Dermatology Practical & Conceptual

Combining Reflectance Confocal Microscopy, Optical Coherence Tomography and Ex-Vivo Fluorescence Confocal Microscopy for Margin Assessment in Basal Cell Carcinoma Excision

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ABSTRACT

Introduction: Recent developments of noninvasive, high-resolution imaging techniques, such as reflectance confocal microscopy (RCM) and optical coherence tomography (OCT), have enhanced skin cancer detection and precise tumor excision particularly in highly aggressive and poorly defined basal cell carcinomas (BCCs).

Objectives: The aim of this pilot study is to assess feasibility and reproducibility of a systematic clinical workflow combining noninvasive (RCM-OCT) and invasive fluorescence confocal microscopy (FCM) imaging modalities in pre- and intra-surgical evaluations of lateral and deep margins of BCC.

Methods: Superficial incisions were made 2 mm beyond the clinical-dermoscopic BCC margins. Lateral margins were then explored with OCT and RCM. In positive margins, a further cut was made 2 mm distal from the previous. A final RCM/OCT-based double-negative margin was drawn around the entire perimeter of the lesion before referring to surgery. The freshly excised specimen was then examined with FCM (ex-vivo) for the evaluation of the deep margin. Histopathologic examination eventually confirmed margin involvement.

Results: The study included 22 lesions from 13 patients. At the end of the study, 146 margins—106 negative (73%) and 40 positive (27%) at RCM/OCT—were collected. RCM/OCT margin evaluation showed an overall sensitivity of 100%, a specificity of 96.3%. The overall positive margins diagnostic accuracy was 98.2%. Reproducibility was evaluated on recorded images and the raters showed a substantial inter-observer agreement on both RCM ($\kappa = 0.752$) and OCT images ($\kappa = 0.724$).

Conclusions: The combined RCM/OCT/FCM ex-vivo approach noninvasively facilitates the presurgical and intrasurgical lateral and deep margin assessment of poorly defined BCCs.

Introduction

Basal cell carcinoma (BCC) is a widely diffused neoplasm in western countries with an increasing incidence as a consequence of inappropriate sun exposure and increased longevity of the population. Although many different minimal to noninvasive procedures have been proposed and applied in selected cases [1], surgical excision remains the recommended treatment option achieving average 5-year disease-free rates of over 98% for BCCs [2].

According to the National Comprehensive Cancer Network (NCCN), the recommended lateral margin for BCCs is 4 mm which should be extended in case of high-risk BCCs, such as sclerosing, infiltrative or micronodular BCCs, because of higher rate of recurrence. Mohs micrographic surgery (MMS) in its original form or in its variants (i.e. spaghetti technique, Tubingen torte, slow-Mohs) represents the best treatment option in terms of margin clearance and recurrence rate in these clinical situations [3]. However, due to MMS highly specialized and expensive requirements, this procedure is not available everywhere.

In the last decades development of non-invasive, high resolution imaging techniques, such as reflectance confocal microscopy (RCM) and optical coherence tomography (OCT), allowed the possibility to explore the tissue in vivo at nearly histologic resolution significantly improving skin cancer diagnostic accuracy. As a consequence, due to shallow imaging penetration the use of these techniques have been proposed also for lateral margin assessment in lentigo maligna and BCC [4,5-10]. Additionally, ex-vivo fluorescence confocal microscopy (FCM) is an emerging imaging technique that allows real-time microscopic examination of freshly excised cutaneous tissue. Thanks to its procedural simplicity and digital histopathologic acquisition rapidity, this tool is mainly applied to intra-operative analysis of the surgical margins of BCC in a MMS-like setting, as it is able to observe the entire skin specimen and both superficial and deeper margins with a very high accuracy [11].

The combined use of highly performing invasive and non-invasive imaging methods may enhance the capabilities for skin cancer detection and precise tumor excision particularly useful in highly aggressive and poorly defined BCCs in order to guarantee a radical treatment whilst saving procedural time and costs.

Objectives

The aim of this pilot study is to assess feasibility and reproducibility of an organized and systematic clinical workflow combining non-invasive (RCM-OCT) and invasive (FCM) imaging modalities in the pre- and intra-surgical evaluation of lateral and deep margins.

Methods

Patients presenting lesions with a confirmed clinical, dermoscopic and RCM diagnosis of BCC were recruited from the outpatient dermatology clinics of San Gallicano Dermatological Institute of Rome and University of Modena and Reggio Emilia.

Inclusion criteria were to present (i) at least one lesion with clinical, dermoscopic or RCM diagnosis of primary BCC; (ii) poorly defined lateral borders and/or clinical features suggesting sclerosing or infiltrating forms; (iii) lesion fully accessible for examination with RCM and OCT; (iv) patients >18 years old (v) patient willingness to participate.

Exclusion criteria were: (i) crusted or ulcerated lesions, (ii) local relapses or previously treated lesions; (iii) lesions located on anatomical sites not allowing a proper evaluation with RCM/OCT (eg nose wings, eyelid margins, auricles etc.); (iv) incapability to understand and sign the informed consent. Written informed consent was collected from all the participants.

Imaging Procedure

Prior to mapping procedure, all patients underwent a clinical, dermoscopic (Dermlite HR, DL4Wmagnification 10x) and hand-held RCM (Vivascope 3000® Vivascope GmbH) evaluation to confirm BCC diagnosis. The lesion mapping procedure consisted of 4 steps, adapted on the "SMART" approach previously proposed for skin tumor mapping, as following [12,13]:

Step 1. Clinical dermoscopic margin marking. After lesion inspection, visible BCC margins were delimited in hexagonal or rhomboidal shape margins around the tumor (depending on the size and shape) to facilitate the subsequent surgical procedure, and marked with ink pen 2 mm beyond the clinically and dermoscopically determined borders.

Step 2. Margin superficial cut. After 1 hour occlusive application of topical anesthetic, (lidocaine 25 mg/g + prilocaine 25 mg/g), a superficial cut was made with a scalpel (blade#15), overlying the dermo-graphic pen ink. In case of bleeding, it was readily arrested with sterile gauze soaked in tranexamic acid.

Step 3. Lateral Margin exploration with non-invasive techniques.

Margins were assessed combining the information from both OCT and RCM. OCT imaging were carried out by Vivosight D-OCT (Michelson Diagnostics) as previously described [14,15]. RCM margins were explored with hand-held RCM Vivascope 3000 in live mode [12,13]. Imaging procedure started from the center of the lesion outwards in radial direction up to the visualization of the superficial cut for each margin with both techniques. A margin was considered "positive" if presenting OCT or RCM BCC specific features less then 1mm inward or outward from the cut. OCT BCC positive features corresponded to the "Berlin Score" system [16], and RCM ones to the features enlisted by Longo et al. (dark silhouettes, bright tumor islands/cords, cleft-like dark spaces, dendritic cells, increased vascularization) [6]. In case of positive margin, a further cut was made 2 mm distal from the previous or at an estimated 2 mm distance from the outermost visible BCC structure, repeating the procedure in case of a further positive margin. A final RCM/OCT-based double negative margin was drawn around the entire perimeter of the lesion before referring to surgery.

All OCT and RCM margin imaging were acquired as a multilayer tiff file and an avi video file, respectively, for reproducibility study (Figures 1 and 2).

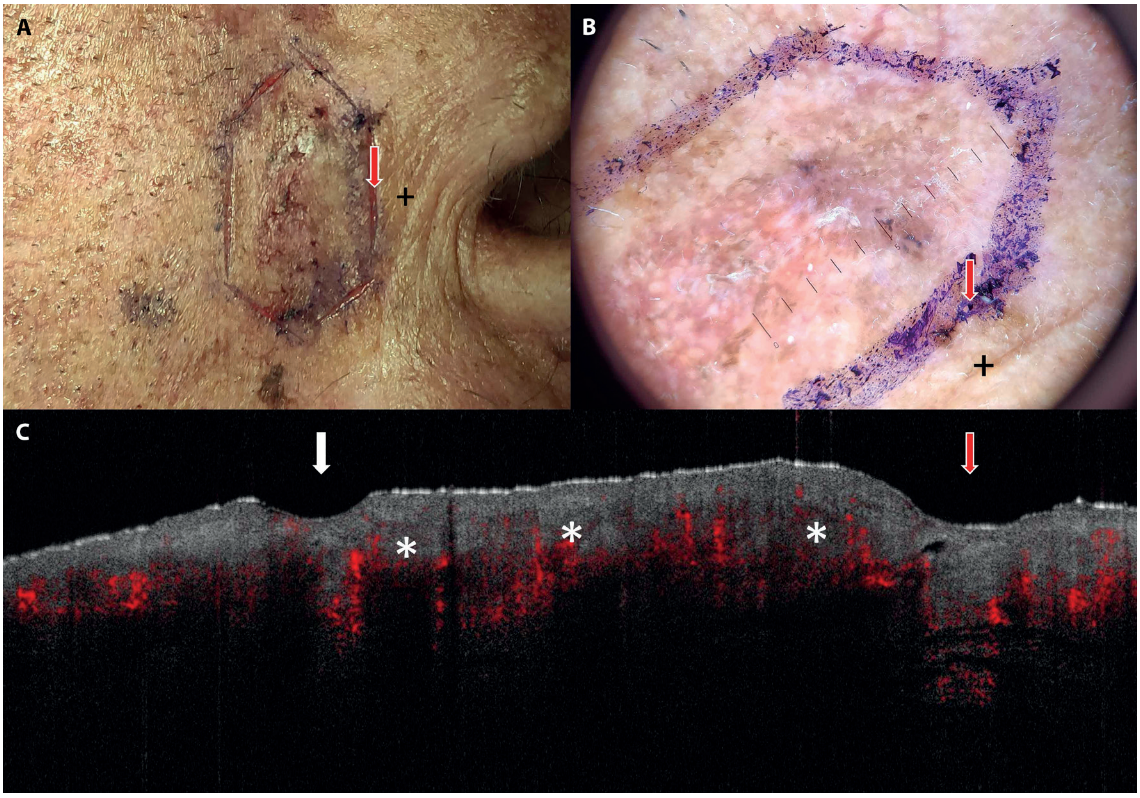

Figure 1. The procedure in clinical (A) and dermoscopic (B) detail. With an ink pen, visible BCC margins were defined around the tumor in a hexagonal or rhomboidal form 2 mm beyond the clinically and dermoscopically confirmed limits. A shallow incision was made over the dermographic pen ink. (C) An OCT scan shows basaloid islands (asterisks) extending beyond the first incision (red arrow). The margin has then been advanced by 2 mm (white arrow).

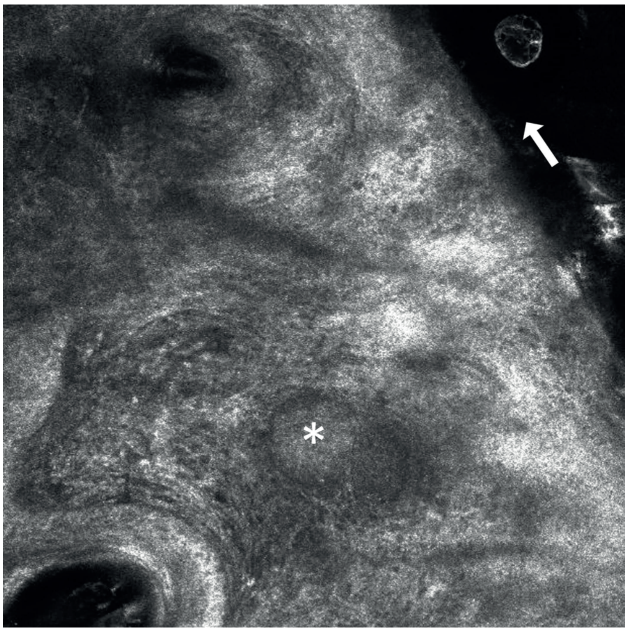

Figure 2. RCM exploration confirmed the presence of basaloid islands (asterisk) near the margin (white arrow).

Step 4. Surgical procedure and deep margin check. Patients proceeded to surgery following the RCM/OCT annotated margins. After specimen excision, the freshly excised specimen was prepared for FCM (ex vivo) imaging procedure for intra-operatory margin evaluation:

- FCM of deep margin: FCM imaging was performed with VivaScope 2500 4th Gen® (MAVIG GmbH) following the previously described procedure. [17] Along the side of the polygonal shaped specimen thin transversal sections were cut from the epidermal surface to the bottom of the excised specimen. The remaining central portion of the specimen and the lateral sections were prepared for FCM imaging. The bottom of the central portion was first imaged for the evaluation of the deep margin. Subsequently each lateral section was imaged positioning the specimen facing the internal side on the device glass plate. A board-certified dermatologist (M.A.), experienced in reading FCM imaging, evaluated BCC margin involvement. In case of BCC positive FCM positive margin, surgical cut was selectively enlarged in the positive sector.

After negative FCM margin confirmation, surgical breach closure is performed. Histopathologic examination was sent to a board-certified pathologist (C.C, A.M.C.) to confirm the diagnosis and margin involvement. (Fig3)

Follow-up study. After 1 year from excision, patients underwent clinical and dermoscopic examination of the scar and its peripheral area in order to identify possible BCC recurrence.

Reproducibility Study

To validate reproducibility of RCM/OCT reading procedure, all the RCM imaging videos and the OCT images from all the margins evaluated were randomized and retrospectively evaluated by two external readers, blinded to any dermoscopic, clinical and histopathologic information.

The external readers were asked to evaluate RCM and OCT margin as positive or negative, separately.

Statistical Analysis

As descriptive statistics, absolute numbers and percentages of true positive, true negative, false positive and false negative margins have been reported along with sensitivity and specificity values. The diagnostic positive margins performance is evaluated on the receiver operating characteristic (ROC) curve and the area under the curve.

The Cohen kappa (κ) statistic has been used to measure the agreement between histologic positive margin with the two "in vivo" instruments. Moreover, κ was also calculated in the evaluation of the agreement between the final operator positive margins and histological positive margins. We evaluated inter-observer agreement for positive margins both RCM and OCT evaluations in relation to the golden standard. The interpretation of agreement adopted here is less than chance agreement ($\kappa < 0$), slight agreement (κ = 0.01-0.20), fair agreement (κ = 0.21-0.40), moderate agreement (κ = 0.41-0.60), substantial agreement (κ = 0.61-0.80), and almost perfect agreement (κ = 0.81-0.99). The interpretation of reproducibility adopted is marginal (κ = 0.00-0.40), good (κ = 0.40-0.75) and excellent (κ>0.75). For all analyses a $P < 0.001$ was considered statistically significant. STATA program version 14 (StataCorp) was used to perform statistical analysis.

Results

The study included a total of 146 margins from 22 lesions from 13 patients, 4 females (30.8%) 9 males (69.2%), median age 71.4 years (range: 47-90 years), enrolled between June 2021 and November 2021 at the San Gallicano Dermatological Institute of Rome (N = 7) and Dermatology Department of Modena (N = 6).

Out of the treated 22 lesions, 12 (54.6%) were localized on the trunk, 3 (13.6%) on the limbs and 7 (31.8%) on the face. The size of the lesion major diameter ranged between 5-15 mm (mean of 8 mm). Histological predominant subtypes resulted in 7 (31.8%) nodular BCC, 8 (36.3%) superficial BCC followed by 3 micronodular BCC (13.6%) and 4 infiltrative BCC (18.2%).

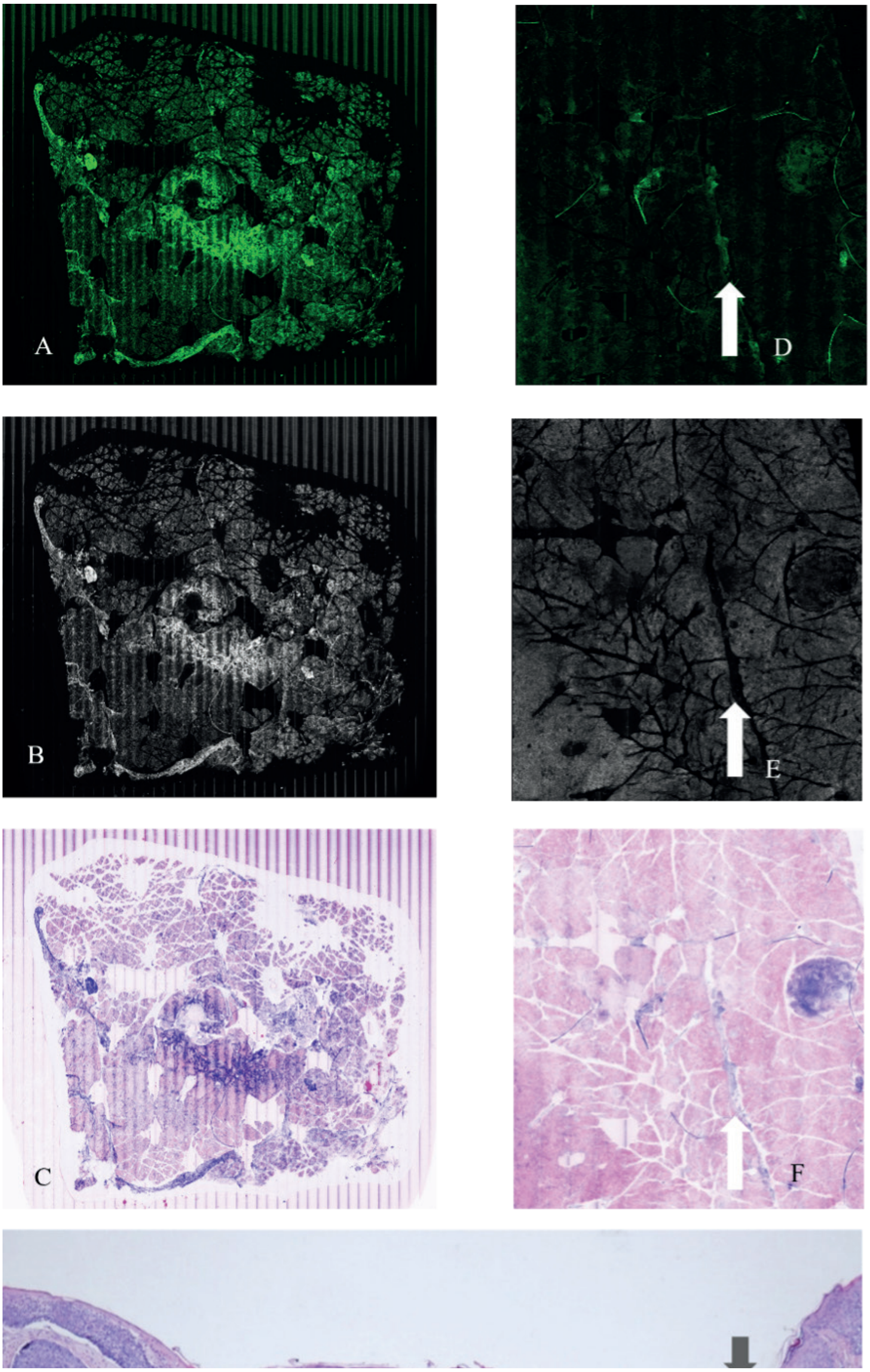

Figure 3. (A) Introperative axial FCM image of an excisional biopsy in fluorescence mode. Reflectance mode (B) and combined mode (C) shoving multiple basaloid islands of a preauricular BCC. En face view highlighting the superficial cut (white arrows) in Fluorescence mode (D) reflectance mode (E) and combined mode (F). Deep margin showed no BCC feature in FCM. (G) Histology image displaying the margin cut (black arrow) close to the BCC.

Lesions were framed into 4 margins (rhombus) in 13 cases (59.1%) or 6 margins (hexagon) in 9 cases (40.9%) depending on the size and the shape of the lesions, thus resulting in a total of 106 first stage margins. 39 margins (36%) were positive, leading to a second stage margin that resulted negative in all cases but one. At the end of the study 146 margin, 106 negative (73%) and 40 positive (27%) at RCM/OCT, were collected. 4 margins were excluded from the study because of poor quality imaging and thus not suitable for reproducibility study.

Concerning histopathology, 33 out of 142 margins were positive. Eight of 22 lesions (36.4%) had all negative margins, 5 (22.7%) had 1 positive margin, 3 (13,6%) had 2 positive margins and 4 (18,2%) had 3 positive margins.

RCM/OCT margin evaluation showed an overall sensitivity of 100% and a specificity of 96.3% and an overall positive margins diagnostic accuracy was 98.2%.

Concerning diagnostic accuracy, the percentages of agreement with histopathology was higher for the first rater, reaching 95.7% accuracy for RCM (κ = 0.89) and 95.1% for OCT (κ = 0.87), than the second one, reaching 91.5% and 89.4% (κ = 0.76 and κ = 0.71), respectively (Table 1).

Reproducibility was evaluated on recorded images, and the raters showed a substantial inter-observer agreement on both RCM (κ = 0.751) and OCT images (κ = 0.724) (Table 2).

Ex vivo FCM deep margin check. All deep margins resulted negative in histopathology as well as in ex vivo FCM imaging.

Follow-up study. After one-year follow-up no recurrences have been observed in clinical and dermoscopic evaluation.

Conclusions

The aim of our study was the evaluation of the impact of the in vivo tumor lateral margin assessment in a presurgical phase using RCM/OCT method combined, and ex vivo tumor deep margin check during the intra-operative phase by means of ex vivo FCM, on BCC excision.

Our experience disclosed that the combined approach, in vivo OCT/RCM + ex vivo FCM, represents a promising new approach to BCC margins identification. Achieving clear narrow margins and attaining the recommended wide safety margins may be complex in some cases, relying only on clinical and dermoscopic criteria. For this reason, we selected a series of BCC showing unclear clinical and dermoscopic margins.

BCC subtypes with aggressive histologic characteristics, poorly defined clinical margins and sites in certain areas, including the H region of the face have been linked to an increased risk of recurrence.

Table 1. Lateral Margin Exploration With Non-Invasive Techniques, Correlation With Histopathology and Reproducibility

		Histology [a]							
		Negative	Positive	% of correct diagnosis	K-value	Level of agreement	AUC	Sensitivity	Specificity
RCM	Negative	105	0	97.2	0.924	Almost perfect	0.982	100	96.3
	Positive	4	33						
OCT	Negative	105	0	97.2	0.924	Almost perfect	0.982	100	96.3
	Positive	4	33						
Rater 1									
RCM	Negative	103	0	95.7	0.888	Almost perfect	0.972	100	94.5
	Positive	6	33						
OCT	Negative	103	1	95.1	0.868	Almost perfect	0.957	96.9	94.5
	Positive	6	32						
Rater 2									
RCM	Negative	104	7	91.5	0.758	Substantial	0.871	78.8	95.4
	Positive	5	26						
OCT	Negative	100	6	89.4	0.713	Substantial	0.867	81.8	91.7
	Positive	9	27						

[a]4 margins histologically result not evaluable.
AUC = area under the curve; OCT = optical coherence tomography; RCM reflectance confocal microscopy.
First and second rater evaluation for RCM and OCT of margins compared to histological diagnoses, the percentage of correct diagnoses, κ value, the level of agreement, the sensitivity, the specificity, and ROC area for both raters.

Table 2. Agreement Between Operators

		RCM rater 1		K-value	Level of agreement
		Negative	Positive		
RCM rater 2	Negative	101	11	0.751	Substantial
	Positive	3	31		
		OCT rater 1			
OCT rater 2	Negative	98	9	0.724	Substantial
	Positive	7	32		
		RCM rater 1			
OCT rater 1	Negative	102	3	0.916	Almost perfect
	Positive	2	39		
		RCM rater 2			
OCT rater 2	Negative	100	7	0.653	Substantial
	Positive	12	27		

OCT = optical coherence tomography; RCM reflectance confocal microscopy.

For these reasons, Mohs surgery was developed for locally aggressive tumors [18-20].

In cases of poorly defined BCCs, Mohs surgery showed great effectiveness. Primary BCC recurrence rates following routine excision versus MMS are 10% and 1%, respectively. In a randomized trial the 10-year cumulative probabilities for recurrence in primary BCCs were 12.2% versus 4.4% with standard excision and MMS, respectively [21].

However, the application of Mohs is limited in several healthcare systems due to technological issues, costs and availability of a dedicated pathologist.

As a result, several methods have been developed, especially in Europe, to use noninvasive methods to detect lateral tumor margins. In a recent meta-analysis, dermoscopy revealed no statistically significant differences in the proportion of complete margin clearance on the first MMS stage between BCCs treated with dermoscopy-guided MMS and those who underwent curettage or visual inspection. However, lateral margin involvement was significantly lower in BCCs that had dermoscopy-guided MMS [22].

The precise assessment of the dermoscopic margins of infiltrative BCC may be very difficult given that these tumors are often more amelanotic and less heavy pigmented than less aggressive subtypes [23].

In one study RCM demonstrated a good global accuracy for primary BCC lateral margin detection with sensitivity and specificity of 95%. However, the study has been done only on superficial BCC-type [24]. To note, in our study difficult BCC in term of clinically definable margins has been included.

OCT displayed a sensitivity of 88.9%-92.6% and a specificity of 96.8%-98.4% on examining BCC-involved margin in 40 BCCs [25]. The major limit of this study is that the histological BCC subtypes were not reported.

However, each of these approaches has its limitations. RCM has excellent lateral resolution (~0.1-0.8 μm) but low tissue penetration power (100–200 μm) while OCT has lower lateral resolution (~5-7.5 μm) but higher penetration power (~1mm).

The combined use of RCT and OCT seems to have multiple advantages: OCT displays very quickly (~10 sec/acquisition) the entire volume of the lesion with a stack of orthogonally oriented images, each of FOV 2 mm. OCT imaging detects dark hypoechoic areas, which indicate the potential presence of BCC. RCM confirms OCT data by visualising BCC features with cellular resolution.

However, none of the non-invasive techniques currently in use enable the vision of deep margin involvement, which is crucial for the possible recurrence of BCC since it might result in infiltration and tumor development in deep tissues.

FCM has been selected for the detection of positive deep margins after surgical excision as RCM and OCT lack to reach the very deep skin layers. The overall sensitivity and specificity of fluorescence mode FCM for detecting BCC with narrow or incomplete margins were 88.0%–96.6% and 89.2%–99.0% respectively, in a large study performed by Bennàssar and colleagues on 80 carcinomas [26]. In more recent devices, reflectance and fluorescence modes may operate simultaneously. The interaction between the two mode increases the identification of BCC features in the fusion mode. The visibility of the tumor and stroma is improved by using acetic acid and acridine orange stains without harming the tissue for further histological investigation [27]. Due to this, we chose to combine RCM and OCT for lateral margin evaluation preoperatively, mutually compensating each other limitation, and to employ FCM intraoperatively for deep margin assessment.

Starting from our study, even if these combined methods are able to determine the exact lateral margins of superficial and nodular BCCs in enface optical sections [28], the both lack to reach the very deep part of the lesions. Adding to the protocol the fast and easy examination with ex vivo FCM of the excised tissue, deep margins can be easily checked with 100% of concordance with histology.

As the primary endpoint is concerned, the overall positive margins diagnostic accuracy was 98,2% as the agreement between positive margins in RCM/OCT and in histological evaluation ($\kappa = 0.9241$). Furthermore, RCM showed a sensitivity of 100% and a specificity of 96.3%, OCT a sensitivity of 96.9% and a specificity of 94.5%. Interestingly, in our study histological examination underlined a high-aggressive BCC subtype unnoticed on dermoscopy in 4 lesions (18.1%). Moreover, in our experience dermoscopically assisted clinical margin detection was accurate in only 36.4% of cases given the 2mm first step margin from the lesion.

Our study is clearly an employee operator, and our observers levels of agreement were generally acceptable. Interobserver evaluation has been made challenging. Neither the clinical nor dermoscopy images were related to the OCT/RCM images.

The investigation, which involved a small number of cases, was conducted in two centers with notable experience in RCM/OCT imaging. The results generalizability must be demonstrated in more patients and across more centers. There is the need of control group for further studies.

The complete procedure might be finished in 35-50 minutes with competent hands, but it might take longer with less experienced hands (>40 min).

Potentially our imaging approach could be extended to intraoperative search for residual cancer [29,30], and post-operative monitoring for local recurrence with the actual limit of a not sterilisable HH-RCM probe with the common methods of sterilization used for surgical devices. Integration of RCM/OCT imaging in Mohs surgery could be considered in a presurgical stage potentially able to save time by reducing the required number of Mohs stages.

Line-field confocal optical coherence tomography (LC-OCT) is a novel technique that combines the technological advantages of reflectance confocal microscopy with OCT in a single instrument.

Compared to the procedures used independently it has a lower resolution, but it allows for quicker switch between diagnostic techniques, facilitating the diagnosis. However, LC-OCT is unable to provide information on the involvement of the deep margin in non-superficial BCC [31,32].

The information provided by the RCM/OCT/FCM combined procedure has all the potential for routinely application in the presurgical and intrasurgical assessment of adequate lateral and deep margin in BCC. This procedure is likely to be most beneficial for difficult BCC of particular areas like the face, where wide margins may be difficult to attain.

Moreover, potentially the FCM can be reserved in very deep BCC in which the deep silhouette is not clearly visible when assessed with in-vivo techniques (RCM, OCT, LC-OCT). This approach could potentially lead to a positive impact on the patient surgical experience, satisfaction, and improve the physician decision-making process. This can decrease patient anxiety, reduce cost by reducing the number recurrences and improve pre-operative surgical planning by discussing appropriate reconstruction options and potential non-invasive treatment options. In summary, the combined approach RCM/OCT/FCM ex vivo noninvasively facilitates both diagnosis and depth assessment, and consequently BCCs may be treated through a "one-stop shop" approach with no need for a biopsy.

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Supplementary material

Video S1. FCM procedure showing the specimen preparation, the stain, and the margins exploration in reflectance, fluorescence and combined mode.