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Sumário / Summary

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Referência da capa: Vénula pós-capilar (diâmetro aproximado: 30 mm) de rede microvascular em mesentério de rato (*Rattus norvegicus*), observada por microscopia intravital de transiluminação. No interior do vaso sanguíneo visualizam-se leucócitos a interagir com a parede vascular. Imagem obtida por Henrique Sobral do Rosário (Instituto de Biopatologia Química – Prof.a Doutora Carlota Saldanha, Faculdade de Medicina de Lisboa; Unidade de Biopatologia Vascular, Instituto de Medicina Molecular)

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COMUNICAÇÃO CRUZADA/INTERLINKED INFORMATION

Conhecer as inter-conexões entre os sistemas que asseguram a reparação da célula endotelial vascular (coagulação/fibrinólise) e dos tecidos danificados ou destruídos (resposta inflamatória) por agentes infecciosos contribui para a interpretação das propriedades reológicas do fluxo sanguíneo. Estas estão intimamente associadas ao meio envolvente como, por exemplo, a qualidade e tipo estrutural do pavimento endotelial. Sendo a célula endotelial uma interface produtora de factores vasoactivos, pró e anti-inflamatórios, pró e anti-coagulantes, é de esperar que ocorra informação cruzada entre os sistemas reparadores/danificadores. Os factores intervenientes da coagulação interferem com a actividade das etapas da resposta inflamatória, e os agentes desta activam a coagulação e influenciam a construção dos depósitos de fibrina.

Aquando da ruptura da placa de ateroma, as células inflamatórias expõem o factor tecidual, dando início à via extrínseca da coagulação, em que a activação das plaquetas pela trombina, traduzida pela alteração de forma e exposição de glicoproteínas activadas (por ex. GPIIbIIIa, GPIb-V), ocasiona a formação de trombos (brancos) plaquetários. As glicoproteínas GPIb interagem com as integrinas αMβ2 (Mac-1) dos leucócitos e com o factor de vonWillebrand do sub-endotélio, enquanto as GPIIb IIIa ligam o fibrinogénio, o qual reforça a agregação plaquetária e estabiliza o trombo branco, no sector arterial, pela sua conversão em fibrina pela trombina. O fibrinogénio e a fibrina estimulam (i) nos monocitos a libertação do factor de necrose tumoral alfa e a IL-1β e, ainda, de quimiocinas, tais como IL-8, e, (ii) nos fibroblastos e células endoteliais, a proteína quimiotáxica para os monocitos. O fibrinogénio é um marcador de fase aguda da inflamação um agente que estabiliza o rolhão de plaquetas e um indutor da agregação eritrocitária. É assim um ligante entre coagulação, inflamação e hemorreologia.

As plaquetas no estado activado induzem a libertação de citocinas e quimiocinas, que levam à estimulação das integrinas nos monocitos e ao seu recrutamento para a placa de ateroma.

Ao interagir com a trombomodulina exposta na célula endotelial, a trombina activa a proteína C que, com o seu ligante natural, a proteína S, bloqueia a activação dos factores V e VIII. A trombina, sendo reconhecida pelo seu receptor (F2R) na célula endotelial e nas plaquetas, induz a libertação da IL-6, que se comporta com factor transformador da inflamação aguda em crónica com agravamento da estase nos capilares. A paragem do fluxo sanguíneo na microcirculação diminui o fornecimento de oxigénio e de metabolitos

aos tecidos e favorece a formação de agregados eritrocitários.

A trombina, ao activar os monocitos a libertarem IL-8, proporciona o acentuar da resposta inflamatória.

Os receptores do activador das proteases (PAR), expostos nas células mononucleares e endoteliais, podem ser estimulados pelos factores da coagulação e induzirem a libertação de citocinas e apoptose nas células inflamatórias, com consequente libertação de microvesículas. Estas são potenciais indutoras de trombogénese e de reacções inflamatórias por exporem, nomeadamente, factor tecidual e fosfolípidos, os quais, possuindo carga negativa, criam uma superfície propícia à activação do factor XII.

O FXIIa estimula o factor XI, dando início à via intrínseca, ou sistema de contacto, e atrai a calicreína plasmática. Esta, ao actuar no cininogénio de elevado peso molecular, origina a bradicinina, que é um potente péptido pro-inflamatório.

De entre as quatro isoformas de PAR, o PAR2 liga o complexo factor tecidual FVIIa, com consequente sobre – regulação dos macrófagos, que libertam citocinas quimiotáxicas para os neutrófilos. A administração de FVIIa a indivíduos saudáveis originou aumento da concentração de IL-6 e IL-8 na circulação sanguínea.

Os factores do sistema fibrinolítico modulam a inflamação, já que a deficiente remoção de fibrina, intra e extravascular, reforça a fibrose e mantém a inflamação crónica. A ligação estabelecida entre o activador do plasminogénio tipo urocinase (u-PA) e os seus receptores na superfície dos neutrófilos medeia a transmigração destes para o espaço intersticial vascular. A ausência de u-PA e dos seus receptores conduz à permanência da fibrina com consequências inflamatórias e hemorreológicas.

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Presidente da SPHM

REFERÊNCIAS

Blood 1996; 87:5051-5060.
Circ Res 2005; 96:1217.
J Mol Med 2010; 88:121-126.
Plos ONE 2010; 5:11 300-11 303.
J Atheroscler Thromb 2010; 17:1-11.
Blood 2010; 116:1593-1603.

HEMORREOLOGIA E MODELOS MATEMÁTICOS / HEMORHEOLOGY AND MATHEMATICAL MODELS

Adélia Sequeira[1]

ABSTRACT

This paper is devoted to a critical review of blood rheology and constitutive models for blood based on phenomenological considerations. The relationship between the multiphase properties and the mechanical characteristics of blood, including shear thinning viscosity, yield stress, tixotropy and viscoelasticity is considered. The significance of the non-Newtonian behavior of blood in the circulation is also addressed.

Key-words: Blood rheology, mathematical models, shear thinning, yield stress, tixotropy, viscoelasticity.

INTRODUCTION

Hemorheology is the science of deformation and flow of blood and its formed elements. This field involves the investigation of both the macroscopic behavior of blood using rheometric experiments as well as its microscopic properties *in vitro* and *in vivo*. It also involves studies of the interactions among blood cellular elements and between these components and the endothelial cells that line blood vessels.

The blood circulation in the cardiovascular system depends not only on the driving force of the heart and the architecture and mechanical properties of the vascular system, but also on the mechanical properties of blood itself. Whole blood is a concentrated suspension of formed cellular elements including red blood cells (RBCs) or erythrocytes, white blood cells (WBCs) or leukocytes and platelets or thrombocy-tes. The non-Newtonian behavior of blood is largely due to three characteristics of the erythrocytes: their ability to form aggrega-tes (three-dimensional microstructures) when at rest or at low shear rates, their deformability, and their tendency to align in the flow direction at high shear rates. A transition in microstructure is found between these two regimes[1,2]. The shape of the erythrocytes can change (deform) both due to in plane stretch-

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ing of its membrane as well as from bending[3].

As will be elaborated below, blood displays a shear thinning viscosity, viscoelasticity, thixotropy and possibly a yield stress. An understanding of the coupling between the blood composition and its physical properties is essential for developing suitable constitutive models to describe blood behavior.

Advances in the field of hemorheology are based on the evidence that they might be the primary cause of many cardiovascular diseases. In fact, hemorheological aberra-tions can easily be considered as a result (or an indicator) of insufficient circulatory function. Basically, pathologies with hematological origin like leukemia, hemolytic anemia, thalassemia or pathologies associated with the risk factors of thrombosis and atherosclerosis like myocardial infarction, hypertension, strokes or diabetes are mainly related to disturbances of local homeostasis. Therefore, the mathematical and numerical study of powerful, yet simple, constitutive models that can capture the rheological response of blood over a range of flow conditions is ultimately recognized as an important tool for clinical diagnosis and therapeutic planning (see e.g.[4,5]).

To better interpret and analyze the experimental data on blood it is helpful to turn to the literature on the rheology of particle suspensions. For rigid particles, a vast amount of published literature exists (see e.g.[6]). However, the study of suspensions of multiple, interacting and highly deformable particles such as blood, has received less attention and presents a challenge for both theoretical and computational fluid dynamicists, (e.g.[7]).

The aim of this paper is to present a brief overview of the rheological properties of blood, including its non-Newtonian characteristics, and review some of the macroscopic mathematical models that have been proposed in the literature to model these features.

PHYSICAL MECHANISMS BEHIND THE MECHANICAL PROPERTIES OF BLOOD

In this section we briefly discuss the physical mechanisms of erythrocytes that have the strongest influence on the non-Newtonian behavior of whole flowing blood at low shear rates.

In the presence of fibrinogen and globulins (two plasma proteins), erythrocytes have the ability to form a primary aggregate structure of rod shaped stacks of individual cells called *rouleaux*. At very low shear rates, the rouleaux align themselves in an end-to-side and side-to-side fashion and form a secondary structure consisting of branched three--dimensional (3-D) aggrega-tes[8]. The biochemical process of rouleaux formation is still unclear. It has been experimentally observed that these stacks will not form if the erythrocytes have been hardened or in the absence of fibrinogen and globulins.

For blood at rest, the 3-D structure formed by the RBCs looks solid-like, appearing to resist flow until a finite level of force is applied. The applied stress needed to initiate flow, e.g. in simple shear, is often referred to as

the yield stress and, under normal conditions, is primarily a function of the hematocrit and the fibrinogen concentration of the plasma[9]. Additional factors such as the red cell shape, deformability and tendency to aggregate also influence the value of the yield stress parameter. As discussed below, the existence of a yield stress for fluids in general and treatment of this yield stress as a material parameter is a controversial issue (see, e.g.,[10,11]).

When blood begins to flow, the solid-like structure breaks into three--dimensional networks of various sizes which appear to move as individual units and reach an equilibrium size for a fixed shear rate. Increases in shear rate lead to a reduction in equilibrium size and lower effective viscosity. In the studies of Schmid--Schönbein[8] at shear rates between 5.8 and 46 s^{-1} each doubling of the shear rate resulted in a decrease in average aggregate size of approximately 50% (see also[12] for further discussion).

Once the chains are broken down to 4-10 cells, they are resistant to further shearing and, at high shear rates they roll, rotate and tumble as units along with individual cells, and the structure visibly recoils elastically. A critical shear rate $\dot{\gamma}_{max}$ is defined as a constant shear rate at which, effectively there are no more aggregates (larger than 15 μm). In whole blood from healthy humans, different values are reported for this critical shear rate, largely in the range of 5-100 s^{-1}. Dintenfass [4] attributed this variation in reported values to the degree of aggregation of the original sample which, as discussed later, is consistent with the thixotropic nature of blood[13]. In diseased states, the critical shear rate can increase substantially. For example, in blood samples from patients with acute myocardial infarctions, the critical shear rate for dispersion was found to be greater than approximately 250 s^{-1} and the average aggregate size was larger than in the controls for all shear rates[8,14].

The process of disaggregation under increasing shear is reversible. When the shear rate is quasi-statically stepped down to lower values, the individual cells form shorter chains, then longer rouleaux and eventually a 3-D microstructure[14]. The finite time necessary for equilibrium of the structure to be reached (both during aggregation and disaggregation) is responsible for the thixotropic behavior of blood at low shear rates (see e.g.[15]). The associated time constants are a function of shear rate. The equilibria are found to be reached more rapidly at higher shear rates and more gradually with lower shear rates (e.g.[4]). For example, in a cone and plate viscometer, for shear rates between 0.01 and 1.0 s^{-1} the equilibrium distributions were found to be reached after time intervals of 20 to 200 seconds.

Accelerating flow has a marked effect on the structure of the aggregate. Under acceleration between fixed shear rates, significant elongation of rouleaux occurs which is not seen when the flow is held at a fixed shear rate. The elongation is particularly evident in rouleaux that bridge larger secondary structures and is found to arise from realignment of the individual cells (sliding of the cells from a parallel stack to a sheared stack) and deformation of individual cells (ellipsoidal defor-

mation and eventually prolate deformation). As a result of these mechanisms, the aggregate length can increase up to three fold[8]. Under a sinusoidal variation of shear, elastic behaviour of the aggregates is observed (e.g.[2,16]). While typical data for whole blood viscosity are obtained from quasi-static shear experiments, small amplitude oscillatory shear experiments are used to measure both viscous and elastic properties in the regime of small deformations from the rest history.

In a Couette rheometer, when blood is subjected to a constant shear rate slightly above $\dot{\gamma}_{max}$, the cells can be seen to rotate. With increasing shear rate, they rotate less and for shear rates above 230 s^{-1} they cease to rotate and remain aligned with the flow direction[8].

For shear rates above 400 s^{-1} the tumbling of the individual cells is completely absent, they lose their biconcave shape, become fully elongated and are transformed into flat outstretched ellipsoids with major axes parallel to the flow direction. At this stage the collision of red cells only occurs when a more rapidly moving cell touches a slower one but there are no further interactions between the cells. Close observation suggests that the changing cells contours are consistent with a tank-treading motion of the cells membranes about their interior, similar to a fluid drop deformation[2,17]. The high deformability of erythrocytes is due to the absence of a nucleus, to the elastic and viscous properties of its membrane and also to geometric factors such as the shape, volume and membrane surface area.

MECHANICAL PROPERTIES OF BLOOD

Measurements of the mechanical properties of whole blood are technically challenging, particularly at low to medium shear rates where denatured protein films, sedimenta-tion and phase separation can lead to erroneous results. At high shear rates, inertial effects can be problematic. As a result, measuring the mechanical properties of blood over a wide range of shear rates (e.g. 0.01 – 500 s^{-1}), can require the use of more than one rheometer. The three most commonly used rheometers for blood are the concentric cylinder rheometer (Couette rheometer), the cone and plate rheometer, and the capillary rheometer. These devices can be used in steady and oscillatory modes to measure the viscosity and linear viscoelastic properties of blood, respectively. An overview of typical blood rheometers and a discussion of some of the challenges which are particular to blood rheometry can be found in[12,15].

Viscosity of blood

The most well studied non--Newtonian characteristic of blood is its diminishing viscosity with increasing shear rate, called shear thinning behavior.

As discussed earlier, the mechanical properties of blood are dominated by the 3-D microstructure formed by the RBCs and general distribution of RBCs in the flowing plasma. At lower shear rates, the behavior is controlled by the effect of the 3-D RBC formations on the flow and the ability of these formations to deform and store

energy. At moderate to high shear rates, these cells are dispersed in the plasma and the properties of the blood are then influenced by their tendency to align and form layers in the flow, as well as their deformation. The importance of RBCs aggregation on blood viscosity at low shear rates was clearly demonstrated by Chien[18] who compared the viscosity of RBCs suspended in heparinized plasma and albumin-Ringer solution (Alb). The normal RBCs aggregation found in plasma does not occur in Alb. Though the viscosity of plasma and Alb were both the same, the viscosity of the RBCs' solution was greatly increased at low shear rates by RBCs aggregation (less than approximately 5 s^{-1}) but unaffected at larger shear rates.

The effect of RBCs deformability on viscosity of suspensions of these cells was also clearly shown in[18]. Chien compared the viscosity of normal RBC in Alb and that of hardened RBC in Alb (presumably at zero shear rate). The ability of normal RBC to deform (change shape and stretch) significantly decreased the viscosity over all shear rates tested. Fig. 1 displays the shear thinning behavior of whole blood as experimentally observed by Chien[18]. Each of these data points represents an equilibrium value obtained at a fixed shear rate.

In addition to shear rate, the aggregate size is a function of cell shape, plasma composition and hematocrit. This is reflected in the dependence of viscosity on these same variables. We do not address quantitative results for these variables here, but refer the reader to references such as[4,19]. Moreover, the viscosity of whole blood is strongly dependent on temperature.

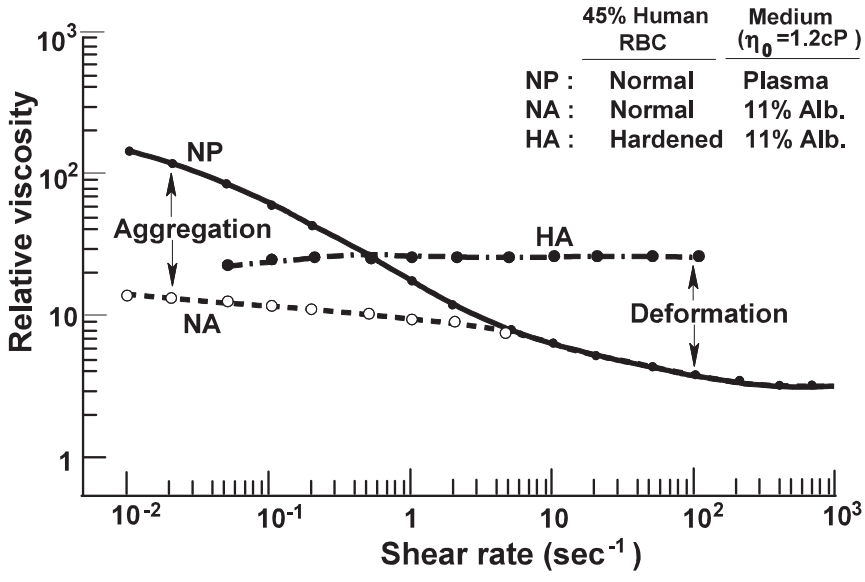

Fig. 1 – Variation of the relative viscosity as a function of the shear rate for normal RBC in heparinized plasma (NP), normal RBC in albumin-Ringer solution (NA) and hardened RBCs in albumin-Ringer solution (HA) at a temperature of 37oC, hematocrit Ht=45% using a Couette viscometer (reproduced from Chien [18], with permission)

Merrill *et al.*[20] found the dependence of blood viscosity on temperature to be similar to that of water for temperatures ranging from 10 to 40°C and shear rates from 1 to 100 s^{-1}. The variation of plasma viscosity with temperature is also known to approximately follow that of water[21]. For these reasons, blood viscosity is often reported relative to the viscosity of plasma or water at the same temperature.

Viscoelasticity and thixotropy of blood

Viscoelastic fluids are viscous fluids which have the ability to store and release energy. The viscoelasticity of blood at normal hematocrits is primarily attributed to reversible deformation of the RBCs 3D microstructures[22]. Elastic energy is due to the properties of the RBC membrane and the bridging mechanisms within the 3D structure. It can also be stored in

the deformation of individual RBC, though this is not believed to play an appreciable role unless the RBC concentration is significantly elevated above normal physiological levels[23]. However, these properties are of relatively small magnitude and to date they have generally only been measured in the context of linear viscoelasticity. By shear rates of the order of 10 s^{-1} the elastic nature of blood is negligible as evidenced by a merging of the oscillatory and steady flow viscosities.

The reader is referred to[22] for a review of the dependence of blood viscoelasticity on factors such as temperature, hematocrit and RBC properties.

While the linear viscoelastic functions are relatively straightforward to obtain in the rheometers referred above, it should be emphasized that blood flow in the circulatory system is rarely in the regime applicable for linear viscoelasticity theory. The linear elastic material constants are indicative of aspects of the microstructure and therefore can be used as a method of quantifying blood properties. However, ultimately, there is a need to consider the finite viscoelastic behavior of blood, if viscoelastic constitutive equations are going to be used to model blood behavior in the circulatory system.

The formation of the three--dimensional microstructure and the alignment of the RBCs are not instantaneous, which gives blood its thixotropic behavior. Essentially we refer to thixotropy as the dependence of the material properties on the time over which shear has been applied. This dependence is due to the finite time required for the 3-D structure of blood to form and breakdown[24].

Yield stress of blood

The behavior of a number of fluids at low shear stress, including blood, has led researchers to believe in the existence of a critical value of stress below which the fluid will not flow. This critical stress level, called the yield value or yield, is typically treated as a material property (constant) of the fluid. An extensive description of methods for measuring yield stress is given in[25]. Briefly, there are two categories of methods: indirect methods and direct methods. In indirect methods, the shear stress versus shear rate curve is back extrapolated to zero, possibly using a specific constitutive model or simple linear approximation. Sometimes the yield stress is set to the lowest measured shear stress[26]. In direct methods, an attempt is made to directly measure the shear stress at which the fluid begins to flow.

Reported values for the yield stress of blood vary greatly ranging from 0.002 to 0.40 dynes/cm^2, see e.g.[4]. This variation has been attributed to artifacts arising from interactions between the RBCs and surfaces of the rheometer[21] as well as the experimental method used to measure the yield stress.

The large range seen for blood is consistent with results for other fluids where this spread is attributed to the experimental methodology, the criterion used to define the yield stress, and the length of time over which the experiment is run[24]. A true material constant should be independent of these factors and these results have called into serious question the treatment of the yield stress as a material parameter. Dintenfass[4] appears to be

the first to question the appropriateness of such a material constant for blood. He suggested that rather than treating the yield stress as a constant, it should be considered as a function of time. This time dependence is consistent with the link between yield stress and thixotropy proposed years later by other researchers[27].

CONSTITUTIVE MODELS FOR BLOOD

As discussed earlier, the presence of the formed elements in blood leads to some significant and fascinating changes in its rheological properties. In this section, we discuss constitutive models introduced to capture one or more of these properties.

We assume that all macroscopic length and time scales are sufficiently large compared to length and time scales at the level of the individual erythrocyte so that the continuum hypothesis holds. Thus the models presented here are not appropriate in the capillary network. For an overview of hemorheology in the microcirculation we refer the reader to the review articles of Popel and Johnson[28] and Pries and Secomb[29].

Since both the shear thinning and viscoelastic properties diminish rapidly as the 3D microstructure of RBCs breaks down, it is important to consider in which flow regimes and clinical situations the non-Newtonian properties of blood will be important. RBCs would need to be subjected to shear rates below 1 s^{-1} per period sufficient for these structures to form and alter the flow. Schmid– Schönbein and co-workers[30] found the half-time for aggregate formation to be 3-5 s for normal blood and 0.5-1.5 s for pathological blood samples. In their experiments, the time for aggregation was measured after samples were exposed to an abrupt drop in shear rate from 460 s^{-1} to approximately zero.

The blood circulation time for humans is on the order of minutes, so for normal blood, the RBC structure will be broken down in the majority of the arterial system and only exist in regions of the circulation where there are stable recirculation zones with shear rates significantly below 1 s^{-1} (see[12] for more details).

Possible locations where the non-Newtonian behavior will be significant include segments of the venous system and stable vortices downstream of some stenosis and in the sacs of some aneurysms. For various pathologies, the 3D microstructure formed by the RBC is substantially stronger than for normal blood. For these patients, it is possible the 3D microstructure will exist in more widespread regions of the circulatory system. Increased RBC aggregation has been observed for patients with infections, trauma, burns, diabetes mellitus, AIDS and other diseases[31]. The quantification of the mechanical response of blood (shear thinning, viscoelasticity) can also be important for patient diagnosis[22].

As a first step towards the macroscopic modeling of blood flow we consider the equations given by

$$\rho\frac{\partial u}{\partial t}+\rho\left(u.\nabla\right)u=-\nabla p+\nabla.\tau \quad (1)$$

$$\nabla.u=0$$

in a domain $\Omega\subset R^3$ representing the lumen of the vessel. These equations express the conservation of linear

momentum and mass (or incompressibility condition) for isothermal flows and are completed with appropriate initial and boundary conditions. Here $u(x,t)$ and $p(x,t)$ denote the blood velocity and pressure in Ω, with $t \geq 0$, ρ is the blood density and τ is the extra-stress tensor accounting for differences in behavior from a purely inviscid, incompressible fluid. To close system (1) we require a constitutive equation relating the state of stress to the kinematic variables such as rate of deformation of fluid elements. The aim of this section is to review some of the macroscopic models suitable for blood flow under certain flow conditions.

Generalized Newtonian and yield stress models

The simplest constitutive model for incompressible viscous fluids is based on the assumption that the extra stress tensor is proportional to the symmetric part of the velocity gradient,

$$\tau = 2\mu D \quad (2)$$

where μ is the (constant) viscosity and $D = (\nabla u + \nabla u^T) / 2$ is the rate of deformation tensor. The substitution of (2) in the linear momentum equation (1) leads to the well-known Navier-Stokes equations for an incompressible viscous fluid. As already discussed this set of equations is commonly used with some justification to describe blood flow in the heart and healthy arteries. However, under certain experimental or physiological conditions, particularly at low shear rates, blood exhibits relevant non-Newtonian characteristics and more complex constitutive models need to be used.

The most general constitutive model of the form $\tau = \tau(\nabla u)$ which satisfies invariance requirements[32] can be written as

$$\tau = \varphi_1(II_D, III_D)D + \varphi_2(II_D, III_D)D^2 \quad (3)$$

where II_D and III_D are the second and third principal invariants of D

$$II_D = \frac{1}{2}\left((trD)^2 - tr(D^2)\right), \qquad III_D = detD$$

And $trD \equiv 0$ for isochoric motions. Incompressible fluids of the form (3) are called Reiner-Rivlin fluids. They include Newtonian fluids as a particular case, corresponding to φ_1 constant and $\varphi_2 \equiv 0$.

The behavior of real fluids impose some restrictions on the material functions φ_1 and φ_2. In fact, there is no evidence of real fluids with non-zero values of φ_2 and the dependence on the value of III_D is often neglected[32]. As a result, attention is usually restricted to a special class of Reiner-Rivlin fluids called generalized Newtonian fluids, for which

$$\tau = 2\mu(\dot{\gamma})D \quad (4)$$

where $\dot{\gamma}$ is the shear rate (a measure of the rate of deformation) defined by

$$\dot{\gamma} \equiv \sqrt{2tr(D^2)} = \sqrt{-4II_D} \quad (5)$$

and $\mu(\dot{\gamma})$ is a shear dependent viscosity function (for isochoric motions II_D is not a positive quantity).

One of the simplest generalized Newtonian fluids is the *power-law*, for which the viscosity function is given by

$$\mu(\dot{\gamma}) = K\dot{\gamma}^{n-1} \quad (6)$$

where the positive constants n and K are termed the power-law index and consistency, respectively. This model includes, as a particular case, the constant viscosity fluid (Newtonian) when $n=1$. For $n<1$ it leads to a monotonic decreasing function of the shear rate (shear thinning fluid) and for $n>1$ the viscosity increases with shear rate (shear thickening fluid). The shear thinning power-law model is often used for blood, due to the analytical solutions easily obtained for its governing equations, but it predicts an unbounded viscosity at zero shear rate and zero viscosity when $(\dot{\gamma}) \to \infty$, which is unphysical.

One of the most successful viscosity laws for blood is an extension of the power-law model due to Walburn and Schneck[33]. In addition to the shear rate, they considered the dependence of the viscosity on the hematocrit (Ht) and total protein minus albumin ($TPMA$) content through the parameter K and n in (6). Using a nonlinear regression analysis they found that shear rate and hematocrit were the two most important factors in decreasing order of importance. Based on these two factors, they formulated the following expressions of K and n,

$$K = C_1 \exp(C_2 Ht),\ n = 1 - C_3 Ht \quad (7)$$

Fig. 2 shows a comparison of viscosity functions $\mu(\dot{\gamma})$ for the power-law model (6) using material constants provided by Kim *et al.*[34] (Kim) and Liepsch and Moravec[35] (LM) for human blood. Representative viscosity curves for the Walburn-Schneck model (WS)[33] with factors depending

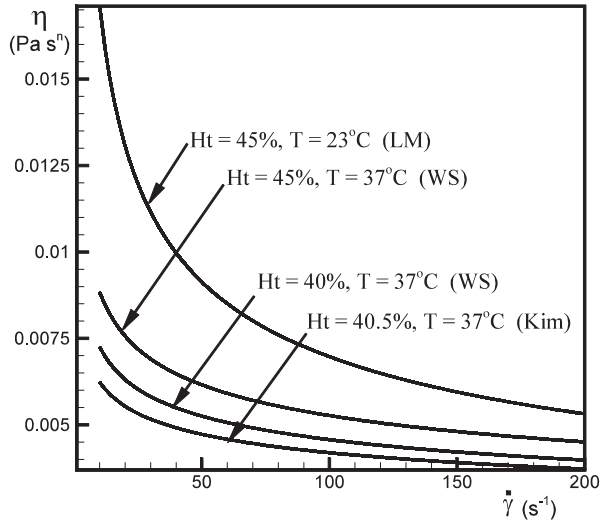

Fig. 2 – Comparison of viscosity functions $\mu(\dot{\gamma})$ for the power-law model (6) using material constants provided obtained by curve fit to experiments.

on hematocrit (7) are also shown. The viscosity functions obtained from[33] and[34] for $Ht = 40\%$ are quite close. In contrast, those in[34] and[35] for $Ht = 45\%$ are substantially different, likely due to the lower temperatures used in[33] compared to those in[35].

Viscosity functions with bounded and non-zero limiting values of viscosity can be written in the general form

$$\mu(\dot{\gamma}) = \mu_\infty + (\mu_0 - \mu_\infty)F(\dot{\gamma})$$

or, in non-dimensional form as

$$\frac{\mu(\dot{\gamma}) - \mu_\infty}{\mu_0 - \mu_\infty} = F(\dot{\gamma})$$

Here μ_0 and μ_∞ are the asymptotic viscosity values at zero and infinite shear rates and $F(\dot{\gamma})$ is a shear dependent function, satisfying the following natural limit conditions

$$\lim_{\dot{\gamma}\to 0^+} F(\dot{\gamma}) = 1 \quad \text{and} \quad \lim_{\dot{\gamma}\to\infty} F(\dot{\gamma}) = 0$$

Different choices of the function $F(\dot{\gamma})$ correspond to different models

Table 1 – Material constants for various generalized Newtonian models for blood

Model	Non-Newtonian viscosity	Material constants for blood
Power Law	$\mu(\dot{\gamma}) = K\dot{\gamma}^{n-1}$	$n = 0.61, K = 0.42$
Powell-Eyring	$\frac{\mu(\dot{\gamma})-\mu_\infty}{\mu_0-\mu_\infty} = \frac{\sinh^{-1}(\lambda\dot{\gamma})}{\lambda\dot{\gamma}}$	$\mu_0 = 0.056Pas, \mu_\infty = 0.00345Pas$ $\lambda = 5.383s$
Cross	$\frac{\mu(\dot{\gamma})-\mu_\infty}{\mu_0-\mu_\infty} = \frac{1}{1+(\lambda\dot{\gamma})^m}$	$\mu_0 = 0.056Pas, \mu_\infty = 0.00345Pas$ $\lambda = 1.007s, m = 1.028$
Modified Cross	$\frac{\mu(\dot{\gamma})-\mu_\infty}{\mu_0-\mu_\infty} = \frac{1}{(1+(\lambda\dot{\gamma})^m)^a}$	$\mu_0 = 0.056Pas, \mu_\infty = 0.00345Pas$ $\lambda = 3.736s, m = 2.406, a = 0.254$
Carreau	$\frac{\mu(\dot{\gamma})-\mu_\infty}{\mu_0-\mu_\infty} = (1+(\lambda\dot{\gamma})^2)^{(n-1)/2}$	$\mu_0 = 0.056Pas, \mu_\infty = 0.00345Pas$ $\lambda = 3.313s, n = 0.3568$
Carreau-Yasuda	$\frac{\mu(\dot{\gamma})-\mu_\infty}{\mu_0-\mu_\infty} = (1+(\lambda\dot{\gamma})^a)^{(n-1)/a}$	$\mu_0 = 0.056Pas, \mu_\infty = 0.00345Pas$ $\lambda = 1.902s, n = 0.22, a = 1.25$

for blood flow, with material constants quite sensitive and depending on a number of factors including hematocrit, temperature, plasma viscosity, age of RBCs, exercise level, gender or disease state.

Table 1 summarizes some of the most common generalized Newtonian models that have been considered in the literature for the shear dependent viscosity of whole human blood. Values for the material constants given in this table were obtained by Cho and Kensey[36] for a compilation of human and canine blood (*Ht* ranging from 33-45%), using a nonlinear least squares analysis.

Yield stress models can be useful to model blood flow in low shear rate regions. Yield stress materials require a finite shear stress τ_Y (the yield stress) to start flowing. A relatively simple, and physically relevant yield criterion is given by $\sqrt{|II_\tau|} = \tau_Y$, where II_Y is the second invariant of the extra stress tensor τ. The most common yield stress model for blood is the Casson's model[37] which, in simple shear has the form

$$\sqrt{|II_\tau|} < \tau_Y \Rightarrow D = 0$$

$$\sqrt{|II_\tau|} \geq \tau_Y \Rightarrow \begin{cases} D = \frac{1}{2\mu_N}\left(1 - \frac{\sqrt{\tau_Y}}{\sqrt[4]{II_\tau}}\right)\tau \\ \tau = 2\left(\sqrt{\mu_N} + \frac{\sqrt{\tau_Y}}{\sqrt[4]{4\,|\,II_\tau\,|}}\right)^2 D \end{cases}$$

The Newtonian constitutive equation is a special case of the Casson equation with τ_Y set to zero, in which case μ_N is the Newtonian viscosity. Through this model blood behaves rigidly until the yield criterion is satisfied, after which it displays a shear thinning behavior.

Other yield stress models like Bingham or Herschel-Bulkley models are also used for blood (see e.g.[12,15]) as well as the constitutive model developed by Quemada[38] using a semi-phenomenological approach, with the apparent viscosity given by

$$\mu(\dot{\gamma}) = \mu_F\left(1 - \frac{1}{2}\frac{k_0 + k_\infty\sqrt{\dot{\gamma}/\dot{\gamma}_c}}{1+\sqrt{\dot{\gamma}/\dot{\gamma}_c}}\varphi\right)^{-2}$$

where μ_F, φ and $\dot{\gamma}_c$ are the viscosity of the suspending fluid, the volume concentration of the dispersed phase and a critical shear rate, respectively.

As discussed earlier, the existence of a yield stress and its use as a material parameter is a controversial issue, since measurements of the yield stress are expected to be quite sensitive to the microstructure of the blood prior to yielding, which is in turn expected to be sensitive to both the shear rate history as well as time[27].

Viscoelastic and thixotropic models

None of the models in the previous section accounts for either the viscoelasticity or the thixotropy of blood. A number of nonlinear viscoelastic constitutive models for blood are now available but because of their complexity we will avoid presenting the mathematical details here, providing instead a summary of the relevant literature.

One of the simplest rate type models accounting for the viscoelasticity of blood is the Maxwell model

$$\tau + \lambda_1 \frac{\delta \tau}{\delta t} = 2\mu D \quad (8)$$

where λ_1 is the relaxation time and $\delta(.)/\delta t$ stands for the so-called *convected derivative*, a generalization of the material time derivative, chosen so that $\delta\tau/\delta t$ is objective under a superposed rigid body motion and the resulting second-order tensor is symmetric[15].

A more general class of rate type models, called *Oldroyd type* models, are defined by

$$\tau + \lambda_1 \frac{\delta \tau}{\delta t} = 2\mu \left(D + \lambda_2 \frac{\delta D}{\delta t} \right) \quad (9)$$

where the material coefficient λ_2 denotes the retardation time and is such that $0 \leq \lambda_2 < \lambda_1$. The Oldroyd type fluids can be considered as Maxwell fluids with additional viscosity. These models contain the previous two models (2) and (8) as particular cases.

Thurston[39] was among the earliest to recognize the viscoelastic nature of blood and that the viscoelastic behavior is less prominent with increasing shear rate. He proposed a generalized Maxwell model that was applicable to one dimensional flow simulations and observed later that, beyond a critical shear rate, the nonlinear behavior is related to the microstructural changes that occur in blood[40]. Thurston's work was suggested to be more applicable to venous or low shear unhealthy blood flow than to arterial flows. Recently, a generalized Maxwell model related to the microstructure of blood, inspired on the behavior of transient networks in polymers, and exhibiting shear thinning, viscoelastici-ty and thixotropy, has been derived by Owens[41].

Other viscoelastic constitutive models of differential type, suitable for describing blood rheology have been proposed in the recent literature. The empirical three constant generalized Oldroyd-B model studied in[42] belongs to this class. It has been obtained by fitting experimental data in one dimensional flows and generalizing such curve fits to three dimensions. This model captures the shear thinning behavior of blood over a large range of shear rates but it has its limitations, given that the relaxation times do not depend on the shear rate, which does not agree with experimental observations. The model developed by Anand and Rajagopal[43] in the general thermodynamic framework stated in[44] includes relaxation times depending on the shear rate and gives good agreement with experimental data in steady Poiseuille and oscillatory flows.

CONCLUSIONS

In this short review the material properties of human blood and in particular its shear viscosity, elasticity and thixotropy have been discussed

in terms of the complex evolving microstructure, and especially of the deforming and migrating red blood cells in their different states of aggregation. We conclude that the most promising rheological models are those developed from an underlying microstructure similar to that of blood. Some existing gaps can be identified as significant challenges for the development of new rheological models to be used in large-scale numerical simula-tions.

1. Although attention has been devoted to the measurement of some of the basic macroscopic rheological properties of blood, especially in flow regimes where non-Newtonian effects are most likely to be observed, there is urgent need for further experimental data to validate the current rheological models and provide a rational basis for the further development of microstructural models.

2. The transition from the non--Newtonian to the Newtonian viscosity character of blood should be better investigated, since a variation on the critical value of the shear rate appears in literature to address this issue. The availability of reliable measurements of quantities such as the velocity, wall shear stress and pressure in well characterized physiological flows (*in vivo*) is essential in CFD studies of blood flow in the cardiovascular system.

3. A suitable account of the mathematical structure of the systems of equations derived from the constitutive models for blood must be considered. These systems, closed with appropriate initial and boundary conditions, are highly nonlinear and the development of well adapted stable, accurate and affordable numerical methods is of the greatest importance. Parallel algorithms and fast large--scale computing platforms, opening up new possibilities in simulation and visualization, become crucial when using non-Newtonian solvers for blood.

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REFERENCES

1. S. Chien, S. Usami, R. J. Dellenback, M. I. Gregersen. Shear-dependent deformation of erythrocytes in rheology of human blood. Am. J. Physiol., 219:136-142, 1970.
2. H. Schmid-Schönbein, R. Wells. Fluid drop-like transition of erythrocytes under shear. Science, 165:288-291, 1969.
3. S. Chien. Red cell deformability and its relevance to blood flow. Ann. Rev. Physiology, 49:117-192, 1987.
4. L. Dintenfass. Blood Microrheology – Viscosity Factors in Blood Flow, Ischaemia and Thrombosis. Butterworth, 1971.
5. G. D. O. Lowe. Clinical Blood Rheology, Vol. I, II. CRC Press, Boca Raton, Florida, 1998.
6. M. C. Roco (Ed.) Particulate Two-Phase Flow. Series in Chemical Eng. Butterworth-Hinemann Publ., 1993.
7. V. Cristini, G.S. Kassab. Computer modeling of red blood cell rheology in the microcircu-lation: A brief overview. Ann. Biomed. Eng., 33(12):1724--1727,2005.
8. H. Schmid-Schönbein, R. Wells. Rheological properties of human erythrocytes and their influence upon anomalous viscosity of blood. Physiology Rev., 63:147-219, 1971.
9. E. W. Merrill. Rheology of blood. Physiol. Rev., 49:863--888, 1969.
10. H. A. Barnes, K. Walters. The yield stress myth? Rheol. Acta, 24:323-326, 1985.
11. P. C. F. Moller, J. Mewis, D. Bonn. Yield stress and thixotropy: on the difficulty of measuring yield stress in practice. Soft Matter, 2:274-288, 2006.
12. A. M. Robertson, A. Sequeira, M. Kameneva. Hemorheology. In: Hemodynamical Flows: Modeling, Analysis and Simulation, Series: Oberwolfach Seminars, Vol. 37, 63-120, Galdi, G.P. et al, Birkhäuser, 2008.
13. A. Barnes. Thixotropy – a review. J. Non--Newtonian Fluid Mech., 70:1-33, 1997.
14. J. Goldstone, H. Schmid-Schönbein, R. Wells. The rheology of red blood cell aggregates. Microvas. Res., 2:273-286, 1970.

15. A. M. Robertson. Review of relevant continuum mechanics. In: Hemodynamical Flows: Modeling, Analysis and Simulation, Series: Oberwolfach Seminars, Vol. 37, 63-120, Galdi, G.P. et al, Birkhäuser, 2008.
16. A. L. Copley, R. G. King, S. Chien, S. Usami, R. Skalak, C. R. Huang. Microscopic observations of viscoelasticity of human blood in steady and oscillatory shear. Biorheology, 12:257-263, 1975.
17. T. M. Fischer, M. Stöehr-Lissen, H. Schmid--Schönbein. The red cell as a fluid droplet: tank tread-like motion of human erythrocyte membrane in shear flux. Science, 202:894-896, 1978.
18. S. Chien. Shear dependence of effective cell volume as a determinant of blood viscosity. Science, 168:977-979, 1970.
19. R. L. Whitmore. Rheology of the Circulation. Pergamon Press, 1968.
20. E. W. Merrill, E. R. Gilliland, G. Cokelet, H. Shin, A. Britten, R. E. Wells, Jr. Rheology of human blood, near and at zero flow. Effects of temperature and hematocrit level. Biophys. J., 3:199-213, 1963.
21. S. E. Charm, G. S. Kurland. Blood Flow and Microcirculation. John Wiley & Sons, 1974.
22. G. B. Thurston. Viscoelastic properties of blood and blood analogs. Advances in Hemodynamics and Hemorheology, 1:1-30, 1996.
23. S. Chien, R. G. King, R. Skalak, S. Usami, A. L. Copley. Viscoelastic properties of human blood and red cell suspensions. Biorheology, 12:341--346, 1975.
24. H. A. Barnes. Thixotropy – a review. J. Non--Newtonian Fluid Mech., 70:1-33, 1997.
25. Q. D. Nguyen, D. V. Boger. Measuring the flow properties of yield stress fluids. Annual Reviews, pages 47-88, 1992.
26. C. Picart, J.M. Piau, H. Galliard, P. Carpentier. Human blood shear yield stress and its hematocrit dependence. J. Rheol., 42:1-12, 1998.
27. P. C. F. Moller, J. Mewis, D. Bonn. Yield stress and thixotropy: on the difficulty of measuring yield stress in practice. Soft Matter, 2:274-288, 2006.
28. A. S. Popel, P. C. Johnson. Microcirculation and hemorheology. Annu. Rev. Fluid Mech., 37:43-69, 2005.
29. A. R. Pries, T. W. Secomb. Rheology of the microcirculation. Clin. Hemorheol. Microcirc, 29:143-148, 2003.
30. H. Schmid-Schönbein, E. Volger, H. J. Klose. Microrheology and light transmission of blood. II– The photometric quantification of red cell aggregation formation and dispersion in flow. Pflüggers Arch, 333:140-155, 1972.
31. G. D. O. Lowe, Ed., Clinical Blood Rheology, Vol. I and II. CRC Press, Boca Raton, Florida, 1998.
32. G. Astarita, G. Marrucci. Principles of Non--Newtonian Fluid Mechanics. McGraw Hill, 1974.
33. F. J. Walburn, D. J. Schneck. A constitutive equation for whole human blood. Biorheology, 13:201--210, 1976.
34. S. Kim, Y.I. Cho, A.H. Jeon, B. Hogenauer, K.R. Kensey. A new method for blood viscosity measurement. J. Non-Newtonian Fluid Mech., 94:47--56, 2000.
35. D. Liepsch, St. Moravec. Pulsatile flow of non--Newtonian fluid in distensible models of human arteries. Biorheology, 21:571-586, 1984.
36. Y. I. Cho and K. R. Kensey. Effects of the non--Newtonian viscosity of blood on flows in a diseased arterial vessel. Part I: Steady flows. Biorheology, 28:241-262, 1991.
37. G. W. Scott Blair. An equation for the flow of blood, plasma and serum through glass capillaries. Nature, 183:613-614, 1959.
38. D. Quemada. Rheology of concentrated disperse systems III. General features of the proposed non--Newtonian model. Comparison with experimental data. Rheological Acta, 17:643-653, 1978.
39. G.B. Thurston. Viscoelasticity of human blood. Biophysical J., 12:1205-1217, 1972.
40. G.B. Thurston. Non-Newtonian viscosity of human blood: Flow induced changes in microstructure. Biorheology, 31(2):179-192, 1994.
41. R.G. Owens. A new microstructure-based constitutive model for human blood. J. Non-Newtonian Fluid Mech., 14:57-70, 2006.
42. K. K. Yeleswarapu, M.V. Kameneva, K. R. Rajagopal, J. F. Antaki. The flow of blood in tubes: Theory and experiment. Mechanics Research Communications, 25(3):257-262, 1998.
43. M. Anand, K. R. Rajagopal. A shear thinning viscoelastic fluid model for describing the flow of blood. Int. J. Cardiovascular Medicine and Science, 4:59-68, 2004.
44. K. R. Rajagopal, A. R. Srinivasa. A thermodynamic framework for rate-type fluid models. 88:207-227, 2000.

INTRAVASCULAR DANGER SIGNALS GUIDE NEUTROPHILS TO SITES OF STERILE INFLAMMATION

B McDonald[1], *K Pittman*[1], *GB Menezes*[1]*, *SA Hirota*[2], *I Slaba*[1],*CCM Waterhouse*[1,3], *P L Beck*[2,4], *DA Muruve*[1,4], *P Kubes*[1]†

Neutrophils are recruited from the blood to sites of sterile inflammation, where they contribute to wound healing but may also cause tissue damage. By using spinning disk confocal intravital microscopy, we examined the kinetics and molecular mechanisms of neutrophil recruitment to sites of focal hepatic necrosis in vivo. Adenosine triphosphate released from necrotic cells activated the Nlrp3 inflammasome to generate an inflammatory microenvironment that alerted circulating neutrophils to adhere within liver sinusoids. Subsequently, generation of an intravascular chemokine gradient directed neutrophil migration through healthy tissue toward foci of damage. Lastly, formyl-peptide signals released from necrotic cells guided neutrophils through nonperfused sinusoids into the injury. Thus, dynamic in vivo imaging revealed a multistep hierarchy of directional cues that guide neutrophil localization to sites of sterile inflammation [**Science 2010;330: 362--360]**.

Ver vídeo: http://videolab.sciencemag.org/54477078001/635318282001/1

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HEMODILUTION THERAPY USING AUTOMATED ERYTHROCYTAPHERESIS IN CENTRAL RETINAL VEIN OCCLUSION: RESULTS OF A MULTICENTER RANDOMIZED CONTROLLED STUDY[1]

A Glacet-Bernard, M Atassi, C Fardeau, JP Romanet, M Tonini, J Conrath, P Denis, M Mauget-Faÿsse, G Coscas, G Soubrane, E Souied

BACKGROUND: Central retinal vein occlusion (CRVO) leads to poor visual outcome in most eyes. Abnormal hemorheology was suspected to play a major role in its pathogenesis. CRVO treatment is still a matter of debate but several studies have pointed out the efficacy of isovolumic hemodilution. The aim of this study was to assess the feasibility and efficacy of hemodilution using automated erythrocytapheresis in recent-onset CRVO.

METHODS: In this prospective randomized controlled multicenter study, 61 consecutive CRVO patients were enrolled when they met the following criteria: CRVO lasting for 3 weeks or less, visual acuity ranging from 20/200 to 20/32, age between 18 and 85 years, no diabetes, no uncontrolled systemic hypertension, no antiplatelet or anticoagulant therapy, hematocrit higher than 38%, and signed informed consent. Patients were randomly assigned to the hemodilution group (n=31) or to the control group (n=30). Hemodilution therapy consisted of one session of erythrocytapheresis on outpatient basis, followed by additional session(s) for 6 weeks if needed. Target hematocrit was 35%. Follow-up was 12 months.

RESULTS: No statistical differences in age, associated risk factors, or CRVO characteristics were observed at baseline between both groups. Mean visual acuity was equivalent to 20/80 in the hemodilution group and to 20/63 in the control group (non-significant difference). In the treated group, mean number of hemodilution sessions was 3.3 (range, 1 to 6), and no major side-effects occurred. At the 12-month follow-up visit, 64.5% of the hemodilution group had visual acuity of 20/40 or better compared to 40% of the control group (p=.048). Visual change was a gain of 1.7 ETDRS line in the hemodilution group versus a loss of 2.3 lines in the control group (p=.007). There was less conversion into an ischemic form in the hemodilution group (11%) than in the control group (50%, p=.004). Mean final retinal thickness was 289 µm in the hemodilution group versus 401 µm in the control group (p=.068).

CONCLUSIONS: This multicenter controlled randomized study demonstrated that automated erythrocytapheresis is a safe and effective tool for performing hemodilution and confirmed that hemodilution therapy can improve the final prognosis of CRVO when applied in the early phase of the disease [**Graefes Arch Clin Exp Ophthalmol. 2010, Oct 17**].

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AGREEMENT BETWEEN ERYTHROCYTE SEDIMENTATION RATE AND C-REACTIVE PROTEIN IN HOSPITAL PRACTICE[1]

I Colombet, J Pouchot, V Kronz, X Hanras, L Capron, P Durieux, B Wyplosz

BACKGROUND: Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are frequently prescribed jointly. The usefulness of this practice is uncertain.

METHODS: All patients with ESR and CRP measured at the same time in an academic tertiary hospital during a 1-year period were included. Concomitant measures of serum creatinine, hematocrit, and anti-Xa activity were recorded to study non-inflammatory cause of increased ESR. Level of agreement between ESR and CRP was assessed with kappa coefficient, and their accuracy was determined in a medical chart review of 99 randomly selected patients with disagreement between both markers.

RESULTS: Among 5777 patients, 35% and 58% had an elevated CRP and ESR, respectively. ESR and CRP were in agreement in 67% of patients (both elevated in 30%, both normal in 37%). A disagreement was observed in 33% (elevated ESR/normal CRP in 28%, normal ESR/elevated CRP in 5%). The kappa coefficient showed poor agreement (k=0.38) between both markers. Review of medical chart showed that 25 patients with elevated CRP and normal ESR had an active inflammatory disease (false-negative ESR). Conversely, 74 patients had elevated ESR and normal CRP-32% had resolving inflammatory disorders, 28% disclosed a variable interfering with the ESR measure (false-positive ESR), 32% had unexplained discrepancies, and 8% had an active inflammatory disease (false-negative CRP).

CONCLUSION: In hospital practice, joint measurement of ESR and CRP is unwarranted. Because of slow variation and frequent confounding, ESR is frequently misleading in unselected patients. When an inflammatory disorder is suspected, priority should be given to CRP. **[Am J Med. 2010; 123(9):863.e7-13]**

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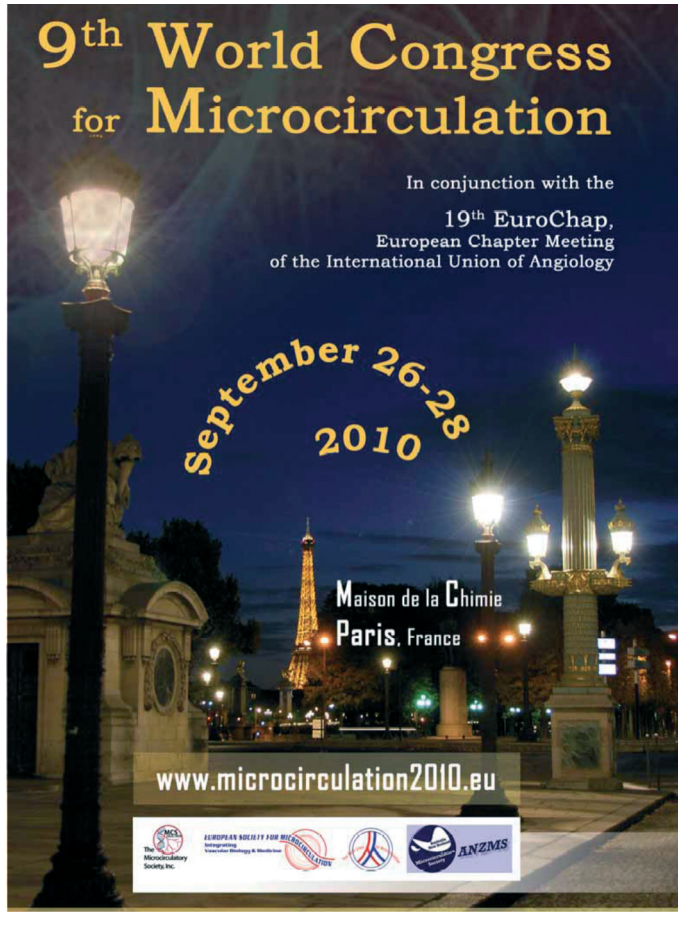
REUNIÕES CIENTÍFICAS REALIZADAS

XXI INTERNATIONAL FIBRINOGEN WORKSHOP

Realizou-se em Roterdão, entre 22 e 25 de Agosto deste ano o XXIth International Fibrinogen Workshop (www.fibrinogen.nl).

A SPHM esteve representada pela sua Presidente com um trabalho intitulado "*Fibrinogen Binding to Erythrocyte Membrane: CD47 a Possible Molecular Target*" da autoria de *S. De Oliveira, V. Vitorino de Almeida, A.Calado, H. S. Rosário, C. Saldanha*. Este trabalho (realizado na Unidade de Biologia Microvascular e Inflamação, Instituto de Medicina Molecular, Instituto de Bioquímica, Faculdade de Medicina, Universidade de Lisboa) insere-se no projecto, em que a Presidente é a investigadora responsável, sendo patrocinado pela Fundação para a Ciência e Tecnologia (FCT). A participação no referido evento foi suportada pela FCT e pela empresa farmacêutica Bayer Portugal.

9TH WORLD CONGRESS ON MICROCIRCULATION



Decorreu de 25 a 28 de Setembro, na *Maison de la Chimie*, em Paris, o *9th World Congress on Microcirculation* presidido por Eric Vicaut, membro da Sociedade Francesa de Microcirculação e da *European Society for Microcirculation*, (ESM). O patrocínio científico destas sociedades foi acrescido com o da *Australia New Zeeland Microcirculation Society* e o da *Asian Union for Microcirculation.*

O prémio Malpighi foi atribuído a Klaus Ley (Fig. 1) que proferiu a primeira lição plenária intitulada *"Leukocyte Rolling"*.

Fig. 1 – Axel Pries secretário da ESM entrega o prémio na presença de Eric Vicaut

A SPHM participou no "*Symposium Microcirculation and Blood Cell Microrheology in Physiological and Pathological State"s*" com duas comunicações: *"Erythrocyte Membrane Binding Fibrinogen"* e "Non-Neuronal Cholinergic System in Human Erythrocytes". Apresentaram estes trabalhos, respectivamente, Carlota Saldanha e Pedro Almeida.

Foram ainda expostos dois posters, intitulados *"Interaction Study at Molecular Level of the Hemorheological Parameters Fibrinogen and Erythrocyte*" e *"Fibrinogen-dependent Signaling in Microvascular Erythrocyte Function Implications on Nitric Oxide Efflux"*, apresentados, respectivamente, por Carlota Saldanha e Pedro Almeida. O programa do evento encontra-se em pdf no site do congresso (http://www.worldmicrocirc.org/).

PRÓXIMAS REUNIÕES MUNDIAIS SOBRE MICROCIRCULAÇÃO

2011 – **8th Asian Congress for Microcirculation**
(October 26-28, Bangkok, Thailand, http://www.worldmicrocirc.org/)

2015 – **10th World Congress for Microcirculation**
(Beijing, China).

RESUMOS APRESENTADOS A CONGRESSO

ERYTHROCYTE MEMBRANE BINDING FIBRINOGEN[1]

C. Saldanha, V. Vitorino de Almeida, H. S. Rosário, J .Pedro Lopes Almeida, S. de Oliveira,

High fibrinogen plasma concentration is considered an independent risk factor for cardiovascular diseases. A positive association between plasma levels of fibrinogen and erythrocyte aggregation tendency has been verified in cardiovascular and metabolic diseases as well as in inflammatory situations.

Studies conducted *in vitro* show that soluble and immobilized forms of fibrinogen favour, by a non-specific mechanism, red blood cells (RBCS) aggregates formation.

The aim of the present communication is to present the effects of fibrinogen on RBCs hemorheological properties, nitric oxide mobilization and the identity of the molecular binding target.

When at different shear stress the erythrocyte deformability (ED) was measured, fibrinogen decreases RBCs ability to deform at high shear stress in dependence of erythrocyte membrane band 3 phosphorylation degree. Otherwise fibrinogen promotes the ED in presence of N-ethylmaleimide.

As RBCs are scavenger for nitric oxide that promoting its efflux at microcirculatory network we have, *in vitro*, verified that fibrinogen abrogate its efflux promoting nitrite, nitrate and S-nitrosoglutatione formation.

The presence of the integrin-associated protein (IAP) or CD47 was identified by us, *in vitro*, as the RBCs membrane molecular target which binds fibrinogen in blood samples taken from healthy humans. When RBCs are separated, *in vitro*, by age, according its life span in blood, we verified a greater binding between fibrinogen and younger RBCs in relation to the interaction with the oldest.

The RBCs signal transduction mechanism mediate by fibrinogen has in course which could brings us novel therapeutically targets.

[1] Comunicação apresentada em 9th WORLD CONGRESS ON MICROCIRCULATION. Estudo realizado na Unidade de Biologia Microvascular e Inflamação, Instituto de Medicina Molecular, Instituto de Bioquímica, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa Portugal

INTERACTION STUDY AT MOLECULAR LEVEL OF THE HEMORHEOLOGICAL PARAMETERS FIBRINOGEN AND ERYTHROCYTE[1]

C. Saldanha, V. Vitorino de Almeida, H. S. Rosário, S. de Oliveira

The bridging model explains the RBC aggregation mediated by adsorbed macromolecules on adjacent RBC surfaces beyond the electrostatic repulsion forces, membrane strain and mechanical shearing.

Besides a huge data about the effect of fibrinogen on erythrocyte aggregation tendency no binding site has been identified as responsible by the erythrocyte membrane fibrinogen interaction.

The aim of this study was to study the interaction between the erythrocytes membrane with different ages, and plasma fibrinogen in presence and absence of anti-CD47.

Human erythrocytes from healthy donors were separated in a percoll discontinuous gradient. Whole population, old, and young RBC were labeled with: human fibrinogen (Alexa488 or 647) and anti-CD47 (PE). Flow cytometry analyses were made at a BD FACS Calibur analyser. RBC fluorescence images were taken in a confocal microscope Zeiss LSM 510Meta and were analyzed in computer program ImageJ.

Our results show the existence of a specific low binding mechanism between RBC membrane and fibrinogen, being higher in younger than in older RBC. We have observed that the presence of the CD47 antibody diminish the interaction of fibrinogen with the membranes of whole population, young and old RBC. These interactions of was visualized by confocal microscopy.

With this study, for the first time, different level of interaction between RBC, with different ages, and fibrinogen has been verified. Younger RBC establishes a higher interaction with fibrinogen than the older ones, even when erythrocyte aggregation indexes are higher in the later. Furthermore, we have observed a decrease in the fibrinogen interaction with all the three studied populations in the presence of anti-CD47 .This data suggests that CD47 could be a possible target for fibrinogen, or at least it may have a role in the interaction of these acute phase protein with red cell membrane.*(Supported by Fundação para a Ciência e Tecnologia: PTDC/SAU-OSM/73449/2006).*

[1] Poster apresentado em 9th WORLD CONGRESS ON MICROCIRCULATION. Estudo realizado na Unidade de Biologia Microvascular e Inflamação, Instituto de Medicina Molecular, Instituto de Bioquímica, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa Portugal

NON-NEURONAL CHOLINERGIC SYSTEM IN HUMAN ERYTHROCYTES: BIOLOGICAL ROLE AND CLINICAL RELEVANCE[1]

J. P. Lopes de Almeida, C. Saldanha

Acetylcholine is well-known in the medical setting as one of the most exemplary neurotransmitters. Its ubiquity in nature otherwise suggests a theoretically diverse spectrum of action and an extremely early appearance in the evolutionary process. In humans, acetylcholine and its synthesizing enzyme, choline acetyltransferase, have been found in various non-neural tissues such as the epithelium, mesothelium and endothelium, the muscle, immune cells and blood cells. The widespread expression of non-neuronal acetylcholine is accompanied by the ubiquitous presence of acetylcholinesterase and nicotinic/muscarinic receptors. Structural and functional dissimilarities are evident between the non-neuronal and neuronal cholinergic systems. An increasing body of evidence throughout the last few years has placed acetylcholine as a major cellular signalling molecule in many pathways. Furthermore, numerous erythrocyte physiological events in microcirculation are strongly regulated by acetylcholine. It is then time to revise the role of acetylcholine in humans. Its biological and pathobiological roles must be evaluated in more detail to eventually achieve novel therapeutical targets. Recent evidence from our Unit has noted significant findings about the non-neuronal acetylcholine in red blood cells, with special regard to (i) the red cell rheology, (ii) plasma ions concentrations (iii) nitric oxide (NO) intracellular translocation and metabolism, and (iv) band 3 protein phosphorylation. Significant correlations among nitrosylated molecules, redox thiol status, NO efflux and hemorheological profile have been explored in human erythrocytes.

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FIBRINOGEN-DEPENDENT SIGNALLING IN MICROVASCULAR ERYTHROCYTE FUNCTION – IMPLICATIONS ON NITRIC OXIDE EFFLUX[1]

Lopes de Almeida JP, Saldanha C

Experimental evidence has shown that plasma fibrinogen plays a key role as a major cardiovascular risk factor, acting directly to trigger erythrocyte aggregation in occlusive vascular disease. However, due to the complex and hitherto unclear interaction between fibrinogen and the erythrocyte membrane, no study has yet evaluated the effects of fibrinogen, under and above physiological range values, on the erythrocyte nitric oxide (NO) mobilization. Taking into consideration the potential NO-derived molecules, we have raised the hypothesis that fibrinogen, under physiological conditions, may act to influence blood flow via erythrocyte NO modulation. In this *in-vitro* study, whole blood samples were harvested from healthy subjects and erythrocyte suspensions were incubated in the absence (control aliquots) and presence of different fibrinogen concentrations and the levels of NO, nitrite, nitrate and S-nitroglutathione (GSNO) were determined. Our results show, after comparing with control aliquot, that the presence of fibrinogen modulate the NO mobilization in erythrocyte by: (1) decreasing the erythrocyte NO efflux levels ($p<0.001$); (2) increasing the levels of intraerythrocytic NO oxidative metabolites namely nitrite ($p<0.0001$) and nitrate ($p<0.0001$); (3) enhancing the formation of GSNO ($p<0.001$). In conclusion, this study gains new insights into an unknown mechanism by which fibrinogen modulates the erythrocyte capacity to supply nitric oxide, which effects on inflammation profiles (generally associated with blood hyperviscosity and hyperaggregation) still need to be elucidated. Also, increased erythrocyte GSNO levels may be associated with platelet NO metabolism, its activation status and hypotension, which may be extremely relevant in the clinical setting as biomarkers.

[1] Poster apresentado em 9TH WORLD CONGRESS ON MICROCIRCULATION. Estudo realizado na Unidade de Biologia Microvascular e Inflamação, Instituto de Medicina Molecular, Instituto de Bioquímica, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa Portugal

FIBRINOGEN BINDING TO ERYTHROCYTE MEMBRANE: CD47 A POSSIBLE MOLECULAR TARGET[1]

S. de Oliveira, V. Vitorino de Almeida, A .Calado, H. S. Rosário, C. Saldanha

During the last decades, several studies have shown that high levels of plasma fibrinogen induce erythrocyte hyperaggregation, suggesting a possible interaction between this protein and red blood cells, (RBCs). Importantly an increased of the thromboembolic risk, in several cardiovascular disorders, is usually associated to both of these hemorheological parameters. There is a theory saying that the fibrinogen interacts with the surface of RBCs due to specific and non-specific mechanisms could be the main trigger to RBC hyperaggregation under inflammatory conditions. We hypothesize that human RBCs are able to specific bind soluble fibrinogen, and a membrane molecular target should be responsible for this phenomenon, although no one until now has pointed out one.

In this work we have applied two different techniques: fluorescent confocal microscopy and flow cytometry. RBCs were isolated and separated in different age fractions from whole blood collected from healthy donors. The data collected in both techniques support the idea that soluble fibrinogen binds to human RBC membrane interacting with it in an age-dependent manner. The youngest RBCs have shown to have higher interaction with soluble fibrinogen than the oldest. Importantly in this work we also point out to a specific molecular target for soluble fibrinogen at RBC membrane, the integrin-associated protein (IAP) or CD47.

Our work describes for the first time a specific and age-dependent interaction of soluble fibrinogen with human RBC membrane and we point out CD47 (human RBC isoform) as a possible molecular target for this acute phase protein. This interaction may well be responsible for a specific mechanism that under inflammatory conditions triggers erythrocyte hyperaggregation.

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