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Comprehensive review on the role of neutrophil recruitment and shear stress in atherosclerosis

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Abstract
Cardiovascular diseases (CVDs) account for nearly 32% of global mortality, with increasing prevalence across all age groups. Atherosclerosis, a key contributor to CVDs, arises from chronic vascular inflammation and is driven by a complex interplay between immune and mechanical factors. Neutrophils play a central role in atherogenesis by releasing reactive oxygen species, proteases, and inflammatory cytokines that damage the endothelium, promote thrombosis, and destabilize plaques. Concurrently, mechanical forces such as wall shear stress (WSS) significantly influence endothelial cell behaviour. Laminar shear stress maintains vascular homeostasis by enhancing nitric oxide synthesis and suppressing inflammatory responses. In contrast, regions exposed to low or oscillatory shear stress exhibit increased expression of adhesion molecules like ICAM-1 and E-selectin, facilitating leukocyte adhesion and transmigration, and contributing to endothelial dysfunction. The mechano-transduction pathways in endothelial cells involve mechano-sensors, signalling intermediates such as MAPKs, and downstream effectors like nitric oxide synthase, collectively determining endothelial responses to hemodynamic forces. Pharmacological suppression of neutrophil recruitment using agents like corticosteroids, mTOR inhibitors, and calcineurin inhibitors has shown potential in mitigating inflammation-driven vascular damage. However, their broad immunosuppressive effects raise concerns regarding infection risk and long-term safety. Integrating the roles of neutrophil-mediated inflammation and shear stress-induced endothelial modulation offers critical insights into the initiation and progression of atherosclerosis, highlighting potential avenues for more targeted and effective therapeutic strategies.

Keywords: Neutrophil, Atherosclerosis, Shear Stress, Neutrophil Recruitment, Cardiovascular Diseases

Introduction
Many studies show that a healthy endothelium is important for preventing atherosclerosis. This is clear from the link between risk factors like smoking, high cholesterol, high homocysteine, low estrogen and high blood pressure with poor endothelial function. Endothelial cells help maintain blood vessel health by responding blood flow and hormones, releasing different proteins and molecule to regulate vascular function. Because of these functions, endothelial cells help to control important processes in blood vessels, such as regulating how plasma lipoproteins pass through, allowing white blood cells to stick, and releasing factors that affect blood clotting, growth and vessel relaxation. When this function are disrupted, it is believed to contribute to the development of atherosclerosis. Like other tissues that sense changes in their environment, endothelial cells react not only to chemical signals in the blood but also to the physical forces from blood flow and the heartbeat. Because of their position in blood vessels, they experience three main mechanical forces: pressure from the blood, stretching of the vessel walls, and shear stress from the flowing blood, these forces come from specific connections between endothelial cells, which apply stretching forces during blood vessel movement, and shear stress the functional forces caused by blood flow. Among these shear stresses is especially important because it triggers the release of substances that affect blood vessels and influences gene activity, cell function and shape [1-5].

The type and strength of shear stress are important for keeping blood vessels healthy over time. Shear stress on endothelial cells depends on how blood flow through the vessels, which is controlled by the heartbeat. In straight parts of blood vessel, blood moves in a smooth and organized way, following the heartbeat. This creates a pulsating shear stress with changing strength, but overall it remains positive. Although steady shear stress and pulsatile stress trigger similar responses in endothelial cells, there are some differences in their effects. When cells experience positive shear stress, they change their shape by aligning their long axis with the direction of blood flow. This realignment helps streamline the cells, reducing resistance and lowering shear stress [7, 8].

Autoimmunity is referred to as a condition where the immune system of the body fails to distinguish between the pathogenic cells and body's own cells, and therefore lead to destruction and apoptosis of its own cells. Any disorder occurring due to this condition is known as auto-immune disorder. The immune system helps to maintain homeostasis and protect our body against any infection but over activity of the immune system can lead rupture and death of healthy cells of the body leading to complex disorders. Common examples of autoimmune disorder are rheumatoid arthritis, erythematosus (lupus), type 1 diabetes mellitus and multiple sclerosis. Autoimmunity can be characterized by the cells (auto reactive T lymphocytes) or products (autoantibodies) of immune system on substrate of the immune system. This may be part of physiological immune response (natural autoimmunity) or pathologically induced immune response, leading to development of clinical abnormalities, termed as autoimmune disorder. Human autoimmune diseases on an aggregate, affects more than 5% of the worldwide population. These disorders can be cell specific or tissue specific where the antigens are targeted in a more systematic manner where multiple tissues are affected. Several factors contribute to autoimmunity including environmental and genetical factors [9-12].

There are five types of white blood cells which can be broadly classified into categories-granulocytes and agranulocytes. Granulocytes which are neutrophils, basophils and eosinophils contains granules in their cytoplasm and have multi-lobed nuclei. Agranulocytes comprising of the monocytes and lymphocytes lacks granules in the cytoplasm and contains single lobed or non - lobed nuclei. Dendritic cells and tissue resident macrophages are monocyte progenitor.

Neutrophils: Neutrophils are the most common type of leukocyte accounting to about 50-70% of the total leukocyte count. These are twice the size of normal erythrocytes and perform the first line defense action of the of the body. Neutrophils are also known polymorphonuclear leukocytes due to the presence of multi-lobular nuclei (2-5 lobes joined by thin filament). The lifespan of neutrophils is about 7-10 hours in the circulation and 1-2 days within the tissues. The cytoplasm of neutrophils contains ribosomes, small numbers of mitochondria, glycogen, and granules of various types. The MCG- stained films of neutrophils when viewed under microscope produce a pink tinged cytoplasm. They contain enzymes like myeloperoxidase, lactoferrin, transcobalamin, collagenase, gelatinase, and lysozymes. These enzymes initiate the pathogenic cell destruction [13].

Eosinophils: Eosinophils comprise of 2-4% of the leukocyte count and have similar size or are sometimes slightly bigger in size than neutrophils. It has a bilobed nuclei and produces immune response by engulfing the antibody-tagged bacteria and their cell debris. Although the primary function of eosinophils can be considered as protecting the body from parasites like tapeworms, pinworms, or flatworms. The eosinophils contain acidophilic granules and the MCG-stained films shows orange color due to their affinity for eosin component of the stain. They contain a weakly basic cytoplasm containing abundant glycogen molecules, a greater number of mitochondria than neutrophil and few numbers of rough endoplasmic reticulum. The crystalline granules eosinophils are made up of eosinophil major basic protein, surrounded by a matrix containing eosinophilic cationic protein, peroxidase, eosinophil derived neurotoxin, plasminogen, RNA, DNA and lipase. It has a lifespan of 1 day in the circulation and 8-12 days within the tissues [14].

Basophils: Basophils are marginally smaller than neutrophils and make up only 1% of leucocytes. The MCG - stained films imparts dark purple granules. The nuclei is multilobed and basophils contain strongly alkaline cytoplasm; containing scattered glycogen particles, Golgi apparatus, few mitochondria and rough endoplasmic reticulum. Basophils have phagocytic activity and upon activation they move to injury sites, degranulates, thus releasing histamine, which results in vasodilation, increased blood flow, and the attraction of additional leukocytes. To maintain healthy blood flow to the area and avoid blood clotting, they also release heparin. The IgE bind to specific membrane receptor and protects the body against helminthic infections. They possess similar characteristics like mast cells and is involved in allergy, chronic inflammation, and anaphylaxis. Along with histamine they also release serotonin and proteolytic enzymes, and IL-4 and IL-13 [15].

Monocytes: monocytes account for about 2-4% of the total leukocyte count and is larger in size than other leukocytes. They have kidney or oval shaped nucleus and has a short lifespan (24 hours) within the circulation and then enter the tissues and differentiate to become macrophages. They are highly mobile and actively phagocytic and defend the body against viruses, parasites, and chronic infections like tuberculosis. On MCG-stained films the cytoplasm appears opaque bluish-grey containing fine azurophilic granules and vacuoles. Other than being phagocytic, the monocytes operate as immune modulators, increasing the inflammatory response by secreting IL-1, IL-6, IL-12, tumour necrosis factor-α, interferon-α, and interferon-β when activated [16]. Heterogeneous granules, glycogen particles, mitochondria, a functioning Golgi apparatus, and brief segments of endoplasmic reticulum are all visible in the cytoplasm upon ultrastructural analysis. They have a 1-3 days intravascular lifespan, however the cells they develop into have a greater life span.

Dendritic cells and macrophages: Tissue-resident macrophages and dendritic cells share a granulocyte/monocyte progenitor. The functions of macrophages include phagocytosis of other senescent or dead cells, removing red cell inclusions, unicellular parasites from erythrocytes, storing iron as ferritin and

hemosiderin, and supplying iron to growing erythroblasts. There are certain negative implications of macrophage activity, particularly in the pathophysiology of chronic disease-related anaemia [17].

Lymphocytes: The smallest leukocytes are lymphocytes, which have a spherical nucleus and are roughly circular in shape. Some have great amount of cytoplasm, with or without granules, while the majority have scanty. As a result, they can be categorized cytologically as small, big, and large granular lymphocytes.

Their characteristics vary greatly upon ultrastructural examination:

There is a lot of free ribosomes, varying levels of rough endoplasmic reticulum, and often a small and dormant Golgi apparatus. The cytoplasm may or may not contain acid phosphatase-positive granules and mitochondria**.** The three functional subtypes of lymphocytes are natural killer cells, T cells, and B cells. Memory B cells and Ig-secreting plasma cells are produced when B cells migrate to the tissues and undergo differentiation. Natural killer cells and T cells are part of both innate and adaptive cellular immunity [18].

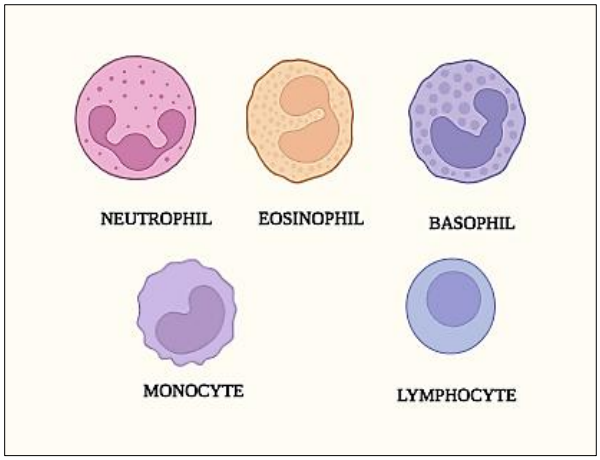

Fig 1: Types of White Blood Cells

Table 2: Examples of Organ Specific Autoimmune Disorder

Organ-Specific Autoimmune Diseases				
Organ	Disease(s)	Major Autoimmune	Self-Antigen Mechanism	References
Adrenal cells	Addison's disease	Cytochrome P-450 antigens	Autoantibodies	[19]
Red blood cells	Autoimmune haemolytic anaemia	Red blood cell membrane proteins	Autoantibodies	[20]
Platelets	Idiopathic thrombocytopenic purpura	Platelet antigens (GP IIb/IIIa)	Autoantibodies	[20]
Stomach	Pernicious anaemia	Gastric parietal cell antigens (H+/ATPase, intrinsic factor)	Autoantibodies/T cells	[21]
Small bowel	Celiac sprue (gluten enteropathy)	Transglutaminase	Autoantibodies/T cells	[21]
Thyroid	Hashimoto's thyroiditis	Thyroid cell antigens (e.g., thyroglobulin)	T cells/autoantibodies	[22]
	Graves' disease	Thyroid-stimulating hormone receptor	Autoantibodies	[22]
Muscle	Myasthenia gravis	Acetylcholine receptors	Autoantibodies	[22]
Pancreatic islets	Type 1 diabetes	Beta cell antigens (glutamic acid decarboxylase, insulin)	T cells (autoantibodies present)	[23, 24]
Hepatocytes	Autoimmune hepatitis	Hepatocyte antigens (cytochrome P450 2D6)	T cells/antibodies	[25]

Atherosclerosis

Atherosclerosis is a chronic, progressive disorder marked by accumulation of cholesterol, lipids, and fibrous elements on the inner lining of the blood vessels. These accumulations when sustain for a prolonged period, leads to the formation of plaques. The deposited plaque hardens further narrowing the arteries, thus restricting blood flow, and reducing oxygen and nutrients to the tissues and organs. This is a major underlying cause several cardiovascular disorders including coronary arterial disorder, stroke, and periphery arterial disorder. Risk factors of atherosclerosis include modifiable factors such as hypertension, elevated cholesterol levels, smoking, consumption of alcohol, obesity, unhealthy diet, and lack of physical exercise. Non-modifiable factors age, gender (men at higher risk), and genetics also come into play [26].

Pathogenesis of Atherosclerosis

The pathogenesis of atherosclerosis is a multistep procedure and develops chronically.

- Endothelial injury: the initial step in atherosclerosis is endothelial injury. This is caused by disease conditions such as diabetes or hypertension, also some habitual factors like smoking. A major reason of endothelial injury is hyperlipidemia i.e. elevated level of lipids (particularly, low density lipoprotein or LDL). Endothelial injury results in increased permeability of lipoproteins and inflammatory cells to endothelial cell lining.
- Oxidation of accumulated lipids: the LDL that permeates through the injured cell gets accumulated in the intimal layer of the arterial wall. These deposited lipids then undergo oxidation, leading to formation of oxidized LDL (oxLDL) from LDL which is highly atherogenic and stimulates pro-inflammatory response.
- Inflammatory cell recruitment: the oxidized LDL, attracts leukocytes (especially neutrophils) which firstly adheres to the endothelium and gradually moves to the intima and firmly adheres to the intimal lining. Once firmly adhered the cells inflammatory cells differentiates into macrophages which engulfs the oxidative LDL and forms foam cells. As the procedure continues, the foam cells accumulate forming a fatty streak, which is the earliest visible lesion in atherosclerosis.
- Formation of atheromatous plaque: continued lipid accumulation and immune cell activation lead to recruitment of more inflammatory cells to smooth muscles. They proliferate in the intimal layer continuously forming fatty streak and allowing its

deposition in the intimal layer. The inflammatory cells also synthesize extracellular matrix proteins like collagen, forming fibrous cap over the lipid core. This process results in the formation of fibro-fatty atheromatous plaque.

- Plaque progression: the recruitment of neutrophils makes the fibrous cap thin and unstable which finally ruptures. The ruptured plaque then gets exposed to the thrombogenic material in the bloodstream, triggering the formation of thrombus which is stable. This acutely blocks the artery, narrow downs the arterial lumen, reduces blood and causes several cardiovascular conditions like myocardial infarction, stroke and can also cause sudden death [27].

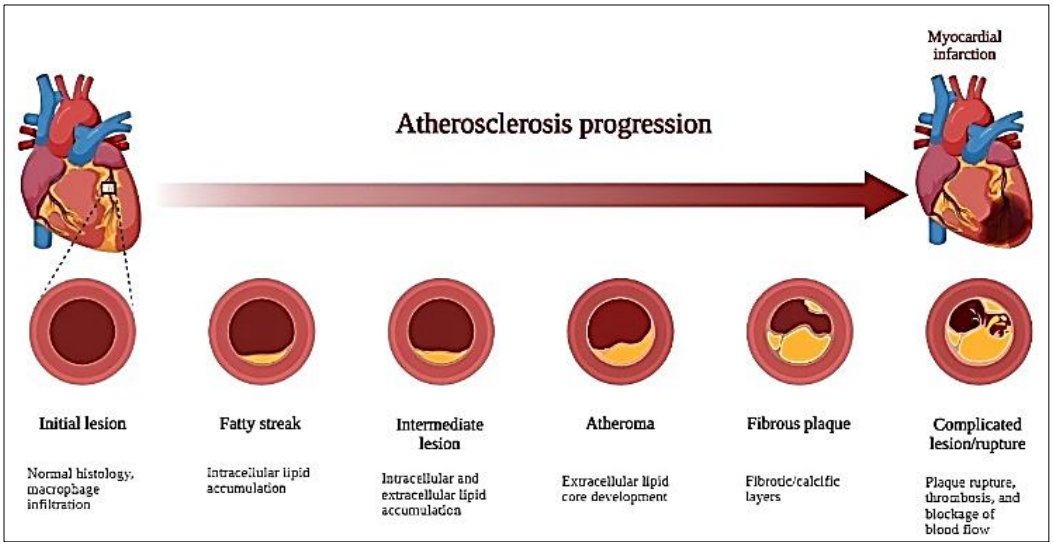

Fig 2: Mechanism of Atherosclerosis Progression

Neutrophil Recruitment in Atherosclerosis

The neutrophil recruitment is a cascade mechanism and is achieved by the following mechanism

Activation of Endothelium: The neutrophil activation process begins with the activation of endothelial cells. This is initiated when inflammatory stimuli like pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) are recognized by the pattern recognition receptors (PRRs) present on resident immune cells. Upon receiving the stimuli, the immune cells produce pro-inflammatory factors i.e. cytokines and chemokines, which initiate activation of endothelial cells nearby the post-capillary venules. Now this activation of the endothelium, induces the secretion of E-selectins and P-selectins on the endothelial surface and increases the vascular permeability.

Rolling: The rolling phase involves transient, low affinity interactions between neutrophils and activated endothelial, primarily mediated by selectins and their ligands. The P-selectin glycoprotein ligand-1 (PSLG-1), expressed on the neutrophils binds to the E-selectin and P-selectin present on endothelium. This causes the neutrophils in the to slow down their speed and roll along the vascular lining, to ensure that the neutrophils sense, interact and respond extensively with the chemokines present on the endothelial surface, particularly the ones bound to glycosaminoglycans. These chemokines activate integrins on the neutrophil surface. Once the binding occurs, chemokines such as CXCL1 and CXCL8 also known as IL-8 bind to their respective G-protein coupled receptors on the rolling neutrophils. This trigger intracellular signaling cascade inducing conformational changes in β2 integrins converting them to a high affinity state from low affinity state. This transition is extremely important for the subsequent step.

Firm adhesion: Firm adhesion is mediated by interaction of activated integrins with intercellular adhesion molecules (ICAM-1 and ICAM-2) and vascular cell adhesion molecule (VCAM-1) present on endothelial cells. This interaction is stable and thus anchors the neutrophils in desired place, preventing the wash away of neutrophils due to sheer force of blood flow. This stage also leads to polarization of the neutrophils.

Transmigration (Diapedesis): the transmigration process involves the migration of neutrophils across the endothelial barrier. In this method the neutrophils squeeze into the endothelial and moves across them to reach the site of infection. This procedure can occur via two methods, namely- paracellular route (between endothelial cells) and transcellular route (through the endothelial cell body). The paracellular route is mediated by the transient opening of the endothelial junction, regulated by molecules such as PECAM-1 (CD31), JAM-A, CD99, and VE-cadherin. These molecules help the neutrophils to reach to the subendothelial space from the endothelial space. Transcellular migration is a less common route and involves formation of transcellular pores, which is dependent upon cytoskeletal remodeling, and vesiculo-vacuolar organelles present with endothelial cells.

Interstitial migration: This phase begins once the neutrophils reaches the tissue. After reaching the tissue, the neutrophils migrate through the extracellular matrix (ECM) towards the site of inflammation. The migration takes place with the help of chemokine gradients like bacterial products, for example N-formyl peptidase. Neutrophils also use integrins and certain metalloproteinases and enzyme to migrate through the extracellular matrix.

Chemotaxis: As the concentration of chemokines increases in the tissue, neutrophils follow the chemotaxis and move towards the site of infection or damage. In addition to phagocytosis of pathogens, neutrophils also release antimicrobial granules and enzymes, producing reactive oxygen species (ROS), and create neutrophil extracellular traps (NETs) to capture and eliminate microorganisms at the site [27- 30].

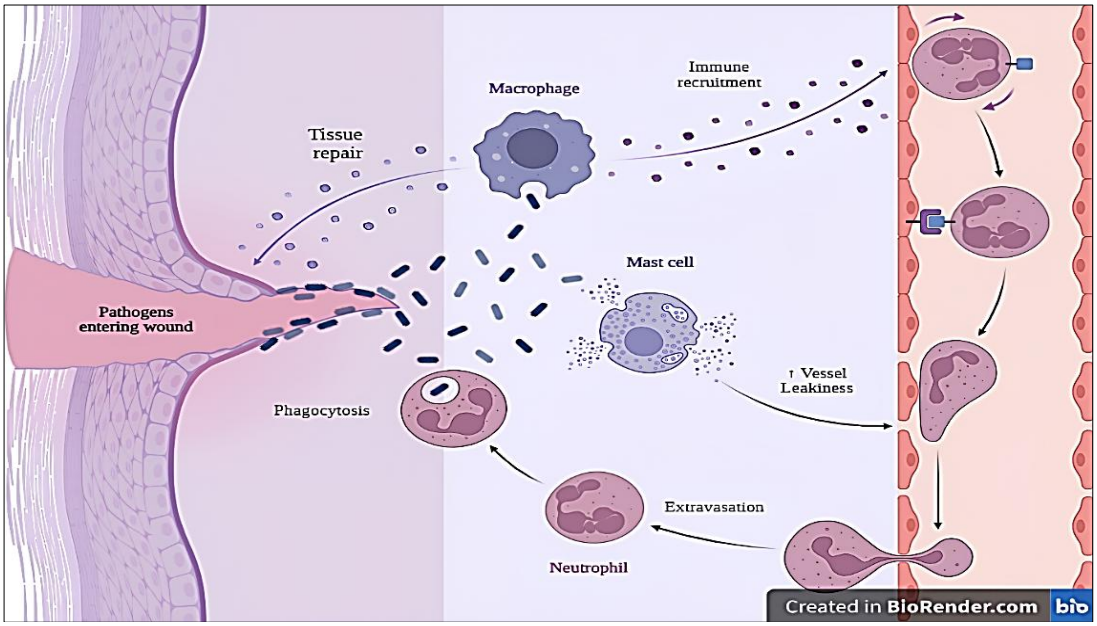

Fig 3: Mechanism of Neutrophil Recruitment

Neutrophils in Atherosclerotic Progression

Neutrophils being the most abundant type of granulocyte (50-70% of the total leukocyte count in human body), is found to the most frequent population of leukocytes circulating in the bloodstream. Therefore, these are among the primary cells that eliminates the microbial pathogens from the bloodstream. This process takes place by phagocytosis and production of various toxic enzymes like ROS, myeloperoxidase, and other proteolytic enzymes. Neutrophil functions occur through signals transmitted by receptors (RIG-1, NOD, C-type lectin). Bone marrow deviated neutrophils has high and low levels of CXCR4 and CXCR2 respectively. These ensure sufficient production of neutrophils [31].

Research studies suggest that patient suffering from atherosclerotic condition has elevated level of neutrophils in their blood circulation. Elevated cholesterol level causes the bone marrow to generate more neutrophils and to express CXCR2 and its ligand CXCL1. The size of atherosclerotic plaque increases when neutrophils are stimulated by impaired CXCL12-CXCR4 expression. Therefore, it is predicted that the progression of atherosclerotic plaque growth is highly influenced by the number of neutrophil counts in the blood stream. Increased number of maintenance factors of homeostasis though neutrophil recruitment is directly proportional to the size of plaque. The number of neutrophils in the blood stream increases during any inflammatory response and secretion of IL-8 attracts these cells towards the necrotic nucleus ensuring its effective function. An increase in neutrophil influx and inflammatory activity readily increases the atherosclerotic plaque progression by various mediators like ROS, leukotriene B4, PTX3, MPO, MMPs, NET, IFN-γ and connexin. In human atherosclerotic lesions, neutrophils generate PTX3 in reaction to inflammatory cues (such as bacterial products, TNF-α, and IL-1). The PTX3 protein also activates the classical immune cascade, which leads to increased tissue damage, phagocytosis, inflammation, chemotaxis, and, ultimately, atherosclerosis progression. MPO, which is abundant in neutrophil primary granules, increases the synthesis of ox-LDL. Increased level of PTX3 and MPOs in individuals with atherosclerosis are two markers set to detect the prognosis of the condition and increase in these two markers leads to increases risk of cardiac failure. Secondary and tertiary granule of neutrophils contain large amount of MMP-2 and MMP-9, which destroy endothelial cells by influencing collagen type IV. Formation of NETs is a unique mechanism of neutrophils helping in the destruction of microbial cells. Inflammation in atherosclerosis can cause production of NETs which leads to thrombosis due to deposition of fibrin protein [32].

The cascade for progression of atherosclerosis due to neutrophil recruitment begins with endothelial injury or endothelial dysfunction, which leads to accumulation of neutrophils to the endothelial cell lining as an inflammatory or protective response. The activated neutrophil upon adhering to the endothelial lining secrete cytokines and chemokines enhancing recruitment of other monocytes and other inflammatory factors. They also secrete ROS and other proteases increasing vascular permeability and allows infiltration of lipid and immune cell entry to the intima. A major progression takes place in this step as the neutrophils other than performing phagocytosis leading to streak of foam cell production, also produces NETs. These activates the local resident immune cells like dendritic cells and macrophages creating a local inflammatory response. NETs also induce activation inflammatory enzymes and promote release of pro-inflammatory cytokines. Histone and proteases which are active constituents of NETs directly induce muscle injury leading to plaque destabilization which on contact with thrombin from the bloodstream forms thrombus, which is the stable form the deposited plaque and

due to hardening it narrows down the blood vessels largely causing coronary artery disorders.

Shear Stress in Atherosclerosis

Role of Endothelial Cells in Thrombosis

The integrity of the endothelial layer is chiefly accountable for inhibiting clotting of blood. When the permeability of blood vessels increases, as when the vessels are inflamed, fluid and significant clot-preventing proteins leak into the surrounding tissues. This reduces the body's own protection against clots and also retards the blood by making its viscosity thicker. Once a clot has occurred, the endothelium allows vessel repair by covering areas of exposure which could otherwise be a focus for ongoing clotting, i.e., injured tissue or ruptured plaques in atherosclerosis (endothelial role). A few studies, including ours, have investigated pathways by which endothelial progenitor cells can cause the reestablishment of blood flow following damage to an artery and assist in the elimination of blood clots from veins. Meanwhile, we have recently discovered that TGFBETA (Transforming Growth Factor Beta), a protein that is released from platelets that have been activated, has no effect on clotting of the blood in damaged arteries. I, in contrast, slow down healing of blood vessels lining and stimulates the formation of thickened tissue within the artery. It is consistent with its established role of suppressing the proliferation of endothelial cells. Additionally, TGFBETA may stimulate these cells to transform into fibrous tissue producing cells. Conversely, activation of growth factor signals encouraging the development of new blood vessels like inhibiting protein tyrosine phosphatase - 1B(PTP1B) can result in excessive cell growth and early aging of endothelial cells, as seen in our research involving gene modified mice and PTP1B inhibitor-treated human cells [33].

Endothelial dysfunction

In addition to the effect of endothelial damage or dysfunction described above, there is a release if a range of factor, WF and asymmetric dimethylarginine (ADME) that play a role in clotting. Asymmetric dimethyl-arginine function as an endogenous endothelial nitric oxide (NO) production inhibitor and in excess. Asymmetric dimethyl arginine can lead to endothelial dysfunction.

Effect of Shear Stress on blood cells

The presence of RBCs plays a strong influence on the wall shear stress, particularly in the microcirculation. The influence is more dynamic with conditions that cause fluctuations in RBC morphology. Note that the high deformability of RBCs is a key component of blood fluidity, and regulates RBC interaction with blood vessel walls and other blood cells. During physiological conditions, the preservation of RBC normal shear forces allow for microcirculatory perfusion and oxygen delivery. But aggregation and structure of RBCs are sensitive to changes in shear, and pathological shear conditions cause irreversible damage to RBCs and endothelial hypoxia. This hypoxia and altered flow can promote endothelial dysfunction, shifting the endothelium towards a prothrombotic phenotype. Oxygenation of tissue is also hindered by the concentration of streaming RBCs in the middle along with that of slow-moving plasma along vessel walls. In trauma patients, also, the RBC aggregation is heightened due to shear activated platelet activation. Such interactions enhance the prothrombotic microenvironment by facilitating platelet-endothelium contact. All these impinge on the relevance of RBCs, whose status of existence impacts the shear stress generated within the endothelium wall interface. However, numerous endothelial flow experiments fail to adequately account for the influence of RBCs on prothrombotic responses.

With a shear stress of 170Nm-2 lasting between only 7 milliseconds, provides procoagulant phospholipids and activates the platelet. Platelet adhesion to sub-endothelium at high shear stresses necessitates rolling, mediated by activated integrins. Abnormally elevated shear induces shedding of GLlba(alpha) and GPVI, and reduce margination and platelet number adhered to VWF(von Willebrand factor) and collagen in a shear- and time dependent manner. This limits effective platelet anchoring to the endothelium under high shear, impairing clot stability. Besides, platelet margination caused by platelet interaction with RBCs within flowing blood is reduced in patients with hemorrhagic shock. It is therefore difficult for platelets to establish strong adhesion with the endothelial wall, a property that prevents blood clotting in these patients. Altogether, these shear related changes affect both cellular components and endothelial response that are essential for thrombosis [34].

Low Endothelial Shear Stress in patients with Hemorrhagic Shock

Tissue injury and hemodynamic alterations drastically alter the prevailing force on the vasculature. Later, vascular endothelium is highly sensitive to alterations in shear stress which induces wall remodeling of the structure. Mechano-transduction may therefore be implicated in the development and progression of endothelial dysfunction when either the forces themselves, or the force measuring device by which forces are measured, are pathological. In critically injured patients with severe blood loss, hypertension and comptonization of the microcirculation autoregulation, tissue injury, aggressive resuscitation, transfusion and changes in viscosity are associated with pathological shear stress on endothelial cells. When the latter are subjected to low, pathologic shear forces, local homeostasis is disrupted and the endothelium initiates remodeling displaying a hyper-coagulant/ prothrombotic and prooxidant condition.

In addition to trauma-related conditions, low shear stress also plays a major role in the pathogenesis of chronic vascular diseases such as atherosclerosis. Regions of arteries exposed to disturbed flow patterns and low shear stress demonstrate enhanced endothelial activation, inflammation and prothrombotic responses, further supporting the critical role of shear stress in vascular health.

Increases in glycocalyx shedding and vascular permeability correlate with more pronounced reduction in blood flow, as shown in the postcapillary venules of rat skeletal muscle and mesentery. Furthermore, loss of endothelial barrier leads to exposure of procoagulant and platelet-activating proteins, shedding of glycocalyx components, hypocoagulability, and release o fibrinolytic factors promoting fibrinolysis. Sepsis research has also found that inadequate shear stress disrupts the shear mediated mechano-transduction and pathological changes [35].

Thus, maintaining physiological shear stress is essential for the endothelium to perform its anticoagulant, anti-inflammatory, and antioxidant functions. Disruption of these mechanical signals is now recognized as a key event in the shift toward a prothrombotic endothelial phenotype.

In other words, healthy endothelium exposed to physiological (~ 15 dyns cm-2) shear stress due to laminar blood flow patterns possesses a resting fusiform smooth morphology, with increased synthesis of anticoagulant/ antithrombotic and antioxidant molecules. But abnormal endothelium due to low shear stress (~5 dyns cm-2) is cobblestone and contains a hypercoaguaent / prothrombotic and prooxidant state [36].

High Endothelial Shear Stress in Patients with Hemorrhagic Shock

Shear stress increase to a pathological degree impacts endothelial function negatively as well. New evidence from mouse and healthy donor human small and large arteries and veins indicates that certain lesions create high shear and elongational flows at the wound edge, where hemostasis is established. Notably, such shear levels not only considerably exceed healthy vessel levels seen during homeostasis but actually damage nearby healthy vessels. Unregulated flow or exaggerated shear stress also dictate glycocalyx thickness and structure. Furthermore, resultant vasoconstriction post-trauma caused by physiologic compensation reactions and/or aggressive resuscitation attempts could also produce increased shear rates potentially capable of altering platelet morphology.

These changes occur even with the imposition of high shear force for a few milliseconds n platelets, impacting receptor-ligand binding interactions and platelet margination. Platelet are thus unable to establish stable adhesion to the wall and engage in thrombus formation. In certain individuals, increased shear activates platelets and enhances their adhesion with fibrinogen. Exposure to supraphysiologic shear stress leads to thining of the platelet membrane and release of microparticles from sheared platelets. These platelet derived microparticles are responsible for increased thrombin generation and the prevention of collagen and adenosine diphosphate-stimulated platelet aggregation. Shear mediated micro-vesiculation is also accompanied by remodelling of platelets receptors, and microparticles carry significantly higher densities of GPIX, PECAM-1, P-selectin, and PSGL-1, adhesion receptors, as well as agonist receptors (P2Y12 AND PAR1) [37].

High shear stress also elevates the release of endothelial microparticles, which disrupts vascular senescence, inflammatory reaction, barrier properties and localized coagulation, thereby enhancing the injurious effect of altered shear stress on the vasculature. Similar to platelet derived microparticles, endothelial microparticles can impact neighboring endothelial cells and worsen local damage. These functional changes can manifest clinically as bleeding and thrombotic side effects. High shear stress not only affects platelets but also induces the release of endothelial microparticles, which act as mechano-sensitive regulators of vascular homeostasis and injury. These microparticles contribute to the development of atherosclerosis and participate in vascular damage in inflammatory disorders. They also modulate endothelial cell responses, including the expression of adhesion molecules and cytokine production, thereby influencing the prothrombotic state of the vasculature.

While hypothermia lowers cellular oxygen requirements and is beneficial to vital organs during cellular ischemia, it worsens coagulopathy, decreases plasma volume, and impairs tissue perfusion. Rewarming increase the metabolic rate and improves tissue perfusion through increased endothelial-dependent vasodilation due to augmented bioavailability of nitric oxide. The exact mechanisms underlying the correlation are yet to be discovered. Rewarming is also correlated with sympathetic nervous system impairment, circulatory failure, and secondary complications.

Proper shear stress is a significant hemodynamic stimulus to vascular adaption; however, shear stress evoked by acute (lower or elevated) temperature is not necessary to prevent endothelial ischemia-reperfusion damage. Notably, various shear rates do not alter flow-mediated dilation for the same heat stimulus. An increase in core temperature caused by whole-body heating has been found to induce significant rises in heart rate and possibly cardiac output in individuals, causing an added cumulative rise in shear stress in the arterial bed [38, 39].

All of these factors can increase already pathological shear forces caused by local vasoconstriction and/or intravascular injection of vasoconstrictors, leading to increased endothelial dysfunction. Moreover, shear stress induced changes in blood viscosity and increased in oxidative stress may reduce nitric oxide bioavailability, enhancing vasoconstriction and impairing endothelial function and flow mediated dilation. An endothelial function in conduit and resistance vessel in influenced by hemodynamic forces in the arterial circulation, preservation and/or enhancement of endothelial homeostasis is necessary to achieve the maximum effect of temperature on shear levels [40].

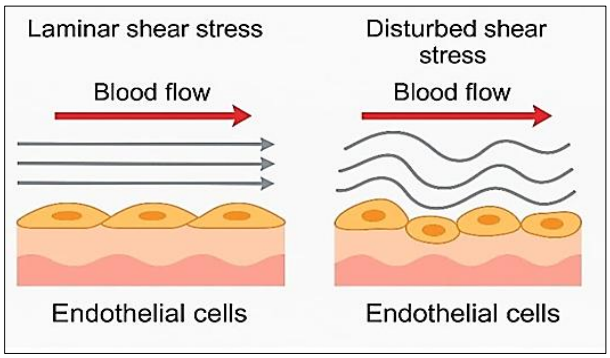

Fig 4: Normal vs Disturbed Blood Flow due to Shear Stress

The major drawback in this treatment procedure is non-specificity of the immunosuppressant class of drugs to the exact cell or receptor. Therefore, chronic use of these drugs can result in extremely weakened immune system, making an individual more prone to diseases and infections. Prolonged use can also cause malignancies, thereby limiting the use immunosuppressants in cardiac disorders. More research is required to make the drugs more target specific and reduce the adverse effects. Therefore, since immunosuppressants plays a significant and promising role in addressing neutrophil recruited cardiovascular disorders, challenges remain to make the therapy more targeted, safe efficacious to provide sustained clinical benefit.

Novel Techniques for Treatment (Allopathic and Biomedical)

Recombinant High-density lipoprotein (rHDL)

The effect of recombinant high-density lipoprotein (rHDL) infusion on endothelial function in diabetes mellitus type2(DM2): specifically, in 7 DM2 patients and 7 normolipidemic controls with similar baseline HDL levels, endothelial function was assessed using venous occlusion plethysmography. Forearm blood flow (FBF) response to intraarterial infusion of the endothelium - dependent and independent vasodilators serotonin (5HT) and sodium nitroprusside, respectively, and the inhibitor of nitric oxide synthase NG- monomethyl -1 arginine werw measured, both before 4 hours after and one weak after infusion of rHDL. Acute HDL increase imoroved endothelial function in DM2 patients but not in controls and that improvement persisted for at least 7 days in spite of return to baseline of HDL concentration. This improvement in endothelial function is significant because endothelial dysfunction is a key early event in the pathogenesis of atherosclerosis. By restoring endothelial responsiveness and nitric oxide bioavailability, rHDL infusion may reduce vascular inflammation and slow the progression of atherosclerotic plaque formation. Thus, rHLD therapy represents a promising novel strategy for the prevention and potential treatment of atherosclerosis, especially in high risk groups such as patients with type 2 diabetes [41-43].

Nadph Oxidase Inhibitors

Inhibition of NADPH oxidase activity, or more effectively by agents like apocynin and diphenyl eneiodonium (DPI), has been shown to have the potential to reduce the generation of reactive oxygen species (ROS), thus reducing oxidative stress and inflammation in atherosclerosis. Apocynin, a plant derived natural compound, blocks NADPH oxidase activation by preventing translocation of the p47 phox submit, with decreased levels of superoxide and inhibited lesion formation in hypercholesterolemic mice,i.e., in the thoraco-abdominal aorota. But apocynin action is cell type specific. It is more an antioxidant than an immediate inhibition of NADPH oxidase in vascular smooth muscle cells and itself gets inhibited through enzyme dependent ways by activation of the enzyme myeloperoxidase (MPO), found in certain inflammatory cells. Apocynin can be incapable of inhibiting NADPH oxidase or may even act with prooxidant action without MPO. While such findings are promising, much of the work so far has been conducted in animal models. Clinical trials and additional work will be needed before learning about the safety, efficacy, and proper use of NADPH oxidase inhibitors in humans.

In general, NADPH oxidase inhibition to prevent ROS production is a therapeutic hope for atherosclerosis. Potential inhibitors like apocynin and DPI have been promising in preclinical models by reducing oxidative stress and slowing disease advancement. Whether their potential can be dependent on specific cellular conditions and stimulation contexts, and thus if they can be therapeutic, will be established in future studies [44-47].

Tie-1 Inhibitors

Tie-1 belongs to the receptors tyrosine kinases and is implicated in endothelial cell dysfunction and vascular inflammation, two variables that are pivotal to the pathogenesis of atherosclerosis.

Evidence has also shown that inhibition of Tie-1 in mouse models reduces atherosclerotic plaque formation in a dose dependent manner. The mechanism is most apparent in regions of vasculature with disturbed shear stress, where they are most susceptible to atherosclerotic conversion. Aside from inhibiting pro-inflammatory marker expression such as ICAM-1 and VCAM-1, Tie-1 inhibition also enhances the activity of endothelial nitric oxide synthase (eNOS), which contributes to better vascular health. Furthermore, Tie-1 suppression has been associated with reduced macrophage invasion of the vascular wall and reduced levels of Rho-associated kinases, both of which play a role in the inflammatory processes responsible for atherosclerosis. These findings suggest that inhibiting Tie-1 can regulate key inflammatory processes without impairing thrombin's function in coagulation, and this is an exciting target for treatment. While these preclinical results are encouraging, keep in mind that Tie-1 inhibitors are in development and not yet approved for use in human clinical trials. Additional research is needed to fully determine their potential effectiveness and safety profiles as an atherosclerosis treatment [48-50].

Conclusion

Atherosclerosis represents a complex interplay between mechanical forces and immune responses. Shear stress, a key biomechanical factor, governs endothelial homeostasis. Under high, steady laminar flow, the endothelium promotes vasodilation, inhibits inflammation, and prevents thrombosis. However, in regions of disturbed or low shear stress such as arterial bifurcations the endothelium adopts a pro-inflammatory, pro-thrombotic phenotype. This dysfunctional state contributes to leukocyte adhesion, lipid accumulation, and plaque formation, ultimately increasing the risk of myocardial infarction and stroke.

Parallelly, the innate immune system particularly neutrophils play a crucial role in initiating and propagating vascular inflammation. Through the release of reactive oxygen species, proteases, and neutrophil extracellular traps (NETs), neutrophils exacerbate endothelial injury and promote atherosclerotic plaque progression and rupture. While these immune mechanisms are vital in acute host defense, their chronic activation leads to maladaptive cardiovascular consequences.

Therapeutic approaches that modulate these pathways hold promise. Pharmacological agents such as statins and anticoagulants improve endothelial function and blood flow, while immunosuppressants including corticosteroids, calcineurin inhibitors, and monoclonal antibodies aim to attenuate neutrophil-driven inflammation. However, the nonspecific nature of current immunosuppressants presents significant limitations, including increased susceptibility to infections and potential malignancies with long-term use.

Future therapeutic strategies must focus on enhancing specificity in targeting pathological immune responses without compromising host defense. An integrated research approach bridging vascular biology, immunology, and pharmacology is essential to develop safer, more effective interventions that restore endothelial integrity and suppress chronic inflammation in atherosclerosis.

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References

1. Libby P, Ridker PM, Hansson GK. Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol. 2009;54(23):2129-2138.
2. Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol. 2011;12(3):204-212.
3. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. Nat Med. 2011;17(11):1410-1422.
4. Soehnlein O. Multiple roles for neutrophils in atherosclerosis. Circ Res. 2012;110(6):875-888.
5. Drechsler M, Megens RT, van Zandvoort M, Weber C, Soehnlein O. Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. Circulation. 2010;122(18):1837-1845.
6. Döring Y, Soehnlein O, Weber C. Neutrophil extracellular traps in atherosclerosis and atherothrombosis. Circ Res. 2017;120(4):736-743.
7. Tabas I, Lichtman AH. Monocyte-macrophages and T cells in atherosclerosis. Immunity. 2017;47(4):621-634.
8. Chistiakov DA, Orekhov AN, Bobryshev YV. Neutrophils in vascular inflammation and atherosclerosis. J Thromb Thrombolysis. 2016;41(3):436-447.
9. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. Science. 2015;349(6245):316-320.
10. Ridker PM. From C-reactive protein to interleukin-6 to interleukin-1: moving upstream to identify novel targets for atheroprotection. Circ Res. 2016;118(1):145-156.
11. Libby P, Lichtman AH, Hansson GK. Immune effector mechanisms implicated in atherosclerosis: from mice to humans. Immunity. 2013;38(6):1092-1104.
12. Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science. 1991;251(4995):788-791.
13. Gimbrone MA, García-Cardeña G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circ Res. 2016;118(4):620-636.
14. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. JAMA. 1999;282(21):2035-2042.
15. Davies PF. Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. Nat Clin Pract Cardiovasc Med. 2009;6(1):16-26.
16. Chiu JJ, Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. Physiol Rev. 2011;91(1):327-387.
17. Caro CG, Fitz-Gerald JM, Schroter RC. Atheroma and arterial wall shear: observation, correlation and proposal of a shear dependent mass transfer mechanism for atherogenesis. Proc R Soc Lond B Biol Sci. 1971;177(1046):109-159.
18. VanderLaan PA, Reardon CA, Getz GS. Site specificity of atherosclerosis: site-selective responses to atherosclerotic modulators. Arterioscler Thromb Vasc Biol. 2004;24(1):12-22.
19. Cuhlmann S, van der Heiden K, Ridger V, Evans PC. Hemodynamics, inflammation and atherosclerosis: insights from mouse models. Curr Opin Lipidol. 2014;25(5):410-416.
20. Passerini AG, Polacek DC, Shi C, Francesco NM, Manduchi E, Grant GR, *et al* . Coexisting proinflammatory and antioxidative endothelial transcription profiles in a disturbed flow region of the human carotid artery. Proc Natl Acad Sci USA. 2004;101(8):2482-2487.
21. Zhou J, Li YS, Chien S. Shear stress-initiated signaling and its regulation of endothelial function. Arterioscler Thromb Vasc Biol. 2014;34(10):2191-2198.
22. Warboys CM, Amini N, de Luca A, Evans PC. The role of blood flow in determining the sites of atherosclerotic plaques. F1000 Med Rep. 2011;3:5.
23. Nigro P, Abe J, Berk BC. Flow shear stress and atherosclerosis: a matter of site specificity. Antioxid Redox Signal. 2011;15(5):1405-1414.
24. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. Nat Rev Mol Cell Biol. 2009;10(1):53-62.
25. Baeyens N, Schwartz MA. Biomechanics of vascular mechanosensation and remodeling. Mol Biol Cell. 2016;27(1):7-11.
26. Kwak BR, Bäck M, Bochaton-Piallat ML, Caligiuri G, Daemen MJ, Davies PF, *et al* . Biomechanical factors in atherosclerosis: mechanisms and clinical implications. Eur Heart J. 2014;35(43):3013-3020.
27. Brown AJ, Teng Z, Evans PC, Gillard JH, Samady H, Bennett MR. Role of biomechanical forces in the natural history of coronary atherosclerosis. Nat Rev Cardiol. 2016;13(4):210-220.
28. van der Heiden K, Cuhlmann S, Luong LA, Zakkar M, Evans PC. Role of nuclear factor κB in cardiovascular health and disease. Clin Sci. 2010;118(10):593-605.
29. Potteaux S, Combadière C, Esposito B, Lecureuil C, Ait-Oufella H, Merval R, *et a*. Chemokine receptor CCR1 disruption in bone marrow cells enhances atherosclerotic lesion development and neointima formation. Arterioscler Thromb Vasc Biol. 2006;26(9):2310-2315.
30. Nourshargh S, Alon R. Leukocyte migration into inflamed tissues. Immunity. 2014;41(5):694-707.
31. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 2013;13(3):159-175.
32. Carbone F, Nencioni A, Mach F, Vuilleumier N, Montecucco F. Pathophysiological role of neutrophils in acute myocardial infarction. Thromb Haemost. 2013;110(3):501-514.
33. Tardif JC, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, *et al* . Efficacy and safety of low-dose colchicine after myocardial infarction. N Engl J Med. 2019;381(26):2497-2505.
34. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, *et al* . Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med. 2017;377(12):1119-1131.
35. Shapiro MD, Fazio S. From lipids to inflammation: new approaches to reducing atherosclerotic risk. Circ Res. 2016;118(4):732-749.

36. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med. 1999;340(2):115-126.
37. Glass CK, Witztum JL. Atherosclerosis: the road ahead. Cell. 2001;104(4):503-516.
38. Frostegård J. Immunity, atherosclerosis and cardiovascular disease. BMC Med. 2013;11:117.
39. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. Cell. 2011;145(3):341-355.
40. Wolf D, Ley K. Immunity and inflammation in atherosclerosis. Circ Res. 2019;124(2):315-327.
41. Heo KS, Fujiwara K, Abe J. Disturbed-flow-mediated vascular reactive oxygen species induce endothelial dysfunction. Circ J. 2011;75(12):2722-2730.
42. Feaver RE, Gelfand BD, Wang C, Schwartz MA, Blackman BR. Atheroprone hemodynamics regulate fibronectin deposition to create positive feedback that sustains endothelial inflammation. Circ Res. 2010;106(11):1703-1711.
43. Liu Y, Chen BP, Lu M, Zhu Y, Stemerman MB, Chien S, *et al* . Shear stress activation of SREBP1 in endothelial cells is mediated by integrins. Arterioscler Thromb Vasc Biol. 2002;22(1):76-81.
44. Xu S, Zeng Z, Chen J, Wu H, Chen S, Chen Z, *et al* . Endothelial dysfunction and oxidative stress in atherosclerosis. Oxid Med Cell Longev. 2017;2017:7609208.
45. Tousoulis D, Oikonomou E, Economou EK, Crea F, Kaski JC. Inflammatory cytokines in atherosclerosis: current therapeutic approaches. Eur Heart J. 2016;37(22):1723-1732.
46. Ye M, Fu H, Hoang A, Mora R, Liu R, Chandra A, *et al* . Inhibition of neutrophil extracellular trap formation by colchicine attenuates myocardial ischemia/reperfusion injury in rats. Sci Rep. 2018;8(1):6565.
47. Lefer AM, Lefer DJ. The role of nitric oxide and cell adhesion molecules on the microcirculation in ischemia-reperfusion. Cardiovasc Res. 1996;32(4):743-751.
48. Yao Y, Wang Y, Zhang Y, Lu X, Liu C, Chen J, *et al* . Endothelial cell activation and injury in atherosclerosis: from pathophysiology to clinical biomarkers. Biomed Pharmacother. 2021;137:111327.
49. Grootaert MO, Bennett MR. Vascular smooth muscle cells in atherosclerosis: time for a re-assessment. Cardiovasc Res. 2021;117(11):2326-2339.
50. Bennett MR, Sinha S, Owens GK. Vascular smooth muscle cells in atherosclerosis. Circ Res. 2016;118(4):692-702.