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In vivo confocal microscopy evaluation of ocular and cutaneous alterations in patients with rosacea

Hong Liang,[1,2,3,4,5] Matthieu Randon,[1,2] Sylvain Michee,[1,2] Rachid Tahiri,[1,2] Antoine Labbe,[1,2,3,4,5,6] Christophe Baudouin[1,2,3,4,5,6]

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[1]Department of Ophthalmology III, Quinze-Vingts National Ophthalmology Hospital, Paris, France
[2]DHU View Maintain, INSERM-DHOS, Center of Clinical Investigations (CIC) 1423, Quinze-Vingts National Ophthalmology Hospital, Paris, France
[3]INSERM, U968, Paris, France
[4]UMR S 968, Institut de la Vision, UPMC University Paris 06, Paris, France
[5]CNRS, UMR 7210, Paris, France
[6]Department of Ophthalmology, Ambroise Paré Hospital, APHP, University of Versailles Saint-Quentin-en-Yvelines, Versailles, France

Correspondence to
Dr Hong Liang, Department of Ophthalmology III, Quinze-Vingts National Ophthalmology Hospital, 28 rue de Charenton, Paris 75012, France; lianghongfr@yahoo.fr

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ABSTRACT

Aims The physiopathology of rosacea and the correlation between ocular and cutaneous rosacea remains unclear. This study analysed ocular and cutaneous rosacea with in vivo confocal microscopy (IVCM).

Methods Thirty-four eyes of 34 patients with confirmed rosacea-associated meibomian gland dysfunction-related evaporative dry eye were enrolled in the study. The ophthalmological investigations included dry eye ocular surface disease index (OSDI), the Schirmer test, tear osmolarity, tear break up time, the Oxford score, infrared meibography for meibomian gland (MG) analysis and IVCM investigation for cornea, MG and skin analysis (cheek, hand). Presences of *Demodex* in the MG and in the cheek were also investigated. We established scores for quantifying the MG alterations in the MG (IVCM-MG) and cheek (IVCM-Cheek), and scores for *Demodex* quantification in the MG and cheek (IVCM-MG-Dex and IVCM-Cheek-Dex).

Results IVCM was relevant for analysing the cornea and MG structures and was also suitable for cutaneous analysis. Exposed skin explorations presented the epidermal and dermal layers clearly. In patients with rosacea, the IVCM-MG alteration scores were correlated with IVCM-Cheek (R^2=0.27 and p=0.0006) and IVCM-MG-Dex was correlated with IVCM-Cheek-Dex (R^2=0.70 and p<0.0001). However, no correlation was found between the IVCM-MG or IVCM-Cheek and the break up time, Schirmer, Oxford and osmolarity evaluations.

Conclusions IVCM could be a safe, effective and reliable tool to quantify alterations of the cornea, MG and cheek glands in patients with rosacea combined with quantification of *Demodex* infections. As a valuable tool for investigating the pathophysiology of the disease, it could be used to assess the effectiveness of therapy.

INTRODUCTION

Rosacea was originally thought to be a skin disorder, but actually combines multiple signs and symptoms including cutaneous and ocular disorders. The physiopathology of rosacea deals with abnormal inflammation, vascular dysfunction, the involvement of several microbial agents such as commensal *Demodex* mites and *Demodex*-associated bacteria *Bacillus oleronius*, *Helicobacter pylori*, *Staphylococcus epidermidis* and so on.[1 2] The prevalence of ocular rosacea varies depending on ophthalmological and dermatological studies, ranging from 6% to 72%, and almost 50% of patients suffering from cutaneous rosacea also suffer from ocular rosacea.[3 4] Approximately, 10 million patients are affected by ocular rosacea in the USA with no gender difference.[5 6] Most particularly, when ocular rosacea occurs in the absence of skin involvement, the disease is often underdiagnosed.[5]

For cutaneous dermatological rosacea, primary and secondary features of rosacea were defined as four subtypes: erythematoid telangiectatic, papulopustular, phymatoid and ocular rosacea with granulomatous rosacea.[7 8] For the ophthalmologist, the diagnosis of ocular rosacea is essentially clinical, such as symptoms of foreign body sensation, dryness, itching, photophobia and tearing with the clinical signs of anterior blepharitis and meibomian gland dysfunction (MGD) with telangiectasia.[9 10] Recurrent ocular rosacea results in the severe bilateral phlyctenular keratoconjunctivitis with painful, bilateral, elevated vascularised corneal lesions.[11] In the most severe cases of rosacea-associated ocular-surface diseases, the cornea could be altered, inducing a decrease in visual acuity, central infiltrates, stromal ulcerations and even perforation if left untreated.[12–14]

In most previously published studies, dermatologists and ophthalmologists investigated the two forms of rosacea separately. It could be advantageous to explore both because close connections exist between cutaneous and ocular rosacea. To better understand the physiopathology of rosacea and the correlation between ocular and cutaneous rosacea, we conducted this study to explore the two rosacea forms using a set of clinical examinations of the ocular surface and in vivo confocal microscopy (IVCM), a technology initially developed for exploring the cornea. After accumulating experience in different ocular surface pathologies, we investigated here the cutaneous and ocular alterations in patients with rosacea. For the first time, we used this non-invasive method to collect data on the alterations of various tissues, including cornea, conjunctiva, meibomian glands (MGs) and cheek skin and evaluated the implication of *Demodex* infestation.

PATIENTS AND METHODS

Thirty-four patients with rosacea were included in this study: 20 females and 14 males, with a mean age of 58.88 years±14.16 (range, 27–81). The diagnosis of rosacea-associated MGD was based on persistent facial redness in the forehead, nose, eyelid, cheek or chin; morphological features of the MG and its duct orifices; and presence of orifice plugging and thickening or absence of expressed excreta. All the patients included presented with ocular rosacea but not cutaneous rosacea. They did not receive a dermatologist's diagnosis because they did not experience symptoms of cutaneous rosacea.

Consequently, the patients did not receive any special systemic treatment for their cutaneous rosacea. We examined the patients under normal conditions without any special cleaning preparation at examination. We chose the most affected eye according to the patients' symptoms when examined. The exclusion criteria were the following: age <18 years, contact lens wear, previous eye surgery and current use or use within the last 6 months of anti-inflammatory eye drops or cutaneous or other medications for any other systemic disease.

This study was conducted at the Clinical Investigations Center (CIC) 1423, Quinze-Vingts National Ophthalmology Hospital, from January 2013 to December 2014 with the approval of the Research Ethics Committee (CCP 5, No 10793). Ten normal subjects were also investigated to compare with patients with rosacea.

Infrared meibography analysis with the meiboscore

We used the OCULUS Keratograph 5M (Oculus, Wetzlar, Germany) to analyse MG alterations. The meiboscore was analysed using a previously published score[15] from 0 to 3: 0, no loss; 1, loss of less than 33%; 2, loss between 33% and 66%; 3, loss of more than 66%.

Cutaneous clinical examination

The redness of the each patient's face was analysed under the same intensity of room luminosity[4]: 0, no redness was visible; 1, mild redness was visible on the forehead, nose, eyelid, cheek or chin; 2, severe redness was visible on the forehead, nose, eyelid, cheek or chin. Papules, pustules or rhinophyma was classified in score 3.

In vivo confocal microscopy analysis of ocular and cutaneous tissues

IVCM images for ocular (cornea and MG) and cutaneous tissues (hand and cheek) were all obtained using the Rostock Cornea Module of the Heidelberg Retina Tomograph (Heidelberg Engineering GmbH, Heidelberg, Germany), which offers images comprising 384×384 pixels covering an area of 400×400 μm.

Before the cornea and MG examinations, one drop of topical anaesthetic (oxybuprocaine 0.4%, MSD-Chibret, Paris, France) combined with one drop of gel tear substitute (Lacrigel, carbomer 0.2%, Europhtha, Monaco) were instilled. The IVCM cornea analysis focused on the basal epithelium layer and inflammatory cell density combined with corneal nerve aspects. Using the Cell Count software (Heidelberg Engineering GmbH), dendritic inflammatory cell density (IVCM-cornea-inf) was quantified at the Bowman layer in the central cornea. For MG analysis (figure 1A), the inferior eyelid was reverted and the objective of IVCM was lightly put in contact with the MG area. We were able to analyse all exposed skin such as cheeks and hands directly, two cutaneous areas that are easy to access. For the cheek (figure 1B) and hand cutaneous analysis (figure 1C), the IVCM lens was placed on the skin without any anaesthesia. No pain and no injury were created after contact with the IVCM lens. For cheek analysis, the patients' cheek was placed in front of the lens. The epidermal and dermal layers of the skin were imaged directly by IVCM, like the IVCM cornea investigation. The hair, follicle, sebaceous gland and sweat gland were analysed. The depths of different layers were determined by the Z mode by turning off the IVCM objective.

IVCM quantification scores for meibomian glands: IVCM-MG

IVCM was used to quantify the MG alterations based on three criteria according to published scores:[16–18] meibum reflectivity (M), inflammation (I) and fibrosis (F). The *M* score: 0, homogeneous hyporeflective meibum; 1, some focal heterogeneous reflectivity; 2, multiple heterogeneous reflectivity; 3 very heterogeneous reflectivity. The *I* score (inflammatory cell infiltration, which presented intraepithelial or interglandular hyperreflective patterns): 0, no hyperreflective patterns; 1, some intraepithelial hyperreflective patterns; 2, numerous intraepithelial hyperreflective patterns; 3, numerous intraepithelial and interglandular points. The *F* score (epithelial fibrosis and rarely interglandular fibrosis): 0, normal epithelium; 1, mild fibrosis; 2, severe fibrosis.

Then we defined three confocal microscopy stages using the three above-mentioned criteria: stage 0, no meibum alteration nor epithelium inflammation (R:0/I:0/F:0); stage 1, meibum alteration without severe inflammation (R:1/2/3, I:0/1, F:0); stage 2, meibum alteration with severe inflammation and no or mild fibrosis (R:1/2/3, I:2/3, F:0/1); and stage 3, fibrosis, atrophy even with a gland density decrease (R:1/2/3, I:1/2/3, F:2).

IVCM quantification scores for cheek glands: IVCM-Cheek

We also scored IVCM-Cheek from 0 to 2 based on our observations of MG aspects: 0, normal reflectivity of skin glands (essentially the sweat gland and sebaceous gland analysis); 1, mild reflectivity of skin glands; 2, severe reflectivity of skin glands with dilation.

IVCM quantification for *Demodex* in MG (IVCM-MG-Dex) and on the cheek (IVCM-Cheek-Dex)

In eyelid lashes/MGs and on the cheek, we also scored the number of *Demodex* observed with IVCM according to our experience in *Demodex* observations:[19] a score of 0, no *Demodex* visible; 1, fewer than two *Demodex* in a follicle or fewer than eight *Demodex* counted; 2, more than three *Demodex* visible in a follicle or more than eight *Demodex* counted.

Statistical analysis

The statistical values of the different parameters (mean value ±SE) were calculated. The correlation between the IVCM-MG versus IVCM-Cheek and IVCM-MG-Dex versus IVCM-Cheek-Dex was determined using a one-way Spearman correlation in GraphPad PRISM (GraphPad Software, La Jolla, California, USA). Probability values $p<0.05$ were considered significant.

RESULTS

Normal cheek, hand and eyelid IVCM analysis

In normal subjects, we used IVCM to analyse different structures including the eyelid, the cheek and the hand. By reversing the inferior eyelid (figure 1A), the superficial epithelium of the inferior eyelid appeared as a hyperreflective mosaic-like structure (figure 1D), and in the deeper layers, the MGs (figure 1G) appeared as grey homogeneous acini. For cheek analysis, the objective of IVCM was put in contact with the cheek's surface without any pressure (figure 1B). We observed the hair and its follicles in the epidermal layer (figure 1E) and in the deeper layers; the larger follicle (figure 1H) showed round keratinised structures. Concerning the IVCM for the hand skin exploration (figure 1C), the superficial epithelium was also observed as hyperreflective patterns (figure 1F) with numerous papillae (figure 1I) in the dermal layer.

Rosacea cheek IVCM exploration with score

The characteristics of normal subjects and patients with MGD are shown in table 1. No significant difference was found

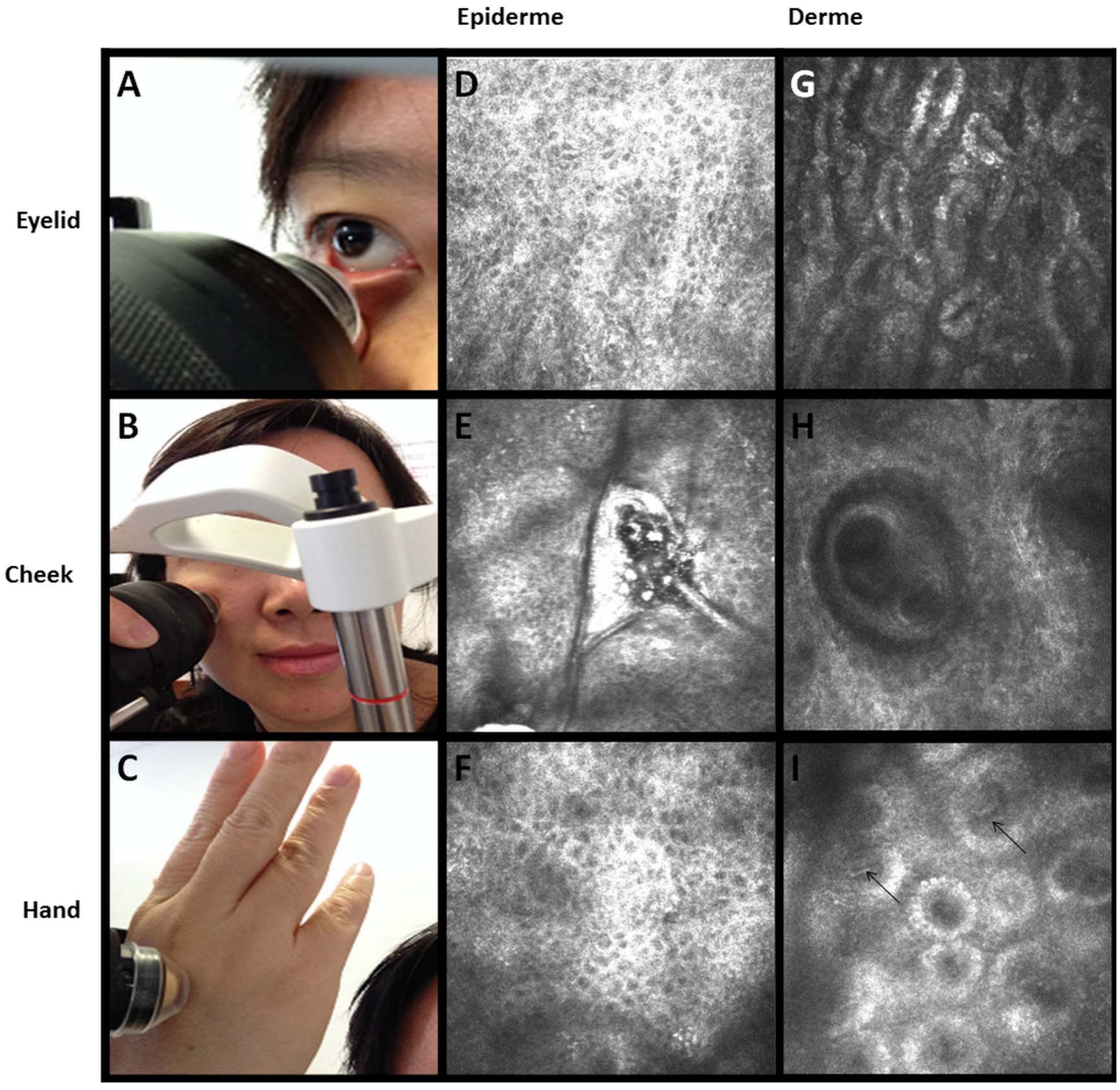

Figure 1 In vivo confocal microscopy (IVCM) for cheek, hand and eyelid explorations in normal eyes. Contact of the IVCM's lens on the surface of the inferior eyelid (A), the cheek (B) and the hand (C). The superficial epithelium of the inferior eyelid appeared more hyperreflective than the cheek cutaneous tissue (D), and the meibomian glands were well organised as acini-form structures (G). In the cheek, we observed the hair and its follicle in the epidermal layer (E), with a follicular structure (H). In IVCM hand skin analysis, the superficial epithelium was observed as hyperreflective aspects (F), and the several tiny papillae appeared as round forms with the visible capillaries (I, arrows).

between gender and age for the control and rosacea groups. In patients with rosacea, we observed obvious redness of the cheek and typical rhinophyma with a score of 3. We also scored IVCM-Cheek from 0 to 2. In normal subjects (figure 2A) or in the patient with mild disease (figure 2B), we observed nearly normal follicle and normal gland aspects. The sweat gland and sebaceous gland appeared dark and grey aspects, with the score of 0. In the moderate disease, a reflectivity of skin glands was observed with the score of 1 (figure 2C, D). We also counted more glands. Finally, in severe rosacea disease, we observed white reflectivity of the skin glands with several gland dilations or the papillae (figure 2E, F for another patient), which curiously resembled the MG aspect in eyelids.

Meiboscore, IVCM-MG/Cheek and IVCM-MG/Cheek-Dex distributions

Of all the patients observed, the distribution of patients with different scores is shown in figure 3A for the MG analysis and figure 3B for the cutaneous analysis. For example, for the Keratograph meiboscore analysis, 5 patients scored 0, 13 patients scored 1, 11 patients scored 2 and 5 patients scored 3.

Table 1 Characteristics of normal subjects and patients with meibomian gland dysfunction (MGD)

	N	Men/women	Mean age (years)	OSDI	SPEED	Schirmer test	Osmolarity
Healthy volunteers	10	5/5	42.50	<10	<5	15.52	294.50
Patients with MGD	34	14/20	61.82	39.98	13.82	10.96	308.40

OSDI, dry eye ocular surface disease index; SPEED, standard patient evaluation of eye dryness.

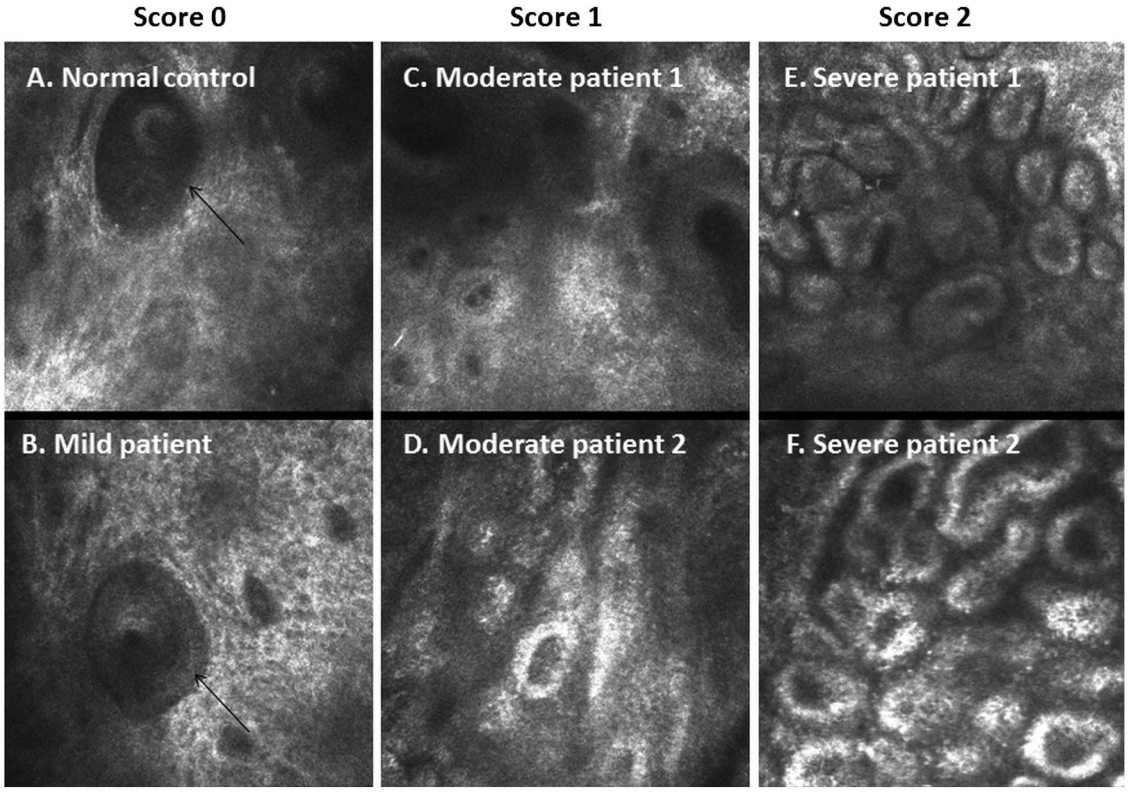

Figure 2 In vivo confocal microscopy (IVCM) for cheek gland explorations with IVCM-Cheek score. We scored cheek gland aspects from 0 to 2 in patients with rosacea with or without obvious redness in the nose and cheeks. In normal subjects (A) or those with minor rosacea (B), we observed normal reflectivity of skin glands with hair follicles (arrows). The sweat gland and sebaceous gland with abnormal *papillae* appeared as dark and grey area sand, with the score of 0. In moderate rosacea diseases (C and D), mild reflectivity of skin glands was observed with the score of 1. Finally, in severe rosacea diseases, we observed a white reflectivity of skin glands with several irregular gland dilations (E and F).

Correlations for IVCM analysis in normal and rosacea patients

Significant differences were found between the normal subjects and the patients with rosacea for IVCM-inf, IVCM-MG, IVCM-MG-Dex, IVCM-Cheek and IVCM-Cheek-Dex analysis ($p<0.0001$) (table 2).

The correlation was significant for the analysis of the IVCM-MG with the IVCM-Cheek ($R^2=0.27$ and $p=0.0006$). A positive correlation was found for the *Demodex* analysis in the MG and in the cheek ($R^2=0.70$ and $p<0.0001$ between IVCM-MG-Dex and IVCM-Cheek-Dex) (see online supplementary data). No correlation was found between the Keratograph meiboscore with the IVCM-MG with $R^2=0.06$ and $p=0.14$.

No correlations were found between the IVCM-MG or IVCM-cheek with clinical indices of the ocular surface (break up time, Schirmer, Oxford and osmolarity) ($p>0.05$ for all correlation analyses).

Cornea, IVCM-MG/Cheek and IVCM-MG/Cheek-Dex investigations

We used IVCM to investigate the correlation between corneal inflammatory infiltration with IVCM-MG/Cheek and IVCM-MG/Cheek-Dex (figure 4). In normal subjects (line 1), the corneal nerves presented a normal aspect with very few inflammatory cells (figure 4A). The MG presented grey homogeneous aspects with no obvious inflammatory infiltration (figure 4B) or *Demodex* (figure 4C). In the cheek skin (figure 4D), a normal follicle aspect was observed and no *Demodex* was observed (figure 4E). In some patients with rosacea, even though the cornea presented a relatively normal aspect (figure 4F, 18 cells/mm^2), the MG presented hyperreflective aspects (figure 4G) with *Demodex* in the follicle (figure 4H) combined with round hyperkeratinised hyperreflective structures. Active proliferative or fibrotic or infiltrative aspects of the sebaceous/sweat glands were observed in the cheek skin (figure 4I). Three *Demodex* were found in the follicle of the cheek (figure 4J). The Demodex was identified as in a previous study.[19] The Demodex body was observed as an elongated structure measuring approximately 350 μm in length. The abdomen's reflectivity was heterogeneous and striated, and the cephalothorax was surrounded by the legs. However, in another patient with rosacea with many inflammatory cells infiltrating the cornea (figure 4K, 412 cells/mm^2), the MG presented an atrophic aspect (figure 4L) with a score of 3. *Demodex* was also observed in this patient in the MG (figure 4M) and the cheek (figure 4O). Some developed hyperreflective glands were also observed in the cheek skin (figure 4N).

We also tried to correlate the Oxford score values or the inflammatory level investigated by IVCM with IVCM-MG/Cheek (data not shown). No correlation was found between IVCM-cornea-inf and the Oxford score ($R^2=0.01$ and $p=0.55$), the IVCM-MG score ($R^2=0.02$ and $p=0.37$) or the IVCM-Cheek score ($R^2=0.01$ and $p=0.50$).

DISCUSSION

In previous studies, IVCM was developed to explore corneal pathologies and also found useful for exploring MGD. Ibrahim *et al*[20] showed the efficacy, sensitivity and specificity of IVCM in the diagnosis of MGD by showing strong and significant correlations with the status of ocular-surface disease: tear functions

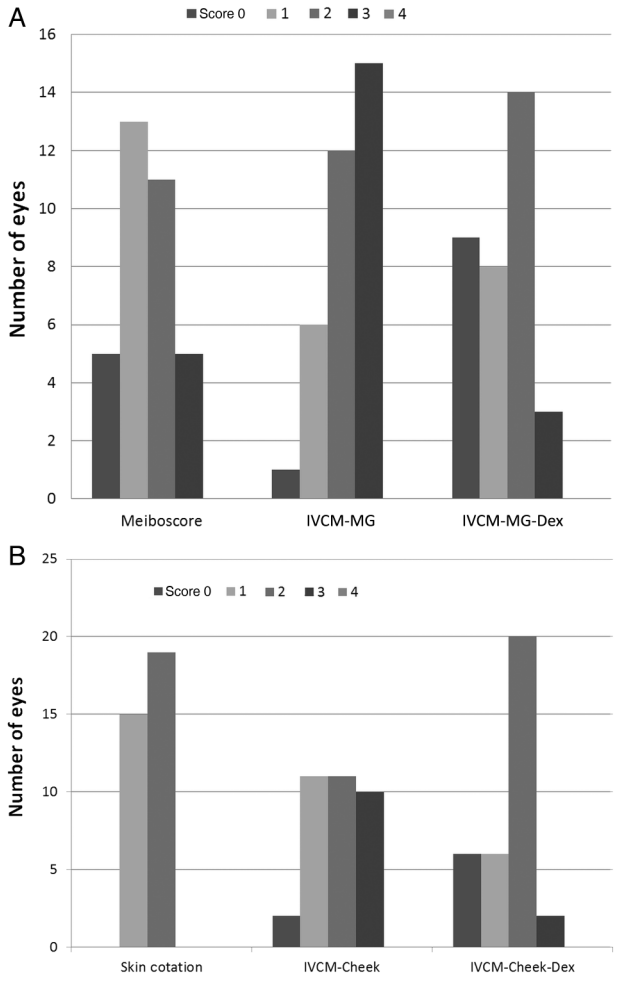

Figure 3 Meiboscore, in vivo confocal microscopy meibomian gland (IVCM-MG) and IVCM-MG-Dex and skin score, IVCM-Cheek and IVCM-Cheek-Dex in patients with rosacea. The distribution of patients with different scores is shown for the MG (A) and the skin (B) analyses.

(such as break-up time, fluorescein staining, rose bengal staining), ocular-surface vital stainings, MG expressibility and MG dropout grades. The importance of IVCM in examining the MG was also elaborated in age-related MG and conjunctival changes, patients with Sjögren syndrome, contact lens wearers, *Demodex* detection, patients with atopic keratoconjunctivitis and graft-versus-host disease.[20–23] However, until now, to our knowledge, no study has been conducted to explore the skin using corneal IVCM. In the present study, IVCM was used for the first time to explore the ocular and cutaneous aspects of patients with rosacea and their gland acini and inflammation infiltration as well as *Demodex*-associated infections. Evaluating cutaneous structures using IVCM analysis is not technically difficult. When the ophthalmologist is used to the corneal IVCM technique in the cornea or the eyelids, the cheek or other exposed skin areas are easier to explore than ocular structures. We focused on the inferior eyelid because it was more frequently involved than the upper eyelid according to a published article.[20] It is most important to use the IVCM lens to explore all the cheek parts thoroughly. The epithelium, melanocytes, wrinkles and glands are the essential patterns to observe. In this study, we concentrated on cheek skin analysis because the cheek is the site the most frequently involved for rosacea subtypes: 100% for flushing and 80% for papulopustules.[23]

Apart from inflammation and vascular problems, rosacea is considered to result from complex mechanisms, involving the innate and adaptive immune defence, neuroimmune relationships, blood vessels (vascular endothelial growth factor) and possibly lymphatic vessel abnormalities. A previous study has shown that there was a significant correlation of facial rosacea with lid margin inflammation levels.[24 25] In the patients with rosacea enrolled in our study, the same pathological inflammatory gland presented the same aspects in the eyelid MG and in the cheek structure, and the IVCM-MG was correlated significantly with IVCM-Cheek. All these data demonstrate the close links between eyelid alterations and cutaneous involvement in patients with rosacea as demonstrated by corneal confocal microscopy. In this study, we found the presence of *Demodex*, which presented at the same time in the ocular and cutaneous alterations of patients with rosacea. Even though the physiopathology implication of *Demodex* in patients with rosacea remains unknown, the literature has shown its importance in the strategy for treating patients with rosacea. A significant correlation was also determined between facial rosacea, lid margin inflammation and ocular *Demodex* infestation with serum immune reactivity of two proteins (62 and 83 kDa) derived from *B. oleronius*.[24]

This study also found that there were no correlations between the corneal alternations evaluated by Oxford score values with IVCM-cornea-inf. The Oxford score reflected epithelial deficiency, which was not correlated to the number of inflammatory infiltration cells in the basal Bowman layer. No correlation between the IVCM-cornea-inf existed with MG alterations, which revealed the discordance of two tissue reactions in the rosacea pathology.

This study initiated the morphological exploration of rosacea in dermatological and ophthalmological investigations, particularly in the comparison of MG and sebaceous/sweat glands in the cheek as well as the *Demodex* quantification in the eyelid and the cheek. In the future, it could be useful to compare the inflammatory biomarkers in the ocular and dermal forms of rosacea. Eventually, our objective is to find the same therapeutic strategy to cure patients with rosacea who often suffer from both forms of rosacea using a certain specific targeted antagonist.

We found limitations to using IVCM to detect the cheek structure, because we could neither perform skin biopsies to compare with IVCM images nor use any specific immunostaining procedure to identify inflammatory cell populations or fibrotic features. For this reason, we could not refine the skin gland score. Due to the direct contact between IVCM's objective with the patients' skin, it was difficult for us to standardise the accurate localisation for each patient. The IVCM lost its high resolution for the deeper structure due to the reflectivity of the dermal structure.

Table 2 IVCM analysis for healthy volunteers and patients with MGD

	Healthy volunteers	Patients with MGD
Oxford	0	0.6±0.52*
IVCM-inf	0.1±0.3	2.33±0.86*
IVCM-MG	0	1.90±1.00*
IVCM-MG-Dex	0	0.9±0.99*
IVCM-Cheek	0.1±0.3	1.40±0.70*
IVCM-Cheek-Dex	0	1.00±0.94*

*p<0.0001, compared with the normal eyes group.
IVCM, in vivo confocal microscopy; MG, meibomian gland; MGD, meibomian gland dysfunction.

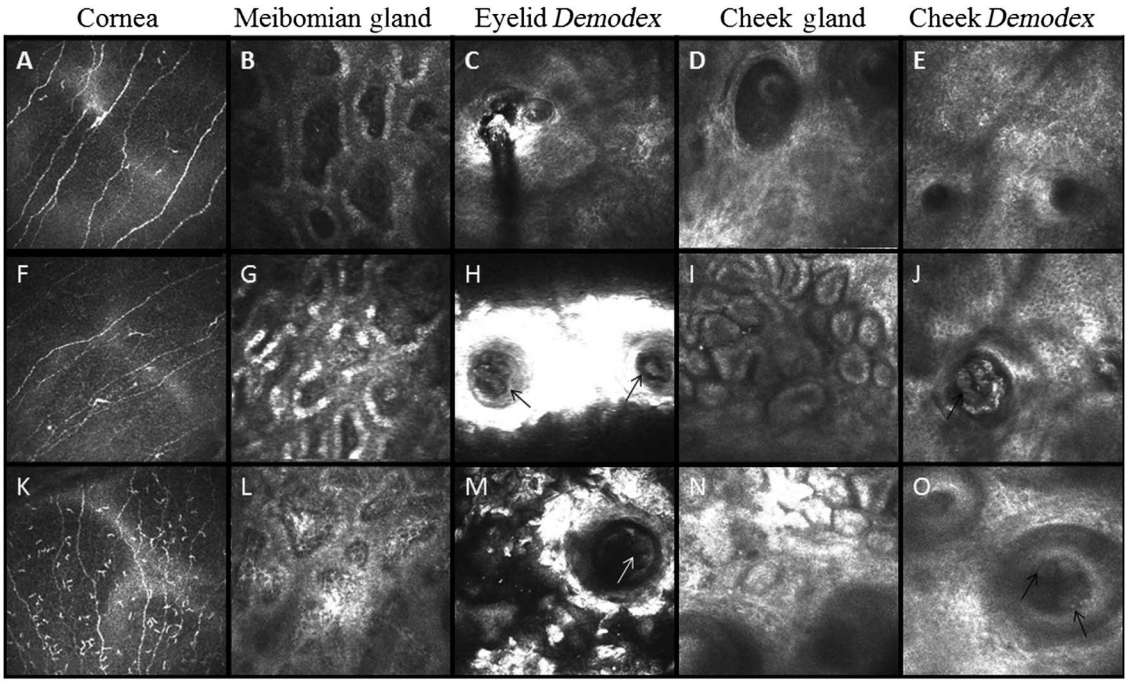

Figure 4 In vivo confocal microscopy (IVCM) in patients with rosacea for cornea, meibomian gland (MG) and cheek analyses. In normal eyes (line 1), the cornea presented few inflammatory cells (fewer than 25 cells/mm^2) with corneal nerves (A). The MG presented homogeneous and grey areas with no inflammatory infiltration (B) and no obvious *Demodex* was observed (C). In the cheek skin (D), a normal follicle aspect was observed without *Demodex* (E). In some patients with rosacea, even though the cornea presented little inflammatory cell infiltration (F), we observed hyperreflective MG (G) with *Demodex* in the follicle (H). The same aspect was observed, possibly a sweat gland, in the cheek skin (I) with three *Demodex* found in the follicle (J). In another patient with many inflammatory cells (K), the MG presented an atrophic aspect (L) with a score 3 with *Demodex* observed in the MG (M) and in the cheek (O). Some hyperreflective glands were also observed in the cheek skin (N).

In conclusion, our study demonstrated the usefulness of IVCM to examine the patient's cheek as a marker of rosacea. We could extend this method to sensitive patients or those experiencing pain, who have not undergone examinations requiring contact with the cornea, or to very young patients, as a surrogate for eyelid examination. The implication of IVCM in other dermatological pathologies, such as seborrhoeic dermatitis, atopy or psoriasis should also be explored when the diagnostic tools are limited or provide mistaken diagnoses.

Contributors Design of the work, or the acquisition, analysis or interpretation of data: HL, CB. The acquisition, analysis or interpretation of data: MR, SM, RT. Manuscript preparation: HL, MR, AL, CB.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval This study was conducted at the Clinical Investigations Center (CIC) 1423, Quinze-Vingts National Ophthalmology Hospital, from January 2013 to December 2014 with the approval of the Research Ethics Committee (CCP 5, No 10793).

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Hong Liang, Matthieu Randon, Sylvain Michee, Rachid Tahiri, Antoine Labbe and Christophe Baudouin

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