Diagnosis of Nonmelanoma Skin Cancer/Keratinocyte Carcinoma: A Review of Diagnostic Accuracy of Nonmelanoma Skin Cancer Diagnostic Tests and Technologies

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BACKGROUND Nonmelanoma skin cancer (NMSC) is the most prevalent cancer in the light-skinned population. Noninvasive treatment is increasingly used for NMSC patients with superficial lesions, making the development of noninvasive diagnostic technologies highly relevant.

OBJECTIVE The scope of this review is to present data on the current state-of-the-art diagnostic methods for keratinocyte carcinoma: basal cell carcinoma, squamous cell carcinoma, and actinic keratosis.

METHODS AND MATERIALS MEDLINE, BIOSIS, and EMBASE searches on NMSC and physical and clinical examination, biopsy, molecular marker, ultrasonography, Doppler, optical coherence tomography, dermoscopy, spectroscopy, fluorescence imaging, confocal microscopy, positron emission tomography, computed tomography, magnetic resonance imaging, terahertz imaging, electrical impedance and sensitivity, specificity, and diagnostic accuracy.

RESULTS State-of-the-art diagnostic research has been limited in this field, but encouraging results from the reviewed diagnostic trials have suggested a high diagnostic accuracy for many of the technologies. Most of the studies, however, were pilot or small studies and the results would need to be validated in larger trials.

CONCLUSIONS Some of these new imaging technologies have the capability of providing new, three-dimensional in vivo, in situ understanding of NMSC development over time. Some of the new technologies described here have the potential to make it from the bench to the clinic.

Mette Mogensen, MD, and Gregor B. E. Jemec, MD, DMSc have indicated no significant interest with commercial supporters.

Nonmelanoma skin cancer (NMSC) is the most prevalent cancer in the light-skinned population, and the critical factor in assessment of patient prognosis in skin cancer is early diagnosis. Serious morbidity in NMSC is often the result of misdiagnosis or underestimation of the biologic potential of the primary tumor.

All diagnostic tests can be accredited with precision and accuracy. Precision, also known as reliability, reproducibility, or repeatability, refers to agreement of the test. If the diagnostic test is performed multiple times on the same subject with the same result, the test is precise. Accuracy describes whether the diagnostic test yields a correct or incorrect answer by correlating the test result with the truth. Thus the accuracy of a test tells us how efficient it is in arriving at the correct diagnosis. The accuracy of a diagnostic test is usually reported in terms of its sensitivity, specificity, and predictive values.

The truth, the correct diagnosis, cannot always unequivocally be reached, and for that reason, a “gold standard” or reference standard is chosen to represent the truth. For skin cancer a biopsy specimen obtained for histopathologic examination to diagnose skin cancer is considered the reference standard. Biopsy may be a time-consuming, expensive, and sometimes a mutilating and painful experience to the patient, especially because only approximately
3% of presumed benign lesions are actually malignant. Furthermore, whereas treatment has hitherto been almost synonymous with surgery, new noninvasive treatment strategies call for noninvasive diagnostics. To assist clinical diagnosis of NMSC and malignant melanoma (MM), an extensive assortment of diagnostic technologies and tests have been developed. The scope of this review is to present available data on the diagnostic accuracy of the different diagnostic methods. NMSC refers mainly to basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and premalignant actinic keratosis (AK).

Accurate diagnostic test assessment involves four phases: Determining the normal range of values for a diagnostic test through observational studies in healthy people (1), diagnostic accuracy assessment through case-control studies (2), assessment of clinical consequences of introducing a diagnostic test through randomized trials (3), and determining the effects of introducing a new diagnostic test into clinical practice by surveillance in large cohort studies (4).

Materials
We have searched for clinical, human studies on the diagnostic accuracy of the following NMSC diagnostic tests and techniques: clinical and physical examination; biopsy techniques; histopathology and molecular markers; high-frequency ultrasonography (HFUS); Doppler sonography; dermoscopy, dermatoscopy, epiluminescence microscopy, incident light microscopy, and skin surface microscopy; optical coherence tomography (OCT); confocal microscopy (CM); near infrared and Raman spectroscopy; fluorescence imaging/spectroscopy; terahertz imaging; electrical impedance; positron emission tomography (PET), computed tomography (CT); and magnetic resonance imaging (MR, MRI). Only studies on diagnosis of NMSC have been selected for this review. Only articles published after 1990 have been included. Articles were restricted to those involving humans.

Methods
MEDLINE, BIOSIS, and EMBASE searches on medical subject headings (MeSH) or similar terms: skin, skin appendages, dermis, cutis, epidermis, integumentum, skin neoplasms, skin tumor and/or tumor, BCC, SCC, Bowen’s disease, AK and/or solar keratosis, keratinocyte carcinoma and physical and clinical examination, biopsy, molecular marker, ultrasonography, Doppler, OCT, dermoscopy, dermatoscopy, epiluminescence microscopy and/or incident light microscopy, spectroscopy, fluorescence imaging, spectrophotometry, colorimetry, confocal microscopy, PET, positron emission tomography, CT, computed tomography, MR, MRI, magnetic resonance imaging, terahertz imaging, electrical impedance and diagnosis, sensitivity and specificity, and diagnostic accuracy. Articles and abstracts have also been retrieved from Web searches on Google Scholar, Ixquick, Scirus, and reference lists of identified studies.
selected studies\textsuperscript{2,6–14} are presented in Table 1. The false-negative diagnostic rates as well as the false-positive rates cannot be ignored as it was demonstrated in a study where 3\% of lesions assessed as benign proved malignant and 40\% of suspected malignancies were benign.\textsuperscript{2} In the reviewed studies the overall sensitivity for clinical diagnosis of NMSC is 56\% to 90\%, and specificity 75\% to 90\%, with highest values for BCC diagnosis.

In studies where positive predictive value (PPV) is mentioned, it must be taken into account that PPV is prevalence-dependent; a study conducted in a high prevalence setting would tend to increase PPV.

Patients themselves may also be involved in the diagnosis of cancer. When patients with nevi and/or melanocytic lesions assessed new and changing moles by performing a skin self-examination, the accuracy was significantly higher with the aid of a baseline digital photography of the skin.\textsuperscript{15} No similar studies have been performed with NMSC.

### Biopsy Techniques

Shave and punch biopsy have been compared in a study including 86 biopsy specimens and subsequent total excision of the tumor.\textsuperscript{16} Punch biopsy was accurate in determining BCC in 81\% of cases, and shave biopsy correctly identified 76\%. In a systematic review of exfoliative cytology in diagnosis of BCC, a meta-analysis showed the pooled sensitivity to be 97\% [95\% confidence interval (CI), 94\%–99\%] and specificity 86\% (95\% CI, 80\%–91\%),\textsuperscript{17,18} however, cytology does not give any information about subtype and none about tumor borders.

### Histopathology and Molecular Markers

Histopathology is a subjective assessment by a dermatopathologist, i.e., data acquisition guided by classification standards and pattern recognition by the human brain. Ideally histopathologic classification of NMSC should be able to identify subtypes that correlate with clinical behavior and treatment requirements. In addition, the classification should be easy to use and reproducible. Determining diagnostic accuracy of the reference standard itself is hampered by the fact that there are several classification systems for NMSC.\textsuperscript{19,20} Difficulties confront pathologists reporting BCCs: Many BCCs have more than one growth pattern, and published data have varied with regard to number necessary to designate the presence of a specific subtype. Furthermore, the accuracy of reporting different subtypes has not been extensively investigated.\textsuperscript{19} In general, interobserver differences cannot be neglected as a potential source of error, with potential clinical consequences for the patient, but also diagnostic researchwise, because all other skin cancer diagnostic techniques must be compared to histopathology, the reference standard. The interobserver differences in this review ranged from 1.2\% to 7\%. In Table 2 results from the reviewed studies are presented.\textsuperscript{21–26}

A current research goal is to define skin cancer by its phenotype in terms of molecular abnormalities as a reference standard for NMSC diagnosis.\textsuperscript{19,20,27–30} To date a molecular marker with a high diagnostic accuracy in NMSC diagnosis has not been identified.

### HFUS and Doppler Sonography

The principle in HFUS is the emission of a pulsed ultrasound (US) from a transducer and registration of the intensity of the echo backscattered from the tissue. The amplitude of the curve reproduces the intensity and time delay of the returning US and is called A-scan. A B-scan is created when the transducer is moved laterally creating a two-dimensional image. Penetration depth and resolution of US is inversely related to the frequency, which makes HFUS suitable in dermatology. Axial resolution at 20 MHz is 50 \( \mu \text{m} \) and lateral resolution is 350 \( \mu \text{m} \). The sonographic characteristics of skin tumors have been widely investigated during the past decades\textsuperscript{31–51} (Table 3). By means of HFUS, skin tumors generally appear as a homogeneously echo-poor area in comparison to the surrounding echo-rich dermis. Because all skin tumors appear echo-poor, HFUS alone is not
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<thead>
<tr>
<th>Authors, year</th>
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<tr>
<td>Whited et al., 1995</td>
<td>Dermatologists blinded to the patient history</td>
<td>50 NMSC patients</td>
<td>Does history influence the clinical diagnosis?</td>
<td>Kappa values −0.04 (blinded) and 0.76 (with history).*</td>
</tr>
<tr>
<td>Leffell et al., 1993</td>
<td>Two dermatologists</td>
<td>53 patients with 143 NMSC lesions</td>
<td>Observer agreement</td>
<td>Kappa 0.78 in AK and 0.38 in SCC.</td>
</tr>
<tr>
<td>Davis et al., 2005</td>
<td>77 pathologists</td>
<td>141 patients with BCC lesions</td>
<td>Observer agreement</td>
<td>ICC was 0.96 for dermatopathologists and anatomic pathologists and 0.65 for fellows.†</td>
</tr>
<tr>
<td>Schwartzberg et al., 2005</td>
<td>Dermatologists complete a questionnaire before biopsy, confidence was plotted level 1–3</td>
<td>102 lesions in 70 renal transplant patients</td>
<td>Effect of additional data</td>
<td>PPV was 80% when additional data was presented.</td>
</tr>
<tr>
<td>Cooper and Wojnarowska, 2002</td>
<td>Dermatologist diagnosis was compared to histopathology</td>
<td>2058 lesions in 809 NMSC patients referred for tumor excision</td>
<td>Diagnostic accuracy</td>
<td>Sensitivity was 90.5% (SCC) and 66.6% (BCC). Specificity was 75.3% (SCC) and 85.6% (BCC).</td>
</tr>
<tr>
<td>Hallock and Lutz, 1998</td>
<td>Plastic surgeons</td>
<td>2582 NMSC lesions excised from 1223</td>
<td>Diagnostic accuracy</td>
<td>Three-fourths of benign lesions were identified (sensitivity 93% and specificity 86%). Only 60% of malignant lesions were identified (sensitivity 73% and specificity 90%). 3% of presumed benign lesions were malignant.</td>
</tr>
<tr>
<td>Ek et al., 2005</td>
<td>Plastic surgeons</td>
<td>835 lesions in 778</td>
<td>Diagnostic accuracy</td>
<td>Sensitivity 91% and a PPV of 71%.</td>
</tr>
<tr>
<td>Har-Shai et al., 2001</td>
<td>Plastic surgeons</td>
<td>493 NMSC patients</td>
<td>Diagnostic accuracy</td>
<td>FPs diagnosed 22% of skin cancer before biopsy, dermatologists diagnosed 87% correctly.</td>
</tr>
<tr>
<td>Morrison et al., 2001</td>
<td>Dermatologists and family practitioners (FP)</td>
<td>190 NMSC patients</td>
<td>Diagnostic accuracy</td>
<td>Sensitivity of the PCPs was 57% (95% CI, 44%–68%) and specificity 88% (95% CI, 81%–93%).</td>
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suitable for differential diagnosis.\textsuperscript{52,53} Overestimation of tumor thickness appears to be a general problem because fibrosis and inflammation generally have the same echogenecity as NMSC. Skin tumor vascularization studies using Doppler techniques are presented in the Table 3 as well. Laser Doppler (LD) devices send a monochromatic laser beam toward the target and collect the reflected radiation. According to the Doppler effect, the change in wavelength of the reflected radiation is a function of the targeted object’s relative velocity. The velocity of the object can thus be calculated by measuring the change in wavelength of the reflected laser light. LD can be configured to act as flow meters.

In short HFUS in NMSC diagnosis is to some extent capable of revealing the three-dimensional size, margins, and relation to adjacent vessels of a suspicious skin lesion. Information on quality (such as solid, cystic, or combined) and information about the inner structure (homogeneous, inhomogeneous, hypo- or hyperechoic, calcification, or necrosis) can be obtained\textsuperscript{54} (Figure 1).

**Dermoscopy, Dermatoscopy, Epiluminescence Microscopy, Incident Light Microscopy, and Skin Surface Microscopy**

In dermoscopy the lesion is examined with a $10 \times$ to $100 \times$ magnification lens placed directly against skin to which immersion oil has been applied to remove scatter of light at the air–skin boundary. Dermoscopy has had the largest clinical impact regarding new skin cancer diagnostic technologies in the diagnosis of pigmented skin lesions, but it has also been used in NMSC. Dermoscopy of NMSC is still at its infancy because consensus regarding diagnostic criteria has not been established yet. Diagnostic accuracy regarding vessels characteristics in BCC and Bowen’s disease, however, seems quite promising, and sensitivity for BCC diagnosis ranges from 87% to 96%, and specificity from 72% to 92% (Table 4).

Dermoscopy and spectroscopy devices, when linked to a video microscope for computer analysis, have
been named “mole scanners.”3 One pilot study on NMSC diagnosis with computerized dermoscopy combined with a two-dimensional in vivo reflectance spectrophotometer included a few BCC and also MM patients.55 Nodular BCC showed a characteristic concentration of perilesional blood vessels combined with slight central fibrosis. Pigmented BCC did not show the erosion of dermal collagens specific for MM.

Dermoscopic features of BCC are arborizing vessels defined as stem vessels with a large diameter, branching irregularly into the finest terminal capillaries. Diameter of the vessels can be up to 0.2 mm, branching irregularly into capillaries of 10 µm.56 Gray-brown lumps, often ovoid in shape, and amber-colored crusts are other dermoscopy features in BCC (Figure 2). In Bowen’s disease, dermoscopic

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**TABLE 2. Histopathology and Molecular Markers**

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<tr>
<th>Authors, year</th>
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<tbody>
<tr>
<td>Lind et al., 199521</td>
<td>Two pathologists</td>
<td>2694 slides</td>
<td>Interobserver differences</td>
<td>Thirty-two major errors were found, involving 1.2% of cases reviewed. Errors were divided into four types: (1) major: errors in diagnosis that could directly affect patient care; (2) diagnostic discrepancies: errors in diagnosis that should not affect patient care; (3) minor: correct diagnosis rendered, but report correction required to add supportive information; (4) clerical: typographical and grammatical errors.</td>
</tr>
<tr>
<td>Olhoffer et al., 200222</td>
<td>Dermatopathologists and pathologists</td>
<td>336 cases</td>
<td>Interobserver differences</td>
<td>Discordance in 5.7% of cases. New management in 18 of 19 severe cases.</td>
</tr>
<tr>
<td>Brochez et al., 200223</td>
<td>20 pathologists diagnosing pigmented skin lesions and NMSC</td>
<td>48 slides</td>
<td>Interobserver differences</td>
<td>Overall sensitivity was 87% (range, 55%–100%) and specificity 94% (range, 83%–100%).</td>
</tr>
<tr>
<td>Trotter and Bruecks, 200226</td>
<td>Two pathologists</td>
<td>Blinded review of 592 histopathology slides</td>
<td>Interobserver differences</td>
<td>Agreement was found in more than 93% of cases.</td>
</tr>
<tr>
<td>Renshaw et al., 2002112</td>
<td>77 pathologists (both dermato- and anatomic)</td>
<td>15 SCC and AK slides</td>
<td>Interobserver differences</td>
<td>Mean ICC* was 0.97 for all pathologists. Agreement was above 70% for 11 of 15 slides.</td>
</tr>
<tr>
<td>Kamiya et al., 200324</td>
<td>Performing reverse transcriptase-polymerase chain reaction (RT-PCR) to amplify keratin19</td>
<td>26 lymph nodes; 10 had histologically proven metastasis, and 16 had none</td>
<td>Molecular marker: micrometastasis of SCC in lymph nodes</td>
<td>This method showed a detection sensitivity of one tumor cell in 10^6 lymphocytes.</td>
</tr>
<tr>
<td>Biesterfeld and Josef, 200225</td>
<td>MIB-1 immunohistometry for the differential diagnosis between KA and SCC</td>
<td>49 keratoacanthomas and 48 SCCs</td>
<td>Molecular marker: proliferation marker MIB-1</td>
<td>If specificity 85% is required sensitivity decreases to 56%. MIB-1 is currently of limited value in SCC diagnosis.</td>
</tr>
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</table>

*Intraclass correlation coefficient (ICC) is an estimate of the interobserver reliability by two-way analysis of variance. Standard criteria for ICC are 0.40 = poor; 0.40–0.59 = fair; 0.60–0.74 = good; 0.75–1.00 = excellent correlation between observers. AK, actinic keratosis; NMSC, nonmelanoma skin cancer; SCC, squamous cell carcinoma.
features are glomerular vessels (90%) and scaly surface (90%). Glomerular vessels are dotted vessels often distributed in clusters mimicking the glomerular apparatus of the kidney. Compressing the blood vessels renders them invisible. Magnification by 30 times or more must be applied to visualize vessels down to 10 μm.

**OCT**

OCT is a novel, noninvasive optical imaging technology. It can provide cross-sectional tomographic images of tissue pathology in situ and in real time. OCT is analog to B-mode US pulse-echo imaging with an optical rather than acoustical reflectivity being measured. In OCT, linear characteristics as scattering, absorption, birefringence, and refractive index are measured to produce images with micrometer resolution. When birefringence characteristics of the tissue are mapped in OCT images, it is referred to as polarization sensitivity (PS) OCT. OCT images can also be enhanced by Doppler function.

OCT provides cross-sectional images of structures below the tissue surface in analogy to histopathology. NMSC and MM has been investigated with OCT with quite promising results. Pilot studies have suggested that OCT can be used clinically in diagnosing NMSC and MM. In a study of 20 BCC lesions, there was an excellent match of histologic features seen on light microscopy with OCT images of superficial, nodular, micronodular, and infiltrative BCCs.

The actual predictive value of OCT in BCC patients could not be calculated from this study. In another study of three patients with superficial BCC and three patients with cutaneous MM, OCT images were compared with histology. The size, allocation, and form of BCC nests seemed to be similar to those in histologic images. In a study of nine patients with 12 BCC lesions, some lesions were distinct in OCT images and BCC subtypes as superficial and nodular could be identified in OCT images. Interestingly scar tissue could be distinguished from BCC with PS-OCT. Therefore, PS-OCT may have

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**TABLE 3. High-Frequency Ultrasonography**

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<tr>
<th>Authors, year</th>
<th>Number and pathology of patients</th>
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<th>Results</th>
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<tbody>
<tr>
<td>Moore and Allan, 2003</td>
<td>181 patients with BCC before and after photodynamic therapy treatment</td>
<td>Thickness of the lesions</td>
<td>BCC thickness predicts outcome 1 year after photodynamic therapy with d-aminolevulinic acid.</td>
</tr>
<tr>
<td>Lont et al., 2003</td>
<td>33 patients with SCC</td>
<td>HFUS diagnosis of SCC of the penis compared to MRI</td>
<td>In SCC of the penis, HFUS provides diagnostic value in staging the disease. PPV for corpus cavernosum infiltration, 67% (HFUS) and 75% (MRI).</td>
</tr>
<tr>
<td>Stucker et al., 1999</td>
<td>16 BCC lesions and 27 MM</td>
<td>Laser Doppler perfusion imaging</td>
<td>Tumor perfusion values higher than surrounding skin. BCC perfusion values similar in the tumor area as opposed to MM, where perfusion was higher in the tumor center.</td>
</tr>
<tr>
<td>Schroder et al., 2001</td>
<td>81 clinically malignant tumors</td>
<td>Laser Doppler perfusion imaging study with and without contrast</td>
<td>Sensitivity 0.75 and specificity 0.79 if three to five vessels were visible in the tumor, and sensitivity 0.58 and specificity 0.88 if a parameter called “percentage vessel area” exceeded 5%.</td>
</tr>
<tr>
<td>Karaman et al., 2001</td>
<td>19 benign, 32 BCC, and 15 SCC</td>
<td>Power Doppler (Doppler independent of angle)</td>
<td>Specificity of 63% and sensitivity 88% in diagnosis based on vascular patterns alone in the lesions.</td>
</tr>
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</table>

BCC, basal cell carcinoma; HFUS, high-frequency ultrasound; MM, malignant melanoma; MRI, magnetic resonance imaging; PPV, positive predictive value; SCC, squamous cell carcinoma.
additional advantages as BCC differ in content of birefringent collagens from normal skin. In a study of two patients with invasive BCC, PS-OCT was used to discriminate tumors from normal skin. In normal skin, a bright band of birefringence signal is seen at middepth of the PS-OCT image, corresponding to the upper reticular dermis. In PS-OCT images of invasive BCC, there was a dramatic alteration in the birefringence signal, it almost disappeared in the ulcerative, invasive BCC and showed a haphazard distribution in the other invasive BCC lesion (see also Figure 2). Furthermore, a gradual transition from normal-appearing tissue to tumor tissue could be detected by PS-OCT at the BCC borders, indicating an ability of PS-OCT to delineate tumor borders (Figure 3).

A study compared OCT and HFUS in diagnosis of eyelid tumors. Examination of 38 patients (BCC 4/38, AK 1/38, and other benign and malignant tumors) showed that OCT was superior in detecting cystic lesions, but due to low penetration of the OCT system in the skin, tumor margins could not be determined.

**CM**

Reflectance CM or confocal scanning laser microscopy has the highest resolution of all optical techniques used in NMSC diagnosis research. Current CM systems have an axial resolution of 1 to 5 μm and a lateral resolution of 0.5 to 1 μm. The penetration depth maximum is 300 μm. CM uses a point source light to illuminate a small spot within a tissue. The pinhole minimizes out-of-focus light reaching the detector, and only confocal light is detected. For in vivo imaging, a plastic cap filled with water must be adapted to the skin. CM has proved a potentially valuable diagnostic aid in BCC and SCC diagnosis, both ex vivo and in vivo (Table 5). Characteristic CM features of NMSC are, in BCCs, abundant blood vessels juxtaposed to BCC cells, sometimes in

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**Figure 1.** HFUS and Doppler images of BCC lesion on the nose. (A) Photo of the BCC lesion. (B) 18-MHz real-time HFUS image of BCC lesion in (A). (C) Doppler image of lesion in (A); vasculature is marked in red and blue. (D) Histopathology slide HE stain from the lesion (A) showing BCC; original magnification, × 20. Images by courtesy of Dr Ximena Wortsman, Chile.
tightly packed nests, and rolling of leukocytes and lymphocytes along the endothelial lining. BCC cells appeared to be oval, elongated with a prominent, monomorphic polarized nucleus. Tumor cells had a high refractive index with dark-appearing nuclei, and the cytoplasm appeared bright.76,77 In SCC, features are irregular epithelial mass with a variable proportion of normal and atypical keratinocytes, along with areas of anaplasia; in AKs, CM revealed hyperkeratosis, lower epidermal nuclear enlargement, and pleomorphism.74 Some features seen in CM images (e.g., uniform polarization of BCC nuclei, margination and rolling of leukocytes) are morphologic features not recognized in BCC histopathology.76,77 In Mohs micrographic surgery (MMS), CM may have a role in analyzing untreated fresh biopsy specimens. Fluorescence fiber-optic CM in vivo is a novel technique, where fluorophore distribution in the skin may illustrate morphologic changes in the epidermis. An application for fluorescence CM is the ability to image fluorescent markers that target specific subcellular molecules including proteins and therefore to monitor specific pathologic and immune processes over time.78

### Near Infrared, Diffuse Reflectance, and Raman Spectroscopy

Light that penetrates the skin surface is variably absorbed by different skin components termed chromophores. The skin components, which subsequently emit radiation, are termed fluorophores. Optical measurements of the skin can therefore be based on the interactions of nonionizing electromagnetic radiation and the skin.73 The absorbed energy may be dissipated as heat (tissue absorption), reemitted as electromagnetic radiation of lower energy with a longer wavelength (fluorescence), or even

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<tr>
<td>Zalaudek et al., 2004, 2005,117</td>
<td>21 SCCs in situ (Bowen’s disease)</td>
<td>Characteristic dermoscopy features</td>
<td>Glomerular vessels with a patchy distribution and scaly surface was found in 90% of all lesions. Arborizing vessels are detected in 52%, sensitivity was 93%, and specificity was 89%–92%. Sensitivity for BCC was 86.7% (95% CI, 76.9%–92.7%) and specificity 71.9% (95% CI, 56.6%–83.3%).</td>
</tr>
<tr>
<td>Zalaudek et al., 2006</td>
<td>71 pigmented BCC lesions</td>
<td>Characteristic dermoscopy features</td>
<td>Internal study regarding diagnostic accuracy, a three-point checklist (asymmetry, atypical network, blue-white structures)</td>
</tr>
<tr>
<td>Argenziano et al., 2004</td>
<td>165 lesions (including 20 BCCs, otherwise mostly pigmented lesions, 150 dermatologists and other doctors</td>
<td>Diagnostic features: vascular structures</td>
<td>Arborizing vessels were seen in 82% of BCCs, with a PPV of 94% (p&lt;.001) SCCs in situ: 13 of 16 lesions showed glomerular vessels, PPV 62%</td>
</tr>
<tr>
<td>Kreusch, 2002</td>
<td>BCC patients, number not retrieved</td>
<td>Diagnostic features: vascular structures</td>
<td>Sensitivity 96%, specificity 91%.</td>
</tr>
<tr>
<td>Otis et al., 2004,120 Neewell et al., 2003</td>
<td>12 patients</td>
<td>Diagnostic features: vascular structures with capillaroscopy</td>
<td>Microvessel area fractions were increased 4.9-fold in BCC and 2.5-fold in AK compared to normal skin. Significant difference between the groups and also between BCCs and SCCs.</td>
</tr>
<tr>
<td>Chin et al., 2003</td>
<td>111 skin samples (including 20 SCCs, 50 BCCs)</td>
<td>Blood vessels counted after immunohistochemistry</td>
<td>Significant difference between the groups and also between BCCs and SCCs.</td>
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AK, actinic keratosis; BCC, basal cell carcinoma; PPV, positive predictive value; SCC, squamous cell carcinoma.
reemitted as radiation of higher energy (Raman scattering), the latter being the least probable event. A spectroscope separates the returned light into individual wavelengths and assesses it. Raman spectroscopy provides molecular information of a sample irradiated with laser light as a small fraction is shifted in frequency (Raman effect). Several studies have described a characteristic Raman spectra in NMSC.79–88 A sensitivity of 97% and specificity of 98% were found in a study of 48 BCCs. In vivo Raman spectroscopy is possible but the precision of the spectra is low. Two ex vivo studies found distinct Raman band differences between BCC and normal skin. Ten samples of BCC suggested this from direct observations of spectral differences, after reducing endogenous autofluorescence by a confocal device, and the two groups were significantly different from each other.89 In an in vitro study of 15 BCCs, Raman pseudo-color maps were compared to skin biopsies. Pseudo-color maps assign areas with similar spectra with the same color and are generated by multivariate statistical analysis and clustering analysis of spectra. A prediction model could classify new tissue samples from BCC lesions from their Raman spectra with a sensitivity of 100% and specificity of 93%.82 Another study suggested that neural network analysis of near-infrared Fourier transform Raman spectra may show some potential for ex vivo NMSC diagnosis.79 An in vivo study of 195 patients with a variety of malignant and benign skin lesions (33 AKs, 32 BCCs) showed promising results for the screening of skin lesions with near infrared spectroscopy. Spectra were compared to histopathology in all lesions; univariate statistics showed significant differences between spectra and healthy skin (normal skin vs. AKs, BCCs, dysplastic nevi, lentigines, banal nevi, and seborrheic keratosis) and also between the spectra themselves. Significant differences, however, are not always diagnostic differences. A pattern recognition technique was applied and successfully discriminated lesions with accuracy higher than 80%.81

Fluorescence Imaging

Specific autofluorescence emitted from malignant tissue upon radiation with a laser, xenon light, or halogen lamp has been used to distinguish normal tissue from cancerous tissue in the head and neck region.90 Fluorescence imaging is an attractive potential diagnostic technique for skin tumor demarcation. In a study of 21 patients with 80 BCCs, the fluorescence intensity from BCCs was significantly lower than surrounding, normal skin.91 In a study of

Figure 2. Dermoscopy image from a BCC lesion (A) and the corresponding clinical photo (B). The dermoscopy image visualizes the arborizing vessel in the upper right section of the lesion. Images courtesy of Prof. Kaare Weismann, MD, DMSc, Horsholm Dermatology, Denmark.
18 patients with 25 NMSC lesions (20 BCCs and 5 SCCs), a fiber-optic–based fluorimeter collected spectral data in vivo and microscopic fluorescence ex vivo. The fluorescence of tryptophan moieties in BCC was $2.9 \pm 1.9$ SD, and for SCC $2.7 \pm 0.9$ SD times larger. A marked loss of fluorescence in the middle of the tumor region was noticed in 78% of the NMSC lesions due to a decrease in collagen and elastin cross-links. Another study of 49 patients (BCC, SCC, AK, and normal skin) compared diagnostic accuracy in laser-induced fluorescence spectroscopy for skin types I to III (Pathak) to determine the skin colors effect on the results. Melanin absorbs fluorescence strongly. Typically normal skin exhibited stronger fluorescence emission than BCC and SCC. The accuracy of classifying NMSC was higher (93%) in Type I skin. It has also been suggested that due to the large variation in fluorescence intensities developing, an algorithm for NMSC is difficult. Bispectral fluorescence imaging combines skin autofluorescence with $d$-aminolevulinic acid (ALA) fluorescence. The agreement between bispectral fluorescence images and the histopathologic tumor boundary of ill-defined BCC in 12 patients with an aggressive BCC undergoing MMS was examined. Only 5 patients had good correlation between histopathology and bispectral images of the tumor, not significant ($p = .057$). Another study ap-
plied two different algorithms in data analysis of bispectral fluorescence and showed promising results in demarcation of skin lesions in 15 BCC patients.94 Also two different fluorescence systems showed a clear demarcation of BCC in 2 patients with several BCC lesions.95 In an in vivo study, 55 patients with oral SCC were studied with respect to endogenous fluorescence. The intensity of the fluorescence significantly corresponded with the pathologic tumor and node categories of SCC \( (p < .01).^{83,96} \)

**Terahertz Imaging**

Terahertz pulsed imaging (TPI) is a novel, noninvasive, imaging modality. It uses pulses of electromagnetic radiation in the frequency range of 0.1 to 10 THz. Water has strong absorption over the entire THz range, and water content in the skin is a source of image contrast.97 TPI has potential use in NMSC diagnosis. A significant difference between the response of THz radiation in normal skin and BCC has been reported.98,99 In a study of 18 BCCs both in vivo (5 BCCs) and ex vivo TPI analysis was performed. In vivo regions of contrast were seen in all THz images and correlated well with histology.100

**Electrical Impedance**

The impedance of the skin is an electrical entity that can be described in complex numbers by resistance and reactance. A pilot study had found statistical difference in electrical impedance values was found between BCC and normal skin.101 This was confirmed in a study of 34 BCC patients.102 Statistical difference in electrical impedance values was found between BCC and normal skin; however, diagnostic accuracy could not be assessed. The electrical impedance system was elaborated further, and the probe equipped with microinvasive electrodes to bypass the barrier function of the high impedance of stratum corneum. The lesions were 99 benign nevi,

### TABLE 5. Confocal Microscopy

<table>
<thead>
<tr>
<th>Authors, year</th>
<th>Number and pathology of patients</th>
<th>Question asked</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonzalez and Tannous, 200276</td>
<td>5 BCCs</td>
<td>In vivo CM compared to histopathology</td>
<td>Characteristic CM features in all specimens.</td>
</tr>
<tr>
<td>Marra et al., 2005123</td>
<td>3 BCCs</td>
<td>CM compared to histopathology</td>
<td>Above confirmed.</td>
</tr>
<tr>
<td>Nori et al., 2004124</td>
<td>152 lesions (83 BCCs and benign)</td>
<td>Diagnostic accuracy of 5 CM criteria for in vivo BCC diagnosis</td>
<td>Sensitivity 93.9% and specificity 78.3% was found using three criteria and 95.7% and 82.9%, respectively.</td>
</tr>
<tr>
<td>Gerger et al., 2005126</td>
<td>20 BCCs</td>
<td>Diagnostic accuracy CM in MMS untreated fresh biopsies</td>
<td>Sensitivity ranging from 44% to 100% and a specificity of 100% for all five criteria.</td>
</tr>
<tr>
<td>Tannous et al., 2003126</td>
<td>5 BCCs</td>
<td>Diagnostic accuracy in vivo CM aluminum chloride contrast</td>
<td>100% sensitivity in Stage 1 MMS and 80% sensitivity in Stage 2.</td>
</tr>
<tr>
<td>Chung et al., 200475</td>
<td>92 BCCs, 23 SCCs</td>
<td>Ex vivo CM of Stage 1 MMS excisions</td>
<td>CM may be an alternative to frozen sections in large nodular BCC. Difficulties in recognizing SCC in situ and poor image quality.</td>
</tr>
<tr>
<td>Sauermann et al., 2002127</td>
<td>12 BCCs</td>
<td>Diagnostic value of CM vascular pattern</td>
<td>Vascular pattern of BCC in CM can be used diagnostically.</td>
</tr>
<tr>
<td>Aghassi et al., 2000128</td>
<td>6 AKs, 1 SCC</td>
<td>CM diagnostic features</td>
<td>CM able to distinguish pathologic features of epidermal neoplasms: 100% show nuclear enlargement and pleomorphism.</td>
</tr>
</tbody>
</table>

BCC, basal cell carcinoma; CM, confocal microscopy; MMS, Mohs micrographic surgery.
28 BCCs, and 13 MMS. Sensitivity for separation of BCC from benign nevi was 96% and specificity 86%, when using the noninvasive probe. The invasive probe had higher diagnostic accuracy only in MM. The choice of electrode can be considered application-dependent. A study of 35 BCC patients compares electrical impedance, transepidermal water loss (TEWL), and LD in diagnosing nodular and superficial BCC. In accordance with other studies, statistically significant differences between electrical impedance and BCC was found ($p < .001$), but no differences between subtypes were found. In addition, TEWL and LD values had similar $p$ values in discriminating BCC from normal skin. Increased TEWL values are ascribed to the decreased barrier function of the skin due to the pathologic processes of BCC. The assumption is that the increased LD values are due to increased angiogenesis and vasodilation in BCC.

**CT, PET, and MR**

CT is based on the X-ray principal, whereas PET is an imaging technique that detects positron release from radioactive substances and provides cross-sectional physiologic information. PET imaging commonly uses 2-deoxy-2-18F-fluoro-D-glucose (FDG), a positron-imaging agent, to measure the metabolic rate of tissue noninvasively. Tumors can be metabolically more active than normal tissue, thus mobilization of the image tracer can be detected by PET scanning. FDG-PET has been investigated in diagnosis of NMSC. Six patients with BCC larger than 1 cm were examined by PET scanning. BCC could only be identified in 3 of 6 patients. Another study compared FDG-PET NMSC diagnosis in patients with head and neck tumors with physical examination, ultrasonography, and CT. In a group of 56 patients (43 SCCs), detecting the primary tumor site with PET had a sensitivity of 95% (95% CI, 80%–98%) and a specificity of 100% (95% CI, 62%–100%). There was no statistical difference between PET and CT diagnosis. MRI makes use of the magnetic properties of hydrogen nucleus (the protons). MRI has been applied to studies of SCC and morphologic information about the shape, the depth, and the location of the tumor between MRI and histopathology are in good agreement. A retrospective study of 33 NMSC patients (20 BCCs, 12 SCCs, 1 mixed) estimated accuracy of MRI and CT; the findings were compared to histopathology. Patients were seen for both primary assessment and follow-up. MR and CT localized the lesions in 29 of 33 patients; of the 4 tumors not identified, three-fourths of patients had mean disease-free survival at 33 months and the fourth patient developed recurrence at 52 months. In a study of 35 patients of whom 18 had perineural spread of BCC and SCC, based on clinical and histopathologic investigation, CT and MR showed that positive perineural spread inversely correlated with 5-year survival rate. Patients who were imaging-positive had a 5-year survival of 50% and for imaging-negative patients it was 86% ($p = .049$). In this study MRI seems to be informative in estimating prognosis.

**Conclusion**

A broad variety of diagnostic technologies are becoming available for noninvasive diagnosis of NMSC. The reference standard, skin biopsy and histopathologic assessment, is, however, not yet to be replaced, because state-of-the-art diagnostic research has only been performed with few of the mentioned new diagnostic methods and technologies. Many of the technologies seem to offer an adequate diagnostic accuracy, especially as a supplement to clinical diagnosis. For all areas of new diagnostic tests and technologies in NMSC the clinical role remains to be established in larger, independent studies.

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DERMATOLOGIC SURGERY

DIAGNOSTIC ACCURACY OF NONMELANOMA SKIN CANCER DIAGNOSTIC TESTS

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