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Review Article

The role of hemoglobin and red blood cells in arterial disease and atherosclerosis: Mechanisms and implications

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Abstract

The Objective is to review the mechanisms by which RBCs and hemoglobin contribute to arterial disease and atherosclerosis and to explore the implications of these mechanisms for disease progression and potential therapeutic interventions. Red blood cells (RBCs) and hemoglobin influence arterial health by affecting blood viscosity and frictional forces on arterial walls. In atherosclerosis, RBC collisions release cytotoxic heme-Fe++, promoting cell death and contributing to disease progression. This local hemolysis is also linked to oxidation in abdominal aortic aneurysm. The regulation of blood viscosity by RBCs is crucial for maintaining regional metabolic requirements and arterial function. Atherosclerosis is a leading cause of cardiovascular diseases, influenced by factors such as hypertension, high cholesterol, smoking, and diabetes. Emerging evidence suggests that red blood cells (RBCs) and hemoglobin play significant roles in the disease's pathogenesis. A comprehensive literature review was conducted, focusing on the interaction between RBCs, hemoglobin, and the arterial wall, including their roles in oxidative stress, inflammation, blood viscosity, and plaque formation.

Keywords: RBC, Hemoglobin, arterial wall, Atherosclerosis, Clotting etc.

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1. Introduction

Arterial disease and atherosclerosis are characterized by the narrowing and reduced blood flow to the organs that results from the accumulation of lipid-rich plaques in the artery walls. The pathophysiology of these disorders is complicated and involves many risk factors. Hypertension, high cholesterol, smoking, diabetes, and hereditary factors are just a few examples. Haemoglobin and red blood cells (RBCs) have emerged as key participants in the pathogenesis and progression of atherosclerosis and vascular disease, according to recent research.[1,2,3,3]

Red blood cells (RBCs), also known as erythrocytes, are the most abundant circulating cells, comprising approximately 90% of blood cells with a concentration of 4.5 × 10^6/mm³. Following RBCs in abundance are polymorphonuclear leukocytes (PMNs) at 8000/mm³ and platelets at 300,000/mm³. However, arterial structures are continuously subjected to mechanical, biochemical, and cellular stress, primarily driven by hemodynamic forces, leading to repeated vascular injuries.[5,5]

This study aims to explore the physiology and pathology of the arterial wall, emphasizing the role of RBCs and their membranous and cytosolic components. By examining observational data from human studies, we highlight the presence of RBCs, hemoglobin, and redox-active iron and analyze how intratissue hemolysis and Fe^{2+}-induced oxidative stress contribute to cellular toxicity and vascular damage. Ultimately, RBCs and their byproducts are consistently observed in both cardiovascular (CV) and non-vascular (NV) diseases, underscoring their significant role in human pathology.[18] Atherosclerosis is a chronic inflammatory disease of the artery wall and the largest cause of death in the western world. Recent studies have

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characterized atherosclerosis as a multifactorial disease due to the many risk factors for cardiovascular disease that have been identified. These include hyperlipidemia, hypertension, diabetes mellitus, male gender, obesity, and a family history of cardiovascular disease. Atherosclerosis begins as a "reaction to insult" that favors immunological activation and endothelial dysfunction and many of these variables encourage processes of inflammation and oxidative stress, which are two fundamental hallmarks of atherosclerosis. The immune system, which includes both innate and adaptive elements, has both beneficial and detrimental effects on the formation of atherosclerotic plaques.[13] Most leukocytes in atherosclerotic lesions are macrophages, dendritic cells (DCs), and activated T and B lymphocytes, and their secretory activity, which includes the release of proinflammatory cytokines and matrix-degrading proteases (Rita Businaro et al.)**,** may be linked to plaque rupture. In particular, immune cells of both innate and adaptive immunity may be activated by various endogenous molecules that have undergone chemical and/or structural modification following oxidative or glycation processes. In this approach the immune system activation gives rise to low level inflammation leading to the delayed development of atherosclerotic disease.[15]

1.1. The arterial wall

Artery walls consist of three layers called the tunica intima, tunica media, and tunica adventitia (**Figure 1**). The outermost layer, known as the tunica adventitia, is responsible for keeping the artery's mechanical and structural properties intact and preventing it from becoming too dilated.[28]

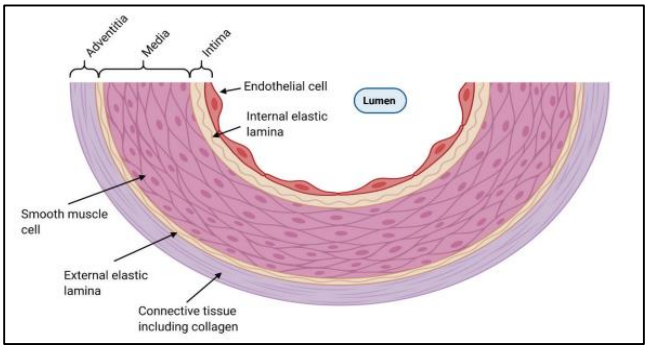

Figure 1: Different layers of the arterial wall

The proportion of each layer varies between vascular beds. Gradual narrowing, atrophy of the tunica medium, and blurring of the boundaries between the three layers characterize the transition from an artery to an arteriole. The inner lining of an artery has only smooth muscle cells and no elastic fibers. Yet, the endothelium monolayer persists throughout the whole circulatory system.[10]

It also has vasa vasorum, which allows the artery to respond to brain stimulation by receiving nutrients and nerve ends**.** The main components of the tunica medium, which are arranged in spiral layers, are vascular smooth muscle cells and elastin fibers. The intima of Tunica is located in the wall's luminal region, close to the bloodstream. The intima consists mainly of the internal elastic lamina and, more importantly, the monolayer of endothelial cells, which will be discussed in more depth below.[23] The endothelium monolayer actively contributes to vascular homeostasis through paracrine mediators such as nitric oxide (NO), bradykinin (KIT), and prostacyclin (PC), and vasoconstrictors such as endothelin-1 (ET-1) and angiotensin II (Ang II).

1.2. Atherosclerosis

Coronary artery disease (CAD), also known as atherosclerotic vascular disease, is the leading cause of cardiac arrest, heart attack, peripheral arterial disease, and stroke.[27] When the endothelium monolayer breaks down, atherosclerotic plaque development begins and progresses over decades (**Figure 2**).

After the endothelium has been compromised, the first step in a pathogenic cascade is for monocytes to move from the bloodstream to the intima. Sub endothelial fatty streaks caused by trapped lipoproteins are a sign that the endothelium has been compromised**.** The increased generation of reactive oxygen species (ROS) is one of the main reasons of this, and this will be described in further detail in the following sections. Foam cells form when monocytes undergo terminal differentiation into macrophages after taking in lipoproteins. This mechanism causes an inflammatory response and endothelial activation of surrounding cells, such as vascular smooth muscle cells, which leads to proliferation of the intima and the creation of a fibro muscular plaque (VSMCs). A fibrous cap will grow over the top of the plaque during the first phases of plaque formation (structural remodelling, inflammatory cell attraction, calcification, and cell death).[25,26] Angina pectoris and limb claudication are symptoms of a gradually narrowing lumen. Depending on the plaque's anatomical position, the fibrous cap covering it may weaken and become more easily ruptured as it expands. It's possible that this might cause a heart attack or stroke. Some of the unknown factors that contribute to the creation of the susceptible plaque include a large necrotic core, increased inflammation, neovascularization, and intraplaque haemorrhage.[17]

Throughout time, several approaches have been investigated to inhibit the development of the susceptible plaque. These treatments may be broadly classified into two categories: those that reduce vascular inflammation and those that treat dyslipidaemia. Even if novel cholesterol targets, such as the proprotein convertase subtilisin/kexin type 9 inhibitor, have earned a stronger place in the therapeutic arsenal, the decades-long success story with statins continues to help patients at high risk for CVD.

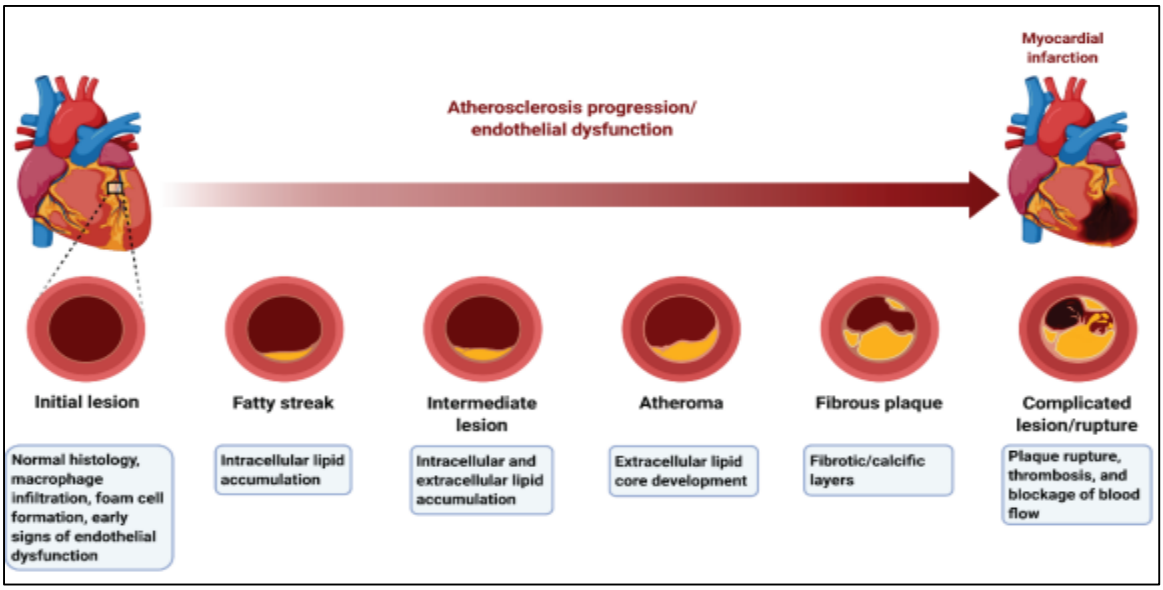

Figure 2: The gradual formation and molecular changes during the development of the atherosclerotic plaque are initiated by dysfunctional endothelium.

Clinical investigations focusing on ROS have not demonstrated any beneficial effects in people with cardiovascular disease**.** It is unknown why this is the case, but it may be because the anti-oxidants used, such as vitamin C or E**,** are too generalized or because treatment was initiated too late in the disease's course.[11,12] As the first positive clinical trial to establish the inflammation theory in atherosclerotic disease, the CANTOS trial found that patients treated with a monoclonal antibody targeting interleukin (IL)-1 had a decreased risk of future cardiovascular events**.** Researchers have shown that colchicine helps patients with acute myocardial infarction and chronic coronary syndrome by acting as a secondary prophylactic measure**.** As a result, it is important to treat vascular inflammation in an effort to avoid atherosclerotic vascular disease. Yet, the objective of these researches was not to find a cure for type 2 diabetes. The risk of cardiovascular events can be lowered not only by pure anti-inflammatory targets, but also by pharmaceuticals that target earlier stages in the continual production of the susceptible plaque, such as defective endothelium.[27]

1.3. The impact of red blood cells on the arterial wall

It is now generally accepted that RBC accumulation in the arterial wall, membrane cholesterol release leading to cholesterol crystals**,** in situ hemolysis, and substantial oxidative stress[28] all play a role in the development of atherosclerosis. Flow impingements have arisen because the circulatory system has evolved from a closed "in-series" system to an open "in-parallel" system, with various variations in the form of the arteries**.** These geometrical shifts lead to a loss of flow laminarity and an increase in the entropy (internal energy instability and dissipation) of the particulate component of the blood, both of which promote the collision of blood cells with the wall and among themselves.[20] Although the hemorheology of circulating cells has a role, the impact of colliding blood cells on the wall is mostly determined by the angle of the bifurcation and the luminal constriction. Collision forces operating on the wall are given by the formula F = m.v, which states that they are proportional to the mass (m) and velocity (v) of the interacting RBCs at each given point. RBCs generate intimal rips, micro-fissures, and the formation of tiny mural hematomas when they collide with the uneven geometry of the wall, either alone or in conjunction with other biomechanical forces operating on the wall. Luminal dilatation is associated with blood stagnation and vortices that promote endovascular coagulation, both of which can lead to collisions between circulating cells.

It has been hypothesized that the phylogeny of circulating cells, which includes hemocytes, innate immunity, and clot formation, may affect the behavior of these cells and the function of fibrinogen in mammals. In vivo, fibrin processing and coagulation are required for both pure intra-tissue bleeding and the creation of mural hematomas.[7,8] This is due to a cell transmembrane protein called coagulation factor III (or TF), which is mostly expressed on smooth muscle cells (SMCs) and adventitial fibroblasts in the arterial wall. TF initiates the extrinsic coagulation pathway. This process converts inactive plasma prothrombin into the thrombin that initiates fibrin reticulation. These tissue and plasma cascades play an active role in physiological hemostasis, which results in the clotting of RBCs.[9]

The majority of thrombi are red (cruoric) because RBCs are present inside the fibrin network, however thrombus development in vivo without RBC entrapment is feasible (white clots comprised of platelets, leukocytes, and fibrin)**.** Platelet activation by collagen-based wall structural features,

such as plaque fissuration and erosion is a common initiating event in CV disorders. The thrombi's secondary fibrin reticulation traps red blood cells inside. Other causes of clotting include blood stagnation, cell collisions due to hemodynamic vortices, and dilated cell walls (aneurysm).[13]

In human atherosclerosis and related diseases, RBC activities are always intertwined with fibrin formation, fibrinolysis, platelet activation, and the trapping of other circulating cells, particularly neutrophils**.** Hemolysis, membrane lysis, and hemoglobin release are all slowed by clotting red blood cells. The oxidation and degradation of the hemoglobin then causes the formed clot to shift color from red to brown and then yellow. Till date, most studies examining the role of RBCs in atherosclerosis have concentrated on the ways in which RBC membranes increase tissue cholesterol in relation to intraplaque hemorrhages and plaque susceptibility**.** We focus on tissue hemolysis in this synthesis because it leads to the release of free hemoglobin and heme, which catalyzes oxidative reactions by donating an electron from Fe^{++}. Beyond the later phases of atherosclerosis in humans and other associated acquired disorders (vulnerable plaque), its pro-oxidant effect is widespread.[21]

Ferric iron (Fe^{+++}), the form of iron stored in ferritin, is chemically neutral and has no direct oxidative capability. Humans with a condition known as hemochromatosis provide a useful example of the distinction between heminic (ferrous) and ferric iron. Hemochromatosis is characterized by excessive iron storage in the liver, pancreas, spleen, and myocardium as a result of increased intestinal absorption of ferric iron**.** Hepcidin directs ferritin and transferrin to take iron from the intracellular and extracellular environments, respectively, keeping the iron in a redox-inactive state. In contrast to hemolysis, which can damage the arterial wall and increase the risk of atherosclerosis, hemochromatosis does not have either of these effects. These empirical observations from humans highlight the striking differences between Fe^{++} and Fe^{+++} in living tissues.[24]

1.4. Atherosclerosis's relation to the immune system and red blood cells

Atherosclerosis, a chronic inflammatory condition of the arterial wall, is the leading cause of mortality in the Western world. Recent studies have shown that atherosclerosis is a complex disease due to the participation of several factors such as hyperlipidemia, hypertension, diabetes mellitus, male gender, obesity, and a family history of cardiovascular disease. Several of these variables increase the inflammatory and oxidative stress pathways that underlie the development of atherosclerosis, including activation of the immune system and endothelial dysfunction**.** Innate and adaptive immune system components can contribute to the development of atherosclerotic plaques. It has been hypothesized that the release of proinflammatory cytokines and matrix-degrading proteases by plaque macrophages, dendritic cells (DCs), and activated T and B lymphocytes is linked to plaque rupture in atherosclerotic lesions**.** Both innate and adaptive immune cells may be activated by various endogenous substances that have undergone chemical and/or structural modification due to oxidative or glycation events. Atherosclerotic disease develops gradually as a result of low-level inflammation brought on by immune system activation.[22]

Intraplaque bleeding is common in vulnerable atherosclerotic lesions**,** and it results in the deposition of red blood cells (RBCs) and the release of hemoglobin (Hb). The accumulation of RBC membranes within an atherosclerotic plaque, which supplies a substantial amount of lipids, is a critical event in the plaque's instability. Evidence suggests that oxidized RBCs have a pathogenic role in atherogenesis, hypertension, coronary artery disease, and stroke**.** The connection between RBCs and the immune system has been examined in the context of essential physiological processes**.** To sustain inflammation in the etiology of atherosclerosis, we recently published evidence that oxidized RBCs have a variety of immunomodulatory functions.[18]

1.5. Arterial calcifications and RBCs

Calcifications of ductile vascular tissues, valvular calcifications, and arterial calcifications and red blood cells (RBCs) or hemoglobin promote bleeding. Calcifications in soft arterial tissues are directly related to the exterior exposure of tissue-cell-derived anionic phosphates (PO_4^{3-}), on which the ionized soluble cationic calcium (Ca^{++}) precipitates. Crystalline hydroxyapatite is synthesized from calcium-phosphate [$Ca_3(PO_4)_2$] through polymerization (mineralization). This passive mechanism rapidly initiates the morphological switch from SMCs to osteoblasts**.** Calcifications in the setting of soft viscoelastic tissues, such as arterial and valvular walls, cause a distortion energy (von Mises stress) at the interface between solid calcifications and elastic tissues, as found by finite element analysis**.** Repetitive microdamage, akin to fatigue, can cause minute rips or macroscopic hemorrhages at the junction between calcifications and elastic tissue, where the deformation shear is highest. Increased susceptibility to plaque breach has been linked to the presence of micro calcifications in the intima**.** If the calcifications develop deeper in the media, the neo-vascularization may rupture (as discussed above), and peri-calcification hemorrhages may appear. Transvalvular transport of plasma lipoproteins, mainly LDL and Lp(a), during diastole produces aortic valve disorders because of the pressure differential between the aorta and the intraventricular diastolic pressure. This explains why aortic valve fibrosa is a common site for tumor development (ventricularis for the mitral valve).[16] The arterial media and the valves in a healthy heart are both avascular structures. Aortic valve disorders are inextricably connected to angiogenesis within the valves, a process facilitated by lipid accumulation in the fibrosa, as in the earliest stages of atheroma. Micro calcifications may form in the fibrosa as a

result of neovascularization tears and hemorrhages, which accelerate the calcification process**.**

Hemoglobin and its derivatives (heme, ferrous iron, free radicals, and NF-kB activation) promote the release of exosomes and the osteoblastic differentiation of valvular interstitial cells and of SMCs in the arterial wall**.** Therefore, in addition to CV risk factors like age, smoking, dyslipidemia, etc., calcifications and RBC/iron are in a vicious loop that promotes the exponential development of valvular and arterial calcifications. These results suggest that NO released from SMCs linked with the cell membrane triggers a switch to an osteoblastic phenotype. This study's authors showed that, in addition to phospholipids, NO released from isolated RBC membranes can induce arterial calcifications in the absence of hemoglobin. RBC walls lacking NO synthase have minimal procalcifying effects. These results indicate that NO release complementarily supports an osteoblastic change in SMCs, in addition to the phospholipid support given by RBC membranes. The amphiphilic pole of phospholipids is exposed when SMCs phagocytose RBCs, leading to the release of numerous microvesicles and exosomes.[2]

2. Results

2.1. Arterial wall structure

The arterial wall comprises three layers: tunica intima, tunica media, and tunica adventitia, with the endothelium playing a key role in vascular homeostasis.

2.2. Atherosclerosis development

Endothelial dysfunction, lipid accumulation, monocyte migration, and foam cell formation are key steps in atherosclerosis. RBCs and hemoglobin exacerbate these processes through oxidative stress and inflammation.

2.3. Oxidative stress and inflammation

Hemolysis of RBCs releases hemoglobin and heme, leading to oxidative stress and inflammatory responses that promote plaque instability.

2.4. Blood viscosity and flow dynamics

RBCs affect blood viscosity and flow dynamics, contributing to endothelial micro-injuries and plaque formation.

2.5. Immune response

RBC-derived oxidative stress activates immune cells, resulting in chronic inflammation and progression of atherosclerosis.

2.6. Arterial calcifications

Hemoglobin derivatives interact with vascular cells to drive calcification processes, affecting plaque stability.

3. Conclusion

Red blood cells (RBCs) in the blood often engage with cardiovascular (CV) tissues, such as the arterial wall, cardiac valves, kidneys, myocardium, and brain, resulting in long-lasting physiological and/or pathological alterations. Red blood cells (RBCs) play a crucial role in regulating blood viscosity and, by extension, the frictional forces that blood exerts on the arterial wall. Tissue hemolysis caused by the acute and chronic local impact of RBCs with the wall is a major source of redox-active iron in disease. In living organisms, Fe++ acts as a primary catalyst for all reactive processes. In this clinical context, protecting SMC, the vascular wall's stromal cell, from iron-dependent oxidative stress and biomechanical stress is the main concern.

4. Source of Funding

None.

5. Conflict of Interest

None.

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