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Author(s):

Nemirova, Svetlana; Orlova, Anna; Kurnikov, Alexey; Litvinova, Yulia; Kazakov, Viacheslav; Ayzvazyan, Irina; Liu, Yu-Hang; Razansky, Daniel ; Subochev, Pavel

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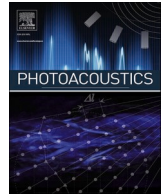
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Scanning optoacoustic angiography for assessing structural and functional alterations in superficial vasculature of patients with post-thrombotic syndrome: A pilot study

Svetlana Nemirova^{a,b}, Anna Orlova^b, Alexey Kurnikov^b, Yulia Litvinova^b, Viacheslav Kazakov^b, Irina Ayvazyan^b, Yu-Hang Liu^{c,d}, Daniel Razansky^{c,d,*}, Pavel Subochev^{b,**}

^a Privolzhsky Research Medical University, 10/1 Minin & Pozharsky sq., Nizhny Novgorod 603950, Russia

^b A.V. Gaponov-Grekhov Institute of Applied Physics, Russian Academy of Sciences, 46 Ulyanov Str., Nizhny Novgorod 603950, Russia

^c Institute of Pharmacology and Toxicology and Institute for Biomedical Engineering and, Faculty of Medicine, University of Zurich, Winterthurerstrasse 190, Zurich 8057, Switzerland

^d Institute for Biomedical Engineering, Department of Information Technology and Electrical Engineering, ETH Zurich, Wolfgang-Pauli-Strasse 27, Zurich 8093, Switzerland

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ABSTRACT

This study highlights the potential of scanning optoacoustic angiography (OA) in identifying alterations of superficial vasculature in patients with post-thrombotic syndrome (PTS) of the foot, a venous stress disorder associated with significant morbidity developing from long-term effects of deep venous thrombosis. The traditional angiography methods available in the clinics are not capable of reliably assessing the state of peripheral veins that provide blood outflow from the skin, a key hallmark of personalized risks of PTS formation after venous thrombosis. Our findings indicate that OA can detect an increase in blood volume, diameter, and tortuosity of superficial blood vessels. The inability to spatially separate vascular plexuses of the dermis and subcutaneous adipose tissue serves as a crucial criterion for distinguishing PTS from normal vasculature. Furthermore, our study demonstrates the ability of scanning optoacoustic angiography to detect blood filling decrease in an elevated limb position versus increase in a lowered position.

1. Introduction

Thrombosis occurring in various locations is of interest to physicians across all specialties, as its effects and consequences can impact all organs and systems. Acute arterial thrombosis leading to heart attacks and strokes remains a leading cause of death, while thrombosis in the lower extremities due to obliterating atherosclerosis and diabetic angiopathy comprises a significant cause of disability. Acute disruption of mesenteric circulation may require emergency surgery, placental thrombosis may lead to miscarriage, and thrombosis of the retinal arteries may result in blindness [1–3]. With venous thrombosis, patients may develop dysfunctions in any affected organs, as well as embolic complications such as pulmonary artery occlusion, which can lead to instant death or

gradually developing changes that significantly reduce the quality of life. Due to the novel coronavirus infection pandemic, there has been an increase in the number of patients with chronic post-thrombotic changes, as evidenced by a pool of publications on COVID-associated thrombosis in the literature [4,5].

The most frequently described post-thrombotic changes occur in the veins of lower extremities. These changes begin from the moment of stenosis or occlusion of the thrombosed vessel and can persist and even worsen throughout the patient's life. Factors that predispose to the development of post-thrombotic disease include damage to the iliac veins, increased body weight of the patient, severity and recurrence of the disease [6,7], which often occurs with the remaining components of the Virchow's triad, including slowing blood flow with

* Corresponding author at: Institute of Pharmacology and Toxicology and Institute for Biomedical Engineering and, Faculty of Medicine, University of Zurich, Winterthurerstrasse 190, Zurich 8057, Switzerland.

** Corresponding author.

E-mail addresses: daniel.razansky@uzh.ch (D. Razansky), pavel.subochev@gmail.com (P. Subochev).

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phlebohypertension, as well as trauma to the vascular machine with long-term inflammation. The formation of post-thrombotic syndrome (PTS) is also associated with the development of venous trophic ulcers, which are more likely to occur in patients with initial disorders of tissue trophism such as diabetes mellitus and varicose veins [8]. An assessment of the state of peripheral vessels that provide blood outflow from the skin could enable an objective evaluation of personalized risks of PTS formation after venous thrombosis. Various methods are available for non-invasive assessment of peripheral vessel morphology and presence of blood flow, such as laser Doppler flowmetry, photoplethysmography, computer-assisted venous occlusion plethysmography, as well as capillaroscopy and optical coherence tomography (OCT) [9–11]. However, these methods do not allow the assessment of larger "intermediate" veins that are not accessible to traditional ultrasound. Currently, vessels of this caliber have largely been overlooked due to the absence of a non-invasive method that allows for longitudinal studies.

Optoacoustic (OA) imaging is a promising approach for obtaining information about the distribution of subcutaneous vessels [12]. As compared to optical imaging methods, OA allows deeper penetration into biological tissues (up to several millimeters) while retaining high spatial resolution in the several tens of microns range. The images are formed by detecting and reconstructing ultrasonic waves generated via absorption of short laser pulses by tissue chromophores, primarily hemoglobin. Scanning optoacoustic mesoscopy systems are well suited for dermatological applications and imaging of superficial vasculature in humans [13]. To obtain a three-dimensional image, divergent radiation is delivered to the tissue through an optical fiber and a raster scan of a focused broadband detector is performed.

Currently, OA is utilized to provide functional and anatomical characteristics of various vasculopathies. For instance, in patients with Raynaud's syndrome, a reduction in blood oxygen saturation levels has been observed when compared to healthy volunteers [14]. Peripheral artery disease patients were found to have low muscle hemoglobin content and oxygenation [15]. Multispectral optoacoustic tomography has proven promising for staging peripheral arterial disease based on muscle oxyhemoglobin concentrations [16]. Differences in dermal vessel density were demonstrated in normal conditions and in port wine stains [17].

Various functional tests based on controlled effect on tissue perfusion are utilized to assess the vascular system condition in different pathologies [18]. Among them changes in limb position are based on decrease in inflow and increase in outflow of blood from the studied organ.

In this work, using raster-scan-based OA mesoscopy we demonstrate, for the first time, the structural and functional alterations in superficial vasculature during repositioning of a healthy limb and in post-thrombotic syndrome.

2. Materials and methods

The main group included four patients (51–66 years old) with anamnestic and clinically identified signs of PTS, as confirmed by ultrasound. In the 2–3 years period prior to the present study, all patients with PTS had suffered occlusive thrombosis of the femoral vein, confirmed clinically and by ultrasound. In all cases, the patients had been treated with oral anticoagulants in a therapeutic dose for up to 6 months. After one year, all consulted a surgeon with complaints of swelling and periodic pain in the lower extremities. Directly upon inclusion in the experimental group, all patients were interviewed and examined to identify characteristic complaints: in all cases, patients reported daily swelling of the lower extremities and pain, which developed during the day with maximum severity in the evening, without visually detectable varicose transformation of the saphenous veins. Ultrasound examination revealed signs of previous thrombosis in the form of thickening of the venous wall, the presence of intraluminal hyperechoic cord-like or ribbon-like structures, partially blocking the lumen of the vein, and fragmentarily creating multichannel blood flow,

fixing deformed valve leaflets with signs of their insufficiency. Exclusion criteria from the study were the presence of skin diseases and hyperpigmentation of the skin in the studied area, anemia, as well as edema and vascular diseases of other etiologies. The control group consisted of four volunteer (21–39 years old) without vascular pathology. Adult men and women who did not have a history of complaints similar to those associated with venous insufficiency of any etiology and who did not have visually or palpably detectable signs of diseases affecting the veins, arteries, and lymphatic vessels at the time of participation in the experiment, were included into the study. They had no history of taking medications or undergoing surgical interventions for vascular diseases, nor pathological ultrasound findings in the arteries and veins of the lower extremities. All experiments were approved by the Ethical Committee of Privolzhsky Research Medical University (Protocol #7 from 06.05.2022).

The scanning OA mesoscopy system, whose detailed description is available elsewhere [19], consisted of two scanning stages ($25 \times 25 \text{ mm}^2$ range; $25 \mu\text{m}$ step size), a pulsed laser source (532 nm wavelength; 1 ns pulse duration; 2 kHz repetition rate) and a digitizer (16 bit vertical resolution; 200 MHz sampling rate). A bright-field OA scanner based a customized spherical wideband (1–50 MHz at 10 dB level) polyvinylidene difluoride detector (BARI-NN Ltd., Russia), integrated with 400 μm diameter multimode optical fiber, was employed for imaging (Figs. 1A,B), providing $<50 \mu\text{m}$ spatial resolution in the lateral dimensions [20].

Examination of all patients was carried out under the same conditions: before the start of scanning, the patient stayed put in a supine position for 10 minutes, then for 10 minutes in each of the following positions: (1) horizontal arrangement of the examined limb; (2) the limb is oriented upward at 30° angle relative to the horizontal plane of the heart; (3) the limb is pointing down at 30° angle relative to the horizontal plane of the heart. For the study, a region of interest was selected 2 cm distal to the medial malleolus, in an area of the most significant effect of phlebohypertension on tissue trophism and the most frequent formation of trophic ulcers of venous etiology. Prior to scanning, a replaceable immersion chamber filled with water was attached under the OA sensor. An immersion OA detector was brought into acoustic contact with the skin through a thin layer of medium-viscosity ultrasonic gel Mediagel (Geltek-Medica LTD, Russia) and transparent film (less than $100 \mu\text{m}$ thick) both located at the bottom of water-filled immersion chamber. Tissue pressing by the immersion chamber was controlled using a Z-stage built into the OA system. To align $\sim 2 \text{ mm}$ visualization depth provided by the laser radiation wavelength (532 nm) with $\sim 2 \text{ mm}$ depth of the field of OA detector, the scanning plane had to be brought in parallel with non-planar skin surface. For convenient lateral alignment, we started each investigation with preliminary volumetric OA imaging ($10 \times 10 \text{ mm}^2$ area with $100 \mu\text{m}$ step), and proceeded by selection of a smaller area for final scanning ($5 \times 5 \text{ mm}^2$, $50 \mu\text{m}$ step). Final scanning required less than 30 seconds, but required for the human to stay in still position to obey motion artefacts.

To improve the signal to noise ratio of blood vessels in OA data, a set of two digital filters was applied to each raw A-scan [21]. Unwanted high-frequency noise and signals from structures smaller than the scanning step size were eliminated by 50 MHz low-pass filter. Low frequency offset signals from the bulk tissues were further suppressed by 0.2 MHz high-pass filter. The filtered 3D datasets were then processed by delay-and-sum reconstruction in Fourier domain [22]. To quantify the parameters of the vascular bed observed in the OA images (blood volume, vessel diameter and tortuosity), numerical data processing methods were applied to the reconstructed datasets. In particular, blood volume was estimated in the 3D data domain using Avizo (Thermo Scientific, USA). First, all the blood vessels observed in the reconstructed 3D images obtained in a horizontally positioned limb were segmented. 3D segmentation was processed by manually adjusting binarization threshold value for each imaged individual. Same pre-adjusted individual threshold values were further used to binarize blood vessels

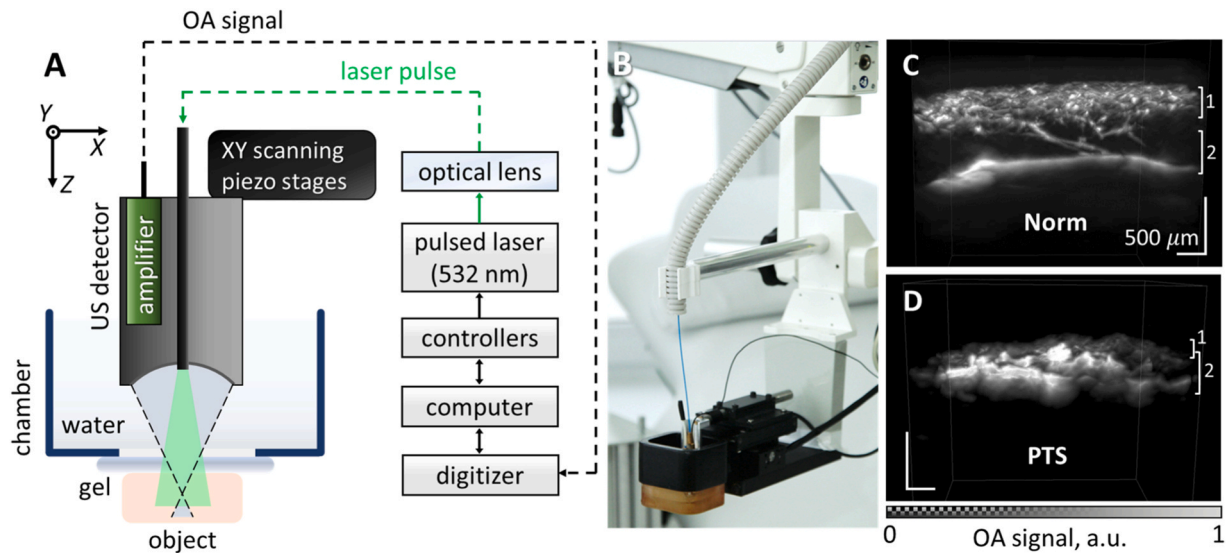


Fig. 1. Scanning optoacoustic angiography of the human foot. (A) Scheme of the experimental setup. (B) Photograph of the experimental setup. (C) Cross-sectional OA images of healthy foot vessels. (D) The corresponding images recorded from a patient with PTS. 1 - vascular plexuses of the dermis, 2 - vessels of subcutaneous adipose tissue.

observed in all the corresponding upward/horizontal/downward limb positions. The values of blood volume were determined for each position, as a percentage of the volume occupied by binarized vessel structures in relation to the total image volume.

The diameters and tortuosity of the blood vessels were estimated in the image domain. The OA images corresponding to each individual were obtained as maximum intensity projections of the reconstructed 3D datasets along the Z direction. Then, contrast enhancement and quadratic interpolation smoothing filters were applied to the images [23]. Binarization and skeletonization of individual blood vessels was provided by automatic adjustment of threshold values using Otsu method [24]. The vessel diameter was calculated using a custom Post-ProGUI Matlab program with a graphical user interface, which allowed for a manual labeling of the individual images by marking the vessel contours to indicate their key rotation points. The vessel tortuosity was then calculated as the relative difference between the length of the labeled vessels and the length of the labeled chords [25].

All measured values were plotted as means \pm SD. For statistical analysis, the IBM SPSS Statistics software was used. T-test for independent samples was performed to estimate the significance of the differences between normal and pathologic tissues. One-way ANOVA with Bonferroni correction was performed to estimate significance of the differences between the limb positions. Statistically significant value was taken as $p < 0.05$. The Pearson correlation coefficient was calculated to assess the correlation between the values of vessel tortuosity and vessel diameter.

3. Results

Exemplary OA images depicting dermal and subcutaneous blood vessels in the foot of a healthy volunteer versus a patient with post-thrombotic syndrome are shown in Figs. 1C and 1D, respectively. Areas with elevated OA signal levels highlight the hemoglobin-containing vessels. Superficially located vessels of the plexuses in the dermis (at up to 1 millimeter depth) have a smaller diameter as compared to underlying vessels in the subcutaneous adipose tissue with up to 300 microns diameter. In normal tissues, subcutaneous adipose tissue vessels and vascular plexuses of the dermis are clearly distinguishable. In PTS, veins of the subcutaneous tissue are sharply dilated and tortuous with the vascular layers overlapping each another and being indistinguishable.

Fig. 2 demonstrates en-face OA images of healthy and PTS vessels of the differently oriented foot. The images of a healthy foot are mostly manifested with small vessels of the dermis with the large deeper located vessels of the subcutaneous adipose tissue being less distinguishable. In a healthy limb elevation causes a decrease in the OA signal associated with the outflow of blood from both small (superficial) and large (deep) vessels (Fig. 2C). Lowering the foot causes an increase in blood supply in the small vessels, which results in a clear manifestation of the superficially located network of small and dense vessels (Fig. 2A). Total blood volume in healthy tissues was not altered significantly by changing the limb position (Fig. 2G). Images of the PTS tissues demonstrate a decrease in the number of small superficial vessels, while subcutaneous large vessels are visualized more clearly. Position-dependent changes are also manifested as an increase of OA signal in the downward limb orientation and a signal decrease in the upward orientation (Fig. 2D-F). Tissue blood volume in patients with PTS changes significantly with change of the extremity position ($p < 0.05$) (Fig. 2G). At the same time, blood volume significantly increased ($p < 0.05$) in PTS patients as compared to the normal values for almost all orientations.

Fig. 3 demonstrates the vessels of subcutaneous adipose tissue of the foot in all the healthy volunteers and PTS patients after manual removal of a layer of small superficial vessels from the OA images. As can be seen from the OA images, healthy tissues are characterized by a regular arrangement of almost straight non-dilated blood vessels (Fig. 3A). Compared with the normal vasculature, the vessels in PTS were found to be curved and significantly dilated (Fig. 3B) with their average diameter increased by a factor of two ($p < 0.05$). Neither vessel diameter nor tortuosity depended on the limb orientation (Fig. 3C,D).

Slight fluctuations in the vessel tortuosity occurred when changing the limb orientation in the control group with the changes being minimal in the elevated limb orientation and more significant when the limb is lowered (Fig. 3D). When examining patients with PTS, attention is drawn to the increase in the vessel tortuosity, which increases in the downward orientation. The degree of vessel tortuosity in PTS exceeded that in normal tissues by approximately 13 times ($p < 0.05$). An increase in the degree of vessel tortuosity may be associated with the rise of their diameter. A strong positive correlation ($r = 0.79$, $p < 0.01$) was revealed between these parameters (Fig. 3E).

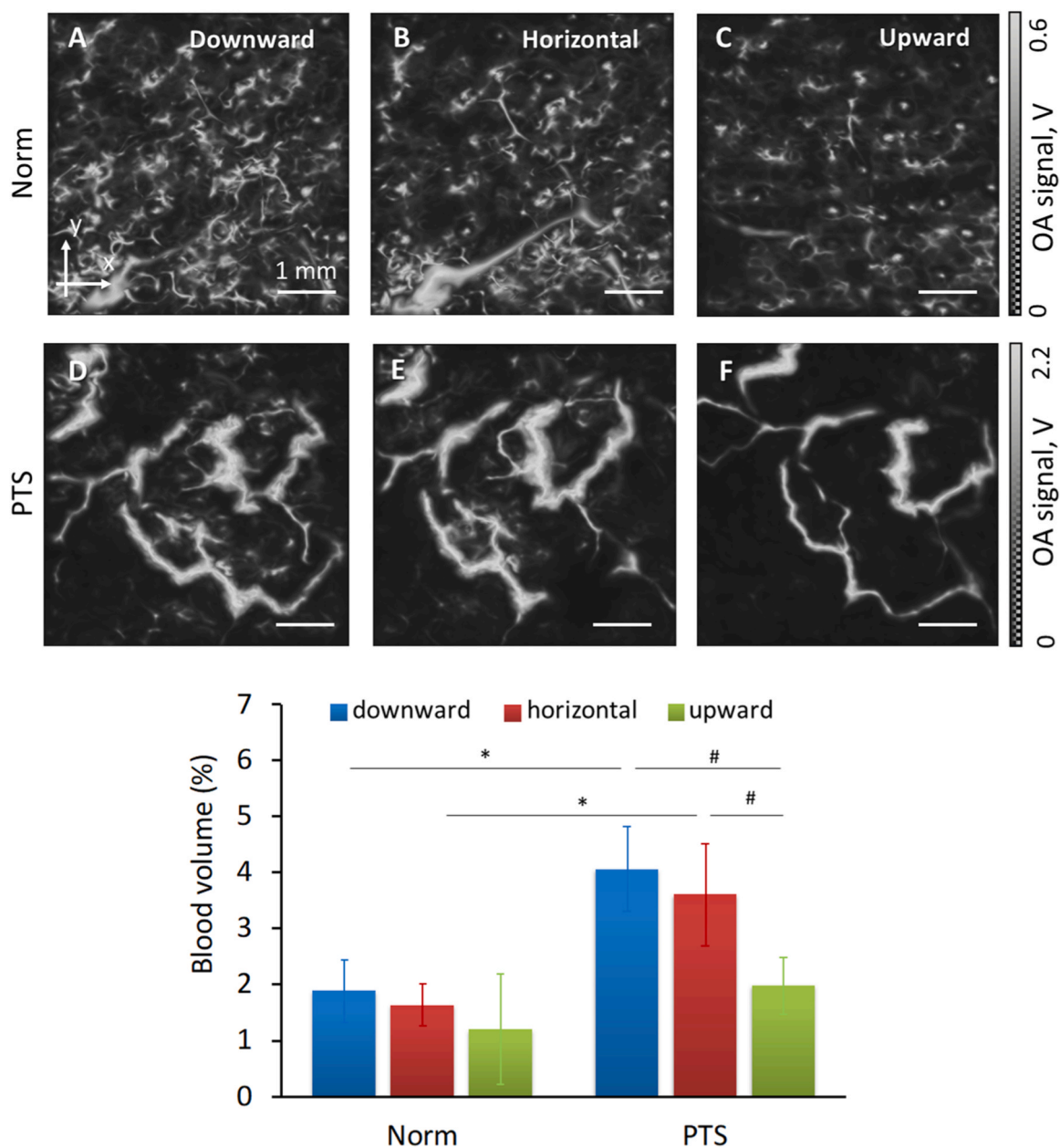


Fig. 2. OA images of the dermal and subcutaneous blood vessels in the foot of healthy volunteers (A-C) versus patients with PTS (D-F). Three limb orientations were considered, namely, downward (A, D), horizontal (B, E) and upward (C, F). The blood volume values calculated from the OA images are shown in (G). * - statistically significant differences between the healthy and PTS groups; # - statistically significant differences between orientations of the extremities.

4. Discussion

Post-thrombotic disease is associated with pathological restructuring of vessels of different calibers. In our work, we used OA to compare the vessels of plexuses in the dermis and subcutaneous adipose tissue of a healthy foot to those of patients with PTS and proposed criteria for diagnosis and follow-up.

The first clinical sign noted in patients with PTS is a violation of the angioarchitectonics in the dermal and subcutaneous adipose tissue layers [26] whose vasculature becomes indiscernible (Figs. 1, 2). In the horizontal foot position, patients with PTS have a relatively more superficial arrangement of larger vessels that compress the peripheral plexuses.

The second parameter is an increase in the vessel diameter in the subcutaneous tissues of the foot in PTS patients (Fig. 2). Vasodilatation is associated with diminished blood drainage through the main deep

veins, which is characteristic of patients with PTS due to partial occlusion of the lumen by organized thrombotic masses and multidirectional flow due to valvular insufficiency and vertical reflux. The thin muscular layer of the peripheral veins does not compensate for the effect of high phlebohypertension [27], the vascular wall appears stretched and the diameter of the lumen increases.

The third parameter detected by OA, which is particularly promising for dynamic observations, was the blood volume. In PTS, against the background of severe phlebohypertension, when lowering the limb, the total volume of the vessels increases not only due to an increase in diameters but also due to the greater tortuosity of the veins. In patients with PTS, there was no significant decrease in the overall density of hemoglobin-containing structures during limb elevation, which indirectly indicates a permanent expansion of the veins in PTS corresponding to a pathological remodeling of the vascular wall.

OA further allows visualizing another key sign appearing in PTS,

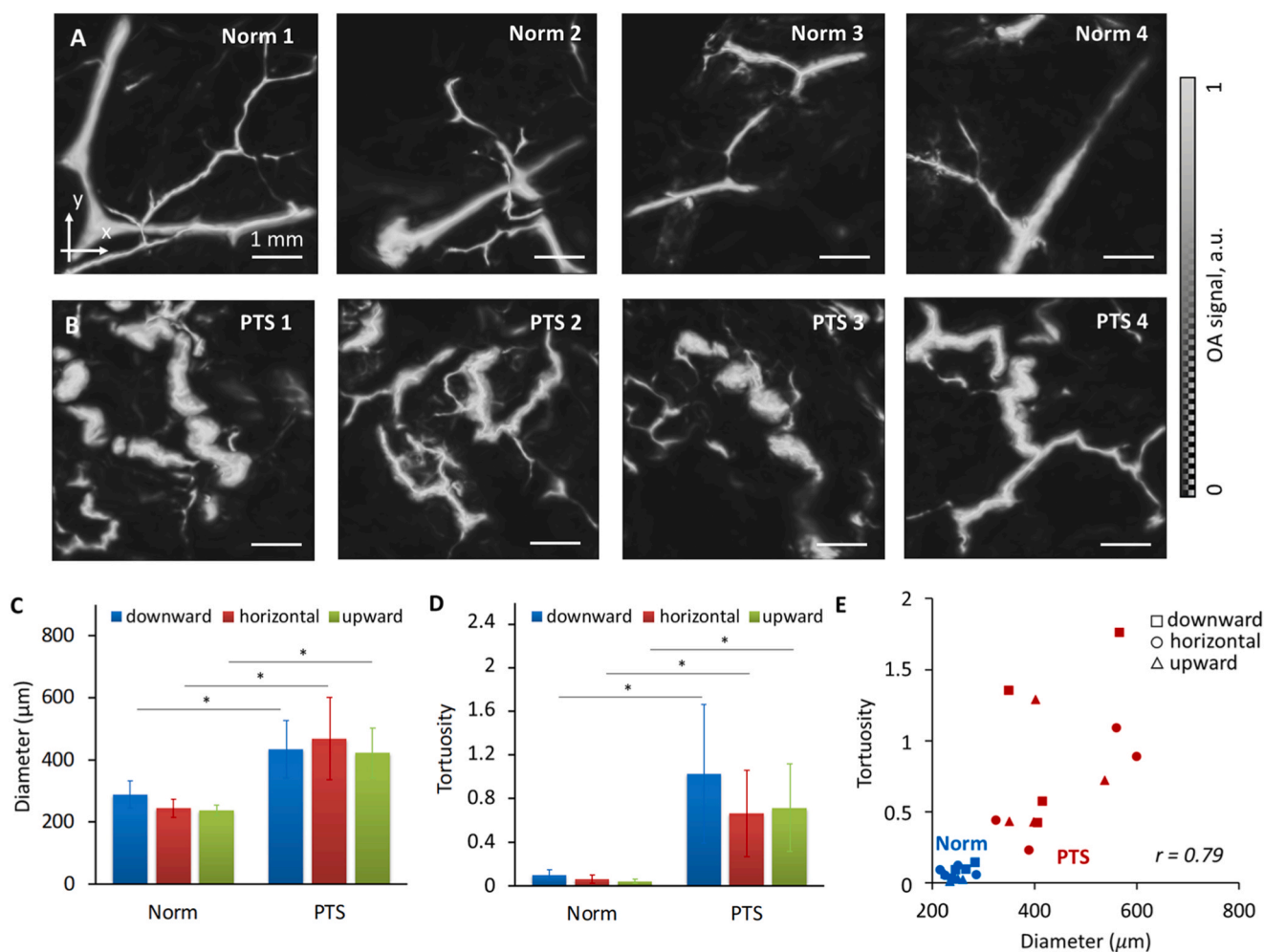


Fig. 3. OA images of blood vessels of subcutaneous adipose tissue of foot in healthy volunteers (A) and patients with PTS (B) in a horizontal limb orientation. Vessel diameter (C) and tortuosity (D) calculated from the OA images in the downward, horizontal and upward limb orientation. Vessel tortuosity values versus vessel diameter values are shown in (E). * - statistically significant differences between healthy and PTS groups.

namely, the tortuosity of subdermal vessels (Fig. 2), which is similar to that described in capillaroscopy in patients with chronic venous insufficiency [28]. This shows that the formation of secondary varicose veins accompanying the PTS development [29] may in fact originate from the subdermal vascular bed. Our OA study revealed that repositioning a healthy limb leads to fluctuations in the vessel blood supply, i.e. the blood volume increases when the limb is lowered and vice versa when it is elevated (Fig. 2).

Changes in diameter of the main vein depending on the limb orientation have previously been described [30]. Our OA study revealed further alterations in the vessels of the dermal and subdermal plexuses, which can be attributed to the pressure increase of the liquid column and expansion of the lumen for the downward limb orientation. Conversely, a decrease in the blood inflow and, accordingly, blood pressure occurs for the upward orientation. Our study clearly shows the effect of an increase in the inner diameter of a vessel (accumulation of hemoglobin in its lumen) on its tortuosity (Fig. 3E). The contour of the inner wall appears stretched in all direction, arguably due to decompensated phlebohypertension.

From a clinical perspective, OA offers several advantages for imaging microcirculatory changes in patients with PTS. In standard ultrasound examinations for PTS, vessels that are located at a depth of 5 mm or less are visible. However, at a depth of 2 mm, it is not possible to differentiate the branching of peripheral vessels due to the limited resolution of the method. In contrast, OA can visualize peripheral vessels with several

levels of branching at this depth, thereby complementing the data obtained from ultrasound. This is particularly valuable because it involves the vessels that drain peripheral tissues where trophic disorders may develop during PTS.

In our study, we demonstrated the capabilities of OA imaging for non-invasive observation of changes in intermediate-caliber vessels, which were previously only accessible through ex vivo methods. Moreover, during ultrasound examinations of patients in PTS group (data not shown), no significant pathological enlargement of the subcutaneous veins was observed, which excluded secondary varicose transformation of the vessels and may lead to an underestimation of the severity of pathological changes. However, the pronounced dilation and tortuosity of the peripheral veins, revealed by OA, provided evidence of early pathological remodeling of the peripheral vessels. Confirming these findings in a larger sample could aid in the early diagnosis of disease progression and help exclude edema of other etiologies.

Superficial small vessels are the primary factor influencing OA signal alterations. In PTS, the signal from the proximal vessels is more pronounced, which is associated with their greater blood supply and a more superficial location of the subdermal veins due to their expansion and compression of the distal vessels of the dermal plexuses.

Importantly, the identified features of the peripheral vascular bed differed from the previously reported age-related changes and were specific for the group of patients with PTS. A gradual decrease in the density and diameter of healthy human skin vessels with age has been

demonstrated in studies by other groups [31,32] and in our previous work [33]. However, the current study reveals an increase in blood volume and vessel diameter in PTS, suggesting that these changes are independent of age-related effects.

5. Conclusions

Our pilot study demonstrated the possibilities of non-invasive OA imaging in differentiating vascular features of the dermal and subdermal vascular plexuses in normal and PTS patients during positional tests. The observations open up new prospects for real-time monitoring of the effectiveness of drugs with angioprotective properties and other methods of prevention and treatment of patients with PTS. Moreover, an important area of application for this method may be the differential diagnosis between PTS, angiopathies and other diseases.

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CRediT authorship contribution statement

Alexey Kurnikov: Writing – original draft, Methodology, Investigation. **Yulia Litvinova:** Visualization, Investigation, Data curation. **Svetlana Nemirova:** Writing – original draft, Methodology, Investigation, Conceptualization. **Anna Orlova:** Writing – review & editing, Writing – original draft, Formal analysis. **Yu-Hang Liu:** Writing – original draft, Visualization, Methodology. **Daniel Razansky:** Writing – review & editing, Writing – original draft, Supervision, Resources. **Viacheslav Kazakov:** Writing – original draft, Investigation. **Irina Ayvazyan:** Writing – original draft. **Pavel Subochev:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pavel Subochev reports financial support was provided by Russian Science Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Svetlana Nemirova (PhD, MD) is a vice-rector of Privolzhsky Research Medical University. She received her Ph.D. degree in medicine in 2007 from Privolzhsky Research Medical University. Her area of scientific interests is prevention, diagnosis and treatment of angiopathy and their complications. She coauthored more than 80 articles, monographs, methodological assays, and patents.



Anna Orlova (PhD) is a senior scientist at the Laboratory of Biophotonics of the Institute of Applied Physics of RAS. She received her Ph.D. degree in biology in 2004 from Nizhny Novgorod State University. The major area of her research focuses on optical and optoacoustic imaging both in clinical and experimental oncology. Her main interests include investigation of tumor vascular microenvironment responses to treatments. She coauthored more than 40 peer-review articles and several book chapters.



Alexey Kurnikov (PhD student) – junior researcher at the Laboratory of ultrasonic and optoacoustic diagnostics, Institute of Applied Physics of the RAS. In 2021, he received a master's degree in radiophysics from Nizhny Novgorod State University named after Lobachevsky. He joined optoacoustic team in 2018 and over the past 6 years has gained extensive experience in the field of optical and acoustic numerical modeling, image reconstruction, design of ultrasonic sensors and optoacoustic systems. His current research focuses on methods of optoacoustic microscopy, tomography and laser-ultrasound imaging. He is a co-author of more than 10 scientific works in peer-reviewed journals such as *Advanced Photonics Nexus*, *Photoacoustics*, *Optics Letters*.



Yulia Litvinova is a student of Radiophysics Faculty of Lobachevsky State University of Nizhny Novgorod and a researcher of Laboratory of ultrasonic and optoacoustic diagnostics, Institute of Applied Physics of RAS. Her current research focuses on optoacoustic image processing.



Viacheslav Kazakov (Tech. Dr.) is a leading researcher of the Laboratory of ultrasonic and optoacoustic diagnostics at the Institute of Applied Physics of RAS (PhD, 1992, acoustics; Dr. Eng., 2009, radio engineering). He specializes in the development of contact and non-contact methods for non-destructive testing of materials and biological tissue.



Irina Ayvazyan graduated from the Kirov State Medical University (medical faculty) in 1974. Since 1997 she has been working as the chief physician of the Clinic of the Institute of Applied Physics of the RAS.



Roy Yu-Hang Liu (PhD) is a Research Fellow at the University of Zurich and ETH Zurich. He obtained his BSc degree in Electrical Engineering from the National Central University, Taiwan, MSc degree in Biomedical Engineering from the National Chiao Tung University, Taiwan, and PhD degree in Neurophotonics from the National University of Singapore. His main research interests include development optoacoustic microscopy and multi-modal imaging systems and their application in brain research and neurological disease.



Daniel Razansky (PhD) holds the Chair of Biomedical Imaging with double appointment at the Faculty of Medicine, University of Zurich and Department of Information Technology and Electrical Engineering, ETH Zurich. He earned Ph.D. in Biomedical Engineering and M.Sc. in Electrical Engineering from the Technion - Israel Institute of Technology and completed post-doctoral training in bio-optics at the Harvard Medical School. Between 2007 and 2018 he was the Director of Multi-Scale Functional and Molecular Imaging Lab and Professor of Molecular Imaging Engineering at the Technical University of Munich and Helmholtz Center Munich. He has published over 300 peer-review journal articles and holds 15 patented inventions in bio-imaging and sensing. He is the Founding Editor of *Photoacoustics* journal and serves on Editorial Boards of a number of journals published by Springer Nature, Elsevier, IEEE and AAPM. He is also an elected Fellow of the IEEE, Optica and SPIE.



Pavel Subochev (PhD) is the head of Laboratory of ultrasonic and optoacoustic diagnostics at the Institute of Applied Physics of RAS. He graduated from Lobachevsky State University of Nizhny Novgorod in 2006 and received his PhD in passive acoustic radiometry from the Institute of Applied Physics of RAS. Since 2012 Dr. Subochev is focused on the development of new instrumentation for optoacoustic imaging of biological tissues.