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Objective melanoma progression

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Background/purpose: Many aspects of the natural history of malignant melanoma (MM) are still unclear, specifically its appearance at onset and particularly how it changes in time. The purpose of our study was to retrospectively determine objective changes in melanoma over a 3–24-month observation period.

Materials and methods: Our study was carried out in two Italian dermatology centers. Digital dermoscopy analyzers (DB-Mips System) were used to retrospectively evaluate dermoscopic images of 59 MM (with no initial clinical aspects suggesting melanoma) under observation for 3–24 months. The analyzer evaluates 49 parameters grouped into four categories: geometries, colors, textures and islands of color. Multivariate analysis of variance for repeated measures was used to evaluate the statistical significance of the changes in the digital dermoscopy variables of melanomas.

Results: Within-lesion analysis indicated that melanomas increased in dimension (Area, Minimum, and Maximum Diameter), manifested greater disorganization of the internal components (Red, Green and Blue Multicomponent, Contrast, and Entropy) and increased in clusters of milky pink color (Light Red Area).

Conclusion: Analysis of the parameters of our model and statistical analysis enabled us to interpret/identify the most significant factors of melanoma modification, providing quantitative insights into the natural history of this cutaneous malignancy.

Key words: melanoma – pigmented skin lesions – dermoscopy – digital dermoscopy analysis – multivariate analysis

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ALTHOUGH THERE have been recent advances in our knowledge of melanoma, many aspects of its natural history are still unclear, specifically its appearance at onset and particularly how and how fast it changes in time (1–4). Some of these aspects have been partly clarified with the advent of epiluminscence light microscopy (ELM), otherwise known as dermoscopy (5). This non-invasive method for more accurate evaluation of skin lesions was introduced in the last 20 years (6–10). While the method is technically simple, it is complex to apply. Many difficulties are due to the subjective nature of dermoscopic interpretation (11, 12). This has led to the development of methods of objective numerical scoring of dermoscopic patterns (13–15). Indeed, the recent introduction of digital-ELM and sophisticated image processing software (Digital Dermoscopy Analysis: DDA) has opened a new dimension in the objective evaluation of benign and malignant pigmented skin lesions (MPSL), allowing the visualization and recognition of slight morphological and colorimetric differences that are difficult to quantify with the naked eye (16–22). As this method is based on a numerical description of clinical images, it allows an unequivocal comparison of skin lesion images in time (18, 23–25).

In the present study, we performed DDA on a series of pigmented skin lesions (digital images) followed for 3–24 months that, after excision, were revealed to be malignant melanomas (MM). The aims were to determine objective changes in MM in this observation period and to determine the existence of numerical variables regarding changes in melanoma that could be useful in the diagnosis of this neoplasm.

Materials and Methods

Institutional approval and patient consent were obtained for all experimental procedures. The study was carried out in two Italian centers, the Department of Dermatology at Siena University and the Istituto Dermopatico dell'Immacolata in

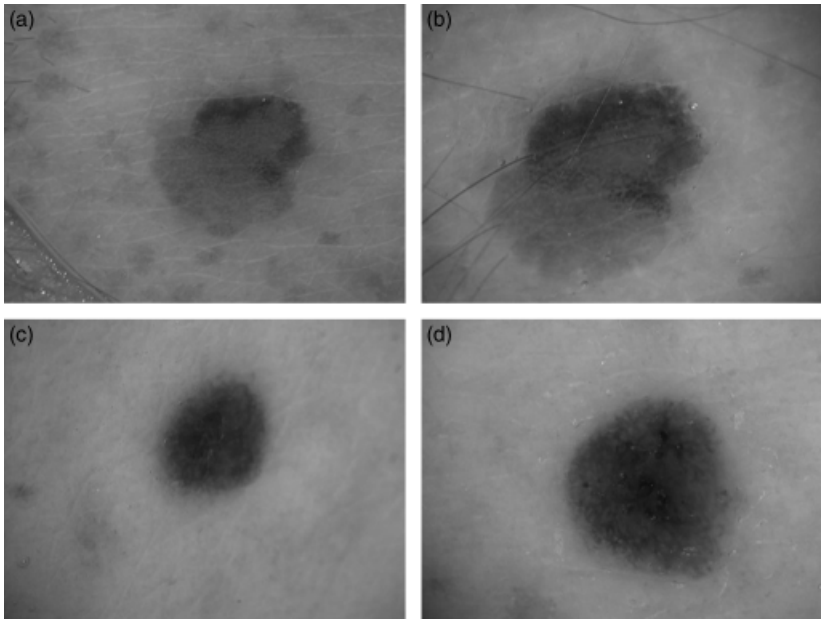

Fig. 1. Two illustrative cases of melanoma (a and c) in our study population followed up in a period from 3 (b) and 24 months (d).

Rome, between September 2006 and September 2009. Fifty-nine MM (with no initial clinical aspects suggesting MM), followed up for 3–24 months before excision, were the subject of this retrospective study (Fig. 1). They belong to a small group of patients who had lesions that were definitely very atypical but at the first observation did not meet the clinical, historical and/or dermoscopic criteria for MM. At each follow-up examination (every 3 months), dermoscopic images of all previously photographed MPSL were obtained, and new and prior dermoscopic images were compared side by side on a split screen by the dermatologists (P. R. and R. B.). All these lesions were removed during follow-up due to their changes, sometimes minimal, observed in time by classical digital dermoscopy (16–40 $\times$) performed by expert clinicians (P. R. and R. B.) (26). After removal, the lesions proved to be MM, with a thickness ranging from 0 (38 MM *in situ*) to 1.2 mm (the median invasion depth of invasive melanomas was 0.5 mm). Histopathological diagnosis was based on NIH Consensus Conference criteria (27).

Measurements

For each lesion, we took into consideration only two images: baseline and the last (the day of excision). The lesions were imaged (magnification $\times$16), stored and analyzed using the DB-Mips System (Biomips Engineering, S.R.L., Via Colleverde, 15-53100 Siena, Italy), a computerized instrument providing a visual database and objective evaluations of pigmented skin lesions. If the lesion and/or the surrounding skin were hairy, the hairs were carefully removed with scissors or a razor. After 3–24 months of follow-up, the clinicians decided to excise the lesions as suspected MM. The lesions were removed surgically and a histological examination was performed. All digital images were analyzed using appropriate algorithms.

Equipment

The DB-Mips System consists of a 3CCD (Charge Coupled Device) PAL Broadcast video camera with 730 lines of image resolution and a 60 db signal-to-noise ratio. The camera is connected to a hand-held 3CCD optical system with five magnifications from 6 to 40, enabling fields from 4 mm to 4 cm in size. The camera was calibrated weekly using a special paper for white balance. Illumination was provided by a 150 W light source at 3200°K. The components of the video signal were connected to a frame-grabber interfaced with a Pentium III 500 MHz personal computer having a magneto-optical drive for image storage. The system ran under Microsoft Windows, and all the software was written in language C/C++.

Digitization and parametrization

The choice of the most useful features to extract from digital images depends on the results of

TABLE 1. Digital dermoscopy analysis (DDA) parameters

Geometric variables	Area, perimeter, maximum and minimum diameters, radius, variance of contour symmetry, circularity, fractality of borders and ellipsoidality
Color variables	Mean values of red, green and blue inside the lesion; deciles of red, green and blue inside the lesion; quartiles of red, green and blue inside the lesion; mean values of red, green and blue of healthy skin around the lesion; mean skin-lesion gradient, variance of border gradient, border homogeneity and border interruptions
Texture variables	Mean contrast and entropy of lesion, contrast and entropy fractality
Islands of color variables	Peripheral dark regions, dark area, imbalance of dark region, total imbalance, green area, light red area, dominant green region imbalance, blue-gray area, blue-gray regions, transition area, transition region imbalance, background area, background region imbalance, red, green and blue multicomponent, number of red, green and blue percentiles inside the lesion

epiluminescence pattern analysis. The variables we chose were dermoscopic parameters currently used in the diagnosis of MPSL. Although the system saves microscope magnifications along with texture analysis, offering an objective evaluation, the different magnifications could confuse clinicians wanting to make subjective comparisons of lesions. In this paper, therefore, we only discuss images with a magnification of $\times 16$. The system used a procedure for digital image processing based on the Laplacian filter for segmentation and a zero-crossing algorithm for border automatic outline (23). It then evaluated 49 parameters (derived from classical dermoscopy) (23, 24) for discriminating power. The parameters, as described previously, belonged to four categories: geometries, colors, textures and islands of color (i.e. color clusters inside the lesion) (Table 1) (24).

Statistical analysis

Multivariate analysis of variance (MANOVA) for repeated measures was used to evaluate the statistical significance of changes in the digital dermoscopy variables (28, 29). Measurements were repeated every 3 months until the lesion was removed (time *T*2) after an initial reference observation (time *T*1). MANOVA included an examination of *within-subject* univariate analysis of each numerical dermoscopic variable and its changes. Statistical significance was set at 95% ($P < 0.05$). MANOVA statistical computations were performed using SPSS software (30).

Results

Fifty-nine melanomas, after a follow-up of 3–24 months (median 16 months), were excised from 58 patients (36 males and 22 females; mean age 47). Most lesions (34 lesions, 57.6%) were located on the trunk, followed by the lower extremities (16 lesions, 27.1%) and the upper extremities (nine lesions, 15.3%).

TABLE 2. Descriptive statistics of principal dermoscopic parameters, including the mean and standard deviation (SD), evaluated at times T1 (initial observation) and T2 (3–24 months after the first observation) for 59 melanomas

	*T*1		*T*2	
Parameters	Mean	SD	Mean	SD
Area (mm^2)	52.29	33.83	65.53	30.04
Perim (mm)	62.94	28.80	68.52	22.42
Ellipsoidality (%)	0.73	0.11	0.76	0.09
Circularity (%)	0.75	0.09	0.75	0.11
Variance of contour sym (%)	3.82	1.53	3.88	1.80
Minimum Diameter (mm)	5.95	2.16	6.88	1.76
Maximum Diameter (mm)	9.63	3.36	11.08	2.80
Skin lesion gradient (%)	9.45	6.94	11.41	7.38
Red average (%)	15.95	4.83	16.00	4.12
Green average (%)	8.59	3.40	9.05	3.54
Blue average (%)	5.41	2.79	6.09	2.79
Contrast (%)	1.22	0.23	1.44	0.53
Entropy (%)	3.20	0.27	3.42	0.40
Fractality of contrast (%)	0.64	0.04	0.63	0.04
Fractality of entropy (%)	0.22	0.05	0.21	0.06
Dark area (%)	0.14	0.04	0.13	0.05
Background area (%)	0.22	0.08	0.20	0.07
Imbalance (%)	0.18	0.10	0.18	0.10
Peripheral dark area (%)	0.25	0.20	0.29	0.19
Light Red Area (%)	0.01	0.02	0.03	0.04
Number of red percentiles (%)	5.27	2.83	5.55	2.48
Number of green percentiles (%)	0.31	0.17	0.30	0.26
Number of blue percentiles (%)	4.00	2.76	5.09	2.71
Red Multicomponent (%)	0.51	0.24	0.68	0.21
Green Multicomponent (%)	0.46	0.20	0.57	0.17
Blue Multicomponent (%)	0.34	0.18	0.42	0.13
Variance of border (%) gradient (%)	5.00	3.28	4.68	2.51
Variance of border (%) interruptions (%)	67.70	17.93	68.96	15.76

The descriptive statistics of the principal dermoscopic parameters used in this study are shown in Table 2, including the means and standard deviations of melanomas at *T*1 and 3–24 months later (*T*2). MANOVA analysis is reported in Table 3, which shows statistical comparisons between observation times. Table 3 illustrates the

TABLE 3. Multivariate analysis of variance (MANOVA): within-lesion analysis of dermoscopic parameters

	Melanomas		
Parameters	*F*	*P*	C%
Area	6.4	S	25.3
Minimum Diameter	8.6	S	15.5
Maximum Diameter	10.0	S	15.2
Contrast	12.1	S	18
Entropy	6.0	S	6.8
Light Red Area	5.3	S	100
Red Multicomponent	21.8	S	34.3
Green Multicomponent	10.6	S	24.2
Blue Multicomponent	4.5	S	22.0

Three- to 24-month differences were evaluated for 59 melanomas. Fisher value, *F*; statistically significant, *S* ($P \leq 0.05$); 3–24-month parameter changes as the percentage of their initial values (time *T*0), C%. Only statistically significant parameter changes are reported.

statistical significance of separate parameter differences for melanomas between times *T*1 and *T*2. The geometric parameters Area, Minimum Diameter and Maximum Diameter increased significantly. Entropy increased significantly ($C\% = 6.8\%$). Light Red Area also increased significantly, i.e. by 100%. Moreover, parameters correlated with the multicomponent pattern (Red, Blue and Green Multicomponent) changed significantly: Red Multicomponent $C\% = 34.4\%$, Green Multicomponent $C\% = 24.2\%$ and Blue Multicomponent $C\% = 22\%$.

Discussion

Clinical examination alone, even by trained dermatologists, has limited sensitivity for melanoma detection. Dermoscopy, which provides enhanced detail of pigmentation patterns and allows visualization of deeper structures, increases the diagnostic accuracy for experienced users, and reduces the overall biopsy rates (8–10). Various dermoscopic algorithms, however, generally have a sensitivity of only 80–90%, possibly because they are based on morphological structures that may not be present in MM (13–15). In these difficult cases of melanoma, a wrong diagnosis leads to inappropriate management and/or delayed treatment. This is why many authors underline the importance of follow-up (16, 18, 21). A further great advantage is that with the advent of digital computer dermatoscopy, the changes during follow-up can be better established (18, 22). Even minor structural/morphological changes such as increase of size, changes in shape and color, signs of regression and the appearance of other differential structures can also be monitored sensitively (16). A further step has been taken with the introduction of DDA, which offers the possibility to assess objectively/numerically these changes (23–25).

The aim of this study was to determine whether variables of change reflecting natural progression could be inferred among MM by DDA. We analyzed the changes occurring over time and endeavored to identify the variables that changed in a statistically significant manner. Differences between the values at *T*1 and *T*2 detected by within-lesion MANOVA were expressed as percentage change *C*% (Table 3). Percentage change (*C*%) of MM determined the significance of variation of individual variables in the course of time. Within-lesion analysis indicated that MM increased in dimension (Area, Minimum and Maximum Diameter), manifested greater disorganization of internal components (Red Multicomponent, Green Multicomponent, Blue Multicomponent, Contrast and Entropy) and increased in milky pink component, if any (Light Red Area).

With regard to the increase in Contrast and Entropy variables, if we imagine a grid superimposed on an MPSL, contrast expresses differences between light and dark squares of the grid and entropy describes how irregular the disposition of light and dark squares is. These two correlated parameters, used to describe lesion texture, provided an indication of the 'order' of patterns within lesions.

Parameters describing the number of different pattern components in the three bands of color (Red, Green and Blue Multicomponent), used to quantify the dermoscopic picture defined as a 'multicomponent pattern,' showed a trend similar to the above geometric parameters: MM showed increases (*C*%) of 22–34%. These were the parameters with the highest percentage changes. This discovery suggests that in cases in which dermoscopic diagnosis is difficult at the first examination, a dermoscopic follow-up of the MPSL will certainly be useful for detecting significant objective changes in the variables associated with the evolution of MM (especially multicomponent pattern variables).

In conclusion, analysis of the parameters of this model and MANOVA enabled us to interpret/identify the most significant factors of modification of MM, providing quantitative insights into the diagnosis of this neoplasm and demonstrating

the utility of an objective/numerical follow-up (31, 32). Our observations strongly indicate that among the various parameters that clinicians should consider in deciding whether or not to remove suspicious but not clearly malignant lesions, the parameter *evolution* and particularly, from the practical point of view, parameter changes in *geometric variables* and internal 'confusion' have decisive weight.

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