Imaging actinic keratosis by high-definition optical coherence tomography. Histomorphologic correlation: a pilot study

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Abstract: With the continued development of non-invasive therapies for actinic keratoses such as PDT and immune therapies, the non-invasive diagnosis and monitoring become increasingly relevant. High-definition optical coherence tomography is a high-resolution imaging tool, with micrometre resolution in both transversal and axial directions, enable to visualize individual cells up to a depth of around 570 µm filling the imaging gap between conventional optical coherence tomography and reflectance confocal microscopy. We sought to determine the feasibility of detecting and grading of actinic keratoses by this technique using criteria defined for reflectance confocal microscopy compared to histology. In this pilot study, skin lesions of 17 patients with a histologically proven actinic keratosis were imaged by high-definition optical coherence tomography just before excision and images analysed qualitatively. The surrounding normal looking skin has been used as control group. In lesional skin, dyskeratotic and atypical keratinocytes could be noticed with this new technique. An atypical honeycomb pattern in variable degree or a disarranged epidermal pattern could be observed. A good correlation between the dimension of atypia and/or disarrangement of the spino- granular layer on en face images and the histopathological grading could be demonstrated. Relevant cross-sectional imaging criteria could be defined for the different histopathological variants of actinic keratoses. The surrounding skin displayed features of photodamage. Using features already suggested by reflectance confocal microscopy, the study implies that high-definition optical coherence tomography facilitates in vivo diagnosis of actinic keratosis and allows the grading of different actinic keratoses lesions for increased clinical utility.

Key words: actinic keratosis – high-definition optical coherence tomography – histopathological grading – reflectance confocal microscopy

Accepted for publication 4 December 2012

Introduction

Actinic keratosis (AK) causes thick, scaly or crusty erythematous patches of the skin. Sites of predilection are areas of chronic, high-dose or intermittently sun exposure (face, neck and forearm) in persons with Fitzpatrick skin phototype I–III (1,2). The etiopathogenetic concept of AK has been referred to as ‘actinic field cancerization’. AK represents one stage in the continuum from subclinical keratinocyte dysplasia to invasive squamous cell carcinoma (SCC) (3,4). SCC requires extensive and intensive therapy, while AK usually can be treated with simpler destructive methods (5). Exact diagnosis is therefore important to segregate premalignant and malignant lesions prior to treatment. The exact progression rates are currently unknown (6).

The diagnosis of AK is actually based on clinical examination and dermoscopy. Conventional dermoscopic criteria have been defined (7,8). Histological evaluation is usually performed in clinically indistinct cases and on suspicion of invasive squamous cell carcinoma. Because of the high prevalence in the population, additional diagnostic options are relevant to pursue, particularly if these are non-invasive as non-surgical treatment modalities such as photodynamic therapy and pharmacological immunomodulation are increasingly being used.

Reflectance confocal microscopy (RCM) is a non-invasive imaging technique that allows the visualization of cellular and subcellular structures of the skin in vivo with near histological resolution to a depth of approximately 200 µm. In contrast to histology, which visualizes vertical sections of the skin, RCM obtains horizontal (en face) optical sections in greyscale. RCM can be used as a diagnostic and monitoring aid in actinic keratosis (4,9–12). RCM diagnostic features of AK have been defined (11).

We have recently introduced high-definition optical coherence tomography (HD-OCT) (13,14). This is a technique based on the principle of conventional OCT, with the ability to carry out optical imaging up to 570 µm deep with a micrometre resolution in both lateral and axial directions, giving it the potential to visualize individual cells (13). HD-OCT is able to provide cross-sectional images like the conventional OCT and en face images comparable to RCM potentially giving the method considerable diagnostic utility (15).

The aim of this pilot study is to implement RCM features of AK with en face HD-OCT images and to compare histological vertical sections of these lesions with the corresponding cross-sectional and en face HD-OCT images and on the basis of this suggest relevant HD-OCT imaging criteria for the in vivo diagnosis and grading of AK. The surrounding skin was used as control group.

Methods

Patients

Seventeen fair-skinned patients (Fitzpatrick types I–III) with a single histologically proven AK lesion each located on the face, neck, arm or trunk were recruited for this pilot study. Signed informed consent was obtained. The group included nine women and eight men with ages ranging from 44 to 81 years. Clinically, AK presented as...
erythematous to brown plaques with a scaly surface. In subclinical cases, only a rough palpation was suggestive for diagnosis. All patients gave informed consent to the study (Table S1).

**High-definition optical coherence tomography**

High-definition OCT is based on the principle of conventional OCT, specifically the ability to carry out optical imaging deep within highly scattering media such as skin, with micrometre resolution in both transversal and axial directions, to visualize individual cells (Skintel®; Agfa Healthcare, Mortsel, Belgium). Instead of a single pin diode, it uses a two-dimensional, infrared-sensitive (1000–1700 nm) imaging array for light detection. This enables focus tracking; the focal plane is continuously moved through the sample. The movements of the focal plane and the reference mirror are synchronized, and the refractive index of the sample is taken into account. This results in a high lateral resolution of 3 µm at all depths of the sample. A high axial resolution (3 µm in skin) is achieved using a broadband thermal light source combined with a special filter. This technology offers high resolution in all three dimensions. Moreover, the system is capable of capturing a slice image and an en face image in real time, as well as fast 3D acquisition. The spectral sensitivity makes it possible to work in the near infrared range above 1000 nm. The field of view is 1.8 × 1.5 mm. The tissue penetration depth goes up to 570 µm, and the total light power at the tissue is <3.5 mW. The system works in direct contact with the skin, using an optical matching gel (Skintel® optical gel; Agfa Healthcare) comparable to ultrasound gel. The interference signal detected by the 2D imaging sensor is digitized, and its envelope of the interference signal is calculated. The result is transferred to a computer and displayed using a grey scale or colour palette, thereby generating an OCT image. Further technical details are discussed elsewhere (13,14).

**Study setting**

Excision under local anaesthesia was performed immediately after HD-OCT imaging, and subsequent histopathological analysis entered the standard histopathological procedure. The HD-OCT images of histologically proven AK lesions were analysed according the criteria for the diagnosis of AK and in RCM. These imaging criteria of AK were implemented on the en face HD-OCT images. These features are displayed in Table S2. Imaging criteria of AK were correlated with the corresponding cross-sectional and en face HD-OCT images. If more than one grade was present in a lesion, the worst age range 44–81 years), contributing a total of 17 biopsy-proven lesions including 2 AKs-KIN III, 9 AKs-KIN-II (2 × IIa, 4 × IIb and 3 × II unspecified) and 6 AKs-KIN-I. If more than one grade was observed in a lesion, the worst grade has been taken into account. KIN-IIa and KIN-IIb often cannot be determined by clinically diagnosed AK would fall into this category. Abnormal keratinocytes display more obvious nuclear enlargement, hyperchromasia and prominent nucleoli. This stage is further divided into KIN-IIa (Fig. 2) where the process spares adnexal structures and KIN-IIb (Fig. 3) where the atypical keratinocytes have involved adnexa, presenting significant acanthosis or budding of keratinocytes into the superficial papillary dermis or areas of atrophy. KIN-IIIA (Fig. 4) represents carcinoma in situ with full-thickness atypia involving the epidermis and adnexal structures. Histological features of this three-tiered grading scale were correlated with the corresponding cross-sectional and en face HD-OCT images. If more than one grade was present in a lesion, the worst grade has been taken into account (4,17).

Secondly, AKs were assessed according to different histopathological variants of actinic keratosis. Atrophic (Fig. 1), hypertrophic (Fig. 2), lichenoid (Fig. 3) and bowenoid actinic keratosis (Fig. 4) have been described (18–20). Histological vertical sections of these variants of AK were correlated with the corresponding cross-sectional HD-OCT images to identify relevant HD-OCT imaging criteria. Furthermore, the correlation between histological/HD-OCT variants and histopathological grading was evaluated (Table S4).

**Results**

Seventeen patients were included in the study (8 men/9 women; age range 44–81 years), contributing a total of 17 biopsy-proven lesions including 2 AKs-KIN III, 9 AKs-KIN-II (2 × IIa, 4 × IIb and 3 × II unspecified) and 6 AKs-KIN-I. If more than one grade was observed in a lesion, the worst grade has been taken into account. KIN-IIa and KIN-IIb often cannot be determined by
Because of the shaving method, the anatomical distribution of the lesions was head (70%), neck (12%), trunk (12%) and arm (6%; Table S1). The implemented RCM features of AK on the en face HD-OCT images are summarized in Table S2.

All lesions displayed an adherent hyperkeratotic scale. Parakeratosis visualized as the presence of polygonal nucleated cells at the stratum corneum was seen in all lesions. Single detached cells in the superficial layer were also constant findings. In early lesions (KIN-I), an atypical honeycomb pattern was confined to the lower 1/3 of the epidermis (Fig. 1). In fully developed lesions (KIN-II), atypical keratinocytes provoking an atypical honeycomb pattern involved mostly the lower two-third of the epidermis (Figs 2 and 3). Severe architectural disarray of the spinous–granular layer in which the honeycomb pattern was no longer visible, was only observed in the two cases of AK graded KIN-III (Fig. 4). Round nucleated bright cells were present in the spinous cell layer (brown arrow). A histological vertical section of this lesion corresponding to the hypertrophic histological variant of AK is displayed.

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Table S3 summarizes the detailed analysis of the en face HD-OCT images of all 17 patients and correlation to histological diagnosis and AK-KIN grading.

The histological vertical sections of different variants of AK were correlated to the corresponding cross-sectional HD-OCT images in order to identify relevant HD-OCT imaging criteria and to correlate them with histopathological grading (Table S4). We could demonstrate a good correlation between histopathology and the cross-sectional HD-OCT imaging of these variants. Relevant cross-sectional HD-OCT imaging criteria could be defined for the different histopathological variants of AK (Table S4). Furthermore, we evaluated the correlation between histological/HD-OCT variants and histopathological grading. The atrophic variant with an atrophic stratum malpighi and typically a substantive overlying hyperkeratosis was only seen in early lesions graded KIN-I (Fig. 1). The hypertrophic variant presented a pronounced parakeratosis, acanthosis and psoriasiform hyperplasia on cross-sectional HD-OCT images. This histological variant was only observed in four of the nine AK lesions graded KIN-II (Fig. 2). An interface inflammatory infiltrate was characteristic for the lichenoid variant. This variant was present in 30% of AK lesions graded KIN-II (Fig. 3). The Bowenoid variant with at least lower two-third thickness atypia but without hypertrophic or lichenoid characteristics was observed in 30% of the AKs graded KIN-III. The Bowenoid variant with full-thickness atypia was only observed in both AKs graded KIN-III (Fig. 4).
which allows diagnosis at an early stage of the lesion. Horn et al. have shown that the value of RCM in the discrimination of AK from surrounding sun-damaged skin offered a high sensitivity and specificity (9,11,26,27). AKs are histopathologically characterized by a proliferation of atypical keratinocytes, starting at the basal layer (18,20) indicating that deep penetration into the skin is a relevant parameter when assessing the utility of a diagnostic method. OCT may be a relevant method as it provides imaging of the entire epidermis and often also of the whole dermis (28).

To stratify degrees of epidermal dysplasia, a three-tiered grading scale has been proposed by Cockerell et al. for AKs that parallel that used for evaluation of cervical dysplasia (4,17). The localized epidermal atypia in AKs reflects a partial dissection of the differentiation programme, whereas a more complete disruption of differentiation is associated with SCC in situ. While the KIN grading criteria evaluate the macroscopic and microscopic features of AKs, identification of genetic and molecular abnormalities associated with these lesions has provided mechanistic insight into their pathogenesis (4).

One of the aims of this study was to investigate the HD-OCT correlates of this histopathological three-tiered grading scale on cross-sectional (Table S4) and en face (Table S3) images obtained by HD-OCT. This new technology has recently been introduced by our group as a possible non-invasive technique for morphological investigation of tissue with cellular resolution filling the imaging gap between reflectance confocal microscopy and conventional optical coherence tomography (13).

In this study, we demonstrated that the RCM diagnostic features of AK could be implemented on the en face HD-OCT images, that is, comparing horizontal section with horizontal sections (Table S3). These features could be divided into two groups. The first group includes features having a good correlation with the histopathological AK-KIN grading. These features are atypical honeycomb and/or disarranged epidermal pattern due to cellular pleomorphism. These patterns do not appear to have clinical or dermoscopic correlates and were unique to RCM assessment of AK and SCC (7). In this study, we could observe the same epidermal patterns beyond the limitations of RCM such as lack of cross-sectional imaging. We could find a good correlation between the dimension of the atypia and/or disarrangement of the spinous-granular layer observed in en face HD-OCT images and the histopathological AK-KIN grading. In early or subclinical lesions (very often only detected by a rough palpation), a mildly atypical honeycomb pattern confined to the bottom third of the epidermis could be noticed. This finding correlated well to KIN-I grading. HD-OCT was, just like RCM, able to identify subclinical AK by visualization of cellular and nuclear atypia within the lower spinous cell layers. The clinical AKs included in this study have an atypical honeycomb pattern involving the lower two-thirds of the epidermis. This was the signature for AKs graded KIN-II. A full-thickness disarranged epidermal pattern was only observed in AKs graded KIN-III. The atypical and dyskeratotic keratinocytes noticed in histopathology of AKs represent the round bright nucleated cells observed in RCM (9,25). In our study, we observed these dyskeratotic and atypical keratinocytes only in lesions graded KIN-II and KIN-III.

The second group includes features observed in variable degree in all patients without any correlation with histopathological (KIN) grading. These features are as follows: hyperkeratotic scale, polygonal nucleated cells in the stratum corneum (as correlates of
parakeratotic cells) and stratum corneum disruption as well as perivascular inflammation. All lesions demonstrated round blood vessels in the superficial dermis which is a typical feature of AK in RCM. Dermoscopic evaluations of the blood vessels have not been performed. Live video recording of white blood cells moving in the blood vessels, which is a very useful tool in RCM, is not possible with HD-OCT (9).

According the histopathological three-tiered grading scale, adnexal involvement is the discriminating factor between KIN-Ila and KIN-IIb. Adnexal involvement observed in en face HD-OCT images correlated well with histopathological AK-KIN grading in six of nine cases. Due to shave biopsy procedure, it was not possible to evaluate the remaining three KIN-II cases because of lack of dermal tissue in the histological specimen. To the best of our knowledge, this HD-OCT feature has not been described for RCM. HD-OCT evaluation of adnexal involvement seems to be a promising non-invasive tool in the clinical grading of AK (Figure S1).

Solar elastosis, represented by lace-like material adjacent to thickened collagen bundles, could be observed in 13 of the 17 cases. Three of the cases without this lace-like material belonged to AK graded KIN-I. This seems reasonable because the most common risk factor for AK is chronic sun exposure. The more severe the photodamage of the skin the higher the likelihood of having a clinical AK. HD-OCT evaluation of solar elastosis can be performed in a very fast way and is a helpful tool in the clinical evaluation of the photodamaged skin (Figure S1).

Additionally, HD-OCT also provides cross-sectional imaging, whereas RCM imaging is only possible in the en face mode potentially giving the former method considerable diagnostic utility. We could demonstrate a good correlation between histopathology and the cross-sectional HD-OCT imaging of AK. Relevant cross-sectional HD-OCT imaging criteria could be defined for the different histopathological variants of actinic keratosis (Table S4). Our study has several limitations including a small sample size and inclusion of only biopsy-proven lesions. Therefore, it remains speculative to suppose that the atrophic histopathological and HD-OCT variant of AK is characteristic for subclinical lesions graded KIN-I. Cutaneous squamous cell carcinoma typically manifests as spectrum of progressively advanced malignancies, ranging from a precursor actinic keratosis to squamous cell carcinoma in situ, invasive and finally metastatic SCC (4). Skin atrophy is one of the characteristic features of photodamaged skin. Furthermore, AKs are predominantly found on sun-exposed surfaces. Therefore, it seems plausible that the atrophic AK variant observed by both methods is only noticed in early subclinical lesions graded AK-KIN-I. Figure S2 presents an overview of cross-sectional images from photodamaged skin via the different grades of AK to invasive squamous cell carcinoma. We have used the surrounding photodamaged skin as a control group. This will make in further studies calculations of sensitivity and specificity of each feature discussed in this paper possible and hence the validation of our findings.

In conclusion, our findings indicate that the AK features of RCM could be implemented on the en face HD-OCT images. Furthermore, a good correlation could be found between histopathological AK variants and corresponding cross-sectional HD-OCT images. On the basis of this relevant HD-OCT imaging criteria for the in vivo diagnosis and grading of AK could be presented. Thereby HD-OCT is also able to identify subclinical AK by visualization of cellular and nuclear atypia within the lower third of the spinous cell layer. In this way, HD-OCT could also be used to survey sun-damaged skin in the setting of field cancerization potentially increasing diagnostic accuracy compared to clinical evaluation alone.

Author contribution
M.A.L.M. Boone designed the research study, performed the study (acquisition, analysis and interpretation of data) and wrote the paper. S. Norrenberg performed the histopathological examination and critically revised the paper. I. Neetens performed the histopathological examination. G.B.E. Jemec and V. Del Marmol revised the paper.

Conflict of interests
The authors have declared no conflicting interests.

References

Supporting Information
Additional Supporting Information may be found in the online version of this article:
Figure S1. Actinic keratosis graded keratinocytic intraepidermal neoplasia-Illb (face of patient #17). Figure S2. Actinic keratosis from photodamaged skin to invasive squamous cell carcinoma. Table S1. Patients characteristics and histological diagnosis with grading and variants of actinic keratosis. Table S2. Reflectance confocal microscopy features of actinic keratosis applied on en face images of high-definition optical coherence tomography. Table S3. Detailed analysis of implemented reflectance confocal microscopy diagnostic features of actinic keratosis (AK) on en face high-definition optical coherence tomography images of all 17 patients and correlation to histopathological AK-keratinocytic intraepidermal neoplasia grading. Table S4. Histological vertical sections of the observed variants of actinic keratoses were correlated with the corresponding cross-sectional high-definition optical coherence tomography images and correlation to histopathological AK-keratinocytic intraepidermal neoplasia grading in all 17 patients.