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Reflectance Confocal Microscopy in the Daily Practice

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Reflectance confocal microscopy (RCM) allows noninvasive imaging of the epidermis and superficial dermis. Like dermoscopy, RCM acquires images in the horizontal plane (en face), allowing assessment of tissue pathology underlying dermoscopic structures of interest at a cellular-level resolution. Thus, clinicians using dermoscopy may find RCM to be particularly useful. Our aim was to show the value of RCM for diagnosis and management decisions related to pigmented and nonpigmented skin neoplasms seen in daily practice. Six cases of clinically and dermoscopically equivocal skin lesions, for which RCM facilitated making the correct diagnosis, are presented. Final diagnoses were made based on histopathologic analysis. Three flat pigmented skin lesions with dermoscopic signs of regression showed distinct RCM features that allowed their correct classification as pigmented basal cell carcinoma, pigmented actinic keratosis, and melanoma on sun-damaged skin. A flat nonpigmented skin lesion on the face, which did not show distinct clinical or dermoscopic features, was correctly diagnosed as basal cell carcinoma based on RCM findings. In addition, the response of a pigmented actinic keratosis and a melanoma in situ on sun-damaged skin to noninvasive topical treatment was monitored using RCM. RCM is a promising and practical imaging tool for the diagnosis and follow-up of pigmented and nonpigmented skin lesions.

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At the "World Congress of Dermatology" in Buenos Aires in 2007, Dr Allan C. Halpern, head of the Dermatology, Department at the Memorial Sloan-Kettering Cancer Center, NY stated that "The Confocal Microscope is a promising research tool . . ." and that "once the unit is in the hands of clinicians, they will find a way to make it useful." Since its development in 1995, the video-rate reflectance confocal microscopy (RCM) has been used to image a variety of skin diseases, particularly pigmented skin neoplasms. Although it was initially used only as a clinical research tool at the university setting, technical improvement of the device and increase in recognition of key RCM features of skin neoplasms have led to a broader commercial dissemination of RCM. The ability to noninvasively assess the epidermis and upper dermis in the horizontal plane (en face) is particularly appealing to clinicians using dermoscopy in daily practice, because dermoscopy also images skin en face. RCM images tissue at cellular-level resolution, allowing assessment of the tissue pathology underlying dermoscopic structures of interest, bridging the gap between dermoscopy and histopathology.[1,2] The increasing interest of the "dermoscopy community" in RCM is well documented by the number of published articles within the past few years. In October of 2008, an RCM device was approved by the FDA "for review by physicians to assist in forming a clinical judgment." The following cases demonstrate the utility of RCM for diagnosis of lesions and for monitoring response to noninvasive topical therapy in daily practice.

RCM in the Assessment of Pigmented Skin Lesions

Melanin is a strong source of contrast in RCM imaging.[3] Key RCM features of nevi and melanomas, as well as of pigmented

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basal cell carcinoma (BCC) and solar lentigines, have been described in the past.[4-9]

Case 1 (Fig. 1)

A 72-year-old man presented with a 4-mm brown papule on his right cheek. With dermoscopy, there was light to gray-brown pigmentation with a structureless eccentric dark brown area. In addition, there were diffuse gray dots/granules, some of which were aggregated, forming linear streaks. A few milia-like cysts and comedo-like openings were also observed. While the clinical and dermoscopic features were suggestive of a seborrheic keratosis undergoing regression, we could not rule out the differential diagnoses of malignant melanoma or pigmented BCC. With RCM, the spinous-granular layers showed a typical honeycomb pattern. At scanning magnification, at the level of the dermo-epidermal junction (DEJ) and superficial dermis, dark silhouettes were observed and interpreted to be tumor islands; these tumor islands were surrounded by a stroma of thickened collagen that housed aggregates of plump-bright, round to triangular cells, interpreted to be melanophages. At higher magnification, dendritic structures were observed within the tumor islands; these bright dendrites were previously shown to represent melanocytes admixed with the tumor islands in pigmented BCCs.[8] Thus, the lesion was diagnosed as pigmented BCC based on the RCM features enumerated. On histopathology, dermal aggregates of pigmented basaloid cells with peripheral palisading were observed, characteristic of a pigmented BCC.

Case 2 (Fig. 2)

A 70-year-old man had a 7 mm slightly raised and poorly circumscribed pink-brown papule on his right midback. Dermoscopy showed a homogeneous pink-to-brown area with aggregated gray dots, focally forming streaks. A subtle brown pigment network was focally detected at the lesion's border. The clinical and dermoscopic features were suggestive of either melanoma on sun-damaged skin or lichen planus-like keratosis. With RCM, an atypical honeycomb pattern with variation in the size of the holes was observed at the spinous-granular layers, correlating to pleomorphism in the size of nuclei of keratinocytes, as well as broadening of the honeycomb's grid, correlating to abnormality in keratinocytes' cytoplasm or intercellular bridges. The epidermal findings were suggestive of an actinic keratosis (AK). At the level of the DEJ, branching tubular structures or "cords" and bulbous projections were seen. Additionally, multiple bright round-to-triangular nonnucleated cells, suggestive of melanophages, were observed. The DEJ findings were characteristic of solar lentigo.[9] The overall RCM appearance in this case was interpreted as an AK in association with a solar lentigo. On histopathology, there were parakeratosis, atypical pigmented keratinocytes in the epidermis, elongation of the rete ridges, and solar elastosis, as well as melanophages in the underlying dermis. The diagnosis was AK in association with solar lentigo.

Case 3 (Fig. 3)

An 83-year-old man presented with a 10-mm gray-brown macule on the left forearm. Dermoscopy revealed a brown reticular pattern with focal gray-brown streaks and some gray-brown dots. The clinical impression was most suggestive of a solar lentigo with focal regression, but a melanoma on sun-damaged skin was considered in the differential diagnosis. With RCM, sheets of round to dendritic nucleated cells and dendritic structures were observed at suprabasal and basal epidermal layers, suggestive of sheets of atypical melanocytes. At the DEJ, bright nucleated cells were observed along the rete ridges as single cells and as aggregates. Bright, round-to-triangular nonnucleated cells, suggestive of melanophages, were seen in the superficial dermis. The overall appearance was of a melanoma on sun-damaged skin. Histopathology showed atypical melanocytes as single cells and as confluent nests at the DEJ, as well as atypical melanocytes in a pagetoid pattern within the suprabasal epidermis, features of melanoma on sun-damaged skin. Atypical melanocytes were also seen in the superficial dermis that also displayed solar elastosis and melanophages. The diagnosis was a melanoma, 0.25 mm in Breslow's depth, with partial regression.

RCM in the Assessment of Nonpigmented Skin Lesions

The value of RCM in the assessment of pink and lightly pigmented skin neoplasms has been shown for BCC, AK, and amelanotic melanoma.[10-15]

Case 4 (Fig. 4)

A 43-year-old man had a 6-mm pink macule adjacent to a brown papule on the left cheek. With dermoscopy, a pink-white area with fine vessels was seen next to a homogeneous brown lesion with multiple comedolike openings and milialike cysts. The differential diagnosis included irritated seborrheic keratosis or BCC adjacent to a seborrheic keratosis. With RCM, a typical honeycomb pattern of the suprabasal epidermal layers was seen at scanning magnification. At the level of the superficial dermis, there were dark silhouettes surrounded by thickened collagen bundles. At high magnification, dark tumor islands and dilated canalicular blood vessels were observed in the superficial dermis. Canalicular blood vessels, running parallel to the horizontal RCM plane of imaging, are a characteristic finding in BCC. Based on the RCM appearance, this case was interpreted as a BCC. On histopathology, aggregates of basaloid cells with peripheral palisading of nuclei were seen within the upper dermis, confirming the diagnosis of BCC.

RCM in the Monitoring of Noninvasive Topical Treatment of Skin Cancer

RCM enables repeated examinations of lesions over time and therefore allows the noninvasive monitoring of the efficacy of nonsurgical therapy of skin cancer.[15-18]

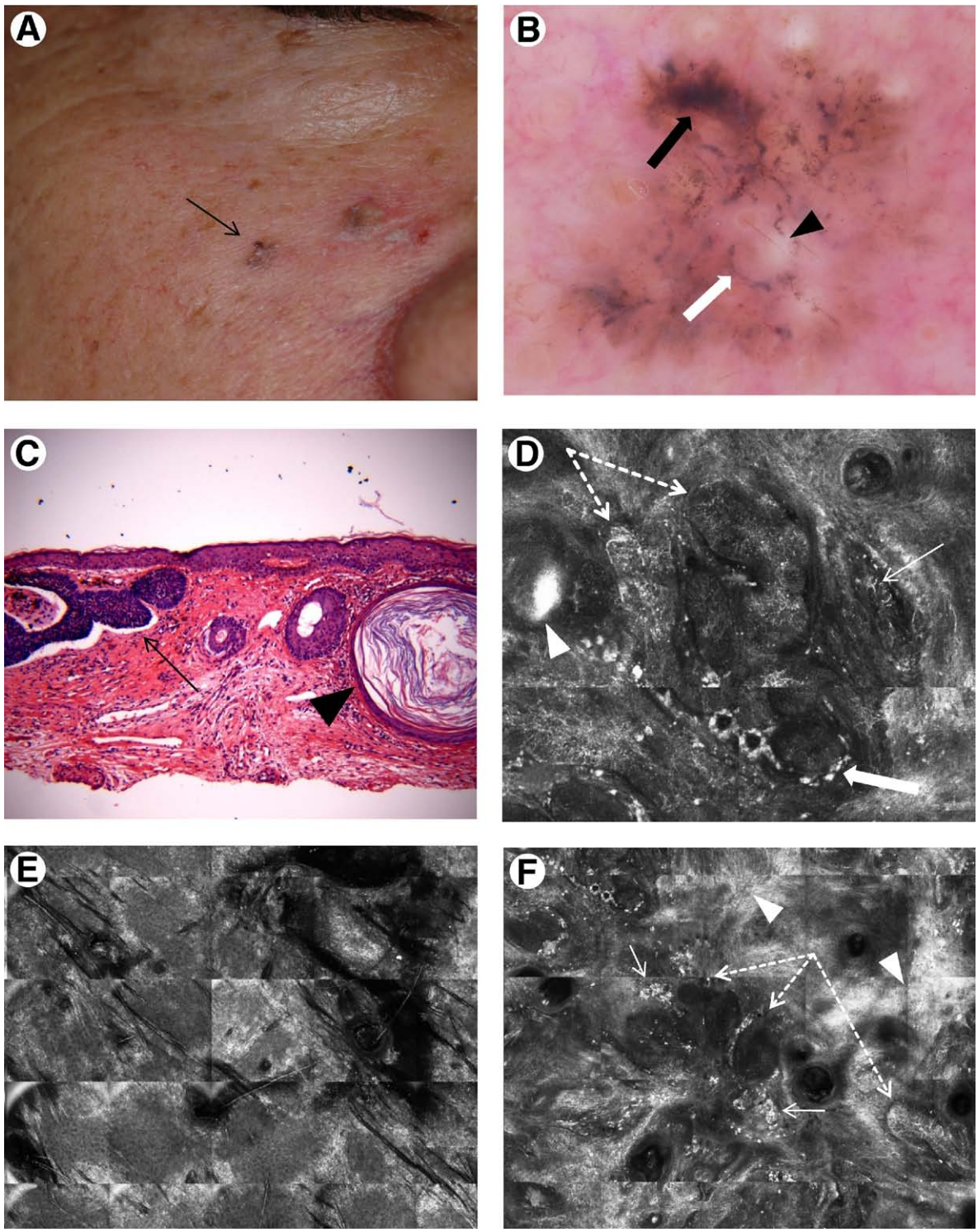

Figure 1 (A) Clinically, a 4-mm light-to-dark brown crusted papule is seen (arrow). (B) Dermoscopy shows light to gray-brown pigmentation with a structureless eccentric dark-brown area (thick black arrow). In addition, there are diffuse gray dots/granules, some of which are aggregated to form linear streaks (thick white arrow). Milia-like cysts (arrowhead) are observed. (C) On histopathology, dermal aggregates of pigmented basaloid cells with peripheral palisading of nuclei (arrow) as well as melanophages and a keratin-filled cyst (arrowhead) within the upper dermis are seen (H&E, magnification ×100). (D) On RCM (0.75 × 0.75 mm field of view, akin to high magnification), tumor islands with peripheral clefting are seen (dashed arrows). Some tumor islands appear dark; others show a bright reflectance due to presence of bright dendrites admixed with pigmented neoplastic cells (thin arrow). Plump-bright round-to-triangular cells, suggestive of melanophages, are seen adjacent to the tumor islands (thick arrow). A large, round, and highly reflective structure, suggestive of a pseudohorn cyst, is seen (arrowhead). (E) At lower magnification (2.5 × 2 mm), a typical honeycomb pattern is observed at the spinous-granular layers. (F) At similar magnification, multiple tumor islands (dashed arrows) surrounded by dense collagen bundles in a fibrotic stroma (arrowheads) and aggregates of melanophages (arrows) are observed within the superficial dermis.

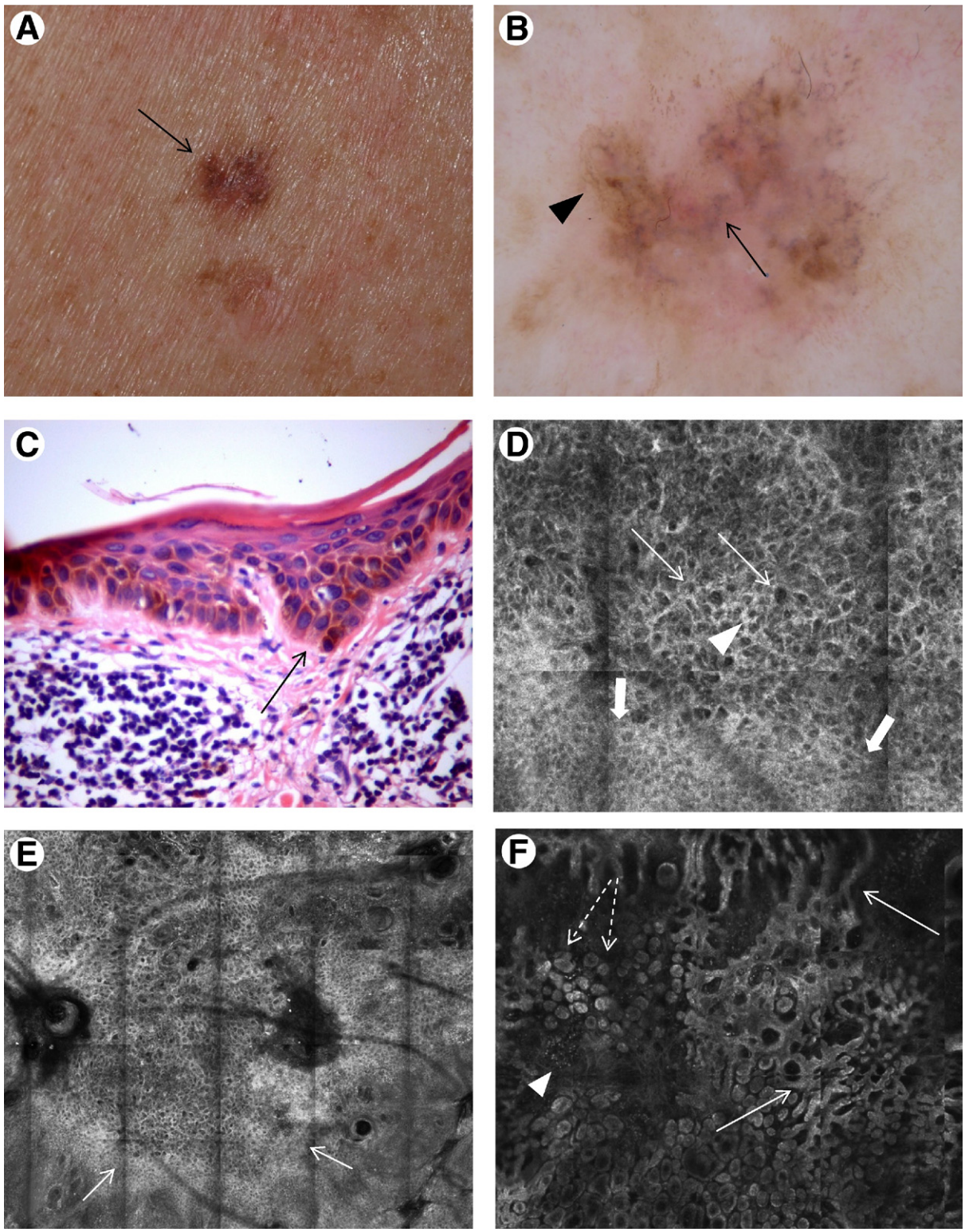

Figure 2 (A) A 7-mm slightly raised and poorly circumscribed pink-brown papule is seen on the close-up clinical image (arrow). (B) A pink-to-brown homogeneous area with multiple gray dots/granules, focally aggregated to streaks (arrow), and a subtle network at 1 lesion border (arrowhead), are seen on dermoscopy. (C) On histopathology, atypical pigmented keratinocytes are seen at basal epidermal layers (arrow) (H&E, magnification ×100). (D) RCM (0.75 × 0.75 mm field of view, akin to high magnification) at the level of the spinous-granular layers shows an atypical honeycomb pattern with variation in the size of the holes was observed (arrows), as well as broadening of the honeycomb's grid (arrowhead). A transition into the typical honeycomb in the surrounding normal skin is shown (thick arrows). (E) At lower magnification (2.5 × 2.5 mm field of view) area focus with an atypical honeycomb pattern is observed at spinous-granular layers (arrows). (F) At the DEJ (2.5 × 2 mm field of view), branched tubular structures ("cords," arrows), bulbous projections (dashed arrows), and aggregates of plump-bright round-to-triangular cells (arrowhead) are found.

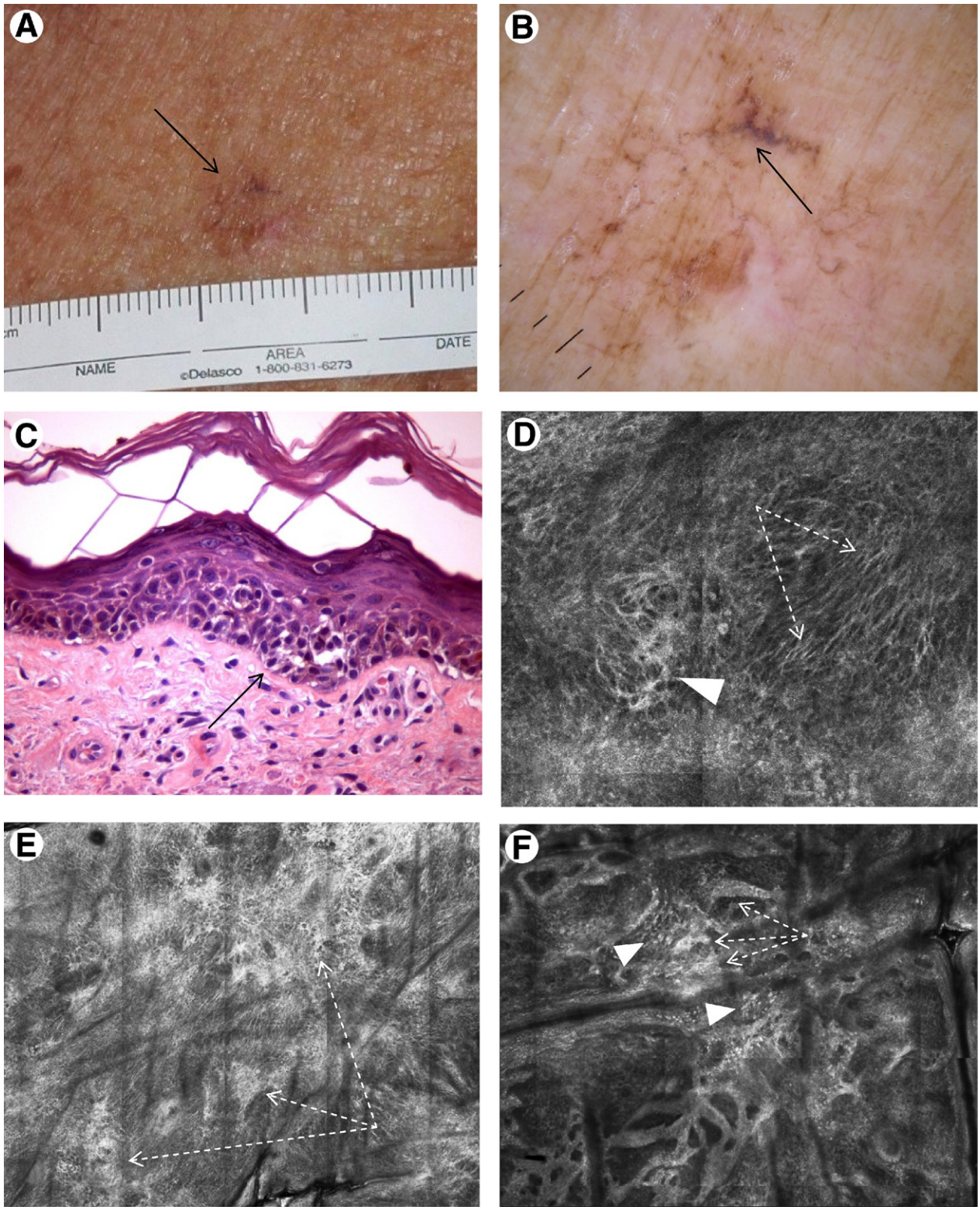

Figure 3 (A) Ten-millimeter gray-brown patch (arrow). (B) A reticular pattern with a focal gray-brown area (arrow) and some gray dots are seen in dermoscopy. (C) Histopathology shows a proliferation of atypical melanocytes at the DEJ as single cells and as confluent nests (arrow) (H&E, magnification ×100). (D) On RCM at high magnification (0.75 × 0.5 mm field of view), round and dendritic nucleated bright cells (arrowhead), suggestive of atypical melanocytes, and bright dendritic processes (dashed arrows) are seen at suprabasal layers. (E) On scanning magnification RCM (2.5 × 2 mm field of view), sheets of bright round and dendritic cells are seen at suprabasal layers. (F) RCM (1.75 × 1.5 mm field of view) at the DEJ level shows thickened rete ridges ("junctional thickening"), compatible with confluent junctional nests, as well as junctional proliferation of bright nucleated single cells (dashed arrows). Multiple bright, round to triangular nonnucleated cells, suggestive of melanophages (arrowheads), are seen within the superficial epidermis.

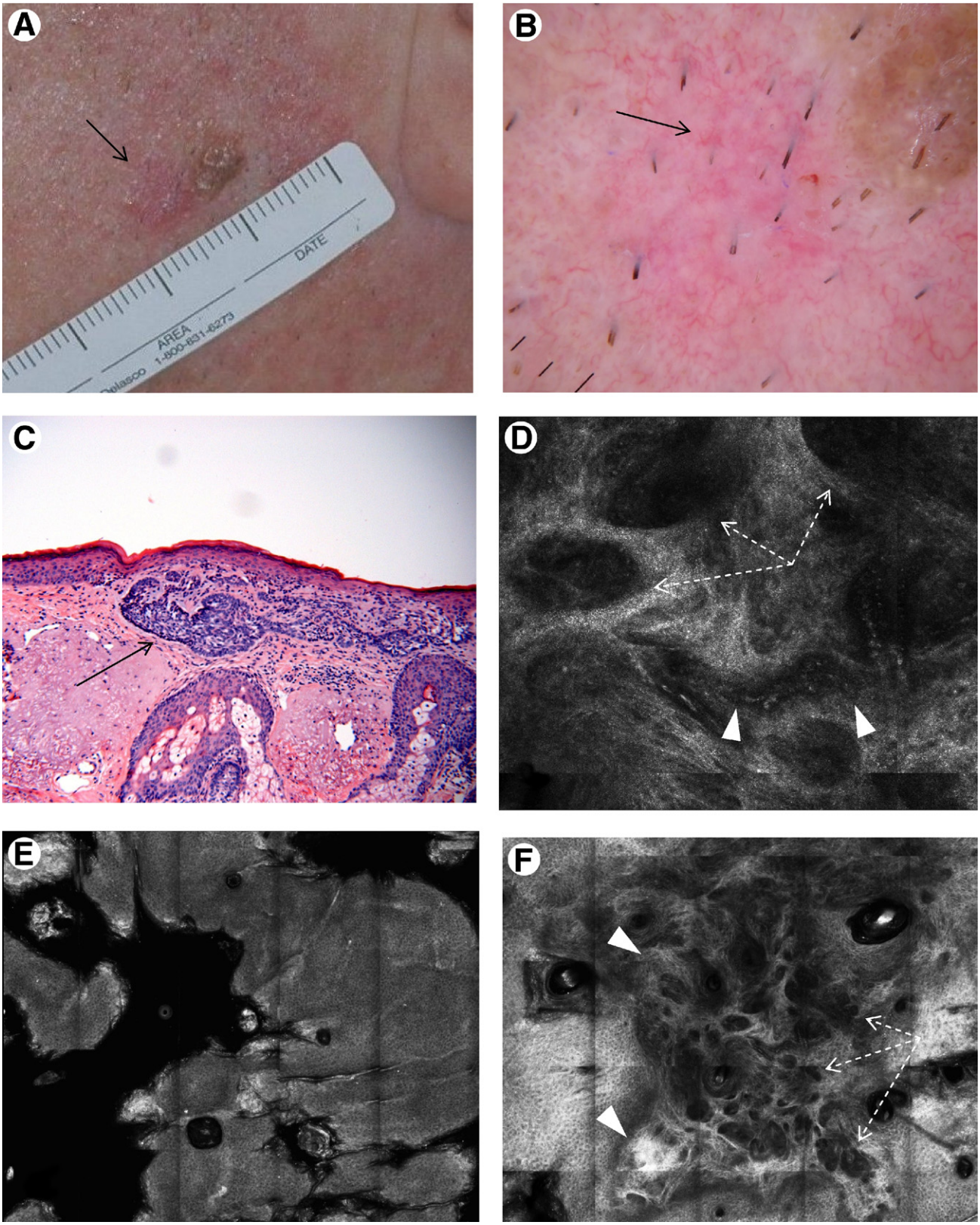

Figure 4 (A) A 6-mm pink macule adjacent to a brown papule (arrow). (B) A pink-white area with fine vessels (arrow) adjacent to a homogeneous brown lesion with comedo-like openings and milia-like cysts is seen in dermoscopy. (C) Histopathology reveals aggregates of basaloid cells with peripheral palisading of nuclei (arrow) (H&E, magnification ×100), features diagnostic of BCC. (D) Dark tumor islands (dashed arrows) and dilated canalicular vessels (arrowheads) are seen on RCM at high magnification (0.5 × 0.5 mm field of view). (E) A typical honeycomb pattern at the superficial epidermal layers is observed on scanning magnification (2.5 × 2 mm field of view). (F) Dark silhouettes (interrupted arrows), which signify tumor islands surrounded by thickened collagen (arrowheads), are seen in the superficial dermis.

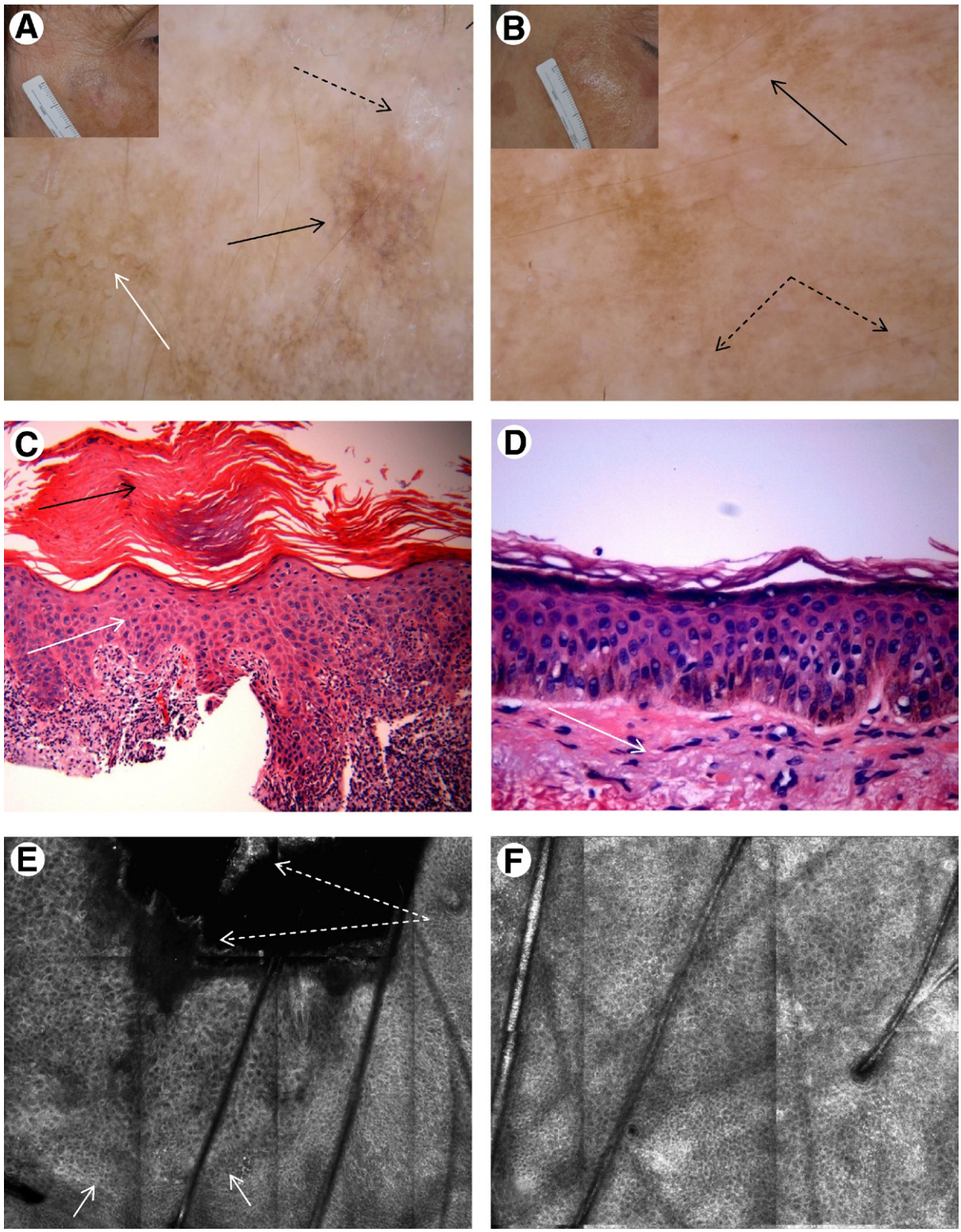

Figure 5 AK with focal superficial squamous cell carcinoma (SCC) before treatment (A, C, E) and after a course of topical treatment (B, D, F). (A) Ill-defined gray-brown scaly patch on the right cheek (inset). A fine scale (dashed arrow), diffuse gray dots/granules (black arrow) and a localized moth-eaten border (white arrow) are observed on dermoscopy. (B) The lesion shows clinically a gray-brown patch with a smooth skin surface (inset). Dermoscopy shows a subtle brown fingerprint pattern (arrow) and some gray dots/granules (dashed arrows). (C) On histopathology (H&E, magnification ×100), keratinocytes with crowded, atypical nuclei and abnormal maturation (white arrow) are seen throughout the thickness of the epidermis that also displays overlying parakeratosis (black arrow). There is dense band-like inflammatory infiltrate in the superficial dermis. (D) Histopathology (H&E, magnification ×100) shows normal epidermis with pigmented basal keratinocytes, as well as solar elastosis in the underlying dermis (arrow). (E) With RCM, there is a focal scale crust (dashed arrows) with an adjacent atypical honeycomb pattern (arrows). There is a typical honeycomb pattern at the periphery. (F) A typical honeycomb pattern is seen on RCM.

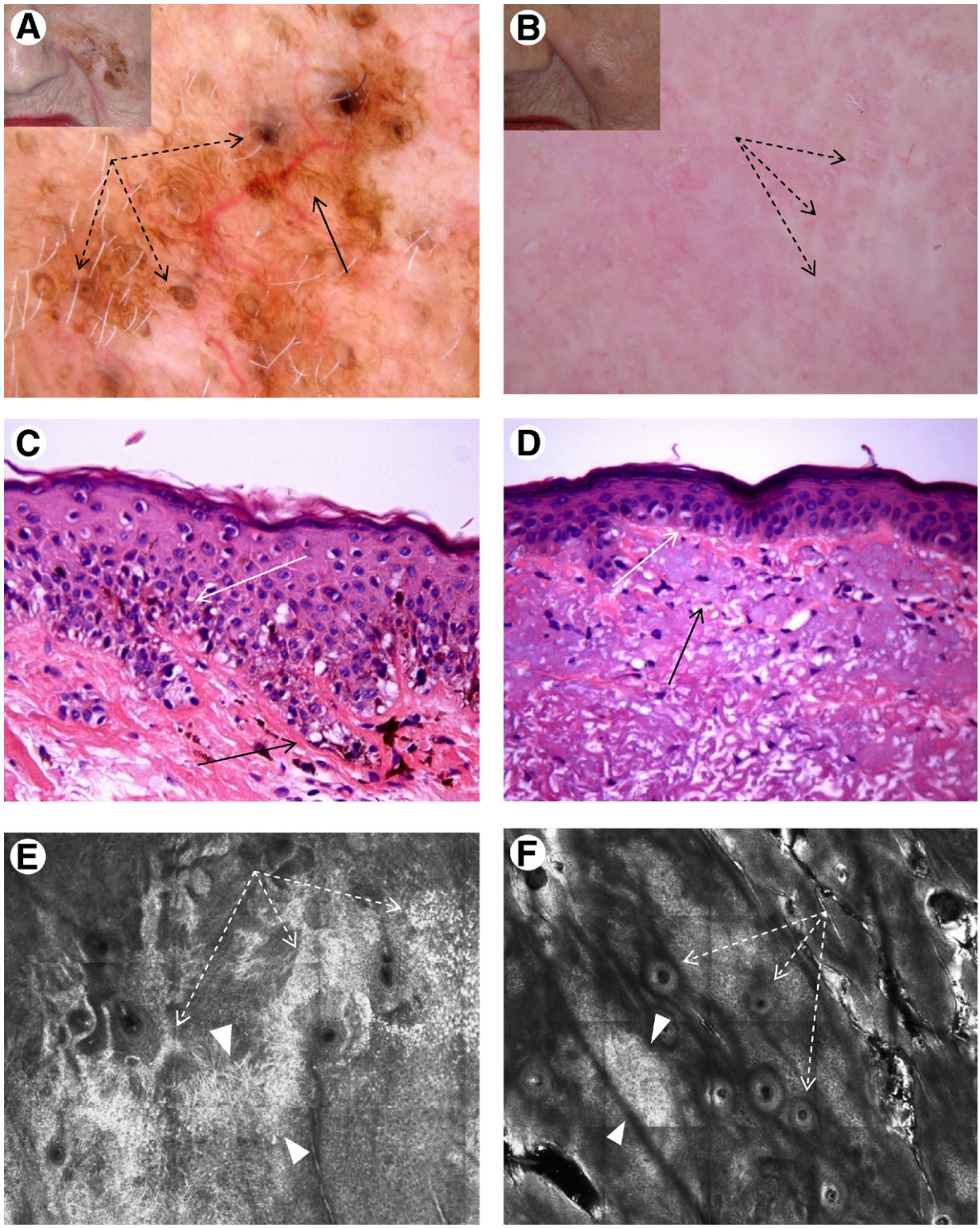

Figure 6 Melanoma on sun damaged skin before treatment (A, C, E) and after a course of topical treatment (B, D, F). (A) A 4 × 3 cm, asymmetric light to dark brown patch on the left cheek (inset). On dermoscopy, a combined reticular and annular disorganized pattern with fingerprinting (arrow) and asymmetrically pigmented adnexal openings (dashed arrows) are seen. Some of the adnexal openings show a gray hue and obliteration. (B) Clinically normal skin of the left cheek 1 year after treatment (inset). The scars seen are the result of previous biopsies. Regular adnexal openings are visualized on dermoscopy (dashed arrows). (C) Histopathology (H&E, magnification ×100) shows a dense proliferation of single melanocytes along the basal layer (white arrow), as well as small aggregates of atypical melanocytes at the tips of rete ridges (black arrow). (D) In contrast, on histopathology after topical treatment (H&E, magnification ×100), a thinned epidermis with sparse single melanocytes (white arrow) and underlying solar elastosis in the dermis (black arrow) are seen. (E) On RCM, round and dendritic cells of different sizes are arranged in sheets at the basal layer (arrowheads), focally surrounding the adnexal openings (dashed arrows). (F) Following topical treatment, a typical honeycomb pattern and normal adnexal openings (dashed arrows) are observed at the DEJ. Areas with thickened and reticulated collagen are seen (arrowheads).

Case 5 (Fig. 5)

A 60-year-old woman presented with an ill-defined gray-brown scaly macule on a mottled gray-brown background. Dermoscopy revealed fine scale, diffuse gray dots/granules, and a localized moth-eaten border. The clinical and dermoscopic features could be interpreted as those of a lichen planus-like keratosis, a pigmented AK, or a SCC. With RCM, a typical honeycomb pattern predominated at the level of the spinous-granular layers. However, focally, a scale crust surrounded by an atypical honeycomb pattern was observed. The atypical honeycomb pattern consisted of a variation in the size of the holes, correlating to pleomorphism in the size of nuclei of keratinocytes, as well as variability in the shape and brightness of the honeycomb's grid, correlating with abnormality in keratinocytes' cytoplasm or intercellular bridges. Multiple bright, round-to-triangular nonnucleated cells, suggestive of melanophages, were seen within the dermis. The RCM diagnosis was suggestive of an AK. A 4-mm biopsy was obtained and the lesion was diagnosed as an AK with a small focus of a superficial, well-differentiated SCC on histopathologic analysis. The patient was offered various treatment alternatives and elected to be managed with a combined topical regimen of imiquimod, tretinoin, and 5-fluorouracil. The combined regimen was applied once daily for 3 weeks followed by a 2-week rest period, and then another 3-week treatment cycle. Eight weeks after the completion of the combination therapy, the skin surface appeared smooth but still showed residual gray-brown pigmentation. With dermoscopy, a brown fingerprint pattern and some gray dots/granules were observed. RCM showed a typical honeycomb pattern of the epidermis. At the DEJ, some cords and bulbous projections, suggestive of a solar lentigo/early seborrheic keratosis, were seen. Bright, round to triangular nonnucleated cells, suggestive of melanophages, were focally noted. The histopathologic findings were consistent with a solar lentigo.

Case 6 (Fig. 6)

A 95-year-old woman had a 4 × 3 cm asymmetric, light-to-dark brown macule on the left cheek. A combined reticular and annular pattern was observed on dermoscopy. The reticular component consisted mainly of fingerprinting, suggestive of solar lentigo. The annular pattern was characterized by asymmetrically pigmented adnexal openings, some of which showed gray areas and obliteration, suggestive of melanoma on sun-damaged skin. With RCM, sheets of round to dendritic bright cells of different sizes, suggestive of atypical melanocytes, were observed at the suprabasal and basal layers. Aggregates of atypical melanocytes surrounding adnexal openings at foci were observed. A descent of atypical melanocytes along adnexal structures was also observed. The RCM findings were consistent with a melanoma on sun-damaged skin. A partial shave biopsy demonstrated melanoma in situ on sun-damaged skin. Due to her age and her multiple medical problems, the patient was given a 16-week course of topical imiquimod, once a day. The treated area showed complete resolution of pigmentation following completion of the treatment course. One year later, the treated area appeared clinically and dermoscopically as normal skin, without evidence of residual or recurrent melanoma. We performed RCM imaging of this area to confirm the absence of atypical melanocytes. On RCM, a typical honeycomb pattern of the suprabasal epidermis was seen; no abnormal melanocytes were seen around the adnexal openings. At the level of the superficial dermis, there was thickened and reticulated collagen, suggestive of solar elastosis. A repeat biopsy was performed to confirm the RCM findings. On histopathology, only solar elastosis was observed, without evidence of residual or recurrent melanoma.

Conclusions

RCM is a promising new, noninvasive imaging tool. Considering the clinical and dermoscopic presentations of equivocal skin lesions, RCM may help in the differential diagnosis as well as in making management decisions.[19,20] The ongoing research in this field may allow future expansion of the use of RCM in a broader variety of skin diseases.

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