Acta Derm Venereol 2011; 91: 137–146

INVESTIGATIVE REPORT

Early Stages of Melanoma on the Limbs of High-risk Patients: Clinical, Dermoscopic, Reflectance Confocal Microscopy and Histopathological Characterization for Improved Recognition

Cristina CARRERA[1], Josep PALOU[1,2], Josep MALVEHY[1,3], Sonia SEGURA[5], Paula AGUILERA[1], Gabriel SALERNI[1], Louise LOVATTO[1], Joan A. PUIG-BUTILLÉ[3,4], Llùcia ALÓS[2] and Susana PUIG[1,3]

Departments of [1]Dermatology, [2]Pathology and [4]Genetics, Melanoma and Dermatopathology Units, Hospital Clínic de Barcelona, [3]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, Spain, CIBER de Enfermedades Raras, Instituto de Salud Carlos III (ISCIII), and [5]Department of Dermatology, Hospital del Mar, Parc de Salut Mar, Barcelona, Spain

Early stages of 36 melanomas on limbs were morphologically characterised. Most occurred in high-risk patients (multiple and/or familial melanoma) attending a referral unit for melanoma and pigmented lesions. None of the tumours was clinically suspicious for melanoma (mean diameter of 4.3 mm). The tumours were classified into four dermoscopic groups: (*i*) prominent network (*n* = 16); (*ii*) delicate network (*n* = 5); (*iii*) hypo-pigmentation with dotted vessels (*n* = 10); and (*iv*) diffuse light pigmentation with perifollicular pigmentation (*n* = 5). Confocal microscopy performed in 12 cases allowed the identification of atypical, single cells within epidermal layers. Histopathology showed marked large atypical cells in a pagetoid spreading pattern in most cases. Significant associations were detected between the third dermoscopic group and naevoid histological appearance and delay in detection, and between the fourth group and lentigo-maligna-like features. Dermoscopy allowed an increase in the suspicious threshold in these difficult melanomas in high-risk patients and enabled the subclassification of early melanomas on the limbs, with a correct confocal and histopathological correlation. Although the biological behaviour of these incipient tumours remains uncertain, the most appropriate treatment seems to be recognition and proper excision. ***Key words: atypical mole syndrome; dermoscopy; dermatoscopy; familial melanoma; melanoma; naevus; reflectance confocal microscopy.***

(Accepted June 23, 2010.)

Acta Derm Venereol 2011; 91: 137–146.

Cristina Carrera, Department of Dermatology, Melanoma Unit, Hospital Clínic Barcelona, IDIBAPS, Villarroel 170, ES-08036 Barcelona, Spain. E-mail: criscarrer@yahoo.es

There is only one effective treatment for malignant melanoma (MM): complete excision of early stage tumours. *In situ* MMs are the only cases with a 100% cure rate after proper surgery, decreasing to 80–85% in thin MM (under 1 mm Breslow). MMs on the limbs are not well characterised in the literature, especially in the early stages, although they appear to be related to different epidemiological settings (e.g. women with intermittent sun-exposure on the lower limbs) (1, 2).

Atypical mole syndrome (AMS), defined by the presence of more than 100 naevi, and/or more than 10 clinical and dermoscopically atypical naevi, and/or previously excised dysplastic naevi, is the most important independent risk marker for developing MM. In addition, naevi are both possible MM precursors and early MM simulators. In fact, the most difficult task in early detection of MM is to differentiate them from the more frequent benign melanocytic lesions. However, systematic excision of atypical naevi has no benefit in preventing MM in these high-risk patients (3, 4).

It is estimated that 10% of cases of MM occur in a familial setting as an autosomal dominant trait. In approximately 50% of these familial multiple melanoma (FamMM) cases a responsible gene can be found, being *CDKN2A* and *CDK4* the two major susceptibility genes most commonly identified. FamMM cases and their relatives, especially when they are affected by AMS and/or are mutation carriers have a very high risk of MM development, even up to 1000 times over general population. To date, no clinical, dermoscopic, or histopathological special feature has been related to tumours in FamMM (5–7). Polymorphisms in *melanocortin 1 receptor (MC1R)* gene, especially the red hair variants (RHV), are considered low susceptibility genes to MM development, increasing the MM risk up to 10 times in respect to wild type (8). We studied the interaction between these low-risk variants among FamMM cases, and found that they can increase the genetic risk in *CDKN2A* (high-risk gene) mutation carriers by up to 14 times and contribute to a less suspicious clinical and dermoscopic appearance of tumours, less colour, and fewer structures (9).

The clinical ABCDE rule fails to recognise MMs that are small (less than 6 mm in diameter) or that exhibit regular shape and homogeneous colour, are symmetrical or undergo unnoticed changes (10, 11). Dermoscopy is now well accepted as a non-invasive technique that improves the accuracy of skin tumour diagnosis (12–14), and is especially useful in the differential diagnosis of MM simulators or hypopigmented MM, avoiding unnecessary excisions (15–17).

 doi: 10.2340/00015555-1021
 ISSN 0001-5555

In vivo reflectance-mode laser scanning confocal microscopy (RCM) is a non-invasive imaging technique that allows real-time skin examination at high resolution and thus improves the diagnostic accuracy in MM and other non-melanocytic tumours (18–20).

We performed a retrospective study of 36 very early MMs on limbs. The objectives of this study were: (*i*) to describe the dermoscopic and *in vivo* RCM features in order to improve their future recognition; (*ii*) to correlate these findings with histopathological characteristics of the tumours that could suggest different types of early MM on the limbs in these very early stages.

MATERIALS AND METHODS

A systematic retrospective review of all thin MMs located on limbs diagnosed in a specialised Pigmented Lesion Unit of a referral hospital between 2005 and 2008.

The inclusion criteria were: (*i*) thin MM (< 1 mm Breslow) proven by histopathological examination, located on limbs; (*ii*) clinical, dermoscopic and histopathological data available; and (*iii*) clinically unsuspicious for MM, defined by no clinical ABCD criteria fulfilled.

Complete clinical patient history was recorded, including familial history, previous melanocytic lesions excised and other MM-associated risk factors. Genetic studies were performed when DNA was available. Exons 1alfa, 1beta, 2 and 3, intronic changes IVS2-105 and –34G>T in the *CDKN2A* promoter, and exon 2 from *CDK4* were studied by PCR single-strand conformation polymorphism (PCR-SSCP) analysis and sequencing (7). *MC1R* was studied by direct sequencing (9).

Clinical and dermoscopic images were taken using digital cameras (Olympus Camedia, Canon G7 and/or Nikon Coolpix 4500) and a polarised dermatoscope (DermlitePhoto®; 3 GEN, LLC.Dana Point, CA, USA). In the case of the high-risk patients included in our digital follow-up protocol (21), Mole Max II (Dermamedical Instruments®), able to detect digital clinical and/or dermoscopic changes in a 6-month follow-up, was an additional tool used in the study. Clinical evaluation was based on ABCDE criteria and dermoscopic pattern analysis (22).

Whenever possible, *in vivo* RCM examination was performed with near-infrared reflectance confocal laser scanning microscopes (Vivascope 1500®; Lucid Inc., Henrietta, NY, USA). The instruments and acquisition procedures, as well as the features studied, have been described previously (23).

Conventional haematoxylin-eosin staining and immunohistochemistry (Melan A, HMB45, Ki67) were performed whenever it was considered necessary. Histopathologically, MMs were classified into one of the following groups according to their characteristics:

- Naevoid MM: predominance of nesting pagetoid invasion of the upper layers of epidermis over solitary cells.
- Paget's disease-like MM: characterised by atypical large

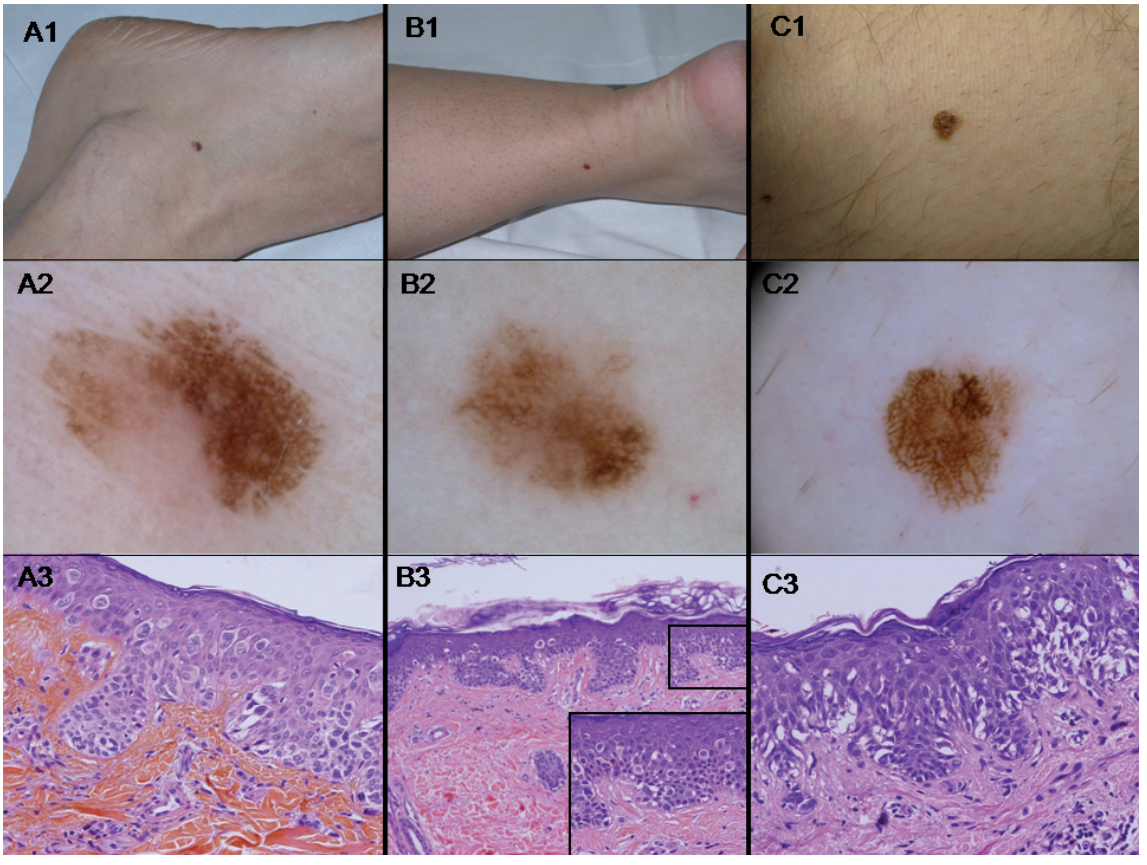

Fig. 1. Dermoscopic group 1: atypical network. Examples of 3 melanomas from group 1. A1, B1, C1: clinical aspect: located on lower limbs, small dark brown lesions, with no malignant criteria A2, B2, C2: dermoscopic images (original magnification ×30). Prominent network pattern, with 2 colours and asymmetrical pigment distribution. Case A is completely asymmetrical in 1 axis. A3, B3, C3: histopathological examination (×20 (B3) and ×40 (A3, B3 inset and C3)). Proliferation of atypical large melanocytes, both solitary and forming discrete nests, in junctural and intraepidermal layers. These 3 cases were *in situ* malignant melanoma.

epithelioid cells invading the whole epidermis resembling genuine Paget's disease.
- Lentiginous MM: melanocytic hyperplasia, with severe architectural atypia and intraepidermal spreading. Small nests can be found on the bottom of rete ridges.
- Lentigo maligna-type: atypical melanocytic proliferation along a faded dermal-epidermal junction and flattened epidermis, with solitary and small nests invading the upper epidermis and characteristic follicular involvement. It may be associated with marked actinic damage.

Statistical evaluation was carried out using SPSS statistical software package for Windows (version 16.0; SPSS Inc., Chicago, IL, USA). A chi-square test was applied for all category features, and Fischer's exact test was applied if any expected cell value in the 2×2 table was < 5. Each group was compared with the other three. Mean and median values were determined for quantitative variables and compared using the Student's *t*-test.

RESULTS

Patient data

Thirty-six tumours from 35 patients in our high-risk patient-set were reviewed. Tumours were assigned, based on overall appearance in dermoscopic analyses, to 1 of 4 groups (for details see below – Dermoscopic examintion): 1, Prominent network (16 tumours, 46%) (Fig. 1); 2, Delicate network with no specific MM dermoscopic features (5 tumours, 14%) (Fig. 2). Melanomas were detected by changes in digital follow-up; 3, Hypopigmented with atypical vessels (10 tumours, 28%), (Fig. 3); and Group 4, Diffuse light pigmentation and perifollicular pigmentation (5 tumours, 14%), (Fig. 4). Patient clinical characteristics are summarised in Table I.

The most remarkable feature was the predomination of women ($n = 29$) over men ($n = 6$), and the presence of high-risk MM history, since 40% had familial MM history, 49% personal MM history, and 17% had multiple primary MMs (MPM) before the current MM diagnosis. The majority of patients (75%) were affected by atypical mole syndrome. Eighteen had been included in our digital follow-up high-risk surveillance programme, which involves total-body photography mapping and digital dermoscopy of atypical lesions every 6 months, as described previously by our group (21).

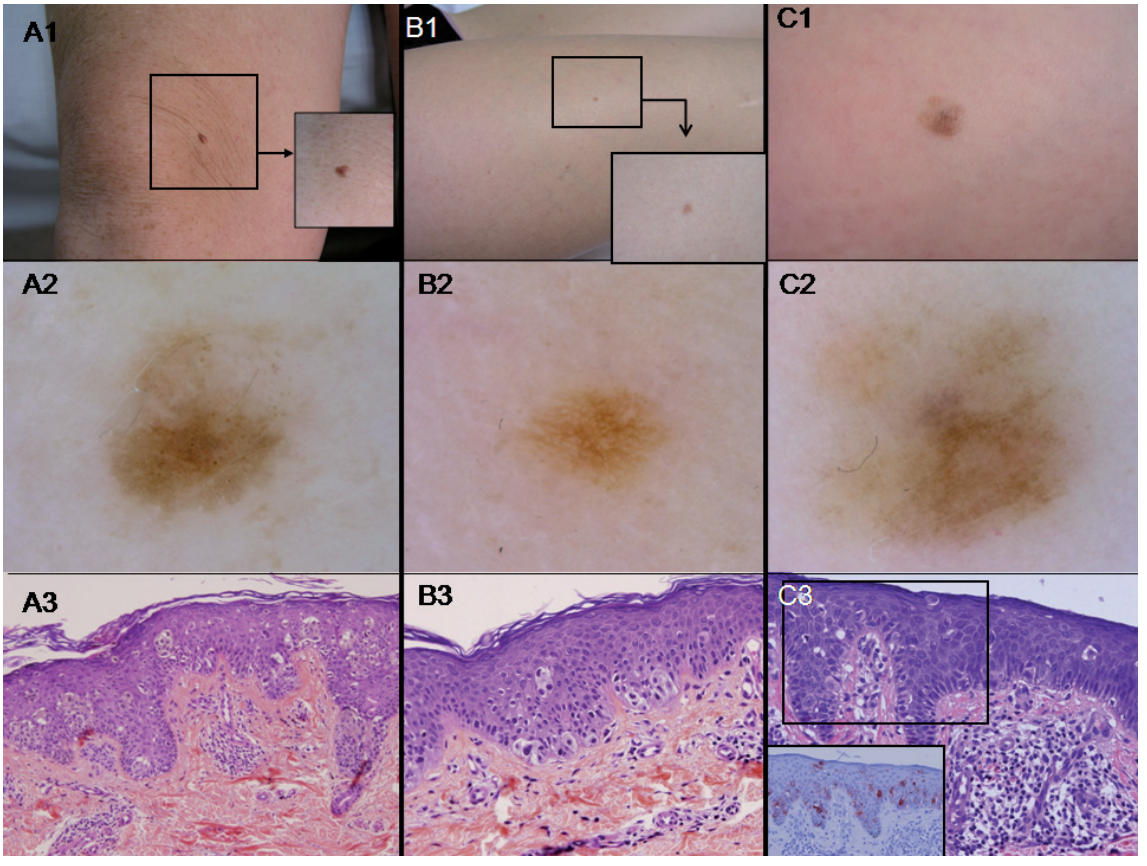

Fig. 2. Dermoscopic group 2: delicate network with changes on digital follow-up. Examples of three melanomas from group 2. A1, B1, C1: clinical aspect: located on lower limbs, the smallest lesions had a completely unremarkable aspect. Case A1 and B1 are mother and daughter, both of them CDKN2A mutation and double-red-hair-variant-MC1R carriers, affected by multiple primary malignant melanoma (MM). A2, B2, C2: dermoscopic images (original magnification ×30). Light-brown very delicate network pattern, with a slight asymmetrical light-brown structureless area in cases A2 and C2 due to a pre-existing naevus. In all cases the lesions were excised due to changes seen in digital follow-up of a very high-risk patient setting. A3, B3, C3 histopathological examination (×20). Proliferation of atypical large melanocytes, both solitary and forming nests, in junctural and intraepidermal layers. All were *in situ* MM. Note the immunohistochemical study in C3 with a more evident pagetoid spreading of Melan-A positive cells.

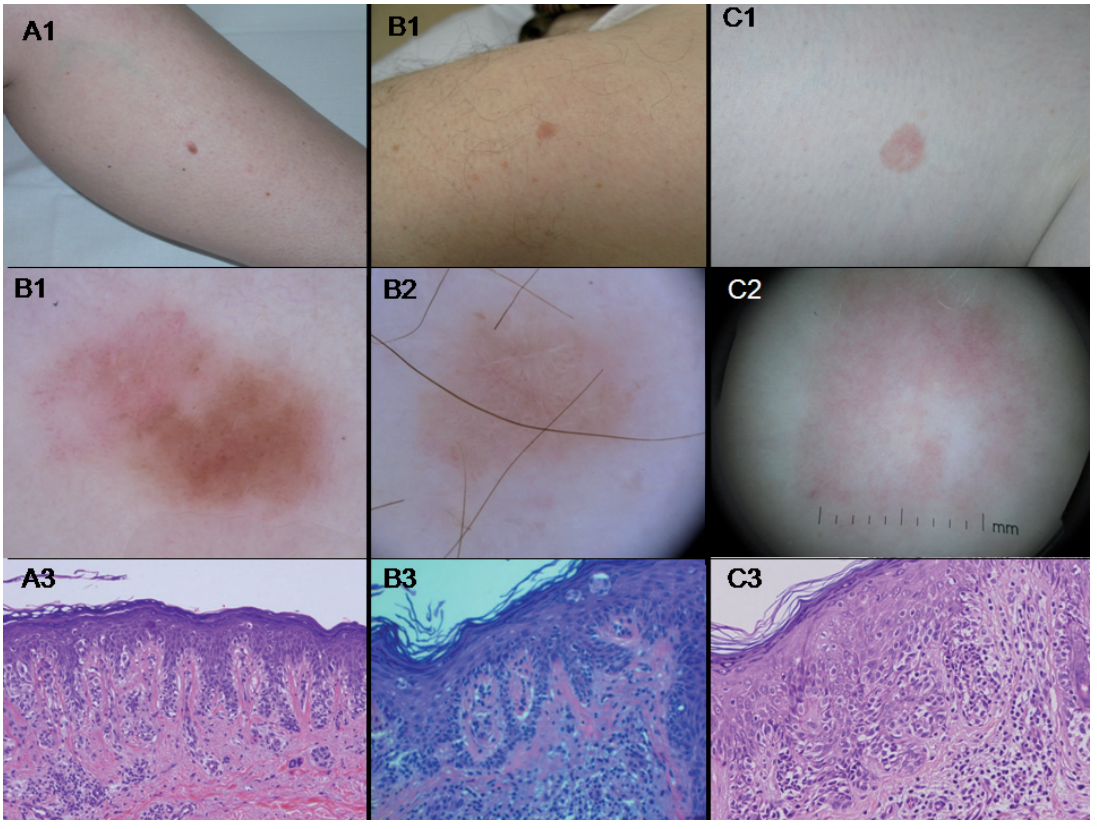

Fig. 3. Dermoscopic group 3: atypical vascular pattern. Examples of three melanomas from group 3. A1, B1, C1: clinical aspect: located on lower limbs, all achromic lesions with erythema. Case C1: albinism type OCA1 in a 34-year-old woman, the largest lesion in the series. A2, B2, C2: dermoscopic images (original magnification × 30). Homogeneous or unspecific pattern, only remarkable by vessels and a light-brown structureless pigmentation. Dotted vessels and whitish linear structures (chrysalides-like) are the only noteworthy features. A3, B3, C3: histopathological examination (× 20). Lentiginous hyperplasia of atypical melanocytes, with mild pagetoid spreading and marked vascular hyperplasia.

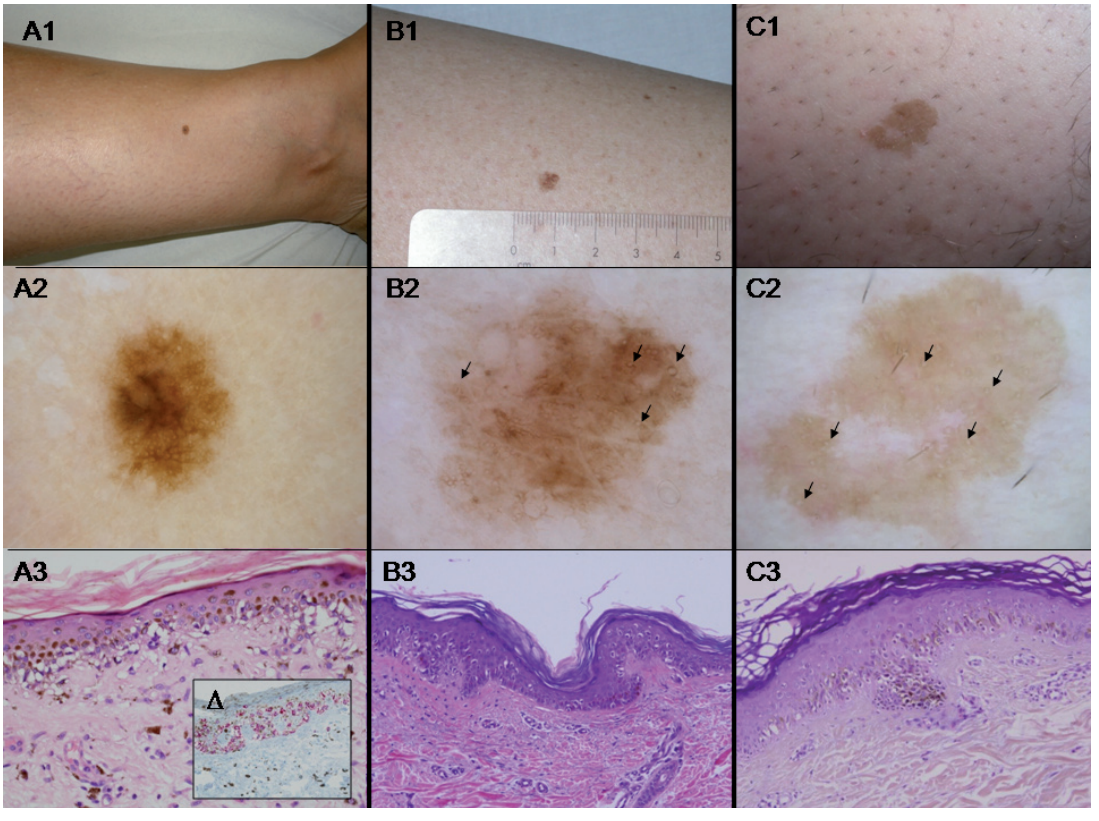

Fig. 4. Dermoscopic group 4: perifollicular pigmentation. Examples of three *in situ* melanomas. A1, B1, C1: clinical aspect: located on lower limbs, the only remarkable feature was irregular borders. A2, B2, C2: dermoscopic images (original magnification × 30). Light-brown structureless pigmentation, with thin and broken pigmented network, and focal hyperpigmentation in case 2. Note some irregular follicular openings (*arrows*). A3, B3, C3: histopathological examination (× 20). Flattened epidermis, with variable elastosis, and proliferation of dendritic melanocytes in both the basal and suprabasal layers. Note the remarkable pagetoid spreading in immunohistochemistry image (Δ) (Melan-A staining).

Table I. *Clinical features of the 35 patients included in this series. Patients were assigned to 1 of 4 groups based on the dermoscopic characteristics of their tumours: 1, "Prominent network"; 2, "Delicate network with no specific MM dermoscopic features"; 3, "Hypopigmented with atypical vessels"; and 4, "Diffuse light pigmentation and perifollicular pigmentation". CDKN2A/CDK4 mutation status was assessed in 21 of the 35 patients. MC1R variants were studied in 20 patients. Multiple malignant melanoma (MM): 2 or more melanomas diagnosed before the present case. Familial MM: 2 or more melanoma cases among first-degree relatives.*

Patient characteristics	Group 1 $n=16$	Group 2 $n=4$	Group 3 $n=10$	Group 4 $n=5$	Total $n=35$
Sex, *n* (%)					
Female	15 (94)	4 (100)	6 (60)	4 (80)	29 (83)
Male	1 (6)	0 (0)	4 (40)	1 (20)	6 (17)
Age (years), mean ± SD	44.7 ± 14.0	40.4 ± 14.7	49 ± 19.3	50 ± 8.3	46 ± 15.4
Atypical mole syndrome, *n* (%)	12 (75)	4 (100)	8 (80)	2 (40)	26 (75)
Previous MM, *n* (%)	7 (44)	3 (75)	6 (60)	1 (25)	17 (49)
Digital follow-up, *n* (%)	8 (50)	4 (100)	4 (40)	2 (40)	18 (51)
Multiple MM, *n* (%)	2 (12)	3 (75)	1 (10)	0 (0)	6 (17)
Familial MM, *n* (%)	6 (37)	4 (100)	2 (20)	2 (40)	14 (40)
Genetic studies performed, *n* (%)	8 (50)	4 (100)	8 (80)	2 (50)	21 (38)
CDKN2A/CDK4 mutation/studied, *n* (%)	4/8 (50)	3/4 (75)	1/7 (14)	0/2 (0)	8/21 (38)
MC1R/studied, *n* (%)					
Any variant	6/8 (75)	3/3 (100)	8/8 (100)	1/1 (100)	18/20 (90)
Red hair variants	4/8 (50)	3/3 (100)	5/8 (62)	1/1 (100)	10/20 (50)
More than one variant	0/8 (0)	2/3 (66)	5/8 (62)	0/1 (0)	7/20 (35)

SD: standard deviation. Note: one patient in group 2 presented with 2 tumours.

Genetic studies. All patients with familial and/or MPM were investigated for major susceptibility MM genes (*CDKN2A*, p14arf, *CDK4*) (as well other patients whose DNA was available**)**. Explicit permission was obtained from all patients tested. Eight of the 21 patients whose *CDKN2A* loci were studied were found to be carriers of known mutations. Six carried the G101W exon 2 mutation (7), the most common in our study population. Polymorphisms in the *MC1R* gene were studied in 20 patients; only 2 of them were wild-type. At least one functional variant was detected in 18 patients, more than one variant in 7, and 13 cases were red hair variant (RHV) carriers and 2 of them had a double RHV polymorphism.

Tumour data

Most of the tumours ($n=33$, 92%) were located on lower limbs, mainly below the knee ($n=28$, 78%). All were less than 6 mm in diameter (except for 2 lesions, 7 and 8 mm in diameter, both lacking pigment, one of them in a patient affected by oculo-cutaneous albinism type 1). The median diameter was 4.3 mm (SD 1.12 mm, range 3–8 mm). On clinical examination none of them fulfilled ABCD criteria for MM suspicion. Only 15 lesions showed mild asymmetry; none presented more than two colours, and borders were slightly irregular in 7 cases.

Dermoscopic examination

Most of the tumours showed two colours and asymmetry in one axis. However, 14 were completely symmetrical and 7 were monochromic. The most frequent overall pattern was reticular pigmented (21 tumours), and no lesion showed a multi-component pattern. An atypical pigmented network was detected in 15 cases, irregular pigment distribution was observed in 20 cases, and atypical vessels in 10 cases. Other worrying, but infrequent, dermoscopic features observed are detailed in Table II.

Based on overall appearance in dermoscopic analyses, tumours were classified into 4 groups (see above):

- Prominent network, characterised by atypical prominent pigmented network with broadened lines and narrow holes.
- Delicate network with no specific MM dermoscopic features.
- Hypopigmented with atypical vessels, with no classical features of MM, but little or no pigment, and dotted vessels and inverse network in several cases.
- Diffuse light pigmentation and perifollicular pigmentation, simulating solar lentigo but with irregular pigmentation of follicule-openings.

Reflectance confocal microscopy (RCM) examination

All the evaluated lesions ($n=12$) were suspicious for melanoma using the second-step algorithm previously described by our group (24). Positive criteria for melanoma were the presence of a pagetoid spread of atypical cells in 8 cases, being roundish in 6 cases, and dendritic in 4 (2 cases showed both cell types) (Fig. 5); the presence of non-edged papillae in eight cases; and the presence of atypical cells in the basal layer in 4 cases and in the dermal papilla in 3.

In the dermis, non-nucleated dermal cells (plump cells) were observed in 4 cases, related to the presence of blue regression (peppering) or melanophages in intense pigmented lesions. Vessels were identified in 2 cases,

Table II. *Clinical and dermoscopic examination of 36 tumours classified by dermoscopic group.*

	Group 1 $n=16$	Group 2 $n=5$	Group 3 $n=10$	Group 4 $n=5$	Total $n=36$
Clinical tumour features					
Site, *n* (%)					
Lower limbs	16 (100)	5 (100)	7 (70)	5 (100)	33 (92)
Upper limbs	0 (0)	0 (0)	3 (30)	0 (0)	3 (7)
In situ malignant melanoma, *n* (%)	13 (72)	5 (100)	5 (50)	5 (100)	28 (78)
Ugly duckling sign, *n* (%)	1 (6)	0 (0)	1 (10)	0 (0)	2 (5)
Size, mm, mean ± SD	4.12 ± 0.9	3.6 ± 0.9	5 ± 1.4	4.4 ± 0.9	4.3 ± 1.12
Clinical asymmetry, *n* (%)	9 (56)	3 (60)	2 (20)	1 (20)	15 (42)
One colour, *n* (%)	4 (25)	2 (40)	7 (70)	3 (60)	16 (45)
Two colours, *n* (%)	12 (75)	3 (60)	3 (30)	2 (40)	20 (56)
Irregular borders, *n* (%)	4 (25)	0 (0)	0 (0)	3 (60)	7 (20)
Dermoscopic tumour features, n (%)					
Asymmetry in one axis, *n* (%)	11 (70)	3 (60)	5 (50)	3 (60)	22 (60)
Only one colour, *n* (%)	0 (0)	2 (40)	4 (40)	1 (20)	7 (20)
Two colours, *n* (%)	13 (72)	3 (60)	5 (50)	4 (80)	25 (69)
More than two colours, *n* (%)	3 (18)	0 (0)	1 (10)	0 (0)	4 (11)
Reticular pattern, *n* (%)	14 (88)	5 (100)	0 (0)	2 (40)	21 (58)
Globular pattern, *n* (%)	1 (6)	0 (0)	1 (10)	0 (0)	2 (5)
Non-specific global pattern, *n* (%)	1 (6)	0 (0)	9 (90)	3 (60)	13 (35)
Atypical network, *n* (%)	14 (88)	0 (0)	1 (10)	0 (0)	15 (42)
Irregular globules, *n* (%)	5 (31)	0 (0)	3 (30)	0 (0)	8 (22)
Radial streaks /pseudopods, *n* (%)	4 (25)	0 (0)	1 (10)	0 (0)	5 (16)
Hyper/hypopigmented irregular areas, *n* (%)	7 (44)	2 (40)	7 (70)	4 (80)	20 (56)
Irregular blotches, *n* (%)	3 (18)	0 (0)	0 (0)	4 (80)	7 (20)
Dotted vessels, *n* (%)	1 (6)	0 (0)	9 (90)	0 (0)	10 (29)
Regression features, *n* (%)	3 (18)	1 (20)	1 (10)	1 (20)	6 (17)
Perifollicular pigmentation, *n* (%)	1 (6)	0 (0)	0 (0)	5 (100)	5 (16)
Negative/inverse network, *n* (%)	0 (0)	0 (0)	3 (30)	0 (0)	3 (8)

with tortuous morphology corresponding to atypical vessels seen under dermoscopy.

Dermoscopic features were the main reason for excision in 31 cases; the remaining 5 cases (dermoscopic group 2) were excised due to minimal changes on digital follow-up in a very-high-risk patient set, despite an unsuspicious clinical and dermoscopic appearance.

Histopathological study

All lesions were evaluated, by 2 independent pathologists (JP and LA).

Twenty-eight tumours (80%) were *in situ* MMs, and the remaining 8 were micro-invasive MMs, Clark II in 5 cases and Clark III in 3 cases. The median Breslow index in these was 0.5 mm. There were only 5 cases

Table III. *Histopathological examination of 36 tumours classified by dermoscopic group. Column headings indicate total numbers and percentages. Note that it was not possible to review the histopathological features of one tumour in group 1 (total of 35 tumours examined), unlike in the clinical/dermoscopic diagnosis (all 36 tumours studied).*

Histopathological features	Group 1 $n=15$	Group 2 $n=5$	Group 3 $n=10$	Group 4 $n=5$	Total $n=35$
Histological classification					
Naevoid malignant melanoma	4 (27)	2 (40)	7 (70)	0 (0)	13 (38)
Pagetoid malignant melanoma	8 (54)	2 (40)	1 (10)	0 (0)	11 (31)
Lentiginous malignant melanoma	3 (20)	1 (20)	2 (20)	0 (0)	6 (17)
Lentigo malignant melanoma-like	0 (0)	0 (0)	0 (0)	5 (100)	5 (14)
Naevus-associated	2 (13)	1 (20)	2 (20)	0 (0)	5 (14)
Marked nest tendency	5 (33)	3 (60)	9 (90)	1 (20)	18 (51)
Marked lentiginous melanocytic hyperplasia	5 (33)	1 (20)	4 (40)	3 (60)	13 (37)
Marked pagetoid spreading	10 (66)	4 (80)	6 (60)	3 (60)	23 (66)
Marked vascular hyperplasia	1 (7)	1 (20)	4 (40)	0 (0)	11 (31)
Marked inflammatory infiltrates	4 (27)	1 (20)	7 (70)	0 (0)	12 (34)
Atypical large cells	6 (40)	2 (40)	2 (20)	1 (20)	11 (31)
Atypical epithelioid-like cells	11 (73)	3 (60)	8 (80)	3 (60)	25 (72)
Histological diagnosis					
Clark I	13 (90)	5 (100)	5 (50)	5 (100)	28 (80)
Clark II	2 (14)	0 (0)	3 (30)	0 (0)	5 (14)
Clark III	1 (7)	0 (0)	2 (20)	0 (0)	2 (6)
Mean Breslow thickness (8 cases), mm	0.41 ± 0.1	–	0.56 ± 0.05	–	0.50 ± 0.1

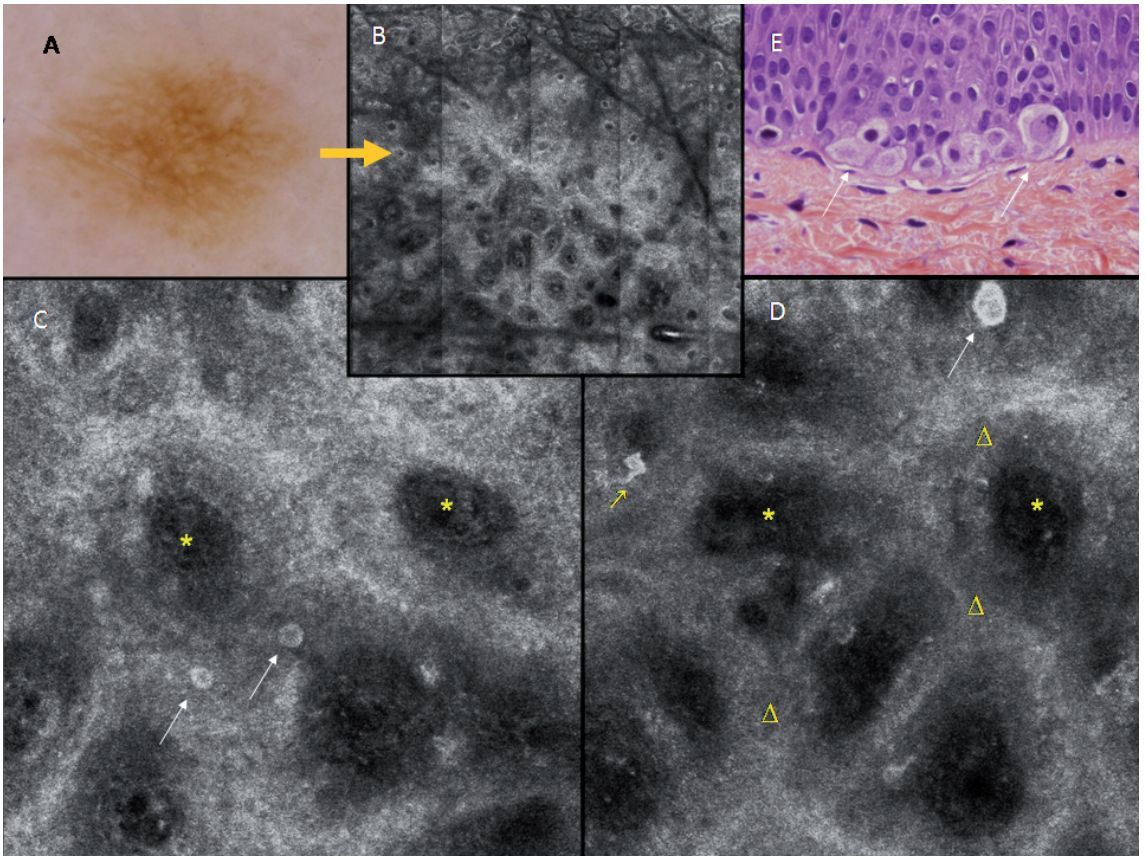

Fig. 5. A: Dermoscopic image of a new pigmented lesion on the knee of a group 2 patient (original magnification × 50). Light-brown, symmetrical pigmented network. B: *In vivo* RCM image sequence in a 4 × 4 mm mosaic: ringed architecture at the dermo-epidermal junction, with (× 30) irregular elongated regular rete ridges with an increased number of refractive cells in the basal layer. C and D: 500 × 500 µm RCM images. Non-edged papillae: dark dermal papillae irregular in size and shape (*), without a demarcated rim of bright cells, separated by interpapillary spaces of different thicknesses (Δ). Scattered atypical junctional nucleated roundish cells at layer (*white arrows*), with a single dendritic cell (*thin yellow arrow*). E: Histopathological examination (original magnification × 200). Atypical large and roundish melanocytes in the dermo-epidermal layer corresponding to the highlighted cells in the RCM and histopathological images.

of MM witn a melanocytic neavus associated in histopathological examination.

Based on the histopathological classification of incipient MMs explained in the Materials and Methods, we were able to divide our cases into groups and to study their possible associations with different dermoscopic groups (Table III). Thirteen cases were classified as naevoid-like MM, with statistically significant associations with marked nesting ($p < 0.001$) and marked vascular hyperplasia ($p < 0.05$). Eleven cases were classified as pagetoid MM-type, with marked pagetoid invasion of the epidermis, and association with very large roundish atypical cells in most cases ($p < 0.03$). Six cases were considered lentiginous MM-type, with this characteristic architecture as the most remarkable feature. And the remaining 5 cases were classified as lentigo-maligna-like MMs. However, not all of these 5 cases showed signs of elastosis.

Defining features of each dermoscopic group

The first group (atypical prominent network) was associated with lesions that were clinically and dermoscopically more pigmented and polychromic ($p < 0.05$). *In vivo* RCM demonstrated that 4 lesions presented striking pagetoid spreading of atypical cells. Histopathologically, group 1 was associated with the most marked pagetoid spreading of atypical solitary cells, so-called Pagetoid-type MM (8 cases, 54% of this group, $p = 0.02$). The diagnosis was *in situ* MM in 13 cases (90%) (Table IV).

The second group (delicate light-brown pigmented network) contained the smallest tumours (mean diameter 3.6 mm), with weak pigmentation, which explains in part the unremarkable aspect of these incipient tumours, and is congruent with *MC1R* variants status. The 3 patients studied had red hair and multiple variants in *MC1R*. Confocal detection of pagetoid cells within the upper epidermis aided the diagnosis in 3 cases. All were *in situ* MMs (Table IV).

The third group (hypopigmented or achromic lesions with atypical vasculature) was the second most frequent pattern, and the only one detected in MM located on upper limbs (30% vs. 0%). The mean size of lesions was slightly larger than the other groups (5 mm ± 1.4 mm), and in two cases the tumour was the reason for consultation because of erythema and pruritus. Most lesions (90%) showed an unspecific overall dermoscopic pattern

Table IV. *Characterisation of each malignant melanoma (MM) subgroup. For each group, the most remarkable features are listed.*

Characteristic features	*n* (%)[a]	Significance (Fisher's exact test)
Group 1 (16 patients, 16 tumours)		
Female	15 (94)	NS
>1 colour clinically	12 (75)	0.04
>1 colour dermoscopically	16 (100)	<0.05
Network global pattern	14 (88)	0.01
Atypical network	14 (88)	0.01
In situ MM	13 (90)	NS
Pagetoid-type MM (histopathological)	8 (54)	0.02
Group 2 (4 patients, 5 tumours)		
Female	4 (100)	NS
Multiple primary MM	3 (75)	<0.01
Familial multiple MM	4 (100)	<0.03
Diameter (mm), median (SD)	3.6 (0.09)	NS (Student's *t*-test)
Network global pattern	5 (100)	<0.01
Diagnosed by changes in digital FU	5 (100)	<0.001
In situ MM	5 (100)	NS
Group 3 (10 patients, 10 tumours)		
Male	4 (40)	0.04
Multiple *MC1R* variants	5 (62)[b]	0.05
Upper limbs	3 (30)	0.02
Only one colour clinically	7 (70)	<0.05
Only one colour dermoscopically	4 (40)	<0.05
Non-specific global pattern	9 (90)	0.01
Dotted vessels	9 (90)	0.01
Inverse negative network	3 (30)	<0.05
Invasive MM	5 (50)	0.03
Naevoid-type MM	7 (70)	0.01
Marked nested tendency	9 (90)	<0.05
Marked vascular hyperplasia	4 (40)	0.05
Marked inflammatory infiltrates	7 (70)	0.01
Group 4 (5 patients, 5 tumours)		
Female	4 (80)	NS
Irregular borders clinically	3 (60)	0.03
Irregular blotches dermoscopically	4 (80)	0.01
Perifollicular pigmentation	5 (100)	<0.001
In situ MM	5 (100)	NS
Lentigo-type MM (histopathological)	5 (100)	<0.001

[a]Unless otherwise indicated. [b]Five cases out of 8 studied.
NS: not significant in Fisher's exact test analysis. FU: follow-up.

($p < 0.001$) and atypical vascularisation, with dotted vessels ($p < 0.001$). In addition 3 cases (30%) presented an inverse network ($p < 0.05$). This group comparing with the other 3, contains the most invasive tumours (*in situ* MM: 50% vs. 88% in the remaining groups, $p < 0.03$; Clark II/III: 50% vs. 9% in the other groups, $p < 0.01$). The third group was statistically associated with the histopathological naevoid MM type ($p = 0.01$), and it was also possible to observe a marked vascular hyperplasia and inflammatory infiltrates (Table IV).

In the fourth group (light-brown structureless and perifollicular pigmentation), a solar lentigo appearance with irregular borders (3 cases) was the most remarkable clinical feature. The dermoscopy criterion for suspicion was pigmentation of the perifollicular openings over a lighter brown structureless pigmentation. These 5 tumours were *in situ* MM with atypical cells invasion of follicles similar to lentigo-maligna-MM but without extensive elastosis (Table IV).

DISCUSSION

Based on this review, mainly dermoscopy, sometimes aided by digital follow-up (DFU) and/or RCM, allowed the excision of 36 early MMs on limbs with unsuspicious clinical aspects.

Our aim was to characterise *in vivo* and *ex vivo* thin MMs on limbs diagnosed over the last 3 years in our unit. A large proportion of the patients in this series belong to a very high-risk MM setting: 49% were affected by previous MPM, 40% had a FamMM syndrome and 75% were affected by AMS. These data are consistent with a population attending a specific pigmented lesions unit in a referral hospital such as ours. The proportion of female patients cannot be explained on the basis of FamMM or MPM (7) and is consistent with the predominant incidence of melanoma on the lower limb in females and on the trunk in males in our general population, as is the case in most countries.

Both primary and secondary prevention strategies are especially important in these families, as the risk of MM may reach 1000 times that in the general population. Early detection of MM without an increase in unnecessary excisions is important in these cases (4–7). To date, the only way to identify this population is through their medical history. However, it would be of great interest to find special clinical, dermoscopic or histopathological features for tumours that form as a result of genetic factors. It has been demonstrated that dermatological surveillance programmes involving total-body photography, digital dermoscopy and *in vivo* RCM are feasible and allow early diagnosis of most *in situ* or micro-invasive MMs, thus avoiding unnecessary excision of benign lesions (optimal ratio benign/malignant) (4, 20, 21, 25). Genetic studies in MM families facilitate the identification of high-risk non-affected individuals who may benefit from specific surveillance programmes. FamMM is a potential pathological candidate for genetic counselling (5–7).

The gender and location of the tumours in this series agree with the well-established higher prevalence of MM on the lower limbs in women (26–28). We also found a higher proportion of male cases among the few upper limb MMs included.

Clinically all lesions were very small and not intensely pigmented, and the clinical "ugly duckling" sign only helped to identify them in only 2 cases. Our series showed that incipient MMs do not usually present the classical malignant appearance and therefore do not fulfil the ABCD criteria. We should, however, assume it is a feasible and useful tool for MM screening among the general population and for use by general practitioners, but not acceptable for use by dermatologists. This clinically unremarkable appearance and the lack of the "ugly duckling" sign in the majority of cases, reminds us that it is important not to clinically pre-select lesions for der-

moscopy (29), especially in high-risk patients. Recently, Zalaudek et al. (30) demonstrated that the time needed for complete skin examination aided by dermoscopy is only one minute longer than for that without, and complete examination with dermoscopy, even in cases with a high naevi count, took approximately 3 min.

In dermoscopic analysis none of our cases showed a multi-component pattern, or marked asymmetry in structure or pigmentation, which are considered clues for recognising MM. This emphasises the importance of finding other dermoscopy features in these early and difficult lesions, such as those we propose in this series, for small, symmetrical and hypopigmented lesions (15–17, 31, 32).

The main open question regards the potential malignant behaviour of these tumours. Obviously, the only way to truly demonstrate the malignant nature of a melanocytic lesion is through the development of metastasis. However, the clinical/dermoscopic and histopathological morphological features of a tumour are usually sufficient to make a diagnosis. As we are now detecting tumours at such an early stage, it is difficult to observe the classical and marked malignant features of more advanced MM. On the other hand, it may be possible that these lesions would never evolve to more invasive MM. Khalifeh et al. (33) reported a series of 11 atypical melanocytic lesions on distal lower limbs, especially on the ankle, which they consider as benign tumours that could be misdiagnosed as MM *in situ*. They concluded that these were benign lesions based on mild cytological atypia, no pagetoid spreading, and no recurrence after a follow-up period of between 4 months and 13 years. These cases showed some similarities to ours, but we found pagetoid invasion in the epidermis in all cases. A benign outcome in such lesions is possible. However; observation of only 11 cases is not sufficient to confirm a benign behaviour.

In agreement with previous studies on RCM in MM, the most frequent features associated with malignancy are the partial or total loss of the honeycomb pattern, pagetoid spreading of roundish or dendritic cells, and irregular or non-edged papillae (24, 34, 35). In our series, despite the unremarkable clinical appearances of the 36 tumours, we were able, based on dermoscopic classification, to establish good correlations between dermoscopic presentation and confocal and histopathological features. In at least two cases in which clinical and dermoscopic features were suggestive of benign lesions or inconclusive, confocal examination according to a 2-step algorithm recently described by our group (24) increased our suspicion and led us to decide on excision instead of follow-up.

Based on our experience, we propose a dermoscopic classification of the early stages of MM on the limbs that could help the further investigation of possible different origins, such as has been proposed in recent observations regarding cutaneous stem cells (36, 37) and MM pathways.

The distribution of *in situ* MMs among the dermoscopic groups was not uniform. Between 90% and 100% of cases in groups 1, 2 and 4 were *in situ* MMs, whereas 50% of MM in group 3 (hypopigmented with atypical vessels) were *in situ* MMs. This may be explained by a delay in diagnosis for more deeply invasive lesions with lesions with greater diameters, which agrees with our observation in a study of MC1R polymorphisms, and which could contribute to a hypopigmented MM aspect with fewer dermoscopic features, thus implying a more difficult early diagnosis (9).

In conclusion, we reviewed 36 cases of very early MMs on the limbs. None of these cases could have been diagnosed by clinical examination alone. Dermoscopy aided by digital follow-up and occasionally by confocal microscopy encouraged us to excise these clinically unsuspicious lesions. The limitation of this retrospective series is that it is not possible to compare these morphological features with those of excised benign lesions, or to confirm the future malignant behaviour of these incipient tumours. Obviously not all thin MMs will disseminate, and not all *in situ* and micro-invasive MMs will become invasive and life-threatening. However, several of the present patients belong to families affected by FamMM, and unfortunately some relatives had died from MM-associated metastasis. Therefore, our aim must be for all MMs in these high-risk patients to be diagnosed at the *in situ* stage. Finally, we can conclude that, despite a banal clinical aspect, melanocytic lesions on the limbs can present some dermoscopic or confocal features that raise suspicion. All of these tumours should be removed or have a short-term follow-up, especially in the case of the very high-risk population attending a referral pigmented lesions unit.

ACKNOWLEDGEMENTS

This work is dedicated to all our willing patients, who have always collaborated and helped us to improve our knowledge of their disease. We are indebted to our dermatologist colleagues, biologists and nurses, who work together on a daily basis and whose effort is not always reflected in investigative papers. We also thank Gillian Randall for her help with the text edition.

This project has been partially supported by Fondo de Investigaciones Sanitarias (FIS), grant 06/0265; Red de Centros de Cáncer C03/10, ISCIII, and the European Union Network of Excellence: 018702 and "The Melanoma Genetic Consortium", National Cancer Institute (National Institute of Health) USA.

The authors declare no conflicts of interest.

REFERENCES

1. Leiter U, Buettner PG, Eigentler TK, Garbe C. Prognostic factors of thin cutaneous melanoma: an analysis of the central malignant melanoma registry of the German Dermatological Society. J Clin Oncol 2004; 22: 3660–3667.
2. Garbe C, Leiter U. Melanoma epidemiology and trends. Clin Dermatol 2009; 27: 3–9.
3. Tsao H, Bevona C, Goggins W, Quinn T. The transfor-

mation rate of moles (melanocytic naevi) into cutaneous melanoma. A population-based estimate. Arch Dermatol 2003; 139: 282–288.
4. Carli P, De Giorgi V, Crocetti E, Mannone F, Massi D, Chiarugi A, Giannotti B. Improvement of malignant/benign ratio in excised melanocytic lesions in the 'dermoscopy era': a retrospective study 1997–2001. Br J Dermatol 2004; 150: 687–692.
5. Bishop JN, Harland M, Randerson-Moor J, Bishop DT. Management of familial melanoma. Lancet Oncol 2007; 8: 46–54.
6. Bergman W, Gruis NA. Phenotypic variation in familial melanoma consequences for predictive DNA testing. Arch Dermatol 2007; 143: 525–526.
7. Puig S, Malvehy J, Badenas C, Ruiz A, Jimenez D, Cuellar F, et al. Role of the CDKN2A locus in patients with multiple primary melanomas. J Clin Oncol 2005; 23: 3043–3051.
8. Goldstein AM, Chaudru V, Ghiorzo P, Badenas C, Malvehy J, Pastorino L, et al. Cutaneous phenotype and MC1R variants as modifying factors for the development of melanoma in CDKN2A G101W mutation carriers from 4 countries. Int J Cancer 2007; 121: 825–831.
9. Cuéllar F, Puig S, Kolm I, Puig-Butille J, Zaballos P, Martí-Laborda R, et al. Dermoscopic features of melanomas associated with MC1R variants in Spanish CDKN2A mutation carriers. Br J Dermatol 2009; 160: 48–53.
10. Wolf IH, Smolle J, Soyer HP, Kerl H. Sensitivity in the clinical diagnosis of malignant melanoma. Melanoma Res 1998; 8: 425–429.
11. Goldsmith SM, Solomon AR. A series of melanomas smaller than 4 mm and implications for the ABCDE rule. J Eur Acad Dermatol Venereol 2007; 21: 929–934.
12. Bafounta ML, Beauchet A, Aegerter P, Saiag P. Is dermoscopy (epiluminescence microscopy) useful for the diagnosis of melanoma? Results of a meta-analysis using techniques adapted to the evaluation of diagnostic tests. Arch Dermatol 2001; 137: 1343–1350.
13. Kittler H, Pehamberger H, Wolff K, Binder M. Diagnostic accuracy of dermoscopy. Lancet Oncol 2002; 3: 159–165.
14. Carli P, de Giorgi V, Chiarugi A, Nardini P, Weinstock MA, Crocetti E, et al. Addition of dermoscopy to conventional naked-eye examination in melanoma screening: a randomized study. J Am Acad Dermatol 2004; 50: 683–689.
15. Argenziano G, Zalaudek I, Ferrara G, Johr R, Langford D, Puig S, et al. Dermoscopy features of melanoma incognito: indications for biopsy. J Am Acad Dermatol 2007; 56: 508–513.
16. Puig S, Argenziano G, Zalaudek I, Ferrara G, Palou J, Massi D, et al. Melanomas that failed dermoscopic detection: a combined clinicodermoscopic approach for not missing melanoma. Dermatol Surg 2007; 33: 1262–1273.
17. Menzies SW, Kreusch J, Byth K, Pizzichetta MA, Marghoob A, Braun R, et al. Dermoscopic evaluation of amelanotic and hypomelanotic melanoma. Arch Dermatol 2008; 144: 1120–1127.
18. Pellacani G, Cesinaro AM, Seidenari S. Reflectance-mode confocal microscopy of pigmented skin lesions-improvement in melanoma diagnostic specificity. J Am Acad Dermatol 2005; 53: 979–985.
19. Gerger A, Koller S, Weger W, Richtig E, Kerl H, Samonigg H, et al. Sensitivity and specificity of confocal laser-scanning microscopy for in vivo diagnosis of malignant skin tumors. Cancer 2006; 107: 193–200.
20. Pellacani G, Guitera P, Longo C, Avramidis M, Seidenari S, Menzies S. The impact of in vivo reflectance confocal microscopy for the diagnostic accuracy of melanoma and equivocal melanocytic lesions. J Invest Dermatol 2007; 127: 2759–2765.
21. Malvehy J, Puig S. Follow-up of melanocytic skin lesions with digital total-body photography and digital dermoscopy: a two-step method. Clin Dermatol 2002; 20: 297–304.
22. Argenziano G, Soyer HP, Chimenti S, Talamini R, Corona R, Sera F et al. Dermoscopy of pigmented skin lesions: results of a consensus meeting via the Internet. J Am Acad Dermatol 2003; 48: 679–693.
23. Scope A, Benvenuto-Andrade C, Agero AL, Malvehy J, Puig S, Rajadhyaksha M, et al. In vivo reflectance confocal microscopy imaging of melanocytic skin lesions: consensus terminology glossary and illustrative images. J Am Acad Dermatol 2007; 57: 644–658.
24. Segura S, Puig S, Carrera C, Palou J, Malvehy J. Development of a two-step method for the diagnosis of melanoma by reflectance confocal microscopy. J Am Acad Dermatol 2009; 61: 216–229.
25. Kittler H, Guitera P, Riedl E, Avramidis M, Teban L, Fiebiger M, et al. Identification of clinically featureless incipient melanoma using sequential dermoscopy imaging. Arch Dermatol 2006; 42: 1113–1119.
26. Clark LN, Shin DB, Troxel AB, Khan S, Sober AJ, Ming ME. Association between the anatomic distribution of melanoma and sex. J Am Acad Dermatol 2007; 56: 768–773.
27. Cho E, Rosner BA, Colditz GA. Risk factors for melanoma by body site. Cancer Epidemiol Biomarkers Prev 2005; 14: 1241–1244.
28. Silva Idos S, Higgins CD, Abramsky T, Swanwick MA, Frazer J, Whitaker LM, et al. Overseas sun exposure, naevus counts, and premature skin aging in young english women: a population-based survey. J Invest Dermatol 2009; 129: 50–59.
29. Seidenari S, Longo C, Giusti F, Pellacani G. Clinical selection of melanocytic lesions for dermoscopy decreases the identification of suspicious lesions in comparison with dermoscopy without clinical preselection. Br J Dermatol 2006; 154: 873–879.
30. Zalaudek I, Kittler H, Marghoob AA, Balato A, Blum A, Dalle S, et al. Time required for a complete skin examination with and without dermoscopy: a prospective, randomized multicenter study. Arch Dermatol 2008; 144: 509–513.
31. Fikrle T, Pizinger K. Dermatoscopic differences between atypical melanocytic naevi and thin malignant melanomas. Melanoma Res 2006; 16: 45–50.
32. Pizzichetta MA, Talamini R, Stanganelli I, Puddu P, Bono R, Argenziano G, et al. Amelanotic/hypomelanotic melanoma: clinical and dermoscopic features. Br J Dermatol 2004; 150: 1117–1124.
33. Khalifeh I, Taraif S, Reed JA, Lazar AF, Diwan AH, Prieto VG. A subgroup of melanocytic naevi on the distal lower extremity (ankle) shares features of acral naevi, dysplastic naevi, and melanoma in situ: a potential misdiagnosis of melanoma in situ. Am J Surg Pathol 2007; 31: 1130–1136.
34. Scope A, Benvenuto-Andrade C, Agero AL, Halpern AC, Gonzalez S, Marghoob AA. Correlation of dermoscopic structures of melanocytic lesions to reflectance confocal microscopy. Arch Dermatol 2007; 143: 176–185
35. Pellacani G, Longo C, Malvehy J, Puig S, Carrera C, Segura S, et al. In vivo confocal microscopic and histopathologic correlations of dermoscopic features in 202 melanocytic lesions. Arch Dermatol 2008; 144: 1597–1608.
36. Zalaudek I, Marghoob AA, Scope A, Leinweber B, Ferrara G, Hofmann-Wellenhof R, et al. Three roots of melanoma. Arch Dermatol 2008; 144: 1375–1379.
37. Grichnik JM. Melanoma, nevogenesis, and stem cell biology. J Invest Dermatol 2008; 128: 2365–2380.