CLINICAL REPORT

In vivo Analysis of Solar Lentigines by Reflectance Confocal Microscopy Before and After Q-switched Ruby Laser Treatment

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Solar lentigines are benign lesions usually found on sun-damaged skin. We investigated twelve cases of solar lentigines through dermoscopy and reflectance confocal microscopy, performed before, and 30 min and 10 days after, a single treatment with a Q-switched ruby laser. At baseline, all lesions showed characteristic features of solar lentigines in reflectance confocal microscopy analysis: regular honeycomb patterns, edged dermal papillae and cord-like rete ridges at the dermoepidermal junction. Thirty minutes post-laser treatment, blurred epidermal intercellular connections, dark structureless areas of different sizes and shapes in the lower epidermal layers, and hyporeflective dermal papillae, reflecting epidermal and dermal oedema, were observed. Ten days post-treatment highly reflective round-to polygonal areas and aggregated granules, representing extracellular melanin, were detected in all epidermal layers featuring regular honeycomb patterns. Reflectance confocal microscopy can be used to visualise dynamic skin processes, allowing non-invasive *in vivo* follow-up of skin lesions after treatment.
Key words: In vivo *reflectance confocal microscopy;* in vivo *confocal laser scanning microscopy; Q-switched ruby laser treatment; solar lentigines.*

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Solar lentigines (SL) appear predominantly on chronically sun-exposed areas of skin, such as the face and the back of the hands. Clinically, they present as irregularly shaped, faint-to-dark brown macules. In dermoscopic analyses, a faint-to-dark brown reticular pattern, often with so-called "fingerprinting", or a homogeneous pattern of pigmentation and sharply demarcated "moth-eaten" borders, is typically seen (1, 2). Clinical and dermoscopic diagnoses can be difficult. SL, especially if regressing, may share clinical and dermoscopic features with pigmented actinic keratosis and lentigo maligna.

Though benign, SL are often considered a stigmatising sign of ageing. Non-invasive laser treatment provides the best cosmetic results, but does not allow histopathological confirmation of diagnosis. Reflectance confocal microscopy (RCM) allows the architecture and cytomorphology of skin lesions to be visualised *in vivo*. In RCM, SL show a regular honeycomb pattern, reflecting the regular epidermal architecture. At the dermoepidermal junction (DEJ), dermal papillae surrounded by a rim of highly refractive monomorphic cells (edged papillae), corresponding to pigmented keratinocytes and melanocytes, are seen. Cord-like rete ridges are also seen at the DEJ, corresponding to the elongated rete ridges seen in histopathological analyses (3).

We investigated the possible application of RCM to pre-procedure assessment of SL and follow-up after a single Q-switched ruby laser treatment.

MATERIALS AND METHODS

Patients

Twelve women with chronic sun-damaged skin (mean age 59.3 years, range 49–69 years) were recruited at the Department of Dermatology, Medical University of Graz, Austria. Twelve light-to-dark brown SL (diagnosed clinically and dermoscopically) were imaged by RCM before a single Q-switched ruby laser treatment. Of these 12 lesions, 10 were located on the back of the hands and the other two on the cheeks. Thirty minutes and 10 days after laser treatment, the lesions were again assessed clinically, and by dermoscopy and RCM. In two patients, additional SL (one on the face and the other on the back of the hand) were biopsied 30 min after laser treatment (after RCM imaging) to enable histopathological and RCM findings to be correlated. Institutional rules governing clinical investigation of humans were strictly adhered to and the study conformed to the Declaration of Helsinki. All patients gave informed consent prior to the start of the study.

Clinical and dermoscopic imaging

Baseline clinical and dermoscopic images were captured using a Dermlite Foto digital imaging system (3Gen LLC, San Juan Capistrano, CA, USA) attached to a Nikon Coolpix 4500 camera (4 MP; Nikon Corporation, Tokyo, Japan).

In vivo *reflectance confocal microscopy imaging*

Confocal imaging was performed using a near-infrared 830 nm *in vivo* reflectance confocal microscope (Vivascope 1000; Lucid Inc., Rochester, NY, USA) as described previously (4, 5).

In our study, the tissue adaptor ring was placed in the centre of the lesion. An automated stepper was used to generate a mosaic grid of 16 (4 × 4) contiguous horizontal images ("Vivablock"; field of view approximately 2 × 1.5 mm) at the level of the upper epidermis, the DEJ and the upper dermis. In areas of specific interest, single images with a field of view of 475 × 350 µm

("Captures") were obtained, and automated vertical sequential imaging from the stratum corneum to the upper dermis (yielding a "Vivastack" image) performed.

Ruby laser treatment

A 694 nm Q-switched ruby laser (TattooStar; Asclepion Laser Technologies GmbH, Jena, Germany) was used, with the energy density set to 3 J/cm^2, to treat solar lentigines. The spot diameter was set to 4 mm, the pulse duration to 40 ns, and the frequency to 2 Hz. The entire area of each lesion was treated once.

RESULTS

Baseline

All lesions presented clinically as faint-to-dark brown uniformly pigmented macules (Fig. 1a, inset). Darker lesions were more common on the back of the hands. In dermoscopic analyses, a reticular pattern, with or without fingerprinting, was seen in 8 cases, and a homogeneous reticular pattern in the remaining 4 cases (Fig. 1a). In RCM, all lesions showed a regular epidermal honeycomb pattern with regular-sized dark nuclei and regular intercellular connections (Fig. 1b). At the DEJ, edged dermal papillae appeared as dark round-to-oval structures surrounded by a rim of bright monomorphic cells (Fig. 1c). Darker lesions contained brighter-coloured cells surrounding the dermal papillae. Cord-like rete ridges presented as bright tubular structures in the lower regions of the DEJ (Fig. 1d).

Follow-up 30 min after ruby laser treatment

Immediately after laser treatment, the lesions clinically presented a subtle white scaly surface ("whitening"). Thirty minutes post-treatment this whitening remained, but was additionally accompanied by discrete erythema and swelling. The dermoscopic appearance remained largely unchanged, despite the presence of a few sca-
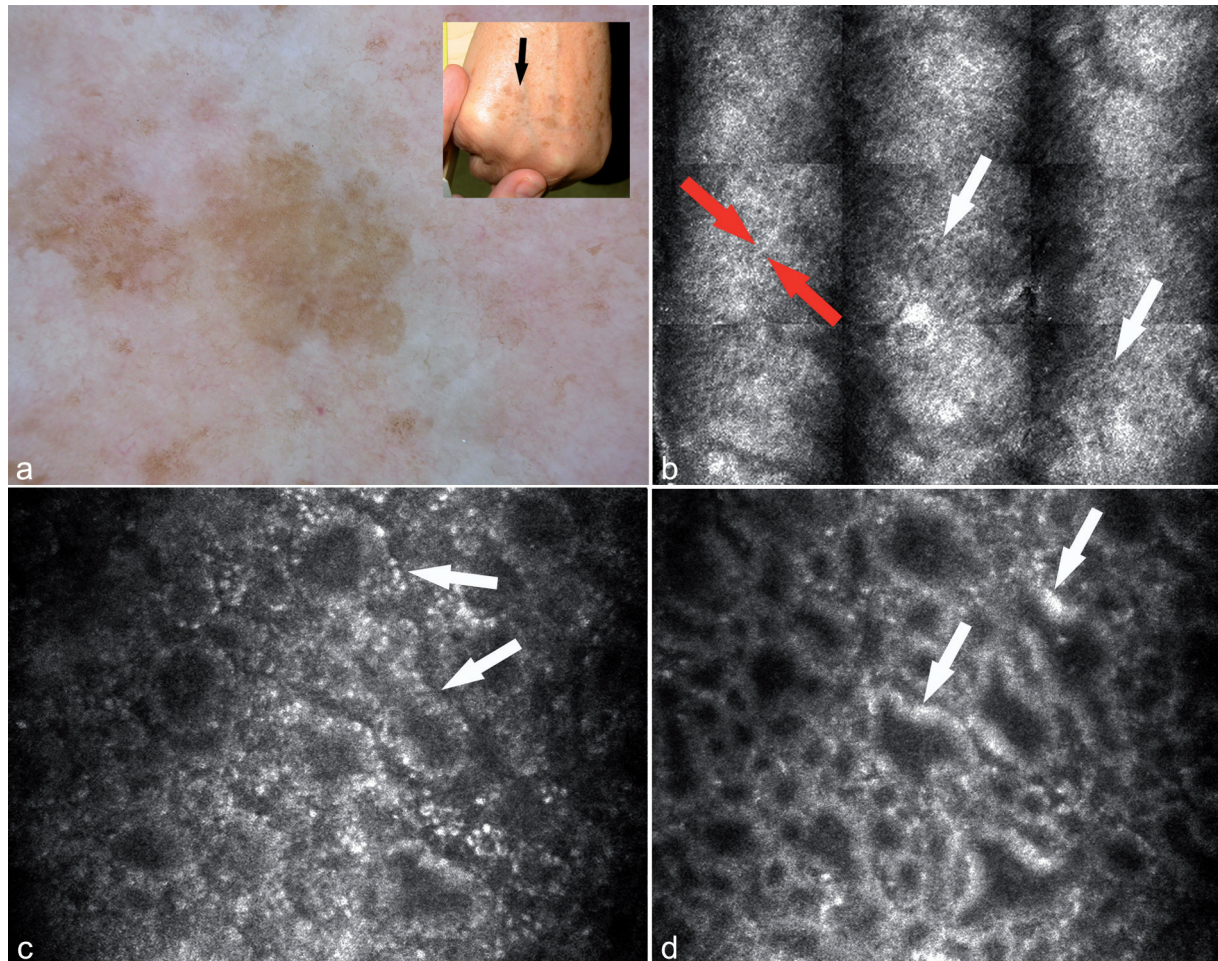
Fig. 1. Solar lentigo before treatment. a) Dermoscopic image of the back of the hand, showing a tan-to-brown-coloured homogeneous reticular pattern. Inset: a tan-to-brown macule on an area of sun-damaged skin (arrow). b) Reflectance confocal microscopy (RCM) image (field of view approximately 1.5 × 1.1 mm) of the upper part of the reticular epidermis, showing a regular honeycomb pattern featuring roundish dark nuclei (white arrows) surrounded by a narrow rim of bright cytoplasm and intercellular connections (red arrows). c) RCM image (475 × 350 µm) of the dermoepidermal junction, showing round-to-oval edged papillae surrounded by a rim of bright monomorphic cells (arrows) corresponding to pigmented keratinocytes and melanocytes. d) RCM image (475 × 350 µm), showing cord-like rete ridges (arrows) in lower regions of the dermoepidermal junction, presenting as bright tubular structures.

les. In RCM, disruption of the stratum corneum was confirmed by the presence of multiple highly reflective round-to-polygonal areas, corresponding to the fine scales (Fig. 2a). The honeycomb pattern showed blurred epidermal intercellular connections (Fig. 2b), while dark structureless areas of different sizes and shapes were observed throughout the epidermis, although they predominated in the lower epidermal layers (Fig. 2c). The dermal papillae were markedly hyporeflective. Histological analysis of the biopsy tissue obtained from two patients revealed epidermal and dermal oedema, presenting as multiple cell debris-filled vacuoles of various sizes, some of them filled with cell debris, at the bases and tips of the rete ridges (Fig. 2d).

Follow-up at ten days

Ten days after laser treatment all lesions presented clinically as scaly areas, often still surrounded by a small erythematous rim (Fig. 3a, inset). Dermoscopic analysis revealed sharply demarcated ("in focus") dark brown blotches and/or dots (Fig. 3a). In RCM, the stratum corneum was found still to be disrupted, with highly reflective polygonal areas. Further bright round-to-polygonal areas and aggregated granules were observed throughout the epidermis, corresponding to extracellular deposits of melanin (Fig. 3b). At the DEJ, non-edged dermal papillae were observed (Fig. 3c), in 9 cases containing a few melanophages (Fig 3d). Bright reflective rims surrounding the dermal papillae were no longer observed at the DEJ. The vacuoles were partially filled with cellular debris and fluid.

DISCUSSION

Solar lentigines are benign skin neoplasms that develop in areas of skin subjected to chronic sun exposure. Various options are available for their removal, in-
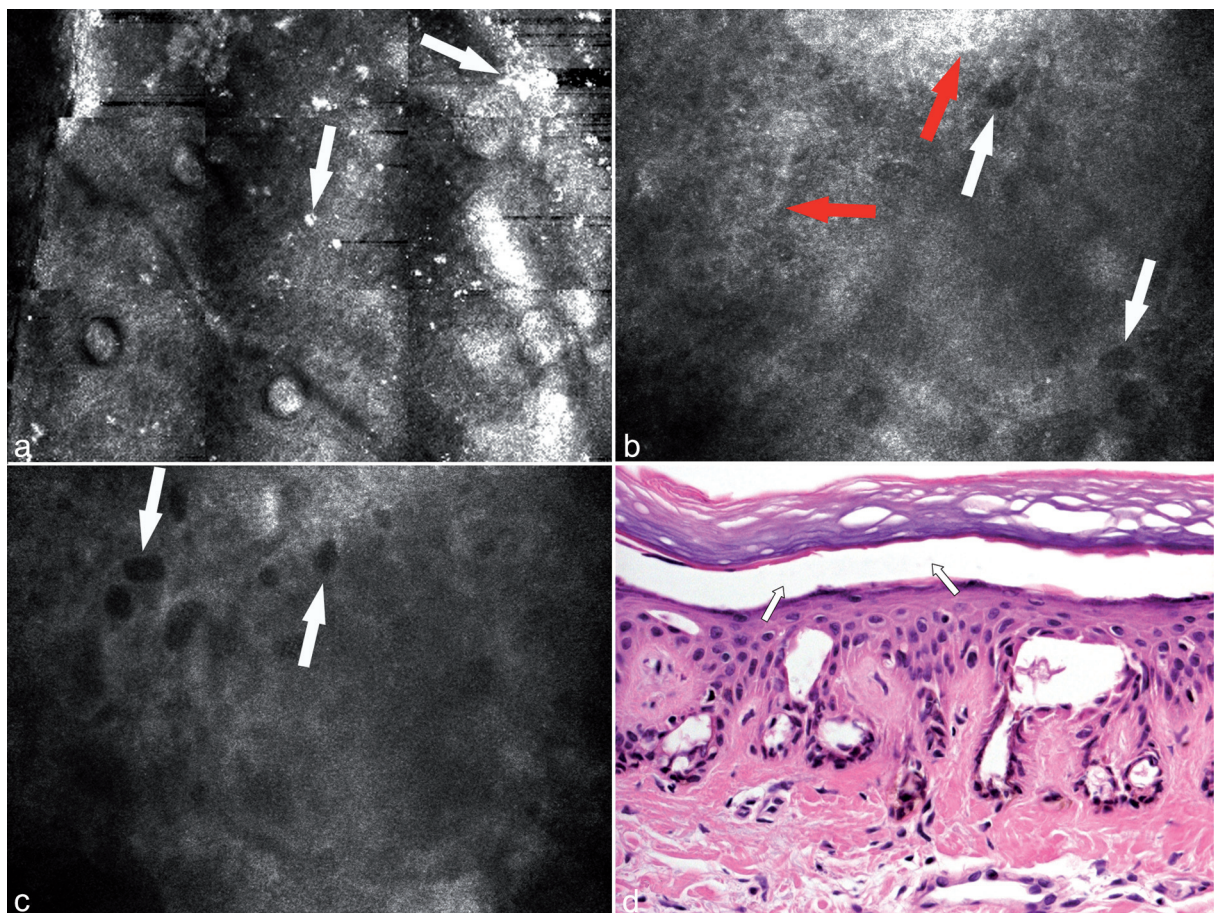

Fig. 2. Solar lentigo 30 min post-laser treatment. a) RCM image mosaic (field of view approximately 1.5×1.1 mm) of the superficial epidermis, showing disruption of the stratum corneum with highly reflective round-to-polygonal areas corresponding to fine scales (arrows). b) RCM image (475×350 µm) of the upper epidermis, featuring blurred epidermal intercellular connections (red arrows) and dark structureless areas of different sizes and shapes (white arrows). c) RCM image (475×350 µm) of the dermoepidermal junction, showing dark structureless areas of different sizes and shapes (arrows). d) Haematoxylin-eosin (HE)-stained tissue section from a shaving biopsy obtained 30 min post-Q-switched ruby laser treatment ($400\times$ magnification). Note the subcorneal split (arrows) and the multiple vacuoles of various sizes in the epidermis, both features attributed to laser treatment. The vacuoles were partially filled with cellular debris and fluid. The epidermis exhibited features of a solar lentigo, including elongated rete ridges whose tips were hyperpigmented, but no increases in the numbers of melanocytes. Note: all images were obtained from the same solar lentigo presented in Fig. 1 except histology which was taken from another lentigo treated with Q-switched ruby laser in the same patient.
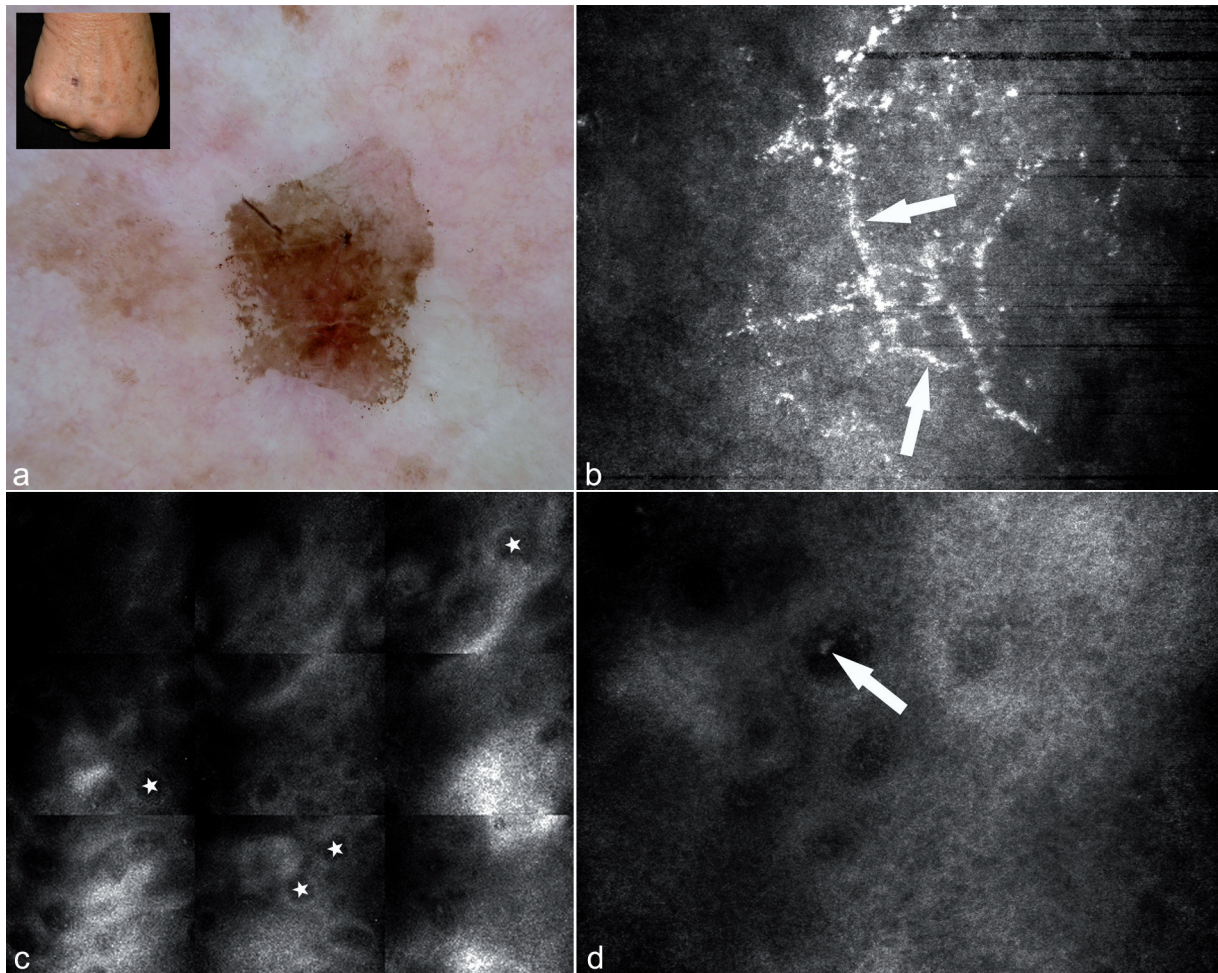
Fig. 3. Solar lentigo 10 days post-laser treatment. a) Dermoscopic image showing a sharply demarcated ("in focus") dark brown blotch and smaller dots. Inset: a dark scaly area on the back of the hand. b) RCM image (475 × 350 µm) showing highly reflective round-to-polygonal aggregates of granules in the epidermal layers, corresponding to extracellular pigment (arrows). c) RCM image mosaic (field of view approximately 1.5 × 1.1 mm) of the dermoepidermal junction, showing non-edged dermal papillae (asterisks) without rims of bright cells. d) RCM image (475 × 350 µm) of the dermoepidermal junction, showing non-edged dermal papillae and a limited number of melanophages (arrow). Note: all images were obtained from the same solar lentigo presented in Figs. 1 and 2.

cluding, among others, lasers (Q-switched ruby laser, Alexandrite laser, Nd-YAG laser, Erb-YAG-laser), intense pulsed light, cryotherapy and topical bleaching substances (trichloroacetic acid, hydroquinone and retinoid acids) (6–13). The Q-switched ruby laser has been shown to be an effective treatment option for SL, associated with a low rate of post-procedure hyperpigmentation, evenness in skin colour, and good acceptance by patients (6, 7, 14). The target pigment for the Q-switched ruby laser is melanin, which is largely present in basal keratinocytes in SL. During the laser procedure, melanosomes are rapidly heated and evaporate within the skin, leaving a conspicuous vapour bubble (vacuolisation), according to the principles of selective photothermolysis (15). Due to its high energy density and short pulse duration, as well as the selective absorption of light, adjacent tissue is minimally affected (6).

Changes in RCM features observed 30 min post-laser treatment in our study correlated well with the histo-pathological findings. Blurred intercellular connections within the epidermis and dark structureless areas, especially in the lower epidermal layers, observed in RCM corresponded well to the epidermal oedema and vacuolisation (caused by the vaporisation of pigment structures) detected histopathologically. The dermal oedema observed in histological specimens appeared as hyporeflective dermal papillae in RCM analyses. The highly reflective granules observed, in RCM, in all epidermal layers at 10 days post-Q-switched ruby laser treatment reflects the migration of extracellular melanin towards the skin surface to be eliminated by regular skin desquamation, as has been previously described following intense pulsed light treatment (13).

Contrary to the observations of Yamashita et al. (13), no activated melanocytes were seen in RCM analyses performed 10 days after Q-switched ruby laser treatment. Moreover, only a limited number of melanophages were detected, by RCM, within the dermal papillae and the upper dermis in our study. These findings

may explain the superior cosmetic results obtained using Q-switched ruby lasers compared to intense pulsed light therapy (6, 7, 14).

In our study RCM was not only used to monitor changes in SL after laser treatment, but also to confirm the diagnosis pre-treatment. In previous reports, RCM has been shown to be a valuable tool for the diagnosis of equivocal pigmented skin lesions, boasting high sensitivity and specificity (16–23). All the lesions we studied displayed typical SL features in RCM analyses, but no features suggestive of malignancy, such as an irregular honeycomb pattern, pagetoid spreading of atypical melanocytes, disorganisation of the dermo-epidermal junction, or irregular melanocytic nests. Our results suggest that RCM could be used as an adjunct in diagnosis before non-invasive treatment to reduce the number of unnecessary biopsies.

In conclusion, RCM appears to be a valuable tool for non-invasive assessment of pigmented skin lesions prior to laser treatment. It can also be used to visualise dynamic skin processes, allowing the *in vivo* follow-up of skin lesions after invasive and non-invasive treatments.

ACKNOWLEDGEMENTS

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