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Acne: morphologic and vascular study of lesions and surrounding skin by means of optical coherence tomography

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Optical coherence tomography study of Acne

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ABSTRACT:

Background: Acne vulgaris is a disease of the pilosebaceous unit, characterized by hyper-keratinization process, comedos formation and inflammatory reactions.

Objective: The definition of the morphology and the vascularization of acne lesions by means of dynamic optical coherence tomography (D-OCT), in order to non-invasively define the alterations occurring during the acne development and patient therapeutic management.

Methods: A set of standardized clinical pictures and D-OCT images were acquired from 114 acne lesions of 31 volunteers, presenting mild to moderate acne and evaluated by experts. Fifteen patients treated with oral antibiotics were followed during time at 0, 20, 40, and 60 days.

Results: Optical coherence tomography enabled to identify vascular and morphological aspects characterizing different types of acne lesions. Oral antibiotic treatment improved the morphologic features and decreased the digitally reconstructed vascular signal during time.

Conclusion: The characterization of acne lesions and the identification of vascular pattern in acne lesions through D-OCT, corresponding to blood vessel dilation and inflammatory associated hyper-vascularization, may have important clinical consequences in the assessment of acne severity, therapeutic decisions and treatment efficacy monitoring.

KEYWORDS:

Acne, Optical coherence tomography, Vascularization, Therapy, Comedogenesis, Microcomedo

Introduction

Acne vulgaris is a disease of the pilosebaceous unit, characterized by hyper-keratinization process, comedos formation and inflammatory reactions.[1,2] There are four well established processes in the development of acne lesions: alteration of the keratinisation process; increased and altered sebum production under androgen control (or increased androgen receptor sensitivity); follicular colonisation by Propionebacterium Acnes and inflammatory mediators released into the skin.[1-5] The growth of a new comedo and the maintenances or remissions of fully developed acne lesions comprise several biological and microscopic processes of great importance for the follow up of patients with acne.[6-8]

There are several techniques available for acne examination, comprising new photographic techniques and biometric analysis of sebum.[9,10] Histopathology represents an optimal method for microscopic morphology lesion evaluation, but is limited due to its invasiveness, cosmetic consequences, and inability to apply to lesion follow-up. Recently, in vivo reflectance confocal microscopy (RCM) has been applied to the study of acne, non-invasively exploring the epidermis and upper dermis, at almost histologic resolution.[11-14] However its penetration power is limited to approximately 200 um depth from the skin surface, being able to

investigate only the epidermis and the upper dermis. OCT represents a promising technique to explore the deeper dermal component.[15] In fact, Baran et al. described the possibility to document local changes in the peripheral blood vessels at the interfollicular region of a selected acne lesion by means of a swept-source OCT system with optical microangiography (OMAG) technique.[16,17] In dermatology, optical coherence tomography (OCT), based on interferometry, was introduced in 1997 and has since been studied in relation to a range of skin diseases and structures providing real time high resolution cross sectional 2D and 3D images. [18,19] OCT is mainly used for diagnosis and monitoring of non-melanoma skin cancer.[20-22] Recently, the introduction of dynamic OCT (D-OCT) allows the description of blood flow in vivo, and visualization of the skin microvasculature in different skin conditions.[19,23] The definition of the morphology of the pilosebaceous units from the epidermis to the deep dermis and the vascular blood flow component with a fast, reliable and non-invasive technique seems useful for a pathophysiological characterization of acne lesions.

In this study we sought to describe the D-OCT morphological features of the different acne lesions and the blood flow changes over time during the therapeutic monitoring of the patients.

Materials and methods

In order to study D-OCT morphology and pathophysiology of acne lesions and of the apparently healthy skin in acne patients was investigated. A total of 31 volunteers, presenting mild to moderate acne were enrolled in this study at the University of Modena and Reggio Emilia between January 2014 and December 2016. The group comprised 5 males and 26 females, ranging in age from 12 to 32 years, with no history of other dermatological or systemic diseases.

Following initial clinical evaluation, 15 patients were prescribed systemic antibiotic therapy, 12 topical therapy and 4 systemic isotretinoin therapy, according to standard clinical practice.[6] The patients treated with systemic antibiotic therapy were followed during time at 20, 40 and 60 days from therapy initiation, with standard clinical pictures and D-OCT image acquisitions of the same areas on the face. Acne lesions (1 to 4 lesions per patients) and apparently unaffected skin of cheeks (1 site per patient) were collected. Acne lesions were identified clinically on the surface and imaged with D-OCT keeping the probe perpendicular to the surface. Lesions were classified as closed or open comedos, papules, or pustules based on their clinical aspect, according with dermatologic semeiology[1]. Per each lesion or unaffected site, 1 'data block' was acquired.

The study was performed in conformity with the ethical guidelines of the Declaration of Helsinki and in compliance with the ethics committee approval (Advance European Project N° 621015 EC 69/14). The standardized clinical pictures were acquired through the Canon G16® (Canon Inc., Melville, NY, U.S.A.) and the Canfield Close-up Scale® (Canfield Imaging Systems, Fairfield, NJ, USA). D-OCT acquisitions were carried out on the same skin area of the right cheek, by means of a commercially available D-OCT (VivoSight®, Michelson Diagnostics Ltd, Maidstone, Kent, UK) and 3D image reconstruction software.[19] D-OCT images were acquired using an automatic built-in software protocol. The device provides vertical and horizontal-plane OCT images, along with functional data (blood flow information), that are immediately displayed on the structural OCT images as an overlay so that the relationship between the vessels and the structural image can be seen. Areas of motion are colored red. A 'stack' of 120 OCT images was captured for each lesion to form a 3-dimensional (3D) 'data block'.

D-OCT descriptors

For the evaluation of D-OCT images, qualitative and quantitative measurements were obtained. An independent examiner using the dedicated VivoSight software measured the diameter of the infundibular opening and the largest lesion diameter. Two expert readers jointly evaluated D-OCT parameters. Absolute and relative frequencies, and mean and standard deviation (SD) for continuous measurements were calculated for each parameter in order to characterize different acne lesions and pilosebaceous units (**Table 1**).

Statistical analysis

Mean (M) and standard deviation (SD) were calculated for acne lesion diameters. Frequencies of the D-OCT patterns were determined. Student T-test and Mc Namar test were calculated in order to compare diameters and D-OCT pattern changes over time (SPSS v. 17 Inc., Chicago, IL, USA). A p-value less than 0.05 was considered significant.

Results

A total of 101 acne lesions were collected and comprised 28 closed comedos, 20 open comedos, 22 papules, 21 pustules. In addition 31 data blocks were acquired in correspondence of apparently uninvolved skin from different areas of the right or left cheek of acne patients. Measurements of the lesion diameter at 1mm depth from the skin surface and at the infundibula are reported in **Table 2**.

Aspect of acne lesions:

Closed comedos are characterized by a reverse v-shaped morphology, delineated by two hypo-echogenic structures, with a variably enlarged infundibulum at the top of the lesion, (infundibular diameter: mean=173 µm, SD± 91 µm; 1mm depth diameter mean 668 µm, SD± 372 µm). A hyper-echogenic area is often found between the two hypo-echogenic boundaries, corresponding to the enlarged sebaceous gland filled with sebaceous material. In

cross-sectional D-OCT images, dermal papillary loops can be recognized as small red dots distributed in close proximity to the dermal epidermal junction (DEJ). In D-OCT en face view, in the proximity of the comedo, the vascular signal is increased, while in correspondence with the core of the comedo, the vascular network is lacking. (**Fig. 1a-c**).

Open comedos are characterized by a rectangualr hypo-echogenic structures at their boundaries, with a large and thick hyper-intense plug at the top of the infundibulum, (infundibular diameter: mean=472 µm, SD± 207 µm; 1mm depth diameter mean 643 µm, SD± 322 µm). The analysis of the vascular component is similar to the one already described for open comedos, with the lack of the vascular signal in the core of the lesion and a normal or increased vascular pattern in the skin around the lesion. (**Fig. 1d-f**).

Papules appeared as dome shaped lesions with an excoriated or intact superficial component (infundibular diameter: mean=317 µm, SD± 217 µm; 1mm depth diameter mean: 730 µm, SD± 408 µm). The rete ridge is often flattened in the central part of the lesion and a hyper-echogenic component in the superficial dermis is often found. The vascular network is intense both in the surrounding dermis and in the proximity of the core of lesion. (**Fig. 2a-c**).

Pustules appeared as dome shaped lesions with multiple central oval cavities containing hyper-intense materials inside the epidermis and the dermis (infundibular diameter: mean=523 µm, SD± 428 µm; 1mm depth diameter mean 1105 µm, SD± 612 µm). The vascular network is prominent in the dermis adjacent to the lesion. Reconstructed vascular flow is often very close to the dermal core of the pustule (**Fig. 2d-f**).

Apparently uninvolved skin in acne patients is similar to the one of healthy subjects unaffected by acne (infundibular diameter: mean=96 µm, SD± 82 µm; 1mm depth diameter mean: 212 µm, SD ±130 µm). Briefly, below the entrance signal that delineates the top of the stratum corneum, the epidermis is seen as a heterogeneous granular textured band of varying thickness, overlying the dermis. Hair shafts can be identified, bulging out of the

infundibulum, under which the pilosebaceous units composed of hair, hair follicle, erector pili muscle and sebaceous gland, take place. The skin of the face is supplied richly with a thin vascular network forming two distinct dermal plexuses. The vascular signal of apparently uninvolved skin in acne patients doesn't differ significantly to the one of healthy volunteer unaffected by acne (**Fig. 3a-d**).

Follow-up of changes induced by antibiotic therapy

The follow up of 15 patients treated with systemic antibiotic therapy allowed the sequential acquisition of 10 open comedos, 10 closed comedos, 10 papules and 10 pustules, showing the early normalization (20 days) of the vascular signal in the inflammatory lesions (papule and pustules) and a significant reduction of the other acne associated patterns over time (**Table 3**).

Discussion

OCT enables the identification of morphological aspects characterizing different types of acne lesions. Open comedos and closed comedos showed variably large infundibulum, with different deeper involvement of the skin. Open comedos have larger infundibular opening delineating lesion borders that develop almost vertically in the dermis, whereas closed comedos are characterized by smaller infundibula, and their border assume an inverse V-shape in the dermis. Larger comedo were more easily associated with the presence of a disarranged granularity of the epidermis and multiple vascular spikes in their dermal core. This could possibly represent the beginning of an inflammatory-shift of the larger or more metabolically active comedos towards their inflammatory stage of evolution.

The papules and pustules were characterized by both an abundant inflammatory reaction in the epidermis and dermis, and increased vascularization. They are both dome shaped lesions but differ for some important features. Papules have an excoriated or intact superficial

component that overlies a hyper-echogenic inflammatory infiltrate in the superficial dermis. Pustules differ from papules because they usually present a more convex surface and contain multiple internal cavities filled with hyper-echogenic materials from the epidermis to the dermis. In both papules and pustules the vascular network is prominent in the dermis, and reconstructed vascular flow is detectable often very close to the dermal core of the lesions, because of an ongoing remodelling process of the microcirculation inside the area. (Fig. 2c,d).

Apparently normal skin of acne patients doesn't reveal significant differences compared with the one of healthy subjects not affected by acne. On the contrary, previously reported observation with RCM showed the presence of small hyperkeratotic plugs in infundibula, identifying a potential pathogenetic substrate of acne. This is likely due to the differences in resolution of the two devices. Infundibular alterations are often small in early lesions and in pre-lesional skin, and, to be identified, they require high-resolution microscopic images with a sharp definition of the different keratinocyte morphologies. On the other hand, D-OCT obtains images with a higher depth of penetration (up to 2 mm) compared to RCM. Additionally, D-OCT provides information of the dermal blood flow, which is of great value in the characterization of the inflammatory process and in the determination of the functional and metabolic features of the skin diseases.[16]

Sequential D-OCT images of acne lesions can show the changes affecting the dermal microcirculation during the comedogenetic process and during their evolution and resolution. Acne inflammatory and scarring process deteriorates the microcirculation network in the inflammation stage, explained by edema formation, leading to damages in the dermal microvasculature, initiating a fibrotic process that ends in hypertrophic scarring.[16,17] The morphologic and vascular alterations occurring during the pathological process of the disease, probably with a central role in the maintenance and worsening of acne, could be non

invasively monitored through sequential D-OCT image acquisition. Along with the development of acne, the progressive resolution of the disease is also of great value for the understanding of the pathogenesis and for the optimal management of the disease. The identification of morphologic and vascular changes during the follow up of acne lesions of patients assuming antibiotic treatment demonstrated an early reduction in the increased vascular network (day 20) and a subsequent normalization of the other acne associated parameters with time (day 60). Although further studies, evaluating the evolution of the different therapies and combinations, are needed, our data seems to favor microvascular D-OCT changes as an important functional parameter in the evaluation of acne associated inflammatory reactions.

Conclusions

Although there are other non-invasive techniques available for the evaluation of the skin physiopathologic parameters, the D-OCT offers a fast and reliable evaluation of the gross morphology of the acne lesions and of the microvascular architecture and changes in blood flow through microscopic imaging, opening a new field in dermatologic research. The characterization of acne lesions and the identification of subclinical alterations at the beginning or during the course of the therapeutic management could have crucial clinical consequences in the assessment of the severity, evolution and management of the patient. The ideal combination with RCM data can allow a complete investigation of acne patients adding high resolution details of skin surface to the lesion morphology overview and microcirculatory architecture. Although further studies are required, D-OCT represents a promising technique for the in vivo skin investigation.

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FIGURE LEGENDS

Figure 1. Closed and Open Comedos

Closed and open comedo aspect upon OCT aquisition. (a) Cross-sectional OCT image characterized by a inverse v-shaped morphology of the closed comedo, with a short infundibular opening (161 µm) (upper dashed line) and a wider diameter at 1mm depth (615 µm) (lower dashed line). (b) Cross-sectional vascular OCT image and (c) reconstructed 3D image of the same closed comedo, showing that the vascular signal is increased in perilesional skin, but in correspondence of the core of the comedo is lacking (ellipse). (d) Cross-sectional OCT image characterized by a rectangualr-shaped morphology of the open comedo, with a large infundibular opening (685 µm) (upper dashed line) and a similar diameter at 1mm depth (743 µm) (lower dashed line). (e) Cross-sectional vascular OCT image and (f) reconstructed 3D image of the same open comedo (ellipse).

Figure 2. Papules and Pustules

Papules and pustules aspect upon OCT aquisition. (a) Cross-sectional OCT image of a papule characterized by intact epidermis, dome shaped morphology and flattening of the rete ridges (dashed ellipse) (b) Cross-sectional vascular OCT image and (c) Reconstructed 3D image of the same papule, showing increased vascularity in the core (dashed ellipse) and in the perilesional skin. (d) Cross-sectional OCT image of a pustule, characterized by a dome shaped morphology and multiple central oval cavities containing hyper-intense inflammatory infiltrate (arrows) (e) Cross-sectional vascular OCT image and (f) reconstructed 3D image of the same pustule (dashed ellipse).

Figure 3. Uninvolved skin of the face

Two different cross-sectional morphological and vascular OCT image of the same hair follicle (a,b and c,d). The epidermis is intact, the infundibular diameter is very short and close to the hair shaft (arrow), rete ridges is regular. In this case, the dermal component of the pilosebacous unit appears slightly enlarged (oval).

Table 1. D-OCT parameters for the evaluation of acne lesions

Table 2. Relative frequencies of D-OCT parameters detected in acne lesions and in apparently uninvolved skin of acne patients.

Table 3. D-OCT parameters detected in 40 acne lesions during systemic antibiotic treatment.

Table 1. D-OCT parameters for the evaluation of acne lesions

D-OCT CRITERIA		
PILO-SEBACEOUS FOLLICLES /ACNE LESION	INFUNDIBULAR DIAMETER	Main axis of the infundibulum/lesion, measured in μm. The measurement was taken at the skin surface.
	1MM DEPTH DIAMETER	Main axis of the lesion, measured in μm. The measurement was taken at a conventional depth of 1mm from the skin surface.
	Structural D-OCT image patterns. (evaluated for presence/absence)	- **Interrupted entrance signal** corresponding to desquamation or detachment of scales from the corneum. - **Increased epidermal thickness,** it corresponds to an increase in the thickness of the epidermal portion of the acne lesion. It is measured from the entrance signal to the dermal epidermal junction, being at least >1.5 times higher than the epidermal thickness at the periphery of the image. - **Vertical Hypoechogenic structures** corresponding to vertical dark areas at the boundaries of the lesion. It can be caused either by edema or by hyperkeratosis on the skin surface, that blocks the interferometric signal. - **Granular hyperechogenic material** corresponding to the collection of organized cells inside the dermal portion of the comedo.
	Vascular D-OCT image patterns. (evaluated for presence/absence)	- **Focal absence of vascular signal,** corresponding to an area where the vascular network in the dermis is absent. - **Hypervascular spikes**, corresponding to the presence of increased blood flow in proximity with the dermal epidermal juncion - **Increased vascular network**, corresponding to a narrowing of the vascular network with a subsequent increase of the vascularity in the dermis.

Table 2. Relative frequencies of D-OCT parameters detected in acne lesions and in apparently uninvolved skin of acne patients.

D-OCT CRITERIA:		**Follicles in acne patients (31 evaluations)**	**CLOSED COMEDOS (28 lesions)**	**OPEN COMEDOS (20 evaluations)**	**PAPULES (22 lesions)**	**PUSTULES (21 lesions)**
INFUNDIBULAR DIAMETER (μm)		96 (SD 82)	173 (SD 91)	472 (SD 207)	317 (SD 217)	523 (SD 428)
1MM DEPTH DIAMETER		212 (SD 130)	668 (SD 372)	643 (SD 322)	730 (SD 408)	1105 (SD 612)
Structural D-OCT image patterns. (evaluated for presence/absence)	**Interrupted entrance signal**	19.3%	100%	25.0%	63.6%	80.9%
	Increased epidermal thickness	12.9%	70.0 %	89.2 %	90.9 %	85.7 %
	Large Hypoechogenic structure	38.7 %	90.0%	57.1%	77.2 %	80.9 %
	Granular hyperechogenic material	0.0%	20.0%	35.7 %	54.5 %	100.0 %
Vascular D-OCT image patterns. (evaluated for presence/absence)	**Focal absence of vascular signal**	16.1%	70.0%	60.7%	9.1%	9.5%
	Hypervascular spikes	25.8%	30.0%	32.1%	63.6%	85.7%
	Increased vascular network	6.4%	20.0%	25.0%	63.6%	90.4%

Table 3. D-OCT parameters detected in 40 acne lesions during systemic antibiotic treatment.

D-OCT CRITERIA:		**T0 (Baseline)**	**T1 (20 days)**	**T2 (45 days)**	**T3 (60 days)**
INFUNDIBULAR DIAMETER (μm)		382.25 μm	342.5 μm	253.5 μm *	184.0 μm *
1MM DEPTH DIAMETER		695.5 μm	310.4 μm *	271.3 μm *	262.5 μm *
Structural D-OCT image patterns. (evaluated for presence/absence)	**Interrupted entrance signal**	60%	62.5%	55 %	40 % *
	Increased epidermal thickness	85%	70.0 %	75.0 %	40 % *
	Large Hypoechogenic structure	85%	87.5 %	70.0 %	40 % *
	Granular hyperechogenic material	40%	20.0% *	0 %	0 %
Vascular D-OCT image patterns. (evaluated for presence/absence)	**Focal absence of vascular signal**	35%	32.5 %	42.5 %	27.5 %
	Hypervascular spikes	57.5%	55.0%	42.5%	42.5 %
	Increased vascular network	55%	22.5% *	25.0% *	20.0% *

* significant reduction from baseline (McNamar's test, p value of 0.05)