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Noninvasive Imaging Techniques for the Diagnosis of Nonmelanoma Skin Cancers

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ABSTRACT Nonmelanoma skin cancers (NMSC), basal cell carcinoma (BCC), and cutaneous squamous cell carcinoma (cSCC) are the most common neoplasms worldwide. Their incidence has been continually rising. This is due to several risk factors such as chronic sun exposure, longer life expectancy, sun-damaged skin, genetic predisposition, and immunosuppression. NMSCs are curable cancers if detected early and treated appropriately. Clinical examination is the first step towards their diagnosis, with accuracy depending on clinician expertise. Dermoscopy has become an irreplaceable diagnostic procedure for clinical examination and improving diagnostic accuracy of skin cancers. However, skin biopsy with histopathological analysis remains the gold standard in establishing a definite diagnosis. Repeated biopsies, however, are not acceptable in patients with multiple suspicious lesions and are often redundant in cases of lesions that are challenging to identify, as they are often benign. Several medical imaging technologies are available as additional tools for noninvasive examination of NMSCs and include reflectance confocal microscopy (RCM), high-frequency ultrasound (HFUS), optical coherence tomography (OCT), Raman spectroscopy, fluorescence polarization, and others. These methods enable clinicians to establish more rapid and accurate diagnoses without the need for invasive biopsies and to achieve optimal treatment for NMSC. RCM an HFUS are discussed along with their clinical applications.

KEY WORDS: noninvasive imaging**,** reflectance confocal microscopy, high-frequency ultrasound, skin cancer

INTRODUCTION

Nonmelanoma skin cancers (NMSC), including basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC), are the most common malignancies worldwide that have taken on epidemic proportions, with a growing prevalence that represents a significant health issue in some countries (1,2). BCC is the most common type and comprises approximately 80% of NMSCs (3,4).

It is estimated that 3.5 million new cases of NMSC are diagnosed each year in the USA, with multiple occurrences in some people (3,5). Incidence rates of BCC in the USA have risen by approximately 2% per year, while the average increase for cSCC is 2-4% per year, with significant increases among women and individuals younger than 40 years (3,6-8).

Causes of these conditions may be found in chronic sun exposure, longer life expectancy, sun-damaged skin, associated with a fair skin type, genetic predisposition (e,g, Gorlin syndrome, xeroderma pigmentosum) and immunosuppression (5,9-11). Exposure to

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Table 1. Reflectance confocal microscopy features of basal cell carcinoma

BCC features	RCM features of BCC
Tumor islands	Round to oval structures at the dermal-epidermal junction that are darker or lighter than the surrounding epidermis or dermis
Blood vessels	Elongated and thickened (appear darker); often oriented parallel to the epidermis
Polarization of nuclei	"Streaming" cells within the tumor islands or overlying keratinocytes whose nuclei are elongated along the same axis
Clefting	Slit-like spaces between the tumor island and surrounding stroma (darker areas)

ultraviolet (UV) light, particularly UVB, represents the greatest risk factor for the development of NMSC with a clear mutational signature of UV radiation (12,13).

BCCs are usually localized, with the potential to infiltrate and damage surrounding tissue, but have low metastasis and mortality rates (14-16). On the other hand, SCCs are high-risk skin cancers with the potential to metastasize and ultimately lead to death (17).

The cure rates of these cancers are high with early detection, accurate diagnosis, and appropriate treatment. Workup of patients with lesions suspicious of NMSC should begin with medical history and clinical examination. Visual inspection should be conducted by a trained physician, given that accuracy has been shown to vary according to the clinician's expertise. The overall sensitivity of clinical examination varies from 56-90%, with a specificity ranging from 75-97% (18).

In recent decades, dermoscopy using a handheld dermatoscope has become the most widely used tool for clinicians to improve diagnostic accuracy of skin cancers and is currently referred as the "dermatologist's stethoscope" (19). Given that it provides rapid *in vivo* visualization of skin morphologic structures up to the papillary dermis, dermoscopic structures have been assessed to correlate well with the underlying histopathologic alterations so the method can be regarded as a link between clinical and histopathological examination (19,20).

The histopathological analysis of a skin specimen obtained by biopsy is considered to be the gold standard in the diagnosis of NMSC and represents the only way to establish a definite diagnosis of a suspicious lesion (21). However, this procedure is invasive and occasionally painful.

The primary aim in diagnosing skin cancer is to minimize false-negative diagnoses to avoid delay in diagnosis and even death in some cases, e.g. in cSCC (22). Nevertheless, multiple biopsies may not be practical or cosmetically acceptable in patients with an extensive history of BCCs or widespread actinic damage (23).

In some cases, it is still challenging to establish a proper diagnosis and to discriminate malignant from benign lesions, as patients often have multiple equivocal and sometimes confluent lesions covering large areas with various clinical presentations (24). Consequently, many biopsies are performed on benign lesions. False-positive diagnoses therefore cause not only unnecessary scarring from a biopsy or excision but also increase patient anxiety while they await the definitive histological results and increase healthcare costs (22). On the other hand, mistaking one skin cancer for another can lead to the wrong treatment or delays in effective treatment. Although BCCs are usually slow-growing and very unlikely to metastasize, delayed diagnosis can lead to larger and more complex surgical procedures with consequently greater morbidity, while the consequences of a falsely negative test in a person with cSCC can be serious and potentially fatal (22).

In addition to rising incidence rates of NMSC, the above has indicated the need for their rapid and efficient management in the sense of developing a

Table 2. Reflectance confocal microscopy features of cutaneous squamous cell carcinoma and actinic keratosis

RCM features of cSCC	RCM features of AK
Scale at the stratum corneum level	Architectural disarray
Atypical honeycomb or a disarranged pattern	Focal areas of atypical honeycomb pattern
Round nucleated cells at the spinous-granular layer Round vessels in the dermis	Keratinocyte pleomorphism

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Table 3. Ultrasonographic features of basal cell carcinoma regarding histological type

Histological type of BCC	Ultrasonographic features
superficial BCC	hypoechoic areas with elongated contours and clear margins
nodular BCC	hypoechoic zones with round or oval outlines and hyperechoic inclusions displaying a punctiform pattern
sclerodermiform BCC	hypoechoic areas, infiltrating dermis, with unclear margins

test that can reduce false positive clinical diagnoses without missing true cases of disease, which is both patient- and resource-beneficial.

Recently, several medical imaging technologies have become available as additional tools for noninvasive examination of NMSCs. In addition to dermoscopy, reflectance confocal microscopy (RCM), high-frequency ultrasound (HFUS), and optical coherence tomography (OCT) provide real-time images at the cellular and subcellular levels, assisting clinicians in making rapid and accurate diagnosis without the need for invasive biopsies and enabling optimal treatment. These methods have proven to be both sensitive and specific and possibly cost-effective. Other modalities include Raman spectroscopy, fluorescence polarization, multiphoton laser scanning microscopy, fluorescence lifetime imaging, and bispectral fluorescence imaging.

Herein we discuss RCM and HFUS in detail with a brief description of technologies behind them as well as the clinical applications and features of NMSCs that can be visualized with these methods.

Reflectance confocal microscopy

Reflectance confocal microscopy (RCM) is a real-time noninvasive optical imaging technique that allows *in vivo* high-resolution visualization of the skin. It is capable of producing cellular resolution from the epidermis to the upper papillary dermal layers (25-28).

The mechanism of RCM is comparable to that of an ultrasound, except the system uses principles of optical reflection instead of sound waves (26). It involves a single point illumination via a laser source of a specific wavelength (23). This light is transmitted through the skin to illuminate a point within the tissue. This light is then reflected back and goes through a pinhole in an optically conjugate plane to form an image in the detector. The pinhole only allows reflected light from the focal point to enter, hence the term "confocal". This produces a black-and-white horizontal (en-face) image, in contrast to conventional colored, vertical histopathology sections (23,24). These grey-scale RCM images thus have a resolution comparable to that of histopathology (26). The contrast in RCM images is dependent on the differences in reflectivity of the tissue, which is an inherent property of the tissue due to differences in molecular structures (23). Structures with a high refractive index appear bright. For skin, melanin and melanosomes have the highest refractive index and therefore appear white, while nonreflective structures appear dark in RCM images. Commercially available microscopes, such as RCM Vivascope 1500, utilize a diode laser source with a near infrared wavelength at 830 nm which is non-harmful to human skin (23). The penetration depth for diagnostic purposes is usually up to 200-300 µm, which corresponds to the superficial dermis. The emitted light can go deeper, but the image resolution is reduced as depth increases (23,24). RCM has proven to be a very valuable tool for evaluating both melanoma and NMSC (29). A growing body of literature has demonstrated that RCM is an additional tool alongside dermoscopy for enhancing accuracy in the diagnosis of skin cancer (29). According to one study, sensitivity and specificity of RCM in the diagnosis of skin cancer is 95.3% and 83.9%, respectively (29). The overall RCM sensitivity for BCC is 92-100% with a specificity ranging from 88% to 97% (30,31).

RCM has proven to be a useful tool in avoiding unnecessary excisions of benign lesions that can look dermoscopically suspicious for skin cancer (25,32). A cost-benefit analysis performed in Europe showed that this led to overall cost savings in the management of both melanoma and NMSC (31). On the other hand, RCM can be useful in identifying the most representative biopsy site within the lesion, bypassing the sampling mistakes or falsely-negative results of the test (33).

Indications for the use of RCM in a clinical setting are dermoscopically challenging lesions located in the head and neck area, allowing skin biopsies that can be disfiguring in such cosmetically sensitive areas to be avoided; lesions on sun-damaged skin, which is related to the fact that the epidermal atrophy and the flattening of the dermoepidermal junction make

these lesions easily explorable with RCM, which has an excellent resolution for flat lesions; regressive lesions; and lesions presenting with unclear BCC dermoscopic criteria, due to the fact that the diagnostic potential of RCM for BCCs has been extensively described (25,29,34,35).

RCM criteria defined for the diagnosis of BCC include tumor islands (round/oval structures at the level of the dermoepidermal junction that are darker or lighter than the surrounding epidermis or dermis), polarization of nuclei ("streaming" cells within the tumor islands or overlying keratinocytes whose nuclei are elongated along the same axis), clefting (slit-like spaces between the tumor island and surrounding stroma; these areas are darker in contrast) and blood vessels (elongated and thickened blood vessels appear darker, often oriented parallel to the epidermis; can contain round white blood cells that appear cleft) (Table 1) (23,36). This is akin to features seen on traditional histology as well as dermoscopy (23).

One study presented RCM features for BCC, which might allow *in vivo* diagnosis of the nodular, micronodular and superficial subtype of BCC, preventing a skin biopsy and resulting in direct proper treatment (34).

RCM can also help differentiate pigmented BCC from melanoma or other pigmented lesions (33).

Under RCM, cSCC is revealed by the presence of scales at the stratum corneum level, which is of limited diagnostic value given that many benign lesions can also present this way. However, other RCM features of cSCC may be helpful for diagnosis and include an atypical honeycomb and/or a disarranged pattern of the spinous-granular layer, round nucleated bright cells in a pagetoid pattern at the spinous-granular layer, and round blood vessels in the center of dermal papillae (Table 2) (37). There are two types of targetoid cells. The first type is a large cell with a bright center and a dark peripheral halo, histologically corresponding to large dyskeratotic keratinocytes separated from adjacent cells by a clear retraction halo. The second type is a cell with a dark center and a bright rim surrounded by a dark halo, histologically corresponding to dyskeratotic keratinocytes containing a pycnotic nucleus (38). RCM is also indicated in the diagnosis and monitoring of actinic keratoses (AK) and monitoring cytological alterations in the cancerization field (24). RCM features of AK are architectural disarray, keratinocyte pleomorphism, and focal areas with an atypical honeycomb pattern (Table 2) (39). There is thus some overlap in the RCM features of cSCC and AK, but architectural disarray at the spinous-granular layer and the presence of nest-like structures in the dermis are highly suggestive of cSCC (40).

Another application of RCM is in Mohs micrographic surgery (MMS). MMS is performed in stages, while being guided by an examination for residual tumor in the peripheral (epidermal) and deep subcutaneous (dermal) margins with frozen pathology (41). However, preparation of frozen pathology at each stage is time-consuming and labor intensive, which is why a noninvasive real-time high-resolution optical imaging approach such as RCM may help to enhance the Mohs procedure by enabling intraoperative detection of residual NMSC tumor directly in the surgical wound on the patient (41,42). RCM has proven to be promising for the detection of BCCs in human skin *in vivo*. This means that it has the potential to define lesion margins before surgical therapy. It has also been demonstrated that it is possible to examine NMSCs in *ex vivo* tissue during MMS without frozen sections, which is time saving (43-46).

RCM has also found application in nonsurgical methods of removing skin cancers (23). One study which evaluated the use of RCM in assessing the efficacy of photodynamic therapy (PDT) in the treatment of BCC and evaluating skin changes following PDT found that RCM was a valuable additional tool for PDT treatment monitoring of BCC (47). Used this way, RCM can help adjust the frequency and duration of treatment for maximum treatment efficacy, leading to an overall treatment cost savings (30,48).

Another study utilized RCM both *in vivo* and *ex vivo* to help guide the laser ablation of BCC (49). RCM was used to detect any residual BCC tumor or complete clearance in tissue sections ablated using either an Erbium-YAG or carbon dioxide laser.

RCM can also be used as a tool for treatment monitoring of AKs and cancerization fields, offering insights into the cellular response to topical treatments (39,50).

There are some disadvantages of RCM, which include the image processing time, limited depth of imaging, and need for extensive training to master image interpretation (31). However, the technology advancements with consequently improved resolution, depth of view, and visualized area as well as incorporation of RCM training into dermatology residencies will lead to improved speed, diagnostic accuracy, and ease of use (23).

RCM is mostly utilized in university hospital centers and in research. However, this noninvasive *in vivo* evaluation of the skin represents a powerful additional tool in everyday practice.

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High-frequency ultrasound

Ultrasound is a noninvasive imaging technique that essentially relies on the measurement of sound wave reflections from the body tissues. A transducer generates a focused beam of sound pulses, measuring the reflections (echoes) produced by structures within the tissue (22). This optical tool translates these sound waves into grey-scale images that can be visualized for interpretation (24). The spatial location of a tissue structure that produced an echo is determined in the lateral direction (parallel to the skin surface) by the position of the sound beam (known) and is determined in the axial (depth) direction by the return time of the echo (measured) and the speed of sound in the tissue (known to a good approximation) (51,52). An important parameter is the range of acoustic frequencies used to form the image. While low frequency ultrasound visualizes the deeper structures of the body, such as the internal organs, high-frequency ultrasound (HFUS) with frequencies of ≥20 MHz has a much lower depth of tissue penetration but produces a higher resolution image of tissues and structures closer to the skin surface (52). Frequencies of 20-25 MHz allow visualization of both the dermis and epidermis, while higher frequencies of 50 MHz and above visualize the epidermis only (52).

In B-mode (brightness mode) ultrasound echography, the image brightness is modulated according to the amplitude of the echoes (echogenicity). This in turn is determined by the values of sound speed and mass-density within an echo-producing structure relative to those values in the surrounding medium and the size, shape, orientation, and number-density of such structures (51). Structural proteins, such as collagen and keratin, are dense and have high sound speed and generate strong echoes (hyperechoic or echogenic) when the fibers are thick, densely packed, and oriented mostly perpendicularly to the ultrasound beam (e.g. reticular dermis). Adipose tissue, highly cellular lesions with little collagen or keratin, and regions where the collagen bundle size is small and oriented mostly parallel to the sound beam (e.g. papillary dermis) generate weak echoes (hypoechoic or echo poor). Liquids (e.g. as in simple cysts) generate no echoes and are referred to as anechoic (53,54).

In dermatological practice, probes with a pulse generation frequency of 20 or 22, 30, 50, 75, and 100 MHz are used (55-57). HFUS provides a high resolution, allowing differentiation between the epidermis, dermis, and subcutaneous fatty tissue as well as visualization of volume formations in various pathological processes (57).

In a retrospective study by Wortsman and Wortsman, the diagnostic value of HFUS was assessed by imaging 4,338 cutaneous lesions along with 130 normal control specimens (58). They found that the ultrasound diagnosis matched the final diagnosis in 73% of cases, with 97% accuracy and 100% specificity.

In BCC, HFUS permits clear visualization of the tumor margins and assists in evaluating whether the tumor is superficial, nodular, or infiltrative (24). In addition, the presence and number of intralesional hyperechoic spots can help in predicting the risk of recurrence in the micronodular, sclerosing variant and morpheaform subtypes of BCC (59).

Regarding ultrasonographic features of BCC, previous studies using the ultrasound probe with a frequency of 20 MHz detected BCC as voluminous, hypoechoic areas, round or oval, with local thickening of the epidermis and the dermis (60,61). However, for the best visualization and determination of the level of invasion, which depends on clinical variants of BCC, it is preferable to use the high-frequency probes (above 20 MHz) (62). A recent Russian study explored ultrasonographic features of the superficial and nodular forms of BCC using 30 and 75 MHz probes (62). In this study, all clinical variants of the BCCs were defined as hypoechoic zones located directly beneath the epidermis and propagating into the dermis at varying depths, which is consistent with the results of other authors. More specifically, superficial BCCs presented as hypoechoic areas with most often elongated contours and clear margins, while the nodular BCCs presented as hypoechoic zones with round or oval outlines and a diffusely-heterogeneous structure (hyperechoic inclusions displaying a punctuation pattern). Sclerodermiform BCCs appeared as hypoechoic areas infiltrating the dermis, with unclear margins (Table 3) (62). The depth of invasion of BCC depends on its histological type and is the greatest with micronodular and infiltrative types, followed by the nodular and superficial type (63).

In this study, hyperechoic inclusions displaying a punctiform pattern were recorded in the structure of 11% of superficial BCCs and in 100% of nodular BCCs. In other studies, these structures were revealed by histological analysis to be calcifications, corneous cysts, and necrosis areas of tumor cells (60,64).

While the use of HFUS in the diagnosis of BCC is well-explored and extensively described, studies on the use of HFUS in the diagnosis of cSCC, especially on ultrasonographic features, are poor. CSCC usually appears as hypoechogenic lesion with an irregular, but quite well-defined border (65). Given that all NMSC lesions appear hypoechogenic in HFUS, this

method alone is not suitable for differential diagnosis (66-68). Dermoscopy is therefore still irreplaceable in early diagnosis of cSCC, and HFUS should be used as a complementary method in preoperative evaluation of the tumor (65).

Generally, HFUS may offer additional diagnostic information when used in conjunction with clinical and/or dermoscopic examination of suspected skin lesion, helping physicians establish more accurate diagnosis and identify lesions requiring excision (22,69). However, more studies investigating this method in diagnosing NMSC in patients with suspicious lesions are needed.

Conclusion

NMSCs are increasing worldwide, but they are highly curable with early and proper diagnosis and appropriate treatment. Visual examination combined with dermoscopy and skin biopsy in suspicious lesions are common diagnostic procedures that sometimes result in unnecessary excisions, since it may be hard to discriminate benign from malignant lesions and a definite diagnosis can only be established through histological analysis. This also requires tissue processing and interpretation time. On the other hand, not recognizing or mistaking one skin cancer for another can lead to delay in diagnosis and effective treatment, resulting in greater morbidity from these cancers and in some cases even death. This led to a need for a rapid and effective diagnostic test with high sensitivity and specificity.

Over the past two decades, novel powerful imaging noninvasive technologies for examination of NMSC have been developed and optimized. In addition to dermoscopy, RCM, HFUS, and others provide real-time images at the cellular and subcellular levels, providing rapid and accurate decisions with challenging lesions. These methods have proven to be both sensitive and specific, and possibly cost-effective.

Ultrasonographic and RCM features of both BCC and cSCC have been proposed to enable *in vivo* diagnosis of these cancers, and even histological subtype assessment in cases of BCC, without the need for invasive biopsies. RCM and HFUS may also be helpful in presurgical evaluation of tumor size as well as in detection of residual tumors in excised specimens. RCM has also proven to be a valuable additional tool for treatment monitoring of BCCs, AKs, and cancerization fields when treated with topical agents.

Continuing technological advancements in the coming years will surely overcome barriers to these methods, such as image processing time, limited depth of imaging, and need for extensive training to master image interpretation, and result in improved speed, diagnostic accuracy, and ease of use.

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