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THE ROLE OF NON-INVASIVE MODERN TECHNIQUES IN THE DIAGNOSIS OF SKIN CANCERS: TELE-REFLECTANCE CONFOCAL MICROSCOPY, TELE-CONSULT AND E-LEARNING

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Abstract: *By developing non-invasive techniques such as dermatoscopy, confocal microscopy (RCM) and confocal endomicroscopy, teledermatology has entered a new age. The benefits of confocal microscopy are multiple by providing real-time dermatologist support for early diagnosis of suspected melanoma lesions that are still in curable stages, preventing unnecessary interventions and postoperative scars for benign lesions. It can also be used as a follow-up method for topical chemotherapy or laser ablation treatments for basal and spinocellular cancers. The utility of tele dermatology in RCM aims at the implementation of confocal microscopy/endomicroscopy as an early diagnostic method in daily practice as well as in research projects.*

Keywords: *tele-reflectance microscopy, tele-dermatology, skin cancer.*

1. Introduction

Dermatology is the branche of medicine in which is very important to visualize the lesions, making it ideal for current telemedicine techniques [21]. The use of the term teledermatology since 1995 has allowed the exchange of clinical information through electronic communications in order to establish a diagnosis to receive a second opinion from another remote specialist [15]. Helps track the evolution, triage or management of chronic lesions. Current data confirm the importance of teledermatology, becoming the second global specialization after teleradiology [15], [32]. Teledermatology has an impressive development through the increased accuracy of the diagnosis, approximately the same accuracy compared to the face-to-face diagnosis with the patient [30, 31].

Increased incidence of cutaneous cancers and melanoma in particular has focused research on the correlation between early excision of malignant

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lesions and increased survival by identifying melanoma as early as possible. Due to the heterogeneity of clinical forms, the diagnosis of skin cancers and melanomas in particular is often difficult to achieve, and therefore any additional information can help and guide a correct diagnosis and appropriate treatment for each clinical form [3, 24]. The use of advanced non-invasive techniques such as dermatoscopy and confocal microscopy allows in vivo imaging of cellular and tissue structures. These procedures increase the accuracy and sensitivity of skin cancer diagnosis [14]. Images obtained through dermatoscopy and confocal microscopy are mainly required for early screening and diagnosis of different types of skin cancer [15]. The purpose of these non-invasive techniques provides real-time clinician support for early diagnosis, especially malignant cutaneous-mucosal lesions, in curable stages, preventing unnecessary interventions.

This article reviews and summarizes current literature regarding to determine the potential role of dermoscopy, reflectance confocal microscopy in the routine clinical practice, and if RCM can improve melanoma detection rates, highlighting successes and barriers of RCM in skin oncology.

Dermoscopy is a non-invasive method that allows us to obtain an enhanced image of epidermis structures, dermal papillae, and once again allows the dermo-epidermal junction to be visualized [24]. Compared to the clinical exam, this diagnostic method increases the accuracy of the diagnosis [3]. By using dermatoscopy you can see pigmentary lesions, suspected benign lesions, and borderline lesions with malignancy potential, increasing sensitivity and specialty in early diagnosis of melanoma. Allows differential diagnosis and diagnosis of benign pigmentation with melanomas, pigmentary basal cell carcinoma, nevus and seborrheic keratoses.

Similar to dermatoscopy, laser confocal microscopy is a new method that allows horizontal sectional images of the skin with penetrating power from the epidermis to the dermal papilla, providing cellular resolution similar to the histopathological examination [9]. Confocal laser microscopy allows visualization of the cyto-architecture of different substrates, making it possible to diagnose with accuracy melanocytic lesions that are usually difficult to interpret, allowing differentiation of melanoma lesions from those of neophytes with dermatological aspects similar to melanomas. Safety margins can also be established. For the interpretation of confocal microscopy images, it is necessary to take into consideration the horizontally viewed sections as well as the impossibility to explore structures in the depth (deeper than 200 μm) [24], [14], [4].

A confocal microscopy session lasts for 5-10 minutes on average, so it is important to provide the patient with a comfortable position, thus avoiding the patient's need to change his position, resulting in immediate changes in the images. The recommended position is with the patient lying on the examination table, and in the case of localized lesions on trunk or upper limbs, the seated position with hands resting on the examination table. Hair removal is recommended in the regions to be examined [29].

2. **Historical Insight**

As early as 1957, Marvin Minsky laid the foundation for confocal microscopy, a technique used to obtain images of the

brain [18]. Shortly, confocal microscopy has become an important tool used to highlight lesions in the skin. Initially, this method used as white light source, now replaced by laser lights [17], [25, 26, 27]. At present, with regard to the diagnosis of certain dermatological disorders, the histopathological examination remains primordial [14].

Confocal microscopy allows to obtain sectional optical images of the tissues without the need for these physical sections, making diagnosis directly through optical sections and endogenous reflection contrast. Through this non-invasive technique, it is possible to observe in vivo, at a high resolution, similar to that of the histopathological examination of the epidermis, dermo-epidermal junction and superficial dermis. Depending on the reflection index of different tissues and cells, horizontal images are obtained [14]. This is possible due to the principle of confocal microscopy, that is, with the help of a metal ring placed in front of the detector, only light focused on the lens of the microscope is accepted, not allowing light outside the plane to penetrate [17], [25].

Confocal microscopy allows both direct in vivo examination of cancerous lesions in the human skin and examination on excised parts. When the examination is done in vivo, either the immersion gel or oil is added to the skin to obtain a reflection index similar to the stratum corneum index. At present, few fluorescein-based dyes are acceptable for highlighting the human skin, but the contrast of reflection can also be achieved by using acetic acid. With regard to the examination of excised tissue, fluorescein based dyes may be used to increase the contrast [25].

The confocal microscope acts on a field narrow enough to compare the size of the lesion, so to visualize the entire formation it is necessary to produce a collage of images, eventually leading to the formation of a mosaic. Image mosaicism allows examination of the entire lesion with the same high resolution of the confocal microscope [16], [25].

Concerning confocal microscopy, contrast refers to the changes occurring depending on the amount of light that can be detected. By using the contrast resulting from confocal microscopy, focused light from the sample level is the one that produces the contrast of the image. The amount of light reflected differs according to the reflection indexes of each structure. This reflectivity depends in turn on the chemical and molecular composition of each structure. The largest source of contrast in the skin is melanin, whose reflection index is about 1.7 compared to the average of 1.3. Also keratinocytes have a high reflection index [17], [25].

3. Data Transmission

Confocal tele-microscopy is an imaging technique that enables the recording and remote transmission of images and microscopic details of skin diseases in order to be diagnosed by specialists remotely or to request a second opinion [19], [33].

Confocal tele-microscopy (Tele-RCM) can be done by two methods. The first consists of storing and sending information, and the second involves a real-time video conference [19]. The main purpose of teledermatology with regard to tele-RCM is to promote and encourage the applicability of microscopy in day-to-

day practice as well as in research projects. Due to the advantages offered by RCM in clinical practice and research, the interest in studying this technique has been enhanced. So many online tutorials have appeared. Each training section contains: a dermatoscopic image of the lesion, two RCM complete mosaic images, clinical data of the patient related to the age and location of the lesion. Thus, through the online platform (skinconfocalmicroscopy.net), the e-learning process can be achieved [34].

In the case of suspicious or difficult to interpret, confocal tele-microscopy can also be consulted and shared with tele-consult. Tele-RCM is based on two components, namely: confocal tele-microscope consultations as well as Tele-RCM training/education programs [5].

4. Clinical Applications

In vivo examination of tissues at a resolution as large as that in confocal microscopy can identify amelanotic cancers. It may also dictate the type of biopsy or may indicate the extension of the safety margins in case of extensive lesions. The biopsy technique can also help with the histopathological examination [16], [27].

The normal appearance of the skin at the confocal microscope highlights the keratinocyte disposition in the form of a honeycomb architecture, being the most striking element of the structure. The cell size varies between 10 and 30 µm for the corneum layer cells, from 20 to 25 µm for the granular layer, and the cells of the spinous layer have a size between 15-25 µm [29]. Using in vivo imaging (RCM) allow the clinician to diagnose more confident skin cancers as: basal cell carcinoma (BCC), squamous cell carcinoma (SCC), or melanoma.

By using the RCM the most representative elements of BCC are: the bright tumor islands (pigmented BCC) or dark silhouettes (non-pigmented BCC) shown as oval, elongated or polycyclic cord-like structures, cleft-like dark spaces between the tumor island and the surrounding dermis and the tumor silhouettes related with the bright collagen bundles in the superficial dermis.

The common features of SCC are: the disarranged honeycomb pattern of the spinous-granular layer with round nucleated cells. Also round blood vessels are going to the dermal papillae perpendicular to the surface. In this type of cancer the RCM has limitations of depth of penetration.

In terms of melanomas, early diagnosis by modern non-invasive methods increases considerably the survival rate [12].

According to histopathological classification, melanomas can be divided into several forms: Superficial Spreading Melanoma (SSM), Nodular Melanoma (NM), Lentigo Malign, and Mucosal Melanoma.

As for SSM, confocal microscopic aspects describe: irregular honeycomb or cobblestone pattern in the epiderm, mild to moderate cytological atypia, nonedged papillae. In the dermis we can observe nests of pleomorphic melanocytes and inflammatory reaction represent by lymphcytes [2], [23].

RCM examination of Lentigo Maligna Melanoma revealed: dendric cells grouping around the hair follicles. In the upper dermis melanophages and reticulated bright collagen bundles are present due to solar damage [2], [9], [20].

Nodular melanoma is characterized by RCM by the presence in the dermis of

some hyporefractive and amorphous nests due to tumor infiltration (cerebriform nets) and large collagen bundles [6].

Regarding the localization of mucosal tumors, they are late diagnosed, often in advanced stages of the disease. From a dermatologist point of view, the most common tumors located in the mucous membranes are malignant melanomas [14], [16].

The technical confocal endomicroscopy is extremely complex and allows examination of the digestive tract mucosal structures at the cellular and subcellular level during the endoscopic examination, while also allowing the differential diagnosis of the localized pigment lesions in the mucous membranes [19]. For example, in over 80% of cases, primary anorectal melanomas are misdiagnosed as polyps, rectal cancer, and adenocarcinoma. Most of the lesions that appear have a polypioid appearance, may have pigment or not. Some of these lesions may also be ulcerated [33, 34], [5, and 12].

Four major RCM features characterize the mucosal melanoma: high density of basal hyper-reflective dendritic cells, presence of pagetoid cells in the epithelium, loss of the normal architecture of the papillae and the sheet-like proliferation in the chorion of atypical cells [28], [10], [8].

Compared to skin localization, confocal endomicroscopy examination of the mucosa provides better resolution and better contrast, more profound penetration into the epithelial and epithelial layers. Cellular morphology at this level can be better viewed due to the absence of the keratinization, pigmentation and thin layer of the epithelium [1], [13].

The principle of endomicroscopy is to integrate a laser confocal microscope in the distal part of a conventional endoscope. During the procedure a laser beam is used which emits an excitation wavelength of 488 nm. Laser scanning technology is adapted to obtain images generated through an optical fiber beam. Optical tissue sections have a size of 7 µm, allowing a lateral resolution of 0.7 µm. The fiber penetrance is between 0 and 250 µm. Thus, using this technology, histological imaging is obtained in vivo, since we can call it a true optical biopsy.

The main advantage is to provide the possibility of an in vivo diagnosis of malignant lesions, offering the possibility of targeting a biopsy, from the most representative area, also facilitating the subsequent anatomopathological examination [13].

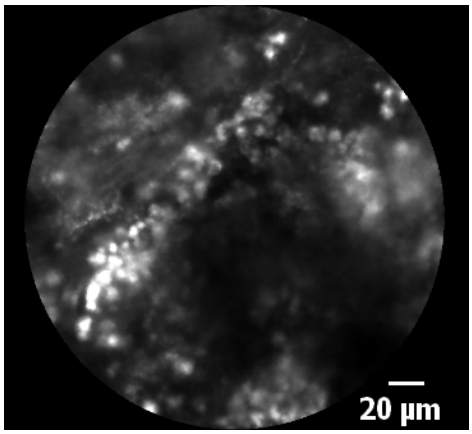

Fig. 1. *CLE image of an anorectal melanoma.*

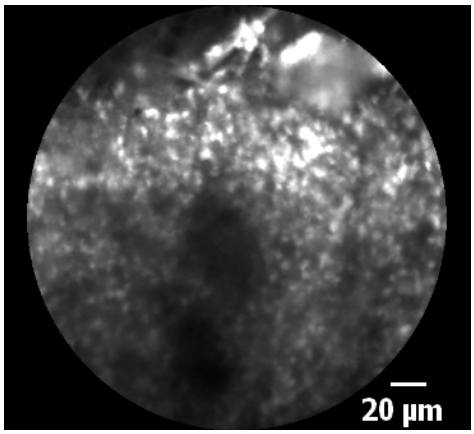

Fig. 2. *CLE image of an anorectal melanoma*

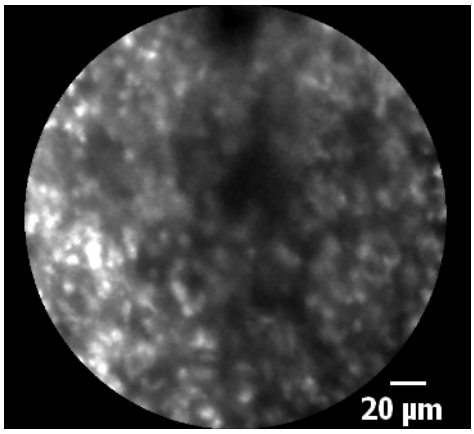

Fig. 3. *CLE image of an anorectal melanoma*

5. Implementations Barriers

Regarding the limitations of these non-invasive techniques, they are most often linked to the high cost and low number of RCM experts / lack of training.

Another limitation is the duration and position of the patient during the examination. Examination of an injury lasts for an average of 20-30 minutes. However, since 2007, it has been mirrored by the handheld RCM device, and depending on the working protocol, the scanning time may be down to 10 minutes.

Often, microscopy images are difficult to interpret, especially in the case of Langerhans / Melanocytes (Langerhans / Melanocytes), but also due to the possibility of examining the epidermis and superficial dermis only up to a depth of 250 microns.

Another limitation consists of evaluating hyperkeratotic or hypertrophic lesions located at the tissue level [13, 11, 7, 22].

Tele-RCM is based on two components: Confocal tele-microscopy consultations and Tele-RCM training programs, thus providing diagnostic methods with increased specificity and sensitivity even in areas without field experts.

6. Conflict of interests

The authors declare that they have no conflict of interests.

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