cutaneous malignant melanoma (CMM) is the most deadly form of skin neoplasm. With an estimated mortality of over 55,000 worldwide in 2012 which is increasing yearly and even with a more rapidly growing incidence, CMM has become a major challenge for modern oncology (1). Detailed studies are required to provide accurate risk stratification and to better identify the groups of patients in need of tailored treatment strategies.

In melanomas, multiple factors with potential prognostic value have been described over the years (2-5). Breslow thickness still remains the most powerful prognostic factor and is a substantial component of every CMM pathology report (6). The presence of ulceration has been considered as the second most important primary tumor characteristic for the purpose of predicting patient outcome (5). With the exception of traumatic disruption of the epidermis, ulceration has been a key element in the last two editions of the melanoma TNM staging guidelines of the American Joint Committee on Cancer (AJCC) - its presence verified in microscopic evaluation of changes in the pT stage from pTxa to pTxb (6,7).

Recently, scientific attention has been drawn towards another microscopic feature of the primary tumor, the mitotic rate (MR). The inclusion of this parameter in multivariate models confirmed its significance in risk stratification, particularly in localized melanomas (8-10). Additionally, several studies have indicated that MR has a higher impact than ulceration (8,11,12). As a valuable parameter that reflects tumor proliferative activity and aggressiveness, MR has recently been introduced into the AJCC melanoma staging system (6). Under

current recommendations, MR is defined as the number of mitotic figures per square millimeter and a value ≥1 upstages the T subcategory of the pTNM classification from a to b, but only at the pT1 level (6). Clinically, this translates into a recommendation for sentinel lymph node biopsy for patients presenting with pT1b, although not for those with pT1a stage tumors (6).

The present study aimed to examine the relationship between key melanoma prognosticators, namely the presence of ulceration and mitotic rate against clinicopathological characteristics and patient survival, and to discuss the results in the context of AJCC melanoma staging recommendations.

Materials and methods

Patients. The study group consisted of 104 patients with CMM, who were diagnosed between 2005 and 2010 and treated at the Lower Silesian Oncology Center in Wrocław, Poland. The group was selected on the basis of tissue material (paraffin blocks and histopathology slides) and the availability of medical documentation. Comprehensive clinical data were obtained from archival medical records. The diagnostic and therapeutic procedures utilized were determined from medical records in the Oncology Outpatient Clinic of the Lower Silesian Oncology Center and data provided by the Lower Silesian Cancer Registry and Civil Register Office. The study was approved by the Institutional Review Board of the Wrocław Medical University, Poland.

The clinicopathological profile of the patients included the following parameters: age and gender, primary tumor location, tumor stratification according to AJCC, presence or absence of nodal (pT or pN) and distant (pM) metastases, information on disease recurrence and sentinel lymph node biopsy (SLNB) procedures (Table I).

Tumor samples and histopathological evaluation. Tumor specimens were fixed in 10% buffered formalin and embedded in paraffin. All haematoxylin and eosin (H&E) stained sections were examined by two pathologists. The parameters of the primary tumor recorded in pathology reports included Breslow thickness, Clark level, growth phase, histologic type, mitotic rate (number of mitotic figures per 1 mm^2), presence of ulceration, lymphangioinvasion, microsatellitosis, intensity of lymphocytic inflammatory infiltrate (TILs, tumor-infiltrating lymphocytes) and microscopic evidence of regression (Table II).

Statistical analysis. Statistical analysis was performed using the Statistica 10.0 and IBM SPSS 21 software packages. Overall survival (OS) was defined as the time between the primary surgical treatment and death, and OS was censored at last follow-up for patients who were still alive. Disease-free survival (DFS) was defined as the time between the primary surgical treatment and the date of relapse. DFS was censored at the last follow-up for patients who survived without disease recurrence or at the date of non-cancer-associated death. Cancer-specific overall survival (CSOS) was defined as the time between the primary surgical treatment and cancer-associated death, and was censored at the last follow-up for surviving patients.

A χ^2 test, exact Fisher's test in the case of 2x2 tables and Spearman's rank correlation were used to analyze the associations between mitotic rate and the presence of ulceration and clinicopathological parameters. Differences between the means were tested with a nonparametric test (Mann-Whitney U test and Kruskal-Wallis test); the log-rank test was used to compare survival in two groups. The overall survival rate was estimated by the Kaplan-Meier method and the influence of explanatory variables on death risk was analyzed by means of the Cox proportional hazard regression. A P-value <0.05 was considered to indicate a statistically significant difference.

Results

MR and the presence of ulceration in 104 melanoma patients. The mitotic activity of the primary tumors was divided into three categories: no mitotic activity (0 mitoses/mm^2), low activity (1-2 mitoses/mm^2) and high mitotic rate (hMR; ≥3 mitoses/mm^2) (Fig. 1A-D). No mitotic activity was detected in 45 patients (43.3%), whereas low MR was observed in 26 patients (25%). High proliferative activity was observed in 33 patients (31.7% of the study group). Analysis of MR in melanomas of various degrees of clinical advancement with regard to pT stage of the primary tumor revealed that only a small percentage of early (pT1 and pT2) tumors exhibited hMR (6 and 10%, respectively). In the advanced disease, hMR tumors accounted for 48 and 70%, respectively in T3 and T4 melanomas (Table I).

Ulceration was observed in 49% of the tumors (Fig. 2A-D). Within the pT1 group of tumors, only a small number (6%) of tissue specimens showed microscopic evidence of ulceration. Interestingly, 30% of pT2 melanomas were ulcerated. Advanced (pT3 and pT4) tumors were characterized by a significantly higher prevalence of ulceration (74 and 91%, respectively) (Table I).

Correlations between hMR and clinicopathological parameters. A high mitotic rate was significantly correlated with higher advancement of the primary tumor (pT; P<0.001), the presence of nodal and distant metastases (P<0.001 and P=0.034, respectively), positive status for sentinel lymph node (P=0.002) and disease recurrence (P=0.041). Furthermore, hMR was strongly associated with deeper infiltration according to Breslow thickness (P<0.001) and Clark level (P<0.001), the presence of ulceration (P<0.001), lymphangioinvasion (P<0.001), microsatellitosis (P=0.012) and histologic type (nodular) of the primary tumor (P<0.001). Interestingly, hMR was related to a lower intensity of lymphocytic inflammatory infiltrate (P=0.01). No other significant correlations were found between hMR and the other analyzed clinicopathological parameters, including gender, age, location and growth phase of the primary tumor and microscopic evidence of its regression (Table II).

Correlations between the presence of ulceration and clinicopathological parameters. The presence of ulceration was significantly correlated with higher advancement of the primary tumor (pT; P<0.001) and with the presence of metastases in sentinel lymph nodes (P=0.001) and regional lymph nodes (P<0.001). It was further demonstrated that the presence of ulceration was associated with deeper tumor

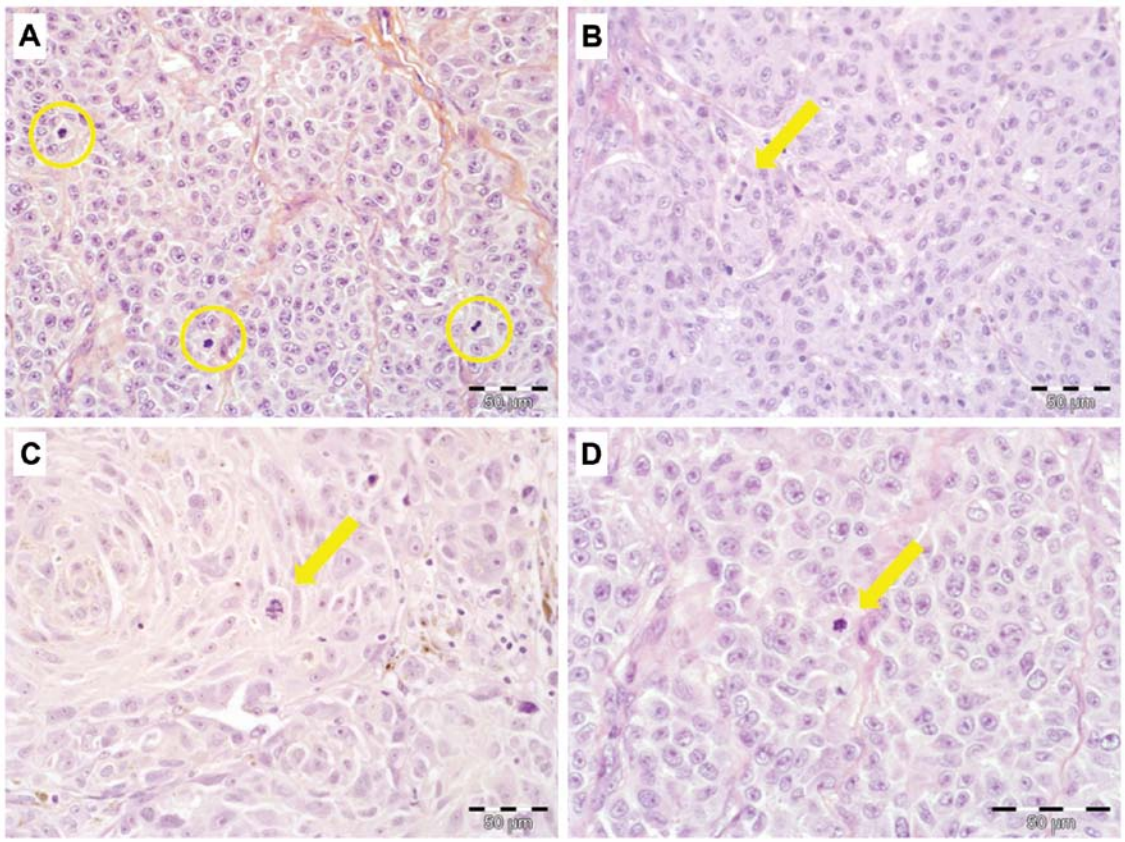

Figure 1. Mitotic activity in cutaneous malignant melanoma. (A) Three pathological mitotic figures (yellow borders) (H&E staining, magnification x200). (B-D) Single mitotic figure in melanoma (yellow arrows). Magnification: H&E staining, x200 in B; H&E staining, x400 in C; H&E staining, x600 in D.

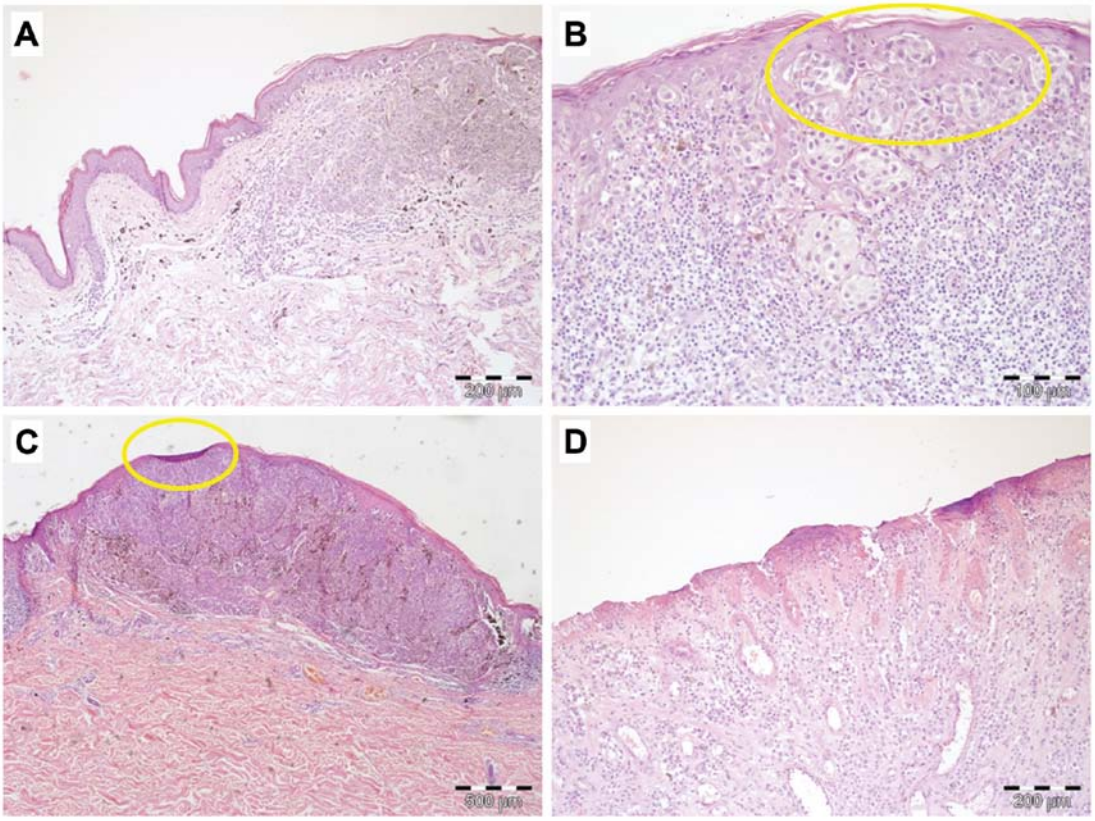

Figure 2. (A) Early superficial spreading malignant melanoma with no evident features of ulceration (H&E staining, magnification x100). (B) Consumption of epidermis was observed as a potential pre-ulcerated status (H&E staining, x200). (C) Artifactual wrapping of the epidermis in nodular malignant melanoma which can imitate the presence of focal ulceration (H&E staining, x40. (D) Diffuse destruction of the epidermis in advanced melanoma (H&E staining, x100).

Table I. Clinicopathological characteristics of the cutaneous malignant melanoma patients.

Clinicopathological characteristics	No (%)	High mitotic rate (≥3/mm^2)	P-value	Ulceration	P-value
All patients	104 (100.0)	33		49	
Age (years)			0.598		0.205
Range (21-79)[a]					
Mean: 56.5±15.4					
Median: 58.5					
Gender[b]			0.576		0.313
Female	60 (57.7)	19		30	
Male	44 (42.3)	14		19	
Primary tumor location[c]			0.494		0.056
Head/neck	15 (14.4)	7		11	
Upper extremities	18 (17.3)	4		8	
Lower extremities	25 (24.0)	7		9	
Trunk	42 (40.4)	13		17	
Hand/foot	4 (3.8)	2		4	
Primary tumor (pT)[a]			**<0.001**		**<0.001**
pT1	34 (32.7)	2		2	
pT2	20 (19.2)	2		6	
pT3	27 (26.0)	13		20	
pT4	23 (22.1)	16		21	
Sentinel lymph node biopsy status (SNLB)[b]	60 (57.7)		**0.002**		**0.001**
No metastases (SNLB$^-$)	48 (80.0)	6		14	
Metastases present (SNLB$^+$)	12 (20.0)	7		10	
Regional lymph nodes status (pN)[b]			**<0.001**		**<0.001**
No metastases (pN$^-$)	86 (82.7)	20		33	
Metastases present (pN$^+$)	18 (17.3)	13		16	
Recurrence[b]			**0.041**		0.093
No	87 (83.7)	24		38	
Yes	17 (16.3)	9		11	
Distant metastases[b]			**0.034**		0.147
No	99 (95.2)	29		45	
Yes	5 (4.8)	4		4	

[a]P-value, Mann-Whitney's U test; [b]P-value, Fisher's exact test; [c]P-value, χ^2 test. Statistically significant results (P<0.05) are indicated in bold font.

infiltration according to Breslow thickness (P<0.001) and Clark level (P<0.001), hMR (P<0.001), lymphangionvasion (P<0.001) and histologic type (nodular and acral-lentiginous) of the primary tumor (P<0.001). Another relationship was revealed between a decrease in lymphocytic inflammatory infiltrate intensity and ulceration of the primary tumor (P<0.001). No further correlations were found regarding the presence of ulceration and other analyzed clinicopathological characteristics (Table II).

Impact of a high mitotic rate and ulceration on the 5-year survival in melanoma patients. In the entire group of 104 patients, hMR was a highly negative prognostic factor, and indicated considerably shorter OS, CSOS and DFS (P=0.002, P=0.004 and P<0.001, respectively) (Fig. 3A, C and E). Similar relationships were observed for ulceration, which also acted as a negative prognosticator for the entire study population (P=0.001 for OS, P=0.003 for CSOS and P=0.003 for DFS) (Fig. 3B, D and F).

An important aspect of this study was to analyze the prognostic significance of hMR and the presence of ulceration in particular pT stages of primary tumor advancement. Notably, hMR appeared to have a statistically significant negative impact on survival in early melanomas in the pT1 (P=0.001) and pT2 subgroups (P=0.006) (Fig. 4A and B). Kaplan-Meier analysis of the remaining subsets (pT3 and pT4) did not reveal

Table II. Correlations between high mitotic rate (hMR) and the presence of ulceration and histopathological characteristics of the cutaneous malignant melanoma primary tumors.

Histopathological characteristics	No. (%)	High mitotic rate (≥3/mm^2)	P-value	Ulceration	P-value
Breslow thickness[a]			**<0.001**		**<0.001**
<1 mm	34 (32.7)	2		2	
1.01-2.00 mm	20 (19.2)	2		5	
2.01-4.00 mm	27 (26.0)	16		21	
>4 mm	23 (22.1)	13		21	
Clark level[a]			**<0.001**		**<0.001**
I	0 (0.0)	0		0	
II	18 (17.3)	1		0	
III	49 (47.1)	10		17	
IV	26 (25.0)	15		22	
V	11 (10.6)	7		10	
Histologic type[b]			**<0.001**		**<0.001**
Superficial spreading melanoma (SSM)	68 (65.4)	11		18	
Nodular malignant melanoma (NMM)	32 (30.8)	20		27	
Acral-lentiginous melanoma (ALM)	4 (3.8)	2		4	
Mitotic rate[a]					**<0.001**
0	45 (43.3)			4	
1-2	26 (25.0)			16	
≥3	33 (31.7)			29	
Ulceration[c]			**<0.001**		
No	55 (52.9)	4			
Yes	49 (47.1)	29			
Lymphangioinvasion[c]			**<0.001**		**<0.001**
No	74 (71.2)	11		25	
Yes	30 (28.8)	22		24	
Growth phase[c]			0.314		0.144
Radial	3 (2.9)	0		0	
Vertical	101 (97.1)	33		49	
Tumor-infiltrating lymphocytes (TILs)[b]			**0.010**		**<0.001**
No	18 (17.3)	10		13	
Nonbrisk	34 (32.7)	13		22	
Brisk	52 (50)	10		14	
Microsatellitosis[c]			**0.012**		0.078
No	98 (94.2)	28		44	
Yes	6 (5.8)	5		5	
Tumor regression[c]			0.495		0.295
No	96 (92.3)	30		44	
Yes	8 (7.7)	3		5	

[a]P-value, Mann-Whitney's U-test; [b]P-value, χ^2 test; [c]P-value, Fisher's exact test. Statistically significant results (P<0.05) are indicated in bold font.

any important differences in 5-year survival with regard to MR values (Fig. 4C and D).

The presence of ulceration also had a prognostic significance for early melanomas, but only for pT1 tumors (P=0.05) (Fig. 5A). Kaplan-Meier analysis of the other groups (pT2-T4) did not show any influence of ulceration on the 5-year survival (Fig. 5B-D).

Multivariable Cox regression analysis. Multivariate analysis confirmed that a high mitotic rate and the presence of distant

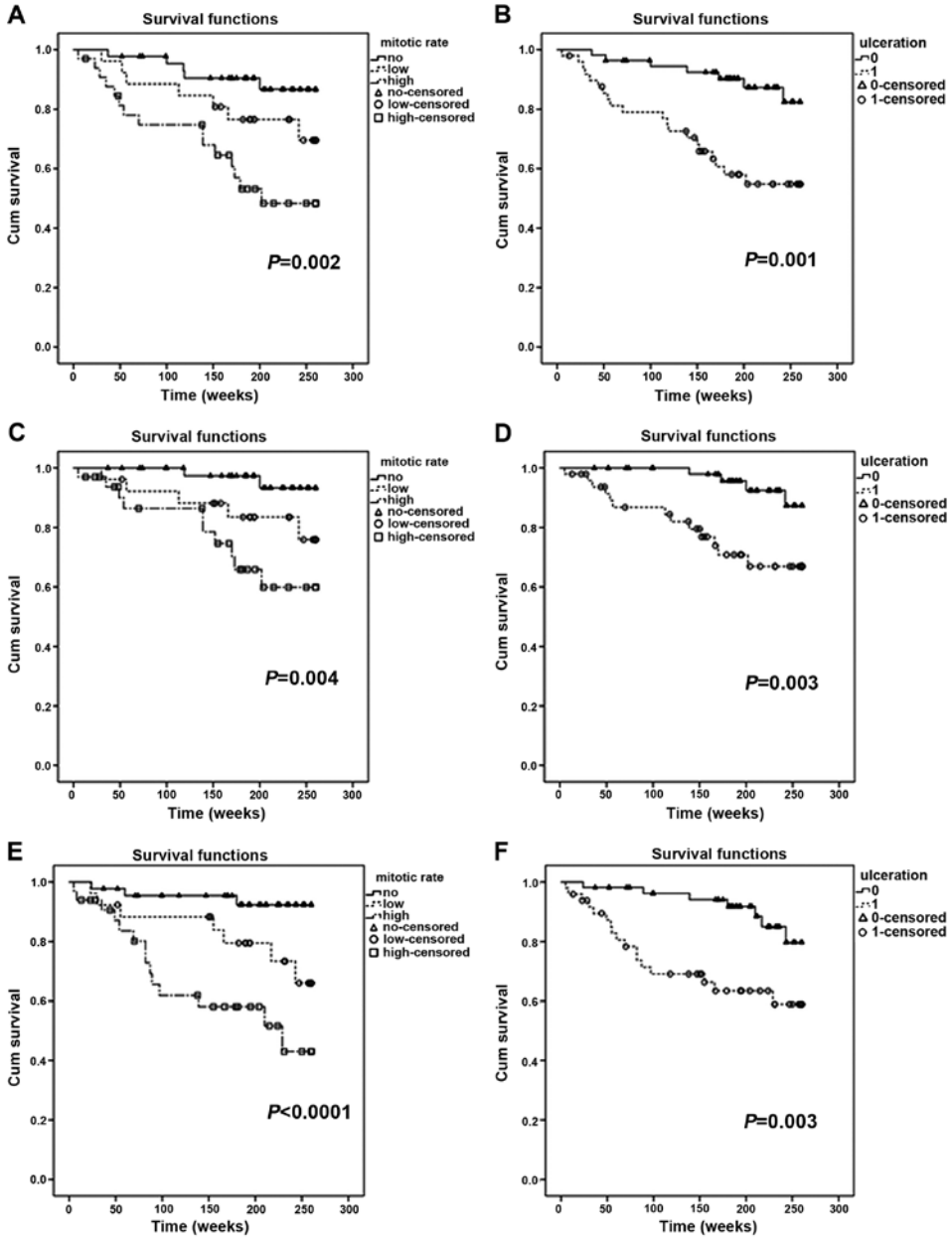

Figure 3. Impact of a high mitotic rate and the presence of ulceration on 5-year survival in melanoma patients in the entire study group of 104 patients (Kaplan-Meier curves). hMR was a highly negative prognostic factor indicating considerably shorter (A) overall survival, (C) cancer-specific overall survival and (E) disease-free survival. Similar relationships were observed for ulceration, which also served as a negative prognosticator for the entire study group in terms of significantly shorter (B) overall survival, (D) cancer-specific overall survival and (F) disease-free survival.

metastases were strongly associated with an unfavorable prognosis (hMR: P=0.005; HR, 1.247; 95% CI, 1.069-1.456; pM: P=0.001; HR, 5.071; 95% CI, 1.883-13.656). Ulceration had no prognostic significance in the Cox proportional hazards model.

Discussion

The aim of the present study was to investigate the relevance of the mitotic rate and primary tumor ulceration for the prognosis of CMM, as well as correlations with other clinicopathological features. In our study group of 104 patients, hMR and the presence of ulceration were highly negative prognostic factors, strongly correlated with shorter overall and cancer-specific overall survival. An important aspect of the study was to analyze the prognostic significance of hMR and the presence of ulceration in particular pT stages of primary tumor advancement. Notably, hMR appeared to have a statistically significant negative impact on survival in early melanomas in the pT1 (P=0.001) and pT2 subgroups (P=0.006), whereas Kaplan-Meier analysis for pT3 and pT4 tumors did not reveal any important differences in 5-year survival with regard to MR values. The presence of ulceration also had a prognostic significance, but only for pT1 melanomas (P=0.05). Kaplan-Meier analysis of other groups (pT2-T4) did not show any influence of ulceration on 5-year survival.

The negative effect of elevated MR on patient survival has been addressed in numerous studies and currently, this

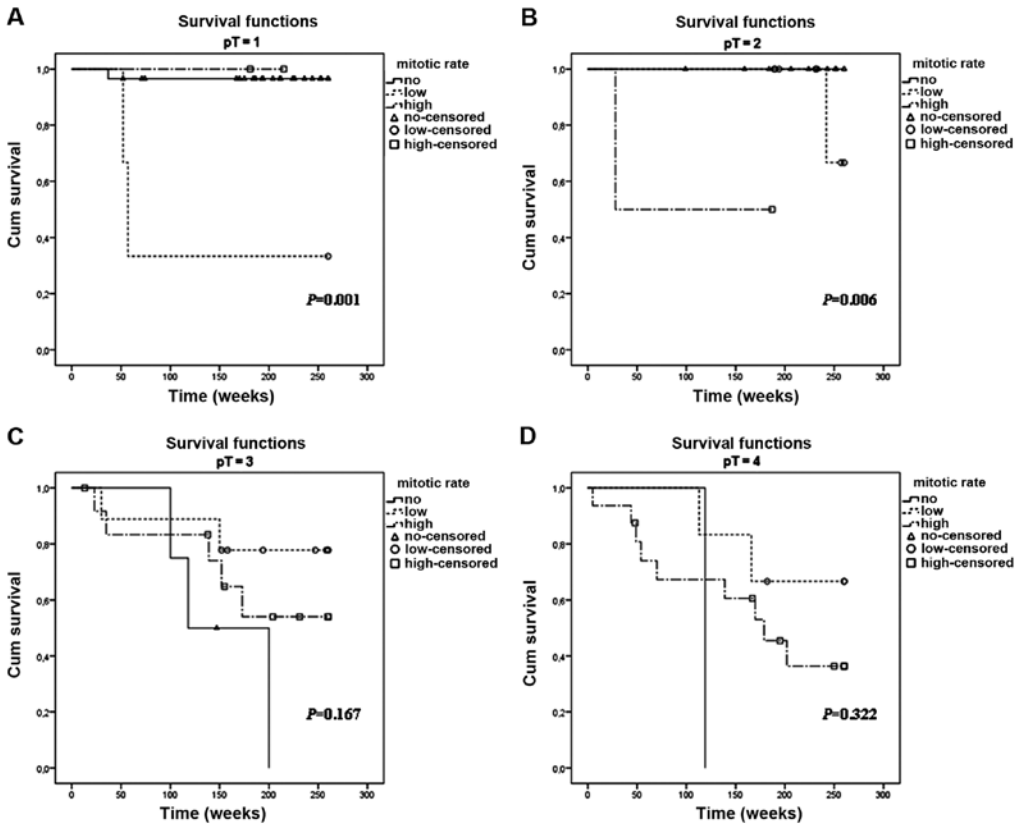

Figure 4. Kaplan-Meier analysis of the prognostic significance of hMR in particular pT stages of the primary tumor advancement. Notably, hMR appeared to have a statistically significant negative impact on survival in early melanomas in both (A) pT1 and (B) pT2 subgroups. (C and D) Kaplan-Meier analysis of the remaining subsets (pT3 and pT4) did not reveal any important differences in 5-year survival with regard to values of MR.

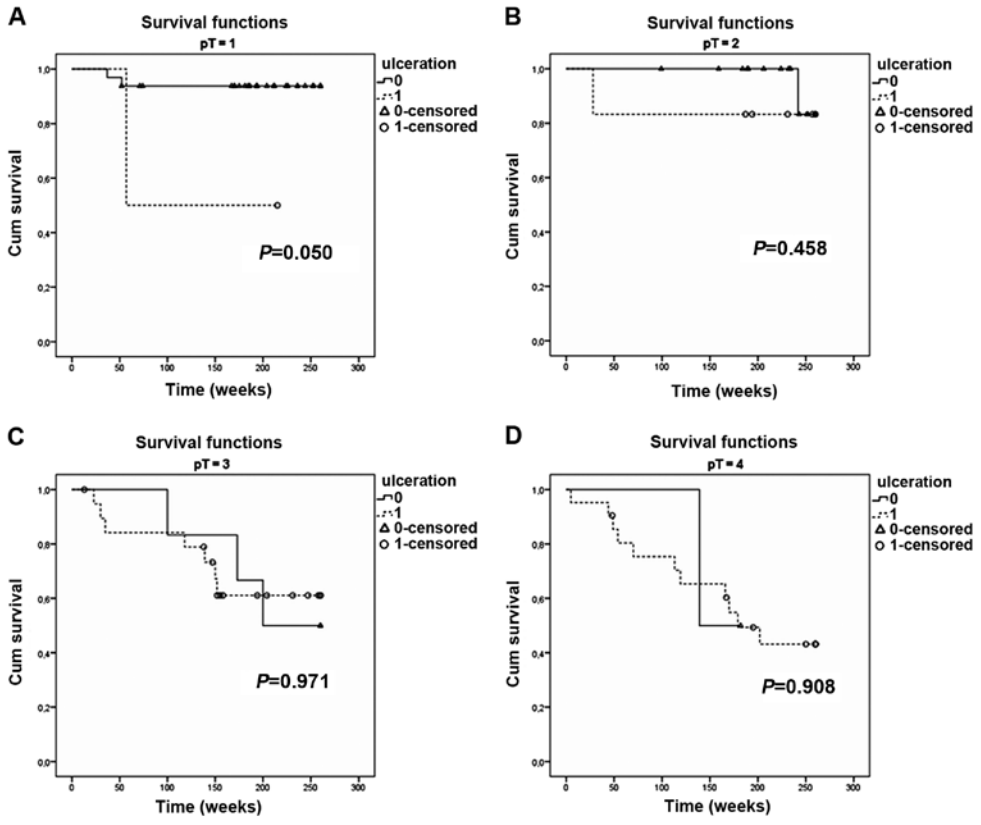

Figure 5. Kaplan-Meier analysis of the prognostic significance of the ulceration in various pT stages of the primary tumor advancement. (A) The presence of ulceration also had prognostic significance for early melanomas, but only in pT1 tumors. (B-D) Kaplan-Meier analysis of other groups (pT2-T4) did not show any influence of ulceration on 5-year survival.

parameter, albeit in a very limited way, affects the TNM staging of CMM (6,8,10-14). However, there are other examples in the literature indicating a far greater significance of MR than that provided by the current version of AJCC recommendations. In the study of Zettersten *et al* increased MR negatively impacted overall survival in a population of patients with thick (>4 mm) CMM (15). Nagore *et al* demonstrated that in 823 localized invasive tumors, MR was the most important prognostic factor of disease-free survival in a multivariable analysis when Breslow thickness was considered as a continuous variable (16).

Considering our results, the mitotic rate emerged as a powerful parameter providing information on patient survival, and on other crucial clinicopathological features. Thus hMR is related to more advanced metastatic cancers and to greater risk of disease recurrence. The relationship between hMR and distant metastases has been recently reported by Murali *et al* (17). Moreover, our study confirms an association between hMR and positivity of SNLB, which supports the validity of the AJCC recommendation to upstage from pT1a to pT1b based on increased MR (8,9,14). An inverse correlation between MR and TIL grade has been noted by Azimi *et al* (18), but refuted by others (16).

Another aspect of our study was the analysis of the prognostic significance of ulceration and investigation of correlations between ulceration status and other clinicopathological parameters. There are examples in the literature which demonstrate that the impact of ulceration on melanoma pathology is unclear. Meanwhile, according to some studies, ulceration loses its role as an independent adverse prognostic factor when MR is included in multivariable models (12,17,19). Eigentler *et al* reported that the presence or absence of ulceration does not influence survival in multivariable analyses of putative CMM prognostic factors among pT1 and pT4 tumors, while it remains significant in intermediate (pT2 and pT3) melanomas. However, MR was not considered in this study (20). It should be said here that determining a universal and coherent definition of tumor-derived (and only tumor-derived) ulceration is another problem, resulting in inter-observer reproducibility that is not entirely satisfactory (21).

In regards to the other correlations observed between ulceration and clinicopathological characteristics, our data are generally concordant with other reports. Whereas no differences relating to the presence of ulceration were found in regards to gender, age and primary tumor location, the findings support the broad consensus that ulcerated lesions are thicker and more deeply invasive (5,22,23). An association between ulcerated melanomas and nodular histologic type has also been previously observed (22). In accordance with our results, the presence of ulceration has been postulated as a predictor of sentinel lymph node involvement (14,24). An interesting aspect reported by Balch *et al* and confirmed by our study is the relationship between scanty lymphocytic infiltrate and the presence of ulceration (25).

Considering the biology of melanoma, MR seems to be a more reliable parameter than the presence or otherwise of ulceration. The value of MR categorizes melanomas into tumors with low or high proliferative potential, thus giving direct information concerning their capacity to infiltrate deeper layers of the dermis and, potentially, to generate regional lymph node and distant metastases. MR is a much more objective parameter than ulceration and its origin is never artifactual. Instead, it always reflects the true biology of the tumor, independently of the infiltration depth and extent of epidermal disruption. In the authors' opinion, ulceration is a valuable parameter that mirrors the invasive potential of melanoma cells, albeit, primarily in moderately advanced (pT1 and pT2) tumors. In these cases, the etiopathogenesis of ulceration, strictly related to the destructive influence of neoplastic melanocytes (so called consumption of the epidermis), is doubtlessly cancer related.

In more deeply infiltrating (pT3, pT4) tumors, ulceration loses its role as an objective parameter associated with the nature of melanoma cells and reflecting their aggressive behavior. In advanced melanomas, the ulceration may be etiologically unrelated to epidermal consumption by cancer cells and may be only a morphological manifestation of a purely mechanical external injury. Although an analogous situation may also occur in non-advanced tumors, it is much less probable.

To sum up, both hMR and ulceration have a very significant influence on the outcome of patients with cutaneous melanoma. However, as stressed above, it is hMR that is a much more objective parameter, more accurately reflecting the biology of a particular tumor. Because of our relatively small study population, further larger-scale investigations are needed to more precisely determine the pathophysiological role and prognostic significance of hMR and ulceration, particularly in patients with early melanoma, in whom more intensive therapy and/or more extensive post-operative follow-up may be justified in order to improve the prognosis.

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