

Non-invasive diagnostic techniques in the diagnosis of squamous cell carcinoma

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Abstract

Squamous cell carcinoma is the second most common cutaneous malignancy after basal cell carcinoma. Although the gold standard of diagnosis for squamous cell carcinoma is biopsy followed by histopathology evaluation, optical non-invasive diagnostic tools have obtained increased attention. Dermoscopy has become one of the basic diagnostic methods in clinical practice. The most common dermoscopic features of squamous cell carcinoma include clustered vascular pattern, glomerular vessels and hyperkeratosis. Under reflectance confocal microscopy, squamous cell carcinoma shows an atypical honeycomb or disarranged pattern of the spinous-granular layer of the epidermis, round nucleated bright cells in the epidermis and round vessels in the dermis. High frequency ultrasound and optical coherence tomography may be helpful in predominantly in pre-surgical evaluation of tumor size. Emerging non-invasive or minimal invasive techniques with possible application in the diagnosis of squamous cell carcinoma of the skin, lip, oral mucosa, vulva or other tissues include high-definition optical coherence tomography, in vivo multiphoton tomography, direct oral microscopy, electrical impedance spectroscopy, fluorescence spectroscopy, Raman spectroscopy, elastic scattering spectroscopy, differential path-length spectroscopy, nuclear magnetic resonance spectroscopy, and angle-resolved low coherence interferometry. (*J Dermatol Case Rep.* 2015; 9(4): 89-97)

Key words:

actinic keratosis, dermoscopy, diagnosis, skin cancer, oral mucous membrane, RCM, spectroscopy, squamous cell carcinoma, ultrasonography, vulva

Introduction

The term non-melanoma skin cancers includes cutaneous lymphomas, adenexal tumors, Merkel-cell carcinoma, and other rare primary cutaneous neoplasms, but is mainly used in clinical practice to define basal cell carcinoma (BCC) and squamous cell carcinoma (SCC).¹ Squamous cell carcinoma is the second most common cutaneous malignancy after basal cell carcinoma with an increasing incidence worldwide.^{2,3} The most frequent presentation of SCC in situ is an erythematous scaly patch or slightly elevated plaque. Invasive SCC is often papulonodular, but can be plaque-like, papilomatous or exophytic. SCC usually arises within a background of sun-exposed skin, most commonly on the bald scalp, face, neck, forearms and dorsal hands. Actinic keratosis (AK) is

a common sun-induced erythematous lesion covered with scale. Historically AK has been described as a precursor of cancer or a precancerous lesion that have potential to develop into SCC. Some authors have postulated that both AK and SCC in situ share the same clinical, histological and molecular features and that they represent a continuum of disease.⁴ Clinical difference of SCC from actinic keratosis, superficial basal cell carcinoma, clear cell acanthoma, amelanotic melanoma, psoriasis, warts or eczema may be difficult. In case of diagnostic difficulties, a confirmatory diagnosis can be achieved by histopathological examination. Although the gold standard of diagnosis for SCC is an invasive biopsy followed by histopathological evaluation, non-invasive and minimally invasive diagnostic tools have obtained increased attention.

Dermoscopy and videodermoscopy

Dermoscopy (dermatoscopy) is a non-invasive diagnostic technique that allows *in vivo* evaluation of colors and microstructures of the epidermis, the dermaepidermal junction and the papillary dermis at 10-fold magnification. The term "dermatoscopy" was used in 1920 for the first time by Saphier, who detailed description of possible applications of skin surface microscopy.⁵ Many different terms have been used in the literature for this technique: dermatoscopy, dermoscopy,⁶ surface microscopy,⁷ incident light microscopy, epiluminescence light microscopy.⁸ Videodermoscopy is a digitalized form of dermoscopy, which allows dermoscopic visualization of structures in high magnification (70-fold and higher).

The dynamic development of dermoscopy and the development of numerous dermoscopic algorithms for distinguishing benign from malignant skin lesions began in the late 80's and 90's of the twentieth century. Several studies comparing dermoscopy examination with conventional clinical examination have documented that the diagnostic accuracy of pigmented lesions, especially for malignant melanoma, is significantly better with dermoscopy when performed by a trained and experienced user.^{9,10} In addition, dermoscopy has been shown to improve diagnostic accuracy of nonpigmented skin malignancies.^{11,12}

Dermoscopic evaluation can be performed with non-polarized light contact dermoscopy, polarized light contact dermoscopy or polarized light non-contact dermoscopy. It is imperative that the users of these techniques should be aware of the subtle differences in color and structures between polarized and non-polarized images to avoid misdiagnosis. In non-polarized light dermoscopy reduction in skin surface reflection is achieved by using an immersion interface between the skin and the lens. Polarized light is usually created with the use of filters. These filters allow the dermoscope to selectively capture backscattered light from deeper levels of the skin and avoid the superficially reflected light. Unlike non-polarized light dermoscopy, polarized light dermoscopy allows visualization of deep skin structures without the necessity of a liquid interface or direct skin contact with the instrument.¹² One of the differences between polarized dermoscopy and non-polarized dermoscopy is the ability to visualize white shiny structures using polarized light contact dermoscopy or polarized light non-contact dermoscopy. Three defined morphologies may be present by white shiny structures and include white shiny lines, white shiny areas and rosettes. White shiny areas are characterized as white shiny clods or larger structureless areas with a shiny, bright white color. Rosettes are defined as four bright white points grouped together akin to a four-leaf clover.¹³ Liebman *et al.* showed that the presence of white shiny lines of any length accompanied by white shiny areas is most suggestive of a diagnosis of BCC, and white rosettes speak in favor of the diagnosis of AK or SCC.¹⁴

Dermoscopic criteria have been described for two superficial forms of squamous cell carcinoma (pigmented and non-pigmented Bowen's disease and interepidermal carcinoma (IEC) and for invasive cutaneous squamous cell carcinoma.

Bowen disease and interepidermal carcinoma are characterized by two types of vascular patterns: classic small dotted vessels and glomerular vessels. Both patterns often appear within the same lesion and are distributed in small, densely packed clusters or groups.¹⁵⁻¹⁹ Pan *et al.*²⁰ reported a clustered vascular pattern, glomerular vessels and hyperkeratosis as the most frequent features of interepidermal carcinoma. The authors showed that the concurrent presence all three features in one lesion allows the diagnosis of interepidermal carcinoma with a probability of 98%.²⁰ In pigmented Bowen's disease, additional dermoscopic features are represented by small brown globules, which are regularly packed in a patchy distribution and by a grey to brown homogeneous pigmentation.¹⁶ In contrast to the dermoscopic patterns of Bowen's disease and interepidermal carcinoma invasive SCC presents a greater polymorphism of vascular structures. One can observe a vascular polymorphism comprised of linear irregular, hairpin and grouped glomerular/dotted vessels over a whitish background with a central mass of keratin or ulceration.²¹⁻²³

Lallas *et al.*²⁴ revealed a significant difference in the dermoscopy pattern between poorly differentiated SCC compared to well- and moderately differentiated tumors. Poorly differentiated SCCs were dermoscopically typified by a predominantly red color, resulting from the presence of bleeding and/or dense vascularity, in the absence of scaling and keratin or other white-colored criteria. In contrast, white-colored criteria, including scales/keratin, white circles, white halos and structureless whitish areas were shown to be associated with well- or moderately differentiated variants. In a study, which included 143 SCCs, Lallas *et al.*²⁴ showed that dermoscopically, the presence of a predominantly red color is associated with a 13-fold increased possibility of poor differentiation, whereas a predominantly white and white-yellow color decreased the odds of poorly differentiated SCC by 97% each. Furthermore, presence of vessels in more than 50% of the lesion's surface, a diffuse distribution of vessels and bleeding are markers of poor differentiation, while scaling is indicative of well- or moderately differentiated tumors.²⁴

Keratoacanthoma is a common low-grade tumor that originates in the pilosebaceous glands and microscopically closely resembles SCC. It is characterized by rapid growth over a few weeks to months, followed by spontaneous resolution over 4-6 months in most cases, but rarely it may progress to invasive carcinoma. Rosendahl *et al.*²⁵ identified several dermoscopic criteria that help differential keratoacanthoma from SCC and other nonpigmented lesions. The most important dermoscopic clues to keratoacanthoma and SCC are white circles, keratin crust/scale, blood spots, and white structureless zones. White circles are observed in both types of lesions but more frequent in SCCs than in keratoacanthomas (60% vs. 25.6%). Keratin crust/scale is present in 79.1% of keratoacanthomas and in 70.0% of SCCs and the presence of central keratin masses is more common in keratoacanthoma compared to SCC (51.2% vs 30.0%). Keratin crust/scale had the highest sensitivity for keratoacanthoma and SCC (79%) and with circles had the highest specificity (87%).²⁵ Lin *et al.*²⁶ have observed white circles in 32% of SCCs and 38% of keratoacanthomas, keratin crust/scale in 90% of SCCs

and all of keratoacanthomas and central keratin masses in 32% of SCCs and 88% keratoacanthomas.

Based on dermoscopic findings, Zalaudek *et al.* proposed a progression model of facial actinic keratosis developing into IEC and invasive SCC. Those AK that progress to increasing atypia tend to display vessels around follicles that become dotted or coiled on higher magnification, then as the lesion develops into SCC in situ the dotted/coiled vessels appear to enlarge, become more convoluted and clustered, and the follicles in this area appear to miniaturize and disappear. In concert with these changes in neovascularization, in other areas of AK the whitish keratotic follicles appear to coalesce, eventually forming the discrete whitish, opaque scaly areas typically seen in IEC. With progression of IEC to invasive SCC, the lesion thickens clinically while dermoscopically hairpin

and/or linear-irregular vessels will appear. Along with these vascular changes, a central mass of keratin forms and ulceration may occur.²⁷

Figure 1 and Table 1 present typical dermoscopy features of SCC.

Reflectance confocal microscopy

Reflectance confocal microscopy (RCM) is a non-invasive optical imaging technique that allows high-resolution visualization images of skin of the epidermis and the superficial level of the dermis in real-time at nearly histologic resolution.²⁸ The technique was first described for skin imaging by Rajadhyaksha *et al.* in 1995.²⁹ RCM relies on a low-power

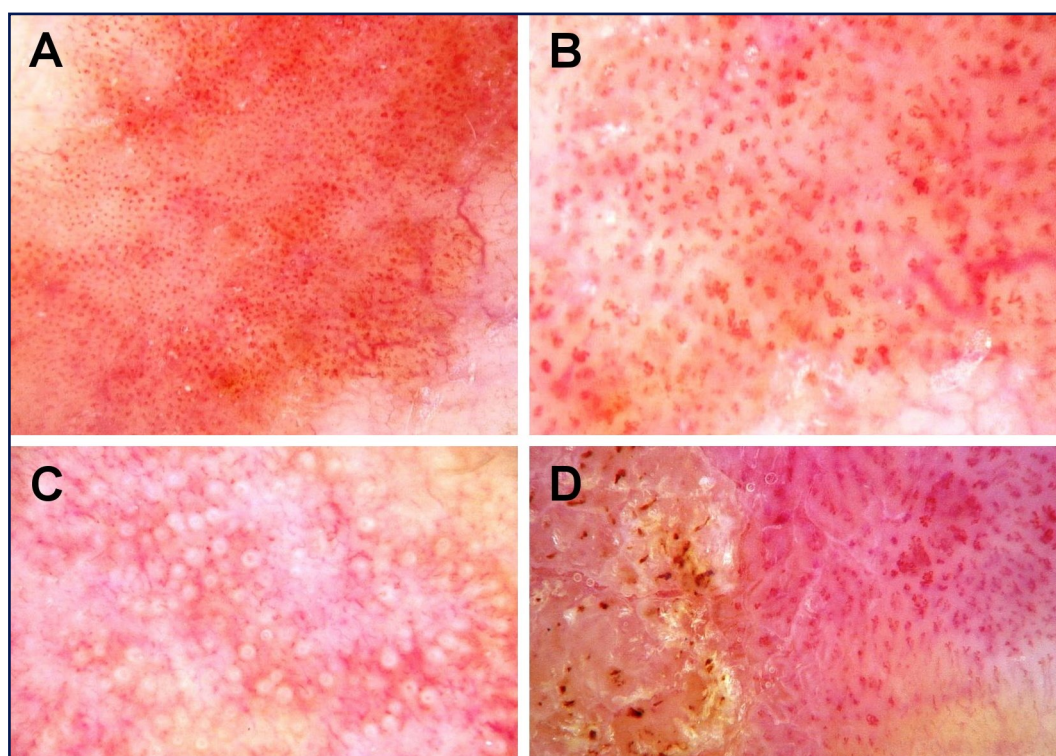


Figure 1

Videodermoscopy of squamous cell carcinoma in situ. 70-fold magnification. (A) SCC in situ with small dotted vessels distributed in packed clusters; (B) SCC in situ with glomerular vessels; (C) SCC in situ with white circles; (D) SCC in situ with dotted and glomerular vessels, and hyperkeratosis.

Table 1: Dermoscopy features of squamous cell carcinoma.

| DERMOSCOPIIC FEATURES OF SCC | |
|------------------------------|----------------------------------|
| Dotted vessels | 3.3–86.6% ^{25,27,69,70} |
| Coiled/glomerular vessels | 42–90% ^{16,25,69} |
| Herpin vessels | 15–38.5% ^{25,27} |
| Keratin crust/scale | 42.3–90% ^{16,25,27} |
| White circle | 60% ²⁵ |
| Ulceration | 17.9–28.6% ^{16,25,27} |

laser that emits a near-infrared light (830 nm), coherent laser beam by which the skin is illuminated. As the laser beam passes through the upper skin layers, it is partially backscattered due to the natural refractive index of microanatomical structures. This backscattered light has to pass through a narrow pinhole, which guarantees that only light reflected from structures "in focus" is detected; light from elsewhere is blocked. After passing the pinhole, the beam is diverted by a semireflective mirror system and, finally, directed to a detector. The obtained data are processed and visualized by special software on a computer screen.³⁰ The basic principles of RCM are based on the different refractive indexes of distinctive intracellular structures. Highly reflective structures appear bright/white, while non-reflective structures appear dark.³¹ In contrast to the colored, vertical conventional histopathology sections, RCM optical slices are horizontal and black-and-white. RCM was reported as a useful tool for *in vivo* assessment of nevi, melanoma, basal cell carcinoma, squamous cell carcinoma, inflammatory diseases, autoimmune bullous disease, bacterial, fungal and viral skin infection.³²⁻³⁹

Under RCM squamous cell carcinoma revealed the presence of scale at the stratum corneum level. The presence of the scale

may be limited diagnostic value because it can be present in many benign lesions. However, within the constellation of other RCM features of SCC, it may be helpful for diagnosis. Under RCM, SCC presents an atypical honeycomb and/or a disarranged pattern of the spinous-granular layer of the epidermis. Round nucleated bright cells in a pagetoid pattern are observed at the spinous-granular layer.⁴⁰ There are two types of targetoid cells. The first type is a large cell with a bright center and a dark peripheral halo. The second type is a cell with a dark center and a bright rim surrounded by a dark halo. The first one corresponds on histologic examination to large dyskeratotic keratinocytes separated from adjacent cell by a clear retraction halo, and the second type corresponds histologically to dyskeratotic keratinocytes containing a pyknotic nucleus.⁴¹ RCM images of SCC revealed in the center of dermal papillae a round blood vessels.⁴⁰ While there is some overlap in the RCM features of SCC and AK, architectural disarray in the stratum granulosum and the spinous layer and the presence of nest-like structures in the dermis are highly suggestive of SCC.⁴²

Figure 2 and Table 2 present typical RCM features of SCC.

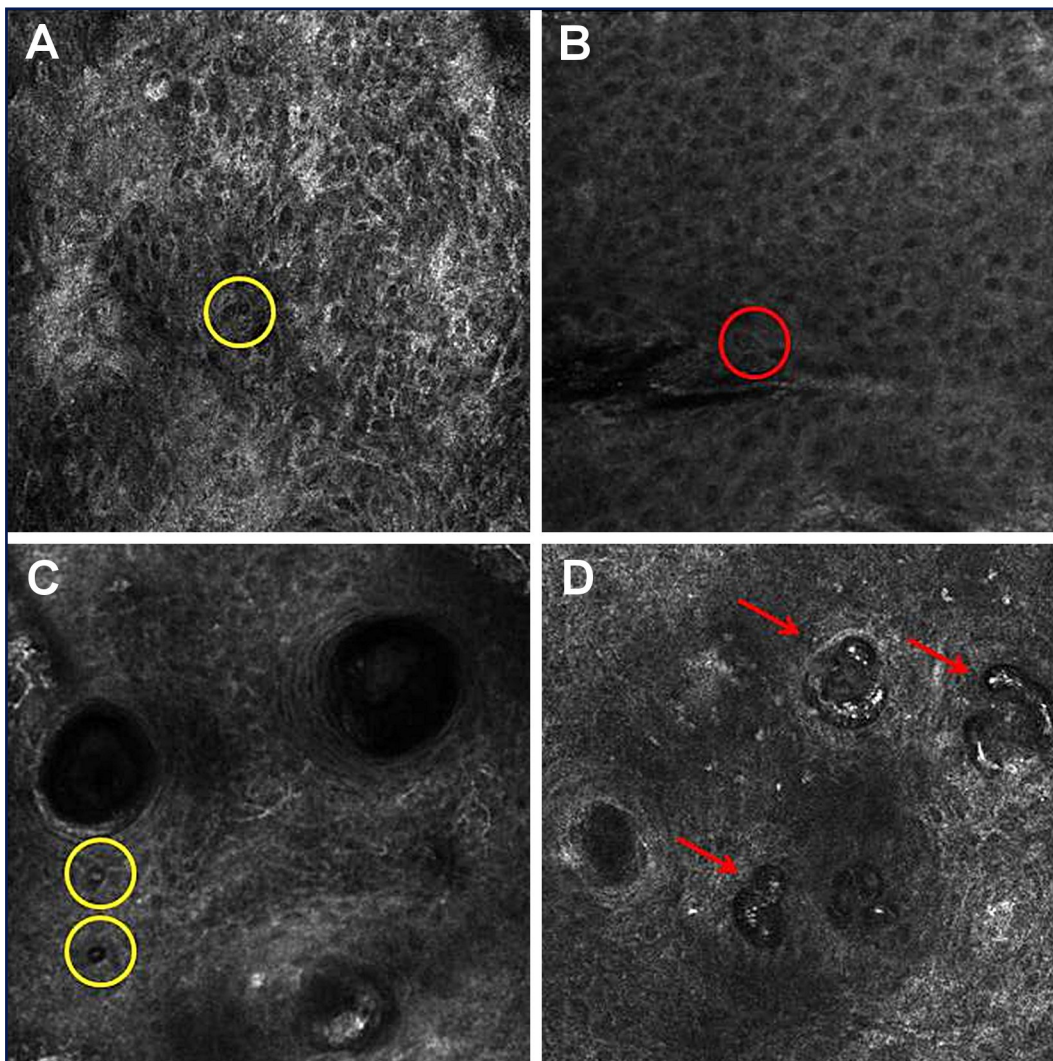


Figure 2

Reflectance confocal microscopy of squamous cell carcinoma.

(A) SCC with a disarranged pattern of the spinous-granular layer of the epidermis and round cell with a bright center and a dark peripheral halo (yellow circle); (B) SCC with an atypical honeycomb and round cell with a dark center and a bright rim surrounded by a dark halo (red circle); (C) SCC with an atypical honeycomb and round cell with a bright center and a dark peripheral halo (yellow circle); (D) SCC with round blood vessels in the dermis.

Table 2: Reflectance confocal microscopy features of squamous cell carcinoma.

| RCM FEATURES OF SCC | |
|-----------------------------------------------------|--------------------------|
| Scale at the stratum corneum level | 95% ⁴⁰ |
| Atypical honeycomb/ a disarranged pattern | 100% ⁴⁰⁻⁴² |
| Round nucleated cells at the spinous-Granular Layer | 50-65% ^{40,41} |
| Round vessels in the dermis | 39-100% ^{40,41} |

High frequency ultrasonography

Ultrasonography is a valuable diagnostic tool widely used in medicine. This is attributed to its versatility, unlimited repeatability with high diagnostic value and the lack of risk to the patients. It provides real-time visual information about benign and malignant process in the skin and subcutis. Since in 1979 Alexander and Miller first time used ultrasonography for measuring skin thickness, great progress in the development of high-frequency scanners occurred. Good correlation between ultrasonographic and histological measurements of melanomas thickness was obtained using transducers of 20-100 MH frequency, which allow a resolution of 80-200 μm and achieve a penetration depth from 1.5 to 8 mm.⁴³

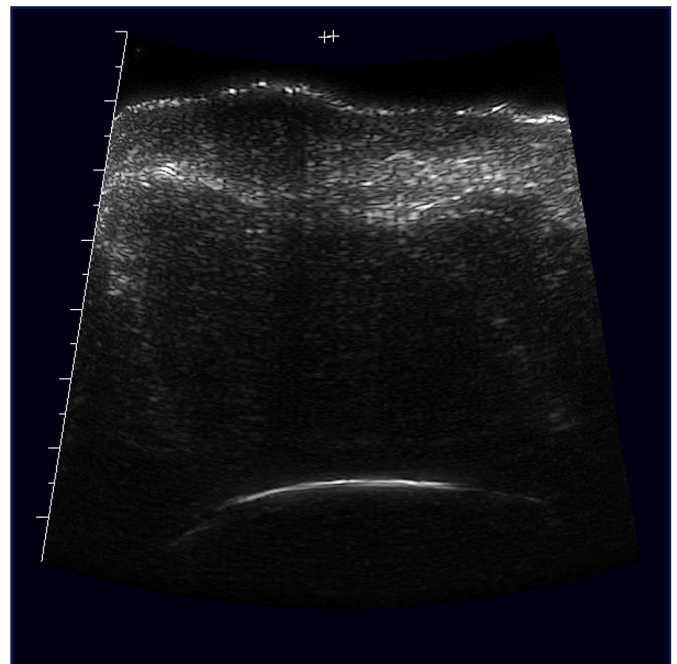
The value of high frequency ultrasonography (HFUS) in clinical dermatology is still being debated. All nonmelanoma skin cancer lesions appeared hypoechogenic in HFUS, suggesting that this method alone is not suitable from differential diagnosis.⁴⁴⁻⁴⁶ However, it gives a clear picture of the size and depth of the tumor. The method should be used as a complementary method in preoperative evolution of the tumor.⁴⁷⁻⁴⁹ Wortsman *et al.*, in a large retrospective study of 4338 ultrasonographic skin examinations, showed that the addition of HF ultrasonography increased the correctness of clinical diagnosis from 73% to 97%.⁵⁰

Figure 3 shows a HFUS image of SCC.

Optical coherent tomography

Optical coherence tomography (OCT) is an emerging technology, which uses infrared light for performing high-resolution cross-sectional imaging. OCT is analogous to ultrasound imaging, except that it uses light instead of sound. OCT systems can generate real-time images of tissue to a depth of about 1.5 mm at a resolution of 10 μm over a field-of-view up to about 5 mm diameter.⁵¹ In dermatology, OCT was introduced in 1995 and is now increasingly used in clinical skin research. It can be used for the investigation of the skin morphology epidermis, dermis, dermo-epidermal junction, hair follicle unit, blood vessels and sweat glands.⁵² The method allows preoperative evaluation of the tumor size in patients with SCC.

Recently, a new non-invasive technique, High-Definition Optical Coherence Tomography (HD-OCT) was introduced permitting the combination of horizontal and vertical images,

**Figure 3**

Ultrasound scan of squamous cell carcinoma: hypoechogenic lesion with irregular, but quiet well defined border.

and therefore a real time three-dimensional (3-D) imaging of nonmelanoma skin cancer. This technique provides an exploration depth up to 570 μm and a lateral and axial resolution of 3 μm , thus allowing the visualization of cytological aspects in their micro-architectural context.⁵³⁻⁵⁵

The presence or absence of an outlined dermo-epidermal junction appeared to be the most powerful criterion to distinguish SCC from AK and normal skin. The absence of the dermo-epidermal junction outline in HD-OCT in SCC lesion appears to be related to irregular budding of the epidermis outstanding into the upper dermis and/or presence of periadenexal collars penetrating through the dermo-epidermal junction. Other features SCC in HD-OCT include hyperkeratosis, parakeratosis, disarranged epidermal architecture on cross-sectional imaging and variability in shape, size and reflective cell in one or more epidermal layers. Moreover, HD-OCT enables the identification of adnexal involvement, based on the presence of the "cocarde image" around the hair follicles.⁵⁶

Table 3 shows typical OCT features of SCC.

Table 3: Optical coherence tomography features of squamous cell carcinoma.⁵⁶

| OCT FEATURES OF SCC | |
|-----------------------------------------------------|------|
| absence of an outlined the dermo-epidermal junction | 100% |
| hyperkeratosis, parakeratosis | 100% |
| disarranged epidermal architecture | 100% |
| the "cocarde image" around the hair follicles | 87% |

Multiphoton tomography

Multiphoton tomography (MPT) employs near-infrared light for multiphoton absorption. There are several endogenous fluorophores in the skin tissue, which can be excited by this process, and their autofluorescence signals can be recorded. The optical resolution of MPT is typically about 0.5 μm in lateral and 1-2 μm in axial direction with a field of view (FOV) of 350 x 350 μm and an imaging depth of 200 μm .^{57,58} Klemp *et al.*⁵⁹ in their study proved that MPT can be a valuable non-invasive imaging method for *in vivo* detection and discrimination of AK and SCC from health skin. They showed that the cell nuclei of AK and SCC were significantly larger compared to healthy skin cells in all cell layers, and cell density in AK and SCC was significantly lower than in the basal and spinous cell layers of healthy skin. In SCC, the cell density was significantly lower than in AK. The nucleus-cytoplasm ratio was significantly higher for AK and SCC than for the healthy skin cells.⁵⁹

Squamous cell carcinoma of the lips and oral mucosa

Among tumors of the oral cavity, oral squamous cell carcinoma (OSCC) comprises 90% of cases. OSCC is seen typically on the lip or lateral part of the tongue usually as a nodule or ulcer, which is white, red, or whitish-red. The five year survival rate of OSCC has not improved significantly in the last three decades and early detection is still a challenge in clinical practice. The literature consists of few articles about dermoscopy of pigmented lesions of the mucosa and the mucocutaneous junction but none of them refer squamous cell carcinoma.^{60,61}

Few emerging techniques may play a role in early detection of SCC of the oral mucosa in future clinical practice.^{62,63} Light-based detection systems are chemiluminescence and tissue fluorescence imaging. Tissue fluorescence spectroscopy is being developed on the basis of the observation that changes in the physical and chemical characteristics of malignant tissues caused by sub-cellular architectural changes make it possible to distinguish normal tissues from malignant tissues. Studies have shown that when an ultraviolet or near ultraviolet light source is used a normal oral mucosa emits more green fluorescence than malignant lesions. The sensi-

tivity of fluorescence spectroscopy technologies was estimated to be up to 81% and the specificity up to 100%.⁶⁴ Raman spectroscopy is based on the Raman effect that occurs when light impinges on a molecule and interacts with the electron cloud and bonds of that molecule. Raman is being investigated as a diagnostic tool for characterizing cancer cells and early malignant changes and distinguishing these cells from normal cells.⁶⁵

In elastic scattering spectroscopy photons hit tissue and are backscattered without changes in wavelength. The relative intensity of this backscattering is influenced by the composition of the interrogated tissue, specifically the relative concentration of scatterers and absorbers. These method detects changes at the subcellular level and may find application in future differential diagnosis of SCC. The sensitivity of this technique was estimated to be 92% and specificity 60%.⁶⁶ Differential path-length spectroscopy is a form of elastic scattering spectroscopy that has fixed photon path length, fixed photon visitation depth and absolute measurement of absorbers. This method gives indirect information about cell biochemistry, intracellular morphology and microvascular properties such as oxygen saturation and average vessel diameter. The sensitivity of this method is 69% and specificity 85%.⁶⁷ Nuclear magnetic resonance spectroscopy uses the magnetic properties of certain atomic nuclei to determine the physical and chemical properties of atoms or the molecules in which they are contained. This technique has been used to identify metabolic signatures of oral squamous cell carcinoma compared with normal tissues. Reflectance confocal microscopy has significant potential to non-invasively diagnose SCC of the lip and reachable OSCC. The confocal images of OSCC revealed markedly disorganized epithelium with haphazardly distributed nuclei.⁶⁸ Optical tomography uses light scattering to construct an image (optical coherence tomography) or to measures the average size of different cell structures (angle-resolved low coherence interferometry).^{62,63} These techniques are being investigated and their potential future value in clinical practice remains unknown.

Squamous cell carcinoma of the vulva

Vulvar cancer accounts for over 5% of gynecological malignancies and for approximately 1% of malignancies in women. The most common vulvar malignancy is squamous cell

carcinoma (over 90% of malignant tumors).⁷¹ The literature about application of non-invasive techniques in differential diagnosis of intraepithelial neoplasia and squamous cell carcinoma is sparse and based mainly on case reports. Dermoscopy has been shown to be a helpful diagnostic aid in a case of Bowen's disease of the vulva that progressed to invasive SCC.⁷² Optical coherence tomography was used to differentiate epidermal thickness of normal vulva tissue and epidermal thickness of vulvar intraepithelial neoplasia.⁷³ Literature data show that optical coherent tomography may be applied for determining appropriate surgical margins in patients operated for vulvar squamous cell carcinoma.⁷⁴

Conclusion

Dermoscopy has become a state of the art technique in early diagnosis of squamous cell carcinoma. Other techniques, such as reflectance confocal microscopy, optical coherence tomography or high frequency ultrasonography are being used with increased frequency as a diagnostic tool or for evaluation of tumor size prior to surgery. Other techniques are under pre-clinical development. The rapid progress in non-invasive skin imaging indicates that pre-surgery histopathological evaluation may become history in near future.

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