Dermatology Practical & Conceptual

Super-High Magnification Dermoscopy Can Help for the Diagnosis of Lentigo Maligna: a Pilot Study on 61 Cases

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ABSTRACT

Introduction: Facial lentigo maligna/lentigo maligna melanoma (LM/LMM) is a significant diagnostic clinical challenge and dermoscopy can help its diagnosis.

Objectives: The following study aimed to evaluate if super-high magnification dermoscopy at 400x can add further details for the diagnosis of the LM/LMM.

Methods: This is a retrospective observational, multicentric study enrolling patients who received a 20x and 400x (D400) magnification dermoscopic examination of facial skin lesions in clinical differential diagnosis with LM/LMM. Dermoscopic images were retrospectively evaluated by four observers for the presence/absence of nine 20x and ten 400x dermoscopic features. Univariate and multivariate analyses were carried out to find predictors of LM/LMM.

Results: We enrolled 61 patients with a single atypical skin lesion of the face, including 23 LMs and 3 LMMs. The presence of roundish and/or dendritic melanocytes ($P < 0.001$), irregular arrangement of melanocytes ($P < 0.001$), irregular in shape and size melanocytes ($P = 0.002$), and folliculotropism of melanocytes ($P < 0.001$) at D400 were more frequent in LM/LMM than other facial lesions. According to the multivariate analysis, roundish melanocytes at 400x dermoscopy were more indicative of LM/LMM (Odds Ratio-OR 49.25, 95% CI 8.75-513.2, $P < 0.001$), and sharply demarcated borders at 20x dermoscopy were more indicative of not-LM/LMM (OR 0.1, 95% CI 0.01-0.79, $P = 0.038$).

Conclusions: D400 can identify atypical melanocyte proliferation and folliculotropism that can help to identify LM/LMM together with conventional dermoscopy data. Our preliminary observations should be confirmed by larger studies.

Introduction

Although cutaneous melanoma represents 4% of the overall tumor amount in the whole body, it is a public health issue, particularly in fair-skinned populations, due to its high morbidity and mortality [1,2-4]. The head and neck region is of particular interest for melanoma; despite accounting for only 9% of the total body surface, it harbors 20% of melanoma cases, mainly of lentigo maligna/lentigo maligna melanoma (LM/LMM) subtype [5]. LM and LMM appear on sun-damaged skin, most often on the head and neck and in elderly patients [6].

Over the last three decades, the global incidence of melanoma has steadily increased, and the current increase in life expectancy of the population could also favor the development of LM/LMM [7,8]. Despite recent advances in both diagnosis and treatment, cutaneous melanoma continues to represent a significant clinical challenge [9]. Earlier detection of melanoma improves the survival rates but the clinical presentation of LM/LMM can be subtle and varied [10]. The differential diagnosis of LM/LMM includes pigmented actinic keratoses, solar lentigines, seborrheic keratoses, and lichen planus-like keratoses [11]. Also, early LM may not exhibit the tell-tale signs of an evolving melanoma (changes in diameter, border, color, and asymmetry), and it is often difficult to distinguish from the surrounding sun-damaged skin [12].

Although histological evaluation, remains the gold standard for the confirmation of the diagnosis, several non-invasive imaging procedures such as dermoscopy and reflectance confocal microscopy (RCM) may improve LM/LMM clinical diagnostic accuracy [13-15]. Moreover, they can help in biopsy site selection, margin delineation, and treatment monitoring.

Recently, our group has shown that dermoscopy at 400x magnification (D400 or super-high magnification dermoscopy) can aid the non-invasive diagnosis of melanoma by observing single pigmented cells [16,17].

Objectives

The following study aimed to evaluate if D400 can add further details for the clinical diagnosis of the LM/LMM and its differential diagnosis with other facial lesions.

Methods

Study Design

Retrospective observational, multicentric study.

Setting

Data were collected on patients who came to the Dermatology department of the University Hospital of Siena (Italy) or Saint-Etienne (France) for a dermatological examination between the 1st January 2018 and 31st December 2020. Data were analyzed from April 2021 to June 2021. The study was conducted according to the criteria set by the Declaration of Helsinki. All data were de-identified before use. All patients gave their consent to the processing of their data.

Participants

We enrolled non-consecutive patients with pigmented skin lesions of the face that needed to be removed or followed up for their atypical clinical and/or 20x dermoscopic features according to a skin imaging expert dermatologist (E.C. or J.L.P.).

Data Sources

For this study, we selected only patients who received a 20x and 400x (D400) magnification dermoscopic examination of facial lesions in clinical differential diagnosis with LM/LMM. These lesions were recorded with the videodermoscope Fotofinder Medicam 1000 (Fotofinder System, Bad Birnbach).

To perform D400, we used the same camera as for 20x video-dermoscopy and we changed the terminal lens. Specialists in skin imaging (E.C. and J.L.P), acquired at least 5 images for each lesion, for a total of 1138 images of 61 skin lesions, as D400 does not show an entire lesion (D400 field of view of 1 mm x 0.5625 mm). We included cases with histological diagnosis or lesions unmodified at clinical and dermoscopic follow-up of at least 12 months.

Variables

For the clinical variables, we evaluated the patient sex and age. A group of 4 dermatologists (E.C, F.G.L., F.F., and V.C.) belonging to the University Departure of Dermatology of the Hospital-University of Siena evaluated the images and defined the dermoscopy variables to be analyzed.

The 20x dermoscopy variables included: benign benchmarks (white and wide follicular opening, reticular or parallel brown lines, sharply demarcated borders, mila-like cysts/comedo-like openings, erythema and red pseudo-network) and malignant benchmarks (polygons/rhomboids/zigzag pattern/angulated lines, annular granular pattern / gray circles, asymmetrical pigmented follicular openings, and follicular obliteration).

The D400 variables were the presence of the pigmented cells and their features, out of focus blue or gray/brown structureless areas, vessels, and their shape (linear/arborizing, and irregular), hyperkeratotic roundish concentric structures, and folliculotropism of melanocytes. Pigmented cells were differentiated into keratinocytes (seen as polygonal brown regular mostly in focus cells, evenly spread and/or inside a network), roundish melanocytes (seen as large

roundish brown-to violet/blue scattered cells; cells were defined as "large" when they were larger than keratinocytes), dendritic melanocytes (large dendritic brown-to-violet/blue scattered cells), and melanophages (large blue-to-violet non in focus cells with a not defined polymorphous shape). Considered cell features were cell color (violet and blue colors are difficult to differentiate with D400 and were considered together; light and dark brown were also considered together because brown is often present with multiple shades in the same structure), shape and size irregularity of melanocytes, and irregular arrangement of single melanocytes.

Statistical Analysis

Descriptive statistics were performed: absolute frequencies and percentages were calculated for qualitative variables and mean and standard deviations for the quantitative ones. The association between qualitative variables and the outcome (ie LM/not LM) and 20x dermoscopy or D400 was evaluated by Fisher exact test. T-test was carried out if the variables were normally distributed (normal distribution evaluated by Kolmogorov Smirnov test) and there was homoscedasticity between variances evaluated by Bartlett test, otherwise Mann-Whitney test was used. Logistic regression was later performed to evaluate variables that were statistically significant at the previous univariate analysis ($P < 0.05$). The best subset of variables was selected by a stepwise procedure based on Akaike criterion. Odds ratio (OR) and 95% confidence intervals (CI) were estimated by logistic regression. The analyses were carried out by R software version 3.6.2.

Results

Participants and Lesion Data

In this study 61 patients, 32 (52%) women and 29(48%) men, with a mean age of 72.3 years (range 44 – 91 years) with a single atypical skin lesion of the face, were selected. The 61 skin lesions included 23 LMs, 3 LMMs, 15 solar lentigines, 12 seborrheic keratoses, 6 lichenoid keratoses, and 2 pigmented actinic keratoses.

20x Dermoscopy

20x dermoscopy features are reported in Table 1. Concerning malignant benchmarks, the annular-granular pattern and gray circles were more frequent in LM/LMM than in the other lesions [Figure1, 19 LM/LMMs (73.1%) and 14(41.2%) $P = 0.019$]; asymmetric pigmented follicular openings and follicular obliteration were not present in any of the benign lesions analyzed, while these features were associated to LM/LMMs ($P < 0.001$). Regarding benign benchmarks, sharply demarcated borders were present in 17 (50%) not-LM/LMM lesions and only three (11.5%) LM/LMMs ($P = 0.002$), while the presence of milia-like cysts/comedo-like openings was detected in seven (20.6%) not-LM/LMM lesions and no LM/LMMs ($P = 0.016$).

400x Dermoscopy

400x dermoscopy features are reported in Table 2. The presence of roundish and/or dendritic melanocytes was mainly observed in skin lesions diagnosed as LM/LMM compared to the other lesions (Figure 1, $P < 0.001$). Roundish melanocytes were seen in four not-LM/LMM lesions (11.4%) and 24 LM/LMMs (92.3%), while dendritic melanocytes were found in seven not LM/LMM lesions (20.0%) and 25 LM/LMMs (96.2%). Pigmented keratinocytes were present in almost all the images analyzed (Figure 2). Besides, LM/LMM showed more frequently than the other facial lesions melanocytes with irregular arrangement ($P < 0.001$) and irregularity in shape and size ($P = 0.002$).

Cell color, out-of-focus bluish or gray/brown structureless areas and vessel presence were not statistically significant

Table 1. 20x dermoscopy features of facial lesions.

	LM/LMM N=26 n(%)	Other[a] N=35 n(%)	P-value
White and wide follicular opening	0 (0.0)	3 (8.8)	0.251
Reticular or parallel brown lines	10 (38.5)	8 (23.5)	0.261
Sharply demarcated borders	3 (11.5)	17 (50.0)	**0.002**
Milia like cysts /comedo-like openings	0 (0.0)	7 (20.6)	**0.016**
Erythema	4 (15.4)	4 (11.8)	0.717
Polygons / rhomboids / zig-zag pattern / angulated lines	3 (11.5)	7 (20.6)	0.491
Annular granular pattern / Gray circles	19 (73.1)	14 (41.2)	**0.019**
Asymmetrical pigmented follicular openings	20 (76.9)	0 (0.0)	**<0.001**
Follicular obliteration	10 (38.5)	0 (0.0)	**<0.001**

[a] This group included 15 solar lentigines, 12 seborrheic keratoses, six lichenoid keratoses, and two pigmented actinic keratoses.

Table 2. 400x dermoscopy features of facial lesions.

	LM/LMM N=26 n(%)	Other[a] N=35 n(%)	P-value
Cell presence:			
Roundish melanocytes	24 (92.3)	4 (11.4)	**<0.001**
Dendritic melanocytes	25 (96.2)	7 (20.0)	**<0.001**
Roundish or dendritic Melanocytes	25 (96.2)	9 (25.7)	**<0.001**
Keratinocytes	26 (100.0)	33 (94.3)	0.503
Melanophages	15 (57.7)	11 (31.4)	0.066
Melanocytes irregular in shape and size	18 (69.2)	10 (28.6)	**0.002**
Melanocyte colour:			
Brown	26 (100.0)	10 (28.6)	0.503
Violet/blue	4 (15.4)	8 (22.9)	0.532
Black	0 (0.0)	1 (2.9)	1.000
Melanocytes distribution:			
Irregular arrangement	20 (76.9)	9 (25.7)	**<0.001**
Out of focus bluish structureless areas	13 (50.0)	12 (34.3)	0.294
Out of focus grey/brown structureless areas	8 (30.8)	9 (25.7)	0.775
Vessels	16 (61.5)	20 (57.1)	0.796
Vessels shape:			
Linear + arborizing	7 (43.8)	6 (30.0)	0.493
Irregular in shape	8 (50.0)	6 (30.0)	0.307
Hyperkeratotic roundish concentric structure	2 (7.7)	10 (28.6)	0.055
Folliculotropism	24 (92.3)	0 (0.0)	**<0.001**

[a] This group included 15 solar lentigines, 12 seborrheic keratoses, six lichenoid keratoses, and two pigmented actinic keratosis.

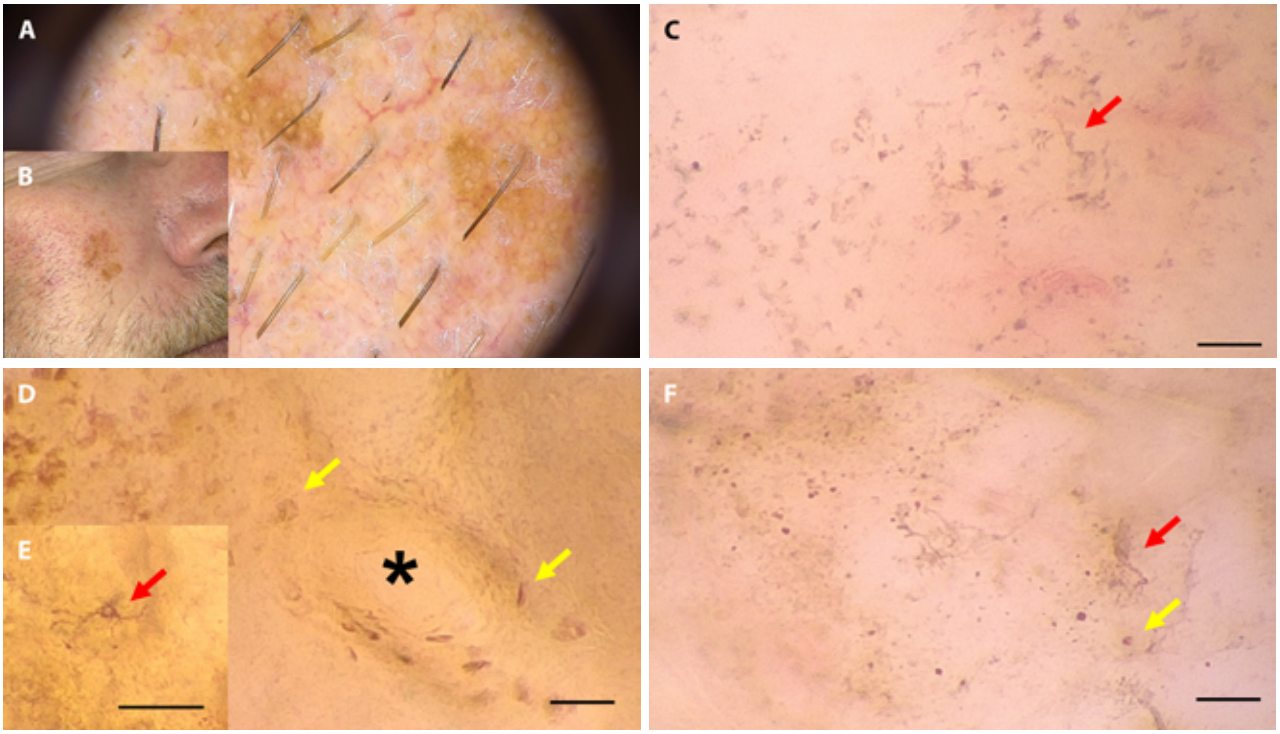

Figure 1. 20x dermoscopy (A), clinical (B) and 400x dermoscopy (C-F) images of a lentigo maligna. 400x dermoscopy shows dendritic (red arrow) and roundish (yellow arrow) melanocytes and melanocytic invasion of a hair follicle (asterisk). Scale bar 100µm.

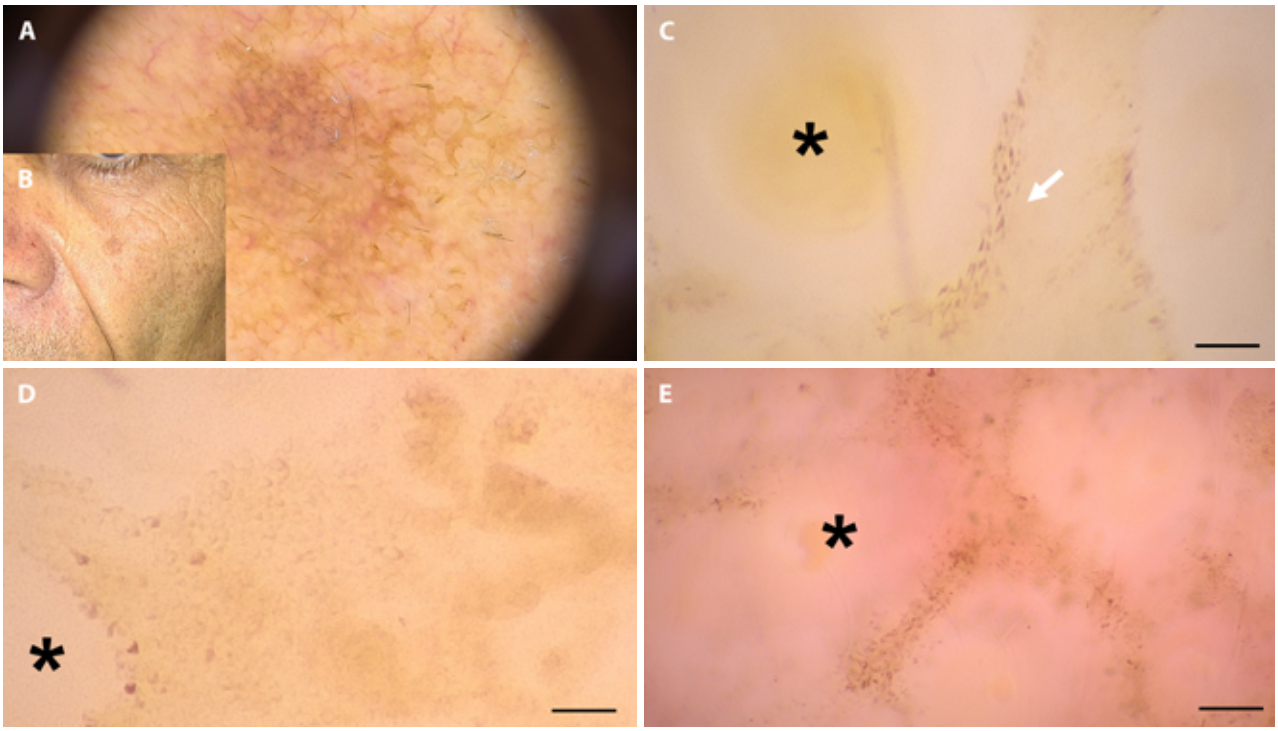

Figure 2. 20x dermoscopy (A), clinical (B) and 400x dermoscopy (C-E) images of a solar lentigo. 400x dermoscopy shows pigmented keratinocytes (white arrow) in the epidermis around hair follicles (asterisk). Scale bar 100μm.

for the differential diagnosis between LM/LMM and benign lesions. Folliculotropism of melanocytes was present in 24 LM/LMMs (92.3%, Figure 1) while it was absent in all benign skin lesions analyzed (P < 0.001). Lastly, hyperkeratotic roundish concentric structures were found in 10 benign skin lesions (28.6%) and 2 LM/LMMs (7.7%), with a P value just above the threshold of statistical significance (P = 0.055).

Multivariate Analysis

The multivariate regression considered the following variables: sharply demarcated borders, milia-like cysts /comedo-like openings, annular granular pattern/ gray circles, roundish melanocytes, melanocyte irregularity in shape and size, irregular arrangement of melanocyte distribution, and follicular obliteration. Although asymmetrical pigmented follicular openings, dendritic melanocytes, roundish or dendritic melanocytes, and folliculotropism were statistically significant at univariate analysis, they were excluded from multivariate analysis since their presence/absence almost perfectly explained the presence/absence of LM/LMM. According to the stepwise procedure, roundish melanocytes at 400x dermoscopy were more indicative of LM/LMM (OR 49.25, 95% CI 8.75-513.2, P < 0.001), and sharply demarcated borders at 20x dermoscopy were more indicative of not-LM/LMM lesion (OR 0.1, 95% CI 0.01-0.79, P = 0.038).

Conclusions

Conventional dermoscopy at 20x magnification can help the diagnosis of LM/LMM and our series found five criteria (demarcated borders, milia-like cysts/comedo-like openings, annular-granular pattern/gray circles, asymmetric pigmented follicular openings, and follicular obliteration) that could help the differential diagnosis in the monovariate analysis [14,15].Our results agreed with a recent study that highlighted how 20x dermoscopic features suggestive of solar lentigo/flat seborrheic keratosis or pigmented actinic keratosis can help to exclude the possibility of an LM/LMM [18]. The presence of sharply demarcated borders, a typical feature of solar lentigo/flat seborrheic keratosis, was the most relevant parameter to exclude LM/LMM in the multivariate analysis (OR: 0.1, 95% CI 0.01-0.79, P = 0.038).

Recently, it has been demonstrated that D400, a new non-invasive skin imaging tool that can reveal pigmented cells in the skin, could also help the clinical diagnosis of cutaneous melanoma, but no data on facial melanoma were available [17]. Our study found that D400 could also have a role in the diagnosis of facial melanoma, namely LM/LMM.

According to our results shown in Table 2, we observed that the presence of roundish and/or dendritic melanocytes in D400 was more frequently associated with LM/LMM than the other pigmented facial lesions (P < 0.001, Figure 1).

Notably, the presence of roundish melanocytes was highly in favor of LM/LMM (OR 49.25, 95% CI 8.75-513.2, P < 0.001). Moreover, the irregular shape and size (P = 0.002) and distribution of melanocytes (P < 0.001) in D400 were also more frequent in LM/LMM.

A distinctive feature of LM/LMM is the invasion of the follicular structures, which can be indirectly appreciated in conventional dermoscopy as pigmentation around hair follicles [19]. As expected, in our series we found annular granular pattern/gray circles (P = 0.019) or asymmetrical pigmented follicular openings (P < 0.001) up to a complete follicular obliteration (P < 0.001) as 20x dermoscopic features associated with LM/LMM. However, as demonstrated by several studies, these dermoscopic features are not specific of LM/LMM [20]. Our case series revealed that D400 can directly show single melanocyte invasion of the hair follicles increasing the diagnostic specificity for facial melanoma: almost all LM/LMMs and no other pigmented facial lesions had visible folliculotropism (P < 0.001) in our series (Figure 1).

Vessel presence and out-of-focus bluish structureless areas, features considered as predictive of melanoma with D400 in our previous study, were not relevant for the identification of LM/LMM [17]. Neovascularization is probably less evident in pigmented superficial melanoma of LM/LMM subtype than in other MM and the presence of bluish structureless areas in non-melanoma lesions of our series could be explained by regression features (aggregates of melanophages) mainly seen in lichenoid keratoses.

The main limitation of this study is represented by the small sample and the fact that the acquisition and the evaluation of the images were dependent on the expertise of the investigators. In addition, correlation with histopathological images and images of other new non-invasive imaging techniques such as RCM was lacking. If we compare D400 to RCM, D400 has less concern of false-positive results given by the presence of dendritic Langerhans cells in the epidermis possibly mistaken for melanocytes under RCM and has a lower cost. However, D400 can miss atypical melanocytes that are not heavily pigmented or deeper located and can show large cells suggestive of atypical melanocytes when multiple keratinocytes are superposed due to the lack of confocal sections.

In conclusion, our study about the use of D400 for the diagnosis of facial lesions found that D400 can identify atypical melanocyte proliferation and folliculotropism that can help the diagnosis of LM/LMM together with conventional dermoscopy data. Moreover, we could assume that D400 could help to direct the choice of the more representative site to perform a biopsy. Our preliminary observations should be confirmed by larger studies.

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