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ORIGINAL ARTICLE

Dynamic optical coherence tomography shows characteristic alterations of blood vessels in malignant melanoma

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Abstract

Background Dynamic optical coherence tomography (D-OCT) allows *in vivo* visualization of blood vessels in the skin and in malignant tumours. Vessel patterns in malignant melanoma may be associated with tumour stage.

Objective The aim of this study was to describe blood vessel patterns in melanomas and to correlate them with stage.

Methods One hundred fifty-nine malignant melanomas were assessed in a multicentre study. Every tumour was imaged using D-OCT prior to surgery and histologic evaluation. The tumour data such as thickness and ulceration as well as the staging at primary diagnosis and a follow-up of at least 40 months resulted in a stage classification. The vessel patterns were assessed according to predefined categories, compared with healthy adjacent skin, and correlated to stage.

Results Melanomas contained more blood vessels in different patterns compared with healthy adjacent skin. In particular, irregular vascular shapes such as blobs, coils, curves and serpiginous vessels were more common in melanomas. In addition, these patterns were significantly more often found in high-risk and metastatic melanomas than in low-risk lesions.

Conclusion In melanomas, the density of the blood vessels is increased, and irregular vascular patterns are more frequent. At higher stages, especially in metastatic melanomas, these atypical vessels are significantly more common.

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Conflicts of interest

Jon Holmes is employed by Michelson Diagnostics, the manufacturer of the D-OCT used in this study. All authors report grants from European Union CIP-ICT PSP Programme, during the conduct of the study. The authors declare no further conflict of interest.

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Introduction

Malignant tumours, especially malignant melanomas, often exhibit a higher density of blood vessels compared with adjacent tissue. These vessels not only feed the growing tumour but also enable haematogenic spread of tumour cells. The process of tumour vascularization proceeds through multiple different mechanisms such as sprouting, intussusceptive angiogenesis, vascular co-option, mosaic vessels, vasculogenic mimicry and bone-marrow-derived vasculogenesis,[1,2] which are driven by interaction between tumour cells, endothelial cells and immune cells in the surrounding tissue.[1] Hypoxia seems to be an important factor for triggering angiogenesis.[3,4,5] Because it plays such an important role, angiogenesis is an interesting target for antitumour therapy.[2,6]

Blood vessels play a role in three out of five steps of metastasis: intravasation, circulation and extravasation of tumour cells.[7] Several studies focused on the quantification of intra- and peritumoral vessels, using immunohistochemistry staining of histologic sections and counting the microvessels.[8] Some studies have shown that intratumoral blood vessel density is associated with

the risk of organ metastasis.[8,9] The results were sometimes contradictory, and a review demonstrated that the microvessel density in melanoma still cannot be recommended as a basis for making clinical decisions.[1]

Existing methods to evaluate blood vessels have limitations. In dermoscopy, atypical blood vessels have been described in malignant melanomas,[10] but pigmentation of the melanoma often obscures the vessels. In histologic sections, the blood vessels collapse after excision, fixation and staining, and furthermore, histology only provides a two-dimensional section and gives little idea of the three-dimensional vessel network in the tumour. It is well known that the architecture of the tumour influences areas of hypoxia and angiogenesis.[3,4,5] Therefore, there is a high and so far unmet need for a non-invasive *in vivo* method to obtain 3D images of skin microvasculature.

Dynamic optical coherence tomography (D-OCT), also called OCT angiography, is a new, non-invasive tool to visualize blood vessels in the skin *in vivo* in three dimensions.

Initial studies have shown distinct vessel patterns in healthy and diseased skin.[11,12] The vasculature allows a differentiation between different types of non-melanoma skin cancer and of subtypes of basal cell carcinoma.[13,14] A case report of a naevus-associated malignant melanoma showed an increased vasculature in the melanoma region compared with the naevus.[15] A study of 127 melanomas showed a strong correlation between vascular patterns and Breslow's thickness.[16]

Here, we report on the relation between qualitative and quantitative aspects of tumour microvasculature with known risk factors for metastasis and stage.

Materials and methods

Consecutive patients with malignant melanomas were recruited for the study after informed consent. D-OCT imaging of the centre of the tumour and of healthy adjacent was performed. Finally, the patients underwent excision of the tumour and, depending on local guidelines, further staging and surgery of the sentinel lymph node.

After surgery, the results of the tumour histology, of the status of sentinel node (if biopsy was performed) of the primary staging (American Joint Committee on Cancer 8th edition[17]) and of follow-up for at least 40 months resulted in a classification as follows:

- Risk group 1 (low risk): *in situ* melanoma and stage IA (thickness ≤1 mm).
- Risk group 2 (intermediate risk): stage IB to stage IIB (intermediate thickness with or without ulceration).
- Risk group 3 (high risk): stage IIC (ulcerated melanoma >4 mm), stage III (lymph node involvement) and stage IV (distant organ metastases).

The D-OCT device VivoSight Dx was developed and CE-marked for the project (Michelson Diagnostics Ltd., Maidstone, UK, www.vivosight.com/researcher/regulatory). All four centres (three hospital settings and a private practice) used the same configuration of the system: lateral resolution <7.5 μm, axial resolution <10 μm (in tissue) and image pixel size 4.3 μm. OCT image depth penetration is ~1 mm, but D-OCT is limited to depth 0.5 mm. Minimum detected vessel diameter is 20 μm due to the speckle variance detection technique with repetitive scans of the same site.

From the centre of each melanoma (respectively, the clinically most relevant site, avoiding scars from previous biopsies), a 6 mm × 6 mm area was imaged in the dynamic mode, taking 30 s, producing 120 cross-sectional images with a spacing of 50 μm between the images. Thus, the scanning process resulted in a three-dimensional image 'block' of volume 6 mm × 6 mm × 2 mm (width × length × depth), in which the brightness of the pixels corresponds to tissue structural details and red pixels correspond to blood vessels. The vessels in D-OCT can only be assessed to a depth of approximately 500 μm, because at greater depths noise in the OCT signal interferes with the speckle variance detection of motion. In addition, the adjacent healthy skin was measured with the same settings at a distance of 5 mm from the visible tumour border.

The images were evaluated by three investigators (JW, SS and NC) independently, all blinded for site and tumour parameters. In case of discrepancy (<10% of the individual OCT pictures), the images were discussed together, and a unanimous consensus was found. All three observers were trained beforehand to apply the parameters.

The previously proposed terminology for standardized description of the D-OCT images, which all investigators were involved in developing, was used.[18] Briefly, the blood vessels were assessed at a depth of 150 and 300 μm in the en face images regarding density, diameter, distribution (regular, irregular or clustered) and direction (no orientation, streaming or radiating) of the vessels. Density and diameter were compared with healthy adjacent skin (less, average or more) being the internal reference. Then, the shapes of the vessels were described using the terms dots, blobs, coils, lines, curves and serpiginous vessels. Each shape was rated 0 = absent or 1 = present. The depths of 150 and 300 μm correspond to the depths of dermal papillae and the reticular dermis in typical healthy skin.

Statistical evaluation was performed using the Wilcoxon test for matched pairs when comparing the melanoma with healthy adjacent skin. Comparison between the tumours was done using the Kruskal–Wallis *H*-tests, followed by the Mann–Whitney *U*-test if there was an indication for significance, which was assumed at $P \leq 0.05$. Due to low case numbers in the individual subgroups, no multivariate logistic regression analysis was performed.

All local ethics committees of the study centres approved the study.

Results

Malignant melanomas

A total of 160 malignant melanomas from 156 patients were evaluated by D-OCT before excision. One patient had three simultaneous melanomas, another two. The mean age of the patients (52 female and 104 male) was 65 years. One patient declined surgery because of his general poor health condition and dropped out of the study, leaving 159 cases for evaluation. In 81 cases, the adjacent skin was also imaged. Twelve melanomas of the included tumours were biopsied before imaging, all others were not. After imaging, the standard procedure of excision with safety margin and – depending on the tumour parameters – sentinel node biopsy and staging were performed in all patients.

This resulted in 23 cases where the sentinel lymph node was excised, thereof seven cases with melanomas of <1 mm thickness because of additional factors such as mitosis or ulceration. Four patients exhibited macrometastasis of the lymph nodes at time point of primary staging before surgery.

Regarding the subtype of melanoma, 107 melanomas were classified as superficial spreading melanomas. Twenty-three were nodular, two were acrolentiginous and 21 were lentigo maligna or invasive lentigo maligna melanomas. Six melanomas could not be classified. Twenty-one melanomas were ulcerated.

Looking at invasion levels, 42 melanomas were classified as *in situ* melanomas and 75 invasive melanomas were up to 1 mm thick. The tumour thickness of 18 melanomas ranged between 1 and 2 mm, of 13 melanomas between 2 and 4 mm, and 11 melanomas were thicker than 4 mm. The tumour thickness according to Breslow was on average 1.29 mm (range from *in situ* melanomas to 18 mm thickness).

During follow-up (range: 40–65 months, median: 51 months after first diagnosis), 15 patients were classified in a higher-risk category due to metastases, resulting in an up-staging.

Finally, 103 melanomas were classified as risk group 1, 30 were classified as risk group 2, and 26 were assigned to risk group 3 from stage IIC with a thick, ulcerated tumour (three cases), stage III (seven cases) with lymph node involvement to stage IV (16 cases) with distant organ metastases.

D-OCT

In all melanomas, it was possible to visualize the three-dimensional vessel network with D-OCT. In the en face view at a depth of 150 µm, dots are the predominant vessel pattern, representing the capillary loops in the papillary dermis, whereas the images at 300 µm show a great variety of vessel patterns.

In 81 cases, the healthy adjacent skin was imaged and evaluated as well. In these cases, the density of blood vessels was lower in 3% of the melanomas, equal in 59% and higher in 38% compared with healthy skin as internal reference. The diameter of the melanoma vessels was equal to healthy skin in 83% and larger than healthy skin in the remaining 17%. Melanin did not seem to have a significant influence on the image quality and signal intensity of the vessels; D-OCT images of vessels in hypomelanotic melanomas did not appear different from those of highly pigmented tumours. Melanomas show a larger number of different vessel patterns compared with healthy skin. At 150 µm, all patterns are more frequently found in melanomas, reaching significance for dots ($P = 0.02$), blobs ($P = 0.002$), lines ($P = 0.04$), curves ($P = 0.002$) and serpiginous vessels ($P = 0.007$). Dots were the most common pattern at 150 µm depth, whereas at 300 µm, all different patterns were found. At that depth, again all patterns were more frequent in melanomas compared with healthy skin at the same depth, reaching significance for coils ($P = 0.0002$), curves ($P = 0.01$) and serpiginous vessels ($P = 0.004$); see Fig. 1. Even in the risk group 1 with *in situ* and thin melanomas, where 42 melanomas were thinner than 150 µm and another 11 cases between 150 and 300 µm, all patterns were more frequent in the melanomas compared with healthy skin, indicating that also subtumoral vessels were affected by the tumour above. Additionally, melanomas show more often an irregular distribution of the vessels ($P = 0.0002$) and an atypical branching such as arborizing vessels or bulges ($P = 0.03$). Fig. 2 shows an example of a melanoma with coils and serpiginous vessels to demonstrate the atypical shapes.

The presence of blobs, curves, coils and serpiginous vessels and the degree of irregular distribution of these patterns correlated significantly with the risk of metastasis. At a depth of 150 µm, significantly more blobs ($P < 0.0001$), more coils ($P = 0.0006$), more curves ($P = 0.007$) and more serpiginous vessels ($P < 0.0001$) as well as an atypical branching ($P < 0.0001$) in risk group 3 compared with risk group 1 were found. At a depth of 300 µm, significantly less dots ($P = 0.046$), more coils ($P = 0.02$), less lines ($P = 0.03$) and more

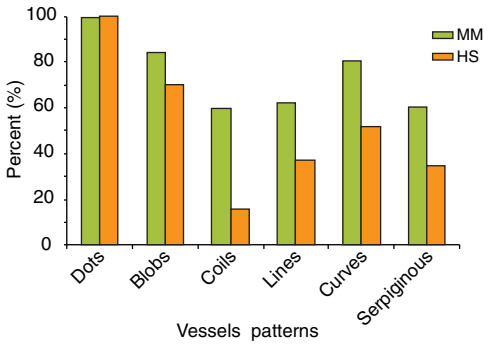

Figure 1 Percentage of vessel patterns in melanomas (MM) and corresponding healthy skin (HS) at a depth of 300 µm, $n = 81$. All patterns except dots are more frequently found in melanomas compared with healthy adjacent skin.

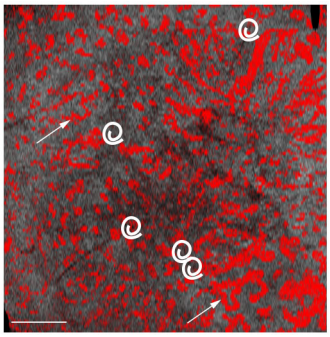

Figure 2 Dynamic optical coherence tomography of a malignant melanoma at the back, tumour thickness 2.2 mm, ulceration, pT3bN0M0, St. IIB, risk group 2. The predominant vessel patterns are coils (marked by small coils) and serpiginous vessels with atypical branching (marked by arrows). En face view at a depth of 300 μm, 6 mm × 6 mm, scale bar 1 mm.

serpiginous vessels ($P = 0.007$) in risk group 3 compared with risk group 1 also reflect this (Table 1, Fig. 3).

In addition, the density of the blood vessels and the diameter were significantly more often increased in high-risk compared with low-risk melanomas at 300 μm ($P = 0.03$ resp. $P = 0.01$); see Fig. 4.

Even in risk group 3, there was a difference between non-metastatic high-risk or lymphogenically metastasized melanomas and those with haematogenic distant metastasis. Only 60% of stage IIC and stage III melanomas showed coils or serpiginous vessels, while 88% of haematogenously spread melanomas in stage IV showed coils and 97% serpiginous vessels.

Table 1 Vessel patterns in relation to risk at a depth of 150 and 300 μm

150 μm	**R1 (*n* = 103)**	**R2 (*n* = 30)**	**R3 (*n* = 26)**
Dots	99% (102)	100% (30)	96% (25)
Blobs	12% (12)	37% (11)	35% (12)
Coils	5% (5)	17% (5)	23% (7)
Lines	11% (11)	13% (4)	23% (6)
Curves	17% (17)	43% (13)	35% (11)
Serpiginous vessels	4% (4)	7% (7)	31% (10)
300 μm	**R1 (*n* = 103)**	**R2 (*n* = 30)**	**R3 (*n* = 26)**
Dots	100% (103)	100% (30)	96% (25)
Blobs	83% (85)	93% (28)	81% (21)
Coils	51% (53)	73% (22)	77% (20)
Lines	69% (71)	53% (16)	46% (12)
Curves	77% (79)	93% (28)	81% (21)
Serpiginous vessels	51% (53)	73% (22)	81% (21)

Risk 1 = MIS to stage IA, risk 2 = stage IB to stage IIB and risk 3 = stage IIC to stage IV. Values as percentage % (*n*). Significant increase compared to R1 is marked light brown at $P < 0.05$ and brown at $P < 0.01$. Significant decrease compared to R1 is marked light green at $P < 0.05$.

Nodular melanomas have coils ($P = 0.01$) and serpiginous vessels ($P = 0.01$) significantly more often compared with the other subtypes. Also, ulceration correlated significantly with the presence of coils ($P = 0.001$) and serpiginous vessels ($P = 0.03$).

Discussion

Dynamic OCT (D-OCT) is a newly developed addition to morphologic OCT that allows a simultaneous display of skin blood vessels *in vivo* and real time.[11] In the present study, we systematically evaluated vessel density and shape in 159 D-OCT scans of malignant melanomas and correlated the findings with healthy adjacent skin, other known categorical risk factors and observed metastasis. We confirmed that D-OCT enables the *in vivo*, real-time and non-invasive visualization of the blood vessel network in malignant melanomas.[11–16] In general, melanomas show more often a higher vessel density and larger vessel diameter, a higher prevalence of distinct vessel patterns, more irregular distribution of vessels and presence of atypical branching and special shapes such as coils and serpiginous vessels compared with healthy skin of the same location as an internal control, even in very thin or *in situ* lesions. This indicates that angiogenesis occurs very early in tumour progression and invasion.

There are also some studies using OCT angiography for the assessment of vessels in choroidal and iris melanomas.[19,20] In the iris, melanomas showed an increased vascularity and more atypical vessels similar to our findings.[19]

Dynamic-OCT results correspond to dermoscopy, where malignant melanomas also exhibit atypical vessels,[10] especially in fast-growing melanomas, indicating angiogenesis in thicker tumours.[21,22] It has been demonstrated that some other dermoscopic features correlate with positive sentinel nodes such as micro-ulceration, pigment blotches and absence of pigment network.[23] It is notable that melanoma vessels are described dermoscopically mainly in hypomelanotic tumours because overlying pigmentation makes it difficult to assess the vasculature. Interestingly, melanin had no influence on the presentation of vessels in D-OCT, because it has a very low absorption coefficient at the D-OCT wavelength (1300 nm) and so is nearly transparent.[24] We had highly pigmented and amelanotic melanomas in our study, both could be evaluated without visible differences.

In contrast to OCT, dermoscopy only allows assessment of very superficial vessels because the detection depth of visible light is limited in a highly scattering medium like skin. Dotted vessels or even areas without visible vessels are described in dermoscopy of melanomas.[10] This is no contradiction to our findings, because in addition to superficial images with increased number or irregularly distributed dots and blobs in melanomas we focus on the vasculature inside the tumour at a depth of 300 μm, which is out of reach of dermoscopy.

In our study, the atypical shapes of vessels such as blobs, curves, coils and serpiginous vessels increase with higher stage of disease.

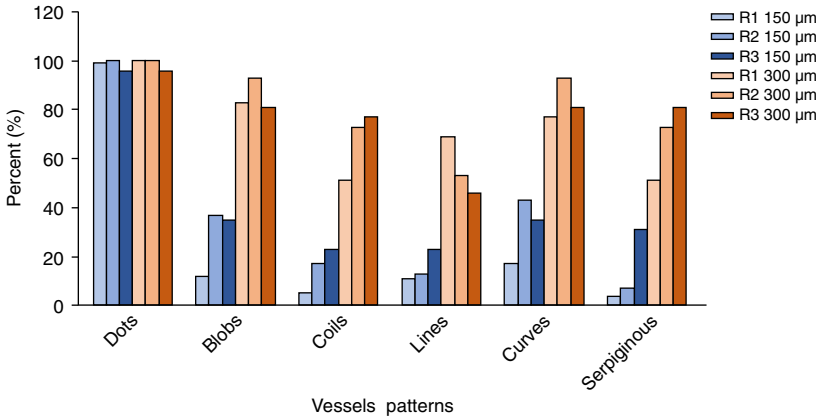

Figure 3 Percentage of vessel patterns at a depth of 150 and 300 µm in different risk classes of melanomas. Risk 1 = MIS to stage IA, risk 2 = stage IB to stage IIB and risk 3 = stage IIC to stage IV. Dots are the predominant pattern at both depths. At 150 µm, all patterns except dots increase with higher stage. At 300 µm, especially coils and serpiginous vessels are more frequently found in medium- to high-risk melanomas compared with lower stage.

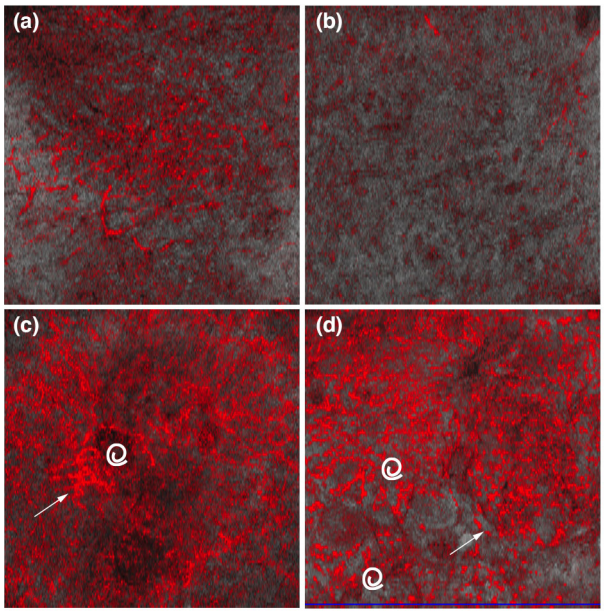

Figure 4 (a) Dynamic optical coherence tomography of a malignant melanoma of risk group 1, St. IA, (b) healthy adjacent skin of (a). The centre of the tumour (a) showed an increased vessel density with polymorphous vessels compared with healthy adjacent skin (b). (c) demonstrates a malignant melanoma of risk group 2, and (d) shows a metastatic malignant melanoma of risk group 3. The vessel density and diameter as well as the number of atypical vessel shapes such as coils (marked by small coils) increase with higher risk. The vessels are distributed irregularly with clustered vessels (arrow). En face view at a depth of 300 µm, 6 mm × 6 mm.

Studies counting blood vessels in hotspots in histologic sections are heterogeneous, but in general agreement with our findings.[8] A meta-analysis showed no clear trend towards a correlation of microvessel density with the risk of metastasis.[1] We propose that this can be largely explained by the differences in methodology. It is very difficult to evaluate the shape of a blood vessel in a cross-sectional histologic slide. Many of the tumour vessels build a horizontal network above and around the tumour in addition to intratumoral vertical vessels.[8] In contrast to histologic sections, D-OCT provides a three-dimensional insight into a tissue block, allowing assessment of vessel patterns in en face and in cross-sectional planes.

In a previous systematic study of a smaller number of melanomas,[16] the increase in the Breslow stage was correlated with more irregular distribution of dotted vessels and presence of curved, coiled and serpiginous vessels in thicker melanomas. The findings suggested that vascular changes described in D-OCT might be correlated with neoangiogenesis related to increased tumour burden and dermal invasion.

In our present study, atypical vessels were not only correlated with tumour thickness. In the subgroup of melanomas with metastasis at the time point of primary diagnosis, the tumour thickness ranged between 1.2 and 10.5 mm. Coils and serpiginous vessels more frequently accompany nodular and ulcerated melanomas, reflecting the fact that tumour thickness and ulceration status correlate with metastasis as well. Both patterns are more frequent in melanomas with haematogenic spread compared to melanomas with solely lymph node metastasis, indicating that they are a marker for neoangiogenesis. No single case of melanoma categorized as

low risk after excision and staging exhibited ulceration, coils and serpiginous vessels all together.

Optical coherence tomography and high-frequency ultrasound can be used to estimate the thickness non-invasively prior to surgery.[25,26,27] Because penetration depth of the signal is limited to about 1 mm, OCT was found to be superior to ultrasound in thinner tumours, but inferior in thicker ones.

Raster-scan optoacoustic mesoscopy (RSOM) is another, so far still experimental option to visualize tumour vessels *in vivo*. A first study of a melanoma model in four mice showed vascular images with a lower resolution and significantly higher detection depth (imaging of vessels at a depth of about 900–2000 μm) compared with OCT. The method is not yet approved for *in vivo* measurements in humans.[28]

Using OCT for risk assessment may include D-OCT not only to assess vessel morphology, but also to estimate tumour thickness. In addition to ulceration, which can be detected by the naked eye, the other risk factors for higher stage and metastasis can be non-invasively assessed prior to biopsy of surgery by integrating D-OCT into the primary staging. Thus, the clinical benefit for patients using dynamic OCT at the time of primary diagnosis prior to surgery is especially for melanomas of the medium-risk category of the preoperative estimation of tumour thickness and the non-invasive detection of additional risk factors due to atypical vessel patterns, which may indicate metastasis.

The study has some limitations: The assessment of the vessel morphology follows a terminology consensus but is nevertheless subjective. Automated image analysis with integrated deep learning may provide a more objective tool to assess blood vessels in the skin. In addition, D-OCT only displays blood vessels, but lymphatic vessels may also play a role in metastatic growth. Multivariable analyses will need more cases to discern whether the D-OCT can provide additional prognostic information to the traditional clinical–pathological risk factors in melanoma.

To conclude, D-OCT enables the assessment of tumour vessels in malignant melanomas. Melanomas exhibit more blood vessels with atypical shapes such as coils and serpiginous vessels with an irregular distribution compared with healthy adjacent skin due to neoangiogenesis of the tumour. In addition, the atypical vessel patterns correlate with stage. Therefore, the assessment of blood vessel morphology in malignant melanomas provides an additional possibility of risk estimation prior to surgery.

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IRB approval

The study has been approved by the local ethics committee of Augsburg (30-14). Informed consent was obtained from all patients.

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