Growth rate of melanoma in vivo and correlation with dermatoscopic and dermatopathologic findings

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ABSTRACT

Objectives: The aim of this study was to calculate the horizontal growth rate of melanoma in vivo and to correlate it with morphologic findings.

Patients and Methods: We searched our database for melanomas for which sequential dermatoscopic images and histopathologic slides were available. The final sample consisted of 50 melanomas of 48 patients (mean age: 50 ± 15 years, 62% females). We calculated the horizontal growth rate in mm^2 per year by morphometric analysis of digital dermatoscopic images. Dermatoscopic and dermatopathologic findings were assessed according to predefined criteria and correlated with the horizontal growth rate.

Results: The median time interval between baseline and follow-up image was 12 months (range: 2–100 months). The majority of melanomas were in situ (n=28, 56%). The mean horizontal growth rate of all melanomas was 5.3 mm^2/year (SD: ± 5.8 mm^2/year). The histopathologic findings of numerous and large epidermal nests were associated with rapid growth. This histopathologic pattern corresponded to a pattern of clods ("globules") dermatoscopically. From a dermatoscopic point of view, melanomas with a main pattern of clods grew significantly faster (mean horizontal growth rate: 10.4 mm^2/year, 95% CI: 6.4-14.4 mm^2/year) than melanomas with mainly a reticular pattern (4.8 mm^2/year, 95% CI: 2.7-7.0 mm^2/year) or with other patterns (2.6 mm^2/year, 95% CI: -0.5-5.6 mm^2/year, p=0.01).

Conclusion: Morphologic characteristics of melanoma are associated with biologic behavior. Large and numerous epidermal nests (corresponding to a pattern of clods dermatoscopically) indicate more rapid growth.

Introduction

Little is known about the growth rate of melanoma in vivo, but it has been speculated that it varies between different types of melanomas. Applying the classification of Clark [1], nodular melanomas are believed to grow faster than superficial spreading or lentigo maligna melanomas. It is known that melanoma subtype per se is not associated with prognosis, but there is some evidence that the growth rate of melanoma is an independent predictor of survival [2]. As Liu et al [3] pointed out there is also a potential indirect impact of the growth rate on prognosis that is related to the penalty associated with diagnostic delay that is particularly severe with rapidly growing melanomas and less severe with slowly growing melanomas.

The growth rate of melanoma in vivo is difficult to measure. It is a dynamic process and neither its direction nor its magnitude can be assessed on a static image or slide. Theoretically, it can be divided in a horizontal and in a vertical growth phase. The vertical growth rate has been estimated indirectly based on information provided by patients and their families, but this method is unreliable. The use of digital dermatoscopy to monitor melanocytic skin lesions has resulted in the availability of sequential dermatoscopic images of melanoma. These images may be used to obtain unbiased measurements of the horizontal growth rate of melanomas in vivo and to identify dermatoscopic and dermatopathologic characteristics that are associated with slow or rapid growth. Identification of these factors would increase our knowledge on the biology of melanoma and would be a step forward towards an integrated classification. It would have important practical implications on early recognition and on the selection of lesions for digital monitoring.

Methods

We included consecutive cases of histopathologically verified melanomas collected between January 1998 and December 2009 for which sequential dermatoscopic images were available. The study was approved by the internal review board of the Medical University of Vienna. All melanomas were initially monitored because of lack of melanoma specific criteria in the setting of patients with multiple nevi. The original sample consisted of 72 cases. Twenty-two cases were excluded because of one of the following reasons: (1) histopathologic slides were not available (n=2), (2) the melanoma was larger than the maximum field of view of the digital camera or was only partly captured all so that the area covered by the lesion could not be calculated (n=9), and (3) review of the histopathologic slides by a board certified dermatopathologist did not result in an unequivocal diagnosis of melanoma (n=11). The final sample included 50 cases. The majority of cases were monitored only once and then excised because of significant changes over time. If melanomas were monitored more than once the observed changes at the first follow-up visit were not considered significant enough to warrant excision. One case was monitored more than once because the patient refused excision despite significant changes of the lesion after the first follow-up visit.

All digital dermatoscopic images were captured with Molemax II™ (DERMA Medical Systems, Vienna, Austria). The device offers a field of view of 10.8 x 8.1 mm. Images were stored in bitmap format with a resolution of 640 x 480 pixels. To capture an image the device has to be in direct contact with the skin. The Molemax II ™ system uses cross-polarization. Since only flat lesions are selected for monitoring, shearing or flattening, which may influence area measurements of raised or polypoid lesions, does not occur. Operators were instructed not to stretch the skin during examination.

The digital dermatoscopic images were analyzed with the image analysis software MELANIE (freely available from the corresponding author on request) that was specifically designed for the purpose of analyzing pigmented skin lesions. The program performs a semiautomatic segmentation of the lesion from the background and computes the area of the lesion in square pixels. After conversion of square pixels into square millimeters, we calculated the horizontal growth rate per year by subtracting the area of the baseline image from the area of the follow-up image and dividing the result by the follow-up time in years.

Assessment of dermatoscopic patterns

For the purpose of this study we used the method of pattern analysis as described by Kittler for dermatoscopic analysis [4]. This method uses a standardized description of pigmented lesions based on patterns, colors, and clues. A pattern is a collection of multiple basic elements of the same type that covers a significant part of the lesion. Basic elements including dots, lines, clods, and circles are given objective geometric definitions. A dot is a tiny round spot (no length, no breadth). A line is a structure with parallel edges where the length is much greater than the breadth. A clod is any solid object larger than a dot and may have any shape. Globules, therefore, are a variant of clods that are small and elliptical. A circle is a curved line equidistant from a fixed point. An area without any of the basic elements dominating is termed structureless. A pigmented lesion may have a single pattern or multiple patterns. We classified the melanomas into the following three categories according to their main patterns: (1) those with mainly reticular lines, (2) those with mainly clods, and (3) and those with other patterns (dots and/or structureless). Baseline dermatoscopic images were evaluated

by 2 observers (H.K. and J.B.) on a computer screen to define their pattern.

Assessment of dermatopathologic criteria

Histopathologic sections of melanomas were examined by a board certified dermatopathologist (H.K.) without knowledge of the dermatoscopic images. We rated four different criteria: upward scatter, nesting, nest size and cell size. With regard to upward scatter and nesting, we adopted the definitions as described by Viros et al [5]. All four histopathologic criteria were defined in advance:

1. Upward scatter was defined as the proportion of intraepidermal melanocytes present above the basal layer, irrespective of whether suprabasal melanocytes were arranged singly or as nests. It was graded from 0 to 3 using the following criteria: 0, essentially all melanocytes situated at the dermo-epidermal junction, with only rare melanocytes in higher epidermal layers; 1, the majority of melanocytes (75%–100%) situated at the dermoepidermal junction, with some present in higher epidermal layers; 2, roughly equal proportions of intraepidermal melanocytes present at the dermo-epidermal junction and in higher epidermal layers; and 3, most (>50%) of the intraepidermal melanocytes situated in the upper layers of the epidermis.

2. Nesting was defined as collections of intraepidermal melanocytes that form clusters of five or more cells. The degree of nesting was quantified as: 0, intraepidermal melanocytes present almost exclusively as single cells with only rare nests; 1, intraepidermal melanocytes predominantly arranged as single cells with no more than 25% of cells in nests; 2, 25%–50% of the intraepidermal melanocytes in nests; and 3, >50% of the intraepidermal melanocytes in nests. A schematic drawing of nesting is shown in Figure 1.

3. With regard to nest size we defined small nests as clusters of five to nine cells, whereas large nests were defined as clusters of ten or more cells. The proportion of large nests
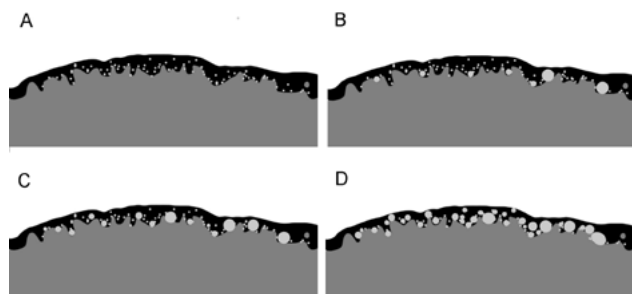

Figure 2. Sequential dermatoscopic images of a melanoma with a pattern of clods. (A) Baseline image; (B) follow up image. The baseline image was taken 5.1 months before the follow-up image. The size at baseline was 32.6 mm^2 and after follow-up 38.9 mm^2. The increase in size was 6.3 mm^2, which corresponds to a horizontal growth rate of 1.1 mm^2 per month.

Figure 1. Schematic drawing of scoring of nesting. A: intraepidermal melanocytes present almost exclusively as single cells with only rare nests (score=0); B: intraepidermal melanocytes predominantly arranged as single cells with no more than 25% of cells in nests (score=1); C: 25%–50% of the intraepidermal melanocytes in nests (score=2); D: >50% of the intraepidermal melanocytes in nests (score=3).
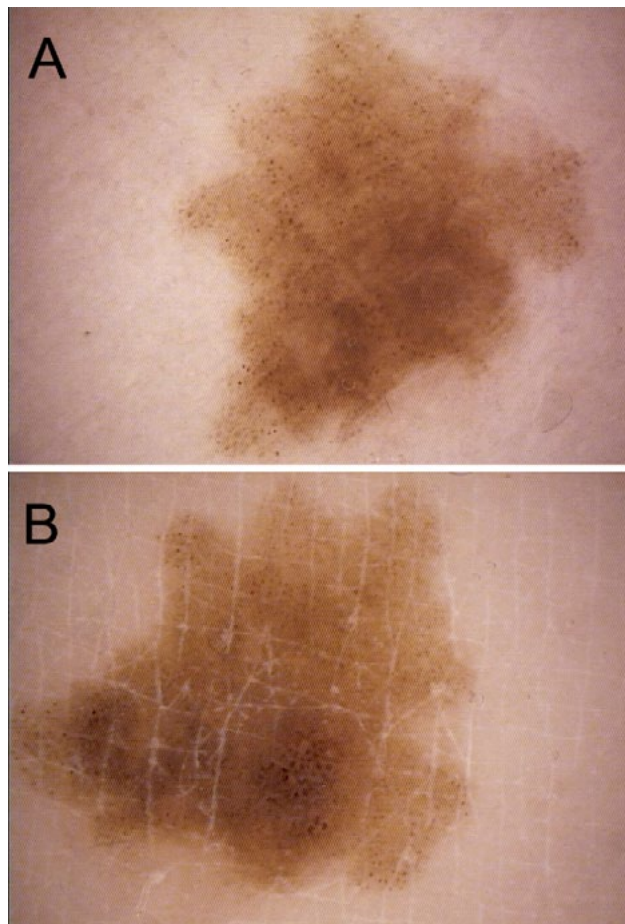
measured against the total number of nests was quantified as: 0, intraepidermal melanocytes present almost exclusively as single cells; 1, intraepidermal nests predominantly arranged as small nests with no more than 25% large nests; 2, 25%–50% of the intraepidermal nests are large nests; and 3, >50% of the intraepidermal nests are large nests.

4. To determine cell size, the diameter of basal keratinocytes was used as a size reference. Cell size referred to the entire cell including the nucleus and cytoplasm. Visual assessment of size was quantified on a continuous rating scale from 1 to 3 using the following definitions: 1, the diameter of tumor cells was similar to or smaller than that of basal keratinocytes; 2, the diameter was larger than that of basal keratinocytes, but not larger than twofold; and 3, the diameter was larger than the twofold diameter of basal keratinocytes.

Statistical analysis

Continuous data are given as mean and standard deviation (SD) unless otherwise specified. We used analysis of variance (ANOVA) for the comparison of the horizontal growth rates

Table 1: Melanomas according to observed pattern

	n	%
Melanomas with a single pattern		
Reticular	13	26%
Clods	2	4%
Dots	0	
Structureless	5	10%
Melanomas with multiple patterns		
Reticular and clods	2	4%
Reticular and dots	8	16%
Reticular and structureless	7	14%
Clods and dots	2	4%
Clods and structureless	3	28%
Dots and structureless	5	10%
Others	3	6%
Total	**50**	**100%**

Table 2: Histopathologic criteria by main dermatoscopic pattern

|	Reticular (n=28)	Dots/Structureless (n=13)	Clods (n=9)
Scatter			
No scatter, almost all melanocytes at the dermo-epidermal junction	9 (32.1%)	6 (46.2%)	3 (33.3%)
75–100% of melanocytes at the dermo-epidermal junction, <25% in higher layers of the epidermis	13 (46.4%)	7 (53.8%)	6 (66.7%)
50–75% of melanocytes at the dermo-epidermal junction, <50% in higher layers of the epidermis	6 (21.4%)	0	0
More than 50% of melanocytes in higher levels of the epidermis	0	0	0
Nesting			
Almost all melanocytes arranged as single cells	8 (28.6%)	5 (38.5%)	0
< 25% of melanocytes arranged in nests	5 (17.9%)	2 (15.4%)	1 (11.1%)
25–50% of melanocytes arranged in nests	10 (35.7%)	3 (23.1%)	1 (11.1%)
>50% of melanocytes arranged in nests	5 (17.9%)	3 (23.1%)	7 (77.8%)
Nest size			
Intraepidermal melanocytes present almost exclusively as single cells	11 (39.3%)	7 (53.8%)	1 (11.1%)
Intraepidermal nests predominantly arranged as small nests with no more than 25% large nests	11 (39.3%)	1 (7.7%)	1 (11.1%)
25–50% of the intraepidermal nests are large nests	3 (10.7%)	3 (23.1%)	1 (11.1%)
>50% of the intraepidermal nests are large nests	3 (10.7%)	2 (15.4%)	6 (66.7%)
Cell size			
Smaller than that of basal keratinocytes	9 (32.1%)	8 (61.5%)	1 (11.1%)
Larger than that of basal keratinocytes, but not larger than twofold	18 (64.3%)	5 (38.5%)	7 (77.8%)
Larger than the twofold diameter of basal keratinocytes	1 (3.6%)	0	1 (11.1%)
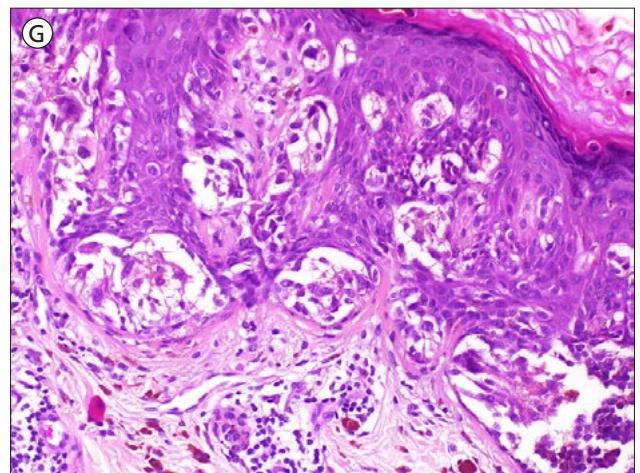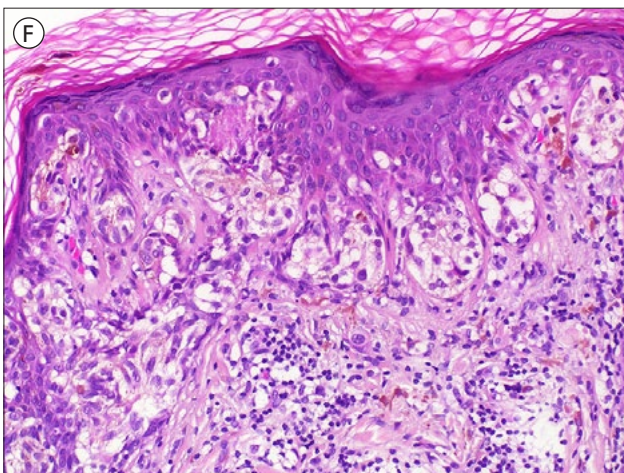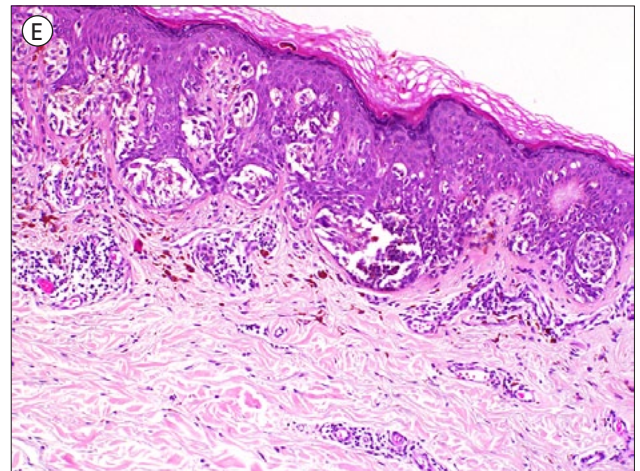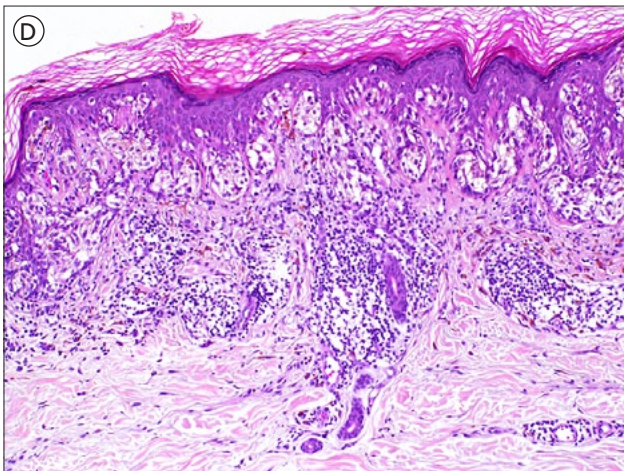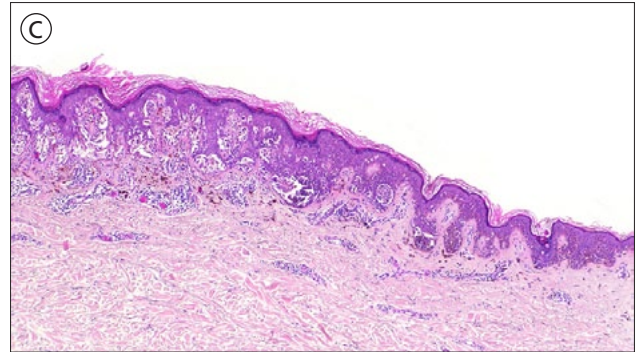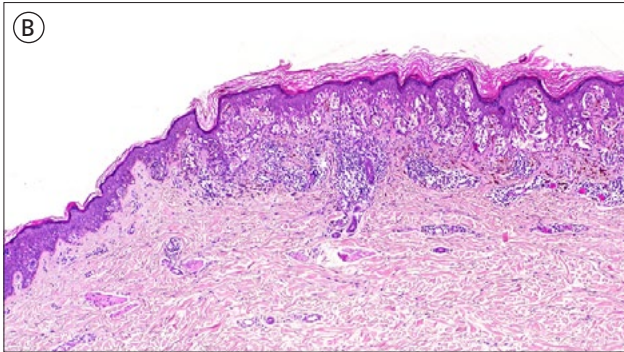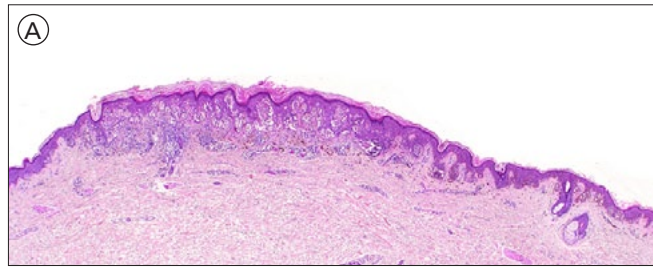
Figure 3. Micrographs of the melanoma shown in Figure 2. The neoplastic melanocytes in the epidermis are mainly arranged in nests. More than 50% of these nests are large.
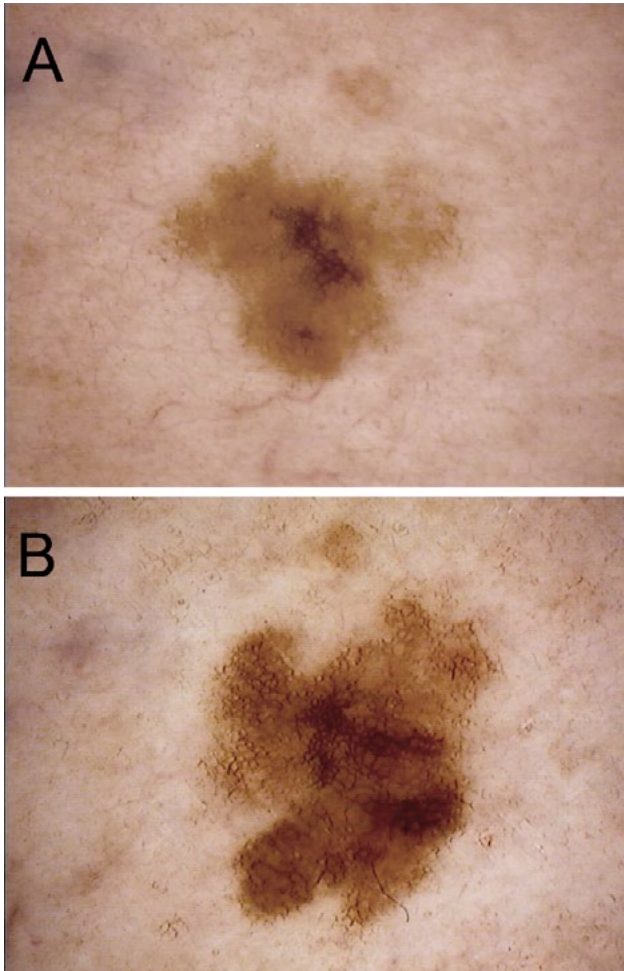
Figure 4. Sequential dermatoscopic images of a melanoma with a reticular pattern. (A) Baseline image; (B) follow up image. The baseline image was taken 35.4 months before the follow-up image. The size at baseline was 12.6 mm^2 and after follow-up 18.8 mm^2. The increase in size was 6.2mm^2, which corresponds to a horizontal growth rate of 0.2 mm^2 per month.

between groups. We adjusted for size differences at baseline by including the size of the melanoma at baseline as a covariate. The Pearson correlation coefficient was used for correlation of continuous data. All given P-values are 2-tailed and a P-value of <0.05 indicates statistical significance.

Results

General data

The final sample consisted of pairs of sequential images of 50 melanomas from 48 patients (mean age: 50 ± 15 years, 62% females). The median time interval between baseline and follow-up image was 12 months (range: 2–100 months). The majority of melanomas were in situ (n=28.56%). The median Breslow thickness of invasive melanomas was 0.50 mm (range: 0.20 to 0.90 mm). Twenty-six melanomas (52%)

were located on the trunk, 12 (24%) on the lower extremities, 7 (14%) on the upper extremities, and 5 (10%) on the head and neck. The original histopathologic diagnosis was superficial spreading melanoma in 38 cases (76%) and lentigo maligna or lentigo maligna melanoma in 8 cases (16%). The remaining 4 cases (8%) were unclassified. Twenty-one melanomas (42%) had one dermatoscopic pattern and 29 (58%) more than one pattern. The frequencies of dermatoscopic patterns are given in Table 1. In 28 melanomas (56%) the main pattern was reticular, nine melanomas (18%) had mainly clods, and 13 melanomas (26%) had other patterns (dots and/or structureless). The mean area size at baseline was 22.6 mm^2 (SD: ± 13.0 mm^2). Melanomas with a main pattern of clods were significantly larger at baseline than melanomas with other dermatoscopic patterns.

Dermatoscopic/dermatopathologic correlation

The dermatopathologic characteristics by dermatoscopic patterns are given in Table 2. The dermatoscopic pattern of clods was associated with nesting and nest size dermatopathologically (Figures 2 and 3). Nests dominated in 77.8% (n=6) of lesions with a pattern of clods and large nests were found in 66.7% (n=5). Reticular melanomas or melanomas with other patterns on the other hand were characterized by a predominance of single cells or small nests (Figures 4 and 5).

Horizontal growth rate

The mean horizontal growth rate of all melanomas was 5.3 mm^2 per year (SD: ± 5.8 mm^2 per year). The annual growth rate did not correlate with patients' age (Pearson correlation coefficient=-0.22, p=0.13) and was similar for females (5.4 ± 5.5 mm^2/year) and males (5.1 ± 6.4 mm^2/year, p=0.86). Superficial spreading melanomas did not grow significantly faster than lentigo maligna or lentigo maligna melanomas (6.2 ± 5.8 mm^2/year versus 3.9 ± 2.5 mm^2/year, p=0.27). We observed no significant differences in the horizontal growth rates of in situ and invasive melanomas (4.3 ± 5.5 mm^2/year for in situ versus 6.4 ± 6.1 mm^2/year for invasive melanomas, p=0.21). Anatomic site was also not associated with the horizontal growth rate.

The horizontal growth rate was significantly related to the type of dermatoscopic pattern. It was highest for melanomas with a pattern of clods (9.6 ± 7.8mm^2), followed by reticular melanomas (5.1 ± 3.9 mm^2), and was lowest for melanomas with a structureless pattern and/or a pattern of dots (2.6 ± 6.4 mm^2, p=0.012, Figure 6). Significant regression occurred in four melanomas, two with a pattern of clods and two with other patterns. The association between dermatopathologic findings and the horizontal growth rate is shown in Figure 7. The scores for scatter, nesting, nest size and cell size were significantly associated with the horizontal growth rate.
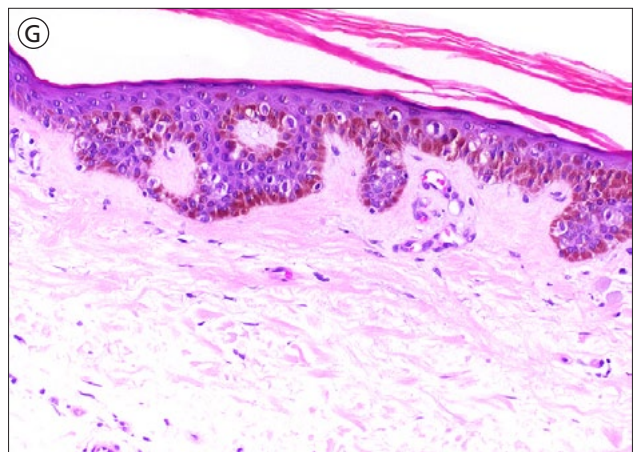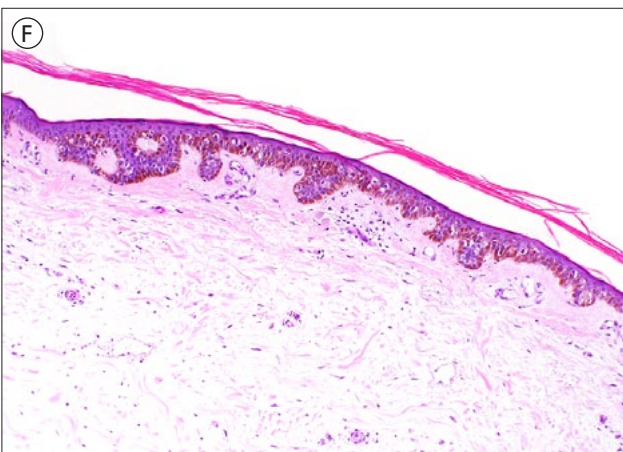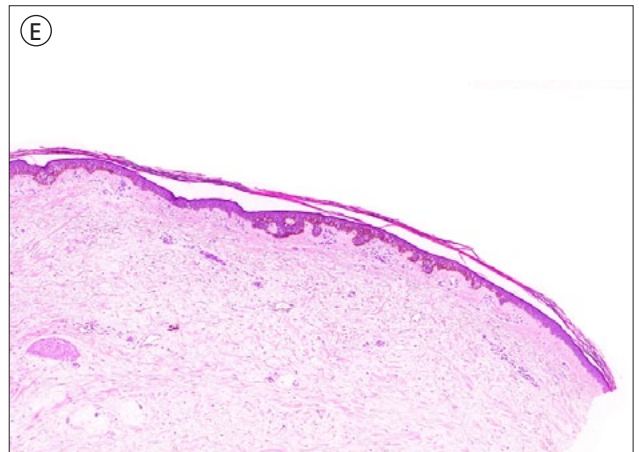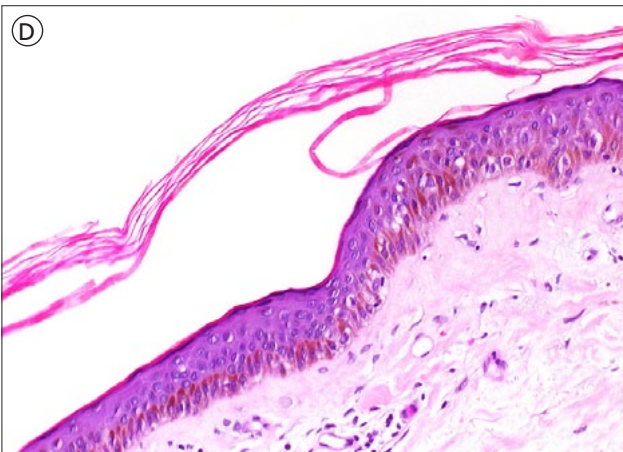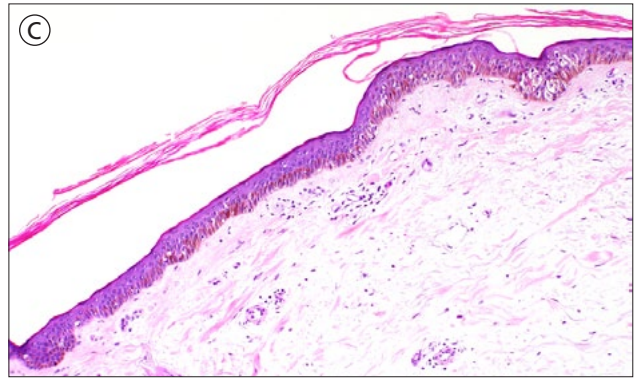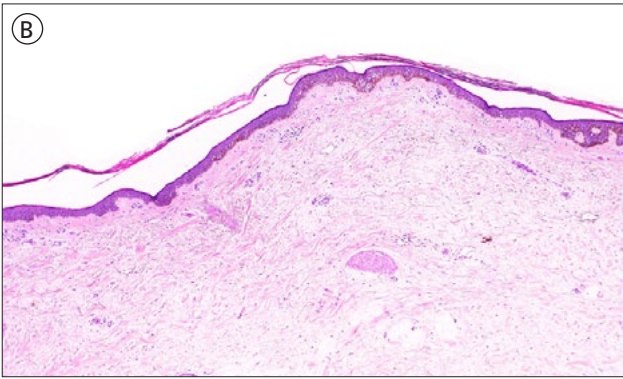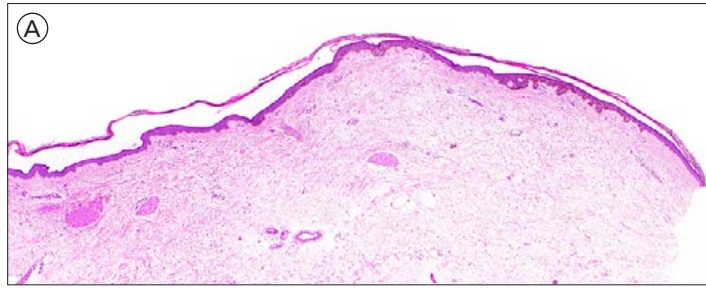
Figure 5. Micrographs of the melanoma shown in Figure 4. The neoplastic melanocytes in the epidermis are mainly arranged as single cells.
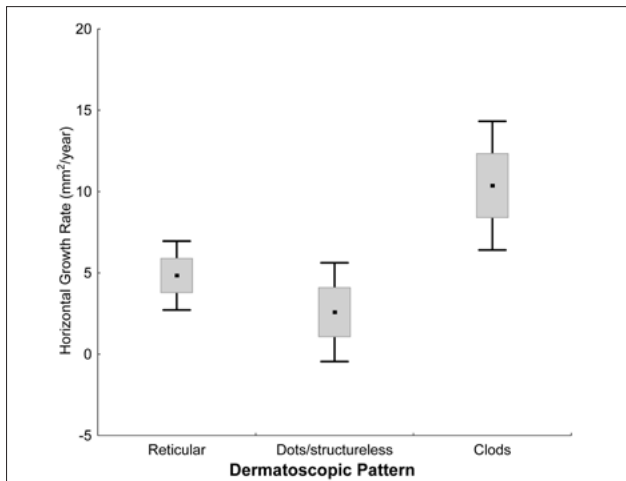
Figure 6. Horizontal growth rate by dermatoscopic pattern: The growth rate was highest for melanomas with a pattern of clods (P for difference: 0.012).
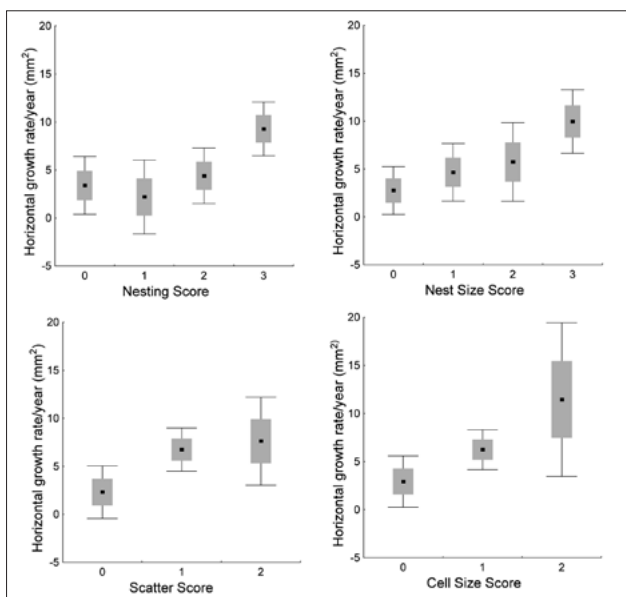

Figure 7. Horizontal growth rate by dermatopathologic findings. The scores for scatter (p=0.035), nesting (p=0.009), nest size (p=0.012), and cell size (p=0.048) were significantly associated with the horizontal growth rate. All p-values are derived from analysis of variance (ANOVA).

Discussion

To date, our knowledge of melanoma development has been based on static, not on dynamic observations. With the rare exceptions of patients who refused excision, it has not been possible to observe the growth rate of melanomas directly. Indirect measurements of the vertical growth rate based on patient recall have been used instead but are believed to be unreliable. By reviewing sequential dermatoscopic images of 50 melanomas that were not excised at the patients' first visit, mainly because of lack of melanoma specific criteria, we were able to obtain unbiased measurements of the hori-

zontal growth rate by morphometric studies. As expected, the melanomas in our sample grew at different speeds. Some grew relatively fast with a horizontal growth rate of up to 21.6 mm^2 per year, others grew slowly or not at all. The horizontal growth rate as observed by us is a net effect of the horizontal spread of proliferating, pigmented melanoma cells minus antagonistic effects due to regression. On average the horizontal growth rate was 5.3 mm^2 per year. This estimate has to be interpreted with caution because it is an extrapolation (some melanomas were monitored for less than one year). Assuming that the growth curve of melanoma is exponential (disregarding the antagonistic effect of regression) we can estimate that it would take approximately 3.5 years for a typical melanoma in our sample to grow from a square with a side length of 0.5 cm to a square with a side length of 1 cm.

Since the range of growth rates was large in our sample, we looked for morphologic criteria that were associated with slow or rapid horizontal growth. In applying the classification of Clark [1] we found that the horizontal growth rate of superficial spreading melanomas was higher than of lentigo maligna melanomas, but this difference was statistically not significant. With the limitation that nodular melanomas were not represented in our sample, the results of our analysis do not support the validity of the classification by Clark, at least with respect to its significance with regard to biologic behavior. The significance of other histomorphologic features was much greater. Nesting and nest size were strongly associated with the horizontal growth rate (Figure 7). Melanomas with numerous and large epidermal nests grew significantly faster than melanomas with mainly single cells or small nests. The significance of this finding is underlined by the fact that melanomas with numerous and large epidermal nests are characterized by a pattern of clods dermatoscopically. Scatter and cell size were also associated with the horizontal growth rate, but these histopathologic characteristics are not associated with dermatoscopic findings and are therefore of limited clinical value.

The melanoma with a pattern of clods shown in Figure 2 grew more than 5 times faster than the melanoma with a reticular pattern shown in Figure 4. Although the increase in size was similar in both cases, the follow-up interval was more than five times longer in the case of the reticular melanoma. The finding that the growth rate of melanomas with a pattern of clods is significantly higher that of those with other dermatoscopic patterns is of great clinical importance. Usually pigmented lesions are selected for monitoring because of diagnostic uncertainty. If lesions with a pattern of clods are selected for digital monitoring, it should be kept in mind that they may turn out to be rapid growing melanomas. Since the penalty associated with diagnostic delay is particularly severe with rapid growing melanomas, digital monitoring for lesions with a dermatoscopic pattern of clods should be per-

formed with caution. The penalty associated with diagnostic delay is less severe with slow growing melanomas. Slow growing melanomas in our sample had a reticular pattern, a structureless pattern, or a pattern of dots. This is in line with the observation of Argenziano et al [6] who found out that the reticular pattern dominates in slow growing melanomas. According to our findings it is relatively unproblematic to select flat lesions with one of these patterns for monitoring. It is, however, indicated to choose a monitoring interval that reflects the slow growth of these types of melanomas. One year would be a reasonable choice.

Our study has several limitations. The most important one is selection bias due to the fact that all melanomas in our series were initially selected for monitoring because of lack of melanoma specific criteria. This may favor the selection of slow growing melanomas and may bias our results. Another limitation is that the vertical growth rate could not be assessed. We consider this a minor limitation because there is no vertical growth as long as melanomas grow in situ. The basal membrane constitutes a natural boundary that impedes vertical growth. After the basal membrane has been penetrated by tumor cells they grow in all directions, and it probably makes no sense to differentiate between horizontal and vertical growth. It should also be kept in mind that the growth rate is a dynamic parameter that is most useful in vivo and to date only the horizontal growth rate but not the vertical growth rate can be measured in vivo. Once a melanoma has been excised and the vertical tumor thickness has been measured, it is of limited value to know how long it took to get there. On the other hand, it would have important practical implications for early recognition and selection of lesions for digital monitoring if we are able to differentiate between rapidly and slowly growing melanomas in vivo by dermatoscopy.

The integration of clinical, dermatoscopic, dermatopathologic, and biologic findings is the only way to profoundly increase our understanding of melanoma. Taken by itself, each of these aspects alone will not be able to fully explain a complex neoplasm like melanoma. Our study and those of others are important initial steps towards an integration of different aspects of melanoma. Viros et al [5], for example, successfully correlated molecular and morphologic aspects of melanoma. With regard to the recent advances in molecular genetics it can be expected that future studies will also integrate molecular aspects, like, for example, the role of activating BRAF mutations, with dynamic biologic findings.

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