Ann Surg Oncol (2015) 22:2978–2987
DOI 10.1245/s10434-014-4349-3

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – MELANOMAS

Tumor Mitotic Rate Added to the Equation: Melanoma Prognostic Factors Changed?

A Single-Institution Database Study on the Prognostic Value of Tumor Mitotic Rate for Sentinel Lymph Node Status and Survival of Cutaneous Melanoma Patients

M. J. Speijers, MD[1], E. Bastiaannet, PhD[1], S. Sloot, MD[1], A. J. H. Suurmeijer, MD, PhD[2], and H. J. Hoekstra, MD, PhD[1]

[1]Division of Surgical Oncology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands; [2]Department of Pathology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands

ABSTRACT

Background. This study aimed to investigate the predictive value of the tumor mitotic rate per mm^2 (TMR) for sentinel lymph node (SLN) status and survival in intermediate and thick cutaneous melanoma.

Methods. Patients treated for stage I and II melanoma with wide local excision and SLN biopsy between May 1995 and May 2013 were analyzed. In case of insufficient data regarding TMR, pathology slides were reanalyzed. Prognostic factors for SLN status and survival were analyzed with the emphasis on TMR, which was analyzed as a continuous variable, dichotomized (median value) and categorized by two methods.

Results. The study analyzed 453 patients with complete TMR data. The median Breslow thickness was 2.20 mm, and 31.8 % of patients had tumor-positive sentinel lymph node biopsies (SLNBs). In the univariate analysis, TMR was associated with tumor-positive SLNB. This association was not significant in the multivariate analysis. Breslow thickness, primary tumor location on trunk and legs, and younger age were associated with tumor-positive SNLB. At a median follow-up of 47 months, 119 patients (26.3 %) had recurrent disease, and 92 (20.3 %) had died of melanoma. In the univariate analysis, TMR could be established as a significant prognostic factor for disease-free and disease-specific survival, but not in the multivariate analyses. Breslow thickness, ulcerated melanoma, and tumor-positive SLNB were significant prognostic factors for survival.

Conclusion. The study was unable to establish TMR as an independent prognostic factor associated with the presence of SLN metastasis. Regarding survival, increasing TMR showed a strong association with decreased survival in the univariate analysis, but this association was rendered nonsignificant by the importance of Breslow thickness and ulceration status in the multivariate model.

Since tumor mitotic rate (TMR) was added as a prognostic factor for thin melanoma to the American Joint Committee on Cancer (AJCC) TNM staging system in 2009,[1] interest in this pathological marker as a potential predictive factor for survival for all cutaneous melanoma patients has been reinstated. A higher number of mitoses in the primary tumor is thought to resemble a more biologically aggressive tumor and thus a decreased disease survival. Recently, the strong prognostic significance of TMR concerning survival for all T stages has been established in an analysis of the AJCC database.[2]

Several single-institution studies reporting data regarding TMR and its predictive properties for sentinel node status show inconsistent results, with mitotic rates varying from strong to weak predictor. In line with published survival studies, TMR has been classified and analyzed differently in most studies.[3–5] Single-institution survival data on the long-term results of the sentinel lymph node biopsy (SLNB) among melanoma patients still are not abundant, especially with incorporation of TMR data in the

First Received: 19 June 2014;
Published Online: 21 January 2015

H. J. Hoekstra, MD, PhD
e-mail: h.j.hoekstra@umcg.nl

analyses and a median follow-up period longer than 5 years.

The last report derived from our institution's melanoma database stated that Breslow thickness, primary melanoma ulceration status, and sentinel lymph node status are the strongest predictive factors for survival.[6] However, these analyses were conducted without taking the TMR into account. The current study aimed to investigate the predictive value of TMR and other current pathologic features of melanoma regarding sentinel lymph node status and melanoma survival.

PATIENTS AND METHODS

Patient Data

All patients with melanoma diagnosed between May 1995 and May 2013 as AJCC stage I and II cutaneous melanoma having a Breslow thickness of 1 mm or more who were treated with wide local excision (WLE) (i.e., 1- or 2-cm excision margin according to Breslow thickness <2 or ≥ 2 mm) and SLNB at the Division of Surgical Oncology in the University Medical Centre (UMC) Groningen, the Netherlands, were entered into a prospective database and studied. Due to a national guideline change in August 2012, patients who had stage 1B melanoma with a Breslow thickness less than 1 mm were offered SLNB also and included in the database.

For this study patients with T1b lesions were excluded from the analyses. The following primary melanoma pathology details were entered into the database: Breslow thickness, ulceration status, Clark level, TMR, and the presence of microsatellitosis, lymphovascular invasion, regression, and tumor infiltrating lymphocytes. The latter four factors were not included in the current study due to a lack of data. Events concerning recurrence and survival were documented. The study protocol and data collection were approved by the institutional review board.

SLNB: Operative Technique and Pathologic Analysis

Before SLNB and WLE of the primary excision site, all the patients were admitted to the hospital. Since the introduction of this procedure at our institution in 1995, the operation and pathology protocol has remained unchanged. The details have been described previously.[7] In summary, the 2-day protocol consists of a lymphoscintigraphy using ^{99m}Tc-nanocolloid injected at the primary excision site on day 1 and WLE of this site and SLNB using Patent Blue dye and a hand-held gamma-probe (Neoprobe® GDS) on day 2.

The harvested sentinel nodes were fixed in formalin and blocked in paraffin. All paraffin-embedded material was evaluated with routine hematoxylin-eosin (H&E) staining. Specific immunohistochemical staining was performed on H&E-negative specimens for the protein S100, the melanoma-associated monoclonal antibody HMB45, and antibodies targeted to the MART-1/Melan-A antigen on melanoma cells. All patients with metastatic involvement of the sentinel lymph node were advised to undergo a completion lymph node dissection (CLND) of the involved regional lymph node basin.

TMR Analysis

Earlier reports from our institution did not include TMR in the analyses due to lack of data. Patients in the database without reliable data on TMR, namely, absent information in general, number of mitoses scored per high-power fields or classified only per group (0–1 mitosis per mm^2, 2–4 or 5 and larger) were filtered out. The original pathology slides of these patients were retrieved from our institution's pathology archive or the pathology department archives of the referring regional hospitals and reanalyzed by the authors (M.J.S. and A.J.H.S.) for TMR per mm^2. The method commonly used by the institution's pathologists for this TMR analysis was the hot-spot method, as described in the recommendations of the 1982 International Pathology Workshop.[8] Following the publications from the Sydney Melanoma Unit by Francken et al.[9] and Azzola et al.[10] and after modification of the AJCC distribution by Thompson et al.,[2] two TMR distributions were used. We analyzed method A (0, 1–4, 5–10, and ≥ 11 mitoses/mm^2) and the alternative method B (0–1, 2–4 and ≥ 5 mitoses/mm^2) as a sensitivity analysis. Additional sensitivity analyses were performed with TMR as a continuous variable and as a dichotomized variable on the median value of 3 mitoses/mm^2.

Follow-Up Evaluation

After primary treatment, patients were entered in a follow-up protocol recommended by the national guideline, with hospital appointments for physical examination every 3, 4, and 6 months in the 1st, 2nd, and 3rd to 5th years after diagnosis, respectively, and then yearly. In case of suspicion for metastatic disease, appropriate additional investigations were conducted.

Statistical Analysis

For the statistical analyses, SPSS (IBM, Arnonk, NY) and Stata (StataCorp, College Station, TX) were used. Multivariate logistic regression was used to assess predictive

factors associated with a tumor-positive SLNB. The follow-up period was defined as the interval between the diagnostic excision of the primary melanoma and the last known date of a clinical follow-up visit or death by any cause. Disease-specific survival (DSS) was calculated similarly, with death due to melanoma as event. Disease-free survival (DFS) was calculated as the time between diagnosis and the first disease recurrence of any type as event. Uni- and multivariate Cox proportional hazard analyses were used to compute factors associated with survival. All variables with p values of 0.10 or lower at the univariate analysis were entered into the multivariate model. All p values lower than 0.05 were considered statistically significant.

RESULTS

Patient and Clinicopathologic Characteristics

The database included 589 patients. For 468 patients (79.4 %), TMR, recorded as the number of mitosis figures per mm^2, was available. From these patients, 15 patients with T1b lesions were excluded. The data for the remaining 453 patients were used in the study analyses. These patients were randomly distributed over the other clinicopathologic characteristics in the database (data not shown). See Table 1 for detailed information on the study population. The median age at diagnosis was 55 years (range 5.7–88.8 years). The median Breslow thickness was 2.20 mm (range 1.01–20.0 mm). Ulcerated primary melanomas were found in 164 patients (36.2 %).

TABLE 1 Characteristics of the study population

	With TMR data	
	n	%
Age (years)		
≤50	172	38.0
>50	281	62.0
Sex		
Female	222	49.0
Male	231	51.0
Location of primary melanoma		
Axial	233	51.4
Head/neck	57	
Trunk	176	
Extremities	220	48.6
Arm	64	
Leg	156	
Breslow thickness (mm)		
1.01–2.0	199	43.9
2.01–4.00	170	37.5
>4.0	84	18.5
Clark level		
II + III	87	19.2
IV + V	362	79.9
Unknown	4	0.9
Ulceration		
Absent	289	63.8
Present	164	36.2
TMR per mm^2 method A		
0	26	5.7
1–4	257	56.7
5–10	131	28.9
≥11	39	8.6
TMR per mm^2 method B		
0–1	119	26.3
2–4	164	36.2
≥5	170	37.5
SLN status		
Negative	309	68.2
Positive	144	31.8

TMR tumor mitotic rate, *SLN* sentinel lymph node

TMR and SLN Status

The study cohort included 144 patients with a tumor-positive SNLB (31.8 %). As presented in Table 2, the univariate analysis showed that increasing Breslow thickness, primary melanoma location on trunk and lower extremity, and increasing TMR with all categorization methods were predictive of SLN tumor positivity. These factors were entered into a multivariate model. Age at diagnosis showed a trend toward significance ($p = 0.08$), with younger age and primary melanoma ulceration associated with higher risks for SLN positivity ($p = 0.1$). Hence, these factors also were entered in the multivariate model.

Multivariate analysis showed that increasing Breslow thickness and primary tumor localization were predictive factors for SLN status. Younger age showed a trend toward significance in the multivariate analysis ($p = 0.06$) and was a significant factor when entered in the model with TMR method B. None of the categorization methods determined TMR to be a significant independent predictor of SLN status. Moreover, in a multivariate model without TMR as a factor, the same factors were associated with SLN positivity.

Prognostic Factors for Survival

At a median follow-up period of 47 months (range 2–199 months), 119 (26.3 %) of 453 patients had at least one disease recurrence (69 patients in the SLNB-group [22.3 %] and 40 patients in the SLNB + group [39.6 %]),

TABLE 2 Factors associated with tumor-positive sentinel lymph node biopsy (SLNB)

Variable	SLNB+ (%)	Univariate OR (95 % CI)	*p* value	Multivariate OR (95 % CI)	*p* value	Multivariate OR (95 % CI)	*p* value
Age (years)							
≤50	36.6	1 (ref)	0.08	1 (ref)	0.06	1 (ref)	0.04
>50	28.8	0.70 (0.47–1.05)		0.66 (0.43–1.01)		0.63 (0.41–0.98)	
Sex							
Female	29.7	1 (ref)	0.3				
Male	33.8	1.20 (0.81–1.79)					
Location of primary melanoma							
Head/neck	21.1	1 (ref)	0.01	1 (ref)	0.005	1 (ref)	0.004
Trunk	38.6	2.36 (1.17–4.78)		2.79 (1.33–5.85)		3.02 (1.42–6.41)	
Arm	20.3	0.96 (0.40–2.31)		1.00 (0.40–2.50)		1.07 (0.42–2.69)	
Leg	32.7	0.27 (0.14–0.50)		2.08 (0.98–4.42)		2.20 (1.03–4.70)	
Breslow thickness (mm)							
1.01–2.0	17.6	1 (ref)	<0.001	1 (ref)	<0.001	1 (ref)	<0.001
2.01–4.00	41.2	3.28 (2.04–5.28)		3.62 (2.17–6.05)		3.91 (2.31–6.63)	
>4.0	46.4	4.06 (2.31–7.13)		4.30 (2.27–8.14)		4.64 (2.44–8.84)	
Clark level							
II + III	26.4	1 (ref)	0.5				
IV + V	33.1	1.38 (0.82–2.33)					
Unknown	25.0	0.93 (0.10–9.37)					
Ulceration							
Absent	29.4	1 (ref)	0.1	1 (ref)	0.4	1 (ref)	0.5
Present	36.0	1.35 (0.90–2.02)		0.80 (0.49–1.30)		0.85 (0.52–1.38)	
TMR method A							
0	26.9	1 (ref)	0.05	1 (ref)	0.8		
1–4	27.2	1.02 (0.41–2.52)		0.83 (0.31–2.17)			
5–10	38.2	1.67 (0.66–4.27)		1.01 (0.36–2.80)			
≥11	43.6	2.10 (0.72–6.13)		1.11 (0.34–3.63)			
Sensitivity analyses with TMR categorized as method B, continuous factor, and cut-off on median[a]							
TMR method B							
0–1	29.4	1 (ref)	0.02			1 (ref)	0.1
2–4	25.6	0.83 (0.49–1.40)				0.57 (0.32–1.02)	
≥5	39.4	1.56 (0.95–2.57)				0.85 (0.46–1.56)	
TMR							
Continuous		1.06 (1.02–1.11)	0.005	1.03 (0.98–1.09)	0.3		
TMR median							
≤3	25.5	1 (ref)	0.002	1 (ref)	0.2		
>3	39.0	1.87 (1.25–2.79)		1.40 (0.87–2.24)			

OR odds ratio, *CI* confidence interval, *TMR* tumor mitotic rate

[a] Sensitivity analyses with alternative TMR classifications entered separately into the multivariate model (last two columns show ORs for TMR method B, ORs for TMR continuous and cut-off on median not shown)

and during the follow-up period, 92 patients died of recurrent disease. The remaining 27 patients (25.2 % of patients with recurrent disease) did not die of melanoma recurrence within the follow-up period. Stratification of these data by SLN status showed that in the negative SLNB group, 24.6 % of the patients with a recurrence and 29.8 % of the patients in the positive SLNB group were still alive at the end of the follow-up period.

Multivariate analyses for survival without incorporation of TMR showed the following prognostic factors to be

TABLE 3 Prognostic factors associated with disease-free survival (DFS) and disease-specific survival (DSS)

Factor	DFS					DSS				
	5-year DFS	Univariate HR (95 % CI)	*p* value	Adjusted HR (95 % CI)	*p* value	5-year DSS	Univariate HR (95 % CI)	*p* value	Adjusted HR (95 %CI)	*p* value
Age (years)										
≤50	74.2	1 (ref)	0.09	1 (ref)	0.1	84.1	1 (ref)	0.09	1 (ref)	0.06
>50	68.1	1.37 (0.94–2.00)		1.36 (0.93–1.99)		75.5	1.44 (0.94–2.18)		1.52 (0.99–2.35)	
Sex										
Female	78.5	1 (ref)	0.006	1 (ref)	0.1	84.9	1 (ref)	0.04	1 (ref)	0.5
Male	63.3	1.67 (1.15–2.41)		1.36 (0.90–2.07)		73.9	1.54 (1.01–2.33)		1.18 (0.74–1.89)	
Location of primary melanoma										
Axial	64.8	1 (ref)	0.05	1 (ref)	0.2	73.8	1 (ref)	0.08	1 (ref)	0.3
Extremities	77.7	0.69 (0.48–0.99)		0.79 (0.53–1.17)		85.5	0.69 (0.46–1.04)		0.78 (0.49–1.24)	
Breslow thickness (mm)										
1.01–2.0	85.0	1 (ref)	<0.001	1 (ref)	0.03	89.0	1 (ref)	<0.001	1 (ref)	0.02
2.01–4.00	68.1	2.11 (1.34–3.32)		1.46 (0.90–2.37)		78.7	2.47 (1.44–4.24)		1.60 (0.90–2.85)	
>4.0	44.8	3.96 (2.43–6.45)		2.11 (1.22–3.65)		59.8	4.62 (2.58–8.24)		2.44 (1.29–4.62)	
Clark level										
II + III	79.7	1 (ref)	0.2	1 (ref)	0.8	85.4	1 (ref)	0.1	1 (ref)	0.4
IV + V	69.4	1.52 (0.89–2.57)		1.11 (0.64–1.92)		78.3	1.90 (0.98–3.67)		1.52 (0.85–2.72)	
Unknown	50.0	2.12 (0.49–9.24)		0.75 (0.17–3.38)		75.0	2.77 (0.60–12.6)		2.40 (1.27–4.53)	
Ulceration										
Absent	81.8	1 (ref)	<0.001	1 (ref)	<0.001	88.5	1 (ref)	<0.001	1 (ref)	0.001
Present	52.7	2.95 (2.04–4.26)		2.46 (1.62–3.72)		64.9	3.03 (1.99–4.61)		2.26 (1.39–3.67)	
SLNB										
Negative	77.4	1 (ref)	<0.001	1 (ref)	0.002	82.8	1 (ref)	0.005	1 (ref)	0.06
Positive	57.3	2.14 (1.49–3.07)		1.85 (1.26–2.71)		72.5	1.80 (1.19–2.71)		1.51 (0.98–2.35)	
TMR method A										
0	91.0	1 (ref)	0.0003	1 (ref)	0.5	89.7	1 (ref)	0.0001	1 (ref)	0.3
1–4	78.5	3.04 (0.74–12.49)		2.84 (0.69–11.75)		84.5	2.33 (0.56–9.65)		2.06 (0.49–8.62)	
5–10	60.9	5.36 (1.30–22.11)		3.06 (0.72–12.91)		75.5	4.05 (0.97–16.9)		2.24 (0.52–9.63)	
≥11	43.4	7.72 (1.78–33.52)		3.05 (0.68–13.69)		54.2	8.20 (1.86–36.1)		3.38 (0.73–15.62)	
Sensitivity analyses with TMR categorized as method B, continuous factor, and cut-off on median[a]										
TMR method B										
0–1	84.4	1 (ref)	0.0002	1 (ref)	0.2	86.5	1 (ref)	0.0009	1 (ref)	0.6
2–4	76.0	1.51 (0.87–2.61)		1.59 (0.89–2.83)		83.6	1.25 (0.68–2.32)		1.17 (0.60–2.26)	

TABLE 3 continued

Factor	DFS					DSS				
	5-year DFS	Univariate HR (95 % CI)	*p* value	Adjusted HR (95 % CI)	*p* value	5-year DSS	Univariate HR (95 % CI)	*p* value	Adjusted HR (95 %CI)	*p* value
≥5	57.5	2.63 (1.58–4.35)		1.55 (0.87–2.77)		70.8	2.47 (1.42–4.29)		1.36 (0.73–2.56)	
TMR										
Cont	–	1.07 (1.04–1.11)	<0.001	1.01 (0.97–1.05)	0.5	–	1.09 (1.05–1.13)	<0.001	1.03 (0.99–1.08)	0.1
TMR median										
≤3	80.6	1 (ref)	<0.001	1 (ref)	0.4	86.7	1 (ref)	0.001	1 (ref)	0.7
>3	60.2	2.05 (1.41–2.96)		1.19 (0.77–1.84)		72.5	1.99 (1.30–3.02)		1.10 (0.68–1.79)	

HR hazard ratio, *SLNB* sentinel lymph node biopsy, *TMR* tumor mitotic rate

[a] Sensitivity analyses with alternative TMR classifications entered separately into the multivariate model. Adjusted HRs for DFP and DSS are shown only for TMR method A

associated with DFS: Breslow thickness, presence of ulceration, and SLN status. For DSS, Breslow thickness and ulceration were significantly associated, with SLN status and age trending toward significance ($p = 0.06$).

The prognostic factors associated with DFS and DSS incorporating TMR in the multivariate model are shown in Table 3. Both TMR methods showed strong significant associations with DFS ($p = 0.0002$) and DSS ($p = 0.0001$) in univariate analyses. Figure 1 shows the Kaplan–Meier survival estimate curves for both DFS and DSS for TMR method A. For DFS ($p = 0.5$) and DSS ($p = 0.3$), TMR method A was no longer an independent significant prognostic factor in the multivariate model. The strongest independent significant prognostic factors for DFS were Breslow thickness (hazard ratio (HR), 1.46 and 2.11; $p = 0.03$) and primary melanoma ulceration (HR, 2.46; $p < 0.001$). A tumor-positive SLNB also was associated with shortened DFS, although the association was not as strong (HR, 1.85; $p = 0.002$). Age older than 50 years at diagnosis showed a trend toward significance (HR, 1.36; $p = 0.1$). For DSS, Breslow thickness (HR, 1.60 and 2.44; $p = 0.02$) and ulceration status (HR, 2.26; $p = 0.001$) could be identified as independent significant prognostic factors. Age at diagnosis (HR, 1.52; $p = 0.06$) and SLNB status (HR, 1.51; $p = 0.06$) marginally failed to reach significance in the multivariate analysis for DSS.

In the multivariate analyses, TMR method B was entered as a sensitivity analysis separately from method A. In this multivariate model, TMR also did not prove to be a significant independent predictor for DFS or DSS, with p values of 0.2 and 0.6, respectively. In the multivariate model with TMR method B, the same prognostic factors were significantly associated with survival, and the hazard ratios showed no important differences. These data were not added to Table 3. Additional sensitivity analyses for TMR performed with TMR as a continuous variable and dichotomized on the median value showed the same overall results, with highly significant hazard ratios at univariate analysis and nonsignificant hazard ratios at multivariate analysis.

Stepwise Analysis of TMR's Prognostic Value

Because TMR was a strong predictive factor for both DFS and DSS in the univariate analysis, stepwise analyses of the prognostic value were performed to investigate which factor or factors caused TMR to lose its statistical significance. These analyses showed that Breslow thickness and primary melanoma ulceration status explained the association between survival or recurrence and TMR. This association was further investigated by stratifying TMR for Breslow thickness and ulceration status (Table 4). The

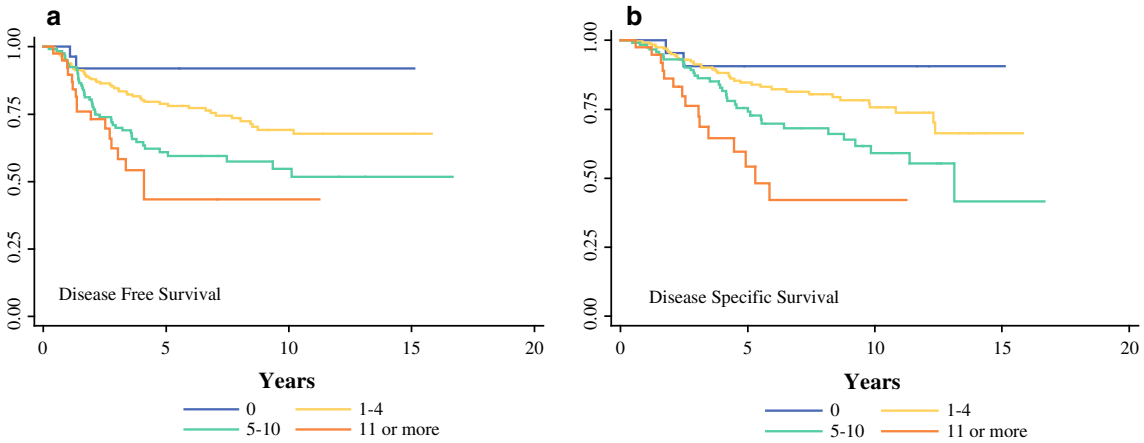

FIG. 1 **a**, **b** Kaplan–Meier survival estimates for disease-free survival ($p = 0.0002$) and disease-specific survival ($p = 0.0001$) according to tumor mitotic rate per mm^2 (TMR) distribution method A

TABLE 4 Number of patients stratified by tumor mitotic rate (TMR) and Breslow thickness or ulceration status

	Mitoses/mm^2	Breslow 1.01–2.0 mm (%)	Breslow 2.01–4.0 mm (%)	Breslow > 4.0 mm (%)
TMR method A	0	17 (8.5)	7 (4.1)	2 (2.4)
	1–4	147 (73.9)	84 (49.1)	26 (30.9)
	5–10	32 (16.1)	61 (35.9)	38 (45.2)
	≥11	3 (1.5)	18 (10.6)	18 (21.4)
TMR method B	0–1	80 (40.2)	30 (17.6)	9 (10.7)
	2–4	84 (42.2)	61 (35.9)	19 (22.6)
	≥5	35 (17.6)	79 (46.5)	56 (66.7)
TMR median	≤3	145 (72.9)	75 (44.1)	23 (27.4)
	>3	54 (27.1)	95 (55.9)	61 (72.6)
	Mitoses/mm^2	Ulceration absent	Ulceration present	
TMR method A	0	20 (6.9)	6 (3.7)	
	1–4	202 (69.9)	55 (33.5)	
	5–10	60 (20.8)	71 (43.3)	
	≥11	7 (2.4)	32 (19.5)	
TMR method B	0–1	103 (35.6)	16 (9.8)	
	2–4	119 (41.2)	45 (27.4)	
	≥5	67 (23.2)	103 (62.8)	
TMR median	≤3	199 (68.9)	44 (26.8)	
	>3	90 (31.1)	120 (73.2)	

percentages of patients with a high TMR increased with greater Breslow thickness and the presence of ulceration.

DISCUSSION

During nearly 20 years of experience, 31 % of our patients had a tumor-positive SLNB. This is a high percentage considering the median Breslow thickness of 2.20 mm in the study population and the percentages reported in other series, which range from 13 to 31 %.[4,5,11,12] This percentage has risen over the years without any significant change in clinicopathologic factors. Possibly the constant reduction of false-negative SLNB is a contributing factor.

First, the UMC Groningen melanoma database was analyzed for factors associated with a tumor-positive SLNB, with special attention given to TMR. A strong association of TMR with SLN status was shown in the univariate analysis for all categorization methods, but particularly when the distribution was according to method

A (0, 1–4, 5–10 and ≥11 mitoses/mm^2), with tumor-positive SLNB at 43.6 % for the highest TMR group. However, all these associations were lost at the multivariate analysis. Increasing Breslow thickness, primary melanoma location on the trunk and lower extremities, and younger age were significantly associated with tumor-positive SLNB. The presence of ulceration did not show a significant association with sentinel node status. Similar results were found in a multivariate model without TMR.

The predictive factors in our model are consistent with the literature. The Sentinel Lymph Node Working Group recently reconfirmed this by analyzing a large worldwide multi-institution database.[13] Sadly, this analysis did not take the TMR into account due to lack of data, as often seems to be the problem when the predictive value of TMR for sentinel node status is studied. If incorporated in the analyses, TMR often is established as a significant predictor for SLNB status by various authors.[3,4,14,15] The fact that a large number of mostly older studies on this topic were conducted without taking TMR into account causes difficulty in the interpretation of its value as a prognostic factor.

Recently, a British study,[16] similar to ours in patient numbers and clinical parameters, concluded that TMR together with Breslow and tumor location is strongly associated with SLN status. In contrast, a post-hoc analysis of the multicenter Sunbelt Melanoma Trail patients with complete data on TMR did not find this factor to be a significant predictor for SLN status in the multivariate analysis.[5] In a study by Fairbairn et al.[17] concerning thick melanomas (>4 mm), TMR could not be established as a prognostic factor for tumor-positive SLNB. However, the majority of the study population had no SLNB at all, and TMR was categorized in 10-mitoses/mm^2 intervals. On the other end of the spectrum, Karakousis et al.[18] concluded that the presence of mitoses in thin melanoma (<1 mm) is predictive for SLN metastasis.

Second, in this study, the focus was on the prognostic value of TMR for melanoma survival in our SLNB database. Accordingly, the aim was to investigate whether the addition of TMR to the survival analysis caused any changes in the prognostic factors for survival established previously for this cohort.[6] As was the case for the association TMR and SLNB, increasing TMR was found to be a strong predictive factor for decreased survival univariately for all categorization methods and particularly for TMR method A. This finding could not be reproduced in a multivariate model for all TMR methods. However, following stepwise multivariate analysis, TMR remains a strong and highly significant prognostic factor for both DFS and DSS until adjustment for Breslow thickness and ulceration status, with TMR method A showing a large difference in hazard ratios. In the multivariate models, our data perfectly followed the AJCC staging manual, with Breslow thickness and ulceration status (i.e., T-stage factors) being the strongest predictors for both DFS and DSS, followed by the N-stage, with SLNB status highly significant for DFS and trending toward significance for DSS. Multivariate models for DFS and DSS without incorporation of TMR showed the exact same results.

In conclusion, the addition of TMR to the survival analyses slightly changed prognostic factors compared with the results published by De Vries et al.[6] Sentinel node status and primary melanoma ulceration showed the strongest association with worsened DFS and DSS for the 429 analyzed patients, followed by higher age and Breslow depth. For the 453 patients in this study, those selected from our institution's database by the presence of TMR data, age was not significantly associated with survival, and the hazard ratios for T- and N-stages were different. Surprisingly, SLN status could not be established as a prognostic factor for DSS, probably due to the limited follow-up time for the patients with TMR data in this study compared with the earlier study from our institution. The fact that the percentage of patients with recurrent disease that died of melanoma showed a small difference stratified for SLN status (75.4 vs 70.2 %) contributed to this finding. The addition of TMR to the multivariate analyses did not change prognostic factors for SLN status nor for survival. Attis and Vollmer[19] also could not determine TMR as an independent factor for overall survival with reexamination of more than 1,200 non-SLNB patients. More importantly, they found that Breslow thickness, ulceration, and mitotic rate all were interrelated, with thickness as the most important factor, thus mimicking the results of the stratification shown in Table 4 and the stepwise analysis.

Mitotic rate has been extensively studied in the past, and a significant association has been established between increasing number of mitoses and decreased survival.[9,10,20,21] This was confirmed in the AJCC melanoma database recently.[2] Further analysis of these data showed differences in prognostic factors for patients at the extremes of age. Moreover, only for these patients was TMR not a significant factor.[22] These results, however, could not be reproduced in our study. The patients in these and older studies were not subjected to SLNB as a staging procedure. As a result, interpretation of the prognostic value of TMR is difficult, and these studies may be less useful in decision making for clinicians routinely performing SLNB nowadays. To date, only the post-hoc analysis of the Sunbelt melanoma group[5] and a recent study from the Mayo Clinic[23] included TMR and SLNB status in their survival analyses, and TMR also was not an independent predictor for survival in these studies. The results of the study by Roach et al.[5] not only were similar to our findings but also established roughly the same

factors as associated with survival. Furthermore, they also suggested that higher TMR seems to be associated with increasing Breslow thickness and the presence of ulceration in intermediate and thick melanoma, as the data in Table 4 suggest.

The current study had limitations. Our institution's melanoma SLNB database currently holds data for 589 patients, a relatively small number compared with other centers. Moreover, for approximately 20 % of our study population, exact TMR data still are missing. The majority of these missing patients were treated with WLE and SLNB before 2006. As mentioned earlier, by exclusion of these patients, the median follow-up period was shortened in this study. However, the median follow-up period for the complete database currently is significantly longer than 6 years.

Finally, there is the problem of the TMR assessment itself. It is known that the interobserver reproducibility of TMR assessment is fairly good.[24] In our database, several pathologists have assessed TMR primarily, with one of the authors reanalyzing the pathology slides of the missing patients. This could have influenced the results. In contrast to other authors, we chose not to use a single cut-off value in our analyses because TMR is a linear variable (e.g. the median number of mitoses per mm^2). But a more linear distribution as used in the AJCC melanoma staging database and an earlier report by the SMU.[2,9,10]

Although statistically established in large single-institution and multicenter studies, data regarding the prognostic value of TMR for survival and SLN status still are difficult to appreciate due to the inconsistent inclusion of SLNB data in survival studies and the use of many different TMR distributions. Moreover, the clinical importance of this factor for the individual patient with intermediate and thick melanomas in our consultation rooms may be limited. Therefore, TMR should be the subject of further study using a standardized categorization method for larger cohorts of patients after SLNB. Moreover, the role of TMR in tumor-positive SLN patients will be of specific interest because Balch et al.[25] found this factor to be the second most powerful predictor of survival. Until these results are available and TMR is established as an independent risk factor in larger single-institution series with a standardized distribution, factors such as T-stage, N-stage, primary melanoma location, and age at diagnosis are best used to predict sentinel node status and melanoma survival.

ACKNOWLEDGMENT The project was partially supported by the Melanoma Sarcoma Groningana Foundation, Groningen, The Netherlands.

DISCLOSURES There are no conflicts of interest.

REFERENCES

1. Balch CM, Greshenwald JE, Soong SJ, et al. Final version of the 2009 AJCC melanoma staging and classification. *J Clin Oncol.* 2009;27:6199–206.
2. Thompson JF, Soong SJ, Balch CM, et al. Prognostic significance of mitotic rate in localized primary cutaneous melanoma: an analysis of patients in the multi-institutional American Joint Committee on Cancer melanoma staging database. *J Clin Oncol.* 2011;29:2199–205.
3. Sondak VK, Taylor JMG, Sabel MS, et al. Mitotic rate and younger age are predictors of sentinel lymph node positivity: lessons learned from the generation of a probabilistic model. *Ann Surg Oncol.* 2004;11:247–58.
4. Paek SC, Griffith KA, Johnson TM, et al. The impact of factors beyond Breslow depth on predicting sentinel lymph node positivity in melanoma. *Cancer.* 2006;109:100–8.
5. Roach BA, Burton AL, Mays MP, et al. Does mitotic rate predict sentinel lymph node metastasis or survival in patients with intermediate and thick melanoma? *Am J Surg.* 2010;200:759–64.
6. De Vries M, Speijers MJ, Bastiaannet E, et al. Long-term follow-up reveals that ulceration and sentinel lymph node status are the strongest predictors for survival in patients with primary cutaneous melanoma. *Eur J Surg Oncol.* 2011;37:681–7.
7. Doting MH, Hoekstra HJ, Plukker JT, et al. Is sentinel node biopsy beneficial in melanoma patients? A report on 200 patients with cutaneous melanoma. *Eur J Surg Oncol.* 2002;28:673–8.
8. McGovern VJ, Cochran AJ, Van der Esch EP et al. The classification of malignant melanoma, its histological reporting, and registration: a revision of the 1972 Sydney classification. *Pathology.* 1986;18:12–21.
9. Francken AB, Shaw HM, Thompson JF, et al. The prognostic importance of tumor mitotic rate confirmed in 1,317 patients with primary cutaneous melanoma and long follow-up. *Ann Surg Oncol.* 2004;11:426–33.
10. Azzola MF, Shaw HM, Thompson JF, et al. Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma: an analysis of 3,661 patients from a single center. *Cancer.* 2003;97:1488–98.
11. Van Akkooi ACJ, de Wilt JHw, Verhoef C, et al. High positive sentinel identification rate by EORTC Melanoma Group Protocol: prognostic indicators of metastatic patterns after sentinel node biopsy in melanoma. *Eur J Cancer.* 2006;42:372–80.
12. Gershenwald JE, Thompson W, Mansfield PF, et al. Multi-institutional melanoma lymphatic mapping experience: the prognostic value of sentinel lymph node status in 612 stage I or II melanoma patients. *J Clin Oncol.* 1999;17:976–83.
13. White RL, Ayers GD, Stell VH, et al. Factors predictive of the status of sentinel lymph nodes in melanoma patients from a large multicenter database. *Ann Surg Oncol.* 2011;18: 3593–600.
14. Kruper LL, Spitz FR, Czerniekcki BJ, et al. Predicting sentinel node status in AJCC stage I/II primary cutaneous melanoma. *Cancer.* 2006;107:2436–45.
15. Mocellin S, Ambrosi A, Montesco MC, et al. Support vector machine learning model for the prediction of sentinel node status in patients with cutaneous melanoma. *Ann Surg Oncol.* 2006;13: 1113–22.
16. Mitra A, Conway C, Walker C, et al. Melanoma sentinel node biopsy and prediction models for relapse and overall survival. *Br J Cancer.* 2010;103:1229–36.
17. Fairbairn NG, Orfaniotis G, Butterworth M. Sentinel lymph node biopsy in thick malignant melanoma: a 10-year single-unit experience. *J Plast Reconstr Aesthet Surg.* 2012;65:1396–402.
18. Karakousis GC, Gimotty PA, Botbyl JD, et al. Predictors of regional nodal disease in patients with thin melanomas. *Ann Surg Oncol.* 2006;13:533–41.

19. Attis MG, Vollmer RT. Mitotic rate in melanoma: a reexamination. *Am J Clin Pathol.* 2007;127:380–4.
20. Ostmeier H, Fuchs B, Otto F, et al. Can immunohistochemical markers and mitotic rate improve prognostic precision in patients with primary melanoma? *Cancer.* 1999;85:2391–9.
21. Schmid-Wendtner MH, Baumert J, Schmidt M, et al. Prognostic index for cutaneous melanoma: an analysis after follow-up of 2,715 patients. *Melanoma Res.* 2001;11:619–26.
22. Balch CM, Soong SJ, Gershenwald JE, Thompson JF, Coit DG, Atkins MB, et al. Age as a prognostic factor in patients with localized melanoma and regional metastases. *Ann Surg Oncol.* 2013;20:3961–8.
23. Stucky CCH, Gray RJ, Dueck AC, et al. Risk factors associated with local in-transit recurrence of cutaneous melanoma. *Am J Surg.* 2010;200:770–5.
24. Scolyer RA, Shaw HM, Thompson JF, et al. Interobserver reproducibility of histopathologic prognostic variables in primary melanoma. *Am J Surg Pathol.* 2003;27:1571–6.
25. Balch CM, Gerschenwald JE, Soong SJ, Thompson JF, Ding S, et al. Multivariate analysis of prognostic factors among 2,313 patients with stage III melanoma: compariscon of nodal micrometastases versus macrometastases. *J Clin Oncol.* 2010;28: 2452–9.