VILNIAUS UNIVERSITETAS
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Baigiamasis darbas

Širdies ir kraujagyslių ligų išsivystymą patologiją lemiantys patofiziologiniai mechanizmai: aterotrombozės ir uždegiminis atsakas (apžvalginis)

Thromboinflammation in Cardiovascular Diseases. Literature review

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SUMMARY

Cardiovascular diseases are the most common reason for death worldwide. They have heterogenous pathophysiologic mechanisms, including augmented oxidative stress, inflammation and activated contact and complement systems. Surplus levels of reactive oxygen species induce damage to cellular macromolecules such as deoxyribonucleotide acid, proteins, and lipids. Increased reactive oxygen species result in decreased nitric oxide availability, vasoconstriction, and the development of pro-coagulant and pro-inflammatory states in blood vessels. In addition, contact and complement systems are involved in coagulation and inflammatory processes that can contribute to the atherosclerotic and thrombotic environment, leading to cardiovascular diseases. Improved knowledge of biomolecular processes triggered by oxidative stress, contact, and complement systems would aid in developing tools for assessing cardiovascular diseases and applying them in clinical settings. Still, some research gaps should be filled by defining the most clinically relevant biomarkers for oxidative stress, contact and complement systems with high sensitivity and specificity for cardiovascular diseases. This work seeks to review the literature on thromboinflammation caused by oxidative stress, contact, and complement systems that lead to cardiovascular diseases and to describe potential biomarkers for estimating the risk and improving diagnosis and potential treatment of cardiovascular diseases targeting oxidative stress, contact and complement systems.

Keywords: cardiovascular diseases, oxidative stress, inflammation, contact system, complement system, biomarkers, drug therapy.

SANTRAUKA

Širdies ir kraujagyslių ligos yra dažniausia mirties priežastis visame pasaulyje. Jas sukelia įvairūs patofiziologiniai mechanizmai: oksidacinis stresas, uždegimas ir kontaktinės bei komplemento sistemų aktyvėjimas. Reaktyviosios deguonies rūšys pažeidžia ląstelių genetinę medžiagą, baltymus ir lipidus bei sumažina azoto oksido bioprieinamumą, dėl ko susiaurėja kraujagyslės ir atsiranda endotelio disfunkcija, pasižyminti prokoaguliacinių ir prouždegimiškų sąlygų atsiradimu. Be to, kontaktinė ir komplemento sistemos prisideda prie krešėjimo ir uždegimo procesų, skatinančių aterosklerozės ir trombozės vystymąsi kraujagyslėse. Gilesnės žinios apie patofiziologinius procesus, kuriuos sukelia oksidacinis stresas, kontaktinės bei komplemento sistemos, padėtų gerinti širdies ir kraujagyslių ligų diagnostiką, šių ligų rizikos vertinimą. Vis dėlto trūksta klinikinių tyrimų, o ir jau padaryti tyrimai neišskiria kliniškai svarbiausių oksidacinio streso, kontaktinės ir komplemento sistemų biologinių žymenų, kurie turėtų didelį jautrumą ir specifiškumą širdies ir kraujagyslių ligoms. Šiame darbe siekiama apžvelgti literatūrą apie širdies ir kraujagyslių ligų patofiziologinius mechanizmus – oksidacinį stresą, kontaktinę ir komplemento sistemas, ir aprašyti galimus biologinius žymenis, padedančius įvertinti širdies ir kraujagyslių ligų riziką ir pagerinti jų diagnostiką bei galimą gydymą.

Raktažodžiai: širdies ir kraujagyslių ligos, oksidacinis stresas, uždegimas, kontaktinė sistema, komplemento sistema, biožymenys, vaistų terapija.

CONTENT

INTRODUCTION

Cardiovascular diseases (CVD) remain a top global issue despite numerous initiatives to reduce their prevalence and impact on human health (1). The most prevalent type of CVD is coronary heart disease (CHD) (2). Research evidence reveals thromboinflammation as a crucial pathophysiological mechanism in developing atherosclerosis-related CVD (3). Thromboinflammation includes such components as oxidative stress, inflammation, complement and contact systems. In the presence of a high degree of oxidative stress and activated contact and complement systems, dysfunction and inflammation arise in blood vessels, leading to the diminished ability of the endothelium to relax blood vessels, endothelial cells (EC) apoptosis, adhesion of monocytes to EC, formation of thrombus and altering the angiogenesis potential of EC (4). The most conclusive term, including all mentioned processes, is endothelium dysfunction. Consequently, due to endothelium dysfunction, atherosclerotic plaques and lesions form and thus lead to cardiovascular diseases. In this thesis, the term 'cardiovascular diseases' refers explicitly to diseases caused by atherosclerotic plaque and lesions in arteries, such as coronary artery disease, including acute coronary syndrome (myocardial infarction) and chronic coronary disease, cerebrovascular disease (stroke, transient ischemic attack, carotid artery stenosis), peripheral artery disease, abdominal and thoracic aortic aneurysm, and intestinal ischemia.

The contact system includes serine proteinase–coagulation factors XII and XI, plasma prekallikrein (PK), and the cofactor kininogen that reacts to pathophysiological factors present in the body (5). The contact system is triggered by surfaces possessing negative charges (6). The complement system is plasma proteins that interact with pathogens to point them for destruction by phagocytes. The complement system has been identified to participate in the pathogenesis of thrombosis, primarily through C5a/C5b-9 (7). Excessive production or accumulation of reactive oxygen species (ROS) causes oxidative stress. ROS comprise oxygen free radicals (superoxide, hydroxyl, and peroxyl radicals) and non-radicals (hydrogen peroxide and hypochlorous acid) (8). Mitochondria are the primary drivers of intracellular oxidant production in most cell types, followed by sources like nicotinamide adenine dinucleotide phosphate oxidases (NOXs), heme oxygenase 1, xanthine oxidase, and cyclooxygenases (9,10). Basal levels of ROS generation are essential for signal transduction pathways, protection against pathological microorganisms and gene expression (11). The body has protective measures against ROS, including enzymatic compounds such as glutathione peroxidase, superoxide dismutase (SOD), and catalase, as well as non-enzymatic compounds like nicotinamide, glutathione and tocopherol (12).

Oxidative stress biomarkers, together with complement and contact proteins, could be utilized to estimate the risk of CVD, or improve the diagnosis. For a biomarker to be clinically beneficial, it must meet at least one of the following criteria: a) demonstrate specificity for a particular disease, b) have prognostic value, and c) be correlated with disease activity (13). Significantly, therapeutically targeting contact, complement systems, and oxidative stress might aid in improving CVD symptoms.

The actuality of this thesis: scrupulous investigation of thromboinflammation helps improve diagnostic tools and target novel drug therapy for cardiovascular diseases.

The aim of this thesis: to investigate the connection between CVD and thromboinflammation.

METHODOLOGY

This thesis conducted a narrative literature search to determine clinical trials and clinical research published between 1999 and 2023. The author accomplished electronic searches in various databases, including MEDLINE (PubMed), Science Direct, Springer and Google Scholar. Different keywords were used, such as cardiovascular diseases, oxidative stress, inflammation, contact, complement systems, biomarkers, and drug therapy. Titles and abstracts were examined, and then full articles were selected for detailed reviewing and analysis.

1. PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL MECHANISMS

1.1. CONTACT SYSTEM

The contact system includes plasma proteins (serine proteinases) that react to pathophysiological factors present in the body, namely coagulation factors XII and XI, plasma prekallikrein (PK), and the cofactor kininogen (5,14). In fact, the contact system is triggered by surfaces possessing negative charges, including natural and synthetic surfaces like collagen, polyphosphates, glycosaminoglycans, nucleic acids, and lipopolysaccharides (6,14).

When activated, coagulation factor XII transforms into α-FXIIa, composed of a heavy and light chain connected by a disulfide bond. Notably, this complex glycoprotein is involved in the coagulation pathway (14,15). The mentioned light chain of α-FXIIa catalyzes and initiates FXI and PK. In contrast, the heavy chain consists of several domains that help in binding to other proteins and surfaces, including fibronectin Type I and II (14,15). Moreover, 75% of the PK in blood plasma forms heterodimeric

complexes with FXI by non-covalent binding. High-molecular-weight kininogen (HK) bridges PK and FXI in these complexes (14,16).

When facing an artificial or endogenous surface, FXII undergoes a structural change, leading to its activation. Furthermore, this activation, in turn, speeds up the conversion of PK into plasma kallikrein (KAL). KAL then activates FXII in a positive feedback mechanism (14,17). Plasma kallikrein, also known as the kallikrein-kinin system (KKS), initiates the conversion of HK into bradykinin (14,18). Alternatively, the endothelial serine protease-prolyl carboxypeptidase can trigger the activation of KKS without the involvement of FXII (14,19). Endothelial cells and platelets are targets of FXII binding. FXII has been observed to activate the complement pathway and the plasminogen – a precursor of fibrinolysis (14,20) (Figure 1).

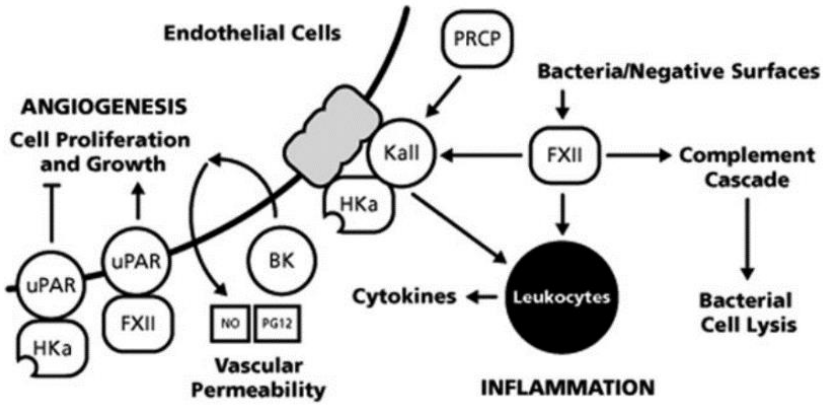

Figure 1. Activated FXII activates the complement system through C1 esterase (C1), leading to the initiation of leukocyte migration and the secretion of cytokines (6). Kallikrein (Kall), urokinase receptor (uPAR), prolylcarboxypeptidase (PRCP), bradykinin (BK), high-molecular weight HK, nitric oxide (NO) and prostacyclin (PG12), factor XII (FXII).

The activation of FXI leads to the initiation of both intrinsic and extrinsic coagulation pathways, resulting in the generation of thrombin (14,21). Thrombin generation becomes independent of factor XI when tissue factor levels are high (6,14). It is essential to mention that thrombin can activate FXI in the same manner as FXII (14,22). Significantly, platelets, as primary endogenous stimulators, release various factors that cause contact system activation. Among these factors are polyphosphate granules released into the surroundings after platelet activation (14,23).

Moreover, the contact system plays a critical role in blood clotting and impacts the breaking down of fibrin. Through a feedback mechanism, thrombin activates FXI, which consequently leads to the generation of thrombin and the production of a thrombin-activated fibrinolysis inhibitor (TAFI). TAFI protects the clot from being broken down through fibrinolysis by eliminating plasminogen from fibrin (14,24). In contrast, FXII causes fibrinolysis, either directly or indirectly, through its ability to induce the proteins responsible for fibrinolysis – urokinase plasminogen activator (u-PA) and tissue plasminogen activator (t-PA) (6,14) (Figure 2).

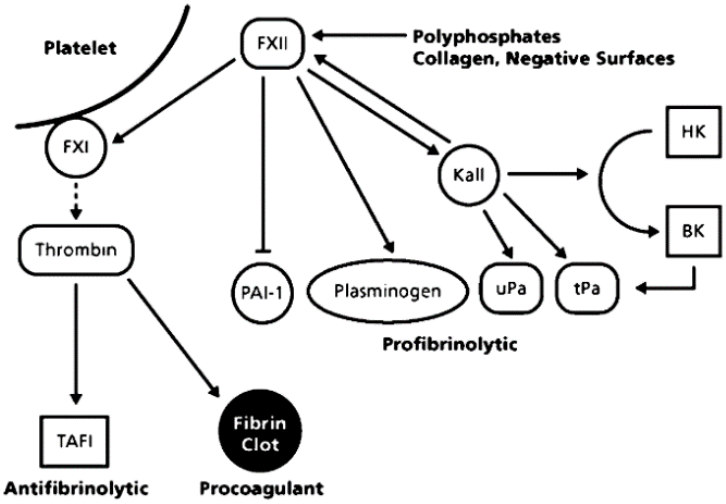

Figure 2. The activation of the contact system lead to both procoagulant and fibrinolytic processes (6). Thrombin-activated fibrinolysis inhibitor (TAFI), kallikrein (Kall), tissue plasminogen activator (tPa), urokinase plasminogen activator (uPa), bradykinin (BK), high-molecular weight kininogen (HK), plasminogen activator inhibitor (PAI-1), factor XII (FXII), factor XI (FXI).

Contact system contribute to a thrombus in the early stages of formation (6,14). Additionally, the formation of the thrombus is impacted by the activation of platelets, the contact system and the extrinsic coagulation cascade, which is triggered by tissue factors released when atherosclerotic plaques on the vessel wall are damaged (14,25). Particularly, thrombin production on the surface of aggregated platelets at the plaque rupture site is a continuous process that causes fibrin formation and stabilizes occlusive thrombi in animal models, primarily through the actions of FXII and FXI (14,26).

Collagen type I, which is abundant in atherosclerotic plaque has been shown to activate the plasma contact system, which stimulates pathways that cause inflammation and play an important role in the development of atherosclerosis (27). Upon activation, the contact system initiates an increased movement of macrophages towards sites of vascular lesion, thus finally leading to the progression of atherosclerosis

(14,28). In addition, FXII triggers neutrophils to move towards the site of injury by activating chemoattractants, as well as causes monocytes to generate interleukin-1 and neutrophil extracellular traps (NET) and causes T-cell differentiation (14,29). Moreover, when FXII is activated, it triggers the classical and alternative cascade of the complement system, which leads to increased vascular permeability, the contraction of smooth muscle cells, and the recruitment of inflammatory cells (14,30). It is essential to mention that activation of the contact pathway initiates the kallikrein-kinin system that triggers the release of BK, increasing blood vessel permeability. BK binds to its receptors B1R and B2R on the endothelial cells and smooth muscle, which releases nitric oxide (NO) and prostacyclin. Consequently, smooth muscle cells relax, and blood vessels dilate (14,31). Besides, neutrophil aggregation and degranulation can be caused by kallikrein and HK. When HK is activated, it can bind to its receptors on both monocytes and neutrophils and induce the release of cytokines, thus contributing to inflammation (14,28).

1.2. COMPLEMENT SYSTEM

The complement system has been identified to participate in the pathogenesis of thrombosis, primarily through C5a/C5b-9 (7). Products of complement component (C5) proteolysis, C5a, and C5b promote the expression of tissue factor, von Willebrand factor (vWF), and P-selectins on the surface of endothelial cells. Various substances, including thrombin, plasmin, DNA, and endothelial breakdown products, activate the complement system (7). These substances are abundant at the site of initial thrombus formation. Additionally, the significance of thrombin as an independent activator of C5 is noteworthy (7). According to in vitro studies, the role of thrombin is indirect - it promotes fibrin formation, leading to an increase in plasmin concentration, which causes C5a production (7). Other studies indicate that thrombin activates the alternative complement pathway through Factor D (adipsin). Factor D, in turn, activates complement component 3 (C3), which cleaves into C3a and C3b (32)

Activated complement (C5a, C3a), directly and indirectly through neutrophils, basophils, and macrophages, promotes thrombus formation. Neutrophils activate platelets, coagulation factors and complement itself, but, on the other hand, suppress anticoagulant and anti-complement factors by secreting the disintegrin and metalloproteinase with thrombospondin type 1 motif, 13 (ADAMTS13), which prevents the formation of ultra-large von Willebrand factor multimers (33). Furthermore, C3a and C5a stimulate neutrophils to release extracellular traps, which activate the contact system. NETs also

contain tissue factors, initiating thrombin production (34). Thrombi associated with NETs are more resistant to fibrinolysis (34).

According to study data, complement system components are frequently found in thrombus histological material (C3, C4, C5a) (32). C3 increases thrombus stability and resistance to fibrinolysis by directly binding to fibrin. Notably, C5b-9 form the membrane-attacking complexes (MAC), which cause the destruction of pathological cells and the formation of anionic surfaces on the membrane, necessary to initiate the coagulation cascade and thrombin production (33). Accordingly, cell destruction promotes procoagulant and proinflammatory processes, including the expression of tissue factor, vWF, cytokines, P-selectins, adhesion molecules (intracellular cell–adhesion molecule – 1 (ICAM-1), vascular cell adhesion molecule – 1 (VCAM-1)), and subsequent neutrophil migration, platelet activation, and faster thrombus formation (33). The classical and alternative complement pathways can lead to platelet activation and aggregation; for example, C3a and C5a activate platelets and promote their aggregation through P-selectin molecules, and C1q also induces conformational changes in platelets through GpIIbIIIa platelet integrins (35). Significantly, activated platelets also initiate the activation of complement systems by expressing mannan-binding lectin (MBL) and associated serine protease (MASP), which activates the leptin-dependent complement pathway and secreting substances from alpha and dense granules that lead to MAC formation (35,36). Besides, MASP can also activate FXII, prothrombin, prekallikrein, and fibrinogen, resulting in coagulation cascade activation (32) (Figure 3).

Complement components such as C5a and C3a cause endothelium to shift towards procoagulant and proinflammatory phenotype by binding to their receptors on endothelial cells (36).

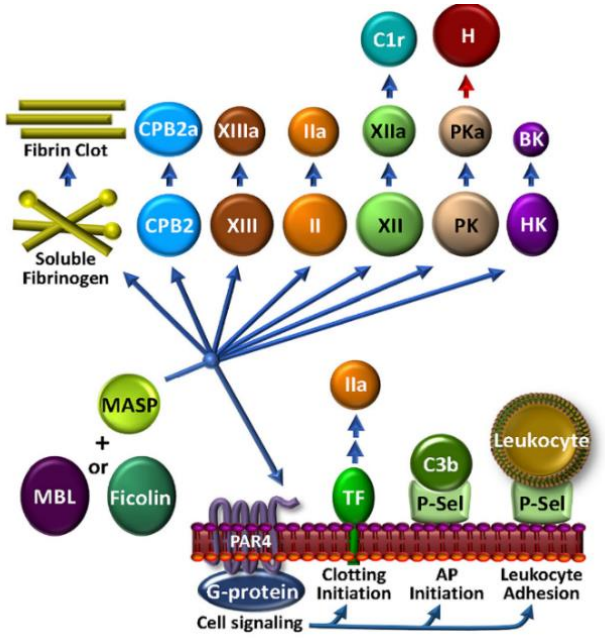

Figure 3. MASP substrates include various molecules influencing coagulation processes: FXII, thrombin, kallikrein, and fibrin. MBL-associated serine protease (MASP), mannose binding lectination (MBL), protease activated receptor 4 (PAR4), procoagulant tissue factor (TF), bradykinin (BK), kallikrein (PKa), factor H (H), activated factor XII (FXIIa), thrombin (IIa), activated factor XIII (FXIIIa), carboxypeptidase 2 (CPB2) (32).

1.3. OXIDATIVE STRESS AND INFLAMMATION

Oxidative stress plays a crucial role in the pathogenesis of CVD by initiating inflammatory and coagulation processes (37). Oxidative stress is caused by surplus of reactive oxygen species (ROS), which include hydrogen peroxide (H_2O_2), superoxide anion (O^{2-}), hydroxyl radical (^{-}OH), nitrogen, and sulfur reactive forms (38,39). In addition, deficient antioxidant systems, including superoxide dismutase, catalase, peroxiredoxin, glutathione peroxidase, tocopherol/vitamin E, glutathione, and nicotinamide can result in oxidative stress too (39,40). Significantly, ROS are the result of mitochondrial metabolism and enzymes such as heme oxygenase 1, xanthine oxidase, and NADPH oxidases (NOXs) (39,41). Oxidized low-density lipoproteins (oxLDL) through the lectin oxidized LDL receptor-1 (LOX-1) primarily activate NOXs (39,42,43). Also, oxidative stress can be caused by various substances such as angiotensin II, endothelin I, aldosterone, cytokines released by monocytes and neutrophils as tumour necrosis factor

(TNF-α), and glycosylated compounds by activating NOXs (39,44,45). Likewise, individuals with arterial hypertension may develop atherosclerosis due to elevated LOX-1 expression caused by increased blood pressure (39,43).

Endothelium dysfunction is defined by the development of procoagulant, proinflammatory, and proliferative properties on endothelium, mainly triggered by oxidative stress, thus eventually leading to atherothrombosis and the associated inflammation (38,39,42).

Notably, the bioavailability of the vasodilators produced by the endothelium, such as nitric oxide, prostacyclin (PGI2) (Rajendran et al., 2013), is reduced due to excessive free radicals. Nitric oxide exhibits anti-inflammatory and antithrombotic properties, inhibits platelet aggregation and protects blood vessels from vasospasms by acting through guanylate cyclase in the membrane of vascular smooth muscle cells (39,46).

The superoxide anion reacts with NO and deactivates it, creating peroxynitrite (ONOO-). Furthermore, the levels of nitric oxide synthase (eNOS), which is responsible for producing NO, are reduced by free radicals. In the same way, ROS lowers the concentration of eNOS substrates and cofactors, specifically L-arginine and tetrahydrobiopterin (BH4). Besides, free radicals increase the amount of dimethylarginine, an inhibitor of eNOS (38,39) (Figure 4.).

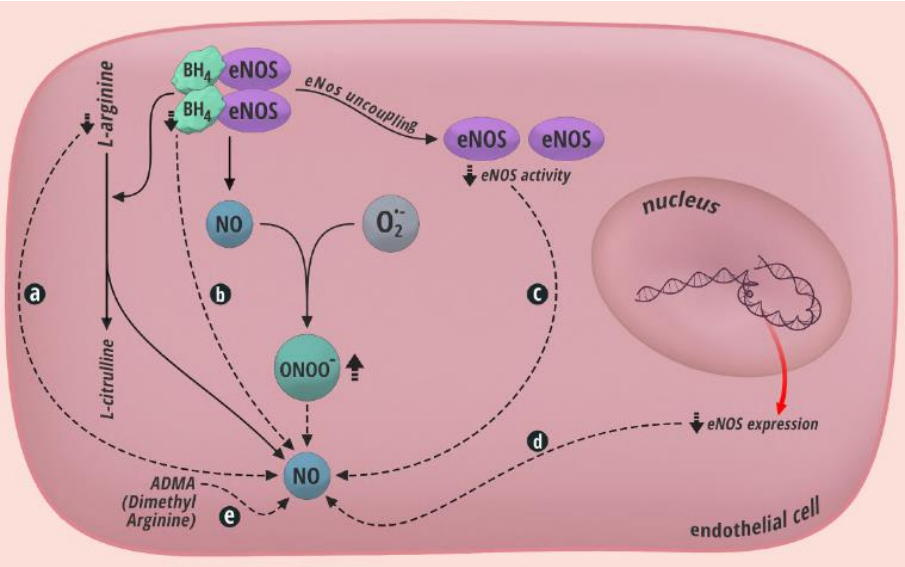

Figure 4. Fundamental mechanisms promoting NO reduction. A) and B) Reduction of eNOS substrates - L-arginine and cofactor BH4. C) Inactivation of NO through reaction with superoxide anion. D) Decreased eNOS expression. E) Dimethylarginine-induced eNOS inhibition. Nitric oxide (NO), superoxide anion (O_2^-); peroxynitrite (ONOO−), endothelial nitric oxide synthase (eNOS), tetrahydrobiopterin (BH4), dimethylarginine (ADMA) (38).

Free radicals break down eNOS dimers. Therefore, NO synthesis becomes less efficient. Significantly, ONOO- nitrifies cellular protein, thus causing mitochondrial dysfunction and cell apoptosis. Mitochondrial dysfunction is characterized by changes in mitochondrial calcium ion homeostasis, such as inhibiting the mitochondrial Na+/Ca2+ pump and alterations in the membrane potential (38,39). Mitochondria are responsible for generating ROS, which can harm the mitochondrial DNA (mtDNA). Furthermore, mitochondria naturally are an internal source of ROS, which contributes to oxidative stress (38,39).

ROS activate mitogen-activated protein kinases (MAPK); therefore, proliferative molecules like fibroblast growth factor, insulin-like growth factor, platelets, and epidermal growth factor are produced in the vascular smooth muscle layer. These growth factors cause smooth muscle cells to move towards the endothelium, leading to their proliferation and the accumulation of type III collagen in the extracellular matrix (39,43). Subsequently, smooth muscle cells can transition to a macrophage-like phenotype and contribute to atherosclerotic plaque formation (39,47).

During oxidative stress, endothelial cells tend to secrete cytokines, such as interleukin 1 (IL-1), interleukin 6 (IL-6), and TNF-α, and express cell adhesion molecules, particularly VCAM-1 and ICAM-1 (48). ROS leads to the activation of nuclear factor κB (NF-κB), which initiates the activation of specific genes that are responsible for producing adhesion molecules such as P and E-selectins, ICAM-1, and VCAM-1, as well as cytokines like IL-6 and TNF-α. Consequently, neutrophils and monocytes activate and move to the damaged vascular tissues. Additionally, the dysfunctional endothelium releases extracellular vesicles that contain microRNAs 155 (miR155) and microRNAs 92a (miR92a), which cause the polarization of monocytes towards the pro-inflammatory M1 phenotype (39,48).

Oxidized LDL with cholesterol crystals and activated platelets stimulate neutrophils to form and release NETs, which promote interleukin 1β (IL-1β) cytokine production by macrophages, further contributing to the migration of neutrophils and T lymphocytes (39,49). Activated T lymphocytes secrete cytokines interleukin 17 (IL-17) and stimulate adaptive immune system cells. Macrophages, which are derived from monocytes, initiate the phagocytosis of lipoproteins through scavenger receptors CD36 and SR-A, resulting in the transformation of lipoproteins into foam cells (39,49). Moreover, pro-inflammatory endothelial adhesion molecules limit macrophage migration from atherosclerotic lesions, while netrins-1 and semaphorin-3E inhibit macrophage chemotaxis by blocking Chemokine (C-C motif) ligand 19 (CCL19) and Chemokine (C-C motif) ligand 21 (CCL21) receptors (39,49). Consequently, the necrotic lipid core, comprised of dead foam cells and efferocytes, starts to form due to macrophage migration inhibition and inefficient efferocytosis (49). Cytokines and Toll-like receptor (TLR) agonists stimulate dendritic cells to mature and express receptors CD11c+, CD11b+, and CD40. Afterwards, dendritic cells present antigens to different subtypes of T lymphocytes using major histocompatibility complex (MHC) I molecules for CD8+ T lymphocytes and MHC II molecules for CD4+ (39,49).

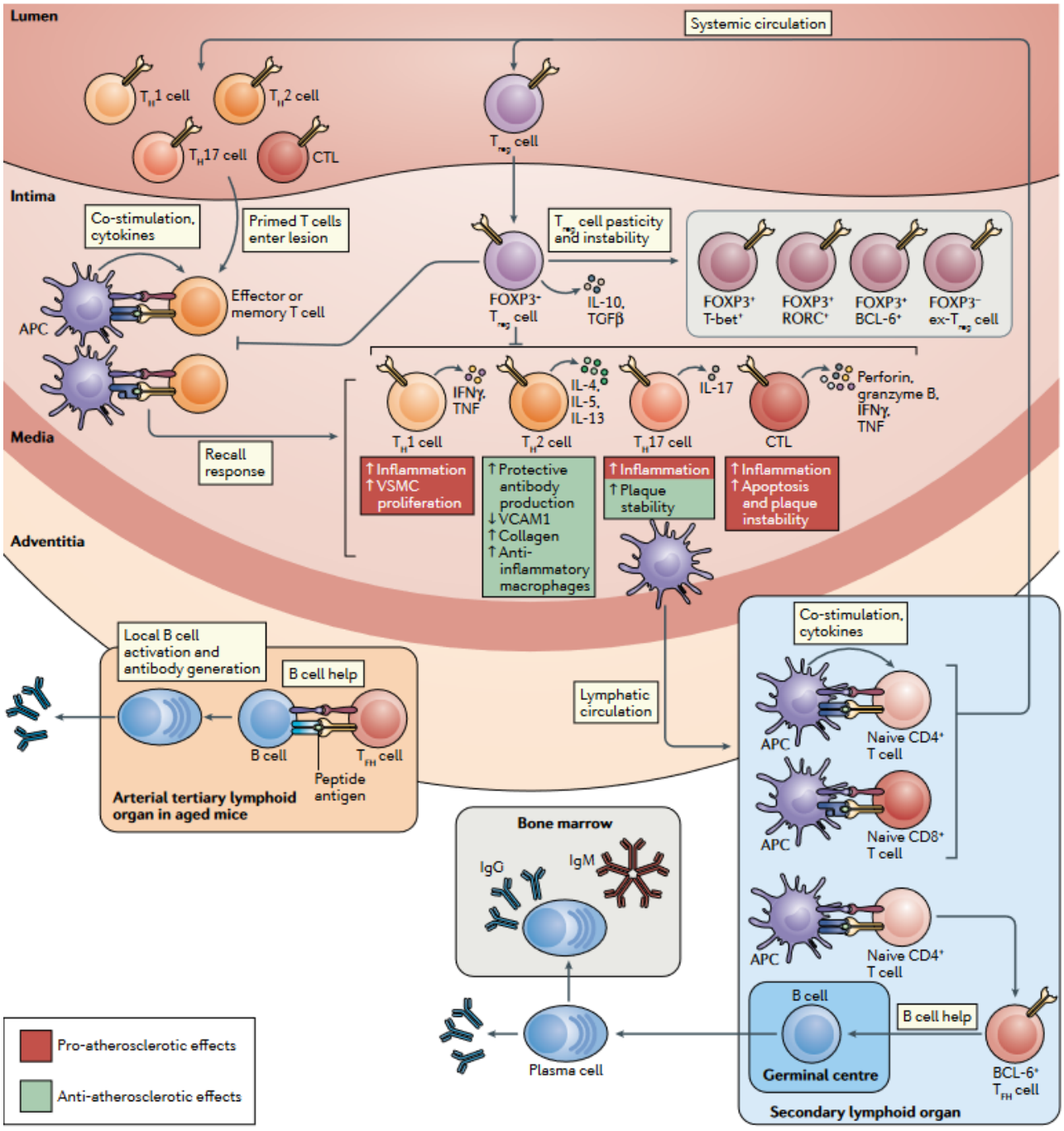

Figure 5. Adaptive immune system in atherosclerosis formation. Antigen-presenting cells (APCs) BCL-6+ T follicular helper (TFH), T helper 1 (TH1), T helper 2 (TH2), TH17 cells, regulatory T (Treg), CD8+ cytotoxic T lymphocytes (CTLs), transforming growth factor-β (TGFβ), interferon-γ (IFNγ), tumour necrosis factor (TNF), vascular cell adhesion molecule 1 (VCAM1), vascular smooth muscle cell (VSMC) (49).

Due to vascular endothelium dysfunction caused by oxidative stress, an atherosclerotic plaque develops and can rupture, leading to clot formation. The risk of rupture is significantly increased in the presence of a large necrotic core of the plaque, a thin fibrin cap (<65μm), pronounced inflammation, or vessel remodelling (39,50). Besides, macrophages express plaque fibrin cap lysing enzymes such as collagenases and gelatinases and, together with T lymphocytes, secrete interferon-γ (IFN-γ), which inhibits collagen synthesis and also induces apoptosis of vascular smooth muscle cell (25,39). After the rupture of an atherosclerotic plaque, various substances such as collagen, tissue factor, and vWF are released. The vWF attaches itself to the glycoprotein Ib-IX-V complex (GPIb-IX-V) receptor complex on platelets, while collagen binds to the glycoprotein Ia/IIa complex (GPIa/IIa) and VI receptors thus activating platelets. Once activated, the platelets change shape and release the contents of their granules, such as adenosine diphosphate (ADP), serotonin, and thromboxane A2, leading to platelet adhesion (39,51). C – type lectin – like receptor 2 (CLEC-2), a newly found protein on the surface of platelets, has been identified as an essential regulator of platelet activation. CLEC-2 triggers platelet activation (25,39). When the atherosclerotic plaque ruptures, tissue factors are released, which activate the external coagulation cascade and platelets thus resulting in fibrin production. Consequently, the formation of blood clots occurs in the blood vessels and causes CVD and even death.

Non-modifiable risk factors for CVD include vascular ageing, which is highly challenging to manage. Systemic oxidative stress is a prevalent feature of aging. Enzymes such as NOXs and monomeric eNOS cause a natural rise in superoxide anion O^{2-} concentration in aging arteries (39,52). Mitochondria generate ROS in the form of H_2O_2 and O^{2-} thus contributing to the development of endothelium dysfunction related to ageing (53). As ageing progresses, mitochondrial capacity to synthesize adenosine triphosphate (ATP) decreases, generating more ROS (53). Ageing reduces the protection capacity of the antioxidant system (52).

The primary vascular dilators also become hydrogen peroxide instead of prostacyclins and NO. Nevertheless, when the concentration of H_2O_2 exceeds, it causes and sustains oxidative stress despite its initial dilating effect (52). As a result of oxidative stress, the process of peroxynitrite inhibits the synthesis of PGI2, thus leading to the elevated level of thromboxane A2, which is known to have vasoconstrictive properties and promotes platelet aggregation (53).

Genes of SASP (senescence-associated secretory phenotype) are activated by ROS, DNA damage caused by mechanical stress (radiation), telomere dysfunction, and cellular stress. These activated genes further activate NF-κB, ensuring the continuous production and secretion of cytokines in vascular cells

(54). The perpetuating processes sustain inflammation in the vascular cells, resulting in lasting alterations such as thickening of vascular walls, instability of formed atherosclerotic plaques, and formation of thrombus.

2. OPPORTUNITIES FOR CLINICAL PRACTICE

2.1. CONTACT SYSTEM IN CLINICAL PRACTICE

As previously stated, prekallikrein (PK), high molecular weight kininogen (HK), FXII, and FXI are contact and kallikrein/kinin systems components. In a population-based study, researchers studied the relationship between plasma HK and PK levels and the likelihood of developing chronic heart disease and ischemic stroke (14,55). Individuals in the cohort group with higher quartiles of HK and PK exhibited higher levels of total cholesterol and diabetes prevalence. In contrast, women in this group showed higher levels of high-density lipoprotein (HDL) cholesterol. However, the study did not establish a significant positive correlation between plasma HK or PK amounts and CVD. Even when comparing the highest decile of HK versus <90th percentile, no correlation was found with ischemic stroke (14,55). In another study, levels of inhibitory complexes, including FXIa-C1-inhibitor (FXIa-C1INH), FXIIa-C1-inhibitor (FXIIa-C1INH), FXIa-antithrombin (FXIa-AT), and kallikrein- C1-inhibitor (kallikrein-C1INH) were measured throughout an acute myocardial infarction (AMI), as well as after 3 and 6 months (14,56). Statistically significant differences were observed in the levels of FXIa-C1INH for most patients, with the highest levels detected upon admission. However, the FXIa-AT, FXIIa-C1INH, and kallikrein-C1INH levels did not change significantly. Besides, the odds ratios determined for FXIa-C1INH, FXIa-AT, and FXIIa-C1INH, respectively, indicated no predictive value of high levels through AMI for the occurrence of recurrent events such as cardiovascular death, recurrent myocardial infarction, ischemic stroke, second coronary intervention, or coronary artery bypass grafting after 3 and 6 months of follow-up (Attachment 1).

FXI has been found to play a vital role in venous thrombosis, acute stroke and myocardial infarction (14,57). The study also revealed that FXII role in VTE, myocardial infarction, and stroke is less significant than of FXI. Several therapeutic strategies are being developed for venous thrombosis to target FXI and its protease form, FXIa. Some of these approaches include 2'-Methoxyethyl DNA ASOs, anti-FXI ASO IONIS-FXIRX Ocosimab, and BMS-986177 (14,58,59). A comparison was made between an

anti-FXI ASO and enoxaparin for VTE prevention in surgical patients. The study found that FXI ASO IONIS – FXIRX at 300 mg provided the best outcomes, with only 4% of thrombi growing in the lower extremity. Moreover, abnormal intraoperative bleeding was rare, even when plasma FXI grades were below 10% (14,58). Another potential treatment is Ocosimab, an IgG that bonds FXIa. Clinical trials have shown that a preoperative infusion of 1.8 mg/kg had more profitable effects than enoxaparin, with a VTE incidence of 11.3% vs 26.3% (14,60). BMS-986177 is a medication currently being tested in phase 2 clinical trials, acting by inhibiting FXIa. However, the therapeutic interference with contact system factors and atherosclerosis-related cardiovascular diseases has not been investigated yet (27).

2.2. COMPLEMENT SYSTEM IN CLINICAL PRACTICE

Activation of complement system proteins causes vascular remodelling and the development of atherosclerosis (61). Intimal thickening and fibrous plaques exhibit elevated levels of C5b-9 compared to normal tissue. Moreover, the levels of C5b-9 in intimal thickenings are greater than those in fibrous plaques, indicating that complement activation occurs directly in the artery wall (62). Additionally, CVD was found to be linked with C3 and C3a in heavy-smoking individuals (63). Another study found that the membrane-attacking complex can play a crucial role in forming plaques and the rupture of aneurysms (64). Recently, it was discovered that high levels of C5b9 can increase the risk of acute ischemic stroke and unstable carotid plaques (65). The mentioned study found that the serum C5b-9 levels were statistically significantly higher in patients with unstable carotid plaques than those with stable ones.

Furthermore, in patients with small infarcts, the C5b-9 levels increased to 814.00 (725,50–919,00) ng/mL and further increased to 857,00 (780,00–968,50) ng/mL in patients with larger infarcts. However, there was no statistically notable difference in the C5b-9 levels between patients with small and large infarcts (Attachment 2). Furthermore, 90-day follow-up demonstrated that the patients with poor outcomes had higher serum C5b-9 levels than those with good outcomes. In contrast, one study found that levels of C1q are not statistically related to CVD but have a linear relationship with carotid intima-media thickness or endothelium dysfunction (66).

Considering complement-targeted therapy, one clinical trial currently uses recombinant human C1 esterase inhibitors to prevent acute ischemic cerebral and renal events after transcatheter aortic valve implantation. Moreover, soluble complement receptor 1 (TP10) was used experimentally in some clinical trials as a potent inhibitor of C3 and C5 convertases in preventing systemic inflammatory response after

cardiopulmonary bypass; however, TP10 was very expensive to produce and never came in clinical utilization, although results revealed its efficacy in inhibiting complement activation during cardiopulmonary bypass surgery (67,68).

2.3. OXIDATIVE STRESS AND INFLAMMATION IN CLINICAL PRACTICE

There are numerous biomarkers for oxidative stress and inflammation in CVD. Still, their clinical applicability is a concern mostly due to no consensus exists on which one is superior. In this thesis, only more specific for CVD and more researched biomarkers will be investigated as F_2 – isoprostanes (F_2 – IsoPs), *trans* – 4 – hydroxy – 2 – nominal (4 – HNE), malondialdehyde (MDA), oxidized low–density lipoproteins (oxLDL), matrix metalloproteinases (MMPs), and C-reactive protein (CRP) (69). F_2 – IsoPs, which arise from polyunsaturated fatty acid (PUFA) peroxidation, are prostaglandin-like compounds characterized by platelet–activating and vasoconstrictive properties (39,70) (Gaggini et al., 2020). Specifically, F_2 – IsoPs are frequently treated as the most credible markers for monitoring oxidative stress *in vivo* because they correlate with the extent of CVD, reliable outcome prediction and chemical stability (39,70). Using mass spectrometry, Shishehbor et al. quantified nine distinct lipid peroxidation products in patients plasma. After this, patients were evaluated by diagnostic coronary angiography to reveal a statistically significant correlation between higher lipoxidation product levels and the existence of CHD. Eventually, results of the study showed that F_2 – IsoPs were significantly higher in those diagnosed with CHD (OR 9,7 in the highest F_2 – IsoPs quartile and plasma levels 1,5 fold higher in CHD) (39,73).

Aldehydes as *trans* – 4 – hydroxy – 2 – nominal (4 – HNE) and malondialdehyde (MDA) are lipid peroxidation products characterized by their rapid reactivity with proteins to form Michael adducts (advanced lipoxidation end products) (39,74). As mentioned above, proteins participating in reaction with aldehydes are residues of apolipoprotein B (apoB) (39,75), and following alteration in its structure by MDA increases its affinity for the scavenger receptors of macrophages and causes their transformation into foam cells (39,76). It is known that 4-HNE provokes cellular oxidative stress and induces the activation of the endoplasmic reticulum stress (39,77). Considering CVD, HNE and MDA as markers are selected in preference to other lipid oxidation products for estimating the risk of acute cardiovascular events, especially myocardial infarction and ischemic stroke (39,78).

The potential of oxidized low–density lipoproteins (oxLDL) as biomarkers in CVD has been reviewed in many studies (39,79). As a biomarker, the most investigated oxLDL component is oxidized

phospholipids (oxPLs), and their elevation is thought to have a significant role in oxLDL-induced vascular inflammation and subsequent coronary, carotid and femoral artery diseases (39,80–83). Dijk et al. evaluated six atherosclerotic lesion types of postmortem carotid endarterectomy by using immunostaining for the detection of oxPLs, malondialdehyde (MDA) and apoprotein an (apo(a)) epitopes (39,84). Results demonstrated that all atherosclerotic lesions, such as necrotic core, fibrous cap, foamy macrophages, and SMC, express oxidization-specific epitopes (39,84).

The most researched and specific inflammatory biomarkers in CVD include matrix metalloproteinases (MMPs) and C-reactive protein (CRP).

MMPs are expressed chiefly in macrophages and also in vascular smooth muscle cells (SMCs) and endothelial cells (85) and play a significant role in inflammation and other physiological and pathological processes such as vascular remodelling, aneurysm formation and, especially, progression of atherosclerosis and plaque rupture (86). Additionally, MMPs have been announced to be upgraded in patients with unstable angina, myocardial infarction and sudden cardiac death (87).

CRP is acute-phase protein hepatocytes produce in response to stimulating cytokines such as IL-6, Il-1 and TNF-α (88). Previous studies extensively studied CRP, treating it as a predictor of future cardiovascular events (89). Currently, CRP is considered a robust but unspecific biomarker in predicting cardiovascular risk. Wang et al. examined the correlation between levels of monomeric CRP (mCRP) and acute myocardial infarction (AMI) (90). Analysis revealed that raised plasma mCRP was extensively detected in AMI compared to patients with both unstable and stable angina.

Different strategies are currently being explored to treat the adverse effects of oxidation stress on cells. Clinical research and trials primarily focus on preventing the formation of harmful molecules like $ONOO^-$ by removing O_2^- before it can react with NO or removing H_2O_2 before it can form OH^- (91) (Table 1). Notably, current antidiabetic cardiovascular drugs, such as sodium-glucose transport protein 2 inhibitors (SGLT2i) and glucagon-like peptide-1 (GLP-1) analogues, are effective in reducing CVD risk by inhibiting inflammatory and oxidative stress mechanisms (92). Based on numerous clinical trials, European Society of Cardiology confirmed updated acute and chronic heart failure diagnosis and treatment guidelines, which recommend SGLT2 inhibitors as agents that reduce cardiovascular death, worsening heart failure and hospitalization due to heart failure (93).

SGTL2i impact on ameliorating thromboinflammation was investigated in many basic and clinical research. Such agents as empagliflozin and ipragliflozin were demonstrated to attenuate ROS, VCAM-1 and ICAM-1 in the abdominal aorta of mice (94). Furthermore, Empagliflozin was revealed to decrease

mitochondrial production of ROS in endothelial cells of diabetic and hypertensive elderly patients (95). Uthman et al. conducted a study to investigate whether Empagliflozin and Dapagliflozin decrease TNF-α induced inflammation in human coronary arterial endothelial cells. Results revealed that SGLT2i inhibited ROS generation and thus diminished inflammation in TNF-α induced coronary arterial endothelial cells (96). Another study demonstrated that uremic serum from patients with chronic kidney disease harms cardiac microvascular endothelial control of cardiomyocytes and that empagliflozin recovers this intercellular crosstalk by reducing ROS and restoring NO levels in cardiomyocytes, improving their relaxation and contraction (97).

Another antidiabetic agent, GLP-1, is currently extensively researched. Different clinical trials and studies have revealed reduced major cardiovascular events in patients treated with GLP-1. In the clinical trial LEADER (Effects of Liraglutide on Cardiovascular Outcomes in Patients With Diabetes With or Without Heart Failure), patients treated with liraglutide had lower risk of cardiovascular death, AMI, or stroke (98).

GLP-1, as well as SGLT2i, demonstrated its antioxidant effect by decreasing ROS in endothelial cells, reducing the accumulation of macrophages in the vascular wall, the expression of VCAM-1, ICAM-1, E-selectins and thus preventing atherosclerotic plaque formation (99). Additionally, statins and angiotensin-converting enzyme inhibitors lower the risk of CVD by decreasing proinflammatory chemokines and cytokines, NF-κB, P-selectins, and monocyte activation (100). They also minimize oxidative stress by reducing NOX and O_2 production and oxidation of LDL.

Table 1. Clinical trials of drugs targeting oxidative stress in CVD.

Antioxidant	Mimic drug	Indications	Clinical status
Glutathione peroxidase	ALT-2074	Diabetes, coronary artery diseases	Phase II
Superoxide dismutase and catalase	EUK-8	Heart-ischemia-reperfusion, cardiomyopathy	Preclinical
Superoxide dismutase and catalase	EUK-134	Stroke	Preclinical

CONCLUSION AND SUGGESTIONS

Many factors contribute to the development and acceleration of cardiovascular diseases. The most essential pathological mechanism is oxidative stress. Others include activated contact and complement systems. Significantly, surplus reactive oxygen species production leads to endothelium dysfunction that affects cardiovascular homeostasis and orchestrates thromboinflammation, which is also influenced by activated contact and complement systems. Augmented thromboinflammation results in cardiovascular diseases. Oxidative stress, contact and complement molecules could be utilized as biomarkers for estimating the risk or improving the diagnosis of cardiovascular diseases. However, none of the researched biomarkers has made it into regular clinical usage due to their questionable specificity, prognostic value, correlation with CVD, unstable nature, lack of valid data, hard detectable levels, and methodological challenges. Many therapeutic drug solutions targeting oxidative stress and complement system in cardiovascular diseases are currently in clinical trials. Notably, current antidiabetic cardiovascular drugs, such as sodium-glucose transport protein 2 (SGLT2) inhibitors, were confirmed in the guidelines of acute and chronic heart failure diagnosis and treatment of European Society of Cardiology due to evident efficacy in reducing CVD risk by inhibiting inflammatory and oxidative stress mechanisms.

It is essential to target further research into the primary pathophysiological cardiovascular disease mechanism—oxidative stress—biomarkers, especially their clinical importance in diagnosing cardiovascular diseases, estimating risk, and identifying utilization opportunities. Further discoveries on oxidative stress and its biomarkers would change the routine clinical approach to cardiovascular diseases. Moreover, drug therapies targeting oxidative stress and their clinical effectiveness on cardiovascular diseases should be explored in clinical research and trials.

Further investigation into contact and complement systems as possible biomarkers for cardiovascular diseases is crucial. Currently, there is a lack of valid clinical research demonstrating the significant role of contact and complement systems in developing thromboinflammation. Despite current clinical drug trials in complement systems, more drug therapy solutions must be explored. Targeting the therapeutic approach in the contact system is essential. No valid clinical study has been done on anti-complement therapy for cardiovascular diseases.

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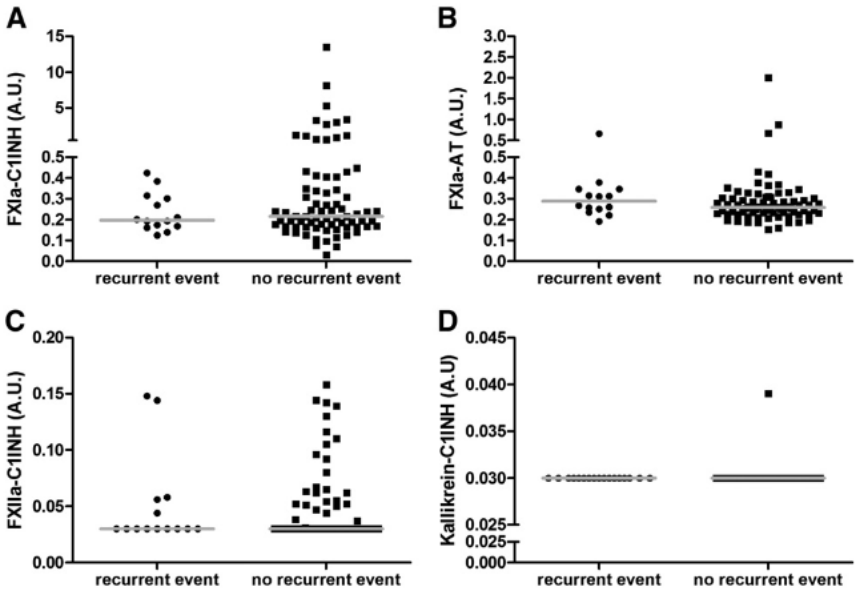
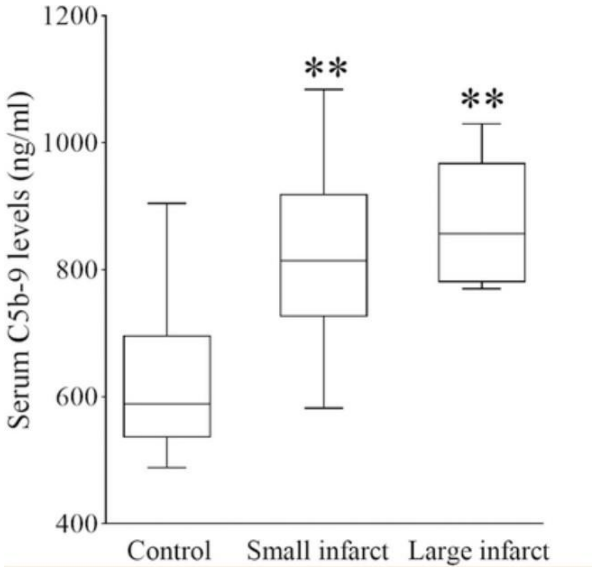
ATTACHMENTS



Attachment 1. Levels of FXIa-C1-inhibitor (FXIa-C1INH) (A), FXIa-antithrombin (FXIa-AT) (B), FXIIa-C1-inhibitor (FXIIa-C1INH) (C) and kallikrein- C1-inhibitor (kallikrein-C1INH) (D) on admission in patients during a first myocardial infarction. The levels of FXIa, FXIIa and kallikrein in complex with C1 esterase inhibitor (C1INH) and FXIa – AT are separated into patients with a recurrent cardiovascular event within the first year and those that did not have a recurrent event. The grey line depicts the median value (56).



Attachment 2. Serum C5b-9 levels in acute ischemic stroke patients with different infarct sizes. The range of serum C5b-9 levels variation in patients with small and large infarct $^{**}p < 0.001$ (66).