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Influence of tumor thickness and vascularity on survival in cutaneous melanoma.

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Abstract

Eighty-eight cases of cutaneous melanoma (CM) were analyzed in order to elucidate the relationship between thickness, angiogenesis, and prognosis. The thickness of the tumor was measured according to the Breslow method, and the microvessels were identified by an immunohistochemical study using anti-factor VIII monoclonal antibody on specimens from 40 patients with superficial spreading melanoma (SSM), and 48 with nodular type (NM). Microvessels were counted in the area of highest density. The overall survival and disease-free period were analyzed retrospectively. The proportion of patients with thicker CMs ($>$ 1.5 mm) increased with age in both sexes. Mean vascular count was statistically significant different only between thinner and thicker tumors in the SSM group ($P < 0.05$). Prognosis was correlated with the thickness of CM ($P = 0.0002$), mean vascular count alone ($P = 0.004$), mean vascular count in association with CM thickness less than 1.5 mm ($P = 0.0005$), and with mean vascular count in NM ($P = 0.02$). These findings suggest that increasing microvessel density indicates a worsening prognosis.

KEYWORDS: cutaneous melanoma, anigiogenesis, thickness, prognosis

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Original Article

Influence of Tumor Thickness and Vascularity on Survival in Cutaneous Melanoma

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Eighty-eight cases of cutaneous melanoma (CM) were analyzed in order to elucidate the relationship between thickness, angiogenesis, and prognosis. The thickness of the tumor was measured according to the Breslow method, and the microvessels were identified by an immunohistochemical study using anti-factor VIII monoclonal antibody on specimens from 40 patients with superficial spreading melanoma (SSM), and 48 with nodular type (NM). Microvessels were counted in the area of highest density. The overall survival and disease-free period were analyzed retrospectively. The proportion of patients with thicker CMs (> 1.5 mm) increased with age in both sexes. Mean vascular count was statistically significant different only between thinner and thicker tumors in the SSM group ($P < 0.05$). Prognosis was correlated with the thickness of CM ($P = 0.0002$), mean vascular count alone ($P = 0.004$), mean vascular count in association with CM thickness less than 1.5 mm ($P = 0.0005$), and with mean vascular count in NM ($P = 0.02$). These findings suggest that increasing microvessel density indicates a worsening prognosis.

Key words: cutaneous melanoma, angiogenesis, thickness, prognosis

The incidence of cutaneous melanoma (CM) is increasing steadily and faster than other malignant tumors worldwide, with this trend also observed in our region [1]. The most important etiological factor of CM seems to be sun exposure, especially intermittent sun exposure. Ultraviolet radiation generates free radicals, damages the DNA with formation of pyrimidine dimers, can cause local and systemic immunosuppression, and alterate the expression of growth factors [2, 3]. The depletion of the earth's ozone layer with the subsequent increase of ultraviolet radiation reaching the skin contributes to the increase in incidence of CM. Currently, the tumor is the leading fatal skin disease [4]. Definite diagnosis of CM can be established only by histopathological examination, which also permits assessment of the prognosis. A number of clinical and histological features influence the outcome of the disease. The level of dermal invasion and tumor thickness in millimeters (Breslow method) are generally accepted as the most important prognostic factors [5-7]. Patients with thicker melanomas generally have more metastases. Recently, degree of vascularization has also been believed to determine the prognosis of this tumor, but reports are contradictory [8-12].

The present study was undertaken in order to investigate the influence of thickness (less or greater than 1.5 mm), tumor type, and vascularization on survival in patients with clinical stage I melanoma localized in the skin without evidence of regional lymph node metastases [13].

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Material and Methods

Clinicopathological parameters. Data regarding 88 patients with clinical stage I melanoma seen and clinically followed at the Department of Dermatology between January 1st, 1994, and December 31st, 1999, were analyzed. In all the patients the diagnosis and thickness (Breslow method) were confirmed by histological examination of hematoxylin-eosin stained sections at the Department of Pathology. Clinical data regarding sex, age, and tumor sites were obtained by reviewing each patient's chart. Patients with lentigo maligna melanoma, with acrolentiginous type, or unclassifiable tumors were excluded. The clinicopathological parameters of 88 patients included in this study are shown in Table 1 and 2. All the patients were followed-up for at least 60 months. All deaths were tumor related.

Immunohistochemical analysis and assessment of microvessel density. The specimens were fixed in 10% formaline, embedded in paraffin, sectioned at 5 μm thickness, and stained with the antibody against F-VIII (DAKO, clone F8/86) using a standard avidin-biotin-peroxydase complex method. The tumor vascularity was quantified and compared in patients with superficial spreading melanoma (SSM) and with the nodular type (NM), and thickness less or more than 1.5 mm determined by light microscopy evaluation of immunohistochemically stained sections.

Areas of the highest neovascularization (hot spots) were found in each tumor by scanning the section at low power, while individual microvessels were counted on a 200x field (area of 0.785 square millimeters) by 3 independent pathologists (GZ, NJ, ZI). The average count obtained by the pathologists was used for the study. Within each hot spot the highest single values were recorded. The microvessel counts per field were converted to microvessel per square millimeters for subsequent statistical analysis. Any endothelial cell or endothelial cell cluster positive for F-VIII and separate from an adjacent cluster was considered a single countable microvessel.

Statistical analysis was performed using one-way Anova and student's t-test.

Table 1 Characteristics of patients with cutaneous melanoma by sites, thickness, type of growth, and sex

	Women	Men	Total
Localization:			
Head	2	6	8 (9 %)
Trunk	18	29	47 (53.4%)
Upper limbs	1	3	4 (4.5%)
Lower limbs	21	8	29 (32.6%)
Thickness:			
< 1.5 mm	32	36	68 (77.3%)
> 1.5 mm	10	10	20 (22.7%)
Tumor growth:			
superficial spreading	26	14	40 (45.5%)
nodular type	16	32	48 (54.5%)
Total	42 (47.7%)	46 (52.3%)	88

Table 2 Number and proportion of patients with thinner and thicker melanomas

	< 1.5 mm	> 1.5 mm	Total
Superficial spreading	30 (75%)	10 (25%)	40
Nodular	38 (79.1%)	10 (20.3%)	48
Total	68	20	88

Results

Eighty-eight cases of primary CM were available for the study: 42 (47.7%) cases occurred in women (range 35–70, mean age 52.5) and 46 (52.3%) in men (range 31–78, mean age 54.5), with a male to female ratio of 1.1:1.0. The most affected site was the trunk with 47 cases (53.4%), and the lower limbs with 29 cases (32.6%). According to thickness, 68 cases (77.3%) had tumors of less than 1.5 mm, and 20 (22.7%) had a thickness of more than 1.5 mm (Table 2). Among the men, 36 patients (78.2%) had thinner melanomas (< 1.5 mm), while in females there were 32 cases (76.2%), with no significant difference ($P > 0.05$). The proportion of patients with melanomas less than 1.5 mm thick decreased significantly with increasing age in both sexes, above 60 years ($P < 0.05$) (Table 3). The proportion of SSM and NM between groups with thickness < or > than 1.5 mm was nearly equal. However, SSM was more frequent in women (87.7%), while NM was more frequent in men (69.6%) (Table 1).

Table 4. shows the mean vascular count in CM. In the SSM type thinner than 1.5 mm, the MVC was 20.9 ± 10.6 (range 4.0–76.0) and in the thicker (> 1.5 mm) was 34.6 ± 16.2 (range 15.6–42.3). This difference was statistically significant ($P < 0.05$). Among the nodu-

lar type, the values of vascularization in the thinner type was 27.2 ± 17.5 (range 7.3–101.0) and in the thicker type was 26.7 ± 17.5 (range 10.6–71.3) ($P > 0.05$).

Patient prognose were analyzed according the thickness, tumor growth, and vascularization of CM. No deaths occurred in the patients group with SSMs thinner than 1.5 mm during the follow-up period of 60 months, while in the patients group with SSMs deeper than 1.5 mm, 30% died (tumor-related); their mean period of survival was 49 ± 8.2 months. Among patients with the nodular type not deeper than 1.5 mm, the lethality was 36.8% (with mean survival of 47.1 ± 9.9 months). In the patients with NMs deeper than 1.5 mm, the lethality was 40% (mean survival 26.0 ± 9.8 months).

The thickness of CM was significantly different between living patients (1.1 ± 0.8 mm) and those who had died of the disease (1.9 ± 1.1 mm) ($P = 0.0002$) (Table 4). The differences were also observed according to angiogenesis; a lower value of microvessel count (23.9 ± 13.5) in living patients versus a higher value (35.1 ± 19.4) in those who had died ($P = 0.004$). Furthermore, mean vascular count was significantly lower in living patients with CMs thinner than 1.5 mm ($P = 0.0005$), while this difference was not observed in the group with CMs thicker than 1.5 mm.

Finally, the statistical analysis of the clinical outcome in cases of SSM revealed no correlation between mean vascular count and death ($P > 0.05$). However, in cases with NM a correlation was seen between vascularization and prognosis, namely the living patients had lower values of microvessel count ($P = 0.02$).

Table 3 Number of patients with cutaneous melanomas of thickness less or more than 1.5 mm regarding the patients' sex and age

	< 1.5 mm		> 1.5 mm		Total
Age	Women	Men	Women	Men	
< 40	–	1	1	–	2
41–50	4	3	2	2	11
51–60	16	17	1	4	38
61–70	12	9	6	4	31
> 70	–	6	–	–	6

Table 4 Mean vascular count in patients with superficial spreading (SSM) and nodular melanoma (NM) concerning thickness < or > 1.5 mm

Thickness:	Mean vascular count	
	SSM	NM
< 1.5 mm	20.9 ± 10.6*	27.2 ± 17.5
> 1.5 mm	34.6 ± 16.2*	26.7 ± 17.5

Table 5 Prognosis of patients with cutaneous melanoma concerning thickness, tumor growth (superficial spreading or nodular type), and mean vascular count

	Alive (N. 67)	Death (N. 21)	*P*
Thickness:	1.1 ± 0.8 mm	1.9 ± 1.1 mm	0.0002
Mean vascular count:	23.9 ± 13.5	35.1 ± 19.4	0.004
Mean vascular count:			
< 1.5 mm	22.2 ± 10.9	38.1 ± 22.8	0.0005
> 1.5 mm	28.6 ± 15.0	28.9 ± 7.7	NS
Mean vascular count:			
SSM	23.7 ± 13.9	30.4 ± 5.4	NS
NM	24.3 ± 13.3	36.1 ± 21.4	0.02

NS, no significance.

Discussion

It is well-known that thickness presents the best histopathologic prognostic predictor in CM [5-7, 14]. The results of this study confirm this observation and furthermore indicate the important prognostic significance of tumor growth pattern. Namely, the prognosis in those with thin melanoma (less than 1.5 mm) was not the same in the group of SSM compared to NM. The best prognose were observed in the patients with SSM (< 1.5 mm), while in the group with NM (< 1.5 mm) tumor-related death was nearly the same as in the group with thicker (more than 1.5 mm) SSM. This finding indicates that a small but significant number of thin melanomas can also metastasize. For this reason, the prognostic significance of tumor vascularization (angiogenesis) was further analyzed in this study.

The role of angiogenesis in distinguishing benign from malignant melanocytic lesions and in their later progression is rather contradictory as described in the literature. Some authors demonstrated that angiogenesis does not distinguish benign (Spitz nevus, common nevi) from malignant melanocytic neoplasms [10, 15]. In contrast, others found an increased number of microvessels in CM in comparison with normal skin [16], and in comparison

with dysplastic melanocytic nevi [8] and Spitz nevi [17]. Increased vascular density has been described in cases of invasive lentigo maligna [18]. The reason for this discrepancy in results obtained by different authors is probably linked with the study of small groups of patients or the use of different immunohistochemical methods.

The results of the present study reveal statistically significant differences in microvessel count between the group having SSMs with thinner melanoma (< 1.5 mm) and that with thicker melanoma (> 1.5 mm): the microvessel count was 20.9 in the former and 34.6 in the latter ($P < 0.05$). These findings indicate that angiogenesis develops in parallel with the thickness of the lesion as it was suggested that the onset of angiogenesis in thin CM is related to development of the vertical growth phase [19]. However, we could not find significant differences in angiogenesis between thinner and thicker NM, nor could we find significant differences in microvessel count between SSM and NM. This is in agreement with some authors [8, 11, 16, 20] although others have suggested that the vertical growth phase of CM may be associated with greater angiogenesis [9, 21, 22].

Tumor angiogenesis may be a prognostic indicator in cutaneous melanoma, as it has been proposed for a number of solid tumors [12]. However, certain studies dispute this point. Namely, some authors found no statistically significant difference between the metastasizing and non-metastasizing tumors with regard to tumor angiogenesis [8, 11]. In contrast, new research focusing on larger groups of patients found greater vascularity in metastasizing patients compared with patients without metastases [23, 24], thus one can conclude that angiogensis may be important in the process of melanoma metastasis. Furthermore, it was found that in thin lesions (less than 0.76 mm), mean vessel number is significantly related not only to metastasis but also to death [10]. Our results are in agreement with these findings. Namely, significant differences in microvessel count were observed between living patients and those that had died, between living patients and those that had died in regard to CMs less than 1.5 mm, and finally between living patients and those that had died in the NM group.

In conclusion, our findings confirm the relevance of thickness to prognosis and also suggest that the angiogenesis of CM may have a prognostic significance, especially in NM and in melanomas thinner than 1.5 mm, and thus can represent a target for antiangiogenic drugs. For this reason larger matched studies are indicated to confirm this observation.

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