

Inflammation and Oxidative Stress in Acute Coronary Syndromes: A Continuum from Plaque Vulnerability to Thrombus Formation

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Abstract

Acute coronary syndromes are caused by plaque rupture and thrombus formation. Inflammation and oxidative stress play a pivotal role in atherosclerosis progression and plaque instability. Recruited inflammatory cells produce several cytokines, chemokines and adhesion molecules stimulating the biosynthesis of further pro-inflammatory factors. Mediators of inflammation are able to trigger several pathophysiological processes involved in plaque vulnerability such as neoangiogenesis, MMPs activity, extracellular matrix degradation, ROS production, lipid peroxidation and others. Several studies investigated the role of markers of inflammation and oxidative stress in the pathophysiology of plaque formation and progression, basis of clinical epiphenomena.

Keywords

Acute coronary syndromes; Inflammation; Oxidative stress; Plaque vulnerability

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Cardiovascular diseases currently represent the first cause of death in industrialized countries with ischemic heart disease corresponding to the zenith of the phenomenon being responsible either for acute cardiovascular events or chronic heart failure (HF) as evolution of acute coronary syndromes. Acute myocardial infarction (AMI) with ST elevation (STEMI) is due to coronary obstruction or occlusion by means of atherosclerotic plaque fissuring and/or rupture. Inflammation and oxidative stress play undeniably a pivotal role in the pathogenesis and progression of atherosclerosis, inducing plaque instability and the subsequent thrombus formation [1-3]. Previous studies have already underlined the ubiquitous presence of platelets, erythrocytes, fibrin and inflammatory cells within the thrombus corroborating, therefore, the crucial role of the inflammatory cascade activation. Thrombi are morphologically classified in: 1) platelets-rich thrombi, with low content of erythrocytes, 2) mixed thrombi, with platelets and erythrocytes, 3) erythrocytes-rich thrombi, with low content of platelets, usually present in smokers with elevated blood levels of oxidative stress markers such as myeloperoxidase and oxidized low-density lipoproteins-(oxLDL) [4-6]. Thrombus composition depends on many factors, such as ischemic time, inflammatory and oxidative state. Furthermore, thrombus composition is strictly related to the period of time between plaque rupture and thrombus progression. Given these premises they are also classified as: 1) fresh thrombi (< 1 day) composed by fibrin layers, platelets, erythrocytes, granulocytes, 2) lytic thrombi (1-5 days) with colliquative necrosis areas and karyorrhexis 3) organized thrombi (>5 days) with smooth muscular cells, connective tissue deposition and growing capillaries [7]. Many studies rely on coronary thrombi composition, focusing on platelets, fibrin and tissue factor role, since this latter has already demonstrated its ability to sustain and promote thrombus formation [8-11]. Accordingly starting from a core which is made of observations, concepts and tools, appears evident that thrombi derive from plaque rupture and/or erosion and are characterized by a dynamic evolution. When the fibrin cap is broken, collagen and tissue factor are exposed to blood flow, inducing platelets activation, conversion of fibrinogen into fibrin and, consequently, thrombus formation [12-15]. During the early stages, platelets aggregate into the lipidic core causing thrombus shape modification and, thus, its protrusion in the vessel lumen. While sometimes a spontaneous lysis follows this early phase, in other cases the evolution of this process move forward with intramural thrombus formation.

Several studies have demonstrated that, sometimes, non-culprit lesion may progress toward rupture with consequent possible coronary thrombosis even in absence of clinical symptoms and myocardial damage [16]. Nevertheless, platelets aggregation, regardless of its pathophysiological process, once activated leads to a progressive partial or complete vessel occlusion. Moreover, in this stage, the intermittent antegrade blood flow may facilitate micro-embolization to the distal vessels with consequent increased resistance in distal circulation that might end up in the "no reflow" phenomenon [17].

Another important aspect of this pathological process is undoubtedly related to the fibrin network formation. Fibrin, in fact, can accumulate over the plaque, trap a large number of erythrocytes, as well as stabilize the thrombus, giving rise to the formation of a red thrombus. The erythrocytes-rich thrombus in this final stage can propagate both proximally and distally further worsening coronary circulation. In this phase, another important actor is the tissue factor (TF), released by macrophages and smooth muscular cells and highly expressed in the advanced atherosclerotic lesions. Plaque rupture, in fact, determines the exposure of TF to blood flow triggering the activation of the coagulation pathways as well as the formation of platelet and fibrin-rich thrombi [18,19]. These findings, thus, suggest that an increased concentration of TF in the plaque is responsible for fibrin-rich high-burden thrombus formation and that monocytes activation is able to increase the platelet thrombogenic potential, leading to acute coronary syndrome [20-22]. Tissue factor is a powerful trigger of the coagulation cascade and it is usually localized in the extracellular space between the adventitial cells of the vessel providing a continuous hemostatic barrier able to induce the coagulation once the vessel wall integrity is interrupted. Moreover, TF after its interaction with factor VII, activates factor X leading to the generation of thrombin. Tissue factor is considered an important regulator of blood coagulation, hemostasis and thrombosis [23,24], and, binding PARs, it is able to modulate inflammation signals. Its presence is well demonstrated in thrombi isolated from patients with STEMI, and several studies already underlined that monocytes and granulocytes are an important source of circulating TF. This protein is potentially active inside the atherosclerotic plaque, partially determining the thrombogenic potential of the plaque [25,26]. Leucocytes can transfer TF to platelets by CD15 receptor, resulting in the formation of pro-coagulant aggregates with high content of leucocyte-derived TF. Besides, it is known that also platelets can further express an active form of this molecule [27].

From these considerations appears evident that inflammation plays a key role in the development of acute coronary syndromes. Systemic levels of acute-phase proteins, especially C reactive protein, correlate in a proportional way with the number of ruptured or eroded plaques evaluated by intravascular ultrasonography (IVUS) as well as inversely correlate with the thickness of the fibrous cap of culprit lesion. Some studies underlined an increased haematic level of C reactive protein in cases of sudden death for AMI, regardless the morphology of the culprit lesions. Furthermore, systemic levels of myeloperoxidase, a protein contained in azurophilic granules and released after neutrophil activation, can predict the outcome of patients with acute coronary syndrome and are considered a marker of plaque vulnerability [28-30].

It is not clear if different levels of myeloperoxidase are related to several presentations of plaque morphology; however, some studies showed, by means of in vivo optical coherence tomography (OCT), that systemic levels of myeloperoxidase in patients with acute coronary syndrome reflect the presence of plaque erosion/rupture.

Likewise high levels of co-stimulatory molecule CD80 are expressed in vulnerable human carotid plaques compared to stable plaques and for this reason CD80 specific radiotracer has been proposed as tool to identify vulnerable plaque [31].

Soluble CD40 ligand (CD40L) is an important pro-inflammation factor likewise involved in thrombus formation. Its expression is increased in the fresh thrombus and it participates to the thrombus formation during early phases of AMI. CD40L is expressed on platelet surface after their stimulation and is cleaved in a time interval of minutes to hours, generating the soluble factor (sCD40). Despite the soluble factor can be also secreted by activated lymphocytes, it is showed that more than 95% of circulating factor is platelets-derived [32,33]. The relation between sCD40L levels and HBTF (high burden thrombus formation) risk in patients with AMI is still not clearly understood; the most accepted hypothesis is that circulating levels of sCD40, considered as a platelet aggregation index, are related to angiographic features of HBTF in patients with STEMI. Additionally, high levels of sCD40L in patients with HBTF are associated with an increased white blood cell count [34,35] such as polymorphonuclear cells and activated platelets, in thrombi removed by patients with AMI. Last, sCD40L concentration is increased during acute phase of myocardial infarction. Plaque composition is also influenced by other factors, such as oxLDL. The association between oxLDL and atherosclerosis progression is a consolidated evidence. OxLDL induce expression of adhesion molecules on endothelial cells, leading to the recruitment of monocytes in the vessel wall intima and are phagocytized by macrophages through scavenger receptors with accumulation in macrophages and formation of foam cells.

Moreover, OxLDL stimulate migration and proliferation of vascular smooth muscle cells, increasing the production of reactive oxygen species (ROS), which in turn promote atherosclerosis with magnification of the inflammatory process [36]. Avogaro et al. [37] identified a negatively charge fraction of oxLDL, partially responsible for ROS formation, while Mello et al. [38] defined the class of minimally oxidized LDL [LDL(-)] by analyzing their electrical mobility. LDLs are increased in patients with diabetes mellitus, hypercholesterolemia, coronary artery disease, and play a central role both in the activation of inflammatory responses as well as in promoting atherosclerosis. Lipid accumulation is associated with endothelial dysfunction, monocyte infiltration and with inflammatory cells migration. These processes, in synergy with the activation of vascular smooth muscle cells and the increased production of extracellular matrix, lead to vessel wall thickening and asymmetric lumen narrowing bringing to remarkable alterations on the structure of intima, media and adventitia. Early atherosclerotic lesions are composed of lipidic excess, cholesterol, extracellular matrix components (especially collagen type I and III), elastine, and proteoglycans. The early lesions usually induce vessel wall thickening without stenosis, whereas in the later stages atherosclerosis leads to asymmetric narrowing due to momentous remodeling of the vessel wall related to the proteolytic degradation of extracellular matrix [39-42]. Leucocytes, activated macrophages, and inflammatory processes are all involved in the progression of early lesions and plaque vulnerability. The recruitment of leucocytes and inflammatory cells is facilitated by endothelial dysfunction, phlogosis and increased expression of adhesion molecules. Recruited inflammatory cells and vessel cells produce several cytokines, chemokines and adhesion molecules stimulating the biosynthesis of other pro-inflammatory factors and both innate and adaptive immunity are involved in this process [43].

Interleukin-8 (IL-8) contributes to the recruitment of white cell subtypes and to the amplification of the inflammatory response through the stimulation of interferon- γ (IFN- γ) synthesis [44,45]. Monocytes and macrophages represent the principal cellular population in atherosclerosis, even if T cells, B cells, dendritic cells and neutrophils are also present in atherosclerotic lesions [46].

Neutrophil activity in unstable lesions has already been demonstrated and it is particularly observed in the rupture sites of atherosclerotic plaques [47]. Neutrophils accomplish many functions, including endocytosis of cellular debris and secretion of proteolytic enzymes like elastase, myeloperoxidase and neutral endopeptidase. These enzymes contribute to plaque vulnerability by extracellular matrix degradation and inflammatory response modulation. Other factors are undoubtedly involved in the complex mechanism of atherosclerosis and its evolution. The production of pro- and anti-inflammatory cytokines and the modulation of specific immunologic mechanisms contribute to the progressions of the disease [48-50]. Several pro-inflammatory mediators, including INF- γ and tumor necrosis factor- α (TNF- α), stimulate cellular response in the atheroma and vessel wall [51-52]. It is demonstrated that blood of patients with vulnerable carotid plaques has increased levels of TNF- α and IL-8 in comparison with patients with stable plaques. Furthermore, several studies have already shown a strong relation between IL-8 blood levels and macrophage accumulation in vulnerable plaques. The extracellular matrix degradation, partially caused by metalloproteinases activation and the inflammatory response represent the main promoters of plaque progression to plaque rupture [53-56]. Smooth muscle cells are anchored on a basement membrane susceptible to metalloproteinases activity that, through enzymatic degradation of the basement membrane, induces the proliferation of the vascular smooth muscle cells (VEMCs).

These processes can be defined as an adaptive response to inflammatory activation that, in this case, ends up as a maladaptive evolution. Extracellular matrix is composed by proteoglycans, collagen and elastin. Proteoglycans are involved in important biological functions such as permeability regulation, interaction with other components of extracellular matrix (especially collagen type I and III), as well as preservation of vessel elasticity and stability. Large part of these components is synthesized by VSMCs, which are essential in maintaining the integrity and homeostasis of vessel wall. Enzymatic proteolysis of extracellular matrix is a pivotal pathophysiological process in tissue repair, chronic inflammatory response and atherosclerosis. Metalloproteinases (MMPs) play therefore a crucial role in these processes and are classified into 3 categories: 1) Matrix MMPs, already known for their role in plaque vulnerability and rupture 2) ADAM family of metalloproteinases 3) ADAMTS, metalloproteinases with thrombospondin domain. Approximately 25 types of MMPs have been described, and 14 are located in the vessel wall. While some of these proteases are secreted by different cells, most of them are expressed on cell surface. MMP-1 is localized in intraplaque haemorrhage, and is considered as an indicative factor of plaque instability [57-59]. Some studies have correlated high levels of MMP-2 and MMP-9 with IL-6 and IL-8 in atherosclerotic lesions, while a high concentration of MMP-9 has been demonstrated in unstable plaque of symptomatic patients [60,61]. Furthermore, an excess of MMPs has been identified in the inflammatory infiltrate of atherosclerotic lesions confirming their

important role in plaque progression [62]. Some studies investigated the pattern expression of MMPs proving that: a) MMP-7 and MMP-12 are often localized on the border of lipidic core, b) MMP-1, MMP-2, MMP-9 are mainly localized in the fibrous cap. Furthermore it has been identified a different expressions of MMPs inhibitors [63]. MMPs are able to promote cellular migration through extracellular matrix, and, particularly, MMP-1 facilitates cellular growth, VSMCs migration playing a key role in tissue remodeling [64,65]. MMP-7 and MMP-3 are involved in extracellular matrix degradation whereas MMP-1, MMP-3 and MMP-9 are the mainly expressed in atherosclerotic vessels. The above mentioned enzymes are all involved in vessel thickening (synthesis and deposition of extracellular matrix) and plaque rupture (extracellular matrix degradation). Protease activity, debris and dead cell clearance and inflammatory infiltrate interplay leading to plaque destabilization and rupture [66,67]. Several studies have described that several cells, including macrophages and inflammatory cells, release MMPs in the plaque, whereas collagen synthesis (which balances and finely tunes extracellular matrix degradation started by MMPs) is sustained almost exclusively by VECMs. Furthermore, in high-grade stenosis, it has been proved a reduction of VECMs, probably related to the involvement of MMPs, especially MMP-7, in VECMs apoptosis [68-70]. Consequently extracellular matrix production decreases while degradation remains constant or increases during atherosclerosis progression. ADAM family is involved in many biological functions such as cellular adhesion, migration, activation or cell surface markers and proteolysis. Twenty-nine ADAM proteases have been so far described, but their role in atherosclerosis pathogenesis and progression is still not totally clear. Increased levels of ADAM 8, 9, 12 and 15 have been found in atherosclerotic carotid arteries, and ADAM 10 and 17 have shown ability to induce TNF- α secretion [71-73]. ADAMTS family is composed by 19 components, and particularly ADAMTS 1, 2, 3, 4, 5, 8, 9, and 13 are expressed into the vessel wall. Almost all these enzymes are able to degrade proteoglycans and ADAMTS 2 and 3 are also able to degrade procollagen I, II and III [74], even if hitherto the role of ADAMTS family in atherosclerosis is not totally clear. Extracellular matrix degradation caused by MMPs, ADAM and ADAMTS, undeniably facilitates the release of mediators capable to induce angiogenesis. Expression of MMP-1, -2, -3, -7, -9, and ADAM-12, -15 in neo-vessels as well as the high permeability of vessels involved in this process allow the accumulation of inflammatory cells in the plaque, leading to plaque instability.

Atherosclerotic plaques are usually nourished by a sprouting of small vessels [75], induced by hypoxic and inflammatory stimuli and therefore pivotal in the process of plaque progressions and vulnerability [76].

Sprouting of neo-vessels is induced by mediators such as HIF-1 (hypoxia-inducible growth factor), VEGF (vascular endothelial growth factor), FGF (fibroblast growth factor), PDGF (platelet-derived growth factor), Ang (Angiopoietin), and Eph (ephrins) [77,78]. Neoangiogenesis is usually activated by VEGF and its receptors VEGFR-1 and VEGFR-2 [79,80] while recruitment of other endothelial components, pericytes, and smooth muscle cells is triggered by PDGF action.

Angiopoietin-1 (Ang-1) reduces vascular permeability and supports vessel stability, and Angiopoietin-2 (Ang-2), antagonist of Ang-1, reduces Ang-1 effects leading to vessel instability [81]. A reduced production of Ang-1 in comparison with Ang-2 expression, has been described as typical pattern of advanced lesions.

Despite neo-vessels are present in almost all atherosclerotic lesions, there are few evidences of causal relation among atherosclerosis progression, neovascularization and plaque vulnerability. Despite is still not clear the role of angiogenic factors in atherosclerosis, molecules such as VEGF and Ang-1 could be able to influence the plaque stability and could be considered as biomarkers in patients with high risk of acute cardiovascular events [82]. More than 97% of neo-vessels identified in the plaque, irrespective of their size, are immature, without the basement constituted by VSMCs, and therefore highly permeable [83].

Placental growth factor (PIGF), homologous of VEGF, is expressed in cardiac tissue and plays a crucial role in neo-angiogenesis, regulating the sprouting of cardiac vessels. PIGF concentration, in fact, increases after hypoxic and ischemic cardiac stimuli contributing to the adaptive and maladaptive angiogenesis. The interaction between PIGF, TNF- α , TNF- α -converting enzyme (TACE) and Tissue inhibitor of metalloproteinases-3 (TIMP-3) plays a pivotal role in inflammatory process of vascular and cardiac tissue [84]. TNF- α is a pleiotropic cytokine involved in the relation between phlogosis and atherosclerosis, and mononuclear cells are its main source, even if this mediator is synthesized also by B cells, T cell, NK, endothelial cells, mast cells and nervous tissue. The regulation of TNF- α production has been related to transcription and translation processes.

TNF- α transcription is induced by Toll-like receptors, T Cell Receptor and Pattern Recognition Receptors regulated by cell activation and inflammatory stimuli. A dysregulation of TNF- α production is involved in several pathological conditions such as cardiovascular diseases, asthma, rheumatoid arthritis, systemic lupus erythematosus, susceptibility to infections, eczema and multiple sclerosis. TNF- α is expressed on cell surface and cleaved by TACE (or ADAM17) with production of the soluble type in the interstitial fluid. Both cell surface and soluble type are active if assembled into trimeric complex. Receptors TNFR-1 and TNFR-2 have shown ability to mediate the functions of TNF- α . TNFR1 is constitutively expressed by immunitary cells and other cytotypes, TNFR2 is expressed after induction on endothelial and hematopoietic cell surface. TNFR2 activates signal of Nf-kB and MAPK, stimulating the expression of pro-inflammatory cytokines, chemokines, adhesion molecules and other factors implicated in phlogosis. Nf-kB is also involved in antioxidants expression and anti-/pro-apoptotic factors production, such as caspase 8.

Moreover, oxidative stress, due to unbalanced production of ROS and antioxidants, has been described as crucial in atherosclerosis induction and evolution, even if the protective effect of some antioxidant factors, such as glutathione peroxidase and superoxide dismutase, appears to be less effective in the process. ROS production, in fact, has shown to be responsible of a) expression of adhesion molecules, b) activation of MMPs, c) proliferation of VSMCs, d) lipid peroxidation, e) endothelial dysfunction, and apoptosis. Moreover several main cardiovascular risk factors, such as diabetes mellitus, hypercholesterolemia, smoking habits and hypertension, have shown important relationship with oxidative stress. NADPH-oxidase is an enzyme responsible for ROS production and its activity can be induced by infections, hypertension and hypercholesterolemia. This enzyme is constituted by 2 cytosolic subunits and 2 transmembrane subunits and its activity is evaluated through the concentration of hydrogen peroxide and anion superoxide. Nitric Oxide (NO), produced by endothelial cells, is an important intra- and inter-cellular mediator, and plays a key role in endothelial function. NO production is induced by chemical and mechanical stimuli and is able to reduce platelet aggregation and leucocyte adhesion as well as to induce the relaxation of VSMCs, leading to anti-aggregation, anti-inflammatory and anti-hypertensive effects.

Finally, several pathophysiological mechanisms are responsible for plaque vulnerability, including phlogosis, increased extracellular matrix degradation and neo-vascularization. These processes interplay leading to atherosclerotic progression, plaque instability and rupture and for this reason atherosclerosis is considered as a highly heterogeneous, complex and multifaceted cardiovascular disease. Therefore, all single mentioned factors should be considered, evaluated and integrated into the development of new diagnostic and therapeutic strategies.

Reference

- 1 Libby P, Ridker PM, Maseri A (2002) Inflammation and atherosclerosis. *Circulation* 105: 1135-1143.
- 2 Ross R (1999) Atherosclerosis: an inflammatory disease. *N Eng J Med* 340: 115-126.
- 3 Kobayashi S, Inoue N, Ohashi Y, Terashima M, Matsui K, et al. (2003) Interaction of oxidative stress and inflammatory response in coronary plaque instability: important role of C-reactive protein. *Arterioscler Thromb Vasc Biol* 23: 1398-1404.
- 4 Rittersma SZ, van der Wal AC, Koch KT, Piek JJ, Henriques JP, et al. (2005) Plaque instability frequently occurs days or weeks before occlusive coronary thrombosis: a pathological thrombectomy study in primary percutaneous coronary intervention. *Circulation* 111: 1160-1165.
- 5 Yunoki K, Naruko T, Sugioka K, Inaba M, Iwasa Y, et al. (2012) Erythrocyte-rich thrombus aspirated from patients with ST-elevation myocardial infarction: association with oxidative stress and its impact on myocardial reperfusion. *Eur Heart J* 33: 1480-1490.
- 6 Rautou PE, Vion AC, Amabile N, Chironi G, Simon A, et al. (2011) Microparticles, vascular function, and atherothrombosis. *Circ Res* 109: 593-606.
- 7 Carol AI, Bernet M, Curós A, Rodríguez-Leor O, Serra J, et al. (2014) Thrombus age, clinical presentation, and reperfusion grade in myocardial infarction. *Cardiovasc Pathol* 23: 126-130.
- 8 Sato Y, Hatakeyama K, Yamashita A, Marutsuka K, Sumiyoshi A, et al. (2005) Proportion of fibrin and platelet thrombi on ruptured and eroded coronary atherosclerotic plaque in humans. *Heart* 91: 526-530.
- 9 Steffel J, Luscher TF, Tanner FC (2006) Tissue factor in cardiovascular diseases: molecular mechanisms and clinical implications. *Circulation* 113: 722-731.
- 10 Uchida Y, Sakurai T, Kanai M, Shirai S, Morita T (2011) Characterization of coronary fibrin thrombus in patients with acute coronary syndrome using dyestaining angiography. *Arterioscler Thromb Vasc Biol* 31: 1452-1460.
- 11 Breintenstein A, Camici GG, Tanner FC (2009) Tissue factor: beyond coagulation in the cardiovascular system. *Clin Sci* 118: 159-172.
- 12 Davies MJ (2000) The pathophysiology of acute coronary syndromes. *Heart* 83: 361-366.
- 13 Grech ED, Ramsdale DR (2003) Acute coronary syndrome: ST segment elevation myocardial infarction. *BMJ* 326: 1379-1381.
- 14 Thim T, Hagensen MK, Bentzon JF, Falk E (2008) From vulnerable plaque to atherothrombosis. *J Intern Med* 263: 506-516.
- 15 Falk E (1991) Coronary thrombosis: pathogenesis and clinical manifestations. *Am J Cardiol* 68: 28B-35B.
- 16 Sato Y, Hatakeyama K, Marutsuka K, Asada Y (2009) Incidence of asymptomatic coronary thrombosis and plaque disruption: comparison of non-cardiac and cardiac deaths among autopsy cases. *Thromb Res* 124: 19-23.
- 17 Henriques JP, Zijlstra F, Ottervanger JP, de Boer MJ, van't Hof AW (2002) Incidence and clinical significance of distal embolization during primary angioplasty for acute myocardial infarction. *Eur Heart J* 23: 1112-1117.
- 18 van der Wal AC, Becker AE, van der Loos CM, Das PK (1994) Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* 89: 36-44.
- 19 Badimon L, Chesebro JH, Badimon JJ (1992) Thrombus formation on ruptured atherosclerotic plaques and rethrombosis on evolving thrombi. *Circulation* 86: 11174-11185.
- 20 Leatham EW, Bath PM, Toole JA, Camm AJ (1995) Increased monocyte tissue factor expression in coronary disease. *Br Heart J* 73: 10-13.
- 21 Hatakeyama K, Asada Y, Marutsuka K, Sato Y, Kamikubo Y, et al. (1997) Localization and activity of tissue factor in human aortic atherosclerotic lesions. *Atherosclerosis* 133: 213-219.
- 22 Ardissino D, Merlini PA, Ariens R, Coppola R, Bramucci E, et al. (1997) Tissue factor antigen and activity in human coronary atherosclerotic plaques. *Lancet* 349: 769-771.
- 23 Badimon JJ, Lettino M, Toschi V, Fuster V, Berrozpe M, et al. (1999) Local inhibition of tissue factor reduces the thrombogenicity of disrupted human atherosclerotic plaques: effects of tissue factor pathway inhibitor on plaque thrombogenicity under flow conditions. *Circulation* 99: 1780-1787.
- 24 Drake TA, Morrissy JH, Edgington TS (1989) Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am J Pathol* 134: 1087-1097.
- 25 Palmerini T, Tomasi L, Barozzi C, Della Riva D, Mariani A, et al. (2013) Detection of Tissue Factor Antigen and Coagulation Activity in coronary artery thrombi isolated from patients with ST-segment elevation acute myocardial infarction. *PLoS One* 8: e81501.
- 26 Hoshiba Y, Hatakeyama K, Tanabe T, Asada Y, Goto S (2006) Co-localization of von Willenbrand factor with platelet thrombi, tissue factor and platelets with fibrin, and consistent presence of inflammatory cells in coronary thrombi obtained by an aspiration device from patients with acute myocardial infarction. *J Thromb Haemost* 4: 114-120.
- 27 Sakai T, Inoue S, Takei M, Ogawa G, Hamazaki Y, et al. (2011) Activated inflammatory cells participate in thrombus size through tissue factor and plasminogen activator inhibitor-1 in acute coronary syndrome: Immunohistochemical analysis. *Thromb Res* 127: 443-449.
- 28 Ridker PM (2001) High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation* 103: 1813-1818.
- 29 Tarzewski J, Torzewski M, Bowyer DE, Frohlich M, Koenig W, et al. (1998) C-reactive protein frequently colocalizes with the terminal complement complex in the intima of early atherosclerotic lesions of human coronary arteries. *Arterioscler Thromb Vasc Biol* 18: 1386-1392.
- 30 Calabro P, Willerson JT, Yeh ET (2003) Inflammatory cytokines stimulate C-reactive protein production by human coronary artery smooth muscle cells. *Circulation* 108: 1930-1932.
- 31 Müller A, Mu L, Meletta R, Beck K, Rancic Z, Drandarov K, et al. (2014) Towards non-invasive imaging of vulnerable atherosclerotic plaques by targeting co-stimulatory molecules. *Int J Cardiol*. Jul 1 174: 503-515.
- 32 Yasojima K, Schwab C, McGeer EG, McGeer PL (2001) Generation of C-reactive protein and complement components in atherosclerotic plaques. *Am J Pathol* 158: 1039-1051.

- 33 Youssef AA, Chang LT, Sheu JJ, Lee FY, Chua S, et al. (2007) Association between circulating level of CD40 ligand and angiographic morphologic features indicating high-burden thrombus formation in patients with acute myocardial infarction undergoing primary coronary intervention. *Circ J* 71: 1857-1861.
- 34 Heeschen C, Dimmeler S, Hamm CW, van der Brand MJ, Boersma E, et al. (2003) CAPTURE Study Investigator: Soluble CD40 ligand in acute coronary syndromes. *N Eng J Med* 348: 1104-1111.
- 35 Schonbeck U, Mach F, Sukhova GK, Herman M, Graber P, et al. (2000) CD40 ligation induces tissue factor expression in human vascular smooth muscle cells. *Am J Pathol* 156: 7-14.
- 36 André P, Prasad KS, Denis CV, He M, Papalia JM, et al. (2002) CD40L stabilizes arterial thrombi by a beta3 integrin-dependent mechanism. *Nat Med* 8: 247-252.
- 37 Avogaro P, Bon GB, Cazzolato G (1988) Presence of a modified low density lipoprotein in humans. *Arteriosclerosis* 8: 79-87.
- 38 Mello AP, da Silva IT, Abdalla DS, Damasceno NR (2011) Electronegative low-density lipoprotein: origin and impact on health and disease. *Atherosclerosis* 215: 257-265.
- 39 Chen CH, Jiang T, Jiang W, Lu J, Marathe GK, et al. (2003) Low-density lipoprotein in hypercholesterolemic human plasma induces vascular endothelial cell apoptosis by inhibiting fibroblast growth factor 2 transcription. *Circulation* 107: 2102-2108.
- 40 Tomasik A, Jachec W, Skrzep-Poloczek B, Widera-Romuk E, Wodniecki J, et al. (2003) Circulating electronegatively charged low-density lipoprotein in patients with angiographically documented coronary artery disease. *Scand J Clin Lab Invest* 63: 259-265.
- 41 Fabjan JS, Abuja PM, Schaur RJ, Sevanian A (2001) Hypochlorite induces the formation of LDL(-), a potentially atherogenic low density lipoprotein subspecies. *FEBS Lett* 499: 69-72.
- 42 Shen CM, Mao SJ, Huang GS, Yang PC, Chu RM (2001) Stimulation of smooth muscle cell proliferation by ox-LDL and acetyl LDL-induced macrophage-derived foam cells. *Life Sci* 70: 443-452.
- 43 Businaro R (2013) Neuroimmunology of the atherosclerotic plaque: a morphological approach. *J NeuroimmunePharmacol* 8: 15-27.
- 44 Zernecke A, Shagdarsuren E, Weber C (2008) Chemokines in atherosclerosis: an update. *Arterioscler Thromb Vasc Biol* 28: 1897-1908.
- 45 Zernecke A, Weber C (2005) Inflammatory mediators in atherosclerotic vascular disease. *Basic Res Cardiol* 100: 93-101.
- 46 Andrés V, Pello OM, Silvestre-Roig (2012) Macrophage proliferation and apoptosis in atherosclerosis. *Curr Opin Lipidol* 23:429-438.
- 47 Naruko T, Ueda M, Haze K, van der Wal AC, van der Loos CM, et al. (2002) Neutrophil infiltration of culprit lesions in acute coronary syndromes. *Circulation* 106: 2894-2900.
- 48 Weber C, Zernecke A, Libby P (2008) The multifaceted contributions of leukocyte subsets to atherosclerotic: lessons from mouse models. *Nat Rev Immunol* 8: 802-815.
- 49 Hansson GK, Hermansson A (2011) The immune system in atherosclerosis. *Nat Immunol* 12: 204-212.
- 50 Manthey HD, Zernecke A (2011) Dendritic cells in atherosclerosis: functions in immune regulation and beyond. *Thromb Haemost* 106: 772-778.
- 51 Plutzky J (2003) The vascular biology of atherosclerosis. *Am J Med* 115: 55S-61S.
- 52 Libby P (2002) Inflammation in atherosclerosis. *Nature* 402: 868-874.
- 53 Pelisek J, Rudelius M, Zepper P, Poppert H, Reeps C, et al. (2009) Multiple biological predictors for vulnerable carotid lesions. *Cerebrovasc Dis* 28: 601-610.
- 54 Newby AC (2005) Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. *Physiol Rev* 85: 1-31.
- 55 Virmani R, Burke AP, Farb A, Kolodgie FD (2006) Pathology of the vulnerable plaque. *J Am Coll Cardiol* 47:C13-C18.
- 56 Libby P, Ridker PM, Hansson GK (2009) Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol* 54: 2129-2138.
- 57 Roy R, Zhang B, Moses MA (2006) Making the cut: protease-mediated regulation of angiogenesis. *Exp Cell Res* 312: 608-622.
- 58 Heider P, Pelisek J, Poppert H, Eckstein HH (2009) Evaluation of serum matrix metalloproteinases as biomarkers for detection of neurological symptoms in carotid artery disease. *Vasc Endovascular Surg* 43: 551-560.
- 59 Nikkari ST, O'Brien KD, Ferguson M, Hatsukami T, Welgus HG, et al. (1995) Interstitial collagenase (MMP-1) expressions in human carotid atherosclerosis. *Circulation* 92: 1393-1398.
- 60 Dollery CM, Libby P. (2006) Atherosclerosis and proteinase activation. *Cardiovasc Res* 69: 625-635.
- 61 Formato M, Farina M, Spirito R, Maggioni M, Guarino A (2004) Evidence for a proinflammatory and proteolytic environment in plaques from endarterectomy segments of human carotid arteries. *Arterioscler Thromb Vasc Biol* 24: 129-135.
- 62 Reeps C, Pelisek J, Seidl S, Schuster T, Zimmermann A, et al. (2009) Inflammatory infiltrates and neovessels are relevant sources of MMPs in abdominal aortic aneurysm wall. *Pathobiology* 76: 243-252.
- 63 Müller A, Krämer SD, Meletta R, Beck K, Selivanova SV, et al. (2014) Gene expression levels of matrix metalloproteinases in human atherosclerotic plaques and evaluation of radiolabeled inhibitors as imaging agents for plaque vulnerability. *Nucl Med Biol* 41: 562-569
- 64 Jaroslav P, Christian R, Stefan O, Alexander Z, Zepper P, et al. (2012) Evaluation of serum biomarkers for patients at increased risk of stroke. *Int J Vasc Med*.
- 65 Pelisek C, Rudelius M, Zepper P, Poppert H, Reeps C, et al. (2009) Multiple biological predictors for vulnerable carotid lesions. *Cerebrovasc Dis* 28: 601-610.
- 66 Silvestre-Roig C, de Winther MP, Weber C, Daemen MJ, Lutgens E, et al. (2014) Atherosclerotic plaque destabilization: mechanisms, models, and therapeutic strategies. *Circ Res* 114 : 214-226.
- 67 Tomey MI, Narula J, Kovacic JC (2014) Advances in the understanding of plaque composition and treatment options: year in review. *J Am Coll Cardiol* 63: 1604-1616.
- 68 Thakore AH, Guo CY, Larson MG, Corey D, Wang TJ, et al. (2007) Vasan RS, D'Agostino RB Sr, Lipinska I, Keany JF Jr, Benjamin EJ, O'Donnel CJ. Association of multiple inflammatory markers with carotid intimal medial thickness and stenosis (from the Framingham Heart Study). *Am J Cardiol* 99: 1598-1602.
- 69 Pelisek J, Hahntow IN, Eckstein HH, Ockert S, Reeps C, et al. (2011) Impact of chronic kidney disease on carotid plaque vulnerability. *J Vasc Surg* 54: 1643-1649.
- 70 Williams H, Johnson JL, Jackson CL, White SJ, George SJ (2010) MMP-7 mediates cleavage of N-cadherin and promotes smooth muscle cell apoptosis. *Cardiovasc Res* 87: 137-146.
- 71 Duffy MJ, McKiernan E, O'Donovan N, McGowan PM. (2009) The role of ADAMs in disease pathophysiology. *Clin Chim Acta* 403: 31-36.
- 72 Seals DF, Courtneidge SA (2003) The ADAMs family of metalloproteinases: multidomain proteins with multiple functions. *Genes Dev* 17: 7-30.
- 73 Van Goor H, Melenhorst WB, Turner AJ, Holgate ST (2009) Adamalysins in biology and disease. *J Pathol* 219: 277-286.
- 74 Porter S, Clark IM, Kevorkian L, Edwards DR (2005) The ADAMTS metalloproteinases. *Biochem J* 386: 15-27.
- 75 Langheinrich AC, Kampschulte M, Buch T, Bohle RM (2007) Vasa vasorum and atherosclerosis - Quid novi? *Thromb Haemost* 97: 873-879.

- 76 Sluimer JC, Daemen MJ (2009) Novel concepts in atherogenesis: angiogenesis and hypoxia in atherosclerosis. *J Pathol* 218: 7-29.
- 77 Carmeliet P (2003) Angiogenesis in health and disease. *Nat Med* 9: 653-660.
- 78 Heilmann C, Beyersdorf F, Lutter G (2002) Collateral growth: cells arrive at the construction site. *Cardiovasc Surg* 10: 570-578.
- 79 Ferrara N (2009) Vascular endothelial growth factor. *Arterioscler Thromb Vasc Biol* 29: 789-791.
- 80 Karamysheva AF (2008) Mechanism of angiogenesis. *Biochemistry* 73: 751-762.
- 81 Lim HS, Lip GY, Blann AD (2005) Angiopoietin-1 and angiopoietin-2 in diabetes mellitus: relationship to VEGF glycaemic control endothelial damage/dysfunction and atherosclerosis. *Atherosclerosis* 80: 113-118.
- 82 Pries AR, Kuebler WM (2006) Normal endothelial. *Handb Exp Pharmacol* 176: 1-40.
- 83 Schröder R (2004) Prognostic impact of early ST-segment resolution in acute ST-elevation myocardial infarction. *Circulation* 110: e506-e510.
- 84 Carnevale D, Cifelli G, Mascio G, Madonna M, Sbroggiò M, et al. (2011) Placental Growth Factor Regulates Cardiac Inflammation Through the Tissue Inhibitor of Metalloproteinases-3/Tumor Necrosis Factor- α Converting Enzyme Axis: Crucial Role for Adaptive Cardiac Remodeling During Cardiac Pressure Overload. *Circulation* 124: 1337-1350.

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