Review Article

In vivo confocal microscopy in the daily practice of the dermatologic surgeon

Perspectivas no uso da microscopia confocal in vivo *na prática do cirurgião dermatológico*

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ABSTRACT

New imaging methods, such as optical coherence tomography, magnetic resonance and high frequency ultrasonography, have been developed for use in dermatology. Among the new methods, in vivo reflectance confocal microscopy, has presented the fastest growth in large cutaneous oncology centers and has generated the greatest number of articles in the literature recently, due to its resolution – similar to histological resolution. In vivo confocal microscopy has been particularly useful given the many limitations of the dermoscopic diagnosis of cutaneous neoplasias, especially of achromic or hypopigmented lesions, lesions in the face or in mucous membranes. Some studies have also suggested considerable utility in the assessment of surgical margins and in determining the best area of the body for carrying out excisional biopsies. This study briefly describes how the confocal laser reflectance microscope works and discusses some of the issues that arise in its use in the daily practice of dermatologic surgeons.
Keywords: microscopy, confocal; early detection of cancer; skin neoplasms.

RESUMO

Novos métodos de imagem, como a tomografia óptica de coerência, a ressonância magnética e a ultrassonografia de alta frequência vem sendo desenvolvidos para uso na dermatologia. Entre estes novos métodos, destacamos a microscopia confocal de reflectância in vivo (MCR) como a que mais tem se difundido nos grandes centros de oncologia cutânea e que recentemente mais tem gerado publicações nos grandes periódicos, por permitir resolução semelhante à histológica. A microscopia confocal in vivo tem encontrado espaço nas muitas limitações que ainda se impõem ao diagnóstico dermatoscópico das neoplasias cutâneas, especialmente lesões acrômicas ou hipopigmentadas, lesões na face ou em mucosas. Ainda, alguns estudos tem sugerido grande utilidade na avaliação de margens cirúrgicas e na determinação do melhor local para a realização de biópsias excisionais. Este trabalho prtetende descrever brevemente o funcionamento do microscópio confocal de reflectância a laser e discutir algumas perspectivas que despontam para seu uso na prática cotidiana do cirurgião dermatológico.
Palavras-chave: *microscopia confocal; detecção precoce de câncer; neoplasias cutâneas.*

INTRODUCTION

Significant improvements in image quality and the clinical applicability of imaging suggest a promising future for the non-invasive screening and diagnosis of skin tumors. [1] Great advances have been achieved with the extensive use of dermoscopy; previously confined primarily to academic settings, dermoscopy is increasingly used in dermatologic practices and is becoming an indispensable tool in the evaluation of pigmented lesions. The use of dermoscopy is known to significantly enhance the precision of clinical examinations in the early diagnosis of melanoma, increasing the accuracy of the diagnosis by up to 20% [2,3] compared to examination with the naked eye.

Melanocytic lesions, however, remain a diagnostic challenge. Given the possibility of melanoma, the goal should always be 100% precision. In order to avoid procedures, biopsies and especially unnecessary surgeries, the diagnostic methods' specificity is crucial. [4,5]

Digital dermoscopy and total body mapping have been used to improve diagnostic accuracy. These techniques allow the evolutionary analysis of suspicious lesions or of the entire tegument of high-risk patients, allowing an early diagnosis of melanoma that is based on timely, comparative analysis of the same area of the body over time.[4]

Although they are still used more frequently in research, new imaging methods – such as optical coherence tomography, magnetic resonance and high frequency ultrasound – have been developed for use in dermatology.[3,5] Among these new methods, the in vivo reflectance confocal microscopy (RCM) stands out. Its use has been the most widespread in the major cutaneous oncology centers and, more recently it has been reported in the literature to obtain a resolution similar to histologic examinations. [5-9] In Brazil, the broader term "confocal microscopy" has become popular, and can refer to other methods, for different purposes from those described in this study, which in the English-language technical literature is known as in vivo reflectance confocal microscopy (RCM).

In vivo confocal microscopy has found space in the many limitations that still hamper the dermoscopic diagnosis of skin cancer, especially achromic or hypopigmented lesions. Some studies have also suggested that the technique can be very useful in evaluating surgical margins and determining the best site to perform excisional biopsies, particularly in large facial lesions.[1,9]

Next, we will briefly describe the operation of the RCM and discuss the emerging perspectives regarding its use in the daily practice of dermatologic surgeons.

IN VIVO CONFOCAL MICROSCOPY

Since it was first described in 1995 by Rajadhyaksha, the RCM technique and its dermatologic applications have been widely studied. Using this method, in non processed tissues are optically sectioned and viewed with sufficient resolution and contrast to allow the in vivo examination of skin lesions at the cellular level, with close-up images of histologic transversal cuts.[6]

The light source is an 830 nm and 35 MW laser, which illuminates a limited area without causing any tissue damage. In the same optical plane, a diaphragm with a detector receives only the photons reflected by the illuminated tissue. The smaller the diaphragm, the greater the resolution and smaller the thickness of the histological view obtained.[10]

The image formation is based on differences in the reflection of light provided by the various components of the skin, as a result of the different sizes of the structures and their refractive indices (Mie's theory). Melanin, keratin and collagen are considered natural contrasts, for they reflect the incident light more intensively and appear as bright structures in the examination.[11]

Vivascope, manufactured by Lucid Inc., is the commercially available microscope. The examination begins by attaching a metal ring to the patient's skin; a dermoscopic image of the lesion is taken with a dermoscopic camera that is connected to the device (VivaCam) (Figure 1). The rigid arm (Vivascope 1500) allows the microscope to be accurately coupled to the ring, aligned in the same direction as the dermoscopic photographs, which are used as a navigation map to guide the recording of images through the microscope in the areas of greatest interest. This mounting system ensures the immobilization of the skin and the stability of the images (Figures 2 and 3).

Horizontal images are recorded in different planes in the tegument. For better characterization and documentation, serial images of the entire lesion at several different levels – stratum corneum, epidermis, dermal-epidermal junction and papillary dermis – should be analyzed. This study provides relevant information about the lesions' architecture and suggests areas where closer examinations should be performed.

Adjacent 500 x 500 m images of each plane are acquired, forming a mosaic of up to 8 x 8 mm, representing the full extent of the lesion to be studied. The mosaics allow an overview of the lesion; sites with more architectural disorganization can be

Figure 1 – A metal ring is attached to the patient's skin and the device's dermoscopic camera (VivaCam) is coupled, in order to take the initial dermoscopic image

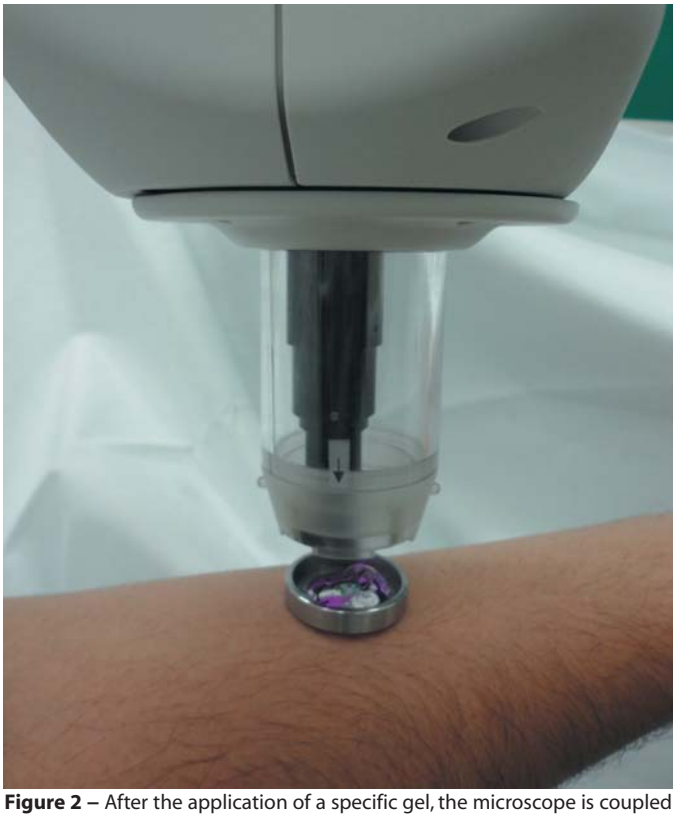

Figure 2 – After the application of a specific gel, the microscope is coupled to the metal ring using a magnet, and is aligned with the dermoscopic image's orientation

Figura 3 - The device allows the acquisition of histological images *in vivo* and a precise and stable navigation of the lesion, in a non-invasive manner

detected. In these locations, vertical navigation allows deeper or more superficial optical cuts, making it more likely that diagnostic patterns or structures are found.

An examination of a melanocytic lesion that is found to be suspicious using dermoscopy can be deepened in areas with, for instance, radial streaming or pseudopodia , and the atypical melanocytes nests can be recorded.[12]

Compared to conventional histopathological examination, RCM has some advantages. Because it is performed in real time, the patient's presence during the examination can be of great value. In addition to the clinical history and skin examination – which are not often requested together with anatomical pathological analyses – the entire lesion and skin (not just a piece of excised tissue) are available for immediate study. Processing or staining the tissue, or the presence of technicians and the preparation of blades, are not necessary. Also, the lack of aggression to the studied tissue allows the histological development of the process to be observed, if desired. Capturing images in real time also allows the cases to be dynamically evaluated. For instance, it is possible to observe microvascular phenomena in the papillary dermis, such as the leukocytary flow and the rolling of the leukocytes adhered to the vascular endothelium.

The quality of the images and the lack of studies are, however, limiting factors for the widespread use of RCM in decision-making. Thus, anatomical pathologic examinations arguably remain the gold standard for the definitive diagnosis of skin lesions.

APPLICATIONS OF CONFOCAL MICROSCOPY IN THE DAILY PRACTICE OF DERMATOLOGIC SURGEONS

There are many possibilities for the use of RCM in the daily practice of dermatologic surgeons. As knowledge about the method deepens, new applications are envisioned.

NON-INVASIVE DIAGNOSIS

Non-invasive diagnosis is the primary function of RCM. Specific and non-specific features of RCM have already been described for various cutaneous, tumoral and inflammatory processes. Well-defined diagnostic criteria are accepted for many skin tumors, such as basal cell carcinoma, [13-15] dermatofibroma, [16] nevi, lentigo maligna and melanoma.[17-25] Aspects observed using RCM for other tumors and lesions, such as angioma, [26] seborrheic keratosis,[27] actinic keratosis, squamous cell carcinoma,[28,29] keratosis lichenoides[30] clear cell acanthoma,[31] trichoepithelioma,[32] porokeratosis,[33] sebaceous hyperplasia,[34] and mycosis fungoides[35] have also been described. Most RCM studies, however, concentrate their efforts on the differential diagnosis of melanocytic lesions, since the strong contrast provided by the melanin in the RCM facilitates the observation of melanocytes.[6]

Dermoscopy has known limitations in the recognition of some types of melanoma that are categorized as featureless (lack of structures), and several algorithms have been developed and revised to help reduce false-negative results.[1,36,37] Although they do not have visible pigment, melanosomes are refracted in con-

focal microscopy, making this method a valuable tool that complements clinical examinations and dermoscopy of achromic or hypopigmented lesions.

In a number of such cases, RCM has proved to be a valuable adjuvant to non-invasive diagnosis. In equivocal clinical and dermoscopy cases, RCM helps to deepen the analysis when there is a suspicion of malignant lesions, as in the case of in situ melanomas that present an atypical dermoscopic pattern but do not meet the diagnostic criteria for melanoma (increased sensitivity).[37] RCM can help confirm a benign diagnosis for lesions that appear benign using dermoscopy, as in the case of compound nevi, keratosis lichenoides or thrombosed angioma (increased specificity).[38] In uncharacteristic lesions that are very small or have an "absence of structures" pattern, RCM can provide additional relevant information for the diagnosis and help determine the best surgical approach.[9] The approach to lesions on the face and in mucous membranes – areas of the body that are challenging for dermoscopy – also benefits from this method.[39] In this manner, many unnecessary diagnostic biopsies can be avoided, and a more assertive therapeutic approach can be used from the beginning, when appropriate.

We conducted multivariate analysis of all parameters displayed in RCM, and diagnostic algorithms were established for those that were statistically significant in differentiating nevi and melanomas.[40] In the Barcelona series,[21] there are two protective or negative criteria for melanoma (dermal papilla well delimited by refractory cells or edged papillae, and typical cells in the basal layer), and two positive criteria for melanoma (round intraepidermal pagetoid cells and atypical cells within the dermal papillae). Establishing a cut point at -2 benignities, and from -1, 0, 1 -2 - malignant, 100% sensitivity is obtained (all melanomas are detected) with 57% specificity (reducing 57% of the extirpations of clinical and dermoscopically doubtful lesions).

The Modena Group's algorithm evaluates equivalent parameters in the dermis and epidermis, however it sets out major and minor criteria. Examining a series of 351 lesions, Pellacani and colleagues obtained lower sensitivity with greater specificity.[40]

POST-OPERATIVE FOLLOW-UP

It is very important to be able to detect any local recurrence after surgical excision when conducting a primary diagnosis of skin cancer. This RCM benefit is evident in many situations, especially in cases of recurrent tumors, such as extramammary Paget's disease.[41]

In the case of basal cell carcinomas excised with compromised lateral margins, a follow-up using RCM gives more confidence to the surgeon and patient when choosing to wait, and allows the early detection of recurrence, which is expected in about 27% of cases of incomplete resection, keeping the deep margins free.[42]

RESPONSE TO CONSERVATIVE TREATMENT AND TREATMENT CONTROL

Non-invasive therapeutic modalities have been well received by dermatologic surgeons, who now employ them extensively for many cancers, for example photodynamic therapy, imiquimod or topic fluorouracil, and phototherapy. These treatment methods spare the patient from invasive procedures – which are considerably painful and sometimes unfeasible due to the extent and location of the lesions – and unattractive scars. Before the advent of RCM, the therapeutic success of these interventions could only be proven by anatomical pathological examination, performed through a skin biopsy.

More recently, the results of a study of 72 patients with histologically proven basal cell carcinomas, who underwent Mohs Micrographic Surgery and adjuvant topical imiquimod and followed up with RCM, showed alignment with the histopathology and a higher positive predictive value than that of isolated clinical examination for controlling the treatment.[42] For the treatment control of lentigo maligna and actinic keratoses with 5% imiquimod, RCM also seems to be very useful.[43-45]

ACTINIC AREAS WITH POTENTIAL FOR CANCER

Regarding the evaluation of actinic areas with the potential for cancer, RCM fills an important gap in the daily practice of dermatologic surgeons. Aging skin that has many signs of actinic damage, scars from previous procedures or poikiloderma is difficult to treat, and identifying incipient neoplasms in this type of skin, with areas designated as cancerizable, is a challenge. Even using dermoscopy, the distinction between actinic keratosis, invasive squamous cell carcinoma, Bowen's disease, basal cell carcinoma, keratosis lichenoides and seborrheic dermatitis in these areas can often rely exclusively on histopathology. RCM's usefulness in evaluating this skin type has been highlighted in recent studies, especially with an increase in non-invasive and preventive treatment options in areas with strong solar damage.[44]

IN VIVO MAPPING OF SURGICAL MARGINS

Precisely determining the boundaries between normal skin and tumorous tissue may often be impossible from a clinical perspective. Lesions of the lentigo maligna and lentigo maligna melanoma (LMM) types, non-pigmented or sclerosing basal cell carcinomas, and amelanotic melanomas, among others, may have poorly defined borders or may even be merged with adjacent skin with actinic lesions. In addition, in some cases of previously operated recurrent skin cancer, the presence of scars and irregular tumor growth also hamper the accurate assessment of the lesion's limits.

In such cases, RCM seems to have great value for surgical planning, as suggested by a study in which it showed a higher accuracy than examinations with Wood light and dermoscopy; its precision was comparable to the results obtained from histopathology, for a better delineation of LMM margins.[46,47]

The focal points for the exam should be selected based on the clinical and dermoscopic examinations, and also with the assistance of Wood light assessments. A more precise delimitation of the borders can be carried out using punch biopsies, guided by RCM. The advantage is that the latter is used in fewer pre-selected points, which can be confirmed through immunohistochemistry.[47,48]

A recent study described using RCM to evaluate surgical margins in Paget's extramammary disease.[41] The intra-operative examination of the margins in shavings was described in 2010 by a New York-based group that used 35% aluminum chloride – used for hemostasis purposes – to enhance the contrast of the structures in vivo.[49] RCM has also been suggested as another useful tool for the Mohs surgeon, in both *in vivo* and ex vivo applications.[50-52]

SELECTING THE BEST SITE FOR BIOPSY

The representativeness of incisional biopsies is a relevant concern in the daily practice of dermatologic surgeons. In order to achieve an accurate and definitive diagnosis, in addition to the adequate processing of the tissue and the correct interpretation of the pathologist, it is essential that the material sent for analysis is representative of the process being analyzed.[9] In extensive lesions with possible isolated areas of alteration that can actually allow diagnosis, it is of paramount importance to assess the best location for the biopsy.

Cases where the extent or location of the tumor make it impossible to implement excisional biopsy as the initial approach also pose challenges for incisional biopsy. For instance, isolated hyperchromic macules on the face of elderly patients may lead to a diagnosis of solar lentigo, lentigo maligna or lentigo maligna melanoma. A lentigo maligna melanoma lesion may present histological features of those three entities at different points. In that setting, dermoscopy of the face – due to the rectification of the epithelial cones and the actinic damage's poikiloderma – may prove difficult, and RCM can be considerably helpful in choosing the best location for the biopsy.44 Following the confirmation of the diagnosis, RCM can also be used to monitor and control the treatment in the case of a non-invasive therapy.[44, 45]

Mycosis fungoides lesions can also be challenging, since many biopsies are required to confirm the diagnosis. In this case, RCM can assist in finding the foci of the most significant epidermal alterations for biopsy, with occasional Pautrier microabscesses, which significantly increases the likelihood of obtaining a conclusive histopathological examination.[35]

CONCLUSIONS

RCM is emerging as a promising and versatile tool to assist dermatologic surgeons in the diagnosis and approach of cutaneous tumors. Compared with dermoscopy, it has demonstrated increased sensitivity and specificity in the clinical diagnosis of melanocytic lesions and doubtful dermoscopies, and many other applications are being studied. Nevertheless, it is important to note that traditional histopathology remains the gold standard for the definitive diagnosis of skin lesions.

RCM still has many limitations, which have been mitigated as more research is performed, and the device has been improved. In 2007, a consensus conference was organized to standardize concepts, and in 2009 an Internet-based study involving six reference centers was conducted to evaluate the reproducibility of those concepts and the derived terminology. 53, 54

The examination of a single lesion takes 5-15 minutes. A clinical examination and dermoscopy are essential to determine what should be assessed by RCM, for lesions with fewer alterations in the initial tests are more likely to present fewer characteristic findings using RCM.40

Another important limitation to be overcome with technical improvements in the near future is the visualization of the dermis, given that the reflection of the light only allows viewing to a depth of 350 m only (i.e., papillary or superficial reticular dermis).

However, while dermoscopy has probably already reached its full diagnostic accuracy potential, we expect great advances in RCM in the next few years.1 As was the case for dermoscopy, we expect RCM become part of the dermatologists' daily practice as an auxiliary method in the diagnosis and treatment of skin cancer. ●

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