

Non-invasive imaging techniques for the *in vivo* diagnosis of Bowen's disease: Three case reports

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Abstract. Bowen's disease (BD) is a relatively frequent non-melanoma skin cancer occurring mostly in elderly people. Until now, the usual way to establish the diagnosis is histopathological examination of a skin biopsy. Dermoscopy and reflectance confocal microscopy (RCM) are modern alternative methods that can be used as quick and non-invasive diagnostic techniques and as follow-up instruments in cases in which a conservative treatment is chosen for the management of BD. There are no very specific dermoscopic criteria for the diagnosis of this disease, but some dermoscopic features (scaly surface, vascular structures and pigmentation) can be found more frequent and can be helpful for the diagnosis. RCM of BD shows an acanthotic epidermis with two types of targetoid cells: the first, a large cell with bright center and dark peripheral halo, the second, a cell with dark center and a bright rim surrounded by a dark halo, related with dyskeratotic cells on histological examination. BD management could be improved by using non-invasive, *in vivo* imaging techniques that allow a fast and easy diagnosis and can be used as follow-up tools. However, larger studies are necessary for the validation of our observations.

Introduction

Bowen's disease (BD), often considered an *in situ* squamous cell carcinoma (SCC) is a relatively frequent nonmelanoma skin cancer occurring mostly in elderly people with photodamaged skin (1). In 3-5% of cases it may progress into invasive SCC, and 10% of invasive tumors may metastasize (2,3). Clinically, it presents most commonly as a slowly growing erythematous or light brown papule or plaque, flat or slightly elevated, with a scaly surface. Due to the lack of distinctive features it can often pose as a diagnostic dilemma. BD has also a pigmented variant that may share overlapping clinical features with melanoma (4,5).

Dermoscopy was shown to be useful in the assessment of pigmented and nonpigmented skin tumors as it allows a rapid and non-invasive recognition of vascular or pigmented structures that are not visible to the naked eye (6,7).

Another imaging technique that allows a non-invasive and in real-time examination of pigmented and nonpigmented skin lesions is the reflectance confocal microscopy (RCM). Its usefulness in the assessment of tumoral (8-10), inflammatory (11-14) or infectious skin lesions has been proven by extensive research (15,16).

In this study, we highlight the usefulness of combining the two imaging methods for a non-invasive and rapid diagnosis of BD. Therefore, we present 3 cases of BD that we have examined dermoscopically and by RCM technique and correlated to the corresponding histological examination.

Materials and methods

The study was conducted in the Department of Dermatology, 'Prof. N.C. Paulescu' National Institute of Diabetes, Nutrition and Metabolic Diseases (Bucharest, Romania) and approved by the Ethics Committee of 'Prof. N.C. Paulescu' National

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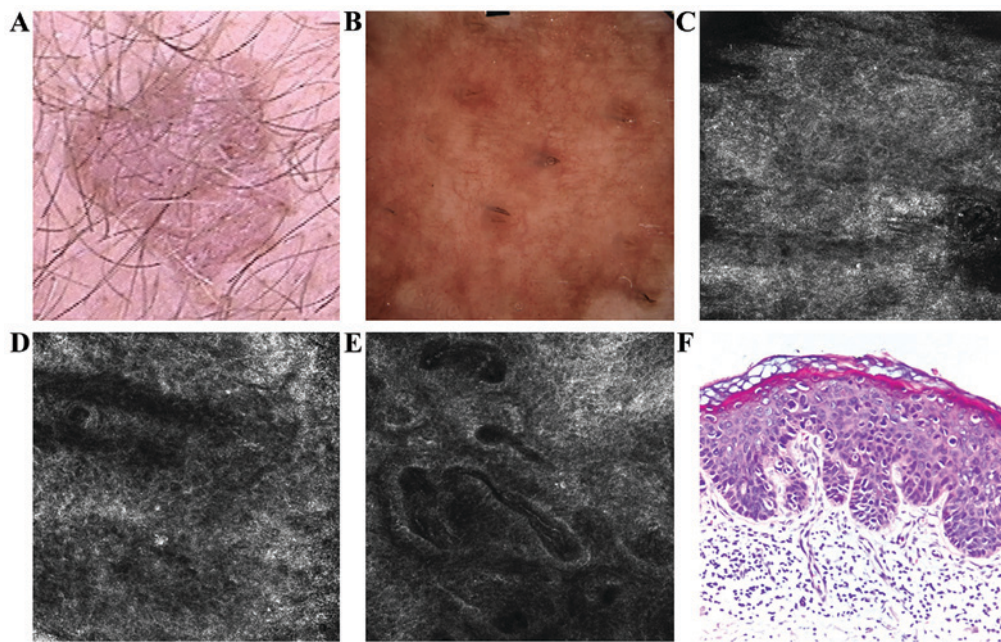


Figure 1. (A) Clinical image illustrated a well-demarcated, erythematous, scaly plaque. (B) Dermoscopic aspect of the lesion revealed vascular pattern with dotted, linear, comma-like and glomerular vessels. (C) RCM examination (500x500 μm) of the stratum corneum showed the presence of bright, polygonal shaped structures corresponding to parakeratotic cells (D) RCM image (500x500 μm) with chaotic appearance of the spinous layer resulted in an atypical honeycomb pattern and sparse target-like cells. (E) RCM image (500x500 μm) of the dermal-epidermal junction revealed enlarged, irregular shaped papillae filled with dilated capillaries, with reduction of the papillary rings normally seen in healthy skin. (F) Histopathological image showed areas of parakeratosis, numerous dyskeratotic keratinocytes, moderate cellular and nuclear pleomorphism and numerous mitosis; moderate hyperemia and lymphocytic inflammatory infiltrate (hematoxylin and eosin staining; x200 magnification).

Institute of Diabetes, Nutrition and Metabolic Diseases, and informed consent was obtained from the patients. Clinical evaluation of the lesions followed the guidelines for diagnosis of BD. In each case, clinical photographs of the lesions and dermoscopic images were acquired using a digital video-dermoscopy system (FotoFinder, Bad Birnbach, Germany) and the VivaScope 1500 VivaCam macro camera (Lucid Inc., Rochester, NY, USA). A commercially available *in vivo* confocal microscope (Vivascope 1500; Lucid Inc.) was used for RCM imaging. All lesions evaluated in our study underwent excision under local anesthesia and histopathological examination was performed after standard hematoxylin and eosin staining of the excised tissue.

Case reports

Case 1. A 78-year-old male, phototype II, presented in above-mentioned Department for the evaluation of a well-demarcated, slowly enlarging, erythematous, scaly plaque of 1.8x1.6 cm, located on the abdomen (Fig. 1A). On dermoscopic examination pink structureless areas and a polymorphous vascular pattern consisting of dotted, linear, comma-like and glomerular vessels were detected (Fig. 1B).

On RCM images, the stacking examination showed a thickened stratum corneum; the presence of polygonal, refractile structures in this layer corresponds to nucleated corneocytes (parakeratosis) and grouped, elongated, bright structures correspond to inflammatory infiltrates (Fig. 1C). Descending to the spinous layer, an atypical honeycomb pattern due to great variations in cell and nuclear morphology, and rare targetoid cells were identified (Fig. 1D). At the dermo-epidermal

junction, there was an increased number of dermal papillae of different sizes and shapes, some of them connecting; the bright halo surrounding dermal papillae seen in healthy skin, corresponding to basal cells, was diminished in the lesion examined. Dilated and tortuous capillaries were seen as large, dark, round to oval or canallicular structures containing bright blood cells (Fig. 1E).

The biopsy showed areas of parakeratosis with absence of underlying granular layer, alternating with areas of orthokeratosis; the epidermis was disorganized with atypical keratinocytes with hypertrophic nuclei; moderate lymphocytic inflammatory infiltrate with rare melanophages in the superficial dermis. The histologic features were consistent with BD (Fig. 1F).

Case 2. A 76-year-old male, phototype III, presented to the above-mentioned Dermatology Department for evaluation of two slowly growing plaques situated on the lateral and dorsal aspects of the thorax respectively. The first lesion was a well-demarcated, flat plaque of 1.5x1.3 cm with irregular borders, showing pink and brown diffuse areas and slightly scaly surface (Fig. 2A). This lesion revealed a dermoscopic pattern characterized by a diffuse, structureless light-brown pigmentation, a few dark-brown irregular distributed globules, conglomerates of glomerular vessels and some linear and dotted vessels (Fig. 2B). RCM images showed a thick stratum corneum with round to polygonal nucleated bright cells corresponding to parakeratotic keratinocytes and intensely refractile round structures corresponding to infiltrating neutrophils (Fig. 2C). Associated with the atypical honeycomb pattern in the spinous layer, there was a high-density

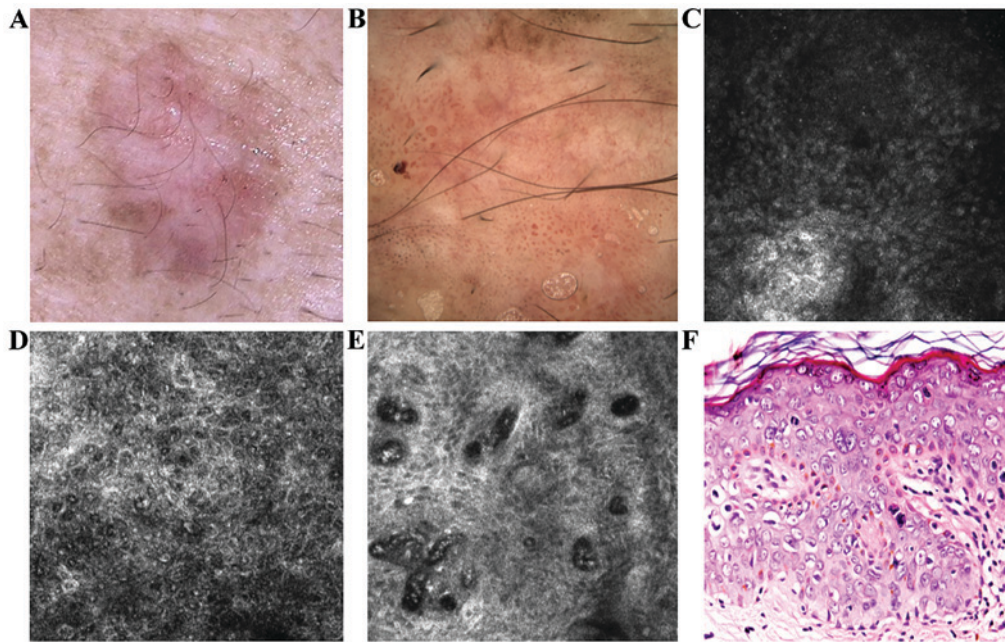


Figure 2. (A) Clinical image of a flat plaque showed pink and brown diffuse areas. (B) Corresponding dermoscopic image showed diffuse, structureless brown pigmentation, some brown globules and an atypical vascular pattern consist of glomerular, dotted and linear vessels. (C) RCM image (500x500 μm) at the level of the stratum corneum where parakeratotic cells were identified as round to polygonal, refractile structures. (D) RCM image (500x500 μm) revealed the atypical honeycomb pattern in the spinous layer, with various shapes and sizes of the cells and nuclei. Many of these cells have a target-like aspect, with a bright center and a dark peripheral halo. Another type of cell with targetoid aspect, with a dark center and a bright peripheral rim, was also identified in smaller numbers. (E) RCM image (500x500 μm) at the level of the dermo-epidermal junction showed enlarged dermal papillae, with bizarre shapes, without the papillary rings of basal cells, inside of which are identified dilated capillaries, some captured in horizontal section, parallel to the skin. (F) Histopathological image displaying important cytonuclear pleomorphism with hypertrophy and nuclear monstrosities and atypical mitosis at different levels in epidermal thickness (hematoxylin and eosin staining; x200 magnification).

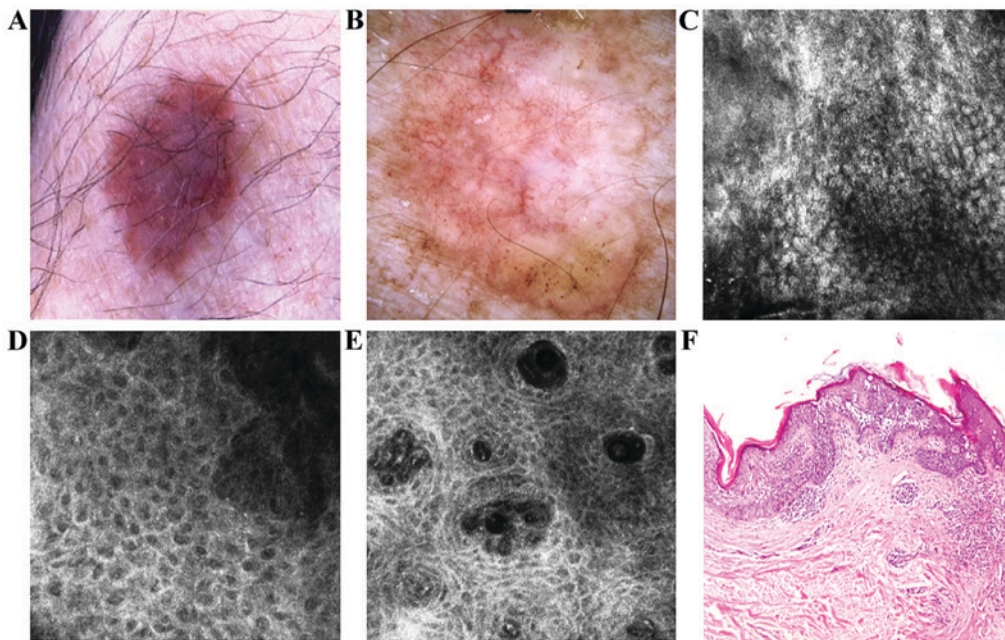


Figure 3. (A) Clinical image of a slightly variegated brown plaque with very fine scaling. (B) Dermoscopic view showed structureless asymmetric brown pigmentation with erythematous background, few brown globules and irregular network in the periphery and an atypical vascular pattern. (C) RCM examination (500x500 μm) revealed parakeratosis in the horny layer. (D) RCM image (500x500 μm) at the level of the spinous-granular layers showed a disarrayed pattern and target-like cells. (E) RCM image (500x500 μm) at the dermo-epidermal junction the dermal papillae are filled with many dilated capillaries. (F) Corresponding histology with epidermal thickening due to proliferation of atypical squamous cells with cyto-nuclear atypia extended through the whole thickness of the epidermis (hematoxylin and eosin staining; x100 magnification).

of targetoid cells, recognizable as enlarged round cells with a bright center and a dark peripheral halo or as enlarged cells

with dark center surrounded by a bright rim (Fig. 2D). RCM examination of the superficial dermis exhibited dysmorphic,

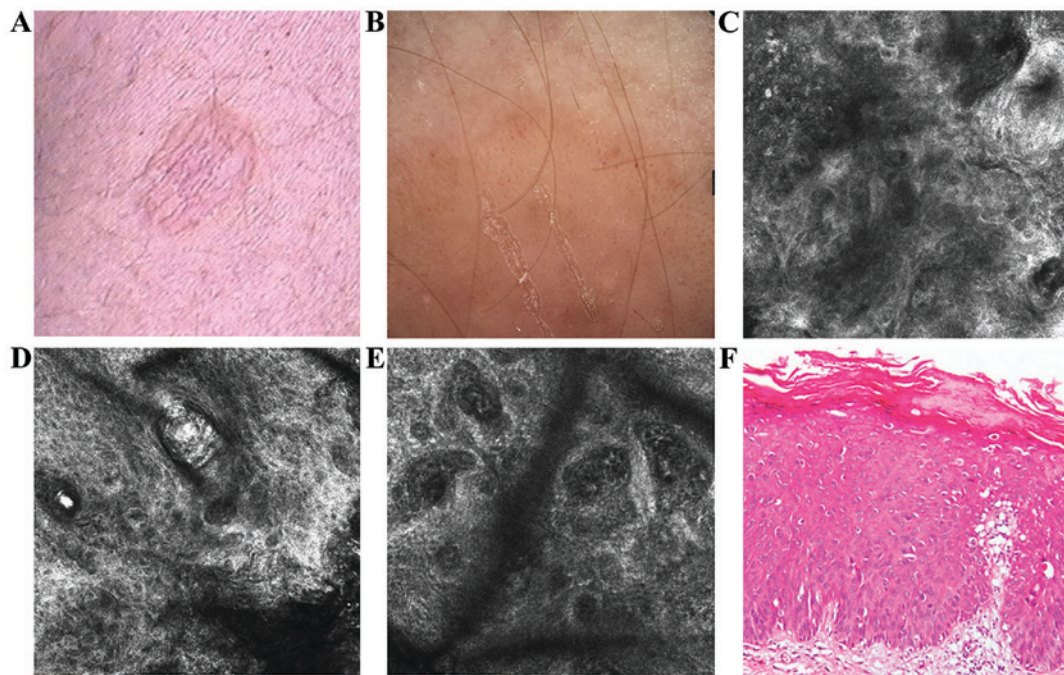


Figure 4. (A) Clinical image showed a scaly, pink and light brown, flat plaque, with well-defined borders. (B) Dermoscopic image showed coil-shaped and dotted vessels and light brown structureless areas on a pink background. (C) RCM image (500x500 μm) revealed a parakeratosis area in the horny layer (upper left). (D) RCM image (500x500 μm) at the level of the spinous layer showing an atypical honeycomb pattern. (E) RCM image (500x500 μm) at the dermo-epidermal junction revealing enlarged dermal papillae with dilated and sinuous blood vessels. (F) Histopathological image showing epidermal thickening with areas of parakeratosis, atypical keratinocytes, moderate pleomorphism and moderate lymphocytic inflammatory infiltrate (hematoxylin and eosin staining; x200 magnification).

large papillae, containing dilated capillaries with sinuous trajectories parallel on the horizontal sections of the skin and with increased blood flow in real-time imaging (Fig. 2E). Histopathological examination of both lesions confirmed the diagnosis of BD (Figs. 2F and 3F).

Naked-eye examination of the second lesion revealed an irregular-shaped, well-demarcated, brown and grey plaque of 1.3x0.8 cm, with fine scales on the surface (Fig. 3A). Dermoscopic examination of the lesion revealed dark-pink and brown diffuse areas overlapped by vessels with varied sizes and shapes, mostly glomerular, dotted and comma-like (Fig. 3B). RCM examination revealed a thick cornified layer with remaining parakeratotic keratinocytes and extensive black areas containing multiple refractile oval and elongated cells (Fig. 3C). The atypical honeycomb pattern with high-density of targetoid cells was also identified (Fig. 3D). Descending to the dermo-epidermal junction it can be noted that the atypical honeycomb pattern is maintained in the lower layers. Enlarged papillae containing a high number of dilated vessels with intense blood flow were also revealed (Fig. 3E).

Case 3. A 56-year-old male, phototype III, without any significant personal and familial medical history, presented to the above Dermatology Department for investigation of a 2x1.5 cm flat plaque, with well-defined borders and a slightly scaly surface, colored in shades of pink and light brown, located on the inner aspect of the left calf (Fig. 4A). The dermoscopic assessment of this skin lesion showed coil-shaped and dotted vessels on a pink to light-brown background (Fig. 4B).

RCM examination showed a thick horny layer with areas of parakeratosis, atypical honeycomb pattern of spinous

layer (Fig. 4C and D). At the dermo-epidermal junction enlarged papillae with dilated and tortuous blood vessels were revealed (Fig. 4E). Histological examination showed typical features of BD (Fig. 4F).

Our results confirm the RCM features of BD, showing a good correlation with dermoscopic and histopathologic findings (Table I).

Discussion

In many cases, BD presents as a diagnostic dilemma due to lack of specific clinical features. At present, the only way to establish diagnostic certainty of BD is by histopathological examination of a skin biopsy. Current trends in skin imaging are focused on non-invasive techniques characterized by rapidity, possibility to evaluate the evolution of the lesions over time by preserving skin integrity, lack of invasiveness and patient comfort (17). The unpredictable evolution of BD to invasive SCC is an important issue especially when the lesion is incorrectly managed as an erythematous-squamous inflammatory skin disease. Given that, until now, very little information related to non-invasive diagnostic techniques for BD can be found in literature.

In 2012, Ulrich *et al* examined ten cases of BD using dermoscopy and RCM achieving results that match our observations (18). In all cases they identified parakeratosis, neutrophilic inflammatory infiltrate within the stratum corneum, atypical honeycomb pattern in the spinous layer extending to the whole epidermis thickness, two types of targetoid cells and dilated, tortuous capillaries. These observations had a good correlation with histopathological examination.

Table I. RCM features of BD and their dermoscopic and histopathologic correlations.

RCM features	Case 1	Case 2		Case 3	Dermoscopic correlation	Histopathological correlation
		Lesion 1	Lesion 2			
Polygonal, refractile structures in the superior layers	+	+	+	+	-	Parakeratotic corneocytes
Atypical honeycomb pattern	+	+	+	+	-	Cyto-nuclear pleomorphism
Targetoid cells	+	+	+	-	-	Dyskeratotic cells
Dysmorphic, large papillae	+	+	+	+	-	Papillomatosis
Dilated, sinuous capillaries	+	+	+	+	Atypical vascular pattern	Dilated capillaries

RCM, reflectance confocal microscopy.

Another study from 2016 highlights through a case report the usefulness of RCM technique in distinguishing between a lesion that presented clinically and dermoscopically as a pigmented BD but histopathological examination proved to be a lightly pigmented melanoma (19). The pigmented version of BD is often a diagnostic challenge whereas the differential diagnosis with melanoma is crucial (20-23).

There are no specific dermoscopic criteria described in the literature for the diagnosis of BD but some features such as scaly surface, vascular structures and pigmentation defined by small brown globules with patchy distribution, reticular pigmentation and homogeneous gray brownish pigmentation, can be found more frequently and although not specific, they can be helpful in making a diagnosis. Moreover, the absence of specific dermoscopic features of the melanocytic lesions was noted such as pigmented network, irregular lines, blue white structures and parallel pattern (21-23).

The typical or atypical vascular component is well-represented BD and consists of regular dotted vessels, linear, bushy, arborizing, hairpin-like vessels and milky red globules. The vascular structures may appear as dots, clods or lines. If they appear as lines, vessels may be straight, curved, looped (one major bend of approximately 180 degrees), serpentine (multiple bends), helical (multiple bends along a central axis), or coiled (multiple tight bends convoluted compactly). The last type is the same as the so-called 'glomerular' vessels described by other authors and correspond to dilated vessels found in the papillary dermis (24,25). Although the arrangement of the dots or vessels as lines is characteristic for BD, it has been described that the linear or serpiginous arrangement of coiled vessels is highly specific for clear cell acanthoma. However, the clinical differential diagnosis of the two is easy. Other authors consider this arrangement in lines of coiled vessels and/or dots (gray and/or brown) is a specific feature of BD and allows differential diagnosis from melanoma (5). The neoangiogenesis (neovascularization) process that was quite common and prominent is clearly correlated with the neoplastic nature of BD (26).

Regarding the amount of pigmentation, BD can appear under different dermoscopic aspects, varying from the absence of pigmented network or scarcely pigmented like a

pseudo-network, irregular diffuse pigmentation or pigmented areas, irregularly distributed dots and globules, or focal/multifocal hypopigmentation. The colors noted by dermoscopy may be due to melanin or other pigments, like hemoglobin (5). The most frequent aspect was a structureless pattern seen in almost half of the cases and shades of brown represented the predominant color. There were variants of this structureless brown pattern with few circles and dots on a brown structural background (5). This pattern is the least specific dermoscopically for BD and when combined with no vessels can lead to misdiagnosis of this condition by dermoscopy. Another common appearance is the asymmetric combination of the patterns, structureless and dots. Usually, the structureless areas are hypopigmented in comparison to the brown found in one-pattern lesions. Furthermore, these areas are mostly located eccentrically. When brown and gray pigmentation is intense, the lesion can be mistaken as a melanoma (27-29).

In a 40-BD cases study performed by Bugatti *et al*, all the patients presented a multicomponent pattern but the most frequent dermoscopic features were the atypical vascular structures represented by dotted vessels followed by absence of pigmented network and focal/multifocal hypopigmentation (30).

In another study performed on 52 patients with pigmented BD in Australia, the authors used Kittler's pattern for dermoscopic analysis which is a standardized description of pigmented lesions based on patterns, colors and clues (5,26-31). The most frequent pattern was asymmetrically structureless combined or not with dots. With regard to pigmentation, brown color was the most frequent, followed by a combination of brown and gray and in a few cases only gray.

Moreover, hypopigmented (pink, skin colored, or white) structureless zones were present in 67.2% of the lesions. In all cases of pigmented BD, the distribution of pigment resulted in a variegated appearance. An important clue was brown or gray dots in a linear arrangement seen in 21.2% of cases and most often at the periphery with the lines oriented radially.

There is a correlation between the presence or absence of melanophages in the dermis and dermoscopic aspects. The absence of melanophages at histopathological examination was correlated with the absence of the gray dots in dermoscopy. If

melanophages were present, the ability to identify gray dots in dermoscopy increases with their histopathological density. As regards inflammation, even if it was abundant in over 45% of cases, the authors were not able to highlight any correlation with dermoscopic features (5).

In BD, dermoscopy can be used as a valuable follow-up tool. In one case, glomerular vessels disappeared after laser treatment and it correlates histologically to the decreased vascularity in the dermal papillae (31). The occurrence of glomerular vessels in BD seen by dermoscopy is unique among skin tumors and therefore can indicate a proper treatment of this condition (24,26,30). Furthermore, there was a generalized decrease in the degree of pigmentation and the absence of small brown globules and reticular pigmentation after laser therapy that corresponded histologically to the loss of melanophages in the papillary dermis (31).

RCM is relatively new imaging tool applied for non-invasive evaluation of the skin, especially in dermatology that provides horizontal scanning of the skin at nearly histological resolution. In normal skin, vascular structures and blood vessels can be visualized in real-time, high resolution examination by RCM. In the dermis, they appear as dark spaces transited by small bright blood cells, process named leukocyte rolling (32,33). The vessels arranged horizontally and perpendicularly to the surface are visualized as canalicular or, respectively, dark spaces. RCM can also be used to quantify the size and the density of the vessels and the flow intensity. Additional information regarding the diagnosis and monitoring of the nonpigmented tumoral lesions can result from the vascular morphology and the pattern's distribution.

Some characteristic features for the diagnosis of nonpigmented tumoral lesions of the skin have been described (32). For *in situ* SCC, RCM shows the vessels traversing through the papilla perpendicular to the horizontal confocal plane, appearing round in cross section (17,32,33). The vascular component is prominent due to increased number, density and vasodilatation of the capillaries that usually traverse the dermal papillae. In real-time an increased blood flow was noted.

On RCM, BD shows an acanthotic epidermis with an atypical/disarranged honey-comb pattern with grainy appearance with variation in cell and nuclear morphology. Two types of targetoid cells were identified: the first, a large cell with bright center and dark peripheral halo, the second a cell with dark center and a bright rim surrounded by a dark halo. At the dermoepidermal junction, non-edged, multiple dermal papillae of different sizes with weakly refractive dense and sparse nests and nucleated atypical cells and dilated round blood vessels were observed (18).

The histopathological examination of BD shows characteristic features: proliferation of atypical keratinocytes with marked nucleocytoplasmic pleomorphism (poikilocarynosis), frequent atypical mitoses and dyskeratosis. There is a correlation between the histological features and the ones observed on RCM. For example, the two types of targetoid cells seen by RCM relate to different morphological types of dyskeratotic cells observed histological. In addition, large cells with bright center surrounded by a dark halo correspond to dyskeratotic keratinocytes separated by a clear retraction halo seen on hematoxylin and eosin sections (33). The second type of targetoid

cells correlates histological to dyskeratotic keratinocytes with pycnotic nucleus. Cells with aggregated nuclei on RCM corresponded to multinucleated keratinocytes seen on hematoxylin and eosin sections. The atypical honeycomb pattern corresponded histologically to poikilocarynosis. Round to oval blood vessels and tortuous S-shaped structures seen on RCM relate to round/oval vessel histologically. Small, bright cells found in the superficial dermis correspond to inflammatory cells seen on histologic sections.

The vascular structure was described in numerous studies (34). Therefore, the vascular morphology was defined as 6 types of vessels: straight linear, curved linear, tubular/canalicular round and polymorph and branching vessels. RCM showed in the majority of cases the presence of curved linear vessels as equivalent glomerular vessels, regular and irregular hairpin vessels in dermoscopic examination. Many studies showed slightly elongated round vessels in dermal papillae in the superficial dermis on RCM examination (35,36). In a study by Incel *et al* performed on 122 tumoral lesions, curved linear S-shaped vessels in actinic keratosis/BD were seen in 93.3% of cases (34). All BD lesions showed this type of vessels as an equivalent of glomerular vessels on dermoscopy (37). The tumoral lesions with vascular polymorphism were associated with higher rates of malignancy (34).

In a study of 10 cases, RCM showed superficial epidermal disruption in all cases, with single, detached and nucleated keratinocytes corresponding to parakeratosis (37). In 7 out of 10 cases, neutrophils within the corneum layer were observed. Also, an atypical honey-comb pattern with variations in cell and nuclear morphology was seen in all cases at the granular/spinous epidermal layers. In this study, in half of the cases targetoid cells with bright center and dark peripheral halo were seen. In all cases, in the center of the dermal papillae, round to oval blood vessels with prominent tortuosity were noted. S-shaped blood vessels were observed in 7 cases. A retrospective analysis of the vertical sections, showed the round to oval vessels within the superficial papillary dermis and S shaped vessels at the lower papillary dermis. In 5 out of 10 cases, small, round, bright cells corresponding to inflammatory cells were observed.

In BD a disorganized pattern of the epidermis with numerous hyper reflective dendritic intraepithelial cells compatible with Langerhans cells and occasional ones around the papillae at the dermo-epidermal junction were revealed on RCM examination (38,39). In time, Langerhans cells had totally disappeared after laser treatment confirming complete remission of the tumor. Hence, RCM may be regarded as a complementary technique for assessing treatment efficacy.

Besides its usefulness as a quick and non-invasive diagnostic method, RCM may be used as a follow-up instrument in cases in which a conservative treatment is chosen for the management of BD. Due to its advantage in preserving skin integrity, allowing multiple examinations of the same area over time, the response to conservative therapy regimen can be evaluated, complete healing can be concluded and relapses can be tracked, aspects which currently are not possible. However, comprehensive studies are required in order to establish criteria applicable in everyday practice.

In conclusion, in this study we demonstrated that BD management could be improved by using non-invasive, *in vivo* imaging techniques that allow a fast and easy diagnosis and to

follow-up the treatment efficacy. However, larger studies are necessary for the validation of our observations.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SLI, DB, CC were responsible for the design of the study and interpretation of the data. They also have revised critically the manuscript for important intellectual content. AB, MAI, SZ were responsible for the data acquisition, selection and analysis and clinical interpretation of the data. MT, SRG, NGI, DN, DC, CT were responsible for the data analysis and interpretation. All authors contributed to the writing of the manuscript. All authors read and approved the final version of manuscript.

Ethics approval and consent to participate

The study was conducted in the Department of Dermatology, 'Prof. N.C. Paulescu' National Institute of Diabetes, Nutrition and Metabolic Diseases (Bucharest, Romania) and approved by the Ethics Committee of 'Prof. N.C. Paulescu' National Institute of Diabetes, Nutrition and Metabolic Diseases (Bucharest, Romania), and an informed consent was obtained from all the patients included in this study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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