Optical Coherence Tomography Angiography for Monitoring Sublingual Microcirculatory Changes: a Prospective Tool for Investigation of Tissue Hypoperfusion in Critical Care Medicine

Anton A. Plekhanov[1*], Ivan A. Ryzhkov[2], Pavel A. Shilyagin[3], Elena B. Kiseleva[1], Konstantin N. Lapin[2], Lydia A. Varnakova[2], Yulia V. Korzhimanova[1], Anna I. Dementeva[1,4], Marina A. Sirotkina[1], Grigory V. Gelikonov[3], Evgeniy V. Grigoryev[5], and Natalia D. Gladkova[1]

[1] Institute of Experimental Oncology and Biomedical Technologies, Privolzhsky Research Medical University, 10/1 Minin and Pozharsky sq., Nizhny Novgorod 603950, Russian Federation

[2] Laboratory of Experimental Research, V.A. Negovsky Research Institute of General Reanimatology, Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, 25/2 Petrovka str., Moscow 107031, Russian Federation

[3] A.V. Gaponov-Grekhov Institute of Applied Physics of the Russian Academy of Sciences, 46 Ulyanov str., Nizhny Novgorod 603155, Russian Federation

[4] Lobachevsky University, 23 Gagarin av., Nizhny Novgorod 603022, Russian Federation

[5] Research Institute for Complex Issues of Cardiovascular Diseases, 6 Sosnoviy blvd., Kemerovo 650002, Russian Federation

*e-mail: strike_gor@mail.ru

Abstract. Using optical coherence tomography angiography (OCT-A), we demonstrate the feasibility of monitoring microcirculatory changes in the clinically important sublingual region, which reflects perfusion disturbances in internal organs of critically ill patients. We present the results of OCT-A monitoring of sublingual microcirculation alterations in both animals and humans induced by external stimuli (modeling massive blood loss and administration of a vasodilator drug). Our findings highlight the strong potential of OCT-A for addressing the challenges of early detection of tissue hypoperfusion in patients. We propose a signal intensity analysis for OCT-A images which might be an effective approach to predict multiple organ failure development, thereby enabling monitoring of the effectiveness of ongoing intensive care.

Keywords: optical coherence tomography angiography; sublingual microcirculation; tissue hypoperfusion; multiple organ failure.

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1 Introduction

One of the key challenges in the intensive care of critically ill patients with systemic inflammatory response syndrome, sepsis, or shock is the lack of effective clinical and laboratory tools for direct assessment of microcirculatory disorders in organs and tissues [1]. It is worth noting that diagnostics and therapy of already existing macro-vascular complications do not guarantee recovery of microcirculation and sufficient regeneration of perfusion in hypoxic organs [2], which is reflected in current trends in maintaining high mortality rates in critically ill patients with the manifestation of shock and multiple organ failure (MOF) [3]. Therefore, it is necessary to develop new clinical tools for direct diagnostics of microcirculatory disorders to identify effective criteria for predicting the manifestation/progress of MOF and timely provision of targeted therapy.

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It has been repeatedly shown that sublingual microcirculation reflects the state of blood supply to internal organs, likely because these vascular beds share a common embryonic origin [4–6]. Direct visualization of sublingual blood vessels with the ability to quantify them can significantly surpass other criteria in understanding the severity of a patient's condition and help physicians make timely optimal decisions. Vital microscopy (orthogonal polarization spectral imaging / side-stream dark field imaging / incident dark field illumination imaging) is widely used for sublingual assessment [7]. However, it has not become standard clinical practice due to the frequent and inevitable occurrence of artifacts (microscope pressure on the tissue being studied, shifting of the scanning area, poor illumination, and uneven focus) [8]. These artifacts require continuous, real-time quality control of the recordings by a qualified specialist. The lack of such a specialist often leads to the rejection of most data due to unacceptable recording quality, even with manual processing. Another limitation of such methods is the time-consuming and labor-intensive manual post-processing of recordings, which is often required because existing automated analysis approaches frequently yield inaccurate estimates of microcirculatory parameters [9]. Furthermore, existing studies evaluating therapies for critical illness complicated by internal organ hypoxia / MOF demonstrated inconsistent results regarding the prognostic value of sublingual microcirculation parameters measured by vital microscopy and highlight the need for further research and for the development of effective criteria / methods for monitoring the treatment being administered [10, 11].

This Letter discusses the possibility of using optical coherence tomography (OCT) technology to monitor changes of sublingual perfusion and diagnose microcirculatory disorders in patients developing shock / MOF. OCT-Angiography (OCT-A), based on interferometric signal recording method [12], offers several advantages for the investigation of oral vascular architecture. The device's built-in beam focusing system shifts along the depth of the object, allowing for stable examination of the structure and microcirculation of biological tissue at various depths up to 2 mm with high spatial resolution up to 10 μm [13]. As previously shown using vital microscopy, the relatively limited visualization depth does not hinder the study of microcirculatory changes in sublingual vessels, which are located beneath the basement membrane of the lamina propria of the oral mucosa [14, 15]. Moreover, OCT-A is a label-free method with high scanning rate and enough fast signal filtration to localize micromovements of scatterers (erythrocytes) and visualize in real time of blood vessels down to the capillary level [16]. Most often OCT-A represents the blood vessel network as a 2D image in *en face* (top view) projection (Fig. 1). Having proven its high efficiency in ophthalmology [17, 18], the multimodal OCT/OCT-A has been widely developed to address problems in various clinical areas [19–21], including growing interest in the use of OCT for diagnosing the oral mucosa [22, 23]. In particular, the feasibility of studying sublingual microcirculation was demonstrated in volunteers (in a sitting position) using an adapted commercial ophthalmological OCT-A system [24]. The study demonstrated the effectiveness of OCT-A and its high agreement with incident dark field illumination microscopy (current gold standard) in assessing perfused vessel density (PVD), confirming the promising potential for OCT-A to address various clinical challenges in critical care medicine.

It is worth noting that the first OCT studies of the oral cavity carried out by our research group were focused primarily on examining the morphology of oral soft and hard tissues with qualitative image evaluation [25]. We subsequently expanded these studies by adding blood flow assessment using the OCT-A modality. To reduce motion artifacts and improve the quality of OCT-A monitoring of oral cavity vessels, we use a non-traumatic vacuum-assisted attachment [26]. This attachment significantly stabilizes OCT-A images even in the absence of dedicated OCT probe fixtures and during manual scanning.

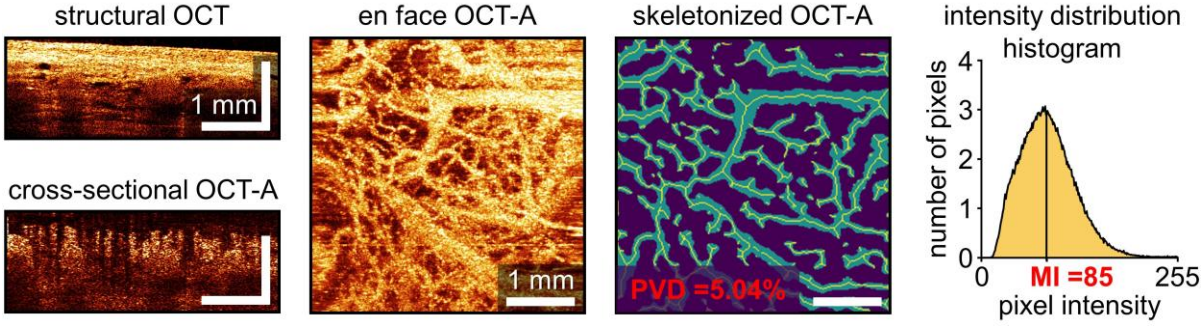

Fig. 1 A demonstration of standard OCT-A imaging via human sublingual vessel assessment. High-frequency filtration of sequential B-scans (structural OCT images) generates a 3D angiographic dataset (from cross-sectional OCT-A images), processing (manual depth selection and maximum intensity projection) of which produces a 2D *en face* OCT-A image of the tissue's vascular network [16]; scale bar size is indicated on images. Subsequent analysis of such images most often relies on skeletonization and characterization of the traced vascular branches; moreover, analysis of the signal intensity distribution spectrum is used less frequently.

In the paper [27], the capability of OCT-A for mapping the microcirculatory bed of oral cavity vessels in the cheek region was demonstrated. The resolution of the method allowed the detection of vascular loops in the papillary layer and a branched vascular network in the reticular layer of the lamina propria, as well as the cessation of blood flow in individual capillaries due to cold exposure. However, quantitative evaluation of OCT-A images, which involved skeletonization of vascular branches followed by PVD analysis, did not allow statistically significant confirmation of the visually observed changes, namely, a decrease in the signal intensity of vessels in the reticular layer during monitoring of cooling. The reduction of blood flow in the vascular loops of the papillary layer also supports the conclusion that there is a decreased blood flow velocity in the reticular layer, while the perfusion map remains preserved. Therefore, given that a drastic pathomorphological alteration of the tissue's vascular pattern (such as that seen in neoangiogenesis monitoring [28, 29]) is not anticipated, it is essential to validate novel analytical approaches capable of detecting subtle physiological changes in blood flow velocity using OCT-A monitoring data from the same oral mucosa regions.

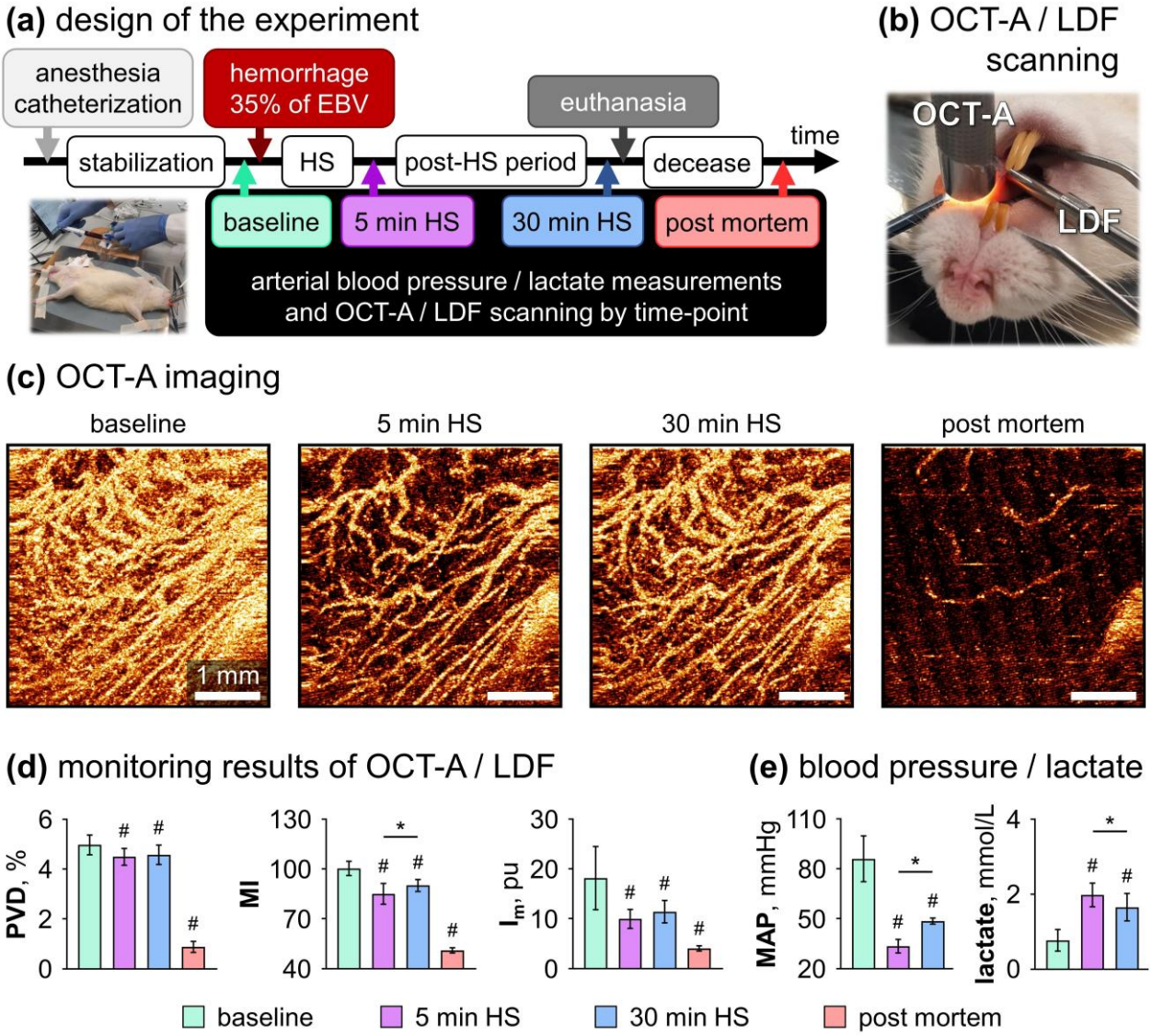

Fig. 2 Sublingual microcirculation changes during modeling of HS in rat: (a) schematic design of the experiment; (b) OCT-A and LDF probes placement for parallel monitoring of sublingual microcirculation; (c) representative OCT-A images before hemorrhage modeling (baseline), 5 min after losing 35% of estimated blood volume and HS onset (5 min HS), 30 min after HS onset (30 min HS), and immediately after animal euthanasia (post mortem); scale bar size is indicated on images; (d) analysis of sublingual microcirculation and diagrams of obtained values (mean ± SD) for PVD and MI according to OCT-A data, and I_m according to LDF data; # – statistical significance ($p < 0.05$) between the time points after hemorrhage modeling and baseline point, * – statistical significance ($p < 0.05$) between 5 and 30 min HS time points, Repeated Measures one-way ANOVA test; (e) analysis of hemodynamic and perfusion/oxygenation parameters – diagrams of MAP values and lactate concentration.

This study is the first to demonstrate OCT-A monitoring of sublingual microcirculatory changes in response to external stimuli. These stimuli were associated with changes in microhemodynamics commonly observed in routine intensive care practice [3]. In animal experiments, we assessed the sensitivity of OCT-A to alterations in sublingual blood flow during massive blood loss accompanied by hemorrhagic shock (HS). While the fact and etiopathogenesis of microcirculatory changes in HS are known [30, 31], OCT-A here allows for the first visualization and demonstration of this effect specifically in the sublingual area, which is of practical interest for subsequent clinical implementation. Furthermore, parallel monitoring of microcirculation using laser Doppler flowmetry (LDF), monitoring of the animal's arterial blood pressure, and biochemical analysis of arterial blood lactate confirmed hemodynamic disturbances and the development of organ ischemia in HS due to massive blood loss. Subsequently, we tested OCT-A monitoring of changes in sublingual microcirculation in volunteers under the influence of the vasodilator nitroglycerin (glyceryl trinitrate, GTN). Parallel blood pressure monitoring confirmed the observed hemodynamic changes upon local administration of the drug. Beyond demonstrating OCT-A's utility for monitoring sublingual microcirculation in both animals and humans, we propose a different approach of OCT-A data (was obtained as described in Ref. [32]) analyzing based on estimation of signal intensity (pixels' grey scale range from 0 to 255) histogram distributions and calculation the value of mean intensity (MI) of image (Fig. 1). This approach yields statistically significant differences (in contrast to PVD analysis) in cases where vascular maps show clear visual differences due to changed flow velocity, despite an unchanged vascular network architecture.

The experimental phase involved studies of sublingual blood flow changes in Wistar rats ($n = 5$) during modeled fixed-volume blood loss. The design of the experiment is presented in Fig. 2(a). The target blood loss volume was 35% of the estimated blood volume, corresponding to class III hypovolemic shock (HS) according to the Advanced Trauma Life Support classification [33]. This class of blood loss typically leads to pronounced tachycardia, hypotension, decreased pulse pressure, and increased respiratory rate, indicating significant tissue hypoperfusion / hypoxia of internal organs [34]. Changes in sublingual vascular perfusion were monitored in parallel using OCT-A (Biomedtech Ltd., Russia) [27] and LDF (LAZMA Ltd., Russia) [35] (probes' placements is demonstrated in Fig.2(b)) before modeling blood loss (time point "baseline"), 5 min after the completion of blood loss and the onset of HS ("5 min HS"), 30 min after the onset of HS ("30 min HS"), and immediately after animal euthanasia (via intra-arterial administration of a lidocaine solution) following the 30-min monitoring period ("post mortem").

Visual analysis of the OCT-A images revealed a sharp decrease in capillary blood flow while vascular architecture remained relatively preserved by the 5 min HS (Fig. 2(c)). By 30 min HS, some increase in signal intensity of blood vessels was observed. This small increase in sublingual perfusion can be explained by two factors: a simultaneous increase in mean arterial pressure (MAP) due to sympathetic activation, increased myocardial contractility and hemodilution (a compensatory response to bleeding); and microvascular vasodilation due to persistent metabolic acidosis [36]. In contrast, following animal euthanasia, a complete cessation of blood flow was observed in the majority of vessels.

Quantitative analysis of the PVD and MI parameters (by OCT-A) and the index of blood microcirculation (I_m; by LDF) revealed statistically significant differences ($p < 0.05$) between the time points "5 min HS" and "baseline" (Fig. 2(d)). Among the evaluated indices, only MI detected the visually observed change in microcirculation between the 5- and 30-min HS time points (85.01 ± 6.23 vs 90.09 ± 3.60, $p < 0.05$), whereas PVD (4.49 ± 0.34 vs $4.57 \pm 0.39\%$, $p = 0.44$) and I_m (9.96 ± 1.90 vs 11.42 ± 2.25 perfusion units, $p = 0.46$) did not differ significantly. These changes are consistent with the statistically significant ($p < 0.05$) increase in MAP (33.50 ± 4.05 vs 48.58 ± 1.81 mmHg) and the decrease in arterial blood lactate concentration (1.98 ± 0.31 vs 1.65 ± 0.36 mmol/L), confirming the development of HS and tissue hypoxia (Fig. 2(e)). Similar trends in sublingual microcirculatory changes have been demonstrated using vital microscopy in critically ill patients with HS who developed MOF or mortality [37]. Finally, the results obtained at this experimental stage, on one hand, directly demonstrate the development of microhemodynamic disturbances in sublingual area during acute blood loss and HS; on the other hand, they establish the efficacy of OCT-A with MI estimation for monitoring sublingual microcirculation changes during the progression of critical conditions.

In the next phase, we evaluated the capability of OCT-A for monitoring changes of human sublingual microcirculation in healthy volunteers ($n = 9$) receiving GTN sublingually (to reduce drug burden and adverse effects half of the therapeutic dose was used). The OCT-probe was firmly fixed to the assessed tissue using a vacuum attachment [26], which prevented displacement of the scanning area during prolonged monitoring (Fig. 3(a)). The predictable pharmacokinetics of GTN (therapeutic vasodilatory effect following a full sublingual dose occurs within 2–4 min, with a mean duration of approximately half an hour [38]) enabled the design of an OCT-A monitoring protocol comprising measurements before GTN administration ("baseline"), and at 5 and 20 min after GTN application ("5 and 20 min post-GTN"). Parallel blood pressure measurements confirmed a decrease in systolic arterial pressure (SAP) at 5 min following GTN administration (111 ± 5 vs 122 ± 8 mmHg, $p < 0.05$), whereas by 20 min (118 ± 4 mmHg) no statistically significant differences compared to "baseline" were observed (Fig. 3(b)).

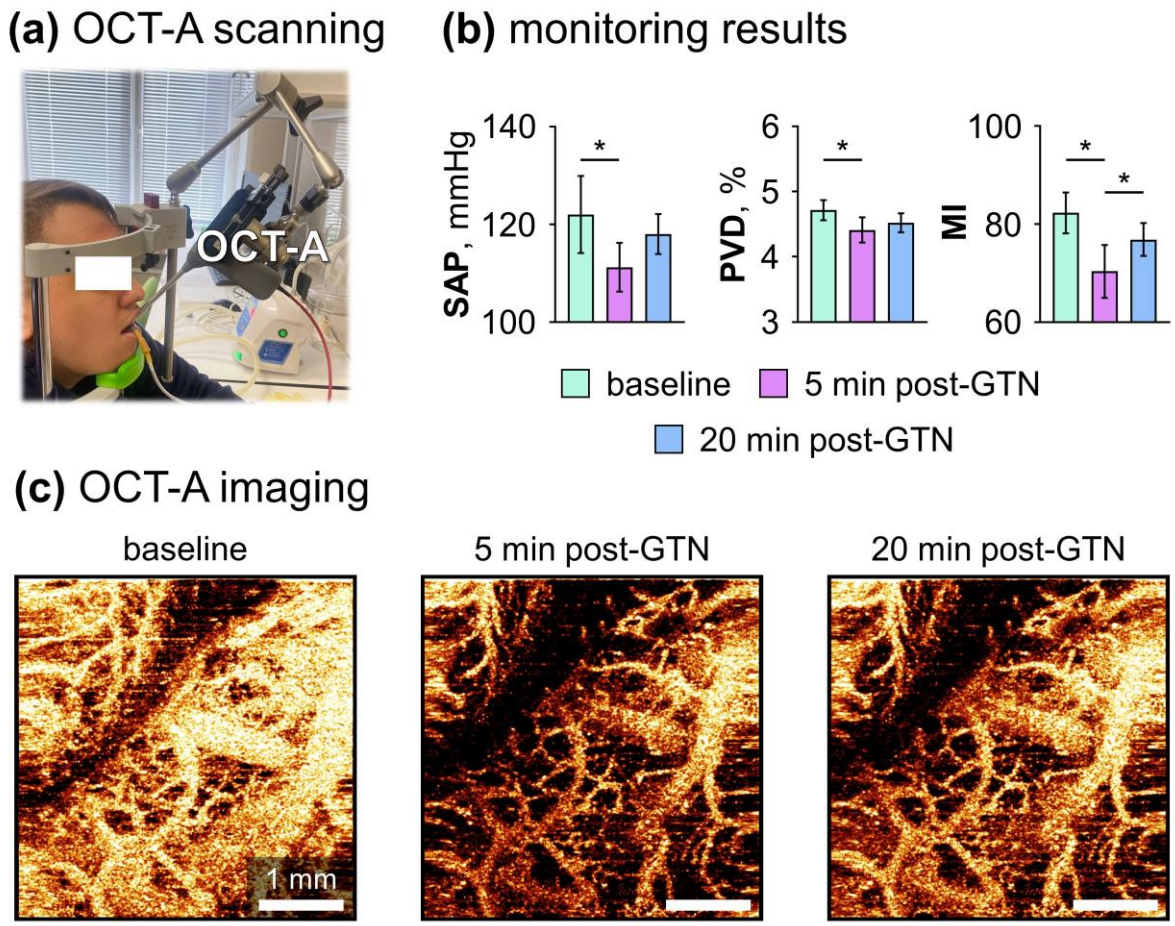

Fig. 3 Changes in human sublingual microcirculation following local GTN administration: (a) demonstration of sublingual OCT-A monitoring of volunteer; (b) diagrams of the obtained values (mean + SD) before GTN administration (baseline), by 5 and 20 min after GTN application (5 and 20 min post-GNT) for SAP, PVD and MI; * – statistical significance ($p < 0.05$) between the monitoring time points, Repeated Measures one-way ANOVA test; (c) representative OCT-A images; scale bar size is indicated on images.

Visual and quantitative (by PVD and MI) analyses of the OCT-A data (Fig. 3(b–c)) demonstrated a statistically significant ($p < 0.05$) decrease in sublingual microcirculation 5 min after GTN application (4.41 ± 0.19 vs $4.71 \pm 0.16\%$; 70.34 ± 5.38 vs 82.24 ± 4.17). Furthermore, the use of intensity analysis and calculation of the MI parameter enables statistically significant confirmation of the restoration of vascular perfusion in the sublingual region when comparing the 5- and 20-min post-GTN time points (70.34 ± 5.38 vs 76.83 ± 3.35, $p < 0.05$). These results confirm the feasibility of longitudinal OCT-A monitoring of microcirculatory changes in the human sublingual region and underscore the need for further development of a novel approach to the analysis of OCT-A data (distinct from the widely used PVD estimation) for assessing physiological (hemodynamic) alterations in vascular network, which are frequently encountered in critically ill patients. This could provide a clear basis for assessing the effectiveness of sublingual microcirculation monitoring in critically ill patients, both for predicting complications and for guiding appropriate adjustments to critical care treatment [39].

It is only fair to note that the sole limitation of OCT-A compared to vital microscopy techniques is essentially the inability to assess the proportion of non-perfused and perfused vessels among their total number. Nevertheless, it should not be overlooked that vital microscopy methods (not yet entered into widespread clinical practice) remain susceptible to artifacts caused by microscope pressure on the tissue or displacement of the scanning area, require precise focusing and the involvement of a skilled operator to ensure correct image acquisition, and often necessitate labor-intensive and time-consuming manual processing of the recorded data [7–9]. In contrast, OCT-A at its current stage of development is substantially less dependent on the above-mentioned factors. Among the advantages of OCT-A are the mitigation of motion artifacts through the use of a vacuum attachment and real-time visual control of acquired images (with the ability to quickly reject and re-collect data), a straightforward and intuitive software interface, the absence of a need for focusing, and the capability for immediate interpretation and assessment of the obtained OCT-A data.

It is also fair to say that future studies of sublingual microcirculation will need to explore how to utilize or modify the OCT-A parameters used (possibly in combination with other detectable features) to achieve sufficient diagnostic power for practical clinical applications. Addressing the diagnostic aspect would require subsequent work dedicated to identifying the most informative combination of OCT-A and clinical parameters capable of high-accuracy state classification in critical care medicine. The development of such methods could involve machine learning and other advanced techniques, followed by rigorous evaluation of their performance metrics (e.g., ROC, AUROC, accuracy, sensitivity, and specificity).

In conclusion, considering recent advances in the development of OCT-A approaches for assessing tissue microcirculation and the results presented in this Letter, there is no doubt regarding the relevance and promise of OCT-A technology for addressing the challenges of early detection of tissue hypoperfusion in critically ill patients and for identifying predictive criteria of MOF development to monitor the effectiveness of ongoing intensive care.

Disclosures

The authors declare no conflict of interest.

Data Availability

Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

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