Chapter

FIBRINOGEN INVOLVEMENT IN HEMORHEOLOGY AND INFLAMMATION

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ABSTRACT

The aim of this chapter is to review the knowledge obtained from; (i) *ex vivo* and *in vitro* studies about the effects of human soluble fibrinogen molecules on blood components behavior and their effects on physiological and pathophysiological conditions, and (ii) *in vitro* studies conducted on experimental animal models.

Ex vivo studies which demonstrated the hemorheogical participation of plasma fibrinogen and its involvement in inflammatory vascular disease will be described. *In vitro* studies of fibrinogen binding to erythrocyte and neutrophils targets and their influence (at normal and high concentrations) on the biorheology properties of erythrocyte (NO metabolism under the external presence of endogenous or exogenous body molecules and by intra erythrocyte manipulation of redox or protein phosphorylation status) and on neutrophils activation will be presented.

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In vivo studies on the presence and absence of soluble fibrinogen in the bloodstream, and to enable the leukocyte recruitment will be presented. The participation of erythrocyte in leukocyte recruitment in inflammation will also be described.

Keywords: erythrocyte aggregation, erythrocyte deformability, nitric oxide, fibrinogen, inflammation

1. INVOLVEMENT OF FIBRINOGEN IN INFLAMMATORY VASCULAR DISEASE-*EX VIVO* STUDIES

Hemorheological and hemostatic factors are linked to normal vascular function and also in case of ischemic events favoring endothelial vascular dysfunction propitiating inflammatory response. Inflammation involves the interplay between inflammatory cells, vascular cells, pro and anti-inflammatory mediators. Inflammation occurs in intra and extra vascular fluid compartments where blood components, hemorheology, vascular hemodynamics, blood coagulation and fibrinolysis play a role. The blood plug stops the blood invasion of foreign infectious agents and inflammation contributes to fighting them and repairing the lesions occurring in tissues. The leukocytes are the agents that are recruited from blood flow at dysfunctional arterial vascular endothelium, for example, with incipient lipid accumulation in the artery wall submitted to disturbed shear stress (absence of laminar shear stress). Absence of normal laminar shear stress may reduce local production of endothelium-derived nitric oxide (NO) [1]. This endogenous vasodilator molecule also has anti-platelet adhesion, anti-inflammatory properties and can limit the expression of vascular cell adhesion molecule (VCAM-1) [1]. In addition to inhibiting natural protective mechanisms, disturbed flow can increase the production of certain leukocyte adhesion molecules (e.g., intercellular adhesion molecule-1; ICAM-1) [2]. The leukocytes roll, adhere, crawl and transmigrate guided by chemokines just to the inflammatory trigger ending (solving) the inflammation (resolution) [3]. When resolution processes fail,

the acute inflammation transforms in chronic inflammation (CI) or in some cases, like unsolved systemic inflammation, conduct to multi organ failure and death as in fatal sepsis [3].

Fibrinogen (Fib) is also present at high plasma levels in inflammatory diseases, thereby being considered an acute phase protein [5] and demonstrated by Lominadze as a pro-inflammatory factor responsible for vascular dysfunction [6].

Also CI can exist from unrecognized auto antibodies such as in systemic lupus erythematosus (SLE) and in rheumatoid arthritis (RA) [7]. Patients with SLE and patients with RA are in risk of vascular disease. In both SLE and RA, patient's decreased values of whole blood viscosity (WBV), decreased erythrocyte deformability (EEI) and increased erythrocyte aggregation (EA) were obtained [7]. Compared to RA, SLE women exhibited significantly higher WBV and higher serum fibrinogen levels, a parameter associated with greater risk of arterial thrombosis as well as with higher mortality in the general population [7]. It is known that the presence of erythrocyte aggregates slowdown the blood flow, reducing the level of capillary density and increasing resistance to blood flow [8, 9, 10]. EA was significantly correlated with several cardiovascular (CV) risk factors (body mass index (BMI), waist circumference, and HDL cholesterol and triglycerides levels) and with inflammation markers (haematocrit, sedimentation rate, and C reactive protein) [7]. No association was found between the values of EA and Fib concentration determined in SLE and RA's patients [7]. Among patients, WBV correlated positively with haematocrit, which are usually lower than those of control subjects. However WBV values measured when patients were in the active state of disease, decreases in parallel to the patient's hematocrit values [7]. These hemorheological disturbances are correlated with cardiovascular risk factors and markers of inflammation being more pronounced in sub-classes of patients with metabolic syndrome. Erythrocyte NO efflux is negatively associated with carotid intima-media thickness [7]. This work showed that whole blood viscosity and erythrocyte NO efflux are independently associated with subclinical atherosclerosis in SLE and RA women without previous CV events. So, it

was confirmed, the existence of a large array of hemorheological abnormalities in SLE and RA and provided evidence that both inflammation and traditional CV risk factors are related to altered rheological parameters of blood [7].

Inflammation is recognized as a contributor to the atherothrombotic process that occurs in vessel walls as being a risk factor of cardiovascular diseases. The existence of an atheroma plaque at macrocirculation induces the decrease of blood flow which is an influent factor in the blood perfusion at microcirculation [11]. The involvement of erythrocyte aggregation and erythrocyte resistance to flow in acute coronary syndromes was observed. [12]. Deficiencies in microcirculation could be undetected if studies were only conducted on the macrocirculatory network [13].

Beyond fibrinogen being recognized as a biomarker of inflammatory diseases, it is also a risk factor of vascular diseases such as acute myocardial infarction (AMI) [14].

The Glasgow Monica epidemiological study, conducted with normal healthy humans, showed an *ex vivo* significantly positive association between the values of plasma Fib concentration and EA values [15].

A hemorheological clinical follow-up was established in Portuguese survivors of acute transmural myocardial infarction consecutively enrolled from 1994 to 1999, with clinical and cardiological evaluation and hemostatic, inflammatory and hemorheological parameters assessment at 6, 12, 24, 36 and 60 months [16, 17, 18-20]. During this period of time, the survivors were divided into two groups of patients according to the occurrence of cardiovascular events. At hospital discharge, those patients that presented higher leukocyte counts, higher leukocyte elastase enzyme activity, higher plasminogen activator inhibitor (PAI-1) values, higher erythrocyte membrane viscosity, lower protein C activity or lower erythrocyte aggregation tendency, were found to develop cardiovascular events [16, 17, 18-20]. The absence of an association between erythrocyte aggregation and fibrinogen verified at hospital discharge appeared at 6 and 12 months in an inverse relationship. The same behavior was obtained between PAI-1 and fibrinogen and between PAI-1 and plasma viscosity.

The decrease of erythrocyte aggregation tendency determined at hospital discharge may result from the membrane rigidity in these patients [16]. The association observed between erythrocyte aggregation and inflammatory response (leukocyte elastase activity) and the hemostatic parameter PAI-1 may explain the occurrence of thrombotic events in the patients' survivors of AMI. Until 60 months of follow-up, some hemostatic, hemorheological and inflammatory disturbances, at hospital discharge, seem to be long-term independent predictors of recurrent cardiovascular events in transmural myocardial infarction survivors [16, 17, 18–20].

The multi-biomarker approach currently used in diseases studies, demonstrates a new association between lymphocytes and oxidized low-density lipoprotein, white blood cell counts and C-reactive protein in patients with AMI, suggesting a specificity of the immune response in AMI towards myocardial inflammation and remodeling [21]. So, monitoring Portuguese patients over time during 40 days after infarct onset, two groups of patients were identified using serial soluble CD40 ligand (sCD40L) alterations: a group of patients showing minor time-variations and sCD40L levels <1.8 ng/ml; and other group presenting a remarkable increase of sCD40L levels. A detailed examination of patients in the two groups was carried out, including clinical, genetic, biochemical, cardiac and inflammatory indicators. Patients with lower sCD40L levels had the highest N-terminal pro b-type natriuretic peptide (NT-proBNP) levels (marker of left ventricular dysfunction) and a higher prevalence of endothelial nitric oxide synthase (eNOS) Glu298-->Asp polymorphism G894T (G894T eNOS polymorphism; associated to poor vascular and endothelium function). The follow-up study of clinical data showed that patients with lower sCD40L levels have a worse recovery after AMI, being indicative of the prognostic value of sCD40L [21].

Besides the metabolic disturbance that characterizes diabetes disease, it is known to be joined with macro-and microvascular abnormalities. In a group of Portuguese diabetic patients, insulin dependence was found and their blood samples with higher values of fibrinogen are significantly positive associated with the degree of retinopathy and plasma viscosity

(PV) [22]. The inclusion of an evaluation of hemorheological parameters in the routine tests performed in the clinical laboratory has been suggested by the same authors [22].

In a group of patients with chronic renal failure being submitted to chronic haemodialysis, the erythrocyte aggregation values do not show significant difference when compared with the control group matched in age and gender, in spite of the increased fibrinogen levels observed in those patients [23]. Apparently, in this study, the fibrinogen effect on erythrocyte aggregation tendency is surpassed by anemia that favors the absence of changes in this red blood cell rheological property.

The same apparent lack of influence in the increased EA by higher plasma Fib concentration was obtained in a group of renal transplant recipients where fibrinogen levels were similar to the control group but the erythrocyte aggregation had higher values in the recipients [24]. Other authors also verified an increase of RBCs aggregation in a group of renal transplant patients [25]. The aetiology of chronic renal failure is diverse and consequently, when patients are submitted to kidney transplantation they do not sustain a homogeneous hemorheological behavior. The recipients, between them, exhibited dissimilarities allied to the occurrence of self-immune system adaptation and to inflammatory responses [26].

In a group of Portuguese hypertensive patients with eye vascular dysfunctions that were admitted to hospital, an increase in both EA values and plasma Fib levels [27] was observed. The RBCs hyper aggregation obtained in these patients may be an underlying present factor in the reduction of peripheral tissue oxygenation verified by other authors in severe hypertensive patients [28]. In general, in hypertension, several structural dysfunctions can occur like heart hypertrophy, smooth muscle fibrosis as well as elevation of artery stiffness, impairment of artery remodeling and microvascular rarefaction may be present at microcirculation with different degrees of severity [29]. It was verified by Jung [30] that patients with long term essential hypertension when evaluated by intravital microscopy and the intramuscular by pO_2 needle electrode, some of them present primary microcirculatory disorders and others secondary disorders [30]. In a previous work with patients with

essential hypertension and renal dysfunction, Jung [31] verified that when the number of capillaries per millimetre of epidermis were increased, the degree of retinal vascular changes (fundus hypertonicus) were more severe. Erythrocyte aggregation and plasma viscosity were elevated in those patients being the degree of retinal vascular alteration associated with plasma viscosity [31].

Changes in myogenic, metabolic, hormonal and trophic regulatory mechanisms present in vessel walls occur especially in those high perfused organs with low vascular resistance such as kidney, heart and brain. In general, the elderly have elevated blood pressure, increased plasma fibrinogen, elevated platelet aggregability and augmented blood viscosity [32-34]. In spite of this evidence we conduct a hemorheological and cardiological study with normotensive elderly people [35]. This group presents both erythrocyte aggregation and fibrinogen values lower than younger gender matched individuals without statistical significance [35] Although, it must be emphasized that the elderly group spent a healthy happy past life with regular and moderate exercise and a balanced intake diet [35].

In women with pregnancy-induced hypertension an increase in RBCs aggregation and adhesiveness was verified despite the absence of an increased fibrinogen level [36]. The authors in this work did not find any association between those hemorheological variables as opposed to other studies who reported that in a longitudinal study conducted with normal pregnant women both parameters increase and are associated [37].

The association between the strength of large RBCs aggregates and the inflammatory status and the high plasma fibrinogen concentration was also observed in patients with inflammatory bowel disease where a detrimental microcirculatory blood flow is present in the intestinal microvasculature [38].

Hemorheological parameters have been evaluated in patients with sickle cell disease (SCD). The results showed increased erythrocyte carboxihemoglobin (COHb) levels, suggesting exaggerated carbon monoxide production by vascular endothelium hemoxigenase, a marker of endothelial dysfunction. The intravascular haemolysis that occur in

patients with SCD may be the cause of the increase of hemoxigenase enzyme activity. A negative association is obtained between COHb and erythrocyte hemoglobin ($r = 0.502$; $p = 0.001$). The high values of methemoglobin (MetHb) obtained in the patients with SCD suggested an oxidative stress environment; higher fibrinogen values were associated in the higher MetHb quartile. Erythrocyte deformability showed, at low shear stress (when assessed by laser difractometry), high values that imply a better tendency to deform and to return to the previous shape. However, the reversibility is retarded in time when compared with the erythrocytes of normal individuals. The erythrocyte deformability values obtained at 6.0Pa and 30.0 Pa are directly associated with hematocrit (Ht). In turn, Ht values associated negatively to carboxihemoglobin and methemoglobin erythrocyte contents. Concerning all patients, the results showed decreased values of Hb and hematocrit; increased values of MetHb in crisis; higher fibrinogen values were associated in the higher MetHb quartile; lower values of MetHb were associated with the higher EA quartile. Lower values of COHb were associated with the higher ED quartile; higher values of ED were associated with the higher EA quartile. The improvement of ED in lower shear stress may be a compensatory mechanism that results from the stasis favoring the lower release of oxygen and less deoxygenated sickle-RBCs (with a more approximate biconcave discocyte shape). These unexpected results may be a consequence of having a broader diversity of erythrocytes shapes and density ranging from irreversible sickle cell to reversible, observed in blood smears of SCD`s patients. In conclusion, the results suggested an oxidative stress environment, exaggerated carbon monoxide production by vascular endothelium hemoxigenase, which is a marker of endothelial dysfunction associated with inflammation and hemorheological disturbances [39].

The appearance of structural changes in connective tissue and smooth muscle and functional changes in venous endothelium, defects in the microcirculatory network with deficient supplies of nutrients in the venous sector are inducers of varicosity appearance [40-41]. In venous disease, the upright position originates a decrease of oxygen partial pressure in tissues, capillary stasis and hypoxia that activates endothelial cells with increased

cytoplasmic calcium. This is crucial for the release of mediators of inflammatory responses, such as platelet anti-inflammatory factor (PAF), leukotriene B4, prostaglandins E2 and D2 inhibitors [42-45]. The release of histamine and serotonin stimulates the migration of leukocytes to the endothelium cells and expression of adhesion molecules in both cells [46]. Moreover, endothelial cells produce cytokines (interleukin-1 beta (IL- 1β), IL-6, TNF-α) and the prothrombotic von Willebrand factor that elicit the participation of monocytes and activated T lymphocytes in the inflammatory response [46]. Inflammation does not occur only in the *post*-capillary venules, but also in the large veins which contribute to a better understanding of the etiology of venous thrombosis and pulmonary embolism [47]. Flow-mediated vasodilation is impaired in patients with spontaneous venous thromboembolism, which is an indicator of endothelial dysfunction with pro-coagulation activity causing red thrombus, rich in RBC captured in the fibrin network at low shear rate (10–100 s^{-1} "stasis") [48,49].

In the venous endothelium of healthy individuals, as happens on the arterial side, there is a release of nitric oxide which regulates and maintains venous tone [50]. The NO secretion by vascular endothelial cells depends on shear stress [51]. In chronic venous insufficiency, the diameter of the vein is increased, decreasing both shear stress and the production of NO by endothelial cells during the initial phase [51]. After the expression of inducible nitric oxide synthase, higher amounts of NO are released to the lumen of the vessel, that, interacting with the superoxide anion (produced by leukocytes and macrophages) produce peroxynitrite that causes tissue oxidation of chronic venous ulcers [52].

During venous thrombi formation, fibrinogen and RBCs are the major components depending respectively from structure, plasma levels and RBCs associations and both from vascular dysfunctional endothelial cells targets and the presence of FXIIIa [53, 54]. Factor XIII activity mediates red blood cell retention in venous thrombi and also contributes to stabilizing (or not) the fibrin network [54].

The Portuguese Society of Hemorheology and Microcirculation (SPHM) with the aim to alert the Portuguese people and family physicians

to the chronic venous insufficiency (CVI) disease of lower limbs, performed in 2000 an epidemiological study [55]. The method for stratifying patients according to the severity of their CVI disease was based on clinical, etiological, anatomic and pathological stands (C.E.A.P).

The results showed a positive association between obesity and the C.E.A.P classes being the urban population associated with lower C.E.A.P classes while the rural one with the highest one [55]. Later a second epidemiological study was performed on the Portuguese population where it was concluded that the body mass index (BMI) is a risk factor of CVI of lower limbs [56].

More studies have been realized by others showing the association between the plasma fibrinogen concentration and the erythrocyte tendency to aggregate in metabolic, inflammatory and vascular diseases [57-64].

2. INVOLVEMENT OF FIBRINOGEN MOLECULE ON ERYTHROCYTE AND NEUTROPHILS PROPERTIES – *IN VITRO* STUDIES

Studies regarding questions about the erythrocyte and neutrophil membrane binding targets for fibrinogen and the contribution and effects of other endogenous and exogenous compounds to the erythrocyte aggregation and deformability in the presence of normal or high fibrinogen concentrations mimicking physiological or hyperfibrinogenaemia conditions are herein described. Also, the contribution of fibrinogen in the neutrophils recruitment by endothelial cells are presented.

2.1. Fibrinogen Influence on Erythrocyte Hemorheological Properties

Antony van Leeuwenhoek described, for the first time, the erythrocyte aggregation phenomena in 1699 in a letter sent to the Royal Society in

London and published in 1672 [65]. The tendency of red blood cells to aggregate and disaggregate is a factor in blood's rheological behavior [8-10]. In turn, generally, in vascular networks with low shear stress, the erythrocyte tendency to aggregate is increased [35, 66-68].

After several works on EA dependence on shear stress, other physiological factors were studied like vessel diameter known as the Fahraues Lindquist effect [69].

In the model of the cross-bridging of the erythrocyte aggregation, each fibrinogen molecule binds simultaneously to two erythrocytes forming a bridge between them originating lose aggregates morphologically similar to a stack of coins with a further three–dimensional structure of rouleaux formation variable in shape and dimensions [70].

β-estradiol is an endogenous circulating compound that *in vitro* decreases EA in spite of the unchanged fibrinogen concentration [71]. A fluorescence study evidenced modification in fibrinogen conformation induced by the presence of β-estradiol that may explain the need of a specific fibrinogen conformation to induce erythrocyte aggregation [72]. Also, modifications on fibrinogen structure resulting from changes in composition interfere with the EA tendency [73].

Higher levels of fibrinogen β chain polymorphism-455G/A were found in men associated with a higher erythrocyte aggregation (EA) tendency when compared to men with the -455GG genotype [73]. The same association has not been evidenced in females [73].

Pursuing our approach to study the influence of fibrinogen in EA in dependence of erythrocyte membrane proteins, we focused on band 3 protein phosphorylation degrees which were manipulated by using inhibitors of protein tyrosine kinase and protein tyrosine phosphatase [74]. Using blood samples from healthy individuals with plasma fibrinogen at normal range, the RBCs aggregation was found to be higher when band 3 is phosphorylated than when dephosphorylated, besides both band 3 states have lower values than the control aliquot. The band 3 protein phosphorylation status becomes an intrinsic factor in this erythrocyte rheological property [74].

Maintaining the plasma fibrinogen levels constant, other blood properties influenced EA like pH, lipoproteins classes, osmolality, and haematocrit [75, 76, 77, 78]. Autologous HDL-C increase EA in relation to LDL-C for the same range of lipoprotein classes concentrations in blood samples collected twice (the first one to obtain the different classes of lipoproteins and the second one to proceed the experimental model of study) from adult men volunteers [76]. Particle size, up to LDL-C diameter values, reinforce erythrocyte tendency to aggregate at the same plasma osmolality (particle number) range of values [76]. A significantly positive association between EA values and high plasma osmolality levels was observed in the dependence of the rise of haematocrit values in blood samples obtained from healthy blood donors [77].

Blood samples of healthy old persons showed higher values of erythrocyte aggregation than healthy young ones having an equal range of fibrinogen levels [79]. The same profile was verified *in vitro* between old and young erythrocytes obtained from blood samples of healthy persons [79]. This observation led to the concept of erythrocyte aggregability which is a RBC property independent of its membrane, structure, composition and internal metabolic status [80]. An adverse oxidative environment was reported to induce an increase of EA and a decrease of erythrocyte deformability by unbalancing the steady state of both oxidative and nitrogen stress [81].

The increase of erythrocyte aggregation intensifies the microvascular flow resistance, fills capillaries (become with no flow) and at lower shear rates like in the venous circulation, the aggregation of RBC affects the blood velocity profile and increases blood viscosity [82-84]. RBC surface properties and structure, such as surface charge and the ability of macromolecules to penetrate the membrane glycocalyx, greatly affect aggregation for cells suspended in a defined medium [85, 86].

Erythrocyte deformability or the reversible shape change of erythrocyte is a biorheological property influent on tissue oxygenation at the microcirculatory network [86].

At normal plasma fibrinogen concentration, the endogenous compounds like adrenaline and acetylcholine when in presence of blood

samples from healthy blood donors induced a significantly increased of ED at high shear stress [87, 88]. Maintaining the unchanged plasma Fib levels and enriched blood samples of healthy men with synthetic LDL/HDL, no variations of ED were found in relation to its absences [89].

For more details, which are out of this review, about the influence of membrane surface properties on ED can be read [90, 91].

When higher fibrinogen concentrations, similar to those found in inflammatory conditions, were incubated with blood samples, no erythrocyte deformability variations were observed [92]. At variance, erythrocyte deformability increases maintaining high fibrinogen levels when RBC membrane protein band 3 is phosphorylated in low shear stress [92]. This increase of ED was not verified when plasma Fib is at a normal range while activation or inhibition of protein kinase C increased or decreased ED respectively [74, 93].

2.2. Fibrinogen Binds Erythrocyte Membrane CD47 Influencing NO Metabolism

It was evidenced that soluble fibrinogen interacts with the erythrocyte membrane in a discrete punctuated pattern and in an age-dependent way, applying flow cytometry and confocal microscopy [94]. A higher interaction is established with younger erythrocyte that decrease in the presence of anti-CD47 antibodies arguing that erythrocyte membrane CD47 is a molecular target for plasma fibrinogen [94]. Other authors using immobilized fibrinogen at a tip in atomic force microscopy, suggested that fibrinogen α chains are recognized by αII β3 related integrin [95]. A study performed in mice identified the Arg-Gly-Asp-Ser (RGD) dependent binding process [96]. Those different targets on erythrocytes membrane which establish weak fibrinogen binding are dependent on its immobilized or soluble structural forms [54].

A different process is the erythrocyte aggregation/disaggregation where each fibrinogen molecule binds simultaneously to two erythrocytes

originating a bridge between them which all together originate stable or unstable *rouleaux* formation with variable shapes and dimensions [70].

In case of high blood viscosity paired with erythrocyte hyper aggregation NO and oxygen delivery to tissue with low oxygen, partial pressure may be compromised by the stasis [97].

A signal transduction pathway was described for the changes in human erythrocyte NO mobilization under the influence of fibrinogen binding to erythrocyte CD47 in the absence and presence of CD47 agonist peptide 4N1K.

An *in vitro* study showed that at soluble Fib in physiological concentrations, in the absence of 4N1K, the NO efflux from erythrocyte decreased and S-nitrosoglutathione (GSNO) levels, a NO reservoir molecule, increased [98].

The phosphorylation of the erythrocyte membrane protein band 3 in the presence of high fibrinogen concentration and in the absence or presence of 4N1K, increased the NO efflux [99, 100]. The scavenging NO RBC ability is not changed when both 4N1K and high fibrinogen levels are present, meaning the dependence of low cyclic adenosine monophosphate associated with adenylate cyclase inhibition by CD47Giα [101].

It was shown, in an *in vitro* model, that lower intra-erythrocyte cyclic adenosine monophosphate (cAMP) is an influential condition to the NO efflux in hyperfibrinogenaemia [101]. These results may be considered a useful therapeutic approach for the storage of blood that is used in transfusions.

During inflammation, both acetylcholine and high levels of fibrinogen normal values of NO efflux from erythrocytes are observed [102, 103]. This influence results from the fibrinogen binding to CD47 of RH complex, associated with $G\alpha_i$ protein, which is influenced by the membrane enzyme AChE molecular conformational states and enzyme complex-active or less active forms [104, 105].

This ability of RBC to scavenge NO acts as a compensatory mechanism against the overproduced NO by endothelial inducible NO synthase [106].

When the inhibitor of the erythrocyte Casein Kinase 2 (a cytosol protein that phosphorylates band 3 protein) is present in the erythrocytes suspensions with high fibrinogen concentration, the NO efflux is maintained as normal, confirming the dependence on band 3 phosphorylation [107]. Interestingly, the forskolin, an activator of the AC enzyme, normalizes the levels of NO efflux from erythrocytes in an *in vitro* model of hyperfibrinogenemia [107, 108]. At normal acetylcholine plasma levels, the erythrocyte NO efflux increases by a signal pathway dependent on membrane band 3 protein phosphorylation, Giαβ protein, acetylcholinesterase enzyme activity and molecular conformations [107].

2.3. Fibrinogen Binds Neutrophils Membrane Influencing Its Biophysical Properties

Beyond the neutrophil multi-ligand receptor Mac-1 for fibrinogen as suggested by others, the plasma glycoprotein, at physiological concentrations binds to another distinct neutrophil membrane receptor [109, 110].

The fibrinogen plasma molecule induces two kinds of human neutrophils responses or sub-populations, namely low and high oxygen free radicals production without interfering with the expression and activation of the Mac-1 integrin [110]. These authors raise the hypothesis that Fib could as well play a role in modulating the adhesive behavior of the neutrophil towards the vascular endothelium under normal physiological conditions [110]. It was observed that fibrinogen molecules shield neutrophils from excessive adhesion towards the vascular wall of endothelium cells obtained from human umbilical veins. In other words, the binding of soluble fibrinogen to the neutrophil membrane, under normal conditions, may by a hypothesis of the authors to loosen putative neutrophil-endothelium interactions, making neutrophils easily detachable from the endothelial wall [111]. Such a putative role for soluble fibrinogen could, for instance, be instrumental by preventing an unwanted accumulation of neutrophils in the vasculature and subsequently, avoiding

thrombus formation and growth [111]. Nevertheless, fibrinogen inflammation activities have been suggested to be affected by the vascular flow and the shear forces, to which the vascular walls are exposed [112].

3. INVOLVEMENT OF FIBRINOGEN MOLECULE ON INFLAMMATION – *IN VIVO* STUDIES

Erythrocytes and leukocytes play an important role in the biomechanical properties of blood and at physiological normal conditions margination of the free flow leukocyte towards the endothelial cell where observed [113]. It was found that the lattice Boltzman solver used is fully adaptive to the measured experimental leukocytes rolling and adhesion, hemodynamics and hemorheological parameters obtained *in vivo* from animal models [114, 115]. The leukocyte margination process was clarified using numerical simulations based on experimental results from the leukocytes' interactions with erythrocyte, evidencing the presence of one region of maximum shear stress and two regions of minimum shear stress on the surface of leukocytes close to the endothelial wall. It was verified that the collective hydrodynamic behavior of the cluster of recruited leukocytes establishes a strong motive for additional leukocyte recruitment. The leukocytes are recruited to the wall with the addition of trapping forces and four stagnant regions surrounding the cell in addition to lateral motions towards the wall. It was found that the shear stress in the endothelium gets higher as the clusters move in the main stream enabling early initialization of the rolling process [114, 115].

Fibrinogen interactions with erythrocytes, leukocytes and platelets which play important functions in the mechanical properties of blood, are responsible for different physiological body functions and diseased states, such as inflammation [116]. Inflammation is a complex process in time and space, including dynamic interaction where the influence of mechanical stresses on the biochemistry of cells has to be taken into account. At the physiological level, fibrinogen promotes reversible leukocyte binding to

endothelium, while the significant increase of fibrinogen level verified during inflammation attenuates leukocyte adhesion to the vascular endothelium derived from its participation in the fibrin network and EA *rouleaux* formation [117].

Using intravital microscopy to observe the post-capillary venules microcirculation in an animal model of inflammation, an increase in the number of rolling and adherent neutrophils after 4 h of inflammation, confirmed the inflammatory state induced by PAF as an inflammatory agent. A decrease in the leukocytes' rolling velocity, as expected, decreased until 6h after the inflammation response slowed down [118]. The vessel vasodilation, an inflammatory characteristic, deformation of neutrophil during rolling along the endothelial wall with formation of tethers was observed [118]. Tethers bind to the endothelium via P selectin/P selectin glycoprotein ligand 1 (PSGL1) forming a temporary anchorage [119]. Other observations of the images allow understanding of the role of leukocytes in the approach to the endothelium cells in case of inflammation [119]. Whereas one free cell is crawling toward the endothelial surface, the shape of the adherent cell is deforming. On the other hand, the presence of the adherent cell, slightly, slows the motion of other rolling cells, helping in the capture of the free leukocyte. The perturbed blood flow, pushing the cell along the endothelial wall is less important in the case of the presence of adherent cells, which could be explained by the promotion of the slow rolling [119]. This interaction is needed and is important to later promote efficient leukocyte adhesion with the endothelial cells, for further transmigration to the injury tissue, to solve the acute inflammatory response. The erythrocyte aggregation verified in these *post*-capillary venules is an additional contributing factor in the leukocyte approach to the vessel wall, necessary to fight inflammation. Also, a decrease in the erythrocyte deformability has been observed during an inflammatory response thus confirming the role of the erythrocyte properties in inflammation [118].

Homozygous fibrinogen ($\alpha^{-/-}$) mice displayed an impaired number of adherent leukocytes when compared to the wild-type. Neutrophil

recruitment is compromised by hypo-fibrinogenemia in acute inflammation *in vivo* model [120].

CONCLUSION

Follow-up clinical studies need to be developed in all acute and chronic inflammatory diseases to standardized immune, hemorheological, hemostatics and inflammatory biomarkers to be included in the routine analysis. Erythrocyte and neutrophil binding to fibrinogen are players of inflammatory response. The erythrocyte CD47 binds fibrinogen. If the over expressed CD47 in sickle cell binds to fibrinogen in the absence and presence of thrombospondin and if the binding is dependent on the patient's steady-state-to-crisis transition is a matter to be explored. The lower EA, verified at the hospital discharge of patients after an AMI, acting as a prognostic biomarker of worse further events needs to be explained. Mimicking hyperfibrinogenemia, *in vitro*, Fib induces NO efflux from erythrocytes when band 3 is phosphorylate and cAMP levels are low.

The identification of other neutrophil membrane targets for fibrinogen beyond MAC-1 needs to be achieved. The process of neutrophil binding to endothelium cells mediated by fibrinogen is another complex issue to be known.

The data obtained from the animal model of inflammation allied to mathematical modeling are useful to understand the influence of the mechanical forces and blood components interaction with vascular networks to further surgical and noninvasive instrumental development.

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