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Challenges for New Adopters in Pre-Surgical Margin Assessment by Handheld Reflectance Confocal Microscope of Basal Cell Carcinoma; A Prospective Single-center Study

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ABSTRACT

Introduction: In vivo reflectance confocal microscopy (RCM) is a useful tool for assessing pre-surgical skin tumor margins when performed by a skilled, experienced user. The technique, however, poses significant challenges to novice users, particularly when a handheld RCM (HRCM) device is used.

Objectives: To evaluate the performance of an HRCM device operated by a novice user to delineate basal cell carcinoma (BCC) margins before Mohs micrographic surgery (MMS).

Methods: Prospective study of 17 consecutive patients with a BCC in a high-risk facial area (the H zone) in whom tumor margins were assessed by HRCM and dermoscopy before MMS. Predicted surgical defect areas (cm^2) were calculated using standardized photographic digital documentation and compared to final defect areas after staged excision.

Results: No significant differences were observed between median HRCM-predicted and observed surgical defect areas (2.95 cm^2 [range: 0.83–17.52] versus 2.52 cm^2 [range 0.71–14.42]; P = 0.586). Dermoscopy, by contrast, produced significantly underestimated values (median area of 1.34 cm^2

[0.41-4.64] versus 2.52 cm^2 [range 0.71-14.42]; P < 0.001). Confounders leading to poor agreement between predicted and observed areas were previous treatment (N = 5), a purely infiltrative subtype (N = 1), and abundant sebaceous hyperplasia (N = 1).

Conclusions: Even in the hands of a novice user, HRCM is more accurate than dermoscopy for delineating lateral BCCs margins in high-risk areas and performs well at predicting final surgical defects.

Introduction

Basal cell carcinoma (BCC) is the most common skin cancer and its incidence is rising worldwide due to chronic UV exposure and aging [1]. While the vast majority of BCCs at sites such as the trunk and extremities can be removed by simple excision or local destructive therapies, tumors located in high-risk areas such as the H zone (central area of the face, around the eyes, nose, lips, and ears) are at greater risk of destructive local spread and recurrence. Effective surgical treatment is essential for guaranteeing tumor-free margins and maximal functional and cosmetic outcomes in BCCs that are clinically ill defined and those in high-risk areas, with an aggressive histologic subtype (micronodular, morpheaform, basosquamous, infiltrative), or a history of incomplete excision.

Mohs micrographic surgery (MMS) with rapid intraoperative histologic confirmation of full tumor margins offers the highest success rates in the excision of facial BCCs [2]. MMS, however, requires advanced surgical and histopathologic skills and support from histotechnicians with experience in this procedure. There may also be financial and resource-related obstacles. Reflectance confocal microscopy (RCM) might facilitate BCC margin mapping prior to MMS as it can be used to delineate lateral tumor margins that cannot be determined clinically or by dermoscopy [3,4]. Studies of the use of RCM in this setting, however, have involved users with more than 5-years experience in image navigation and interpretation [3,5-9]. In addition, most of the lesions investigated were located on flat, even surfaces on the face or trunk, where natural skin folds and bony prominences, such as those found in the H zone, do not interfere with navigation. With the recent approval of new Current Procedural Terminology (CPT) codes for RCM imaging and evaluation in the United States and the advent of lower-cost RCM devices, the number of users is expected to increase [10,11].

Objectives

The aim of this study was to investigate the performance of a handheld RCM (HRCM) device operated by a user with 1-year experience in this technique for lateral margin assessment in BCCs in high-risk locations in a real-life clinical setting.

Methods

We prospectively included consecutive patients with non-pigmented, ill-defined, biopsy-proven BCCs located in high-risk areas of the face treated with MMS at our dermatology department between August 2020 and September 2021. The study was approved by the hospital ethics committee, and informed written consent was obtained from all participants prior to enrolment. The study was conducted according to the principles of the Declaration of Helsinki.

The target lesions were imaged using the Vivascope 3000 HRCM device (MAVIG/Caliber ID), which has a horizontal resolution of ~1 µm, optical sectioning of ~3 µm, and a field of view of 0.75 × 0.75 mm. The images were captured in vivo before surgery by an investigator (NR) with 1-year experience who had attended an RCM course and received 2 months practical training at a reference unit.

The clinical margins were first determined by dermoscopy and marked on the skin using a silver paint marker (Edding 780 creative 0.8 mm, Edding International. These markings facilitated RCM navigation and margin calculation since silver ink can be visualized by RCM [6]. The margins were then determined using HRCM and the original silver markings readjusted to the distance of one field of view (0.75 x 0.75) between the last inside tumor island to the internal side of the silverpen delineation. Using a method previously described by a member of our team (OY) [12], we produced standardized photographic documentation containing digital images of the lesions before and after dermoscopy, before and after RCM, and during and after MMS. These images were then calibrated in ImageJ (NIH, available from http://imagej.nih.gov/ij/) using anthropometric measurements and a surgical ruler placed in the image field. The same software was used to calculate surgical defect areas predicted by dermoscopy and HRCM. A 3-mm margin was added to the predicted values as the MMS protocol at our hospital requires histologic clearance of at least 3 mm. Images of the final surgical defect were obtained before surgical reconstruction and the area was re-measured to compare it with the dermoscopy- and HRCM-predicted areas.

The surgeons who performed MMS (AJ, JB, GC) were blinded to the dermoscopy and HRCM calculations, and, as per protocol, extended the dermoscopic margin by 3 mm

during the first excision stage. The excised specimen was frozen and sectioned for microscopic examination of lateral and deep margins and, where necessary, the process was repeated until achievement of full histologic clearance.

Statistical Analysis

Descriptive statistics were used to describe the characteristics of the cohort, lesion size, and predicted and observed surgical defect areas. Normal distribution was checked using the Kolmogorov-Smirnoff test. Since most of the variables were non-normally distributed, non-parametric tests were used. The Wilcoxon test was used to compare the final surgical defect area and the areas predicted by dermoscopy and HRCM. All analyses were performed in SPSS version 22 (IBM corporation).

Results

Seventeen consecutive patients (9 men and 8 women) agreed to participate in the study and underwent complete BCC excision by MMS (Table 1). The median age at the time of HRCM examination was 70 years (range: 46–86 years). Thirteen tumors were located on the nose, 2 on the temple, and 1 each on the ear and inner canthus of the eye (Figure 1). Four patients had previously undergone conventional surgery at the same site and had had positive margins or experienced recurrence. Another 4 patients had been treated with cryotherapy, curettage, or topical imiquimod and 2 had undergone radiotherapy. Salvage MMS was performed in one patient in whom oral vismodegib had been discontinued after 4 months due to adverse effects. Fourteen patients (82.3%) had an infiltrative BCC component on histology, 2 had a nodular/superficial subtype, and 1 had a superficial, undetermined subtype (Table 1).

A median of 2 MMS stages (range: 1–4) were needed to achieve tumor-free margins after margin delineation with dermoscopy (Table 1). Dermoscopy underestimated the final surgical defect area by a median of 1.18 cm^2 (1.34 cm^2 [range: 0.41–4.64] versus 2.52 cm^2 [range: 0.71–14.42]; $P < 0.001$). There were no statistical differences between the HRCM-predicted area and the final area (2.95 cm^2 [range: 0.83–17.52] versus 2.52 cm^2 [range: 0.71–14.42]; $P = 0.586$). HRCM, however, overestimated defect size in three cases and underestimated it in four (Figure 2). Of the 3 patients with overestimated defect areas, 1 had been previously treated by radiotherapy (patient #12), another had a purely infiltrative component (patient #14), and another had prominent sebaceous hyperplasia mistaken for tumor islands by the confocalist (patient #3). Of the 4 patients with underestimated defect areas, 1 had been treated with conventional surgery (patient #11), 1 with curettage (patient #16), 1 with radiotherapy (patient #9), and 1 with imiquimod (patient #5). These treatments had resulted in scarring at different levels of the epidermis and/or dermis.

Conclusions

The main limitation of this study is its small sample size (17 consecutive patients), which was partly due to the COVID-19 pandemic, as fewer operations were performed and fewer patients agreed to participate in the study due to fear of infection by severe acute respiratory syndrome coronavirus 2. Another notable limitation is the decreased resolution offered by RCM at depths of greater than 200-250 µm. Deep margin assessment with this technique is thus suboptimal, particularly for purely infiltrative tumors, deep tumors, and tumors located under scar tissue [13]. Finally, since this was a real-life bedside study involving live imaging, we were unable to assess the difficulty of each case and compare the performance of the novice confocalist with that of an expert. Another limitation is that there is yet no follow-up of the cases available.

The use of in vivo RCM imaging for the bedside diagnosis and histologic subtyping of BCC and other skin cancers has gained popularity in the past decade [13-15]. Its usefulness in the assessment of lateral margins has been demonstrated in nodular and superficial BCCs located on flat surfaces such as the cheek, forehead, and trunk [3,5,6]. Candidates for MMS, however, usually have lesions in high-risk areas of the face, where performance of wide-probe RCM is complicated by skin elasticity and the presence of concave and convex surfaces. HRCM, by contrast, offers advantages in uneven locations, as it allows for free-form navigation. It also presents challenges, however, especially for new users working in real-life settings, as unlike wide-probe RCM, it does not have mosaicking capabilities. Image acquisition is therefore heavily user dependent.

This prospective study analyzed the use of HRCM in the assessment of BCC margins prior to MMS in clinical practice at a single institution. Our results suggest that, even in the hands of novice operators and in challenging locations such as the nose, HRCM outperforms dermoscopy. In more complex cases, however, such as tumors previously treated with radiotherapy, surgery, or imiquimod, it may produce less accurate results due to the presence of scar tissue impeding visualization of tumor structures. Other difficulties include recognition of infiltrative BCC components or BCC mimickers, such as sebaceous hyperplasia, hair follicles, and eccrine glands (Table 2). Novice users need to be familiar with normal skin structures and aware of the limitations of HCRM. We believe that image-reading challenges can be overcome by ensuring that training programs, in addition to focusing on pathologic characteristics of tumors, include content on normal skin structures, mimickers, and RCM limitations. This knowledge should shorten the learning curve. Rapid feedback from experienced confocalists via image-sharing platforms in cases of doubt could also be very

Table 1. Summary of demographic and BCC characteristics and comparison of surgical defect areas predicted by HRCM and dermoscopy and observed after MMS.

Case	Sex	Age (y)	BCC location	BCC subtype	Treatment before RCM	Dermoscopy-predicted surgical defect area (cm^2)	HRCM-predicted surgical defect area (cm^2)	Final surgical defect area (cm^2)	No. of MMS stages
1	M	83	Nasal tip	Nodular /infiltrative	Surgery in 2012, vismodegib for 4 months in 2017	4.727	4.687	4.364	1
2	M	42	Nasal ala	Nodular/adenoid/ micronodular	Surgery with positive margins in 2019	1.082	0.803	0.818	2
3	M	86	Nose	Superficial multifocal/ nodular/infiltrative		2.114	4.148	2	1
4	M	76	Nose	Superficial/nodular		2.191	3.326	3.468	3
5	M	68	Tip/dorsum of nose	Superficial multifocal/ nodular	Imiquimod	3.614	2.718	3.968	2
6	F	68	Nasal ala	Infiltrative/micronodular		2.725	2.947	2.874	1
7	M	84	Ear	Nodular/infiltrative	Cryotherapy	2.149	2.249	2.125	2
8	F	71	Nose	Infiltrative		1.72	1.87	1.794	1
9	M	70	Nasal ala	Infiltrative/micronodular	Radiotherapy	2.612	3.261	7.647	4
10	F	46	Temple	Infiltrative/superficial	Surgery in 2015 cryotherapy, imiquimod	6.351	8.426	8.423	2
11	M	82	Nose	Infiltrative/superficial/ nodular	Curettage + electro-coagulation	6.559	5.379	7.124	1
12	F	52	Nasal tip	Superficial/undefined	Radiotherapy	0.977	6.83	0.71	1
13	F	68	Nasal ala	Micronodular		2.509	2.505	2.357	3
14	F	73	Temple	Micronodular/infiltrative		5.13	17.519	14.416	4
15	F	78	Nasal tip	Infiltrative/micronodular		1.044	1.06	1.105	3
16	M	60	Inner canthus of eye	Infiltrative	Surgery in 2020	1.753	1.832	2.52	1
17	F	53	Nasal ala	Infiltrative		1.106	1.115	1.03	1

BCC = basal cell carcinoma; F = female; HRCM = handheld reflectance confocal microscopy; M = male; MMS = micrographic Mohs surgery.

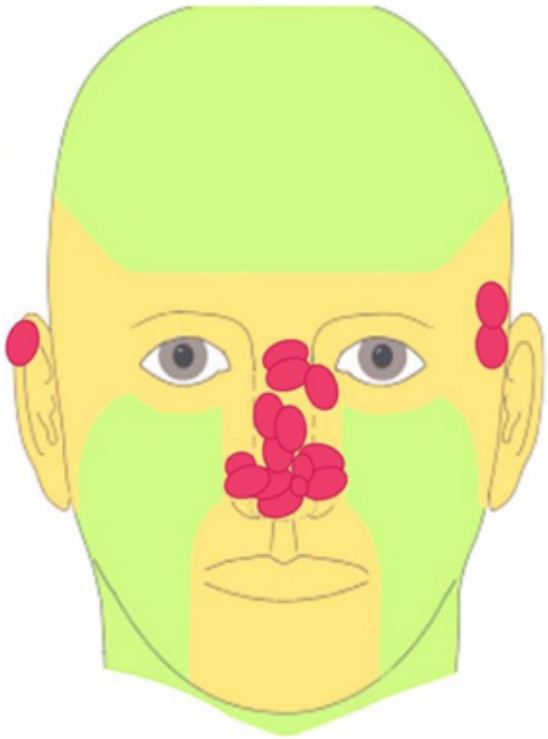

Figure 1. Locations of basal cell carcinomas (red circles) included in the study. High-risk areas are shown in orange.

valuable. Scouting biopsies are useful in complex cases as they can help differentiate tumors from benign structures or scar tissue, especially when working at depths of greater than 200-250 µm. A summary of the above challenges and proposed solutions is given in Table 2.

Although HRCM has cellular resolution, it presents some technical challenges. Navigation in the horizontal plane, for example, can be problematic due to loss of reference points and the impossibility of building an overall mosaic of the lesion. Visualization thus is restricted to a small field of vision (0.75 × 0.75 mm or 1 × 1 mm depending on the generation of microscope). Patient breathing and movement can also result in abrupt motion changes that can distort images. Distortion can also occur when navigating skin folds or bony prominences where it is impossible to establish a flat contact (Table 2). Newer multimodal systems such as combined RCM and optical coherence tomography (OCT) and line-field confocal OCT allow for deeper tissue imaging and improved accuracy in the delineation of lateral and deep tumor margins [10,17]. The problem of reference point loss could be overcome by using in vivo wide-field imaging to guide the horizontally moving device over the skin surface

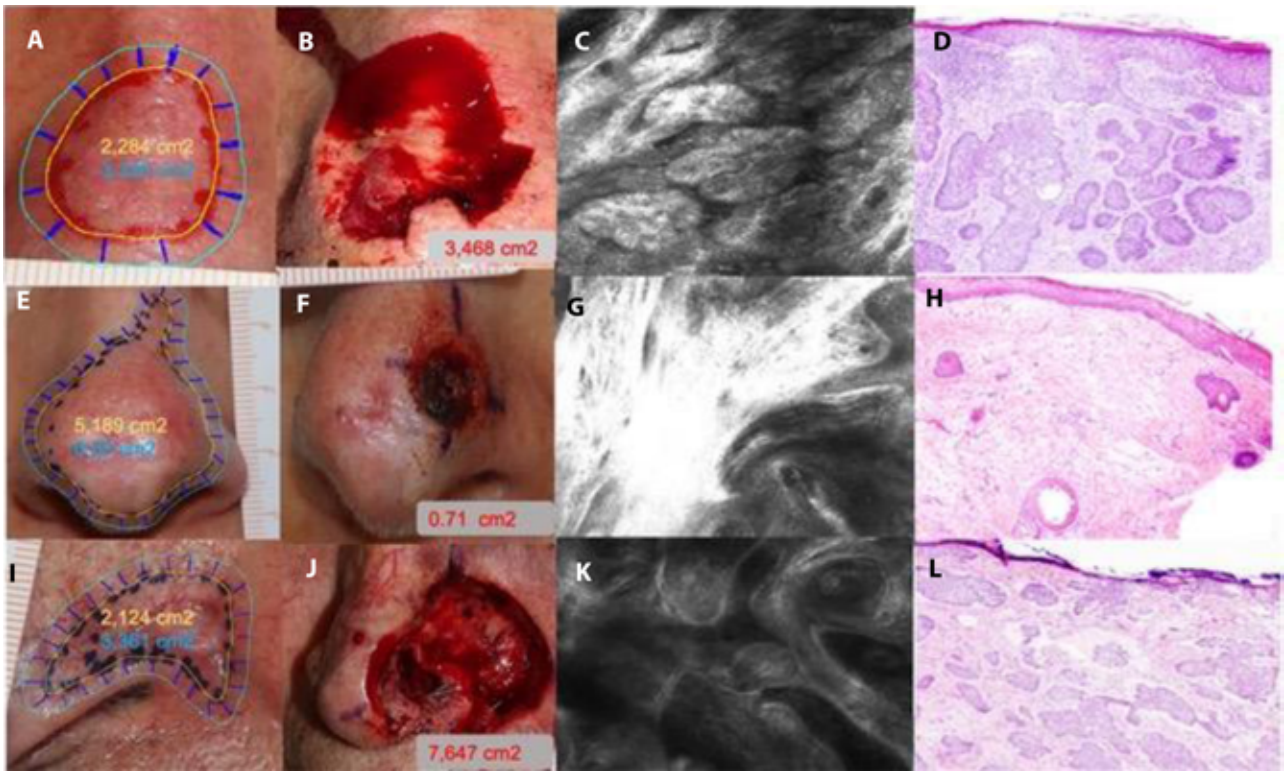

Figure 2. Representative cases of basal cell carcinoma (BCC) showing good agreement between surgical defect areas predicted by handheld reflectance confocal microscopy (HRCM) and the defect areas after Mohs micrographic surgery (MMS) (A-D), overestimated defect areas (E-H), and underestimated defect areas (I-L). (A) Similarity between the HRCM-predicted surgical defect area (blue line) and the final area (B) in a BCC with evident tumor islands seen by RCM (C) and a nodular subtype identified in the intraoperative frozen section (D). (E) HRCM-predicted surgical defect area in a BCC previously treated with radiotherapy that was significantly larger than the final defect area after MMS (F). HRCM images of radiation-induced dermal fibrosis (G) were mistaken for collagen surrounding deep tumor islands, but these were ruled out by histology (H). The HRCM–predicted surgical defect area in (I) was significantly smaller than the final area (J) in a superficial, infiltrative BCC visible by HRCM (K) and histology (L).

Table 2. Basal cell carcinoma margin delineation by HRCM: limitations, challenges for new users, and proposed solutions.

Image-reading challenges/limitations	Proposed solutions
Infiltrative subtypes (no clear tumor islands or clefting but dark silhouettes that appear as imprints embedded in bright collagen)	Training in image reading Scouting biopsies Combined use of OCT Platforms for consulting with RCM experts
Limited penetration depth for delineating deep margins	Combined use of OCT to explore vertical planes
Prominent sebaceous hyperplasia, hair follicles, and eccrine glands that can be mistaken for tumor islands in facial BCC	Training in image reading Scouting biopsies Platforms for consulting with RCM experts
Tissue distortion due to previous biopsies and treatments	Training in image reading Scouting biopsies
Technical challenges associated with horizontal plane navigation	**Proposed solutions**
Loss of reference points due to small field of vision (0.75 × 0.75 mm or 1 × 1 mm) and lack of automated mosaicking capabilities	Integration of videomosaicking algorithm into native HRCM software Use of in vivo wide-field imaging to guide HRCM navigation Use of a robotic arm
Image distortion due to patient breathing and movement	Use of a a robotic arm Artificial intelligence image modeling to restore image aberrations
Loss of direct contact with HRCM device over natural skin folds and bony prominences	Smaller-diameter HRCM lens to facilitate direct contact with the skin Use of a robotic arm

FOV = field of view; HRCM = handheld reflectance confocal microscopy; OCT = optical coherence tomography; RCM = reflectance confocal microscopy.

(Table 2) [18]. HRCM video-mosaicking can also be used to create static images from dynamic videos, enabling improved navigation and interpretation and facilitating comparisons between confocalists [8,12,19]. A subsequent image processing step is necessary, however, as in vivo video-mosaicking is not currently available in native HRCM software. Other options for more precise imaging over uneven surfaces include the use of robotics or HRCM devices with a smaller optical lens for improved skin contact.

Although the number of RCM users is growing worldwide and will continue to grow following the recent approval of RCM CPT codes in the United States, expert users are still limited in number. We have shown that an HRCM device operated by a novice user in real-life clinical practice performs well in the presurgical assessment of BCC margins. We have also offered some suggestions on how performance can be further improved. RCM users often receive limited training in image acquisition and interpretation. They typically learn by experience and feedback (based on pathology reports, for example). While this also has positive effects, the learning curve could be shortened by designing certificate training courses and mentoring programs, establishing image-sharing platforms for consultations between novice and experienced users, and integrating novel technical advances, such as multimodal imaging and artificial intelligence. Nevertheless, our findings show that, even in difficult conditions, HCRM operated by a single, novice user, performed well in the delineation of BCC margins and provided very useful data for application in everyday clinical practice.

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