

THE USE OF *IN VIVO* REFLECTANCE CONFOCAL MICROSCOPY AND DERMOSCOPY IN THE PREOPERATIVE DETERMINATION OF BASAL CELL CARCINOMA HISTOPATHOLOGICAL SUBTYPES

MIHAI LUPU*, CONSTANTIN CĂRUNTU**, ***, IULIA SOLOMON****, ALEXANDRA POPA****, CRISTINA LISIEVICI****, CARMEN DRĂGHICI****, LAURA PAPAGHEORGHE *****, VLAD MIHAI VOICULESCU****, *****, CĂLIN GIURCĂNEANU****, *****

Summary

Due to its worldwide high prevalence, its profoundly negative psycho-social impact, and the significant financial consequences associated with diagnosis and treatment, basal cell carcinoma (BCC) has become a genuine public health issue. Early diagnosis and swift treatment continue to play key roles in the prevention of physical and psychological damage associated with tumor progression as current guidelines emphasize the importance of different therapeutic approaches based on tumor location, dimensions and histopathological subtype. Pre-therapeutic determination of BCC histopathological subtype by means of non-invasive methods could reduce the number of diagnostic invasive procedures, reduce morbidity, and financial costs associated with treatment.

In vivo confocal reflectance microscopy (RCM) is a relatively new, non-invasive, imaging technique capable of quickly delivering high-quality horizontal optical sections through the skin, with image resolutions close to those of conventional histology slides. This case series includes 4 non-consecutive patients with a total of 6 tumors. The evaluation protocol included clinical examination, dermoscopy, RCM examination, and excisional biopsy of the lesions followed by histopathology examination.

Our results show that the presence of tumoral aggregates as cords connected to the epidermis are suggestive for the diagnosis of superficial BCC. In nodular BCC we have encountered mostly large tumoral islands and tumoral cords. As a distinctive RCM element in infiltrative BCC cases we noted the presence of intradermal hyporefractile areas surrounded by hyperrefractile dense collagen bundles.

In conclusion, a good correlation between the structures observed through RCM and those present on histopathological examination was observed. The corroboration of keratinocyte architecture observed through RCM and dermoscopic criteria allowed for the distinction between histopathological BCC subtypes suggesting that this type of complementary approach could significantly improve the precision of the pre-therapeutic diagnosis, thus lowering the need for invasive diagnostic procedures.

Keywords: Carcinoma, Basal Cell; Microscopy, Confocal; Keratinocytes; Dermoscopy; Early Diagnosis; Histopathological Subtype.

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- * Department of Dermatology, MEDAS Medical Center, Bucharest, Romania.
 ** Department of Physiology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.
 *** Department of Dermatology, "Prof. N. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania.
 **** Department of Dermatology, "Elias" University Emergency Hospital, Bucharest, Romania.
 ***** Ambulatory, "Colțea" Clinical Hospital, Bucharest, Romania.
 ***** Department of Dermatology and Allergology, "Elias" Emergency University Hospital, Bucharest, Romania.

Introduction

Basal cell carcinoma (BCC) is the most frequent malignancy world-wide, with incidence rates on the rise by about 10% per year [1, 2]. Due to its high prevalence, its profoundly negative psycho-social impact, and the financial consequences associated with its diagnosis and treatment, basal cell carcinoma has become a genuine public health issue [2–4].

Although usually without lethal potential, these tumors with relatively homogenous histology are locally invasive and can determine important sequelae and morbidity, mostly due to their frequent facial location. Key roles in preventing the tisular damage related to BCC evolution are played by early diagnosis and prompt and correct treatment.

Current guidelines [5] advocate different approaches to the treatment of basal cell carcinoma based on tumor location, dimensions, and tumor type. A special importance is granted, in the international literature, to choosing the appropriate BCC treatment method according to the tumor's histopathological subtype. Thus, first line treatment options for superficial BCCs (sBCC) include non-surgical methods, while surgical excision is recommended for nodular BCCs (nBCC), and infiltrative BCCs (iBCC) are best treated by Mohs micrographic surgery techniques [2, 6]. Therefore, knowing the BCC histopathological subtype represents a key element in choosing the optimal therapeutic course.

Conventional histopathological examination following surgical excision is currently considered the most precise method of establishing BCC histopathological subtype [7], mostly due to the fact that dermoscopy guided punch biopsies are subjected to sampling errors. Preoperative assessment through non-invasive techniques of the BCC histopathological subtype can reduce the number of invasive diagnostic procedures, reduce morbidity, and ultimately reduce treatment associated financial costs.

It has been proven that dermoscopy helps increase both sensitivity and specificity of BCC diagnosis and it can discriminate between BCC and other cutaneous tumors [8–11]. Recent studies have correlated dermoscopic criteria to certain BCC histopathological subtypes [12–14],

but unfortunately the diagnostic accuracy of this method is not, in any way, exceptional.

Reflectance confocal microscopy (RCM) is a relatively new, non-invasive, imaging technique capable of quickly generating horizontal optical sections through the skin with resolutions close to those of histological slides [15]. Reflectance confocal microscopy is becoming more and more popular every day, as it is proving useful in both the evaluation of melanocytic and non-melanocytic tumors, and also inflammatory diseases [16–18]. In this case series we aim to evaluate the utility of RCM in the preoperative determination of BCC histopathological subtype using conventional histopathological examination as reference.

Materials and methods

Four non-consecutive patients with a total of 6 tumors have been included in this case series. The patients were enrolled following a clinical diagnosis of basal cell carcinoma in the Department of Dermatology and Allergology of the “Elias” Emergency University Hospital in Bucharest and the Department of Dermatology of MEDAS Medical Center in Bucharest during the month of August 2017.

The present study was conducted in conformity with the principles of the declaration of Helsinki, all patients enrolled having given informed consent prior to being subjected to the evaluation protocol described herein.

The evaluation protocol for each patient included: personal and familial medical history with an emphasis on risk factors, clinical examination, dermoscopy examination, confocal reflectance microscopy examination, and excisional biopsy followed by histopathological examination.

Clinically, the lesions were characterized as either flat, elevated or nodular.

Dermoscopic images were captured using the integrated dermoscope VivaCam (Caliber ID, Rochester, NY). Dermoscopic diagnosis was based on previously described criteria for the diagnosis of BCC [8, 9].

RCM examination was carried out using the commercially available reflectance confocal microscope VivaScope 1500 (Caliber ID,

Rochester, NY) which uses an 830 nm laser diode with a maximum power of 20 mW, allowing for cutaneous imaging without causing any damage to the analyzed tissues. The RCM examination was guided on the lesion surface using the dermoscopic image initially captured with the VivaCam. Five level VivaCubes (spaced at 30 μm in depth) were executed in the center of each lesion, starting at the stratum corneum. Individual VivaBlocks were set for an area of 8x8 cm or 16x16 individual images. Consecutively, individual VivaStacks were executed in other areas of interest up to a depth of 200 μm , at which diffraction had determined a significant loss in image resolution. The detailed RCM examination protocol using the VivaScope has been previously described [15, 19]. RCM image manipulation, which consisted of minor contrast and/or luminosity adjustments, has been done using the free image processing software ImageJ [20].

Criteria of interest during the RCM examination included: (1) ulcerations, (2) epidermal streaming (keratinocyte nuclei polarization and orientation along a common axis), (3) small tumoral islands (diameter < 300 μm), (4) large tumoral islands (diameter > 300 μm), (5) tumoral cords connected to the epidermis, (6) dark silhouettes (intradermal hyporefractile areas), (7) peritumoral clefting, (8) peripheral palisading, (9) "onion-like" structures, (10) dilated blood vessels, (11) hyperrefractile peritumoral fibrosis, (12) hyperrefractile inflammatory cells.

All tumors subjected to the evaluation protocol were then surgically excised, fixed in formalin, haematoxylin and eosin stained, and underwent histopathological examination which represented the reference for further analysis. The histopathological subtype classification used in this case series included superficial, nodular, and infiltrative BCC.

Results

1. Superficial BCC

Clinically, superficial BCCs in this case series fell under the flat lesions category. Dermoscopic images showed short superficial telangiectasia,

red-white astructural areas, multiple small ulcerations and leaf-like structures (Fig. 1a).

RCM examination revealed anastomosing tumoral cords, horizontal and dilated blood vessels, large hyperrefractile cells corresponding to melanophages, and small, moderately refractile cells corresponding to inflammatory cells (Fig. 1b). Epidermal streaming was obvious at the level of the stratum spinosum (Fig. 1c). Tumoral islands with peripheral palisading and infiltration with dendritic structures could also be seen. In this BCC subtype the tumoral cords observed were connected to the epidermis (Fig. 1d).

2. Nodular BCC

Clinically, nBCC lesions were either elevated or nodular. Dermoscopy revealed large arborizing vessels, short telangiectasia and blue ovoid nests (Fig. 2a).

RCM images at the level of the dermis showed: large tumoral islands (diameter > 300 μm) containing moderately refractile white dots and dendritic structures, hyperrefractile peri-tumoral fibrosis, and clefting in the shape of hyporefractile peritumoral areas corresponding histopathologically to mucin deposition (Fig. 2b). Tumoral islands of different shapes and sizes, ulcerations, and inflammatory infiltrates could also be seen (Fig. 2c,d).

3. Infiltrative BCC

Clinically, iBCC lesions were found to be flat. Dermoscopy showed red-white astructural areas and arborizing vessels (Fig. 3a).

RCM examination at the level of the stratum spinosum showed keratinocytes with elongated nuclei, distributed along the same axis, an element called epidermal streaming (Fig. 3c). Lobulated tumor islands with peripheral palisading containing numerous dendritic structures could be seen at the level of the dermis (Fig. 3d). Maybe the most characteristic clue for the RCM diagnosis of iCBC, dark silhouettes surrounded by hyperrefractile areas, representing tumoral islands surrounded by peritumoral fibrosis, could be seen at the level of the dermis (Fig. 3b).

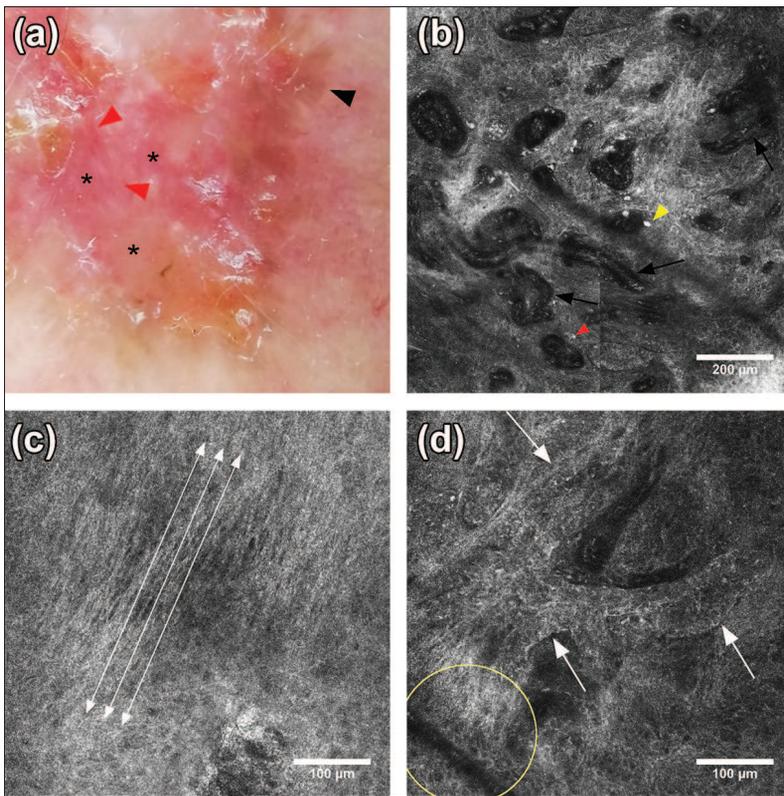


Fig. 1. Dermoscopy and RCM in superficial BCC: (a) Dermoscopy image of a portion of the lesion showing short superficial telangiectasia (red arrowheads), red-white astructural areas (black asterisks), multiple small ulcerations and leaf-like structures (black arrowhead); (b) RCM block (1x1 mm) showing anastomosing tumoral cords, dilated horizontal blood vessels (black arrows), hyperrefractile cells representing melanophages (yellow arrowhead), and small, moderately refractile cells representing inflammatory cells (red arrowhead); (c) RCM image (500x500 μm) at the level of the stratum spinosum illustrating the polarization along the same axis of elongated keratinocyte nuclei, phenomenon called epidermal streaming (white double arrows); (d) RCM image (500x500 μm) showing tumoral cords (white arrows) with peripheral palisading containing numerous dendritical structures. The tumoral cords are connected to the epidermis (yellow circle).

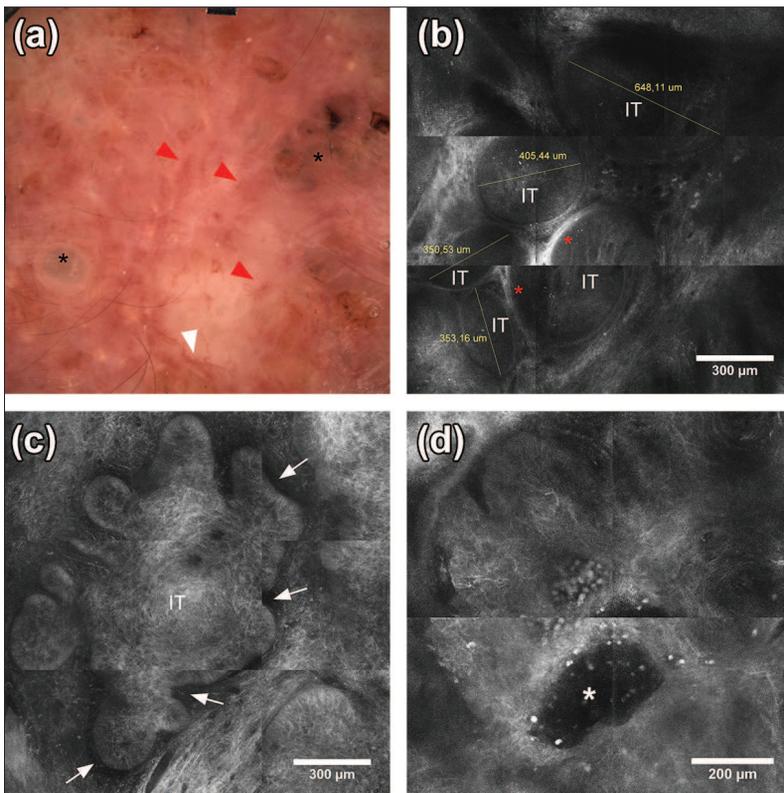
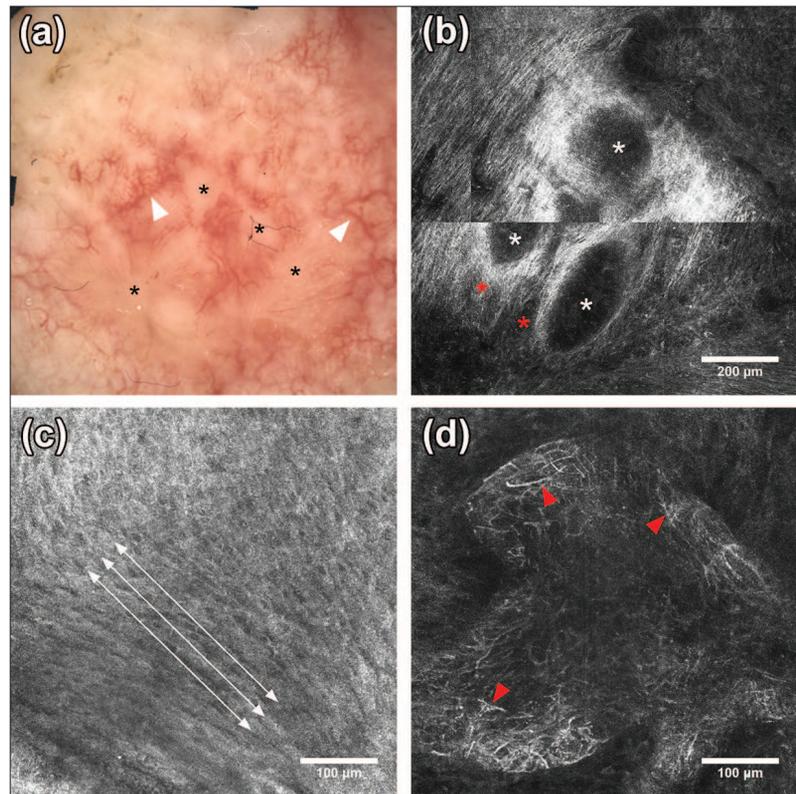


Fig. 2. Dermoscopy and RCM in nodular BCC. (a) Dermoscopy image showing arborizing vessels (white arrowhead), fine telangiectasia (red arrowheads) and blue ovoid nests (black asterisks); (b) RCM block (1,5 x 1,5 mm) at the level of the dermis: large tumoral islands (IT) (diameter > 300 μm) containing moderately refractile dots corresponding to inflammatory cells, surrounded by hyperrefractile areas of peritumoral fibrosis (red asterisks); (c) RCM block (1,5x1,5 mm) at the level of the dermis showing a large multi-lobulated tumoral island (IT) displaying peripheral palisading and peritumoral dark areas of clefting (white arrows); (d) RCM block (1,5x1,5 mm): lobulated tumoral island, ulceration (white asterisk) and hyperrefractile dots corresponding to inflammatory cells.

Fig 3. Dermoscopy and RCM in infiltrative BCC. (a) Dermoscopy image showing astructural red-white areas (black asterisks) and arborizing vessels (white arrowheads); (b) RCM block (approx. 1x1 mm) at the level of the dermis: dark silhouettes (white asterisks) corresponding to tumoral islands surrounded by hyperrefractile areas of peritumoral fibrosis (red asterisks); (c) RCM image (500x500 μm) at the level of the stratum spinosum illustrating epidermal streaming (white double arrows); (d) RCM image (500x500 μm) showing a multi-lobulated tumoral island, showing dark areas of peritumoral clefting, peripheral palisading and containing numerous dendritical structures (red arrowheads)



Discussions

Due to its high world-wide prevalence, basal cell carcinoma is one of the most studied skin cancers. Previous studies have portrayed the presence of tumoral islands and tumoral aggregates in the form of cords as the most important reflectance confocal microscopy criteria for the diagnosis of BCC [21, 22].

The data from this case series supports previous conclusions regarding RCM diagnostic criteria for BCC [21–23], demonstrating at the same time the usefulness of this non-invasive imaging technique for the determination of BCC histopathological subtype.

Therefore, the presence of tumoral aggregates in the form of cords connected to the epidermis is suggestive for the diagnosis of superficial BCC. On the other hand, in nodular BCCs we have observed mostly large tumoral islands (diameter >300 μm) showing peripheral palisading and containing dendritic structures, and tumoral cords. The dark, hyporefractile clefting areas surrounding tumoral islands corresponding to mucin deposition on histopathological exami-

nation were much more pronounced in nBCC when compared to superficial or infiltrative BCC. Although hyper-vascular areas were a constant regardless of the histopathological type, nBCC blood vessel caliber was larger when compared to the other two subtypes.

In the case of iBCC we observed, in addition to other criteria (thin tumoral cords, hyper-vascularization), the presence of intradermal dark silhouettes surrounded by hyperrefractile dense collagen bundles. This specific finding is considered by some authors as a key element for infiltrative BCC RCM diagnosis [24].

Peripheral palisading and inflammatory infiltrates were encountered in all three histopathological subtypes, while “onion-like” structures corresponding to milium cysts were rare occurrences.

Conclusions

Analysis of the data from this case series has shown a good correlation between the structures observed through RCM examination and those

determined by histopathological examination. Keratinocyte architecture visible on RCM examination corroborated with dermoscopy have allowed for discrimination between BCC histopathological subtypes in the examined patients, suggesting that this type of complementary approach could significantly improve pre-therapeutic diagnosis precision. This aspect is extremely important for clinicians, considering that the current guidelines suggest therapeutical approaches based on the histopathological subtype of BCC.

The major limitation of this study is the small number of patients, although this is only a pilot

study for determining the opportunity for a much larger study aimed at ascertaining the sensitivity and specificity of BCC histopathological subtype determination through RCM in the current clinical practice of romanian dermatological clinics.

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Conflict of interest
NONE DECLARED

Correspondance address: Lupu Mihai
Department of Dermatology, MEDAS Medical Center, Bucharest, Romania
email: lupu.g.mihai@gmail.com

