Defining atherothrombotic risk in peripheral artery disease

SUMMARY

This thesis aimed to better understand why some patients with peripheral artery disease (PAD) suffer from cardiovascular events despite current medical treatment strategies, while others do not. In this thesis the risk of cardiovascular events and mortality was explored through the characterization of high-risk patients, combined with the unraveling of important pathophysiological pathways driving cardiovascular events and mortality in PAD. Biomarkers related to these pathways could be used to identify patients at increased risk of such events, enabling clinicians to improve their management strategies to prevent cardiovascular events and mortality.

Chapter two provides an overview of the pleiotropic effects of coagulation enzymes and current evidence on pathophysiological pathways in atherosclerosis and atherothrombosis. Although platelet activation and formation of fibrin are crucial in thrombosis, inflammatory pathways and pleiotropic effects of the coagulation system substantially drive the process of atherosclerosis. Cell signaling in these pathways predominantly occurs via protease-activated receptors (PARs), which can be activated by a wide range of proteins, including coagulation factors IIa (thrombin) and Xa. Upon activation, downstream signaling leads to pro-inflammatory effects and a hypercoagulable state, thereby accelerating atherosclerotic plaque formation. Hypercoagulable effects are mainly exerted through platelet PAR1 and PAR4, while pro-inflammatory responses are mostly seen after vascular PAR1 and PAR2 activation. Inhibition of PAR signaling pathways, for example by the use of PAR1 antagonist vorapaxar, could be explored as a way to lower cardiovascular events and mortality. In this chapter, therefore, we also discussed how to improve vascular protection beyond the prevention of thrombosis. Clinical efficacy to be gained from the search to further decrease cardiovascular events might lie in the use of a low dose of a direct oral anticoagulant (DOAC) combined with antiplatelet therapy. More clinical studies on DOACs in secondary cardiovascular prevention are however necessary to investigate efficacy and safety, especially in subgroups such as patients with peripheral artery disease.

Provided by the insights of chapter two and the knowledge that the use of DOACs may lower the cardiovascular event and mortality risk in PAD, we explored a possible common biochemical background in patients with PAD and patients with non-acute deep vein thrombosis (DVT), as the latter patient group greatly benefits from treatment with a DOAC. In **chapter three**, we assessed these possible common pathways by using biomarkers reflecting the interplay between platelet and endothelial activation, neutrophil activation, and inhibition of inflammation. Neutrophil activity was an important driver of cardiovascular events, although more activity was recorded in PAD patients as compared to DVT patients, even with dampening by lipoxin A4. We found no differences in platelet activation, keeping in mind that PAD patients use antiplatelet therapy. A hypercoagulable state was observed in PAD patients with higher d-dimer levels, but venous thrombosis appeared to be more strongly dependent on blood coagulation activity.

The investigated pathways, as discussed in chapter three, did not appear to play pivotal roles in the cardiovascular event and mortality risk in PAD patients. We therefore decided to create an overview in **chapter four** of all investigated plasmatic biomarkers in association with cardiovascular outcome in PAD. During the last decades, various biomarkers have been investigated concerning cardiovascular outcome in PAD. This systematic review identified promising candidate biomarkers representing different pathophysiological processes in PAD, with biomarkers playing important roles in inflammatory signaling (hsCRP, NLR), the coagulation cascade (fibrinogen, d-dimer), and cardiac (patho)physiology (NT-proBNP, hs-cTnT). These biomarkers should not be used solitary but added to risk stratification models with multiple biomarkers from different pathophysiological pathways, as atherosclerosis is a multifactorial disease. Now that more potent pharmacological interventions are becoming available to target specific mechanisms such as inflammation and hypercoagulability, biomarker-supported risk stratification may help identify PAD patients that may benefit most from intensified treatment. To facilitate this, management studies should address the value of biomarker panels combined with traditional risk factors and patient characteristics.

Following the prior chapters, we conducted a clinical study to further dissect the cardiovascular event and mortality risk in PAD patients. **Chapter five** describes the patient population of the prospective cohort study that we performed as a continuation of the already gathered biomarker evidence. In this observational study, we aimed to find patient characteristics associated with cardiovascular risk in order to find targets for improved management. The patient population had prevalent PAD with a chronic state of atherosclerosis, defined by multiple affected vascular beds, decreased renal function, and lower hemoglobin levels. All patients were treated according to current guidelines with lipid-lowering agents, antiplatelet therapy, and antihypertensive drugs. We observed suboptimal low-density lipoprotein (LDL) levels, indicating that lipid-lowering strategies should be intensified to improve the lipid profile further. Antiplatelet agents were found to be inadequate despite high medication adherence, as platelet reactivity was insufficiently decreased in patients experiencing cardiovascular events. The addition of anticoagulant treatment in the form of a DOAC may counteract the remaining prothrombotic state in PAD patients. This so-called dual-pathway inhibition is, however, still hindered due to concerns about the number of pills per day and the increased risk of major bleeding. Nevertheless, more research is needed on alternative treatment strategies such as dual antiplatelet therapy or combinations with anticoagulant drugs.

Although various biomarkers have been identified as predictors of cardiovascular outcome in PAD, as summarized in chapter four, none have been implemented yet in clinical management. One explanation may be that unexplored biomarkers may play more pivotal roles in cardiovascular risk prediction. Therefore, in **chapter six**, a subgroup of the cohort study was investigated to discover new predictive biomarkers in addition to the already known biomarkers, as presented in chapter

four. Using proximity extension assay technology, we explored the association between a broad set of cardiovascular biomarkers and cardiovascular risk and identified interleukin-6, PAR1, tumor necrosis factor receptor superfamily receptor 11A, and galectin 9 as promising biomarkers to aid in risk stratification. These proteins are involved in prominent atherosclerotic biological processes including activation of endothelial cells, positive regulation of acute inflammatory responses, leukocyte chemotaxis, and platelet activation. This semi-quantitative biomarker discovery can be seen as a first step to improving risk stratification in PAD with new protein biomarkers. Quantitative assays are required to confirm the association with cardiovascular outcome. Potent biomarkers can then be implemented in risk stratification models.

As the association between hypercoagulability and cardiovascular is established, it remains to be elucidated which mechanisms are responsible for this prothrombotic state. To elucidate potential mechanisms, we focused on pathways leading to this state in PAD in **chapter seven**. Activation of the intrinsic pathway, assessed by coagulation enzyme:inhibitor complexes and thrombin generation, was not different in PAD patients at increased risk for cardiovascular events and mortality. This observation may be the result of a profound coagulation activity with systemic atherosclerosis as primary driver of coagulation. It could be that platelets, rather than the hypercoagulability, are responsible for the actual occurrence of cardiovascular events.