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INVESTIGATIVE REPORT

High-Resolution Laser Doppler Perfusion Imaging Aids in Differentiating Between Benign and Malignant Melanocytic Skin Tumours

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Malignant melanomas are characterized by heterogeneity and asymmetry as well as by a higher density of blood vessels than benign pigmented tumours. The aim of this study was to evaluate the benefit of high-resolution laser Doppler perfusion imaging (LDPI) in the differential diagnosis of pigmented skin tumours. One-hundred-and-eighty-nine patients were examined with the LDPI, 22 with malignant melanomas, 39 with clinically suspicious dysplastic melanocytic naevi and 27 with basal cell carcinomas. Following examination, the tumours were excised and examined histologically. A control group of 101 melanocytic naevi showed clinically and, with epiluminescence microscopy, definitely benign criteria. These naevi were not excised. In malignant melanomas there was a 3.6 ± 1.5 times higher perfusion than in healthy skin. The corresponding figures for clinically suspicious melanocytic naevi and basal cell carcinomas were 2.2 ± 1.1 and 2.0 ± 0.7, respectively. The increase in flow in malignant melanomas was significantly higher than in clinically suspicious melanocytic naevi and basal cell carcinomas ($p < 0.001$). All malignant melanomas showed at least 1.8 times higher flow values than healthy skin. When this value is taken as the basis for the diagnosis "benign or malignant", the LDPI proved a sensitivity of 100% and a specificity of 85%. If only the distinction between malignant melanomas and clinically suspicious naevi is considered, the specificity is reduced to 48%. There was no correlation between tumour thickness and increase in the mean perfusion of malignant melanomas ($r = 0.14$; $p = 0.5$). High-resolution LDPI can be used as an additional automatic screening method. ***Key words: laser Doppler imaging; perfusion; skin cancer.***

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Histologically, there are twice as many blood vessels in primary malignant melanomas than in benign naevi (1–6). Using the well-established, one-dimensional laser Doppler flowmetry, significant differences in perfusion between the tumour margin of malignant melanomas and clinically benign melanocytic naevi have been demonstrated (7). Two-dimensional laser Doppler perfusion imaging (LDPI), in contrast to one-dimensional laser Doppler flowmetry, allows assessment not only of the perfusion of one single point, but of the whole tumour surface without any skin contact (8).

Studies with a commercially available laser Doppler perfusion imager have already indicated a different perfusion pattern between malignant melanomas and melanocytic naevi (9). However, owing to the low lateral resolution of only 1 mm of this laser Doppler imager, the area of interest could be covered by only a few points of measurement. Furthermore, because of the large area of one measurement point, perfusion values of the tumour and the healthy surrounding skin were added up and a prototype with a 5 times higher lateral resolution (200 μm) was developed.

In a collective of 189 histologically controlled skin tumours of different dignity and 101 benign melanocytic naevi, the selectivity of the new measuring device was analysed. The aim of this study was to establish a discrimination level based on the differences of blood perfusion between malignant melanomas and melanocytic naevi.

PATIENTS AND METHODS

Patients

One-hundred-and-eighty-nine patients were examined, including 22 with malignant melanomas (tumour thickness 1.34 ± 1.06 mm, from 0.2 to 3.7 mm), 39 with a clinically suspicious melanocytic naevus and 27 with basal cell carcinomas (Table I). Immediately after the examination, these tumours were excised and analysed histologically. One-hundred-and-one benign melanocytic naevi, which were neither clinically suspicious nor epiluminescence microscopy suspicious, were examined with the LDPI without additional histological diagnosis.

Methods

An updated version of the commercially available LDPI was used (Lisca Development, Linköping, Sweden) and an improved lateral resolution of 220 μm was achieved by modifying the software and hardware. The measuring principle is based on the Doppler effect and measures the change in frequency of the monochromatic radiation reflected by the erythrocytes. The

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Table I. *Examined skin tumours (excluding 101 non-suspicious melanocytic naevi)*

Tumour type
Malignant melanomas ($n = 22$)
Superficial spreading ($n = 12$)
Nodular ($n = 6$)
Lentigo maligna ($n = 1$)
Acral lentiginous ($n = 1$)
Cutaneous metastases ($n = 2$)
Clinically suspicious naevi ($n = 39$)
Compound ($n = 24$)
Dermal ($n = 9$)
Junctional ($n = 6$)
Basal cell carcinomas ($n = 27$)
Solid ($n = 16$)
Ulcerated ($n = 3$)
Superficial ($n = 3$)
Sclerosing ($n = 3$)
Cystic ($n = 2$)

area to be examined is scanned in meandering fashion, so that an area of 4096 measurement points is covered (10). With a distance of 5 cm between the scanner and the skin surface, the examined area is 1.4×1.4 cm – chosen so that both skin tumour and adjacent healthy skin were covered.

The laser beam is reflected by the erythrocytes, which allows recording of the returning signal by a detector positioned in the scanner head and thus conversion to an electrical signal, proportional to the tissue perfusion. The underlying intensity of perfusion values is expressed on a scale of different colours extending from blue (low perfusion values) over green and yellow to red (highest perfusion values). For calculation, a finer scale (4096 levels) is used. Measurement points without enough light reflection are coded as grey areas in the image. The background threshold was set to 5.81.

Before measurement started, a climatization period of 10 min was respected. Each tumour, and the healthy tissue next to it or the healthy skin on the contralateral side, was measured in order to rule out the possibility that the differences in laser Doppler flow between the different tumour entities were due to regional variations of blood flow in different areas of the body. A dimension-less index (the relative increase in tumour circulation versus healthy skin) was calculated. It was assumed that regulation of the blood flow was equal in the tumour and the healthy skin with similar alterations to the ambient temperature, emotional stimuli, etc. The Biological Zero could not be quantified, because only a minor number of skin tumours were located at the extremities.

The individual perfusion values and their standard deviations were determined as the average value within a region of interest. This region of interest was rectangular in shape and was selected as large as possible, corresponding to the clinically visible size of the skin tumour. The same size and shape of the region of interest was evaluated in healthy skin.

Immediately after the LDPI examination, the clinically suspect tumours were excised. The obviously benign melanocytic naevi were not excised. The indication of the excision was provided before the LDPI examination by a dermatologist not involved in the study.

The diagnosis was secured histologically and the density of blood vessels (low, normal, high) and the inflammatory infiltrate between and under the tumour nests (none, low, moderate, marked) was examined.

The study was approved by the ethics committee of the faculty of medicine, Ruhr-University Bochum.

Statistics

Differences between the groups were examined with the *t*-test for independent samples for significance and a correlation according to Pearson was calculated to examine relations between parameters (SPSS 8.0 for windows, SPSS, Germany).

RESULTS

Individual examples

Patient 1. A 69-year-old patient with a nodular malignant melanoma on his back. In the laser Doppler perfusion image, the melanoma was perfused 6.14 times higher than healthy skin (Fig. 1). The standard deviation of the perfusion pattern of the malignant melanoma was 11.92 times higher and the coefficient of variation 1.94 times higher than of normal skin, thus reflecting the heterogeneity of the malignant melanoma. On histology, a low inflammatory infiltrate between the tumour nests and a moderate infiltrate under the tumour nests were apparent. The density of blood vessels was higher than in the surrounding healthy skin.

Patient 2. The laser Doppler perfusion of a suspicious naevus on the back a 46-year-old patient showed a flow value of 0.18 ± 0.04 AU (1.38 times higher perfusion than normal skin). The standard deviation in the clinically suspicious naevus was 2.25 times higher compared to healthy skin; the coefficient of variation was 1.63 times higher (Fig. 2). The histological preparation showed a compound naevus without inflammatory infiltrate and changes of blood vessels.

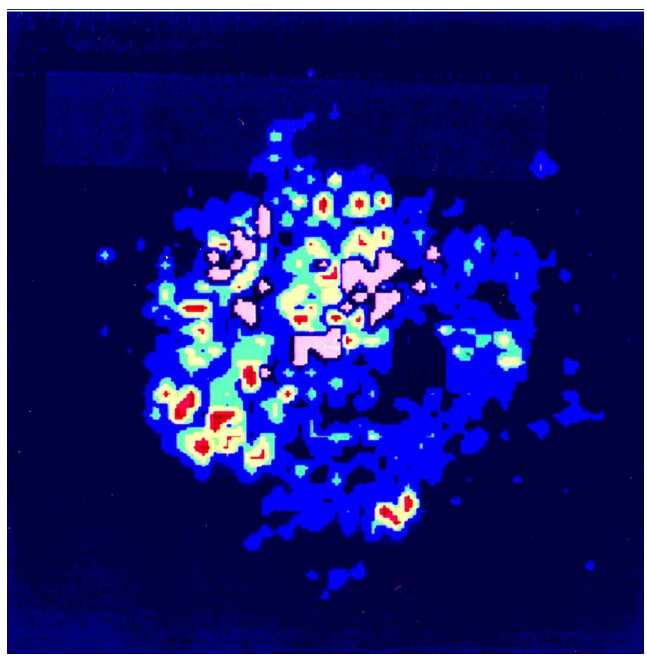

Fig. 1. Laser Doppler perfusion image of a malignant melanoma. The laser Doppler flow is increased in the tumour to a mean of 2.27 ± 1.43 AU in comparison to healthy skin with 0.37 ± 0.12 AU. AU: arbitrary units.

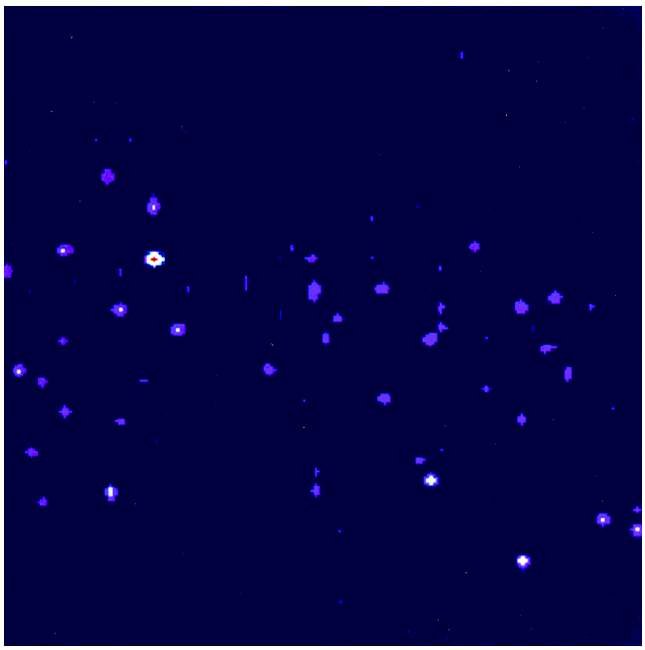

Fig. 2. Laser Doppler perfusion image of a clinically suspicious melanocytic naevus. Laser Doppler flow in the tumour 0.18 ± 0.09 AU, in the adjacent healthy skin 0.13 ± 0.04 AU.

Patient 3. An 89-year-old with an ulcerated basal cell carcinoma retroauricularly. The tumour showed a flow of 0.93 ± 0.63 AU. The basal cell carcinoma showed a 1.6 times higher perfusion compared to healthy tissue. The standard deviation was 3.5 times higher in comparison to healthy skin; the coefficient of variation was 2.21 times higher (Fig. 3). In the histological preparation, an ulcerated basal cell carcinoma with a moderate inflammatory infiltrate between and under the tumour nests was apparent, and the density of blood vessels was higher than in healthy skin.

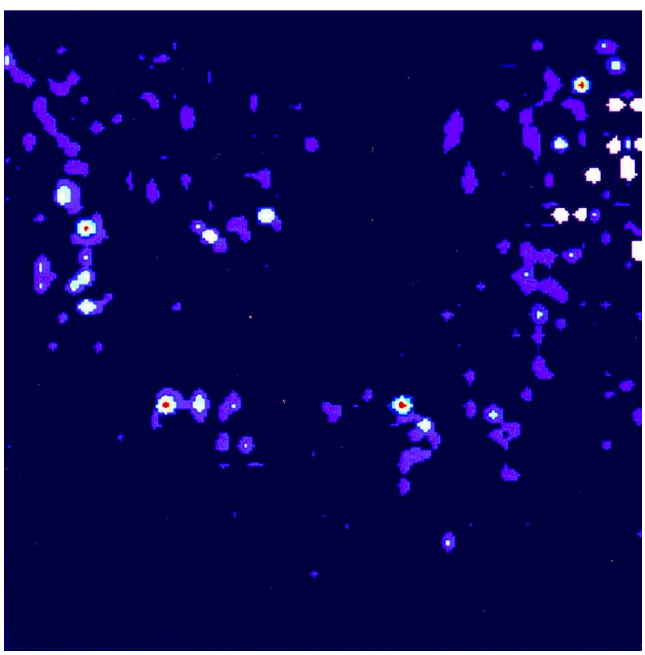

Fig. 3. Laser Doppler perfusion image of an ulcerated basal cell carcinoma. Laser Doppler flow in the tumour 0.93 ± 0.63, in the healthy skin 0.58 ± 0.18 AU.

Overall results

The laser Doppler flow in malignant melanomas was 3.62 ± 1.53 times higher than in healthy skin. In clinically suspicious naevi the flow was increased 2.27 ± 1.1 times and in basal cell carcinomas 2.05 ± 0.77 times higher values than in healthy skin, but this increase was significantly lower than in malignant melanomas ($p < 0.001$). In clinically non-suspicious melanocytic naevi, the quotient of laser Doppler flow in the tumour and in healthy skin was 1.04 ± 1.34 AU (Fig. 4). In clinically suspicious naevi the flow was higher than in clinically inconspicuous melanocytic naevi ($p < 0.001$) but in comparison to basal cell carcinomas, no significant differences were apparent ($p < 0.445$).

Heterogeneity of the perfusion pattern in the area examined was analysed with the coefficient of variation (standard deviation/mean value). Clinically suspicious naevi had a higher coefficient of variation (2.45 ± 1.37) than healthy skin ($p < 0.001$); the same was true for basal cell carcinomas (2.2 ± 1.17) and malignant melanomas (2.12 ± 1.07; $p < 0.001$ for both).

The coefficient of variation for the clinically inconspicuous melanocytic naevi was not significantly higher (1.06 ± 0.53) than in healthy skin ($p > 0.05$).

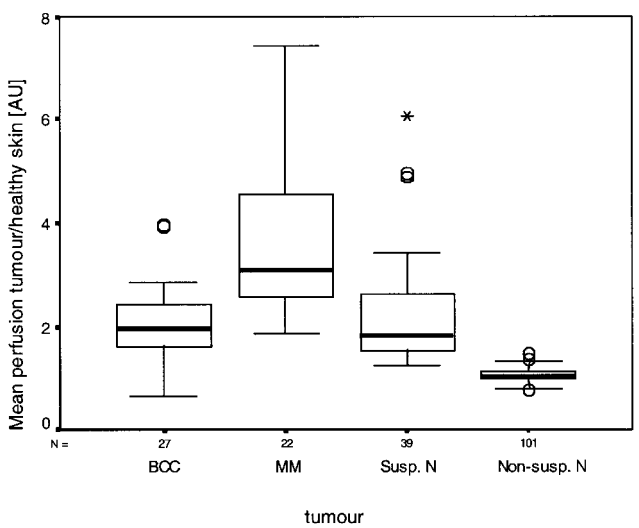

Fig. 4. Quotient of the laser Doppler flow in the skin tumour and the adjacent healthy skin. Significantly higher quotients in malignant melanomas than in BCC and melanocytic naevi ($p \leq 0.001$). No significant difference between BCC and clinically suspicious melanocytic naevi ($p \leq 0.445$). ° = values that are in a 1.5- to 3-fold box length distance from the box and * = values that are in a more than 3-fold box length distance from the box. BCC = basal cell carcinoma; MM = malignant melanoma; susp. N = clinically suspicious melanocytic naevi; non-susp. N = clinically non-suspicious melanocytic naevi.

Threshold determination

All malignant melanomas examined had at least a 1.8 times higher laser Doppler flow than healthy skin. Taking this value as the threshold level, malignity was recognized in 100% of cases. Only by using the threshold level could all clinically non-suspicious naevi be determined as benign. Of the clinical suspicious naevi, 19 (48.7%) were classified as benign, while 20 (51.3%) were falsely classified as melanomas. As for the basal cell carcinomas, 12 (44.4%) showed a score under the 1.8 level.

For the 189 skin tumours examined, a sensitivity of 100% and a specificity of 85% was found to detect the melanomas among all other skin tumours.

Tumour thickness and histological evaluation of the inflammatory infiltrate

The histological examination did not show any correlation between the intensity of the inflammatory infiltrate and the average tumour perfusion in any skin tumour. The tumour thickness and the flow increase in the tumour did not correlate either ($r = 0.14$; $p = 0.5$).

DISCUSSION

Even with a lateral resolution of ≥ 1 mm, the LDPI showed a higher perfusion in basal cell carcinomas of the eye-lid (11) and in superficial carcinomas of the breast (12) in comparison to healthy skin on the surface. However, the difference between malignant melanomas and clinically suspicious naevi was not significant (13). This can be explained by the lower lateral resolution of the traditional LDPI, which is not sensitive enough to distinguish between benign and malignant tumours.

In contrast to benign melanocytic naevi, a higher blood supply in malignant melanomas and basal cell carcinomas could be demonstrated using a one-dimensional laser Doppler flowmeter (7). A problem using the one-dimensional examination technique was in selecting representative measuring points in these examination areas that are often fairly heterogeneous. In addition, the one-dimensional laser Doppler touches the skin. Measuring might be impossible within ulcerations and also of very darkly pigmented tumours (7). This problem could be solved with the two-dimensional measuring system used in this study. The thinnest melanoma examined in our collective had a thickness of 0.2 mm according to Breslow. In the high-resolution LDPI, even this thin malignant melanoma showed a higher flow signal in comparison to the surrounding healthy tissue. A probable explanation for the perfusion in thin melanomas that can also be readily detected is the measurement depth in skin tissue of the laser Doppler imager in the order of 0.2–0.3 mm (14).

The laser Doppler is therefore superior to Doppler ultrasound, which could only register the flow in skin tumours thicker than 0.8 mm (14). It was thus concluded that neo-vascularization begins from a tumour thickness of 0.8 mm onwards (16). The blood flow identified using Doppler ultrasound in a few malignant melanomas of < 0.8 mm was explained to be caused by a higher blood flow due to inflammation or regression. The laser Doppler flow in the malignant melanomas did not increase with increasing tumour thickness ($r = 0.14$; $p = 0.5$). This finding corresponds to histological examinations that could not prove an increasing density of blood vessels with increasing tumour thickness (6, 17). Possibly, the higher inflammatory infiltrate in thin melanomas leads to a higher increase of the inflammatory blood flow. Due to the fact that in our own collective no correlation between the tumour perfusion and the inflammatory infiltrate was found, the inflammatory flow increase does not seem to have a great influence on the laser Doppler flow signal of the tumours.

For the first time, laser Doppler flow signals of clinically highly suspicious naevocytic naevi as well as completely inconspicuous naevi were compared with malignant melanomas. Since the clinically definitely benign melanocytic naevi showed, without exceptions, far lower flow increases than the malignant melanomas, the LDPI seems to be suitable as an automatic screening method for pre-selecting possibly malignant lesions. Because 48% of the highly suspicious naevi showed lower flow values than melanomas, the LDPI can also provide additional information dealing with diagnostically difficult naevi. Tumours with laser Doppler flow values above the critical level should be thoroughly examined clinically and with epiluminescence microscopy.

All melanomas showed an increase in laser Doppler flow of at least 1.8 times in comparison to healthy skin. Therefore a relative value which is calibrated individually for each subject on healthy skin, peritumorally or contralaterally, may be relatively insensitive to variation in temperature in the examination room and to a different level of acclimatization of the patients to the conditions of examination. The calculated values of sensitivity and specificity are influenced by the mixture of different kinds of tumours in the group examined. If, for instance, more basal cell carcinomas had been included, the specificity would have been lower, i.e. the value reflects not only the discriminating ability of the method but also the different tumours included in the study. Therefore, further validation studies making prospective use of this value will be necessary.

In summary, high-resolution LDPI is an important non-invasive technique that provides additional information about the dignity of pigmented skin tumours and is therefore of clinical importance. Apart from epiluminescence microscopy (18) and sonographic determination of the tumour thickness (19), information

about the perfusion of skin tumours can also be used for pre-operative diagnoses.

High-resolution LDPI technology is not aimed to replace conventional skin tumour diagnosis, but instead is intended as an aid to the methods already existing.

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