Biomarkers in ischemic cardiac syndromes

van der Zee, P.M.

Citation for published version (APA):
van der Zee, P. M. (2010). Biomarkers in ischemic cardiac syndromes.

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 1

General introduction and outline of the thesis
General introduction

Mechanisms of stable and unstable coronary syndromes

Both stable and unstable coronary syndromes are manifestations of atherosclerosis, a systemic condition. Although the first atherosclerotic lesions may already occur in adolescence (1), clinical symptoms usually take place at a later age, with high morbidity and mortality in the Western world (2). As a manifestation of atherosclerosis, accumulation of atheromatous plaques within the walls of the coronary arteries, i.e. coronary artery disease, is the most common cause of coronary heart disease (3). Plaque formation may result in narrowing of any of the coronary arteries, resulting in stable disease with symptoms as a result of insufficient oxygen and nutrient supply to the myocardium. In addition, a plaque of any thickness may erode or rupture. Underlying tissue factor (TF) may then come into contact with blood, triggering the cascade of coagulation and hemostasis. As a result, a thrombus develops in a relatively short lapse of time, may cause critical narrowing, leading to non-ST-elevation acute coronary syndrome, or complete occlusion, leading to ST-elevation myocardial infarction. Both conditions of unstable coronary artery disease thus share a similar pathogenesis.

Our views of atherosclerosis, progression to unstable disease and the pathogenesis of myocardial ischemia and necrosis have evolved remarkably over the past 50 years. Current opinion assigns an essential role to inflammation as a common pathway linking risk factors such as dyslipidemia to plaque formation and the tendency to erosion and rupture.

Subsequent thrombus formation is a complex process of successive enzymatic events and counter mechanisms that eventually determine evolution to unstable clinical disease. As mentioned above, thrombus formation starts with exposure of TF from subendothelial tissue. It should be noted that also blood components such as monocytes and endothelial cells may expose TF upon activation (4). TF initiates the coagulation cascade by formation of the tissue factor-factor VIIa complex, requiring a procoagulant membrane containing anionic phospholipids. The complex in turn activates factor X. Further downstream activation is counterbalanced by an endogenous inhibitor of this complex, tissue factor pathway inhibitor (TFPI). TFPI inhibits TF in a two-step mechanism. It binds to the active site of activated factor X (factor Xa), thus inhibiting its proteolytic capacity, followed by inhibition of the catalytic activity of TF-factor VIIa complexes by formation of the quaternary complex TF-factor VIIa/TFPI-factor Xa (5). Human ex-vivo and rabbit in-vivo experiments have shown that TFPI reduces thrombogenicity of disrupted atherosclerotic plaques (6,7). In contrast to stable disease, levels of TFPI decrease and levels of the TFPI/Xa complex increase downstream from the coronary arteries in acute coronary syndromes, indicating involvement in thrombus formation (8). Moreover, systemic levels of TFPI are increased in acute coronary syndromes when compared to stable disease (9-11). Factor Xa will convert some prothrombin into thrombin. This process is strongly autocatalytic, because thrombin will activate platelet-released factor V to factor Va and the Xa–Va complex will convert
prothrombin much faster than factor Xa alone. Prothrombin fragment 1+2 (F1+2) is released from the amino terminal end of prothrombin during its conversion to thrombin and is used as a marker of thrombin production. Another marker of thrombin production are thrombin-antithrombin complexes. Antithrombin is a liver-produced circulating glycoprotein that binds to the proteases of the coagulation cascade (12). Its most important effect is inactivation of thrombin, but it also inactivates factors IXa, Xa, XIa, and XIIa (13). Thrombin–antithrombin (TAT) complexes are formed when antithrombin binds and irreversibly inhibits the active site of thrombin (14,15).

Thrombin converts fibrinogen into the end product of coagulation, fibrin. Fibrin is a polymerized protein that forms a network which, in conjunction with platelets, results in a hemostatic plug. Intravascular fibrinolytic activity is regulated by a balance between plasminogen activators such as tissue plasminogen activator (t-PA), released by vascular endothelium, and endogenous inhibitors of plasminogen activation, such as plasminogen activator inhibitor-1 (PAI-1) and α2-antiplasmin (16).

Next to coagulation, plaque erosion or rupture leads to activation of blood platelets. Exposure of subendothelial constituents of the vessel wall such as collagen and plaque lipid content initiates platelet activation and aggregation (17). In addition, platelet activation occurs in response to thrombin formation (18). Activated platelets release epinephrine (19), and adenosine diphosphate (ADP) (20), leading to further amplification of platelet activation, vasoconstriction, and the eventual formation of a thrombus. The thrombus may lead to reduction of coronary blood flow or even total occlusion. However, the process of thrombus formation is repetitive and dynamic with a delicate balance between thrombosis and thrombolysis (21,22), and eventual occlusion may take days or even weeks (23). It can be estimated that in only 15%–20% of all episodes of acute plaque disruption, a thrombotic complication will occur in the absence of antithrombotic treatment (24). Thus, development to unstable disease and resulting myocardial damage may not only result from the local predisposition of a plaque to rupture or erosion (“vulnerable plaque”), but may also be a consequence of properties of the blood (“vulnerable blood”).

Several studies have shown associations between coagulation and coronary events. Circulating tissue factor levels as well as TFPI levels are increased in patients with acute coronary syndromes when compared to stable disease (10,25,26). Levels of soluble tissue factor are associated with increased cardiovascular mortality. Circulating TAT (27) and F1+2 levels (28) are increased in acute coronary syndromes when compared to stable disease. Patients with a decrease of circulating F1+2 and TAT levels in response to treatment have a lower short-term ischemic event rate (8,29). Increased PAI plasma concentrations are associated with common risk factors for coronary artery disease such as hypertension and particularly diabetes (30) and increased PAI activity predicts subsequent coronary events in patients with angina pectoris (31).
Also, several studies have shown associations between platelet activity and future coronary events (32,33). Direct and indirect methods for assessment of platelet function are available (34). Direct methods include flow cytometric analysis of platelet membrane glycoproteins that, on activation, either become exposed (e.g., P-selectin or CD63) or change conformation (e.g., glycoprotein IIb-IIIa), as well as measurements of platelet secretion products in plasma or urine. Whereas platelet flow cytometry requires fresh samples and is labor-intensive, secretion products are either not very specific for platelets (e.g., soluble P-selectin (35)) or are sensitive to sampling and handling artifacts (e.g., thromboglobulin) (36).

Indirect methods are functional platelet assays, which evaluate the ex-vivo platelet function thought to be dependent on platelet activation status in vivo. In this way, both reversible (e.g., aggregation and adhesion) and irreversible platelet activation (i.e., the secretion response) can be studied. Although rapid platelet function assays have been developed, correlations with direct assays are poor (37). Discrepancies between methods trouble clinical applicability, for example in monitoring of anti-platelet therapy. For example after aspirin therapy, the various methods show a wide range in platelet reactivity and activation (38). PFA-100, a test that assesses cessation of blood flow by a platelet plug, is widely used for monitoring aspirin response, but not sensitive to clopidogrel therapy (39). These findings suggest that such functional assays do not necessarily reflect the actual in vivo activation status.

A particular blood component that has been studied extensively for its association with cardiovascular outcome is C-reactive protein (CRP). CRP is an acute phase protein mainly synthesized in the liver under the control of interleukin 6 (40), and a risk factor for cardiovascular disease (41-43). CRP can contribute to development of atherosclerosis by activating endothelial cells with induction of the expression of adhesion molecules facilitating monocyte recruitment into the arterial intima (44-46). CRP may also facilitate LDL uptake in macrophages, transforming them into foam cells in the vessel wall, a major event in the progression of atherosclerosis (47).

CRP also induces tissue factor expression on endothelial cells (48) and macrophages (49), which may lead to unstable disease by promoting a pro-coagulant state. In addition, CRP may be detrimental during acute myocardial through activation of the complement (50-52). CRP aggravates myocardial damages in ischemia reperfusion (53) and peak levels of CRP and complement activity during myocardial infarction predict development of heart failure better than estimates of infarct size as measured by necrosis markers (54,55).

A cellular derivative that may play an important role in progression to unstable disease and in myocardial damage during infarction are microparticles. Microparticles are small vesicles (0.1 to 1 μm) released from cells after activation, apoptosis, or exposure to shear stress (56). The main source are platelets (57). By exposing negatively charged phospholipids and binding sites for various coagulation factors, they support the assembly and optimal function of coagulant enzyme complexes (58,59). They can also expose tissue factor (60), which has been shown to be active in
vitro (61) as well as in vivo (62). Moreover, microparticles from myocardial infarction patients (but not healthy individuals) cause endothelial dysfunction in vitro by impairing the endothelial nitric oxide transduction pathway (63). Thus microparticles may contribute to progression to unstable disease.

In addition, microparticles provide a surface for binding of CRP (64,65), SAP (66), IgG (67) and IgM (68,69), enabling activation of the complement system by binding of C1q. Thus, microparticles may be an important mediator in CRP and complement related myocardial injury during myocardial infarction.

Risk stratification

As blood components may contribute to pathogenesis of coronary artery disease and development of unstable disease, clinical assessment of these components may be used for detection of patients at risk for future cardiovascular events. Risk stratification is essential to select adequate treatment for patients at increased risk. For example, in healthy individuals with elevated CRP levels, lowering these levels with statin therapy decreases the risk of future cardiovascular events (70). In addition to the above mentioned contributors to atherosclerosis and unstable disease, signs of organ damage or dysfunction produced by cardiovascular disease may be used for risk stratification. This may be performed by cardiac imaging or by measurement of blood components reflecting cardiac or renal dysfunction.

In cardiac imaging, coronary artery disease may be demonstrated directly by showing the anatomical substrate, i.e. coronary calcifications in coronary computed tomography (CT) and coronary stenosis and plaque rupture in coronary angiography. Coronary angiography is therefore often used as gold standard. The priciples of indirect imaging of coronary artery disease are based on its consequences, i.e. myocardial ischemia or even necrosis. These can be detected either trough abnormal flow-reserve and viability (myocardial perfusion scintigraphy, late enhancement in magnetic resonance imaging) or decreased systolic contractility (myocardial perfusion scintigraphy, magnetic resonance imaging and echocardiography). Because of its non-invasiveness and practicality, dobutamine stress echocardiography (DSE) provides an attractive technique in the clinical setting of suspected acute coronary syndrome. Intravenous infusion of dobutamine increases muscular contractility and heart rate, increasing myocardial demand for coronary perfusion. In case of coronary stenosis with abnormal flow reserve the supply will not meet increased demand, resulting in regional reduction in contractility. In patients with stable coronary artery disease, DSE provides independent prognostic information on future adverse coronary events (71-73).

A relatively new marker for myocardial dysfunction are natriuretic peptides. In particular B-type natriuretic peptide (BNP) and the inactive portion of the prohormone, N-terminal B-type
natriuretic peptide (NTproBNP) have proved clinical value. The diagnostic and prognostic value of these natriuretic peptides is well established in patients with a variety of cardiac conditions, such as heart failure (74), stable angina pectoris (75,76), and acute coronary syndromes (77). With ventricular myocardial wall stress (78-80), and myocyte hypoxia (81) as stimuli for production and release of BNP and NTproBNP, NTproBNP levels also depend on renal clearance (82). NTproBNP is thus not only marker of cardiac dysfunction but also of renal impairment. Renal impairment may result from reduced renal perfusion by atherosclerotic disease, directly by renal arterial atherosclerotic lesions or indirectly by reduced cardiac output in heart failure. Furthermore, renal impairment may be the result of conditions associated with atherosclerosis, i.e. diabetes mellitus and hypertension. Therefore, renal impairment is a non-specific marker in atherosclerotic disease. However, even minor impairment predicts cardiovascular morbidity and mortality (83,84) and risk of death after myocardial infarction (85). In a clinical setting, renal function is usually estimated using plasma levels of creatinine, a nucleic break-down product. Levels of creatinine do however not only depend on renal clearance, but, understandably, also on muscle mass (86). This problem is partially overcome by estimation of glomerular filtration rate after a correction for body weight and gender (87). More labour-intensive is the calculation of glomerular filtration rate by sampling 24 hour urine creatinine. Cystatin C, a protein produced by all human nucleated cells (88), is now emerging as a more specific marker than creatinine (89). Studies have failed to relate serum levels with any disease state besides those affecting the glomerular filtration rate (90). Several reports have suggested that the serum concentration of cystatin C may be a better predictor of outcomes of cardiovascular disease than GFR estimates based on the serum creatinine concentration (84,85,91,92).

Myocardial ischemia
In addition to guidance of therapy based on the prognostic value of the different biomarkers, biomarkers may also be helpful in making diagnosis in patients with anginal complaints. One of the major problems with establishing the diagnosis of coronary artery disease is that symptoms are variable and perceived very differently by individual patients. In addition, many other conditions mimic complaints related to coronary artery disease. Patient history taking, physical examination and electrocardiography and also exercise electrocardiography have limited diagnostic value (93-95). Alternatively, imaging techniques are costly, time consuming and labour-intensive. Computed tomography, scintigraphy and angiography also entail radiation exposure. In addition, because of the invasive nature of coronary angiography, this technique holds risk of procedure related complications. Measurement of biomarkers however is safe and inexpensive. Whereas myocardial necrosis can be demonstrated with high sensitivity and specificity by troponins and CKMB (96), diagnosis of myocardial ischemia through blood tests remains a challenge. Markers that have been studied in this respect are natriuretic peptides, ischemia-modified albumin, and myeloperoxidase.
NTproBNP and BNP may be markers of ischemia either directly through production and release after myocyte hypoxia or indirectly by impaired systolic and/or diastolic ventricular function in myocardial ischemia leading to increased wall stress. Increased baseline levels have been reported in patients with inducible myocardial ischemia (97,98) and exercise-induced myocardial ischemia has been reported to induce immediate increases in circulating BNP and NTproBNP (99).

Albumin has been reported to undergo structural modifications in conditions of tissue ischemia, affecting its capacity to bind cobalt in vitro (100). Several studies have shown increased levels of this modified albumin after percutaneous coronary revascularization with transient myocardial ischemia (101-103), and in chest pain patients with myocardial ischemia (104,105).

Myeloperoxidase is released after neutrophil activation (106). Increased systemic levels can be found in patients with chest pain or acute coronary syndromes and especially patients with increased levels of myeloperoxidase are at increased risk for future coronary events (107-110). As exercise-induced muscle ischemia in claudication (111) and experimental myocardial ischemia and reperfusion lead to systemic neutrophil activation (112), and systemic levels of myeloperoxidase increase immediately after coronary stenting with transient myocardial ischemia (113,114), myeloperoxidase has been suggested as a marker for myocardial ischemia in chest pain patients.
Outline of the thesis

Mechanisms of stable and unstable coronary artery disease
The first part of the thesis concerns cell-derived microparticles in various conditions of atherosclerotic disease. Chapter 2 describes the cellular origin and expression of activation markers on microparticles by flow cytometry. Healthy individuals were compared with patients with unstable coronary disease (non-ST elevation myocardial infarction and ST-elevation myocardial infarction) and patients with stable disease, i.e. stable angina pectoris and peripheral arterial disease (with a high atherosclerotic burden). Furthermore, numbers of platelet microparticles expressing activation markers were correlated with soluble markers of platelet activation and coagulation activation. Chapter 3 describes the potential mediating role of microparticles between inflammation and complement activation and inhibition in myocardial infarction. In healthy individuals and in patients with myocardial infarction, activators and inhibitors of complement, and complement activation products were measured in fluid phase and on microparticles in plasma.

Risk stratification
The focus the second part of the thesis is risk stratification in suspected or confirmed acute coronary syndromes. In chapter 4 to 6, risk stratification performed in an emergency room setting of patients with chest pain suspected for an acute coronary syndrome but normal or non-diagnostic electrocardiogram (the DESIRE study cohort), is being investigated. Chapter 4 compares hemostatic markers in patients with an acute coronary syndrome, confirmed by positive plasma troponin T concentrations or serial ECG changes, with controls in whom an acute coronary syndrome was ruled out.
Although an acute coronary syndrome can be ruled out in patients with chest pain, these patients may still be at increased risk for future adverse cardiovascular events. The additive value of NTproBNP and dobutamine stress echocardiography for prediction of long-term cardiovascular mortality in these patients from the DESIRE study cohort are described in chapter 5 and 6, respectively.
Chapter 7 describes the additional predictive value of cystatin C for recurrent myocardial infarction and mortality in a cohort from a large multicenter study, the ICTUS trial. In this trial, high risk patients with non-ST elevation myocardial infarction were randomized to either an early invasive strategy or a selective invasive strategy with angiography performed only in refractory angina or recurrent myocardial ischemia. Patients with renal dysfunction tend to be under-treated in clinical practice despite their increased risk (115). Especially these patients with a higher baseline risk may benefit from more aggressive therapy.
Myocardial ischemia

The third part of the thesis describes a cohort of patients from the out-patient clinic, referred for detection of myocardial ischemia by perfusion scintigraphy after exercise. Serial blood sampling was performed before exercise at baseline, at maximum exercise and every hour until 6 hours after exercise. In chapter 8, the serial changes in levels of cobalt binding albumin were measured and correlated to the presence of exercise-induced myocardial ischemia and to levels of total albumin. In chapter 9, changes in NTproBNP after exercise were correlated with baseline levels, clinical parameters, exercise levels, as well as biochemical and scintigraphic parameters, i.e. variables of left ventricular volumes, ejection fraction and extent of myocardial ischemia. In chapter 10, changes in plasma myeloperoxidase concentrations after exercise were correlated with clinical and biochemical parameters, exercise levels, and the presence of myocardial ischemia.
References


34. Michelson AD. Methods for the measurement of platelet function. Am J Cardiol 2009;103:20A-26A.


49. Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. Blood 1993;82:513-520.


