

The red blood cell in vascular occlusion

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*This article is dedicated to the blessed memory
of Professor Amiram Eldor*

Key Words

erythrocyte aggregation, erythrocyte deformation, erythrocyte adherence

Abstract

Red blood cells (RBC) have unique flow-affecting properties - namely, aggregability, deformability and adherence to endothelial cells (EC) - which play major roles in blood flow. Under normal flow-induced shear stress RBC are dispersed, their adherence to EC is insignificant, and they are sufficiently deformable to enable tissue perfusion. However, in pathological conditions that are associated with low-flow states (e.g., trauma, ischemia), elevated plasma components (mainly fibrinogen), or altered RBC properties (e.g., hemoglobinopathies, oxidative stress, inflammation, diabetes), RBC flow properties are altered and present a circulatory risk.

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Red blood cells (RBC) have special flow properties that play a major role in hemodynamics, and their normal function is essential for adequate blood flow and tissue perfusion in large and small blood vessels. Abnormal deviations in RBC

flow properties have been linked to the pathophysiology in numerous diseases associated with circulatory disorders. These abnormalities evolve from biochemical changes in the cell membrane and content. Both the hemodynamic behavior as such, and the related biochemical changes, may affect the function of other cells of the vascular system. All-in-all, alterations in RBC structure and function may independently and synergistically impair blood flow and induce vascular occlusion. RBC function in the vascular system and circulatory disorders is reviewed herein.

RBC flow properties

RBC properties that determine their hemodynamics conventionally relate to their self-aggregability, deformability and potential adherence to endothelium.

RBC aggregability refers to their ability to form multicellular aggregates, normally in a rouleaux shape, in the presence of plasma proteins or other macromolecules. The extent of aggregation is determined by opposing forces: the repulsive force between the negatively-charged cells, the cell-cell adhesion induced by the presence of the plasma proteins, and the disaggregating shear force generated by the blood flow [1]. Studies in animal models have suggested that RBC aggregation as such contributes to vascular resistance to flow. At the same time, the aggregation, particularly in veins, may be accompanied by RBC migration to the center of the blood vessel, thus forming an RBC-free layer near the vessel walls. This

might exert opposing effects on vascular resistance, as it increases viscosity in the center of the vessel and reduces it near the walls. The resultant effect on vascular resistance might vary with type and size of blood vessels. For example, it has been found that in resting muscle 60% of venous vascular resistance is attributed to RBC aggregation, and it was concluded that the flow-induced disaggregation makes a major contribution to the concomitant reduction in vascular resistance [2].

Normally, the blood flow is sufficient for dispersion of the aggregates before entering the capillaries, which is essential for adequate tissue perfusion. However, in pathological states, which are associated with low-flow states or altered RBC properties, larger and stronger-than-normal RBC aggregates may form, and these might be resistant to disaggregation by the blood flow. In addition, RBC aggregation, especially in low-flow states, parabolically enhances blood viscosity, resulting in increased resistance to blood flow and further reduction of flow rate. Thus, enhanced aggregability has the potential to hinder and even block blood flow in small blood vessels, leading to reduced tissue perfusion, ischemia and infarct. In addition, the migration of RBC aggregates to the center of blood vessels, might attenuate oxygen diffusion through the cell-free layer to the vessel wall [3].

Apart from the direct hemodynamic effects, RBC aggregation might influence the function of other cells of the vascular system. Elevated aggregation can (1) activate endothelial cells (EC) due to the subsequent increase in shear stress at the vessel wall; (2) facilitate platelet migration to the vessel wall and their interaction with EC; and (3) facilitate the margination of white cells to the vessel walls and their adhesion to endothelium [4].

Taken together, elevated RBC aggregation has the potential to impair blood flow and contribute to vascular occlusion in more ways than one. Indeed, increased RBC aggregation has been observed and implicated in the pathophysiology of numerous diseases with circulatory disorders, such as cardiovascular diseases, inflammation, diabetes, hyperlipidemia, sickle cell disease, thalassemia and trauma [1]. In line with that, epidemiological studies have pointed to RBC aggregability as being a strong cardiovascular risk factor.

RBC deformability refers to the ability of the cells to adapt their shape to the dynamically changing flow conditions in order to minimize their resistance to flow, and to enable their passage through small blood vessels. Under normal conditions, RBC deformability allows individual red blood cells, the mean resting diameter of which averages 7 μm , to traverse nutritive capillaries with diameters no more than 3–5 μm , thus supplying the tissues with oxygen. Decreased deformability will result in impaired perfusion and oxygen delivery in peripheral tissues.

RBC deformability is important for adequate flow in large vessels as well; In response to fluid shear forces, erythrocytes

deform from the resting biconcave into ellipsoid shapes and align themselves with their long axes parallel to the fluid stream [5]. Therefore, the ability of the cell to deform allows a reduction of the bulk viscosity in the larger vessels, and allows blood to remain fluid even at high hematocrits.

RBC deformability has been postulated to be a major determinant of red blood cell survival [5]. Passing through the spleen, the red blood cells must traverse extremely narrow endothelial slits with a diameter of 0.5–1.0 μm , which makes the spleen an effective filter. Reduction in RBC deformability, as with RBC aging, may impair their passage and lead to splenic sequestration and destruction.

RBC deformability is determined predominantly by the cytoskeleton and the intracellular viscosity. Thus, structural changes in RBC and subsequent impaired deformability contribute to hindrance of blood flow, particularly in low-flow states [5]. Reduced RBC deformability has been implicated in microcirculatory disorders observed in various diseases, particularly in diabetes, and hemoglobinopathies, such as sickle cell disease, thalassemia and malaria [5].

RBC adherence to endothelial cells (EC) of the blood vessel walls (hereafter ‘adherence’) has been considered in recent years to be a prominent catalyst of blood vessel occlusion, particularly in the microcirculation. Normally, RBC adherence to EC is insignificant. However, in many pathological conditions; alterations in RBC membrane make them adherent to EC. Accordingly, enhanced RBC adherence, which may block capillaries, has been implicated in pathophysiology relating to RBC abnormalities such as in sickle cell disease, cerebral malaria, diabetes, and thalassemia, and was found to correlate with the occurrence and severity of vaso-occlusion [6].

RBC adherence to the vessel wall, which sequesters them from blood in large vessels, might impair local flow patterns and shear stress, and thus might activate EC. In addition, oxidized RBC, such as those formed in hemoglobinopathies or diabetes, can apply oxidative stress on EC and activate them.

RBC biochemical factors affecting hemodynamics

RBC flow properties are determined by biochemical components of their membrane and cellular content, and changes in these factors in pathological conditions are responsible for altering their hemodynamic behavior. In general, it seems that different biochemical factors independently affect the different flow properties, but some might exert a multiple effect. In addition, the biochemical changes can affect the vascular system independently of their effect on RBC rheology. These aspects of RBC are discussed below.

Sialic acid (SA): As noted above, the extent of RBC aggregation at a certain shear stress, is the result of the repulsive force between the negatively-charged cells, and the cell-to-cell

adhesion induced in the presence of plasma proteins. Therefore, the level of surface sialic acid, which is the main contributor to the surface charge, is the major factor in RBC aggregation, and the aggregation is particularly sensitive to changes in surface sialic acid level [1].

On the other hand, the role of RBC surface SA in their adherence to EC is not clear, as disparate results have been reported. It has been suggested that surface SA does not play a role in RBC deformability, but a recent study has suggested that decreased SA reduces RBC deformability.

Membrane phospholipid composition and in/out distribution: Several studies have suggested that changes in membrane lipid composition and the in/out phospholipid (PL) distribution alter RBC flow properties [7]. The in/out PL distribution in RBC membrane is a major factor in determining the RBC shape (mainly through their interaction with the cytoskeleton) [5]. Decrease in PL/cholesterol ratio has been reported to increase RBC aggregability [7], and increased sphingomyelin (SM) ratio has been shown to be associated with elevated RBC aggregation and adhesion to EC. It has been suggested that changes in membrane lipid composition and distribution might also modulate the cell deformability, but this seems to be indirect, via effects on the cytoskeleton [1].

Of the RBC membrane lipids, PS at the cell surface seems to play a special role in the function of blood cells. Normally, PS is located at the membrane inner leaflet, but it is translocated to the outer leaflet in hemoglobinopathies and oxidative stress states [8]. The exposure of PS, which can bind to different proteins, at the RBC surface, has been clearly shown to induce RBC adhesion to EC. On the other hand, although PS binds to fibrinogen, which is a potent inducer of RBC aggregation, the possible influence of cell surface PS on RBC aggregability is not clear.

In addition to its effect on RBC flow properties, the exposure of PS at the RBC surface also affects other cells of the vascular system, EC and platelets in particular. By inducing RBC/EC interaction, PS activates EC at the vessel wall, and thus contributes to the formation of inflammatory conditions. The adherence of pathological RBC, such as thalassemic or sickle cells, has been correlated with increased thrombosis incidence. This might be consequent to EC activation, or due to hemodynamic hindrance at the vessel wall, which might elaborate platelet/EC interaction. However, PS exposure may activate platelets directly; RBC with PS at their surface, such as thalassemic and sickle RBC, induce hypercoagulability, and this was attributed to direct PS-induced activation of platelets [8].

Band-3: Another membrane component that seems to play an important role in RBC/EC interaction is band-3. This is a major membrane protein of RBC, which is known to be an ion exchanger, but growing evidence suggests that it is involved in RBC adhesion to endothelium [9]. In particular, it has been suggested that the adhesion is induced by clustered, rather than

monomeric, band-3, and that the clustering can be induced by oxidative stress. To examine this hypothesis, we determined the adherence to EC of RBC following their treatment with inducers of band-3 clustering, specifically acridine orange or Zn^{++} . It was clearly found that the band-3-clustering treatment induced RBC adhesion to EC, in a dose-dependent manner.

Adhesion molecules on RBC: RBC membrane contains a number of molecules that in other cells (white cells, EC) are involved in cellular adhesion [9]. Yet, under normal conditions, RBC adhesion to EC is insignificant. It has been assumed that these molecules at the RBC surface are involved in hematopoiesis and clearance of RBC [9], but their possible role in intercellular interaction is questionable. Some adhesion molecules, such as CD36 and CD44 are found in pathological RBC, such as sickle cells, and in reticulocytes, but disparate results have been reported as to their existence in normal mature RBC [9]. CD44 is a receptor for hyaluronic acid, and binds also to fibronectin. It can thus mediate RBC attachment to vascular extracellular matrix (under damaged endothelium), and accelerate thrombus formation.

Spectrin cross-linking: As noted above, RBC deformability depends predominantly on the cytoskeleton, mainly spectrin state. Cross-linking of spectrin and/or subsequent membrane rigidity are characteristic of RBC subjected to oxidative stress, such as in hemoglobinopathies and in inflammatory conditions.

Hemoglobin: Hemoglobin determines the RBC intracellular viscosity and thus plays a role in the cell deformability. Increased hemoglobin concentration and polymerization are associated with increased intracellular viscosity and respective reduced cell deformability [1]. Abnormal hemoglobin may strongly impair RBC properties in hemoglobinopathies, which are usually associated with oxidative stress. For example, in β -thalassemia, oxidative stress is exerted by the imbalance between the hemoglobin chains. The excessive α -globin adheres to the cell membrane (inner leaflet), and induces further alterations in the cell membrane, leading to increased membrane rigidity.

Interrelationship between RBC flow properties

The observation that some biochemical factor in RBC might be involved in the control of more than one flow property, led to the assumption that under certain pathological conditions, change in one property is accompanied by similar change in another one(s). This was assumed particularly for RBC aggregation and adherence to EC, which, being intercellular interactions, are governed by cell-surface factors. However, experimental data suggest that RBC aggregability, deformability and adherence may differentially change in dis-

parate modes or remain unchanged independent of each other. This is demonstrated in Table 1, which summarizes the change in RBC aggregability, adherence and deformability, for diverse pathological conditions, conventional blood banking procedures, and experimental inflammatory conditions [1,5,6,10,11, 12].

Summary: RBC in vascular occlusion:

The findings and considerations delineated above, suggest that RBC are involved in the control of circulatory processes by a number of diverse mechanisms, and pathological RBC may facilitate vascular circulatory disorders and hemostasis, by both their hemodynamic behavior and by direct biochemical activations, as illustrated in Fig. 1.

Elevation of RBC aggregation, and subsequent increased blood viscosity, may hinder blood flow in large vessels and occlude microvessels; facilitates EC and platelet activation by modulating shear stress and attenuating flow rate at their environment (which increases intercellular collision); and promotes white cell margination and adhesion to vessel wall endothelium.

RBC adhesion to EC sequesters RBC; occludes microvessels, capillaries in particular; impairs flow pattern at the vessel wall and activates EC.

RBC with reduced deformability (increased rigidity) may occlude microvessels, capillaries in particular; attenuate blood flow and change its pattern; and facilitate platelet activation.

The accompanying biochemical changes in RBC membrane and content may further affect cells of the vascular system. In particular, oxidized RBC, such as those obtained in hemoglobinopathies, exert oxidative stress on the vascular system, and thus may activate EC and platelets. PS and adhesion molecules at the RBC surface play a special role in these processes, as they mediate adhesion of oxidized RBC to EC, and subsequent EC activation, and furthermore, PS contributes to blood coagulability as it can directly activate platelets.

All-in-all, pathological changes in RBC structure and hemodynamic functions, may act independently and synergistically in inducing circulatory disorders and vascular occlusion.

Table 1. Aggregability, adherence and deformability of RBC under pathological conditions:

Disease	Aggregability	Adherence to EC	Deformability
Myocardial Infarct	↑	No data	↓
Inflammation	↑	↑	↓
Angina	↑	No data	↓
Bacterial Sepsis	↑	No data	↓
Diabetes	↑	↑	↓
β-Thalassemia Major	↑	↑	↓
β-Thalassemia Intermedia	No effect	↑	↓
Phenylhydrazine-treated	No aggregation	↑	↓
Cerebral Malaria	↑	↑	↓
High Blood Pressure	↑	No data	No data
Stroke	↑	↑	↓
Sickle anemia	↑	↑	↓
Storage	↑	↑	↓
γ-irradiation	No effect	↑	↓
PDVI-treated	↑	No effect	↓
H ₂ O ₂ -treated	No aggregation	↑	↓

↑ = increase; ↓ = decrease.

Phenylhydrazine - used for induction of β-Thalassemia phenotypes.

PDVI - photo dynamic virus inactivation

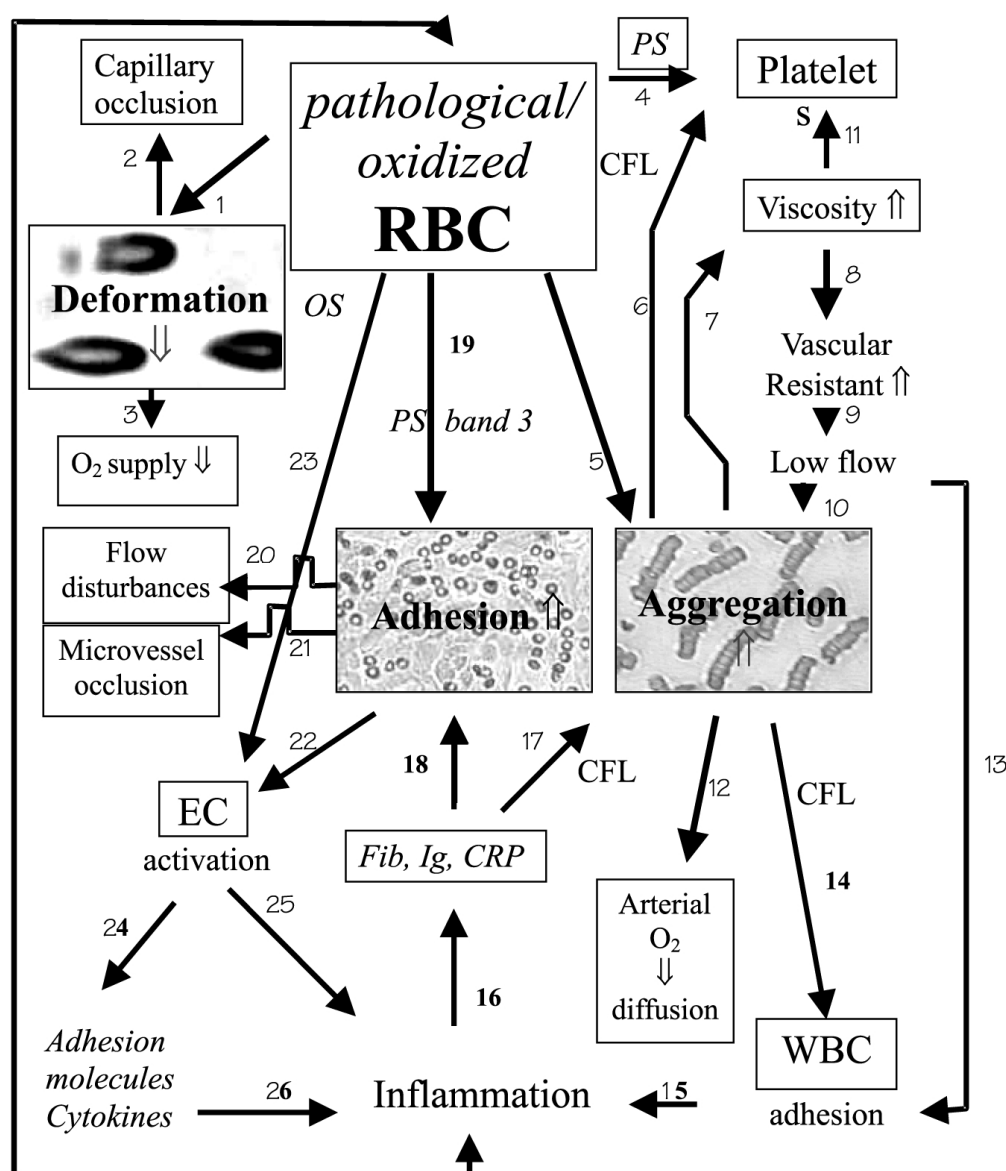


Fig. 1. Functions of the vascular system affected by RBC: Respective to the numbers presented, the Figure shows that: (1) Pathological, mainly oxidized RBC have reduced deformability, which leads to capillary occlusion (2), and attenuates O₂ supply (3), [1]; (4) RBC oxidation induces translocation of PS to the cell surface which facilitate platelet activation [8]; (5) Pathological/oxidized RBC have enhanced aggregability [11], which might form a cell-free layer (CFL) near the vessel walls, resulting in enhanced platelet interaction with the vessel wall (6) [13], and reduced O₂ diffusion (12) [3]; (7) RBC aggregation strongly elevates blood viscosity [1], which increases vascular resistance to flow (8) and decreased flow rate (9). This further increases RBC aggregation (10), thus initiating a self-accelerating “viscous cycle” that leads to vasoocclusion and ischemia [14]. (11) Increased blood viscosity exerted by RBC aggregation facilitates platelet migration to and interaction with the vessel walls; (14) RBC aggregation, by forming the CFL, facilitates WBC migration to the

vessel wall [4], and together with the reduced flow rate (13), promotes their adhesion to the endothelium [15]; (15) WBC adhesion to EC forms inflammatory conditions, as expressed by inflammatory agents (16) which further propagate RBC aggregation (17) [10] and adhesion to EC (18) [17]; (19) Pathological/oxidized RBC, with clustered band-3 and PS [16] at the cell surface adhere to EC, and induce flow disturbances (20) and micro-vessel occlusion (21) [6]. (22) RBC adhesion to EC, as well as direct interaction of oxidized RBC with EC (23) activates EC to express adhesion molecules and cytokines (24), thus inducing inflammatory conditions (25), which might be associated with the production of inflammatory agents (16) that further propagate RBC aggregation (17) and interaction with EC (18). (27) In turn, inflammatory conditions, often associated with the production of reactive oxygen species, might induce the formation of pathological/oxidized RBC, and further contribute to RBC-induced hemodynamic disorders.

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