Optical coherence tomography imaging of non-melanoma skin cancer undergoing imiquimod therapy

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Purpose: To explore the application of optical coherence tomography (OCT) imaging of basal cell carcinomas (BCC) and actinic keratosis (AK) before, during and after imiquimod treatment and the ability of OCT to predict treatment outcome.

Methods: The study subjects were 20 patients with biopsy-verified BCC (9) or AK (11). Patients were OCT-scanned before, after 1 and 4 weeks of imiquimod treatment and after 3 months. Lesions were identified clinically and with OCT. Thickness and morphology of the lesions were recorded at each visit. Any remaining lesions were biopsied at follow-up.

Results: Complete data sets were available for 16 patients (8 women and 8 men aged 52–82 years), four in-compliant patients were excluded. OCT identified all lesions. Previously suggested OCT-criteria identified 5/8 BCCs. Crusting, ulceration and active treatment significantly reduced image quality. All BCCs cleared, but at follow-up residual structures were seen clinically in 4 cases. OCT and histology both ruled out residual BCC. For AKs significant thinning occurred after 1 week of treatment ($P = 0.04$). Imiquimod cleared 2/8 AKs, and significantly decreased the thickness of all lesions ($P = 0.02$).

Conclusions: OCT could identify superficial BCC and AK before treatment. Monitoring during imiquimod treatment revealed impaired image quality most likely caused by inflammation, crusting and ulceration. On follow-up, OCT showed thinning of AKs indicating effect of treatment. All treated BCCs cleared, but where residual tissue was suspected clinically this could be ruled out by OCT.

Key words: optical coherence tomography – monitoring – non-melanoma skin cancer – imiquimod – basal cell carcinomas – actinic keratosis – non-invasive treatment – imaging

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Accepted for publication 18 July 2013

The incidence of non-melanoma skin cancer (NMSC) has increased within the last 60 years, and is today the most common type of cancer in the Western populations. A 3–8% yearly increase in incidence of NMSC has been reported since 1960 worldwide. Incidence of basal cell carcinoma (BCC) alone is increasing by 10% per year worldwide, suggesting that prevalence of this tumour will soon equal that of all other cancers combined (1). Worldwide, the incidence for NMSC varies widely with the highest rates in Australia [>1000/100,000 person-years for BCC] (2).

The concept of field cancerization offers a possible explanation of the common clinical presentation of NMSC that can occur in a UV damaged skin area with simultaneous multiple NMSC-precursors, second primaries and local recurrences (3). The aim of treatment is therefore often not only cancer elimination of the individual tumour but also management of the adjacent clinically uninvolved skin, maintenance of the function of the area treated and a satisfactory cosmetic result.

Imiquimod is a topical, non-invasive therapy approved for the treatment of superficial BCC and actinic keratosis (AK). It is an immune modulating agent that binds to Toll-like receptor 7 (TLR-7) on antigen presenting cells. After binding to the ligand, interferon-alfa, -gamma and interleukin-12 are produced and both innate and adaptive immune responses are activated (4, 5). Clinically, an inflammatory reaction surrounding the dysplastic keratinocytes occurs (6). The ultimate cosmetic result is generally excellent, and may provide a patient-generated strong and independent indication for the use of imiquimod. Before starting a therapy, it is relevant to know the thickness and extent of the lesion in order to choose the most appropriate therapy. Traditionally, diagnosis and follow-up are done by clinical examination and biopsy.
Diagnosing skin cancer in the clinic may be a challenge. NMSC is based on the clinical diagnosis and a skin biopsy. The clinical diagnostic sensitivity for NMSC is 56–90% and the specificity is 75–90% (7, 8), and the histological interobserver agreement is described as moderate (9). Although skin biopsy remains the gold standard for the diagnosis of NMSC, it is often not appropriate as biopsies are invasive, and may be scarring and painful procedures. Conceptually, it can further be discussed if an invasive procedure is appropriate to monitor a non-invasive treatment, thereby negating one of the principal advantages of non-surgical therapy.

Optical Coherence Tomography (OCT) is a non-invasive, in vivo imaging technique. It provides cross-section images of tissue with <10 μm resolution to a depth of up to 2 mm. The system is based on interferometry, where low coherent infrared light (1305 nm) is projected into the tissue. The reflection of light is processed, and the sum of the different light refractions produces an image analogue to ultrasound, but at a significantly higher resolution (10). OCT imaging has been applied for the diagnosis and assessment of tumour thickness of NMSC (10–14). The aim of this study was to investigate if OCT imaging could be used to monitor BCCs and AKs treated with imiquimod.

Materials and Methods

Twenty patients (9 patients with BCC and 11 with AK) from the Department of Dermatology, Roskilde Hospital, Denmark were consecutively included in a prospective study. Four patients were subsequently excluded as they were not fully compliant with the treatment. Therefore, the results from 16 patients (8 patients with BCC and 8 patients with AK) could be evaluated. All patients, 8 women and 8 men, were skin type II–III, and their age was 52–82 years. The BCCs and AKs were located on the temporal region, the dorsal hand, the chest and the back. In addition, one AK was found on the thigh. The protocol was approved by the Regional Ethics Committee for Zealand (27.956), and conducted in agreement with the Helsinki declarations, following informed consent by all patients. The examinations took place in 2011–12 and the patients were examined as follows: (i) Clinical examination of the skin lesion (ii) selection of the area to be OCT-scanned using the Michelson Diagnostics Ltd, UK (iii) a digital photo of the lesion (iv) a 2-mm punch biopsy was taken from the lesion in all patients using the departments standard procedure to verify the diagnosis. All biopsies were read by a skilled dermatopathologist.

Patients with BCC were instructed to apply imiquimod on the lesion five times weekly, patients with AK three times weekly. Both groups were treated for 8 weeks. The patients were OCT-scanned four times: before treatment (visit 1), 1 week after the treatment was started (visit 2), 4 weeks after the treatment was started (visit 3) and 3–4 weeks after the treatment was completed (visit 4). At the final visit, all patients were assessed clinically by a dermatologist blinded for the OCT-scanning result.

All OCT scans and interpretations of the OCT images were performed by the same authors CAB and MM. The OCT-probe was placed at the same point of the lesion at every scan. In cases where more lesions in a patient had been scanned and biopsied, only one of these lesions was included in the final results by random choice.

At follow-up (visit 4), biopsies were taken from lesions in which residual tumour was suspected either clinically or by OCT imaging. All BCCs disappeared on treatment and dynamic changes could therefore not be calculated.

Statistics

Paired t-test was performed by a software from GraphPad Prism (version 4, GraphPad Software, San Diego, CA, USA). P-value < 0.05 was considered statistically significant.

Image analysis

The images were interpreted according to morphological parameters characterizing BCC and AK respectively. The core parameters of BCC in OCT were the presence of hyporeflective ovoid structures with a black layered rim and occasionally a surrounding white band (11, 13–15). The structures are thought to represent BCC-islands (dark ovoid structures) with palisading (black rim) and surrounding tumour stroma (white band). Architectural disarray in the epidermis (disrupted layering) seems to be a characteristic finding in BCC as well as AK in OCT images (10, 11, 13, 14, 16, 17). However, disrupted layering in BCC can be thickened as

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well as thinned epidermis whereas AK has only thickened epidermis. Both types of lesions can furthermore appear with white streaks and dots in OCT if scale or hyperkeratosis occurs (10, 12, 13). Cysts are occasionally seen in BCCs characterized by rounded black (hyporeflective) structures in the dermis with a white (hyperreflective) rim (17).

Inflammation can blur the image and influence the image quality negatively (18). The image quality can also decrease due to pronounced hyperkeratosis (13) or ulcers.

Results

Basal cell carcinomas

Visit 1 – baseline/before imiquimod therapy

All lesions presented characteristic OCT features of BCC. Dark/hyporeflective island-structures with black rims were seen in five cases and in three of these cases, an additional surrounding white rim was seen. In two cases, the image appeared slightly blurred and the white bands could not be assessed. Interestingly, we observed that the profound signal of an island-structure appeared enhanced in two cases. The thickness of the island-structures including a white rim was 0.33–0.51 mm.

Three cases presented a different OCT image. No island-structures were seen. Instead cysts >2 mm in diameter were observed in two cases. In one case, disrupted layering due to crusts of an ulcer influenced the image with hyperreflective streaks and dots and no structures in the dermis could be assessed.

Disrupted layering was observed in 6/8 BCC-lesions. Thickened epidermis was seen in three cases, crustae from ulceration in two cases. In two cases, a thinned epidermis above a presumed BCC-island caused the disrupted layering.

Visit 2 – after 1 week of imiquimod therapy

In general, the images at this visit were of poorer quality and appeared more blurred and were more influenced by shadows from ulcers than previously. Dark/hyporeflective island-structures with black rims were observed in only 3/5 cases. A white halo could only be found in one case, where all other dermal structures were obscured. The identified island-structures (including any ring) measured 0.30–0.72 mm.

The images of the three cases with non-characteristic BCCs in OCT basically appeared unchanged compared to visit 1. Disrupted layering was unchanged. However, crustae from ulcers occurred in twice as many cases compared to visit 1.

Visit 3 – after 4 weeks of imiquimod therapy

Only one patient presented characteristic BCC in OCT images. Part of the image was blurred and the island-structure appeared dissolved (Fig. 1 c). In the remaining four cases where island-structures had previously been identified, the quality of the images was strongly influenced by shadows of ulcers and/or inflammation.

All lesions except from one appeared with disrupted layering.

Visit 4 – at 1-month follow-up, 3–4 weeks after the end of imiquimod therapy

Dark/hyporeflective island-structures with a black rim, white halo or intensified underlying signal were not identified in any of the OCT images at follow-up in spite of a general sufficient image quality. New structures were seen in 4/5 cases, while the OCT image had normalized completely in one. These new structures had not been observed at any previous visit. In 3/5 patients, these new structures appeared as cyst-like structures (white ovoid structures with a hyporeflective, dark core with white halo). In one case, these structures were observed at the same location as the BCC-island-structures seen at earlier visits. The new structures were not associated with an intensified signal profound of the lesions (Fig. 1c). Histologically, these new structures were identified as epidermoid cysts. In 1/5 cases, the new structure appeared as a hyporeflective unspecific area in the dermis and was histologically identified as inflammatory tissue.

Two of the three atypical cases with no BCC-islands in the OCT image had changed compared to the previous visits. In one case, a hyporeflective defined/delineated structure in the dermis was observed. It had no black rim and no surrounding halo. Histologically, it was identified as inflammatory tissue. The other case appeared with disrupted layering because of round hyporeflective structures in the epidermis. These were histologically identified as keratine cysts. The third atypical case that at baseline had presented with an ulcer appeared greater at
follow-up. Therefore, there were basically no changes in the OCT image compared to the previous visits. Disrupted layering because of an ulcer only occurred in this patient at follow-up. The crusts were removed and the OCT image of the underlying skin appeared blurred. Histology showed no signs of BCC, but inflammation was identified.

Though OCT showed no island-structures, 4/8 cases were clinically assessed to be possible
residual BCC, while one case had questionable residual BCC on clinical examination. Histologically, no BCC was found in any of the cases.

Actinic keratosis
Visit 1 – baseline/before treatment with imiquimod
All AKs appeared with disrupted layering in the OCT image. Hyperreflective streaks and dots occurred primarily with hyperkeratosis, but were also seen in crustae of ulcers or scale. In contrast to disrupted layering of BCC-lesions where thinning occurred epidermis in AKs was only thickened or crusts from wounds appeared. In some cases, the thickness of a hyperkeratosis was very pronounced and the signal profound of the lesion disappeared. In these cases, we assessed the thickness of a hyperkeratosis to be >2 mm in lack of a more precise estimation. The thickness of the AKs were measured to be 0.16 to >2 mm in the OCT image.

Visit 2 – 1 week with imiquimod therapy
As seen at visit 1, all AKs appeared with disrupted layering. Again, the hyperreflective streaks and dots were predominantly caused by hyperkeratosis. Crustae of ulcers did not occur more frequently at this visit compared to visit 1. The thickness of the AKs measured at this visit was 0.17 to >2 mm and a significant decrease was observed ($P = 0.04$). Only in one case an OCT image appeared blurred, however this had no influence on the measurement of the lesion.

Visit 3 – 1 month with imiquimod therapy
In all AK-lesions, disrupted layering was observed. In one case, an image appeared blurred, but this had no effect on the measurement. The lesions measured 0.16 to >2 mm in OCT. No significant change in the thickness compared to the previous visits was observed.

Visit 4 – 3–4 weeks after end of imiquimod therapy
At 1-month follow-up, disrupted layering was observed in only 3/8 patients. Two of the three lesions were clinically assessed, OCT-imaged, and assessed by histology. The thickness of the two lesions was 0.26 and 0.29 mm. In the third case, thickened epidermis occurred because of a small wound/scratched epidermis, but histology did not show any signs of AK.

The OCT images of the remaining five patients did not display disrupted layering and therefore did not show any OCT signs of AK. This was in accordance with the clinical assessment.

Though the AK lesions did not seem to decrease linearly at each visit, we found an overall significant decrease in the thickness measured before and after the treatments ($P = 0.02$).

Discussion
This study suggests that OCT imaging may be a valuable tool at follow-up after imiquimod therapy by correctly identifying recurrences. In accordance with other studies (10, 12) AK and superficial BCC (deepest point of lesion observed in a depth of 0.72 mm) could be identified in OCT images. In most cases, BCCs could be identified as dark/hyporeflective islands-structures with black rims and sometimes a halo representing BCC-islands, their palisading border and surrounding tumour stroma as suggested by previous studies. Furthermore, we observed that presumed BCC-islands in two different patients appeared to have a ‘window-effect’ through which the signal appeared to reach more deeply into the dermis. To the best of the authors’ knowledge, this has not been described previously, and may be another OCT characteristic of specifically BCCs. Disrupted layering with thickening as well as thinning, occurred in several BCC-lesions before treatment. Crusts from ulcers and inflammation seemed to be the main reason for disrupted layering in BCCs during treatment. It is furthermore speculated that the inflammation associated with the treatment reduces image quality during therapy and prevents meaningful interim imaging.

In contrast, all AKs imaged by OCT could be identified by thickened epidermis and often hyperkeratosis which consequently led to disrupted layering.

Only the thickness of BCC-lesions that appeared with well-defined island-structures was measurable in OCT. AKs could be measured but hyperkeratosis and resulting OCT image artefacts decreased the value of some OCT images.

OCT may have clinical implications for patients treated with imiquimod. At follow-up,
OCT could be used to invalidate clinical suspicion of BCC based on the missing island-structures with a black rim.

It was not possible to monitor a gradual disappearance of a BCC or AK lesion during a treatment. It is speculated that this may be due to either technical or biological factors. During the visits, we observed that the identification of the lesions were highly dependent of the image quality. Mogensen et al. described that factors like inflammation can blur the image and thereby impair image quality (18). The image quality at interim visits in our study seemed to deteriorate particularly because of hyperkeratosis, ulcers and inflammation (blurred images) induced by imiquimod. Especially for BCCs, the image quality seemed to worsen as the imiquimod dosage proceeded (visit 1–3), possibly reflecting the higher imiquimod dosage. It was only possible to observe and follow island-structures throughout a full treatment in one case (Fig. 1a–d). These observations may, however, also just suggest that the effect of the treatment is not linear.

Imiquimod did not seem to influence the image quality of the AK-lesions to the same degree as seen in BCCs, perhaps because the inflammatory response was weaker due to lower imiquimod dosage. The more superficial location of the lesions in the epidermis may also make the AKs less susceptible to inflammation. Earlier clinical studies have suggested that the more frequently imiquimod that is applied the better the response, and the more efficient the final result (19, 20). Interestingly, we observed that in OCT, BCCs could be either resolved completely or modified by the treatment (Fig. 1c). The more efficient therapy for BCCs seemed to include a risk of new ‘scar-like’ structures or ulcers in the skin that could be misinterpreted clinically and consequently lead to a risk of treating the patient unnecessarily. Epidermoid cysts appearing in the skin because of the strong inflammatory response of imiquimod has been suggested in a previous study (21).

OCT imaging does have the potential to monitor BCCs in the upper dermis and AKs before and after a treatment with imiquimod in this pilot study. Furthermore, OCT seems to have the potential, albeit smaller, of studying the tumour dynamics during a non-invasive treatment. These aspects may have clinical significance as OCT can be used to positively identify new structures appearing in the dermis after treatment which are not clinically accessible.

Acknowledgements

The authors thank Michelson Diagnostics for making available the Vivosight® OCT system for this study.

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