Summary and conclusions

***Part 1**, "**Vulnerable Blood**",* focusses on the additional value of several serum biomarkers for the prediction of MACE on a relatively long term (4 to 10 years of follow-up). These markers are traditionally measured once at the start of follow-up and hence assumed to reflect a constant cardiovascular risk, in a similar way as traditional risk models incorporate clinical risk factors.

Chapter 2 describes a multimarker model in which Troponin (Tn), Interleukin-10, myeloperoxidase and placental growth factor predict 4-year MACE rates in 1090 patients with non ST-elevation acute coronary syndrome. This model was able to stratify a seemingly homogenous study population into a relative low risk (6.0% event rate when all markers were normal) to a very high-risk subgroup in which the MACE rate was 35.8% when three of four biomarkers were abnormal.

Chapters 3 and 4 focus on patients undergoing a percutaneous coronary intervention and conclude that C-reactive protein (CRP) is associated with long-term MACE. Lipoprotein A may be of interest with respect to short-term prognosis after PCI.

***Part 2**, "**Vulnerable Period**",* focusses on serum biomarkers as well, but here the train of thought is to capture the dynamics of coronary pathophysiology, i.e. that the risk of MACE within an individual patient is not constant, but variable over time. Hence repeated biomarker measurements are explored in the BIOMarker study to identify the Acute risk of a Coronary Syndrome (BIOMArCS), in order to evaluate whether fluctuations in biomarker levels can predict the risk of an imminent MACE within the days to weeks to come.

Chapter 5 describes the rationale and study design of the multicenter, prospective BIOMArCS study, together with the baseline clinical characteristics of the 844 enrolled patients presenting with ACS, either with or without ST-elevation and at least one additional cardiovascular risk factor.

The paradigm of the *vulnerable period* is based on the concept that individual patients with CAD actually do not have a constant risk over time. Long periods of stability, with minimal plaque progression and low risk of CV events, are alternated by periods of increased plaque instability and rapid plaque progression, during which the risk of sudden plaque disruption and thrombotic coronary occlusion within short time spans is high. This is a complex and multifactorial pathophysiological process in which temporal variations in distorted lipid metabolism, vascular inflammation, endothelial dysfunction, increased thrombogenicity and myocardial ischaemia play an important role. In order to be able to capture a signal of changing risks over time, frequently repeated measurements are required of markers that reflect the above-mentioned pathophysiological

processes. In BIOMArCS venapuncture was performed every 2 weeks during the first six months and every month thereafter during 1-year follow-up in order to evaluate the obtained repeated biomarker information for risk prediction.

As per the inclusion criteria, every BIOMArCS patient had endured an index ACS in order to qualify for enrollment. Hence it is of great importance to understand the Tn release patterns and stabilization after the index ACS prior to any further evaluation of this marker. **Chapter 6** describes the Tn washout patterns as measured with current high-sensitivity assays. Troponin levels stabilized in approximately 2 weeks after the index event. Intriguingly, low individual variation but large between-patient differences were observed thereafter. Using the first samples taken one month after ACS, we were able to compose a patient specific reference value for approximately 80% of the patients with just two measurements.

Chapter 7 describes the primary results of BIOMArCS through an evaluation of repeated measurements of high-sensitivity Troponins, N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and high-sensitivity C-reactive protein. In this cohort of asymptomatic post ACS patients with preserved ventricular function and guideline based treatment for secondary prevention, we observed that those patients with persistently elevated Tn and NT-proBNP levels were at a significantly higher risk of MACE during 1-year follow-up. In addition, 20% of the patients had asymptomatic, isolated TnI peaks of 10 pg/mL, which was associated with a 2.9 fold increased risk of MACE during follow-up. Similarly an asymptomatic, isolated CRP peak of 10 mg/L, was also associated with a 2.9 fold increased risk of MACE during follow-up.

In ***part 3***, *"**Vulnerable Plaque**"*, the centre of interest is around invasive coronary imaging (coronary angiography, intravascular ultrasound (IVUS) and near-infrared spectroscopy (NIRS)) for the prediction of MACE, as well as cross-sectional analyses evaluating the relation between these imaging techniques and serum biomarkers.

Chapter 8 describes the design and rationale of ATHEROREMO, the European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis study. This large, prospective, observational cohort study was designed to evaluate the relation between novel circulating biomarkers, coronary plaque characteristics as determined by intravascular ultrasound (IVUS) and near-infrared spectroscopy (NIRS) and clinical outcome. IVUS and NIRS imaging was performed in a 40 mm long non-stenotic segment of a nonculprit coronary artery in patients referred for angiography due to stable angina pectoris or ACS.

Chapters 9 and 10 evaluate the prognostic value of the novel intracoronary imaging modality known as near-infrared spectroscopy. This technique is capable of assessing lipid core-containing plaques, which can subsequently be quantified as a lipid core burden index (LCBI). Patients with a relatively high LCBI had a 4-fold risk of MACE during 1-year follow-up (**chapter 9**). A similar association was observed when follow-up was extended to 4 years (**chapter 10**).

Chapters 11 and 12 describe the prognostic value of IVUS assessments of non-stenotic segments during 1 and 4 years of follow-up in 581 patients. Similarly to the NIRS findings, the overall conclusion here is that a higher amount of plaque burden within the coronary vessel wall is predictive of higher MACE rates. In addition, these NIRS and IVUS studies confirm that imaging of a relatively short and angiographically non-stenotic segment does indeed provide prognostic information, thus suggesting that the vessel wall characteristics in these segments seem to reflect a global measure of intracoronary disease burden, despite being short and relatively unobstructed.

Whereas chapters 9-12 rely on a detailed characterization of a short segment of the coronary vessel wall, **chapter 13** focusses on the predictive value of lumen characteristics as obtained by coronary angiography of all three epicardial vessels. The SYNTAX score II, a long-term mortality prediction model for patients with left main and/or three vessel disease, is evaluated in the setting of only one- and two-vessel disease. Syntax score II also proved to be an independent significant predictor for 4.5-year mortality in this non-left main or three vessel disease cohort.

The interlink between chapters 9-13 is that the anatomy of coronary atherosclerosis, whether it is obtained from vessel wall characterization or lumenography, contains prognostic information even after adjustment for those traditional clinical risk factors that form the backbone of current risk prediction models for patients with coronary artery disease.

The following chapters 14-19 describe the crosslink between the *vulnerable plaque* and *vulnerable blood*; the associations between coronary plaque characteristics and serum biomarkers are evaluated. With our epidemiologic studies, we do not aim to unravel the biologically mechanistic intricacies of the interplay between serum proteins and coronary plaque. Nevertheless, it is intriguing to observe that serum biomarkers as C-reactive protein (**chapter 14**) and Troponin (**chapter 15**), that have been described as independent predictors for MACE in Parts 1 and 2 of this thesis (in **chapters 2, 3, 4 and 7** respectively), are also associated with IVUS plaque characteristics. In the following chapters, we describe the relation between plaque characteristics and serum proprotein

convertase substilisin/kexin type 9 (**chapter 16**), serum chemokines (**chapter 17**), serum cytokines (**chapter 18**) and Von Willebrand factor (**chapter 19**). Finally, **chapter 20** is somewhat different as it does not evaluate a potentially fluctuating serum biomarker, but a genetic trait; haptoglobin polymorphisms are evaluated in relation to coronary plaque characteristics.

Accurate risk prediction is important to understand future risks of CAD patients, but clearly prediction alone will not alter the outcome. For that purpose, intervention studies are required in those deemed at high risk. Such studies, often combined with the search for those patient subsets to derive most benefit from the interventions, are described in ***Part 4***, *"**Intervention Studies**"*.

Chapter 21 is part of the Integrated Biomarker and Imaging Study 2 (IBIS 2), and describes the effects of a lipoprotein-associated phospholipase A2 inhibitor on repeatedly measured Tn levels.

Chapter 22 describes the main outcome of the IBIS 3 study, a prospective, non-randomized trial in which the effect of high intensity statin therapy is evaluated through repeated IVUS (n=164) and NIRS (n=103) measurements of a non-stenotic segment of a nonculprit coronary artery at baseline and after 1 year follow-up. High intensity rosuvastatin therapy resulted in a neutral effect on necrotic core and LCBI.

Chapters 23 and 24 describe 8726 patients enrolled in the randomized, placebo controlled EUROPA trial, which evaluated the efficacy of perindopril in patients with stable CAD. In **chapter 23**, models are constructed to evaluate the treatment effect of perindopril in terms of 5 year absolute risk reduction for MACE, based on clinical variables (Europa score) and clinical and pharmacogenetic variables (Europa-GEN score). **Chapter 24** describes the PERindopril GENEtic (PERGENE) risk model, which incorporates clinical and pharmacogenetic variables. On the basis of this cost-effective risk scoring model a very wide range of gradients of absolute treatment benefit on ACE-inhibitor therapy was demonstrated in a seemingly homogenous trial population. The PERGENE score could identify patient subgroups with an annual number needed to treat (NNT) as low as 29, as well as patients with an annual NNT as high as 521.

Finally **chapter 25** describes a single center, primary PCI experience in 4352 patients, and more specifically whether the outcome differs between those treated during regular office hours and those treated during so-called off-hours. Short and long-term mortality were similar in both groups.

In conclusion, this thesis focusses on patients with CAD. Despite current optimal medical therapy, their residual risk for MACE on a group level is evident. Equally evident is the observation that the persons that comprise this heterogenous group of CAD patients,

differ in their individual risk profile. Serum biomarkers, coronary imaging techniques and (pharmaco)genetics can all be successfully deployed in order to aid further risk stratification, i.e. identification of those at a higher, or perhaps even very low risk of a coronary event. The selection of any one of these techniques differs from one instance to another, and depends on the formulation of the particular research question that needs to be answered.