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Handheld In Vivo Reflectance Confocal Microscopy for the Diagnosis of Eyelid Margin and Conjunctival Tumors

Elisa Cinotti, MD, PhD; Aurélie Singer, MD; Bruno Labeille, MD; Damien Grivet, MD; Pietro Rubegni, MD, PhD; Catherine Douchet, MD; Frédéric Cambazard, MD, PhD; Gilles Thuret, MD, PhD; Philippe Gain, MD, PhD; Jean Luc Perrot, MD

IMPORTANCE The clinical diagnosis of conjunctival and eyelid margin tumors is challenging, and new noninvasive imaging techniques could be valuable in this field.

OBJECTIVE To assess the diagnostic accuracy of handheld in vivo reflectance confocal microscopy (IVCM) for the diagnosis of eyelid margin and conjunctival tumors.

DESIGN A prospective observational study was conducted at University Hospital of Saint-Etienne from January 2, 2011, to December 31, 2016 (inclusion of patients until December 31, 2015, and follow-up until December 31, 2016). A total of 278 consecutive patients with eyelid margin or conjunctival lesions were included. Conjunctival lesions were diagnosed with a conventional clinical examination using a slitlamp and by handheld IVCM. Final diagnoses were established by histopathologic examination for 155 neoformations suspicious for being malignant through clinical and/or IVCM examination that were excised and on follow-up of 12 months or longer for the remaining 140 lesions.

MAIN OUTCOMES AND MEASURES Sensitivity, specificity, and positive and negative predictive values for malignant tumors of the conjunctiva and eyelid margin were calculated using clinical examination with slitlamp and handheld IVCM.

RESULTS In the 278 patients (136 [48.9%] females; mean [SD] age, 59 [21] years), a total of 166 eyelid margin and 129 conjunctival lesions were included in the analysis. Of the 155 excised neoformations with a histopathologic diagnosis, IVCM showed higher sensitivity compared with clinical examination conducted with the slitlamp for malignant tumors of the eyelid margin (98% vs 92%) and conjunctiva (100% vs 88%). The specificity for malignant eyelid margin tumors was higher for IVCM than for slitlamp examination (74% vs 46%), but slightly less for malignant conjunctival tumors (78% vs 88%). Analysis of all neoformations (155 excised and 140 in follow-up) confirmed these differences in the diagnostic accuracy of the clinical examination and IVCM. The presence of hyperreflective Langerhans cells mimicking malignant melanocytes was the main cause for misdiagnosis of malignant conjunctival tumors with IVCM.

CONCLUSIONS AND RELEVANCE Handheld IVCM could be a useful tool for the identification of malignant conjunctival tumors. Further studies are required to confirm the usefulness of this device and identify possible features that can differentiate Langerhans cells from malignant melanocytes to prevent the misdiagnosis of melanoma using IVCM.

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Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Elisa Cinotti, MD, PhD, Department of Medical, Surgical, and Neurological Science, Dermatology Section, University of Siena, S Maria alle Scotte Hospital, Viale Bracci 16, 53100 Siena, Italy (elisacinotti@gmail.com).

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The diagnosis of eyelid margin and conjunctival malignant tumors is one of the biggest challenges in clinical ophthalmology, especially in an early phase. Differential diagnosis encompasses a large spectrum of conditions, from benign tumors to inflammatory diseases that are difficult to identify by clinical examination.[1] The need for surgical excision to reach a histologic diagnosis is frequent, with potential functional and aesthetic consequences in this sensitive area.

Apart from the slitlamp biomicroscope that can provide up to 40 × magnification, no other devices are routinely used to help in the clinical diagnosis of eyelid margin and conjunctival lesions.[1] In vivo confocal microscopy (IVCM) is a promising noninvasive tool in this area, providing horizontal greyscale images up to 1000 µm in depth with cellular resolution. Two in vivo reflectance confocal microscopes are available to explore the eye surface: a 4-slit scanning confocal microscope (Confoscan; Nidek Technologies) and a laser-scanning confocal microscope (Heidelberg Retina Tomograph; Heidelberg Engineering GmbH). However, due to their limited ease of handling, both microscopes are used mostly to examine the cornea; rarely are they utilized for the conjunctiva or eyelid margin. Our group recently used a skin-specific, handheld reflectance confocal microscope in examination of the conjunctiva[2-9] and presented preliminary data[4,9] on its diagnostic accuracy. These pilot studies suggest that this device could be efficiently used for noninvasive diagnosis of conjunctival tumors. Herein, we present a large series of conjunctival and eyelid margin tumors examined by handheld IVCM and evaluate the diagnostic accuracy of this technique for these lesions.

Key Points

Question What is the diagnostic accuracy of handheld in vivo reflectance confocal microscopy for diagnosis of conjunctival tumors?

Findings In an observational study of 295 lesions, in vivo reflectance confocal microscopy showed respective sensitivity and specificity values of 100% and 93% for conjunctival and 98% and 90% for eyelid margin malignant tumors.

Meaning These data suggest that in vivo reflectance confocal microscopy has good diagnostic accuracy for conjunctival tumors and could help clinicians to diagnose these lesions correctly.

Methods

Patients and Setting

A total of 278 consecutive patients were included in the study, with 136 (48.9%) females; mean (SD) age was 59 (21) years. Of these patients, 160 (83 females, 77 males; mean age, 65 years; range, 10-95 years) presenting with 166 eyelid margin lesions and 118 (53 females, 65 males; mean age, 51 years; range, 7-94 years) presenting with 129 conjunctival lesions were recruited at the Dermatology Department of the University Hospital of Saint-Etienne between January 2, 2011, and December 31, 2015 (inclusion of patients until December 31, 2015, and follow-up until December 31, 2016). Institutional review board approval was obtained from the University Hospital of Saint-Etienne. Informed consent was obtained orally during the first consultation and before the examination. Patients were instructed not to wear make-up and facial cream; if necessary, these cosmetics were removed before the examination.

Examined Lesion Diagnosis

Clinical (eTable in the Supplement) and IVCM diagnoses of all 295 lesions were prospectively established by a team of 3 dermatologists (E.C., B.L., and J.L.P.) and 3 ophthalmologists (A.S., D.G., and G.T.). A slitlamp examination was performed to establish the final clinical diagnosis.

For IVCM, the diagnostic criteria reported in **Table 1** were adopted. Surgical excision and histopathologic diagnosis were performed in 155 cases (99 eyelid margins and 56 conjunctivae) suspicious for malignant tumors under clinical and/or IVCM examination. The remaining 67 eyelid margin lesions and 73 conjunctival lesions were not excised because they were chronic and did not present any features suggestive of malignancy with clinical and IVCM examination. In addition, these lesions did not show any change following further clinical and IVCM monitoring for 12 months or more.

In Vivo Reflectance Confocal Microscopy Examination

Examination with IVCM was carried out with a handheld reflectance confocal microscope for skin imaging (VivaScope 3000; Caliber I.D.) equipped with an 830-nm diode laser that is not harmful to eyes and does not induce ocular glare (class 1B classification; Center for Devices and Radiological Health). Each image corresponds to a horizontal 920 × 920 µm section up to 250 µm in depth from the epithelial surface to the stroma with a high optical resolution (horizontal and vertical axis: 1.25 µm and 5 µm, respectively).

Before the examination, topical anesthesia was administered using oxybuprocaine hydrochloride, 1.6 mg/0.4 mL (Laboratoires Théa), and tetracaine hydrochloride, 1% (Laboratoires Théa), applied in the inferior conjunctival fornix of the eye, and a transparent ophthalmic gel of carbomer 974P (Laboratoires Théa) was applied to the ocular region to be examined. A disposable sterile transparent film (Visulin; Paul Hartmann AG) was applied to the tip of the IVCM for the first 217 lesions. For the remaining 78 lesions, the tip of the camera was disinfected by applying a layer of chlorine dioxide foam (Tristel Duo; Tristel Solutions Ltd) and by using ethanol wipes (Cidalkan; Alkapharm) before and after application of the foam. Examinations were performed with the patients in a supine position.

Statistical Analysis

Sensitivity, specificity, positive predictive value, and negative predictive value of the clinical examination and IVCM for malignant tumors were calculated for (1) the 155 lesions that were excised considering the histopathologic diagnoses as the criterion standard and (2) all 295 lesions considering the histopathologic diagnoses plus the diagnoses performed after the follow-up of 12 months or more as the criterion standard. Sensitivity, specificity, positive predictive value, and negative predictive value of clinical examination and IVCM for the different types of malignant tumors (basal cell

carcinoma [BCC], squamous cell carcinoma [SCC], melanoma [MM], and mucosa-associated lymphoid tissue [MALT] lymphoma) were calculated only for the 155 lesions that had histopathologic diagnoses.

Results

The diagnostic accuracy of the clinical and IVCM examinations for the different types of eyelid margin and conjunctival malignant tumors is reported in **Table 2**. In vivo reflectance confocal microscopy was more sensitive and specific than clinical examination with the slitlamp considering both the 155 excised lesions with a histopathologic diagnosis and the entire series of 295 lesions (lesions with histopathologic diagnosis and lesions with diagnosis based on the follow-up).

Diagnostic Accuracy for the 155 Excised Lesions

Higher sensitivity of IVCM compared with clinical examination conducted with the slitlamp was identified both for malignant tumors of the eyelid margin (98% vs 92%) and conjunctiva (100% vs 88%). Specificity was higher for IVCM than clinical examination for the malignant eyelid margin tumors (74% vs 46%), but not for malignant conjunctival tumors (78% vs 88%). No complications, such as ocular inflammation, infections, and/or mechanical trauma, were observed using IVCM. The time required for the IVCM examination was 5 to 10 minutes per lesion, whereas less than 5 minutes were necessary for slitlamp examination.

Eyelid Margin Lesions

Histopathologic examination of the 99 excised eyelid margin lesions showed 60 malignant tumors, including 49 BCCs, 4 SCCs, 7 MMs, and 39 benign tumors (18 dermal nevi, 1 solar lentigo, 3 epidermal cysts, 3 seborrheic keratoses, 1 actinic keratosis, 7 adnexal tumors, 3 compound nevi, 1 melanoacanthoma, 1 pyogenic granuloma, and 1 viral wart). Of the 99 excised lesions, 68 were for possible clinical differential diagnosis of BCC, and their histopathologic diagnoses were 49 BCCs, 1 solar lentigo, 9 dermal nevi, 6 adnexal tumors (3 hidrocystomas, 1 hamartoma, 1 trichoepithelioma, and 1 nodular hidradenoma), 2 epidermal cysts, and 1 actinic keratosis. Slitlamp examination failed to identify 5 BCCs (sensitivity, 90%) and misdiagnosed BCC in 21 cases (specificity, 46%). In vivo reflectance confocal microscopy agreed with histopathologic findings in 48 of 49 BCCs, showing sensitivity of 98% for BCC. However, IVCM misdiagnosed BCC in 3 cases of dermal nevus, 1 case of nodular hidradenoma, and 1 case of actinic keratosis, showing specificity of 74%.

Twenty-one cases were evaluated for possible clinical differential diagnosis of MM, and their histopathologic examination showed 7 MMs, 8 dermal nevi, 3 compound nevi, 1 melanoacanthoma, 1 epidermal cyst, and 1 adnexal tumor. Slitlamp examination diagnosed all MMs but misdiagnosed MM in 8 cases (specificity, 43%). In vivo reflectance confocal microscopy diagnosis was in agreement with the histopathologic examination in all 7 cases of MM, showing sensitivity of 100% for MM, but it misdiagnosed MM in 2 dermal nevi, 1 melanoacanthoma, 1 adnexal tumor, and 1 compound nevus, showing specificity of 64%.

Ten cases were evaluated for possible clinical differential diagnosis of SCC, and histopathologic examination indicated 1 basosquamous carcinoma, 3 SCCs, 1 dermal nevus, 1 viral wart,

Table 1. In Vivo Reflectance Confocal Microscopy Features Used to Diagnose Eyelid Margin and Conjunctival Lesions

Eyelid Margin and Conjunctival Lesions	Features
Actinic keratosis	Parakeratosis, hyperkeratosis of the stratum corneum of the epidermis, and atypical honeycomb pattern of the spinous-granular layer of the epidermis
Adnexal tumors	Hidradenoma: small dark silhouettes, cystic areas and duct-like structures; possible dilated blood vessels
	Trichoepithelioma: islands of uniform basaloid cells, sometimes showing peripheral palisading and small keratinous cysts lined by a stratified squamous epithelium
	Hidrocystoma: cysts lined by 2 layers of cells
Basal cell carcinoma	≥2 Of the following criteria: (1) dark silhouette, (2) lobular nests or trabecular structures of tightly packed cells, (3) peripheral palisading of elongated cells, (4) peritumoral clefts, (5) convoluted and dilated blood vessels, (6) polarized elongated keratinocytes (streaming) of the overlying epidermis
Epidermal cyst	Cystic cavity filled with keratin surrounded by a normal epithelium
Melanoacanthoma	Widespread proliferation of homogeneously distributed dendritic melanocytes in an acanthotic epithelium
Melanoma	Large dendritic or roundish hyperreflective cells at the epithelial-stromal junction and/or in the stroma associated with the possible presence of large pagetoid cells
Mucosa-associated lymphoid tissue lymphoma	Normal epithelium and abundant small hyperrefractive roundish cells corresponding to lymphocytes in the stroma
Nevus	Junctional nevus: hyperreflective, homogeneous, medium-sized (10-20 μm), roundish cells organized in nests at the epithelium-stromal junction, with the absence of (1) pagetoid cells, (2) atypical cells at the epithelium-stromal junction, and (3) disarrangement of the epithelial layers
	Dermal/subepithelial nevus: hyperreflective, homogeneous, medium-sized (10-20 μm), roundish cells organized in nests in the stroma, with the absence of (1) pagetoid cells, (2) atypical cells at the epithelium-stromal junction, and (3) disarrangement of the epithelial layers
	Compound nevus: features of junctional and dermal/subepithelial nevus
	Epithelial cystic nevus: same features as common nevi plus stromal pseudocystlike structures partly filled with monomorphous material
Pinguecula	Absence of epithelial atypia and subepithelial presence of degenerated stromal collagen that presented with a coiled shape; possible increased leukocytes (small, hyperrefractive, roundish homogeneous cells) in the stroma
Primary acquired melanosis	Hyperreflective cells confined to the basal layer of the epithelium and/or small pagetoid dendritic cells
Primary acquired melanosis with atypia	Hyperreflective, large dendritic and rarely roundish cells throughout the epithelium
Pterygium	Absence of epithelial atypia and subepithelial presence of a fibrovascular proliferation; possible increased leukocytes (small, hyperrefractive, roundish homogeneous cells) in the stroma
Seborrheic keratosis	Widening and interweaving of the epidermal rete ridges ("polycylic papillary contours") and horn pseudocysts
Solar lentigo	Hyperreflective basal keratinocytes
Squamous cell carcinoma	Disarranged pattern of the spinous-granular layer of the epidermis

Table 2. Diagnostic Accuracy of the Clinical Slitlamp Examination and Handheld IVCM

Characteristic	Sensitivity (95% CI)		Specificity (95% CI)		PPV (95% CI)		NPV (95% CI)	
	Clinical Examination	IVCM	Clinical Examination	IVCM	Clinical Examination	IVCM	Clinical Examination	IVCM
Neoformations With Histopathologic Diagnosis[a]								
Eyelid margin malignant tumor	92 (80-97)	98 (94-100)	46 (30-63)	74 (58-86)	72 (61-82)	86 (74-92)	78 (56-92)	97 (81-100)
BCC	90 (77-96)	98 (94-100)	37 (17-61)	74 (54-93)	79 (65-87)	91 (79-96)	58 (29-84)	93 (66-100)
SCC	100 (40-100)	100 (40-100)	83 (36-99)	100 (52-100)	80 (30-99)	100 (40-100)	100 (46-100)	100 (52-100)
MM	100 (56-100)	100 (56-100)	42 (18-70)	64 (36-86)	47 (22-73)	58 (29-84)	100 (52-100)	100 (63-100)
Conjunctival malignant tumor	88 (74-100)	100 (83-100)	88 (76-99)	78 (60-90)	84 (63-95)	77 (58-90)	90 (73-97)	100 (83-100)
SCC	100 (68-100)	100 (68-100)	40 (73-83)	100 (46-100)	79 (49-94)	100 (68-100)	100 (20-100)	100 (46-100)
MM	73 (39-93)	100 (68-100)	96 (79-100)	74 (53-88)	89 (51-99)	61 (36-82)	90 (72-97)	100 (80-100)
MALT lymphoma	100 (20-100)	100 (20-100)	NA	NA	100 (20-100)	100 (20-100)	NA	NA
Eyelid margin and conjunctival malignant tumors	90 (82-96)	99 (93-100)	65 (52-75)	76 (64-85)	75 (65-83)	83 (74-90)	85 (72-92)	98 (89-100)
Neoformations With Histopathologic and Follow-up Diagnosis[b]								
Eyelid margin malignant tumor	92 (81-97)	98 (90-100)	80 (71-87)	90 (83-95)	72 (61-82)	86 (74-92)	94 (87-97)	99 (94-100)
Conjunctival malignant tumor	88 (67-97)	100 (83-100)	96 (90-99)	93 (86-97)	84 (63-95)	77 (58-90)	97 (91-100)	100 (95-100)
Eyelid margin and conjunctival malignant tumors	90 (82-96)	99 (93-100)	88 (83-92)	92 (87-95)	75 (65-83)	83 (74-90)	96 (92-98)	100 (97-99)

Abbreviations: BCC, basal cell carcinoma; IVCM, in vivo reflective confocal microscopy; MALT, mucosa-associated lymphoid tissue; MM, malignant melanoma; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value; SCC, squamous cell carcinoma.

[a] Analysis of 155 lesions.

[b] Analysis of 295 lesions.

3 seborrheic keratoses, and 1 pyogenic granuloma. Slitlamp examination diagnosed all SCCs (sensitivity, 100%), but misdiagnosed SCC in the case of a viral wart (specificity, 83%). In vivo reflectance confocal microscopy agreed with the histopathologic examination except for 1 basosquamous carcinoma that was considered a BCC (sensitivity and specificity of 100% if we consider that both SCC and basosquamous carcinoma are malignant tumors).

Conjunctival Lesions

Histologic examination of the 56 excised conjunctival lesions showed 24 malignant tumors (11 MMs, 11 SCCs, and 2 MALT lymphomas) and 32 benign tumors (11 compound nevi, 6 epithelial cystic nevi, 2 subepithelial nevi, 3 junctional nevi, 6 primary acquired melanoses, 3 pinguecula, and 1 foreign body reaction). Sixteen cases were evaluated for possible clinical differential diagnosis of SCC, and their histopathologic examination showed 11 SCCs, 3 pingueculas, and 2 compound nevi. Slitlamp examination indicated sensitivity and specificity for SCC of 100% and 40%, respectively (3 pingueculas that presented as whitish nodules were diagnosed as SCC). In vivo reflectance confocal microscopy agreed with the histopathologic examination in all cases and had 100% sensitivity and specificity for SCC.

Thirty-eight cases were evaluated for possible clinical differential diagnosis of MM, and histopathologic examination showed 11 MMs, 2 subepithelial nevi, 6 epithelial cystic nevi, 9 compound nevi, 3 junctional nevi, 1 foreign body reaction, and 6 primary acquired melanoses. Slitlamp examination missed 3 MMs (sensitivity, 72%) and misdiagnosed 1 case of subepithelial nevus (specificity, 96%). In vivo reflectance confocal microscopy showed sensitivity of 100% for MM, but it misdiagnosed MM in 7 benign lesions (1 epithelial cystic nevus, 3 primary acquired melanoses without atypia, and 3 compound nevi), showing specificity of 74% for MM.

Two cases were clinically suspicious for MALT lymphomas, and histopathologic examination confirmed this diagnosis. Slitlamp and IVCM examinations agreed with the histopathologic diagnosis in both cases.

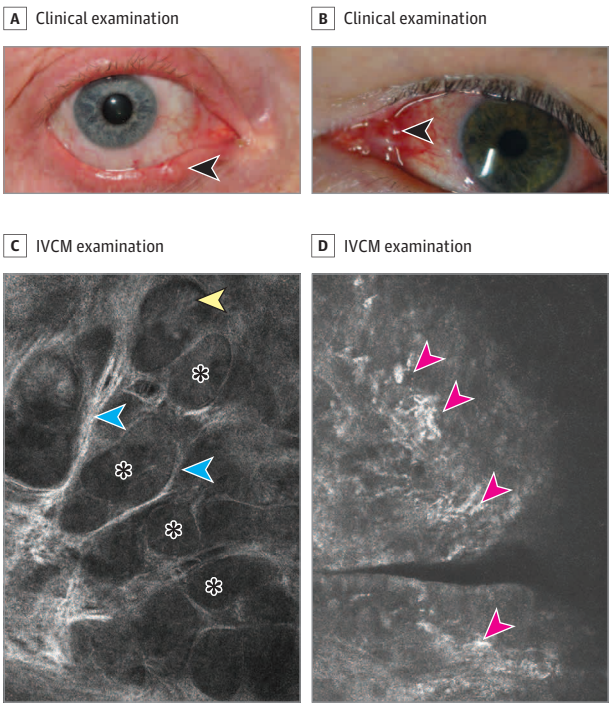
Diagnostic Accuracy for All 295 Lesions

For the entire series of lesions, we noted the same sensitivity results as for the series of 155 excised lesions. Specificity increased for both the clinical and IVCM examination because all lesions that were considered benign based on these 2 examinations did not show any sign of malignancy during the follow-up period and were counted as true-negative.

Discussion

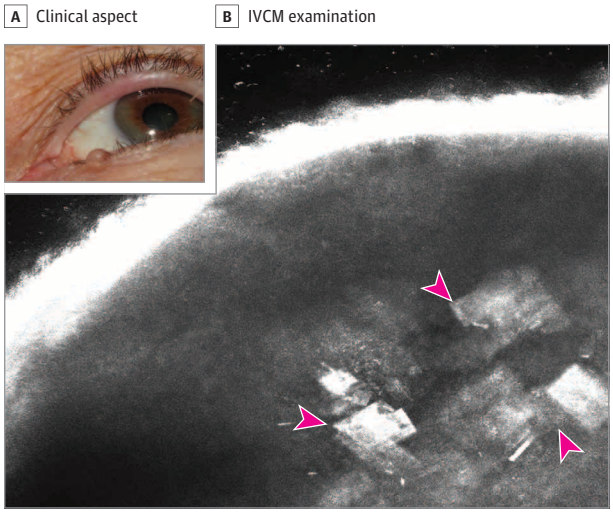
In this study, IVCM with a handheld dermatology microscope proved to be useful to diagnose conjunctival tumors. In addition, IVCM was more sensitive and specific than clinical examination performed with the slitlamp for diagnosis of eyelid margin tumors. Concerning conjunctival tumors, IVCM was more sensitive than clinical examination performed with the slitlamp, but had slightly less specificity for MM. Overall, IVCM did not fail to identify any malignant conjunctival tumors and missed only 1 eyelid margin BCC, whereas clinical examination with the slitlamp failed to identify 5 BCCs of the eyelid margin and 3 MMs of the conjunctiva (**Figure 1**). The BCC that was missed by IVCM examination was diagnosed as foreign

Figure 1. Basal Cell Carcinoma and Melanoma That Were Clinically Diagnosed as Benign and That Were Correctly Diagnosed Using In Vivo Reflectance Confocal Microscopy (IVCM)



Clinical presentation of the basal cell carcinoma (A) and the melanoma (B) indicated with black arrowhead; IVCM, showing a typical aspect of basal cell carcinoma with tumor islands (asterisks) with peripheral palisading cells (yellow arrowhead) surrounded by hyperreflective collagen (blue arrowheads) (C); and a proliferation of large and irregular hyperreflective cells in the stroma suggestive of malignant melanocytes (red arrowheads) (D).

Figure 2. Basal Cell Carcinoma of the Eyelid Margin That Was Misdiagnosed as Foreign Body Granuloma



A, Clinical aspect. B, Hyperreflective, crystal-like bodies shown by in vivo reflectance confocal microscopy (IVCM) on the superficial part of the eyelid margin (red arrowheads).

body granuloma because of the presence of crystal-like bodies (**Figure 2**). The hyperreflectance of these bodies probably hindered the visibility of the deeper layers of the stroma with the BCC. We also tried to analyze the make-up products (foundation and facial creams) used by the patient with IVCM to evaluate a correspondence between potential make-up residues and these structures, but we could not find any correlation. An alternative hypothesis about these crystal-like bodies is that they might correspond to cholesterol crystals similar to what has been reported in an epidermal cyst.[10] We did not observe the presence of similar bodies or any other structures suggestive of exogenous material in the other lesions.

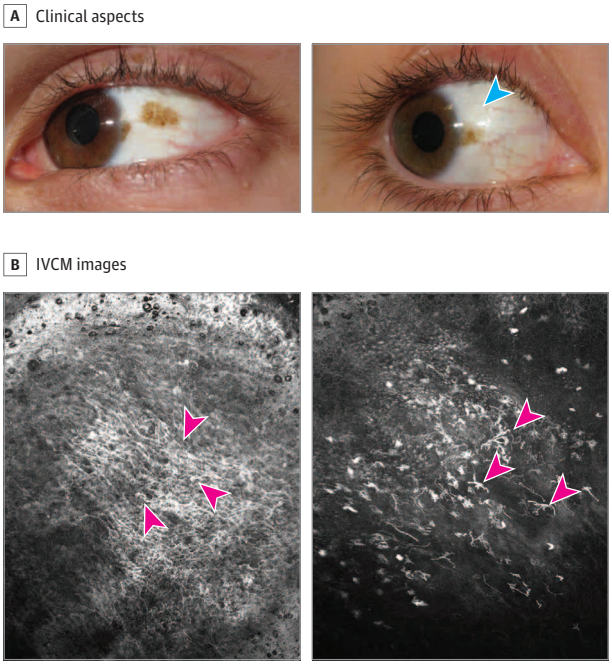
One lesion clinically suggestive of SCC of the eyelid margin was diagnosed as BCC with IVCM but was identified as basosquamous carcinoma on histopathologic examination. Although the IVCM diagnosis was not accurate, it was considered correct for the calculation of IVCM sensitivity because the lesion was diagnosed as malignant and the treatment did not change. Retrospective examination of this case found the absence of the peripheral palisading of elongated cells typical of BCC and of enlarged vessels that are usually present in both BCC and SCC.

Specificity of IVCM was high but did not prevent us from excising some benign tumors. One limitation of this technique could result from the use of a disposable sterile plastic film applied to the tip of the IVCM from the beginning of the study until May 2015 (first 217 lesions), which reduced the quality of the images. Establishing the right diagnosis for these lesions was more difficult as demonstrated by the fact that all of the false-positive cases of the eyelid margin tumors (MMs and BCCs) were imaged using this interface.

Retrospective analysis of the IVCM images of the 5 tumors misdiagnosed as BCCs showed only dark silhouettes and convoluted dilated blood vessels (eFigure 1 in the Supplement); these features might be nonspecific for a diagnosis of eyelid margin BCC different from cutaneous BCC.[9] In these cases, dark silhouettes were either not reflective or were hyporeflective. Retrospective evaluation of images that were hyporeflective found blurred cells inside the islands (corresponding to melanocytic nests) without the peripheral palisade of BCC (eFigure 1 in the Supplement). The adhesive film applied to the tip of the microscope could have hampered visualization of the individual cells inside the silhouettes with the consequent incorrect differentiation between melanocytes and cells of BCC.

Lesions that were incorrectly diagnosed as MM by IVCM mainly showed large, hyperreflective, dendritic cells at the epithelial-stroma junction and/or in the upper layers of the epithelium, mimicking pagetoid cells of MM (**Figure 3**). These cells corresponded to Langerhans cells[11] that could be common in mucous membranes[12,13] and could lead to a wrong diagnosis of MM. In some nevi, sheets of large, hyperreflective, roundish cells were present in the stroma, mimicking a deeper proliferation of malignant melanocytes (eFigure 2 in the Supplement). These cells corresponded to benign melanocytes of nevi that were distributed in sheets instead of being organized in typical well-defined nests, leading us to the diagnosis of MM (eFigure 2 in the Supplement). Moreover, technical difficulties explained the misdiagnosis of 2 compound nevi: IVCM images were too superficial and showed only large dendritic

Figure 3. Primary Acquired Melanoses Without Atypia That Were Misdiagnosed as Melanoma



A, Clinical aspects. The blue arrowhead points to the lesion. B, Handheld in vivo reflectance confocal microscopy (IVCM) images. Numerous large, hyperreflective, dendritic cells (red arrowheads) are visible in the epithelium.

cells in the epithelium and no melanocytic nest. In addition, examination with the handheld device could be hampered sometimes by patients' movements, which could prevent the exploration of the entire tumor.

In this study, we obtained images similar to those described for the previous in vivo reflectance confocal microscopes used to examine the ocular surface.[14-17] However, the handheld device allows imaging of a larger field of view (920 × 920 µm vs up to 400 × 400 µm) and allows better handling that can be particularly helpful for examination of the lateral part of the conjunctiva and the eyelid margin and can facilitate its use in clinical practice.[4,9]

Compared with optical coherence tomography (OCT) and high-frequency ultrasound biomicroscopy (UBM), which have proven to be of assistance in the diagnosis of many ophthalmic pathologies, IVCM offers higher resolution.[17] Anterior segment OCT[17,18] and UBM[19] do not allow the differentiation of single cells because they feature resolution of only 18 and 25 µm, respectively. Although ultrahigh-resolution OCT features resolution as low as 3 µm, details of single cells are not seen.[20-22] Ultrahigh-resolution OCT and UBM allow the identification of the silhouettes of tumors and some architectural features, such as the presence of cystic areas,[18,23] but not cytologic details.[22,24] These techniques are less suitable than handheld IVCM to explore the lateral margin of the conjunctiva.[18,24] Ultrahigh-resolution OCT and UBM have the advantage over IVCM to provide cross-sectional images as in histopathologic examination in contrast with en face images[17] as well as a comprehensive scan of a tumor because of their larger field of view and deeper penetration.[17] However, some degree of optical shadowing of deeper structures can occur in ultrahigh-resolution OCT, particularly in the case of pigmented lesions.[18,20] At present, it is difficult to compare handheld IVCM with ultrahigh-resolution OCT and UBM, because the diagnostic accuracy for conjunctival tumors of the latter techniques has been studied in smaller series[18-20,22,24,25] and there are only case reports for their comparison with IVCM.[5,15]

For all of these high-resolution imaging techniques, the patient's cooperation is essential to avoid blurred images linked to eye movements. For IVCM conducted when the patients are in a supine position, the operator needs experience to continuously adapt the position of the camera and place it on the ocular surface without pushing excessively.

Limitations

The main limitation of our study is that the diagnoses were established during a joint consultation of skilled dermatologists and ophthalmologists who are experts on conjunctival tumors and cutaneous and ocular IVCM—a chance synergy that may be seldom. Nevertheless, for IVCM, the importance of the skill of the clinicians in both image acquisition and interpretation has been demonstrated in several domains, with a variable learning curve length.[26-30]

This study has also shown the need to clean the lens accurately instead of applying the sterile adhesive film, because the film reduces the image quality. Another drawback of IVCM is its high cost, which limits its widespread use but is accessible for tertiary care centers.

Conclusions

Our study shows that skin-specific, handheld IVCM is a fast, noninvasive, reliable tool for in vivo diagnosis of eyelid margin and conjunctival lesions. Moreover, IVCM has the advantage of allowing repetition of examinations of the same tumors at different times during follow-up. This technique has shown sensitivity for conjunctival malignant tumors and greater specificity than clinical examination conducted with the slitlamp for the diagnosis of eyelid margin tumors. The main limitation of IVCM for the diagnosis of conjunctival tumors is that it does not allow dependable identification of either Langerhans cells or malignant melanocytes. Further studies should be performed to identify possible features that can differentiate malignant melanocytes from Langerhans cells.

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Author Affiliations: Department of Medical, Surgical, and Neurological Science, Dermatology Section, University of Siena, S Maria alle Scotte Hospital, Siena, Italy (Cinotti, Rubegni); Department of Ophthalmology, University Hospital of Saint-Etienne, Saint-Etienne, France (Singer, Grivet, Thuret, Gain); Department of Dermatology, University Hospital of Saint-Etienne, Saint-Etienne, France (Labeille, Cambazard, Perrot); Biology, Engineering and Imaging of Corneal Graft Laboratory, EA2521, Jean Monnet University, Saint-Etienne, France (Grivet, Thuret, Gain);

Department of Pathology, University Hospital of Saint-Etienne, Saint-Etienne, France (Douchet); French University Institute, Paris, France (Thuret).

Author Contributions: Drs Cinotti and Singer had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of data analysis.
Study concept and design: Cinotti, Gain, Perrot.
Acquisition, analysis, or interpretation of data: Cinotti, Singer, Labeille, Rubegni, Perrot.
Drafting of the manuscript: Cinotti, Singer.
Critical revision of the manuscript for important intellectual content: Cinotti, Labeille, Grivet, Rubegni, Douchet, Cambazard, Thuret, Gain, Perrot.
Statistical analysis: Cinotti, Singer.
Administrative, technical, or material support: Perrot.
Supervision: Rubegni, Gain, Perrot.

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