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Modern non-invasive diagnostic techniques in the detection of early cutaneous melanoma

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Abstract

Over the past few years melanoma has grown into a disease of socio-economic importance due to the increasing incidence and persistently high mortality rates. Melanoma is a malignant tumor with a high tendency to metastasize. Therefore, an extremely important part of the therapeutic process is to identify the disease at an early stage: *in situ* or stage I. Many tools for early diagnosis of melanoma are available today, including dermoscopy, videodermoscopy and *in vivo* reflectance confocal microscopy. Other methods such as high frequency ultrasound, optical coherence tomography and electrical impedance spectroscopy may serve as additional diagnostic aids. Modern imaging techniques also allow the monitoring of melanocytic skin lesions over months or years to detect the moment of malignant transformation. This review summarizes the current knowledge about modern diagnostic techniques, which may aid early diagnosis of melanoma. (*J Dermatol Case Rep*. 2014; 8(1): 1-8)

Introduction

Cutaneous melanoma accounts for approximately 2% of skin cancers and is responsible for approximately 1% of deaths caused by oncological reasons.[1] Approximately 50-80% cutaneous melanomas develop "*de novo*", other cases develop within pre-existing nevi.[2]

The prognosis of melanoma depends on the depth of invasion at the time of diagnosis. If diagnosed at an early stage, defined as melanoma *in situ* or stage I according to AJCC, with a thickness according to Breslow scale of less than 1 mm, melanoma shows a 5-year overall survival rate of over 90%.[1] Thus, early diagnosis and excision is crucial for prognosis in patients with cutaneous melanoma. A success of recent years is an increasing number of melanomas diagnosed at an early stage. This is related to the dynamic development of advanced diagnostics techniques, predominantly dermoscopy, and the rising awareness among physicians and patients about the need of dermoscopy screening.[2,3] Videodermoscopy is a digitalized form of dermoscopy. Other diagnostic methods include reflectance confocal microscopy, high frequency ultrasound, optical coherence tomography and electrical impedance spectroscopy.

Dermoscopy (and videodermoscopy)

Dermoscopy (dermatoscopy, epiluminescence microscopy) is a non-invasive imaging technique that uses optical magnification and fluid immersion or cross-polarized lighting to visualize skin lesions at 10-fold magnification. Videodermoscopy is digital dermoscopy. The method allows dermoscopic visualization of structures in high magnification (70-fold and higher). The term videodermoscopy was occasionally used in publications to exemplify that digital equipment was used to perform dermoscopy, but currently the word "dermoscopy" is used most commonly for both, regular and digital dermoscopy.

The dynamic development of dermoscopy and the development of numerous dermoscopic algorithms for distinguishing between benign and malignant skin lesions started in the late 80's and 90's of the twentieth century. The interest in dermoscopy is still growing, which is reflected in numerous publications, conferences and clinical studies coordinated by the *International Dermoscopy Society*. A ten-year multicenter study showed that the use of dermoscopy decreases the number of unnecessary excised benign melanocytic lesions and increases the number of detected melanomas.[4,5]

It has been calculated that a dermoscopy examination all patient's pigmented skin lesions takes on average only 72 seconds, however some experts indicate that this may be significantly longer.

The principle of dermoscopy screening is examination of all pigmented lesions, not only lesions preselected by clinical examination. Additional diagnostic techniques, such as videodermoscopy, ultrasonography or reflectance confocal microscopy usually are used after a preselection by the specialist or on the basis of dermoscopy examination.[6,7] The examination of all pigmented skin lesions, not only lesions preselected by the patient or the clinician is important for several reasons. Patients are usually concerned about palpable skin lesions, including seborrheic keratoses or dermatofibromas, and ignoring small, flat melanocytic lesions, which may be early melanomas. These small lesions may not appear suspicious on clinical examination even to experienced clinicians. Another reason for the examination of all pigmented lesions is to determine the dominating dermoscopic pattern of a patient's nevi. This allows to identify lesions which differ in pattern from other lesions in the same patient (the ugly duckling sign). The concept of the "ugly duckling" is based on the observation that nevi in the same individual tend to resemble one another, and that melanoma often deviates from this nevus pattern. There are specific conditions, in which melanocytic nevi may develop an unusual dermoscopy structure, for example during pregnancy, childhood or following extensive exposure to UV radiation. Some experts do not recommend routine examination of pigmented nevi up to two months after the last intensive sun exposure.[8] An important part of a dermoscopy screening is to examine in detail of the whole body of the patient, including special locations such as scalp, palmes, soles, interdigital spaces, scalp and available mucous membranes.[1]

Regular dermoscopy screening allows to reduce the risk of failing melanoma. Additionally it decreases the number of surgical excisions of melanocytic nevi. The measure of a good expertise in dermoscopy is the *number needed to excise* (NNE). This is an index for the number of excisions of skin lesions performed for every melanoma diagnoses. For GPs a desirable NNE is 20-40, for physicians practicing in primary care skin cancer clinics is 19-28, for dermatologists: 4-18.[6,9]

On the basis of the observation that in adult patients benign lesions remain stable whereas melanoma tends to grow and change over time, digital follow-up of melanocytic lesions has been proposed as a strategy to recognize melanoma. Stratification of the risk of developing melanoma is of great importance to establish the best strategies and follow-up methods. Short-term follow-up (after 3 months) may be the only way to recognize early melanoma. Meta-analysis of 14 studies on digital dermoscopy follow-up shows that chances to detect a melanoma during surveillance increase as the length of follow-up extends, so surveillance must be maintained over time in high-risk population.[10] The intervals between successive dermoscopy examination vary. Most frequently the first check-up is performed 3 months after initial examination, further follow-up is carried out every 12 months.[4,11] Short-term follow-up is oriented towards assessment of single melanocytic lesions, while medium (every 6 months) and long-term (every 12 months) follow up is focused on monitoring of multiple lesions. The lesions selected for short-term follow-up usually fall into two categories: moderately atypical lesions without a history of change and mildly atypical nevi with a history of change. Long-term follow-up is used for the surveillance of patients with atypical mole syndrome or high nevi count among other risk factor.[10] Haenssle *et al*. recommend follow-up every 3 months for patients with familial atypical mole and melanoma syndrome (FAMM) and every 6-12 months for those with multiple atypical nevi.[12] Argenziano *et al*. and Salerni *et al*. in two independent studies have found that in 58,3-65% of patients with slow growing melanoma the only dermocopic abnormality was enlargement of the lesion (more than 2 mm in 3 years — Argenziano *et al*.).[11,13] Many experts, including Argenziano *et al*., suggest that in most cases only one follow-up visit should be conducted. If the dermatologist remains unsure about the nature of the lesion during the first follow-up visit, the lesion should be excised.

The sensitivity of diagnosing melanoma increases by approximately 20% and the specificity by about 10% when using dermoscopy (as compared to the naked eye examination).[14] The sensitivity of dermoscopy for detection of melanoma is estimated to be 85%-100%, depending on the experience of the dermoscopist. The specificity of the method reaches 90%.[14]

Dermoscopic features in the diagnosis of different types of melanoma

There are many dermoscopic algorithms, but in daily practice dermoscopy experts usually follow a "10 second rule of Argenziano". This rule is based on the assumption that if classifying a lesion as malignant versus mild takes the investigator more time than usual, surgical excision or a short-term follow-up should be recommended. There are however numerous dermoscopic algorithms, which are especially helpful for the inexperienced dermoscopists. These are: the pattern analysis by Pehamberger, the ABCD rule by Stolz, the ABC rule modified by Kittler, the Menzies method, the seven-point checklist by Argenziano, the CASH algorithm and other.[4,6,15,16] Figures 1 and 2 show typical dermoscopy features of thin versus thick melanomas. The images were taken with a digital dermoscope (videodermoscope).

Dermoscopy diagnosis of lentigo maligna based on criteria defined by Stolz *et al*. has a 89% sensitivity and a 93% specificity. At least one of the classical Stolz criteria is present in 87% of cases: hyperpigmented follicular opening (51% of cases), annular-granular pattern (42% of cases), pigmented rhomboidal structures (69% of cases), obliterated hair follicles (13%) — according to Pralong report of 125 cases. Pralong *et al*. decribed also three original criteria: increased density of the vascular network (58% frequency), red rhomboidal structures (40%) and target-like patterns (41%). About 68% of lentigo maligna melanoma characterized the presence of at least three colours. In 45% of cases

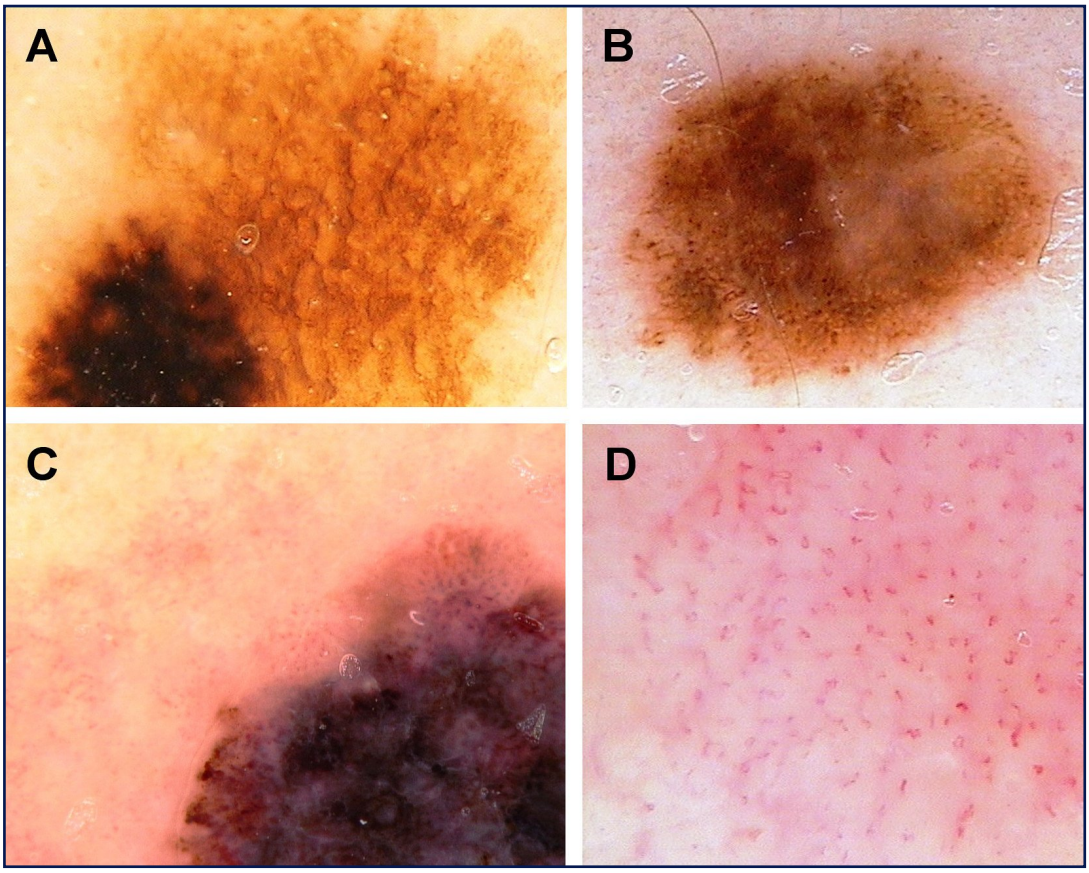

Figure 1

Videodermoscopy of thin melanomas (Breslow < 1 mm). A – melanoma in situ with asymmetric pigment network; B – melanoma 0,5 mm Breslow thickness with atypical dots and small regression area; C – melanoma in situ with blue-gray area, atypical peripheral dots and erythematous halo; D – melanoma in situ with numerous point blood vessels and hairpin vessels.

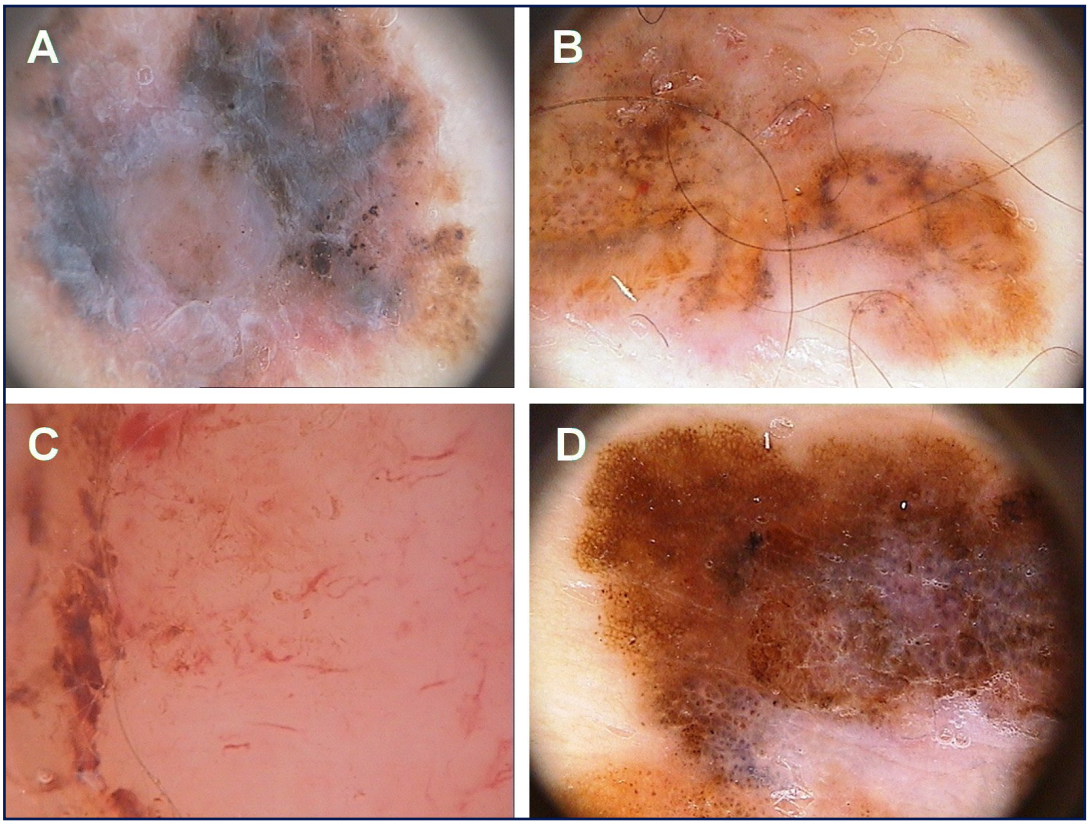

Figure 2

Videodermoscopy of thick melanomas (Breslow > 1 mm). A – melanoma 1,9 mm Breslow thickness with blue-whitish veil and atypical globules; B – melanoma 1,5 mm Breslow thickness with atypical network, asymmetry in both axes and regression area; C – hypomelanotic melanoma 5 mm Breslow thickness with irregular linear blood vessels; D – melanoma 8 mm Breslow thickness with atypical network and regression area.

revealed the presence of regression, among which dominated peppering (35%) and the presence of white scar-like structureless areas (10%).[17,18]

The most common type of melanoma, the superficial spreading melanoma has a composite dermoscopic appearance. The most of the above-described dermoscopic structures, on the basis of which diagnostic algorithms were developed, occurs in superficial spreading melanoma. The most commonly observed structures include: a blue-whitish veil, blue-gray areas, irregular dots and structureless areas. Regression-associated dermoscopic features and abnormalities of blood vessel structures are also observed in these lesions.[19]

The most characteristic features of nodular melanoma are atypical vascular pattern, blue-whitish veil and co-presence multiple colors. Pigment is usually visible dermoscopically, even in hypomelanotic tumors, and often occurs periferally. Argenziano *et al*. developed the "blue-black rule", which indicates that the presence of a combination of blue and black color within the lesion, is a simple dermoscopic clue to recognize pigmented nodular melanoma with a 84.6% sensitivity and a 80.5% specificity.[20] The frequency of atypical blood vessels is approximately 70%. Ulceration is present in half the cases of nodular melanoma.[17,21]

Acral melanomas are located on palms, soles, fingers, toes and in the nail unit. The dermoscopic features of melanomas located on the palms and soles were described Saida *et al*.[22] The two most prevalent features were irregular diffuse pigmentation and the parallel-ridge pattern with a sweat glands infiltration.[22] Presence of the latter feature allows detection of melanoma with the diagnostic 86.4% sensitivity and 99% specificity. The occurrence of four or more colors within the lesion is frequent (65% of cases). Almost half of acral melanomas have polymorphic vascular pattern with combinations of milky-red areas (95%), linear irregular vessels (49%), dotted vessels (43%) and hairpin vessels (41%). Approximately 43% of acral melanomas show a combination of three or more types of blood vessels.[22] In the case of acral melanoma of the nail unit, the most prominent pattern is the presence of irregular lines (70%). Nail dystrophy occurs in 43% of cases. The periungual pigmentation (Hutchinson's sign) is present in 25% of the lesions, including 9% of cases, when it is only dermoscopically visible.[17]

One of the most characteristic features of amelanotic melanoma is the presence of atypical, polymorphous blood vessels. Primary amelanotic melanoma should be strongly considered if more than one shade of pink, dotted and serpentine vessels or predominant central vessels are present (sensitivity 70%, specificity 60%). Jaimes *et al*. described the presence of serpentine vessels (85% of cases), dotted irregular vessels (75% of cases), linear-irregular vessels (35%) and milky-red areas (80%) in amelanotic melanoma. In 55% of amelanotic melanomas a subtle light brown pigmentation located peripherally was observed. This feature is associated with better prognosis. Similarly, polymorphous vessels and dotted vessels are also associated with melanoma of relatively better prognosis. In 50% of cases blood vessels are scattered throughout the lesion, in 20% of cases are located centrally, and in 5% of cases are located peripherally.[23] According to Menzies *et al*., the vascular dermoscopic criteria for diagnosis of amelanotic melanoma include: blood vessels in the central part of the lesion, hairpin vessels, milky red-pink areas, more than one shade of pink, and a combination of dotted and linear irregular vessels. Remnants of pigmemtation in conjunction with listed above vessel types and their distribution are the key to diagnosis of amelanotic and hypomelanotic melanoma.[21]

The so-called "desmoplastic melanoma" is devoid of the characteristic dermoscopic features. The unusual clinical and dermoscopic features often lead to a delay in diagnosis. These features may include scar-like areas and structures characteristic of dermatofibroma.[17] Dermoscopically, desmoplastic melanoma has at least one melanoma-specific structure, the most frequent being atypical vascular pattern. Peppering or other features of regression are seen in 24-44% of cases. Multiple colors (at least 4) are often considered a dermoscopic criterion of melanoma, also in the desmoplastic type.[24,25]

Reflectance confocal microscopy

In vivo reflectance mode confocal microscopy (RCM) is a non-invasive optical imaging technique illustrating the epidermis and papillary dermis at histological resolution and good contrast in real time.[26,27] The confocal laser scanning microscope is a variant of a microscope, in which the light source is a low-power laser that emits near infrared light, projected through a lens system. It uses multiple mirrors to scan the laser across the sample and "descan" the images across a fixed pinhole and detector. A focused laser beam scans the surface of the specimens and penetrate into the tissue. The intensity of remitted light is recorded. The skin imaging is based on different reflection indexes of individual cells and anatomical structures of the skin. This gives the image its contrast. Melanin and keratin produce the strongest contrast (reflection index = 1.7 and 1.5 respectively). The images are horizontal and parallel with the surface of the skin.[28,29] The imaging depth is in the relation to the wavelength of the employed laser light but limited to about 200 µm, which corresponds to the papillary dermis. RCM is especially valuable in diagnosis of amelanotic melanomas and in case of "collision tumors" with the presence of two or more coexisting neoplasms in the same skin lesion, for example melanoma and basal cell carcinoma.[28,29] Pellacani *et al*. have developed algorithm for the diagnosis of melanoma based on a reflectance confocal microscopy imaging, which is based on the presence of: irregularity in shape and distribution, nonedged contours of dermal papillae and cellular atypia, mild or marked at the dermoepidermal junction (major criteria) and the presence of pagetoid cells (large roundish nucleated cells with refractive cytoplasm and dark nucleus) within superficial layers of the epidermis or widespread pagetoid infiltration thorough the lesion, cerebriform clusters in the papillary dermis and nucleated cells within dermal papilla (minor criteria).[26,30] Considering lesions with a score 2 (1 major criterion and 1 minor criterion) a 91% sensitivity and 68% specificity in diagnosing melanoma was obtained.[26,31] According to Longo *et al*., the sensitivity of the RCM for the

diagnosis of melanoma is 96.5% and the specificity is 94.1%.[32] Segura's *et al*. two-step method for the diagnosis of melanoma is based on identifying melanocytic versus non-melanocytic lesions in the first step and distinguishing between melanoma and nevi in the second step.

In this algorithm 4 features differentiate melanoma from nevi: two features of the dermoepidermal junction are "protective" (edged papillae and absence of basal cell atypia; for each of them -1 point is given) and two features of the epidermis and papillary dermis are "risk" factors (roundish pagetoid cells and atypical nucleated dermal cells; for each of them +1 point is given). The outcome of ≤-1 results in detection of melanoma with sensitivity of 100% and specificity of 57,1%. For the result of ≥0 the number are 86.1% and 95.3% respectively.[33]

Figure 3 shows typical reflectance confocal microscopy images of cutaneous melanoma depending on the thicknes of the lesion according to Breslow score.

Reflectance confocal microscopy in different types of melanoma

In about 60% of *in situ* melanomas disarrangement of the granular and spinous layers of the epidermis is observed. Roundish and dendritic pagetoid cells are visible in the superficial layers of the epidermis and blurred dermoepidermal junction (dermal papillae without a demarked rim of bright cells).[34]

RCM of superficial spreading melanoma shows epidermal disarray, numerous roundish and dendritic pagetoid cells thorough the epidermis and disarray at the dermoepidermal junction. The presence of roundish pagetoid cells in the epidermis between the stratum corneum and the basal layer has a sensitivity of 77.9% and a specificity of a 81.4% for indicating melanoma.[34] At the dermoepidermal junction nonedged papillae are seen. In the papillary dermis nonaggregated atypical nucleated cells (atypical melanocytes), nonnucleated irregularly shaped bright dermal cells (plump cells) corresponding to melanophages and dense cellular clusters corresponding to melanocytic nests are present.[34]

In 70% of cases of nodular melanoma in RCM imaging shows nucleated cells, which are polygonal, polymorphic in size and shape, arranged in an extended atypical honeycomb pattern. These features are present in the granular and spinous layers of the epidermis. Epidermal disarray or pagetoid spread.[30,31] In over 80% of cases of nodular melanoma atypia of basal cells is observed. Melanophages (plump cells) and atypical melanocytes are present within the papillary dermis.

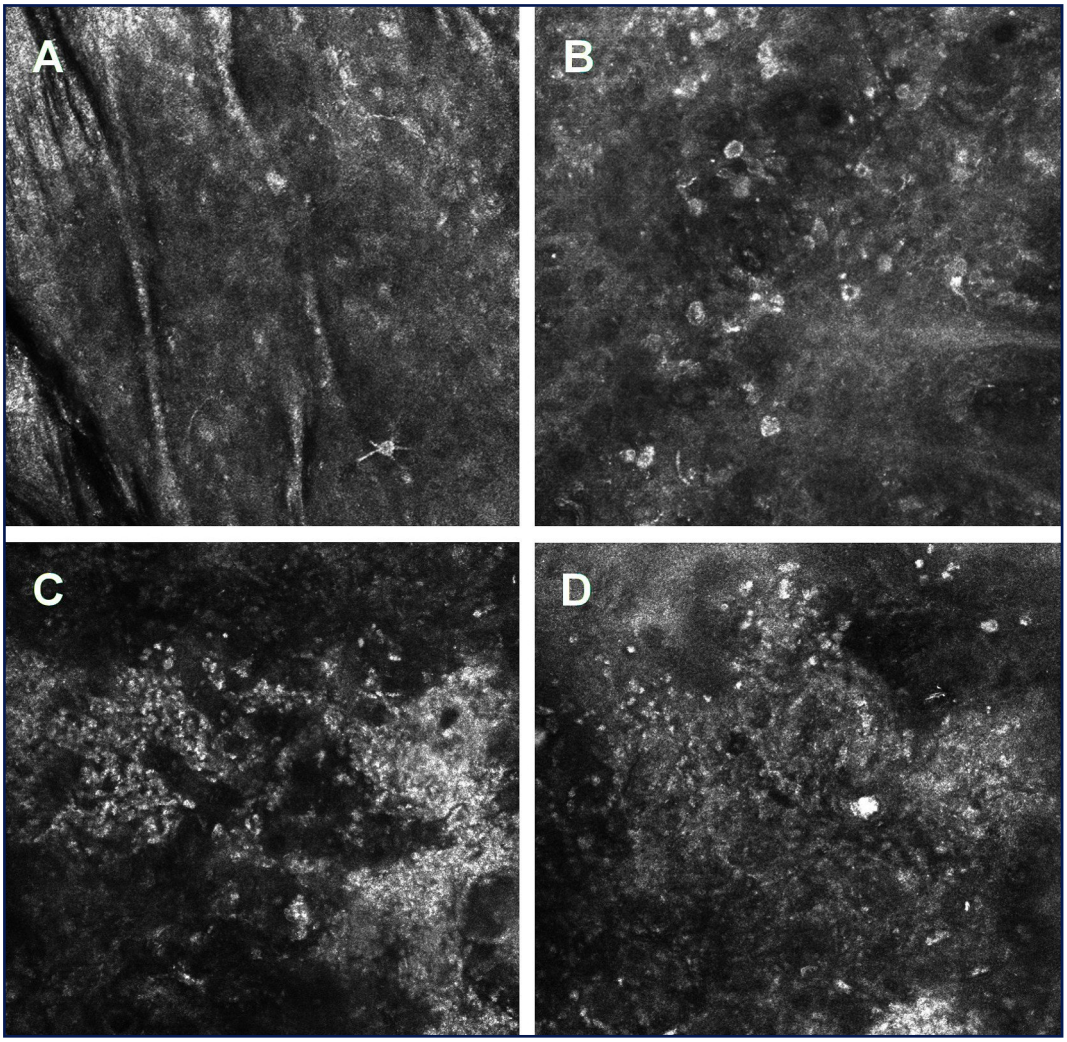

Figure 3

Reflectance confocal microscopy of melanoma.
A – melanoma in situ with the presence of a few dendritic cells in superficial layers of the epidermis;
B – melanoma in situ with the presence of numerous atypical pigmented cells and disarray of the epidermis;
C, D – melanoma 3 mm Breslow thickness (C) and 1,3 mm Breslow thickness (D) with the total disorganization of the epidermis structure and the presence of polymorphonuclear bright cells.

In 60% of cases cerebriform cells in the reticular layer of dermis are observed. In 90% of nodular melanomas enlarged, tortuous blood vessels are present.[30]

RCM imaging of lentigo maligna shows epidermal disarray with partial or complete loss of the honeycomb pattern, pleomorphism of melanocytes and the presence of roundish or dendritic pagetoid cells. Atypical melanocytes or nests of melanocytes surround adnexal openings and infiltrate of adnexal structures at a dermoepidermal junction. Atypical melanocytes occur also in the upper layers of the dermis, where melanophages and thickened collagen bundles may be seen.[35] Guitera *et al*. developed a melanoma diagnosis algorithm on the basis of RCM. This comprised two major features: nonedged papillae and round large pagetoid cells >20 µm (2 points for each), and four minor features: ≥3 atypical cells at the dermoepidermal junction in five 0.5 x 0.5 mm^2 fields, follicular localization of atypical cells, and nucleated cells within the dermal papillae (1 point for each), and one (negative) feature: a broadened honeycomb pattern (for -1 point). A score of ≥2 resulted in a sensitivity of 85% and specificity of 76% in the diagnosis of lentigo maligna.[36]

RCM is a useful non-invasive diagnostic technique in amelanotic melanoma.[27,31] The diagnostic accuracy of the RCM method was significantly superior for amelanotic and light-colored lesions compared with partially and completely pigmented lesions. Epidermal disarray, extender honeycomb pattern, atypical melanocytes are present in superficial layers of the epidermis, basal layer and within the papillary dermis. Different size and shape cell clusters are visible on the cross-section of the lesion. There are also cerebriform nests and tortuous, dilated blood vessels. Single dispersed cells or dishomogenous nests are present at the dermoepidermal junction. Cells forming a tumor mass are poorly demarked from each other. Dermal papillae usually are invisible.[26]

High frequency ultrasonography

In recent years there has been rapid development of ultrasound techniques, including improvement of gray-scale harmonic imaging and transducers using higher frequencies, which allows better resolution.[37] Transducers of 20, 75 or 100 MHz frequency have been developed. A resolution of 80-200 µm and penetration depth from 8 to 1.5 mm is possible.[38] High resolution of the method allows histological resolution in imaging of individual layers of the epidermis and dermis, cutaneous appendages and blood vessels.[39] The standard 7.5-15 MHz sonographic equipment can be used for the assessment of the skin but does not precisely differentiate the structures smaller than 1 mm. The highest resolution high-frequency ultrasound devices (20-100 MHz) showed a 99.4% correlation with pathology in an analysis of superficial spreading melanoma.[40] Jasaitiene *et al*. indicate that the correlation between the thickness of melanoma assessed by ultrasound examination and histopathological examination in the case of devices with a frequency of 20, 75 and 100 MHz is from 0.895 to 0.99, whereas for the devices of 7.5 MHz the "r" value is 0.76.[38] In ultrasound examination melanoma is a solid, hypoechogenic, richly vascularized structure, well demarked from surrounding structures. Accurate assessment of tumor vasculature allows optional color Doppler. Inflammatory infiltrates, which are hypoechogenic as melanoma, may be difficult to differentiate from the tumor, what is a disadvantage of ultrasonography. For this reason, the thickness of the lesion observed in the ultrasound examination may be overestimated.[38,41] The underestimation of tumor thickness however, can occur in case of hyperkeratotic lesions causing the loss of echo effect and the difficulty of accurate assessment of the deeper layers of the skin. The diagnostic accuracy of ultrasound may be limited in palmar and plantar melanoma.[42]

Optical coherence tomography

Optical coherence tomography (OCT) is non-invasive visualization technique, which uses infrared light. It enables the imaging of structures with a resolution 3-15 µm at a depth of up to about 1 mm. OCT is an interferometric tomographic imaging technique that generates cross-sectional 2D and 3D images of backscattered or back-reflected light from the tissue in real time. The most common use of OCT is in the diagnosis of various diseases of eye, gastrointestinal tract, vascular tissue, dental tissue and skin. In dermatology, OCT was introduced in 1995 and is now increasingly used in clinical skin research. Optical coherence tomography proved to be an effective imaging technique for the investigation of skin morphology: epidermis, dermis, dermoepidermal junction, hair follicle unit, blood vessels and sweat glands. Unlike ultrasound, OCT may be a useful diagnostic technique also for hyperkeratotic lesion and locations, such as lesions on the palms and soles and difficult to examine location, for example oral mucous membrane. The limitation is a tumor thickness, because it is no possible to examine the lesions exceeding 1 mm. OCT was used for monitoring non-surgical treatment (imiquimod) of lentigo maligna.[43-45] The results of these studies showed a good correlation between OCT and pathology. In OCT melanoma is characterized by marked architectural disarray and presence of vertical, icicle-shaped structures.[43] Hinz *et al*., on the basis of 26 cases of skin melanomas analysis by using HFU and OCT revealed better agreement for OCT and histopathology concerning tumor thickness measurements than for ultrasound. There was a higher median difference (0.16 mm) between HFU and histopathology tumor thickness measurements compared to the measurements by OCT and histopathology (median difference 0.08 mm). Both HFU and OCT tend toward tumor depth overestimation compared to histopathological *ex vivo* tumor measurement.[45]

Electrical impedance spectroscopy

Electrical impedance spectroscopy (EIS) is a non-invasive technique to image various types of cancer. The method is based on the observation that frequency dispersions in the

electrical conductivity and permittivity of malignant tissue is different compared to normal tissue. This technique has been also called "electric biopsy". The method is still under development and the potential application of EIS in dermatology remains uncertain.

Conclusions

Development of advanced non-invasive diagnostic techniques allows tissue imaging *in vivo* and contributes to more accurate diagnosis of skin diseases, including melanoma. Videodermoscopy allows the analysis of digital records, what is important in monitoring and diagnosing skin malignancies, including melanoma, also in histologically doubtful cases.[46] Development of more advanced high-resolution imaging techniques (RCM, OCT, HFU, EIS) allows imaging of the skin structures with nearly histological resolution and gives the possibility of "non-invasive *in vivo* biopsy".

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