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PRACA POGLĄDOWA
REVIEW

Blood rheological properties and methods of their measurement

Właściwości reologiczne krwi oraz metody ich pomiaru

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ABSTRACT

From the physical point of view, blood is a multi-phase and multi-component system; plasma is the dispersion medium and the morphotic elements are the dispersed phase. Flow rate analysis is essential to determine the correct rheological properties of blood. Slow blood flow can lead to increased erythrocyte aggregation, which is due to fibrinogen and globulins. The deformability of red blood cells is also important, especially during flow through the capillaries, where they must adapt to the smaller diameter of the vessels.

Viscosity is defined as the internal resistance to flow; if we consider blood as a component of two parallel layers, then viscosity is described by the friction of two adjacent layers. The liquid layers move at different velocities parallel to each other, and a velocity gradient is created. To create it, a force is needed to move the layers, which is referred to as shear stress.

Erythrocyte aggregates are observed physiologically as well as in the course of some diseases such as ischemic heart disease, myocardial infarction and atherosclerosis. There are two types of factors inducing the formation of aggregates: the external factors include the plasma protein concentration, hematocrit and shear forces; the internal factors are the shape and deformability of the erythrocytes in addition to the properties of the cell membrane. Also in hyperfibrinogenemia, erythrocyte aggregation, plasma viscosity and microvascular resistance increase.

The laser-assisted optical rotational cell analyzer (LORCA) is used to test the deformability and aggregation of erythrocytes. It combines the techniques of syllectometry with ektacytometry. The formation of a three-dimensional structure of red blood cells has a significant impact on the measurement of blood viscosity and low shear rate blood flow.

KEYWORDS

rheology, blood viscosity, erythrocyte aggregation, erythrocyte deformability

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STRESZCZENIE

Krew, z fizycznego punktu widzenia, to układ wielofazowy oraz wieloskładnikowy; osocze jest fazą zwartą, a elementy morfotyczne są fazą rozproszoną. Aby określić prawidłowe właściwości reologiczne konieczna jest analiza prędkości przepływu. Podczas wolnego przepływu może dochodzić do wzrostu agregacji krwinek, za co odpowiadają fibrynogen oraz globuliny. Ważna jest także odkształcalność krwinek czerwonych, szczególnie podczas przepływu przez naczynia włosowate, gdzie muszą się dostosować do mniejszej średnicy naczyń.
Lepkość określa się jako wewnętrzny opór przepływu; jeśli będziemy rozpatrywać krew jako składową dwóch warstw, które są równoległe, to lepkość opisywana jest przez tarcie dwóch sąsiadujących ze sobą warstw. Warstwy cieczy przemieszczają się z różną prędkością równolegle względem siebie i powstaje gradient prędkości (szybkość ścinania). Aby go wytworzyć, potrzebna jest siła poruszająca warstwy, określana jako naprężenie ścinające.
Agregaty z krwinek są obserwowane zarówno fizjologicznie, jak i w przebiegu niektórych chorób, takich jak choroba niedokrwienna serca, zawał mięśnia sercowego i miażdżyca. Wyróżnia się dwa rodzaje czynników sprzyjających tworzeniu się agregatów: zewnętrzne – stężenie białek osocza, hematokryt oraz siły ścinania, i wewnętrzne, które stanowią kształt i odkształcalność erytrocytów oraz właściwości błony komórkowej. Przy hiperfibrynogenemii wzrasta agregacja erytrocytów, lepkość osocza i opór mikronaczyniowy.
Laserowo-optyczny rotacyjny analizator krwinek czerwonych (*laser-assisted optical rotational cell analyzer* – LORCA) służy do badań odkształcalności oraz agregacji erytrocytów. Łączy techniki sylektometrii z ektacytometrią. Tworzenie się trójwymiarowej struktury krwinek czerwonych ma istotny wpływ na pomiar lepkości krwi oraz przepływu krwi przy niskiej prędkości ścinania.

SŁOWA KLUCZOWE

reologia, lepkość krwi, agregacja erytrocytów, odkształcalność erytrocytów

Introduction

Blood rheology mainly regards the viscosity of blood and its flow through blood vessels [1]. Blood viscosity depends on the plasma viscosity, hematocrit, and the rheological properties of erythrocytes (their aggregation and elongation) [2,3,4] constituting about 99% of the blood components [5], while leukocytes and platelets, due to their lower number, do not affect blood fluidity [6]. Plasma is a fluid whose viscosity is not dependent on the shear rate; it is conditioned by the presence of proteins: immunoglobulins, lipoproteins, and fibrinogen [7,8]. Under physiological conditions, changes in blood viscosity directly affect the endothelium, which then regulates blood viscosity and blood vessel diameter by releasing vasoactive factors [9].
From the physical point of view, blood is a multi-phase and multi-component system; plasma is the liquid phase (a diluted solution of electrolytes, proteins, and lipids) and the morphotic elements are the dispersed phase (maximum content about 50%) [10]. Whole blood is considered a non-Newtonian fluid with a variable viscosity [1]. To determine the correct rheological properties, it is necessary to analyze the flow velocity [11], which is used in such methods as atomic force microscope (AFM) analysis, Raman spectroscopy and the laser-assisted optical rotational cell analyzer (LORCA) [6,7].
During slow flow, erythrocyte aggregation may increase, which is due to fibrinogen and globulins [5]. The deformability of red blood cells is also important, especially during flow through capillaries, where they must adapt to the smaller diameter of the vessels [3]. Deformability is associated with adenosine triphosphate (ATP)-dependent metabolism, which ensures the transport of ions in the cell, adenosine triphosphate deficiency leads to an increase in the intracellular calcium concentration (structural changes occur in the membranes), sodium (an increase in the blood cell volume) and erythrocyte deformability disorders [5,12]. Changes in blood viscosity depend on the shear rate and hematocrit value; at low hematocrit levels the curve is relatively flat (Figure 1) [11].

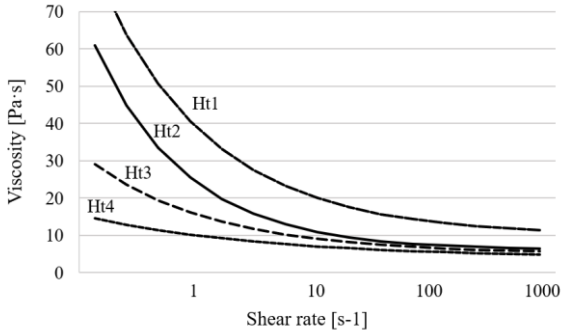

Fig.1. Dependence of blood viscosity on shear rate at different hematocrit levels (Ht4 < Ht3 < Ht2 < Ht1) (adapted from ref. [11] with permission of the Via Medica).
Ryc. 1. Zależność lepkości krwi od szybkości ścinania przy różnych poziomach hematokrytu (Ht4 < Ht3 < Ht2 < Ht1) (zaadaptowano z ref. [11] za zgodą Via Medica).

Red blood cells

In 1 mm^3 of blood, the number of red blood cells in men is on average 5 million (range: 4.3–5.8) and in women 4.5 million (range: 3.9–5.1) [13]. During hematopoiesis in humans, approximately 10^{10} red blood cells are produced per hour, and their production is tightly controlled by macrophages. Erythrocytes have a biconcave shape with a diameter of 6–8 μm, a thickness of ~2 μm, a volume of ~90 fl, and an area

of ~136 μm^2 [10,14]. The age of red blood cells is up to 120 days and they are eliminated by macrophages residing in the spleen and liver [15]. A loss of membrane deformability may result in the initial entrapment of old or damaged erythrocytes in the spleen, which precedes their degradation by macrophages [16].

Red blood cells are distinguished by specific mechanical properties: mechanical and osmotic resistance as well as deformability. Erythrocytes have the ability to elongate and return to their original shape because their cytoplasm regains its original shape and after the cessation of forces acting on it, also by storing elastic energy in the erythrocyte membrane. The intracellular concentration of hemoglobin affects the viscosity of the cytoplasm, which gives red blood cells viscoelastic properties. The deformability of erythrocytes also depends on the mechanical properties of the cell membrane (its cytoskeleton), the size and geometry of microvessels [10]. When the deformability of red blood cells changes, the viscosity of the blood changes. Increased erythrocyte aggregation causes an increase in blood viscosity [3], especially when shear forces are low. In contrast, at high shear forces, the higher blood viscosity results from the reduced deformability of erythrocytes [14,17], which is an indicator of perfusion disorders, capillary occlusion [18] and ischemia; it can also lead to hypoxia of the organs and impair their physiological functions [1,6].

Blood viscosity

In 1929–1931, Fahraeus and Lindqvist demonstrated that dynamic blood viscosity is reduced by the axial accumulation of erythrocytes in the bloodstream. The plasma is located at the periphery of the bloodstream, while the red blood cells accumulate axially in its center. In capillaries less than 300 μm in diameter, blood viscosity decreases as the diameter of the vessels decreases. Below a certain diameter, the opposite effect is observed, meaning that the viscosity increases again as the diameter of the vessel decreases [19]. Viscosity is defined as the internal resistance to flow; if we consider blood as a component of two layers that are parallel, then viscosity is described by the friction of two adjacent layers [5,6]. Blood flow in the vessels is largely laminar (excluding heart valves and pathological vasoconstriction), thus viscosity is crucial in relation to vascular resistance. The tangential force to any layer of flowing liquid can be described by the formula [11,20]:

$$F_s = \eta A \frac{\Delta v}{\Delta x} \quad (1)$$

where:

F_s – force that acts tangentially to a layer of area A [N]; η – dynamic viscosity coefficient [Pa·s]; A – surface area of a given liquid layer [m^2]; Δv – velocity difference between the top and bottom layer [m/s]; Δx – difference in the distance between the layers [m].

The liquid layers move at different velocities parallel to each other, and a velocity gradient (shear rate) is created. To create it, a force is needed to move the layers, which is referred to as shear stress. Hence, viscosity (η) is the ratio of shear stress (τ) to the shear rate (γ) [1,4]:

$$\eta = \frac{\tau}{\gamma} \quad (2)$$

In the middle part, the friction is the lowest (the parabolic flow profile), thanks to which the layers move faster than those located at the wall of the vessel, where the friction is greatest. The higher the velocity gradient of the layer (this is the result of the interaction of the friction force, which is directed in the opposite direction to the blood flow) and the viscosity coefficient of the liquid, the higher the shear stress [6,11,21].

The volume of liquid flowing out of a cylindrical tube laid horizontally is determined by Poiseuille's law; it is directly proportional to the pressure difference and the radius of the vessel, and inversely proportional to the viscosity coefficient and the length of the vessel [4,7,11,22]:

$$Q = \frac{\Delta p \pi r^4}{8 l \eta} \quad (3)$$

where:

Q – volume flow rate [m^3/s]; Δp – pressure difference [Pa]; r – vessel radius [m]; l – vessel length [m]; η – dynamic viscosity coefficient [Pa · s].

The above law applies to blood that flows through vessels with a diameter greater than 1 mm; for vessels with a smaller diameter, the structure of the blood and changes during flow must be taken into account. The blood viscosity coefficient at 20°C is 0.23–0.28 P and is 3 times higher than the viscosity of water. At 37°C, the viscosity of blood is 2.5 times lower than at 0°C [11] owing to the increased deformability of red blood cells [5]. It also depends on macro-rheological factors such as the plasma viscosity and hematocrit in addition to micro-rheological factors, which include the deformability and aggregation of red blood cells [23].

Red blood cell aggregation

Aggregation is a reversible, natural physiological process that consists of the formation of aggregates or rouleaux [18], whose dispersion and formation depend on shear forces [8,24]. Erythrocyte aggregates are observed both physiologically and in the course of some diseases [25]. There are two types of factors inducing the formation of aggregates. The external factors include the plasma protein concentration (lipoproteins, fibrinogen, immunoglobulins, macroglobulins), hematocrit, and shear forces. The

internal ones are the shape and deformability of the erythrocytes and the properties of the cell membrane [3,5]. Large blood proteins or their characteristic configuration (macroglobulins, immunoglobulins, fibrinogen) adhere to the surface of erythrocytes, which is negatively polarized. When this linkage is strong enough, it can lead to adjacent red blood cells sticking together and forming a chain, causing increased aggregation of red blood cells [1,6].

Tissue hypoxia and acidosis favor the formation of erythrocyte aggregates. An increase in the mass of red blood cells, and a decrease in the plasma volume causes an increase in the hematocrit, resulting in an increase in blood viscosity [1]. High levels of fibrinogen also lead to the increased aggregation of red blood cells, increased resistance in microcirculation, and plasma viscosity [3,17]. Rheological disorders of the blood manifest themselves mainly in microvessels. Aggregation is one of the most important factors contributing to a decrease in flow velocity and even stopping it, despite maintaining the pressure gradient [6].

Fibrinogen

Fibrinogen, a high molecular weight protein (340,000 Daltons) [1] synthesized by fibroblasts and hepatocytes, determines complex hemorheological properties. It is mainly found in plasma (with an average concentration of 2.0–4.5 g/l). In hyperfibrinogenemia, erythrocyte aggregation, plasma viscosity, and microvascular resistance grow. In hypertension, stroke, myocardial infarction, coronary artery disease, and ischemia, increased aggregation can be also seen [26], which is closely related to the level of fibrinogen [5,18]. In the 1950s, fibrinogen was associated with cardiovascular disease, in the 1980s it was recognized as a risk factor for coronary heart disease [27], and in the 1990s as a predictor of cardiovascular disease [28]. The risk of coronary artery disease rises with the level of fibrinogen, which is also related to calcification and the severity of changes in coronary arteries. High levels of fibrinogen raise the risk of myocardial infarction and death [23,29].

The factors associated with an increase in the fibrinogen concentration and disorders of hemorrhagic properties include diabetes, age, male sex, obesity, smoking, menopause, microalbuminuria, polymetabolic syndrome, and lipid disorders [30]. The above-mentioned risk factors are accompanied by endothelial dysfunction [3,31]. In addition, the blood viscosity rises, increasing the time of blood stagnation, atherogenic particles are deposited, which penetrate the inner membrane, and consequently, atherosclerotic plaques are formed [32]. Factors that reduce the level of fibrinogen and improve the rheological properties of blood include statins, fibrates, allopurinol, heparin, acetylsalicylic acid, and exercise [23].

Plasma viscosity increases with renal failure, and proteinuria may contribute to the development of nephropathy. The loss of blood proteins in urine is accompanied by an increase in fibrinogen concentration. Increased plasma viscosity accompanies pathological conditions conducive to atherogenesis, such as visceral obesity, polymetabolic syndrome, diabetes [24], hypercholesterolemia [33,34], and hypertension [3,5,23].

Atherosclerotic changes in the coronary arteries, carotid arteries and lower limbs are correlated with hemorrhagic disorders. Complications after myocardial infarction, cardiogenic shock and circulatory failure are more often seen in patients with high levels of C-reactive protein (CRP is an acute-phase protein) and fibrinogen [29]. In hypertension, left ventricular hypertrophy is positively correlated with the fibrinogen concentration as well as plasma and blood viscosity. In diabetes, high plasma viscosity is associated with the risk of developing retinopathy [35], diabetic nephropathy, and microangiopathy [23,24,26,27].

Physiological factors affecting the rheological properties of blood

In the elderly, an increment in fibrinogen levels, increased blood viscosity and a decrease in erythrocyte deformability may be observed, which contribute to a drop in blood flow velocity and to the development of cardiovascular diseases [34,36]. In addition, there may be increased oxidative stress, disturbances in the activity of antioxidant enzymes in erythrocytes [26], including a decrease in the activity of superoxide dismutase and catalase as well as a rise in the concentration of malondialdehyde (MDA), which is a marker of lipid peroxidation [5,6]. In people leading a sedentary lifestyle who had undertaken regular physical training, a decrease in blood viscosity and improvement in the elastic properties of erythrocytes can be observed, which reduces the risk of cardiovascular complications [3,22,30,37,38].

Blood rheology testing is important for athletes. Physical effort shortens the lifespan of red blood cells because it leads to increased hemolysis; old red blood cells are replaced with new erythrocytes, which more efficiently transport oxygen to the working muscles. Long-term, regular training causes a decrease in fibrinogen levels, which is closely associated with a reduction in erythrocyte aggregation. This has a positive effect on the cardiovascular system and results in a rise in the elongation index of red blood cells [3,39].

After intense physical exercise (which depends on the mode, intensity, duration of exercise, and physical fitness), there is an increase in blood viscosity, a decline in the deformability of red blood cells, an increment in hematocrit in addition to erythrocyte aggregation [22,40]. Eating meals after exercise leads to growth in

the stiffness of the red blood cell membrane, and thus to a further decrease in their deformability. The position of the body during exercise can also change the hemorheological properties. A decrease in plasma volume occurs when changing the position from sitting to standing; body dehydration also contributes to hemoconcentration [2,41]. Prolonged dehydration increases the stiffness of the cell membrane and its destabilization, thus it is important to hydrate the body during and after exercise to reduce excessive blood viscosity [6].

The increased aggregation of erythrocytes and growth in blood viscosity also occur when the temperature in the limbs drops, which contributes to the formation of frostbite. Treatments using low temperature: cold water baths, and systemic cryotherapy affect the rheological properties of blood [42]. For example, a cold water bath could reduce plasma fibrinogen levels compared to the baseline. On the other hand, the use of whole-body cryotherapy leads to an increment in the elongation of erythrocytes and a decline in their aggregation [6,43].

There is a strong correlation between the lipid accumulation product (LAP) index, which is a marker of cardiovascular disease risk, and blood viscosity. A too high content of adipose tissue in the body (e.g. in the abdominal cavity) contributes to greater aggregation of erythrocytes and an increase in blood viscosity by raising the level of hematocrit. The hemorheological properties also change in conditions such as multiple sclerosis [43], ischemic stroke [18], sepsis, and hematological diseases [6].

Clinical significance

Changes in blood viscosity have been described in cardiovascular diseases such as myocardial infarction, ischemic heart disease, hyperlipoproteinemia, and atherosclerosis [32,35]. Circulatory system disorders and changes in the hemorrhagic parameters may occur [18,26,44]. Hyperlipoproteinemia may reduce the coronary flow reserve (increased coronary blood flow) due to elevated blood viscosity and could lead to a greater incidence of coronary artery disease. There is a positive correlation between blood viscosity and total cholesterol, triglycerides [25,34], low-density lipoproteins (LDLs) [45], uric acid, fibrinogen, interleukin-6, the von Willebrand factor, body mass index (BMI) and smoking. On the other hand, it is negative between the high-density lipoproteins fraction (HDLs, which reduce the aggregation of erythrocytes) and blood viscosity [5]. In addition, the BMI positively correlates with leukocytosis, the fibrinogen level, CRP, the erythrocyte sedimentation rate, and aggregation [23,29]. In people suffering from hypertension, a higher value of blood viscosity is observed, which is associated with blood vessel wall damage [3,11].

Diabetes negatively affects the structure of the erythrocyte cell membrane, which in people with diabetes reduces the deformability of erythrocytes, increases the stiffness of red blood cells and erythrocyte aggregation [2,26] as well as endothelial dysfunction [46], leading to disorders in the regulation of blood viscosity [5] and a rise in the hematocrit [47].

Microvascular angina is characterized by positive electrocardiographic stress tests, the absence of coronary stenosis (coronograph), and chest pain. Increased plasma and whole blood viscosity may be an important factor in the development of this disease. The pathomechanism of exercise pain and ischemic changes has not been explained, the causes of which include psychosomatic disorders, impaired coronary microcirculation, endothelial dysfunction [48], and sympathetic-parasympathetic balance disorder. Endothelial dysfunction can lead to an impaired viscosity-lowering mechanism; factors that may cause it include elevated levels of asymmetric dimethylarginine, nitric oxide synthase disorders, estrogen deficiency in women, elevated levels of the endogenous synthase inhibitor, nitric oxide, endothelin-1, and hyperinsulinemia [49]. Dysfunction is not limited to coronary circulation, it may also affect peripheral arteries, including the brachial artery [11].

Increased plasma viscosity may significantly reduce blood flow in small blood vessels [50] and it depends on the content of lipids and proteins [49]. Patients with the above-mentioned disease have an elevated level of fibrinogen, which increases the aggregation of erythrocytes at low flow velocity. Hyperlipidemia, a decrease in coronary flow reserve, and an increment in blood viscosity may be the cause of recurrent ischemic episodes. In a study of statin therapy, its beneficial effect on exercise test parameters, a decline in the concentration of CRP, total cholesterol, fibrinogen, triglycerides, apolipoprotein B, LDL, and also a drop in the number of chest pain episodes were shown. This may depend on the beneficial effect of statins on the endothelium, the reduction in blood viscosity, and the positive effect on microcirculation [11,45].

Blood rheological measurement methods

Among rheological measurements, we can distinguish measurements of the elongation index and erythrocyte aggregation. Measurements of erythrocyte elongation are carried out in devices called rheometers [7], which operate by rotating a cell suspension in polyvinylpyrrolidone (PVP). The solution with blood cells is introduced between the wall of the outer cylinder and the inner cylinder rotating in the middle. Then the deformability of the erythrocytes is recorded by passing a laser beam through the solution.

A computer program creates a graph, which illustrates the shear stress and calculates the elongation index (EI) [12,43,51]. In normal erythrocytes, the graph takes a curved shape, the line of stiffened erythrocytes is flattened (Figure 2) [52].

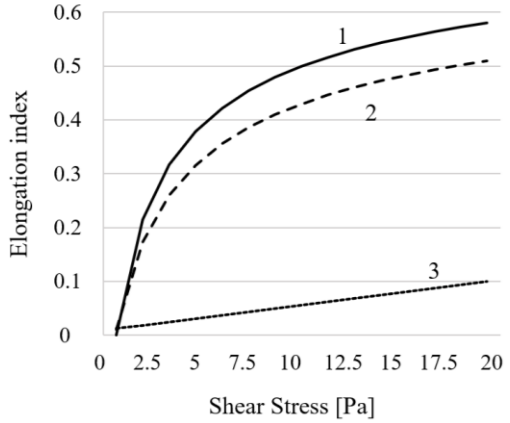

Fig. 2. Elongation index for red blood cells at various shear stresses on example of erythrocyte incubation in *tert*-butyl hydroperoxide (TBHP). The first line represents healthy red blood cells, the second line – cells treated with 0.5 mM TBHP, and the third line – cells treated with 0.75 mM TBHP. TBHP induces oxidative stress in erythrocyte cell membrane and increases its stiffness (adapted from ref. [52], figure's author – Gábor Závodszky).
Ryc. 2. Indeks elongacji dla czerwonych krwinek przy różnych naprężeniach ścinających na przykładzie inkubacji erytrocytów w wodoronadtlenku tert-butylu (TBHP). Linią numer 1 zaznaczono zdrowe czerwone krwinki, linią numer 2 krwinki traktowane 0,5 mM TBHP, linią numer 3 krwinki traktowane 0,75 mM TBHP. TBHP indukuje stres oksydacyjny w błonie komórkowej erytrocytów oraz wzrost jej sztywności (zaadaptowano z ref. [52], autor ryciny – Gábor Závodszky).

Evaluation of the rate of rouleaux formation and dispersion of erythrocytes in the blood can be performed by aggregometry [7], which is the measurement of the adherence of adjacent erythrocytes. The spontaneous aggregation of red blood cells in whole blood is a reversible physiological phenomenon that plays a significant role in blood flow at low shear rates and significantly increases blood viscosity. Places particularly susceptible to the formation of erythrocyte aggregates are small blood vessels where the shear rate is usually low. Ultimately, this leads to a decrease in blood flow velocity, which as a consequence leads to the hypoxia of cells and tissues [43]. Erythrocyte aggregation can also be measured by cytometry, which also allows one to study the interaction between different blood components [53].

Laser diffractometer

This method is utilized to assess the deformability of red blood cells. A diluted red blood cell suspension is sheared in a microchannel under continuously decreasing pressure ranging from 20 to 0 Pa. A direct laser beam passing through the sample is blocked, and the diffraction pattern created by the cells is recorded by a CCD camera for later analysis. The deformation of red blood cells under the shear stress generated in the microchannel causes elongation of the diffraction pattern in the direction perpendicular to cell stretching. The EI – defined as the ratio of the difference between the long and short axes to their sum [54], characterizes the ability of red blood cells to deform when passing through capillaries and thin blood vessels [55].

Computational fluid dynamics

Used for imaging flows in biomedical and biological systems, it can be employed to visualize the distribution of velocity vector fields, wall shear stress, the dynamic reactions of variable vessel geometry, and pressure distribution. Every practical application of computational fluid dynamics (CFD) involves three steps: pre--processing, processing, and post-processing. Pre--processing involves preparing the object for calculations and defining the geometric model of the investigated object (determining the characteristics of blood, in particular, its rheological properties). The data source may involve coronary angiography combined with intravascular ultrasound (IVUS) or computed tomography angiography (CTA). In CFD calculations, numerical grids are used; a properly constructed grid (which reflects the architecture of, for example, the arteries) is the basis for obtaining an optimal numerical simulation. Processing – this is the actual computational part in which the problem is solved numerically using a solver. The differential equations are selected and the methods of solving them. The basis of the CFD method is the Navier-Stokes transport equation [56]. In its general form, it is a differential equation containing the derivatives of functions of multiple variables; in special cases they have analytical solutions for which the flow variables (volume as a function of time) can be calculated. Post-processing – analysis of the received data and presentation of them in graphical form (charts, histograms, animations). If the simulation concerns pulsatile flow, the distribution of shear stresses in the cardiac cycle and the presentation of instantaneous flow fields are imaged [57].

Viscosity of the erythrocyte interior – Morse method

This method uses tempamine (as a spin label) in electron paramagnetic resonance (EPR). The viscosity of the cell interior is determined by comparing the movement of the spin label in the cell to its movement in water:

$$\eta = \frac{t_{c(\mathrm{PLASMA})}}{t_{c(\mathrm{H_2O})}} \eta_{\mathrm{H_2O}} \qquad (4)$$

where:
η – dynamic viscosity coefficient [Pa · s]; $t_{c(\mathrm{PLASMA})}$ – rotational correlation time for tempamine in plasma;

$t_{c(H2O)}$ – rotational correlation time for tempamine in water; η_{H2O} – water viscosity equal to 1 cP.
The value of the rotational correlation time, which reflects the motion of the spin label, is consistent with:

$$t_c = kw_0\left(\sqrt{\frac{h_0}{h_{-1}}} - 1\right) \quad (5)$$

where:
t_c – rotational correlation time; k – constant equal to 6.5 × 10^{-10} s/G; w_0 – width of the mid-line of the EPR spectrum; h_0 – height of the mid-line of the EPR spectrum; h_{-1} – height of the high-field line of the EPR spectrum [58,59].

Dielectric spectroscopy method

This method uses measurement of the electrical capacitance (C_L) of blood samples as a function of time [60]. At the beginning, the sample is pushed through the measurement chamber by applying pressure with a microsyringe (a needle with a diameter of approximately 0.27 mm). During the flow, erythrocytes separate due to shear stress. An appropriate value of blood flow intensity is forced to obtain a minimum value of electrical capacitance (the complete separation of erythrocytes in the sample). Then the blood flow is stopped, and a program is initiated to control the measurement of electrical capacitance (C_L) as a function of time and then it calculates the relative permittivity (ε_L) of the sample:

$$\varepsilon_L = \frac{k}{\varepsilon_0}(C_L - C_R) \quad (6)$$

where:
k – chamber constant (m^{-1}); ε_0 – vacuum permittivity ($\varepsilon_0 = 8.85 \times 10^{-12}$ F/m); C_L – electrical capacitance of the sample (F); C_R – electrical capacity of the wires that connect the measuring chamber with the analyzer (F).
After 250 seconds from stopping the blood flow through the measuring chamber, the measurement of electrical capacitance (C_L) ends because its value no longer changes [61].

Laser-assisted optical rotational cell analyzer

Constructed in the late 1990s, LORCA is used to test the deformability and aggregation of erythrocytes. It has a laser diode and a camera coupled with a computer system. It combines the techniques of syllectometry with ektacytometry (using laser diffraction viscometry to measure the deformability of red blood cells subjected to increasing shear stress or an osmotic gradient at a constant value of applied shear stress) [62]. The formation of a three-dimensional structure of red blood cells has a significant impact on the measurement of blood viscosity and low shear rate blood flow. The deformability of red blood cells is significant in their flow through capillaries with a diameter two times smaller than the diameter of erythrocytes [10]. The result of the deformation of red blood cells in large arteries (high flow velocity) is a decrease in the blood viscosity and a decrease in resistance. A slight increase in blood cell stiffness may cause disturbances in macro and microflows [63].

Red blood cell aggregation parameters

The blood sample is incubated and mixed with air (oxygenated) at a ratio of 1:3 (the volume of blood to the volume of air) for 15 minutes. 1.5 ml of blood is poured into the measuring chamber of the analyzer, which is a space between two transparent cylinders; the outer one rotates at a given shear rate, relative to the inner resting cylinder. The measurement is made when the chamber with the sample reaches a temperature of 37°C. The method is based on the phenomenon of laser light scattering by erythrocytes, the intensity of which depends on the level of red blood cell aggregation. The light source is a laser diode placed in the inner cylinder [51]. Backscattered light falls on 2 photodiodes, which are later analyzed by a computer. A curve of scattered light intensity versus time is created (Figure 3) [12,43,63,64,65,66].

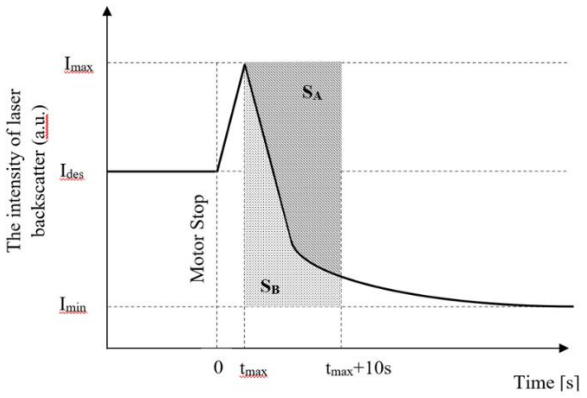

Fig. 3. Syllectogram – scattered light intensity versus time curve for red blood cells (adapted from ref. [63]).
Ryc. 3. Sylektogram – krzywa zależności natężenia światła rozproszonego od czasu dla krwinek czerwonych (zaadaptowano z ref. [63]).

Curve description:
1. Time t < 0 – blood sample subjected to initial shear at a certain velocity, which leads to the complete disaggregation of erythrocytes (their maximum deformation); the scattered light intensity is approximately constant (I_{des}).
2. Time equals 0 – the motor responsible for the rotation of the outer cylinder is turned off.
3. Interval 0 < t < t_{max} – the erythrocyte shear rate reaches 0 after time t_{max}; the blood cells return to their normal spherical shape; maximum light scatter value (I_{max}).
4. Interval t_{max} < t < (t_{max}+10 s) – partial reconstruction of blood cell aggregates; the intensity of scattered light decreases exponentially.

5. Time t > (t_{max}+10 s) – the maximum increase in erythrocyte aggregation; the light scattering takes a minimum value (I_{min}) [63,67].

The computer reads the aggregation parameters I_{des}, I_{max}, I_{min}, t_{max}. Then more complex quantities are calculated to compare blood cell aggregation:

1. A_{mp} (aggregation amplitude)= I_{max} - I_{min}.
2. $T_{1/2}$ – erythrocyte aggregation recovery rate time.
3. *AI* – aggregation index which represents a measure of aggregation intensity [43,64,65].

$$AI = \frac{S_A}{S_A+S_B}100\% \quad (7)$$

where:
S_A and S_B – surface on the syllectogram above and below the curve, the range of scattered light intensity over a time interval (t_{max} – t_{max} + 10 s) [12,63].

Measurement of erythrocyte deformability

The test sample is blood (25 µl) dissolved in 5 ml of a 0.14 mM PVP buffer solution, which is placed in the measuring chamber. Measurements are made at 37°C. The laser light passes through the layer of red blood cells, it is diffracted and gives a diffraction pattern on the screen. The shape of the image depends on the shear stress that acts on the erythrocyte during rotation. For low values, it takes the shape of a circle, and with increasing shear stress, the obtained image takes the shape of an ellipse. The camera records the diffraction image, and the program measures the lengths of the long (*a*) and short (*b*) axes and calculates the EI [12,51,63,68,69]:

$$EI = \frac{a-b}{a+b} \quad (8)$$

Conclusions

Rheological tests of blood can provide an abundance of valuable information on blood viscosity and data on blood flow in blood vessels, and it is also worth checking the level of hematocrit and fibrinogen. Their determination should be included in the routine examination panel in order to assess the risk of developing many diseases. In Table I it can be seen that elevated aggregation of red blood cells can occur in many diseases such as myocardial infarction, stroke, diabetes, ischemia, coronary artery disease, and hypertension. In addition, the formation of erythrocyte aggregates is favored by a high level of fibrinogen, adipose tissue, and intense physical exercise (at which the blood viscosity increases, while erythrocyte deformability decreases). Diabetes, dehydration, and older age contribute to the increased stiffness of red blood cell membranes. Factors that may reduce blood viscosity include a lower hematocrit, greater erythrocyte deformability, and a reduced fibrinogen concentration. The above-mentioned risk factors and disease entities testify to the great diagnostic possibilities of blood rheology measurements.

Table I. Factors and disease entities affecting increase or decrease in aggregation, elongation, and viscosity
Tabela I. Czynniki oraz jednostki chorobowe wpływające na wzrost bądź spadek agregacji, elongacji oraz lepkości

Blood	Erythrocyte aggregation [5,6,23,29,40,43]	Red blood cell deformability [6,43]	Blood viscosity [3,6,11,14,23,29]
Factors and diseases that increase a given parameter	Slow blood flow Increased globulin concentration Hyperfibrinogenemia Stroke Myocardial infarction Hypertension Ischemia Coronary artery disease Intense physical effort Drop in temperature in limbs High body fat content Diabetes	Whole-body cryotherapy Hydration of body during and after exercise	Increased erythrocyte aggregation Decrease in deformability of erythrocytes Intense physical effort Drop in temperature in the limbs High body fat content High hematocrit Older age Increased fibrinogen levels Microvascular angina
Factors and disease entities causing decrease in a given parameter	Whole-body cryotherapy Increased HDL levels	Intense physical effort Prolonged dehydration Diabetes Older age	Low hematocrit Increased erythrocyte deformability Increase in temperature in limbs Decrease in fibrinogen levels

HDL – high-density lipoprotein

Author's contribution

Study design – P. Dolibog
Data collection – P. Dolibog, T. Pryzwan
Manuscript preparation – T. Pryzwan, B. Pietrzyk, K. Kierszniok
Literature research – T. Pryzwan
Final approval of the version to be published – P. Dolibog

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