Research Article | Vol. 10, No. 8 | 1 Aug 2019 | BIOMEDICAL OPTICS EXPRESS 3974
Biomedical Optics EXPRESS

Optical assessment of alterations of microrheologic and microcirculation parameters in cardiovascular diseases

ANDREI E. LUGOVTSOV,[1,2,*] YURY I. GURFINKEL,[3] PETR B. ERMOLINSKIY,[1] ANASTASIYA I. MASLYANITSINA,[1] LARISA I. DYACHUK,[3] AND ALEXANDER V. PRIEZZHEV[1,2]

[1]*Department of Physics of Lomonosov Moscow State University, 1-2 Leninskie Gory, Moscow, 119991, Russia*
[2]*International Laser Center of Lomonosov Moscow State University, 1-62 Leninskie Gory, Moscow, 119991, Russia*
[3]*Medical Research and Education Center of Lomonosov Moscow State University, 27-10 Lomonosovsky pr-t, Moscow, 119991, Russia*
**anlug@biomedphotonics.ru*

Abstract: In this work, we compare the blood aggregation parameters measured in vitro by laser aggregometry and optical trapping techniques in blood samples with the parameters of blood rheology measured in vivo by digital capillaroscopy in the nail bed capillaries of patients suffering from the hypertension and coronary heart disease. We show that the alterations of the parameters measured in vivo and in vitro for patients with different stages of these diseases are interrelated. Good agreement between the results obtained with different techniques, and their applicability for the diagnostics of abnormalities of rheological properties of blood are demonstrated.

1. Introduction

A typical adult human body comprises more than 100 billion blood vessels. More than 99% of these vessels, microvessels and capillaries, comprise the microcirculatory network [1]. This network operates with numerous control and compensatory mechanisms aimed at pathological changes occurring in this complex system. The earliest detection of microcirculation disorders is the best way to prevent possible complications of cardiovascular diseases and thus provide a well-timed start of treatment. An illustration of this thesis could be the result of our study on the early detection of impaired microcirculation in patients with prehypertension [2]. Based on perennial investigations [3] Mchedlishvili concluded that the blood movement in capillaries is primarily dependent on the "structure" of the flowing flux that determines the resistance to blood advancement in the microvessels rather than on the well-known hydrodynamic relationships characteristic of the larger blood vessels.

In human body, the processes of red blood cells (RBC) aggregation and disaggregation are in a state of relative equilibrium. However, mechanisms for restoring the equilibrium after alterations become not sufficiently effective with age. This is especially pronounced for patients suffering from arterial hypertension (AH) and coronary heart disease (CHD). Different physiological factors are involved in functional and structural alterations of microcirculation in AH. Activation of the renin-angiotensin-aldosterone system, enhanced growth of the smooth muscle cells of the media, remodeling of the extracellular matrix, and elevated collagen and fibronectin deposition accompany the development of this disease [4]. Hypertension significantly influences the microcirculatory vascular bed, restructures it and deteriorates its functioning [5]. Detection of early signs of microcirculatory changes in AH allows for new approach to diagnosis and treatment of hypertensive patients, and as a result, reduces the risk of possible complications [6,7].

#365039 https://doi.org/10.1364/BOE.10.003974
Journal © 2019 Received 15 Apr 2019; revised 10 Jun 2019; accepted 11 Jun 2019; published 15 Jul 2019

Biomedical Optics EXPRESS

Much of the research in clinical and experimental cardiology focuses on the problems associated with myocardial contractility and perfusion deterioration due to lesions of the coronary arteries. However, quite recently, an increasing interest of researchers was directed to rheologic and microcirculatory disorders in heart diseases. This is due to the growing awareness that the delivery of oxygen and nutrients to the heart tissue mainly depends on the coronary microcirculation and rheological properties of blood [8–10].

The study of RBC aggregation parameters is also important for modern cardiology because in the last decade, high-performance oral anticoagulants have appeared in the arsenal of cardiological drugs that have a powerful effect on the coagulation properties of blood [11]. Along with antiplatelet agents, these drugs, in addition to their benefits, have a high risk of inducing bleeding. Administration of the antiplatelet and anticoagulants agents into blood is based on general assumptions about the average doses of the drug, which often do not fully comply with the requirements of the personalized medicine. As a rule, monitoring the effectiveness of the treatment carried out is not sufficient, especially when using the so-called New Oral Anticoagulants (NOAC), for which the necessary laboratory tests have not been developed yet. However, overdose and uncontrolled intake of anticoagulants and antiplatelet agents can cause serious hemorrhagic complications [12,13]. On the other hand, insufficiently effective antiplatelet therapy leads to complications of different kinds, associated with the risk of increased thrombosis.

One of the techniques for in vivo studying the rheological properties of blood is digital capillaroscopy – an optical in vivo microscopy technique allowing for direct visualization of the superficial skin microvessels and tissue surrounding them. Original image-processing software provides non-invasive quantitative assessment of static and dynamic parameters of microcirculation [14].

It also allows for estimating the density of the capillary network, the degree of perivascular tissues oedema, the diameters of the capillary sections. In addition, this technique provides an opportunity to assess the capillary blood velocity, as well as visualize the presence of blood aggregates. The RBC aggregates formation usually occurs against the background of the capillary blood flow velocity reduction and stasis [15,16].

To measure the aggregation parameters on a large number of RBCs the diffuse light scattering method based on registration and subsequent analysis of intensity of light scattered from the layer of whole blood is used. This method is implemented in several commercial systems: laser-assisted optical rotational cell analyzer LORCA (Mechatronics, Netherlands) [17], laser aggregometer and red blood cell deformometer LADE (Rheomedlab, Russia) [18,19], and fully automatic erythrocyte aggregometer FAEA (Myrenne, Germany) [20]. During the last two decades, a new device RheoScan (RheoMeditech, Seoul, Korea) for studying RBC aggregation in whole blood samples in vitro was developed [21]. Another optical approach for studying heart failure severity in humans suggested was based on imaging of the viable epidermis around the nailfold capillaries [22]. To measure RBC aggregation on the level of individual cells laser tweezers (laser traps) are used [23].

The aim of this study is to analyze the relationships of the results obtained in vivo with capillaroscopy and in vitro with RBC aggregometry and laser tweezers in order to assess the pathologic alterations of the microrheologic properties of blood in patients with such wide-spread cardiovascular diseases as arterial hypertension (AH) and established coronary heart disease (CHD).

2. Materials and methods

In this work, we used three optical techniques for assessing microrheologic and microcirculatory parameters of blood: laser aggregometry, optical trapping and manipulation of individual red cells, and vital digital capillaroscopy.

In vitro measurements of microrheologic properties of blood that characterize the aggregation of erythrocytes were carried out on large ensembles of cells. Measurements were

Biomedical Optics EXPRESS

performed on whole blood samples (macrolevel), and also on the singe cell interaction level (cellular microlevel) in highly diluted suspension of RBCs in blood plasma. Detection of diffuse light scattering from a layer of whole blood (the method of laser aggregometry) allows us to get the parameters of RBC aggregation that characterize the average time of aggregate formation as well as determine the relative amount of aggregated cells. Using the method of optical trapping, we can manipulate individual cells without mechanical contact, measure the forces of RBC interaction during their aggregation and the aggregation time. In vivo visualization of blood microcirculation with digital capillaroscopy technique allows us to determine visually the presence of blood aggregates and stasis in blood flow, and calculate the capillary blood velocity (CBV).

Comparison of the results obtained using these three in vitro and in vivo techniques can clarify the effects of microrheological parameters on capillary blood flow, and also shed light on the possibility of using in vitro measurements to characterize native blood microcirculation.

2.1 Laser aggregometry technique

In order to assess the aggregation parameters on whole blood samples we used laser aggregometer RheoScan which utilizes the method of diffuse laser light scattering (laser aggregometry) [19,21].

Disposable microcuvettes that were used during the experiment consist of a small flat reservoir for whole blood with a thickness of 300 μm and diameter of 5 mm. Inside the reservoir there is a thin magnetic stirrer bar that can rotate when an electrical field is present inside the microcuvette thus stirring its contents. Whole blood samples of 8 μl were administered into the reservoir using a micro-doser for each measurement.

The process of measurement begins after the microcuvette is put inside the device. It records the time dependence of the intensity of laser light (wavelength of 633 nm) scattered forward by the blood sample, regarded as the RBC aggregation kinetics (Fig. 1). First, the bar rotates at a high speed, thus creating an external shear stress that destroys all the aggregates that have already appeared in the sample. Then, the rotation is abruptly stopped (time t = 0) and the process of spontaneous aggregation of erythrocytes at rest (without any external shear stress) in our sample begins to take place. Laser beam shines on the blood reservoir and is scattered by the individual cells and the newly forming aggregates. After some time the intensity of light scattered in forward direction increases because of the increase of the average size of the scattering centers due to the formation of aggregates. This process of spontaneous aggregation is accomplished after approximately 2 minutes when the scattered light intensity reaches its maximum value indicating that almost all the cells have aggregated (Fig. 1).

Most of aggregation parameters measured with the techniques based on light scattering are significantly influenced by the hematocrit of the blood samples due to the dependence of scattered light intensity on the volumetric concentration of scattering centers – red blood cells [21]. In this work, all measurements by laser aggregometry were implemented in the RheoScan device and were performed on whole blood samples after normalizing the hematocrit to the value of 40% by autologous blood plasma.

Biomedical Optics EXPRESS

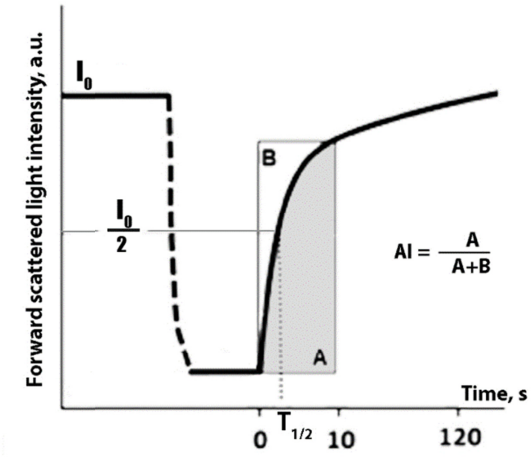

Fig. 1. The kinetics of spontaneous aggregation of RBCs recorded by RheoScan. I_0 is the intensity of light scattered forward by the layer of whole blood in the state with maximum aggregation of erythrocytes; $T_{1/2}$ is the characteristic time of aggregates formation. AI is the aggregation index.

Using the aggregation kinetics, we can determine the characteristic time of aggregate formation in seconds (the time it takes for the intensity of light scattered forward to reach half of the maximum level). Also, we can calculate the aggregation index (AI) that describes the number of cells that have aggregated in 10 seconds. This parameter is defined as the ratio of the area under the kinetics curve to the total area under and above the curve for the first 10 seconds of spontaneous aggregation.

2.2 Optical trapping and manipulation

Two-channeled system of optical trapping was used to measure the interaction forces of two single cells (aggregation forces of a pair of cells) and the time of complete aggregation for two cells (aggregation time). The layout of the experimental setup is provided in Fig. 2.

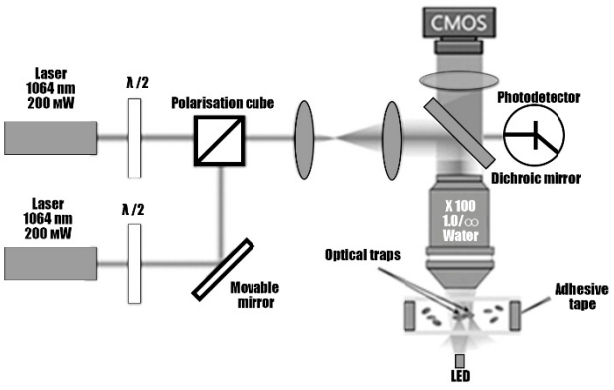

Fig. 2. The schematics of the two-channeled system of optical trapping. The calibration of the setup was performed in flow conditions by comparing the optical trapping force with the viscous friction force (see Visualization 1).

Two Nd:YAG lasers (1064 nm, 200 mW each) were used. The position of the lasers, beam-splitting cube and the lens system were all aligned with high precision in order to get

Biomedical Optics EXPRESS

the needed intensity gradient in the focal area of the beams (the positions of laser traps are shown with arrows in Fig. 2). Upon passing the lens system the beam is split by the dichroic mirror, the larger part of the intensity being directed to the aperture of the camera lens Olympus (x100, N.A. = 1, water immersion), while the smaller part – to the photodetector to measure its power. The power of each beam can be changed using half-wave plates that are connected to electric motors with small steps of rotation. One beam is always stationary, while the second one can be moved by rotatable mirror. This way we are using 2 areas of trapping: one is stationary, one can move inside our sample. A lens and CMOS-camera are installed vertically and are illuminated by white light from under the sample.

The cuvette is placed on a motorized platform. It consists of an object and a cover glass plates with the distance between them equal to 100 μm created by double layer of adhesive tape on the sides. The suspension of erythrocytes is diluted in plasma (1:1000 proportion) and is placed between the glass plates using a micro-doser. After that it is sealed off by Vaseline to prevent unwanted currents and evaporation.

Before any measurements can take place, a calibration procedure must be performed to find one-to-one relation between the trapping force F_{trap} and the beam power. It was made by matching F_{trap} with the force of viscous friction acting on the trapped cell when the platform was moved with a controlled speed relative to the trap. Calibration procedure is described in details in [24]. The maximum trapping force achieved was 15 pN with the laser power being 30 mW in the focal area. The aggregation force F_A is defined as the trapping force F_{trap} which is minimally sufficient for preventing the two cells from overlapping during the aggregation process.

The procedure of measuring F_A and aggregation time consisted of several steps (Fig. 3).

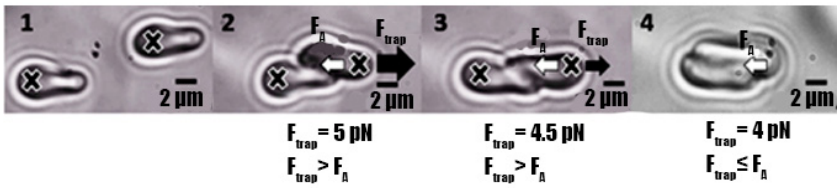

Fig. 3. Photographs illustrating step by step the method of RBC aggregation force measurement. Crosses indicate the positions of the traps, white and black arrows point in the direction of the forces applied (F_A is white, F_{trap} is black). Interaction of two single RBCs results in formation of a doublet aggregate (see Visualization 2 and Visualization 3).

During the first step two RBCs were trapped with the beams and were aligned parallel to each other by moving the platform. So, one cell was trapped by the stationary beam, the other one by the movable trap. In the second step the erythrocytes were moved closer together until their interaction area reaches approximately 40% of the area of each cell. In the final step the movable beam power was slowly decreased until the trapping force (black arrow in Fig. 3) was no longer sufficient to resist the spontaneous aggregation force (white arrow) between the two cells. After that the cell can escape the trap and complete the aggregation (due to $F_{trap} \leq F_A$). At this moment the laser power is recorded and F_A is calculated. Also, the aggregation time needed for full overlapping of the cells is measured.

For each sample the measurements of F_A and aggregation time were carried out on at least 15 pairs of cells. The values were averaged to get the final results. All experiments with optical traps were conducted at room temperature in a temperature-controlled room.

2.3 Vital digital capillaroscopy (VDC)

Following a minimum 15 minutes seated rest, the in vivo microvascular measurements were conducted between 9 and 11 am in a quiet temperature-controlled room (the temperature was maintained between 22 and 23.5 °C), with the subject in the seated position and the left hand at the heart level. All participants were required to refrain from smoking and caffeinated

Biomedical Optics EXPRESS

drinks one day before the examination. Capillary blood flow velocity (CBV) was measured in the eponychium of the fourth or third finger of the left hand.

Skin temperature was measured at the dorsal middle phalangeal area of the tested finger of the left hand by medical precision thermometry; the mean skin temperature was 33.2 ± 1.7 °C with no significant differences in the studied group.

Nail fold capillaries were visualized using the digital capillaroscope Kapillaroskan-1 (AET, Russia) equipped with high speed CCD-camera (1/3'' monochrome progressive scan IT CCD sensor, resolution 640 x 480 px, frame rate 200 fps full frame), TM-6740GE (JAI, Japan). The nail bed illumination was achieved with a LED-based illuminating system. Two ranges of the total magnification (125x) and (400x) were used to visualize the nail bed capillaries. 125x magnification was used for obtaining the panoramic images of the capillaries, while more detailed imaging of single capillaries was performed at 400x total magnification and included the measurement of static parameters (capillary length and diameters in different parts) and CBV in different parts of the capillary. We also recorded the presence or absence of blood aggregates in the capillaries (Fig. 4) from the analysis of obtained digital microscopy images and video movies.

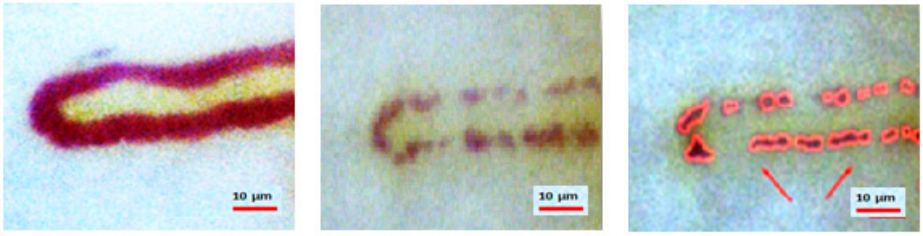

Fig. 4. Visualization of RBCs in the capillary bed: normal laminar capillary blood flow with absence of RBC aggregates – Aggregates = 0 (left image); capillary blood flow with presence of RBC aggregates – Aggregates = 1 (central image); results of image processing for detection of aggregates (red contours) in the capillary bed (right image).

For determining of the CBV after recording the video fragment the program stabilizes the dynamic images of the capillaries and then processes the images in the specified region of interest in the offline regime. The tracks of specific spots (RBCs) differ in the level of light intensity. The program marks them and then recognizes in the next frame. The program determines the average velocity along the axis of the capillary over 5 seconds long time intervals (500 frames). The CBV is estimated in 6 capillaries and the results are averaged. We estimated CBV only in the capillaries of the first line, where the capillaries are located within one layer. Thus, the obtained values of CBV are not affected by the movement of blood in the vessels lying above and below the investigated capillary. Detailed procedure of CVB measurements is described in [25]. Usually, at rest in healthy people not taking caffeine-containing substances and drinks on the eve of the study, the average CBV varies in the range from 800 to 1500 µm/s.

In this paper, we make a decision about the two states of the presence or absence of RBC aggregates in the capillaries based on visual processing of the nail bed images and videos obtained with VDC technique in vivo. The criteria of distinguishing RBC aggregates presence in the capillary blood flow can be formulated as follows:

1. Blood aggregates are clusters of blood cells that form autonomous conglomerates separated by plasma gaps.
2. Conglomerates do not merge when moving along the capillary bed.
3. The appearance of aggregates in most cases is accompanied by slowing down of the capillary blood velocity.

Biomedical Optics EXPRESS

Thereby, the determination of the presence of RBC aggregates in the capillary blood flow was performed by visual assessment of nail bed capillaries images and videos according to the scale: the absence of aggregates in the capillaries — 0, the presence of aggregates in the capillaries — 1. In case of visual detection of at least several distinguished clusters of RBC in the flow we considered the state as Aggregates = 1, otherwise the Aggregates state was indicated by 0 (Fig. 4).

In this work, registration the CBV dependence on time was used to reveal the presence of a stasis, as a sign of a pronounced deterioration in the rheological properties of blood. The minimum duration of the blood flow halting in the capillary, which in our study is classified as stasis, is 0.25 sec. Generally, the duration of stasis can reach several seconds.

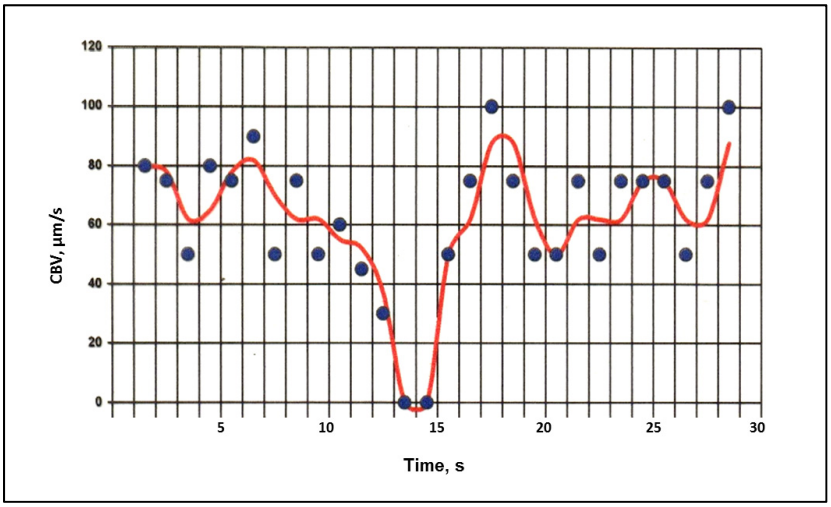

Fig. 5. Time dependence of CBV measured in vivo by VDC for a patient with CHD.

Figure 5 demonstrates the dependence of CBV on time during 30 seconds of measurements in a patient with CHD. In the center of the graph, one can see a drop in CBV to zero. The duration of this stasis was 1.16 sec. In our study, the presence of a stasis in the capillaries was assessed as "1" if CBV remained about zero more than 0.25 sec in at least one visualized capillary; otherwise the absence of a stasis was evaluated as "0". Accordingly, the higher CBV, the lower probability of the stasis.

2.4 Blood samples

All in vitro measurements were carried out using samples of whole human blood and erythrocyte suspensions. In our experiments, we have taken into account the latest recommendations for hemorheligical laboratories made by an international expert team to standardize hemorheological methods [26]. The samples were kept at 37°C during the experiment and were used only during the first 3 hours after blood draw from the cubital veins of donors on an empty stomach. All blood samples were collected into containers (Vacuette EDTA K3E tube, 1.8 mg/ 1 ml of blood, total volume 4.5 ml) with EDTA as an anti-coagulant agent.

Laser aggregometry measurements was performed with whole blood. A highly diluted erythrocyte suspension was used for measuring the time and force of aggregation by the laser tweezers. The dilution was made with autologous poor platelets plasma with the final erythrocytes concentration of about 0.1%. Plasma was acquired by centrifuging in a following manner: firstly, centrifuging for 10 minutes at 170g, then centrifuging twice for 10 minutes at 3000g.

Biomedical Optics EXPRESS

2.5 Experimental groups and statistics

Overall, 88 patients with arterial hypertension were enrolled in the study. They were divided into two groups. First group enrolled only patients with hypertension without cardiovascular complications (n = 48) and second group included patients (n = 40) with established coronary heart disease (CHD). Comparison of clinical backgrounds between hypertensive patients with and without coronary heart disease (CHD) is presented in Table 1.

The average age of all patients was 62.8 ranging from 24 to 87 years, 51.7% of them were males. In the group of hypertensive patients without CHD, females prevailed - 28 persons (58.4%). In the group of patients with CHD males prevailed (63.4%). The body mass index (BMI) for the groups was significantly higher than normal value, but not statistically significantly different. The mean age was also higher in the group of patients with CHD. The left ventricle ejection fraction in the group with CHD was statistically lower in comparison with group of hypertensive patients without CHD.

Table 1. Comparison of clinical backgrounds between patients with and without coronary heart disease (CHD)

	Overall (n = 88) (%)	Hypertensive patient without CHD (n = 48) (%)	Hypertensive patients with CHD (n = 40) (%)
Male	46 (51.7%)	20 (41.6%)	26 (63.4%)
Age. years (range)	62.8 (24-87)	60.4 (24-83)	67.7 (52-87)
BMI (kg/m2)	29.7 ± 5.4	29.5 ± 5.3	29.9 ± 5.6
Current smokers	9 (10.1%)	4 (8.3%)	5 (12.5%)
Systolic BP (mmHg), Office blood pressure	143.0 ± 26.8	147.7 ± 31.5	138.1 ± 32.9
Diastolic BP (mmHg), Office blood pressure	85.9 ± 16.0	88.3 ± 18.5	83.3 ± 19.9
Heart rate (bpm)	73.2 ± 18.5	76.3 ± 23.4	69.8 ± 16.6
LV ejection fraction (%)	59.0 ± 7.2	59.5 ± 4.9	56.9 ± 8.9
Previous myocardial infarction	13 (14.6%)	-	13 (32.5%)
Angina pectoris	32 (35.9%)	-	32 (80%)
Bypass grafts	4 (4.5%)	-	4 (10%)
Stents	7 (7.9%)	-	7 (17.5%)
Diabetes mellitus	17 (19.1%)	7 (14.6%)	10 (25%)
Aspirin	42 (47.2%)	21 (43.8%)	21 (52.5%)
Clopidogrel	3 (3.4%)	0	3 (7.5%)
Anticoagulants	10 (11.2%)	0	10 (25%)
Diuretics	48 (53.9%)	25 (52%)	23 (57.5%)

Standard Student T-test was used to analyze statistical difference between sets of values measured from compared groups. The difference was considered statistically significant when $p < 0.1$.

The study was approved by the ethics committees of the Medical Research and Education Center of Lomonosov Moscow State University. All donors were informed on the purpose of the study and agreed to participate in this study.

3. Results and discussion

Blood samples from every patient were investigated by methods of laser aggregometry and optical trapping. Microrheologic parameters of RBC aggregation (aggregation index, characteristic time of aggregate formation, aggregation force and time) were measured in vitro. Before blood was drawn from the patients all of them underwent the microcirculation measurements in vivo by means of vital digital capillaroscopy (VDC) technique.

Biomedical Optics EXPRESS

To compare in vitro and in vivo techniques and assess the consistency of these methods we presented the measured microrheologic parameters for both experimental groups of patients (AH and CHD) in the terms of subgroups depending on the results of measurements of the native capillary blood flow with VDC. Three parameters were obtained by VDC that is why 3 types of subgroups where considered: (1) by distinguishing in visual detection of the absence (aggregates = 0) or presence (aggregates = 1) of aggregates in the capillaries; (2) by distinguishing in CBV with values lower or higher than 800 μm/s; (3) by distinguishing in visual detection of stasis absence (stasis = 0) or presence (stasis = 1). The results for experimental groups distinguished by mentioned subgroups are shown in diagrams (Fig. 6-9) where the mean values of measured parameters, standard deviation with error bars, and statistical significance (*$p < 0.05$ and **$p < 0.1$) are indicated on the diagrams.

Left diagram in Fig. 6 demonstrates that the number of aggregated RBCs in the blood sample — aggregation index AI, measured with RheoScan was significantly increased ($p = 0.04$) in patients with hypertension in the presence of blood aggregates detected using VDC. For patients with CHD, significant differences were also obtained for this parameter ($p = 0.09$). Measurements of the aggregation index (AI) in the presence and absence of the capillary blood flow stasis did not reveal significant differences for patients with AH ($p = 0.12$), while for patients with CHD statistically significant differences were found ($p = 0.04$). Aggregation index measured with RheoScan for individuals with characteristic capillary blood flow velocity below and higher than 800 μm/s measured by VDC, was significantly different in patients with AH ($p = 0.02$) and CHD ($p = 0.096$). Thus, the tendency is that larger numbers of aggregates detected with RheoScan, were accompanied with reduced CBV, determined by the VDC (Fig. 6).

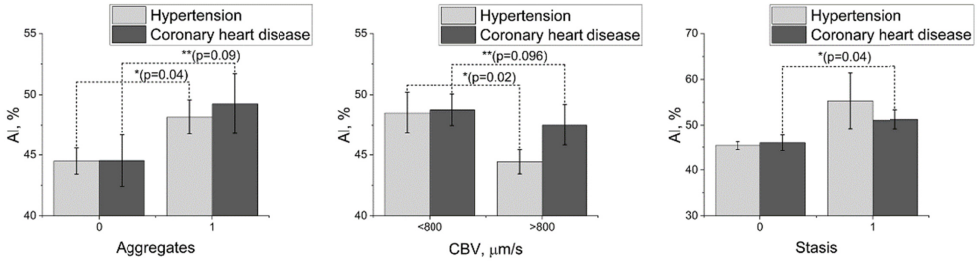

Fig. 6. Aggregation index (AI) measured in vitro by laser aggregometry technique for groups of patients with AH and CHD depending on the presence or absence of aggregates in the capillaries, capillary blood velocity and presence or absence of stasis in blood flow detected by VDC.

First diagram in Fig. 7 represents a diagram showing the presence or absence of aggregates detected by vital digital capillaroscopy (VDC) of the nail bed and the parameter $T_{1/2}$, characterizing the average or characteristic time of aggregates formation in a whole blood sample obtained using diffuse light scattering with the aggregometer ReoScan. In patients with AH, the characteristic aggregation time measured in vitro is significantly reduced ($p = 0.03$) in the case of presence of aggregates in vivo (more aggregates - less time for their formation). For patients with CHD, there were no significant alterations of this parameter. The characteristic time $T_{1/2}$ of aggregation measured with RheoScan is inversely proportional to the average rate of aggregates formation. For groups of patients with AH ($p = 0.01$) and CHD ($p = 0.097$), significant differences in $T_{1/2}$ parameter were found for individuals with CBV higher than 800 μm/s measured by VDC (Fig. 7). Measurements of $T_{1/2}$ parameter revealed significant differences in groups without and with the capillary blood flow stasis (right part of Fig. 7) both for patients with AH ($p = 0.08$), and for patients with CHD ($p = 0.02$).

Biomedical Optics EXPRESS

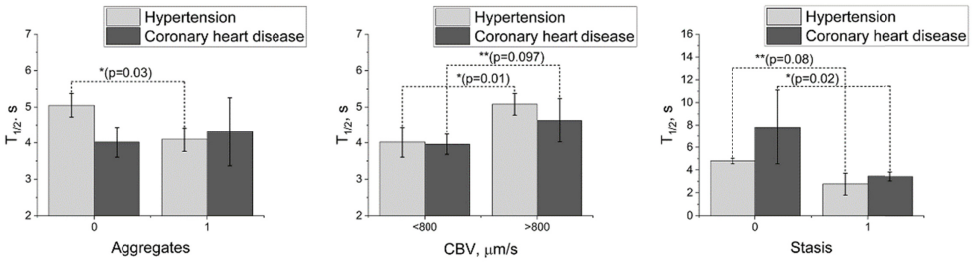

Fig. 7. Characteristic time of aggregates formation measured in vitro by laser aggregometry technique for groups of patients with AH and CHD depending on presence or absence of aggregates in the capillaries, capillary blood velocity and presence or absence of stasis in blood flow detected by VDC.

Figure 8 relates the time of aggregation of a pair of cells measured with the optical trap (OT) to the absence or presence of blood aggregates detected by the VDC in patients with AH and patients with CHD. The former parameter is significantly reduced in the hypertensive patients ($p = 0.008$) and patients with CHD ($p = 0.01$) in the presence of blood aggregates detected by the VDC. Measurements conducted with OT on individual RBCs showed significant differences in aggregation time for patients with AH ($p = 0.006$) and CHD ($p = 0.06$) in relation to the CBV parameter obtained by the VDC. That is, the higher the CBV, the slower the formation of aggregates. In the case of absence and presence of stasis (Fig. 8, right image), the aggregation time measured on individual RBC with OT revealed no significant differences for the group of hypertensive patients, whereas for the group of patients with CHD, statistically significant differences were obtained ($p = 0.03$). These results support the reasonable assumption that the slowing down of blood flow leads to the formation of blood aggregates, and the presence of aggregates in the capillary bed may lead to the appearance of stasis.

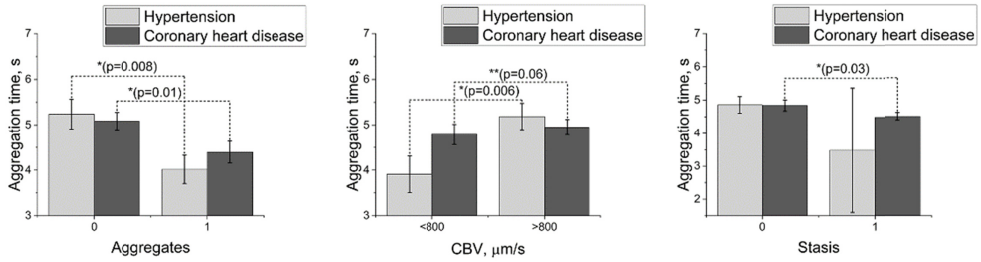

Fig. 8. Aggregation time measured in vitro by optical trapping for groups of patients with AH and CHD depending on presence or absence of aggregates in the capillaries, capillary blood velocity and presence or absence of stasis in blood flow detected by VDC.

Figure 9 shows the aggregation forces for experimental groups and subgroups. When measuring the aggregation force with the OT and relating it with the microcirculation indices obtained by the VDC we found no significant differences between the values for the groups with AH and CHD, in case of absence of blood aggregates. There are no indicated significant differences between the aggregation force and CBV for hypertensive patients and patients with CHD. No significant differences for the forces were obtained in relation with the parameter of stasis measured by VDC.

Biomedical Optics EXPRESS

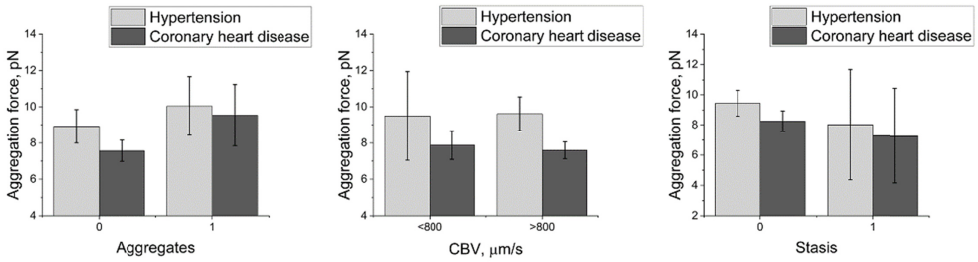

Fig. 9. Aggregation forces measured in vitro by optical trapping for groups of patients with AH and CHD depending on presence or absence of aggregates in the capillaries, capillary blood velocity and presence or absence of stasis in blood flow detected by VDC.

The performed study has demonstrated that there is a high level of statistically significant differences of information about the rheological properties of blood in the capillary bed that can be obtained with the vital digital capillaroscopy method in vivo with the information that can be obtained with the selected optical techniques, i.e. laser scattering aggregometry and optical tweezers, in vitro. The obtained statistical results of comparative studies of blood rheological properties using three different methods allow us to conclude about the applicability of these methods in clinical practice in particular when treating patients with cardiovascular diseases, arterial hypertension and coronary heart disease. For example, our previous results of comprehensive studies with optical trapping and laser aggregometry techniques performed in [27,28] confirm the hypothesis of enhanced RBC aggregation and impairment of blood flow in patients with hypertension and CHD in comparison with normal value.

Blood rheology largely depends on the aggregation state of blood components, in particular, red blood cells and platelets. This work is focused on the characterization of the aggregation properties the former cells. It is known that the activation and aggregation state of the platelets affects blood rheology not only directly by raising blood viscosity and reducing its fluidity but also indirectly influencing the aggregation of RBC. However, this effect is out of focus of this work.

The need to evaluate the rheological properties of blood has increased significantly in recent years due to the widespread use of new oral anticoagulants and powerful antiplatelet agents acting on both platelets and RBC. This use is aimed at the prevention of reocclusion of the coronary arteries because of stenting and bypass operations. With an insufficient dose of drugs, RBC aggregates continue to circulate in the blood, which greatly reduces the supply of tissues with oxygen and other important substances.

Another important aspect characterizing the capillary blood flow is the interaction of blood cells with the vascular wall. It should be remembered that capillaries are living tubes consisting of endothelial cells, endotheliocytes, that in addition to enabling the blood transport, play the role of a kind of paracrine organ, since nitrogen monoxide, endothelin-1 and other vasoactive substances directly and indirectly affect blood aggregation properties.

RBCs interact with endotheliocytes, glycocalyx and blood plasma, containing a large set of various chemical elements circulating in the bloodstream. Most of these factors are absent when conducting measurements with an optical trap and with the RheoScan aggregometer. In addition, substances-stabilizers like EDTA are added to the blood sample in order to prevent blood from coagulating during the test.

Definitely, one of the advantages of the VDC method is its non-invasiveness, the absence of any unpleasant sensations in patients during the study. Among the limitations of this method is the lack of ability to detect *in vivo* the interaction of individual blood cells in the flow and to reveal the strength of RBC aggregates, as we do with an optical trap or RheoScan aggregometer. However in future, combining VDC with other types of laser microscopies and, possibly, with optical tweezers it may become possible to overcome these limitations.

Biomedical Optics EXPRESS

4. Conclusion

In this work, a series of in vitro measurements of RBC aggregation parameters with blood samples obtained from the patients suffering from hypertension and coronary heart disease were performed. Aggregation index and characteristic time of aggregates formation were measured by laser aggregometry technique in whole blood samples comprising large populations of RBCs. In addition, forces and time of aggregation on individual cells level were obtained with an optical trap. The capillary blood velocity, presence or absence of RBC aggregates and stasis in blood flow were assessed in vivo with vital digital capillaroscopy. The relationships between the parameters measured in vitro demonstrate good agreement of the results for the patients distinguished into subgroups in accordance with VDC data obtained in vivo. In particular, impairing of capillary blood flow in case of AH or CHD leads to unconditional deterioration of aggregation parameters of blood cell.

Our results also clearly demonstrate the possibility of using laser aggregometry and optical trapping for estimating the alterations of microrheologic and, consequently, microcirculation parameters. Alterations of RBC aggregation parameters measured in vitro can be used to evaluate the alterations of vital capillary blood flow parameters in human body.

Funding

Russian Science Foundation (18-15-00422); Russian Foundation for Basic Research (17-02-01200).

Acknowledgments

Russian Science Foundation (18-15-00422) grant supported the preparation of blood samples and measurements; Russian Foundation for Basic Research (17-02-01200) grant supported the collection of medical background information about patients and its statistical analysis.

The authors thank Ms Alexandra Pigurenko for technical assistance in data processing and analysis.

Disclosures

The authors declare that there are no conflicts of interest related to this article.

References

1. G. W. Schmid-Schonbein, “What is the relevance of the microcirculation in cardiovascular disease?” Microcirc. Cardiovascular Disease **2000**, 1–13 (2000).
2. Y. I. Gurfinkel, M. L. Sasonko, and A. V. Priezzhev, “Digital capillaroscopy as important tool for early diagnostics of arterial hypertension,” Proc. SPIE **9448**, 117 (2014).
3. G. Mchedlishvili, “Basic factors determining the hemorheological disorders in the microcirculation,” Clin. Hemorheol. Microcirc. **30**(3-4), 179–180 (2004).
4. E. Agabiti-Rosei, “Structural and functional changes of the microcirculation in hypertension: influence of pharmacological therapy,” Drugs **63**(1), 19–29 (2003).
5. B. I. Levy, G. Ambrosio, A. R. Pries, and H. A. Struijker-Boudier, “Microcirculation in hypertension: a new target for treatment?” Circulation **104**(6), 735–740 (2001).
6. M. Roustit and J. L. Cracowski, “Non-invasive assessment of skin microvascular function in humans: an insight into methods,” Microcirculation **19**(1), 47–64 (2012).
7. C. De Ciuceis, E. Porteri, D. Rizzoni, N. Rizzardi, S. Paiardi, G. E. Boari, M. Miclini, F. Zani, M. L. Muiesan, F. Donato, M. Salvetti, M. Castellano, G. A. Tiberio, S. M. Giulini, and E. Agabiti Rosei, “Structural alterations of subcutaneous small-resistance arteries may predict major cardiovascular events in patients with hypertension,” Am. J. Hypertens. **20**(8), 846–852 (2007).
8. G. D. Lowe, A. J. Lee, A. Rumley, J. F. Price, and F. G. Fowkes, “Blood viscosity and risk of cardiovascular events: the edinburgh artery study,” Br. J. Haematol. **96**(1), 168–173 (1997).
9. K. Rakusan, “Microcirculation in the heart during cardiovascular disease,” Microcirc. Cardiovascular Disease **2000**, 103–116 (2000).

Biomedical Optics EXPRESS

10. I. Tikhomirova, E. Petrochenko, A. Muravyov, Y. Malysheva, A. Petrochenko, V. Yakusevich, and A. Oslyakova, "Microcirculation and blood rheology abnormalities in chronic heart failure," Clin. Hemorheol. Microcirc. **65**(4), 383–391 (2017).
11. L. Staerk, T. A. Gerds, G. Y. H. Lip, B. Ozenne, A. N. Bonde, M. Lamberts, E. L. Fosbøl, C. Torp-Pedersen, G. H. Gislason, and J. B. Olesen, "Standard and reduced doses of dabigatran, rivaroxaban and apixaban for stroke prevention in atrial fibrillation: a nationwide cohort study," J. Intern. Med. **283**(1), 45–55 (2018).
12. T. Nantsupawat, S. Soontrapa, N. Nantsupawat, D. Sotello, S. Klomjit, S. Adabag, and A. Perez-Verdia, "Risk factors and prevention of dabigatran-related gastrointestinal bleeding in patients with atrial fibrillation," J. Arrhythm. **34**(1), 30–35 (2018).
13. H. Ullrich and T. Gori, "Antiplatelet therapies in patients with an indication for anticoagulation," Clin. Hemorheol. Microcirc. **64**(3), 273–278 (2017).
14. Y. I. Gurfinkel, A. V. Priezzhev, M. L. Sasonko, and M. I. Kuznetzov, "Importance of image processing in digital optical capillaroscopy for early diagnostics of arterial hypertension," BioPhotonics, 2015 International Conference Proceeding, Florence, Italy, 20–22, 1–4 (2015).
15. A. Bollinger and B. Fagrell, *Clinical Capillaroscopy: A Guide to its use in Clinical Research and Practice* (Hogrefe & Huber, 1990), pp. 31–52.
16. B. Fagrell and M. Intaglietta, "Microcirculation: its significance in clinical and molecular medicine," J. Intern. Med. **241**(5), 349–362 (1997).
17. M. R. Hardeman, J. G. G. Dobbe, and C. Ince, "The Laser-assisted Optical Rotational Cell Analyzer (LORCA) as red blood cell aggregometer," Clin. Hemorheol. Microcirc. **25**(1), 1–11 (2001).
18. A. V. Priezzhev, O. M. Ryaboshapka, N. N. Firsov, and I. V. Sirko, "Aggregation and disaggregation of erythrocytes in whole blood: study by backscattering technique," J. Biomed. Opt. **4**(1), 76–84 (1999).
19. A. Priezzhev, K. Lee, N. Firsov, and J. Lademann, "Optical study of RBC aggregation in whole blood samples and single cells," Chapter 1 in *Handbook on Optical Biomedical Diagnostics*, V.V. Tuchin, ed. (SPIE Press, 2016), pp. 5–36.
20. H. Kiesewetter, H. Radtke, R. Schneider, K. Mussler, A. Scheffler, and H. Schmid-Schönbein, "[The mini-erythrocyte aggregometer: a new apparatus for the rapid quantification of the extent of erythrocyte aggregation]," Biomed. Tech. (Berl.) **27**(9), 209–213 (1982).
21. S. Shin, Y. Yang, and J. S. Suh, "Measurement of erythrocyte aggregation in a microchip stirring system by light transmission," Clin. Hemorheol. Microcirc. **41**(3), 197–207 (2009).
22. E. A. Shirshin, Y. I. Gurfinkel, S. T. Matskeplishvili, M. L. Sasonko, N. P. Omelyanenko, B. P. Yakimov, J. Lademann, and M. E. Darvin, "In vivo optical imaging of the viable epidermis around the nailfold capillaries for the assessment of heart failure severity in humans," J. Biophotonics **11**(9), e201800066 (2018).
23. K. Lee, M. Kinnunen, M. D. Khokhlova, E. V. Lyubin, A. V. Priezzhev, I. Meglinski, and A. A. Fedyanin, "Optical tweezers study of red blood cell aggregation and disaggregation in plasma and protein solutions," J. Biomed. Opt. **21**(3), 035001 (2016).
24. A. Y. Maklygin, A. V. Priezzhev, A. V. Karmenyan, S. Y. Nikitin, I. S. Obolenskii, A. E. Lugovtsov, and K. Lee, "Measurement of interaction forces between red blood cells in aggregates by optical tweezers," Quantum Electron. **42**(6), 500–504 (2012).
25. Y. I. Gurfinkel, A. V. Priezzhev, M. L. Sasonko, and M. I. Kuznetzov, "Importance of image processing in digital optical capillaroscopy for early diagnostics of arterial hypertension," BioPhotonics, 2015 International Conference Proceeding, Florence, Italy, 20–22, 1–4 (2015).
26. O. K. Baskurt, M. Boynard, G. C. Cokelet, P. Connes, B. M. Cooke, S. Forconi, F. Liao, M. R. Hardeman, F. Jung, H. J. Meiselman, G. Nash, N. Nemeth, B. Neu, B. Sandhagen, S. Shin, G. Thurston, and J. L. Wautier; International Expert Panel for Standardization of Hemorheological Methods, "New guidelines for hemorheological laboratory techniques," Clin. Hemorheol. Microcirc. **42**(2), 75–97 (2009).
27. P. B. Ermolinskiy, A. E. Lugovtsov, A. I. Maslyanitsina, A. N. Semenov, L. I. Dyachuk, and A. V. Priezzhev, "Interaction of erythrocytes in the process of pair aggregation in blood samples from patients with arterial hypertension and healthy donors: measurements with laser tweezers," J. of Biomedical Photonics & Eng. **4**(3), 030303 (2018).
28. P. Ermolinskiy, A. Lugovtsov, A. Maslyanitsina, A. Semenov, L. Dyachuk, and A. Priezzhev, "In vitro assessment of microrheological properties of erythrocytes in norm and pathology with optical methods," Ser. Biomech. **32**(3), 20–25 (2018).