Biomarkers in ischemic cardiac syndromes

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Summary of the thesis

This thesis comprises several studies on biochemical markers in ischemic myocardial syndromes. Most often a consequence of atherosclerosis and coronary artery disease in particular, ischemic myocardial syndromes constitute a significant burden of morbidity and mortality in the Western world. As symptoms are perceived very differently by patients and many conditions may mimic myocardial ischemia, biochemical markers measured in the peripheral blood may add diagnostic and prognostic value to patient history, physical examination, (exercise) electrocardiography and cardiac imaging. Biochemical markers may either reflect causes of myocardial ischemia (progression of atherosclerotic disease, pro-coagulant activity) or consequences of myocardial ischemic disease (organ dysfunction).

Part one

The first part of the thesis concerns cell-derived microparticles. These may contribute to pro-coagulant activity and may play a role in myocardial injury after myocardial infarction.

Chapter 2 describes the cellular origin and expression of activation markers on microparticles in plasma 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, the latter having a high atherosclerotic burden. Furthermore, numbers of platelet microparticles expressing activation markers were correlated with soluble markers of platelet and coagulation activation. The majority of microparticles were platelet derived. While total numbers of platelet microparticles were similar between the groups, microparticles of patients with peripheral arterial disease and myocardial infarction showed increased numbers of platelet microparticles expressing activation markers. There was no correlation with markers of coagulation activation (prohrombin fragment 1 + 2 and TAT). The significance of these findings was confirmed by in vitro tests. Activation of platelets by thrombin receptor-activating peptide did not result in increased total numbers of released microparticles but did result in increased numbers of platelet microparticles expressing activation markers. Thus, increased systemic platelet activation in myocardial infarction and peripheral arterial disease can be assessed by measuring platelet microparticles expressing activation markers rather than total numbers of platelet microparticles or its maximal increase. In chapter 3 the potential mediating role of microparticles between inflammation and complement activation and inhibition in myocardial infarction is studied. In healthy individuals and patients with myocardial infarction, activators (CRP, IgG, IgM, SAP) and an inhibitor (C4bp) of complement, and complement activation products were measured in fluid phase and on microparticles. CRP-associated complement activation occurs on the surface of microparticles in healthy individuals. In myocardial infarction patients, increased levels of microparticle-bound CRP and fluid phase CRP were found, with a strong correlation between
both. However, in myocardial infarction, microparticle-associated complement activation appears to be IgG-dependent and not CRP-dependent. In patients with myocardial infarction, levels of microparticle-associated IgG correlated strongly with levels of microparticle-associated C4bp. Possibly, complement inhibitors such as C4bp prevent excessive tissue injury from CRP-induced complement activation.

**Part two**
The second part of the thesis focuses on risk stratification in patients with suspected or proven non-ST elevation acute coronary syndrome. Since patients at higher risk for future cardiac events may benefit from a more aggressive treatment, identifying these patients is important. In chapter 4 to 6, risk stratification was performed in the DESIRE study cohort. This cohort consists of patients presented at the cardiac emergency department with a normal or non-diagnostic ECG and chest pain suggestive of an acute coronary syndrome (ACS). Patients underwent a rule-out protocol with serial troponin T measurements and serial ECGs. In chapter 4, the levels of markers of haemostasis were assessed in a case-control study within the DESIRE study cohort. Patients with ACS established by either a positive troponin T result or serial ECG changes were compared to age- and sex-matched controls without ACS. There was no difference in plasma concentrations of F1+2, TAT complexes, PAI, and tissue factor and TFPI activity between patients with an ACS and controls. Plasma concentrations of F1+2 and TAT complexes were slightly higher in the subset of troponin T patients with an ACS than in controls. However, both the positive and negative predictive value were low. Significantly higher PAI plasma concentrations were observed in the troponin T negative ACS subjects than in controls, but not in troponin T positive ACS cases. The study shows that an increased haemostatic state may play a role in the development (het lukt me niet om hier een commentaar veld in telassen. Ik vraag me af of je dit mag zeggen “development” je meet immers na het acute moment) of an acute coronary syndrome, but that measurement of the above mentioned haemostatic markers in systemic blood has no diagnostic utility.

In chapter 5, the long-term prognostic value of NTproBNP is studied. Cardiovascular mortality during a 9 year follow-up was low (6%) in the DESIRE cohort of non-ACS patients. Elevated NTproBNP levels on admission, however, were an independent risk factor for cardiovascular mortality in this cohort of relatively low risk patients. Patients with increased levels of both NTproBNP and CRP had an 18% risk of cardiovascular mortality, compared to 1% in patients with normal NTproBNP and CRP levels. NTproBNP is a marker of increased ventricular wall stress, which may be a consequence of ongoing myocardial ischemia or previous myocardial infarction. A subsequent decrease in stroke volume in these disease states results in an unfavorable activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system. Thus, NTproBNP identifies patients who might benefit from neurohormonal antagonism treatment (i.e. ACE-inhibitors and beta-blockers). In chapter 6, the long-term value of dobutamine stress
echocardiography in the DESIRE cohort is described. Dobutamine stress echocardiography (DSE) was performed after ruling out ACS. Of the performed DSE 7% were positive for myocardial ischemia. Whereas a previous study has shown a strong short-term prognostic value of DSE for cardiac events including revascularization, a positive DSE did not predict long-term future cardiovascular mortality. Moreover, in a subset of patients referred for myocardial perfusion scintigraphy (MPS), a positive MPS was not predictive for long-term future cardiovascular mortality. The lack of prognostic value of DSE may be caused by the lack of sensitivity to detect relatively limited amounts of myocardial ischemia. This might be more likely to occur in patients without evidence of ischemia on the ECG and normal levels of cardiac necrosis markers.

Chapter 7 studies the prognostic value of renal dysfunction as measured by cystatin C in 1128 patients with a non-ST-elevation myocardial infarction enrolled in the multicenter ICTUS study. Cystatin C was an independent predictor for future cardiac events and even mild to moderate renal dysfunction was associated with a higher risk of death and spontaneous MI.

Part three

This part describes the associations between exercise, exercise induced myocardial ischemia and levels of biochemical markers that have been suggested to become elevated secondary to myocardial ischemia. In out-patients referred for detection of myocardial ischemia by symptom-limited myocardial perfusion scintigraphy, peripheral blood samples were drawn at baseline, maximum exercise and every hour until 6 hours after exercise. Chapter 8 describes an immediate decrease of plasma “ischemia-modified albumin” (IMA) levels after exercise in both patients with and without myocardial ischemia. Whereas there was no difference in baseline levels or changes after exercise between patients with and without myocardial ischemia, a strong correlation between baseline levels of IMA and levels of albumin was demonstrated. Previous results on the diagnostic value of high IMA concentrations in patients with chest pain are, at least partly, attributable to the dependency of IMA on the concentration of serum albumin, as low albumin concentrations are associated with a higher risk for future adverse cardiac events. Chapter 9 describes the rapid increase of plasma myeloperoxidase concentrations after exercise. MPO levels and absolute changes in MPO did not differ between patients with and without ischemia at any time point. Thus, exercise related immediate increases in MPO levels do not reflect myocardial ischemia. Chapter 10 shows an immediate as well as a secondary increase in NTproBNP levels after exercise. Although the absolute increases in NTproBNP levels were higher in myocardial ischemia patients, multivariate analysis showed that not the extent of myocardial ischemia but baseline NTproBNP levels were an independent determinant of exercise induced immediate increases in NTproBNP levels. For secondary increases, again baseline NTproBNP levels, renal function, and end systolic volume, but not the extent of myocardial ischemia, were independent determinants. In turn, independent determinants for baseline NTproBNP levels were renal function and end systolic volume, but not the extent of inducible myocardial ischemia. Thus,
increased baseline NT-proBNP and exercise induced increases in NT-proBNP levels may reflect wall stress relative to impaired systolic and/or diastolic ventricular function due to ongoing myocardial ischemia or previous myocardial infarction, rather than myocardial ischemia per se.

Conclusion and recommendations
Increased systemic platelet activation measured as activation of markers on microparticles using flowcytometry is present in patients with myocardial infarction. Measurement of platelet activation rather than coagulation activation may be helpful in early identification of high-risk patients, and monitoring anti-platelet therapy. However, as flow-cytometry of microparticles is time-consuming and labour intensive, more feasible methods of measuring platelet microparticles expressing activation markers should be developed. Our study on microparticles and complement activation suggests an important mediating role of microparticles between inflammation and complement activation and inhibition in tissue damage in myocardial infarction. Microparticles are thus a potential therapeutic target. Possible reduction of tissue damage in myocardial infarction by inhibiting microparticle mediated complement activation can be evaluated in animal models, requiring development of specific inhibitors of microparticle formation or binding of complement to microparticles.

Cystatin C and NT-proBNP were strong predictors for long-term cardiovascular mortality. Whether ACS patients with increased levels of cystatin C should undergo a more aggressive approach, and ruled-out ACS patients with increased levels of NT-proBNP benefit from ACE inhibition and beta-blockers, can be evaluated in future clinical trials.

Several markers that were suggested to be indicative for myocardial ischemia in previous studies, failed as such using myocardial perfusion scintigraphy as gold standard for inducible myocardial ischemia. Future research on finding myocardial ischemia markers should focus on pathophysiological changes in myocyte hypoxia that might lead to systemically measurable effects, rather than indirect and aspecific changes such as neutrophil activation, conformational albumin changes and increased ventricular wall stress.