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Summary

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Platelets play main roles in thrombosis and haemostasis. The interaction of several extracellular matrix components and coagulation-generated ligands with platelet receptors triggers activation and thrombus formation. In this thesis, I investigated acute and persistent mechanisms of platelet activation, mainly through the platelet receptors glycoprotein VI (GPVI) and protease activated receptor 1 (PAR1), which are interesting and prospective targets for novel antiplatelet drugs.

Chapter 1 provides background information on platelet activation and thrombus formation, as well as on the ligands and platelet receptors involved in these processes. Furthermore, the interplay between platelets and coagulation is introduced in this chapter and variability in the efficacy of antiplatelet therapy is discussed. Then, the review in **Chapter 2** describes the process of clonal haematopoiesis of indeterminate potential (CHIP) as pre-malignant state in which somatic mutations in hematopoietic stem cells lead to clonal expansion of a subset of cells. **Chapter 2** provides an overview of genes associated with clonal haematopoiesis and altered platelet production or functionality and its relation to the risk of thrombosis or bleeding. Hence, this chapter illustrates how the functional status of platelets can be altered by intrinsic platelet factors.

In the next chapters, novel mechanisms of platelet activation are investigated following specific ligand-receptor interactions. In **Chapter 3**, the aim was to determine the thrombogenic activity of collagen-like peptides and fibrillar collagens through GPVI and the tyrosine kinase Syk. In platelet suspension, only collagen-like peptides containing the GPVI-activating sequence GPO, and Horm-type collagen evoked Syk-dependent platelet activation. Instead, immobilised collagen-like peptides induced Syk-dependent platelet activation and thrombus formation, independent of the GPO sequence. Taken together, **Chapter 3** showed that GPVI-dependent signalling through Syk supports thrombus formation on collagen-like peptides, regardless of a GPO sequence. Next to collagens, GPVI has other ligands, such as fibrin(ogen) and laminin. In **Chapter 4**, we studied the role of coagulation-generated ligands in platelet activation,

spreading and thrombus formation under flow conditions. Interestingly, coagulated plasma, supported platelet activation (integrin αIIbβ3 activation, P-selectin exposure) and aggregation, independent of thrombin. We revealed that coagulation factor (F)XIIIa induced platelet activation via GPVI. Furthermore, this chapter provides evidence that the anticoagulation factor APC evoked platelet activation responses through the platelet thrombin receptor PAR1. Coated on a surface, FXIIIa and APC enhanced platelet adhesion under flow and the combination of FXIIIa and APC resulted in synergistic platelet activating effects. Strikingly, FXa-driven platelet activation appeared to be completely dependent on thrombin activity. In general, immobilisation of the (anti)coagulation factors evoked greater platelet-activating effects, compared to the soluble form of these factors. In **Chapter 5**, we studied the reversal of platelet activation following stimulation through GPVI and the G-protein coupled receptors (PAR1 and $P2Y_{1/12}$) and the platelets' potential to become re-activated. Using multicolour flow cytometry and electron microscopy, it was shown that platelet activation through GPVI is persistent, while platelet activation through PAR1 and $P2Y_{1/12}$ is rather transient. After long-term stimulation through PAR1 and $P2Y_{1/12}$, the platelets started to return from filopodia-protruding to a disc-shaped morphology, whereas GPVI-induced platelet stimulation resulted in prolonged activation. In addition, after previous stimulation, platelets were desensitised for the agonists they were stimulated with previously, but could be restimulated via another pathway. We were able to demonstrate that platelets regained their potential to contribute to thrombus formation after prior stimulation with the GPCR agonists TRAP6 or ADP, but not after GPVI stimulation.

In **Chapter 6**, platelet responsiveness to agonists (CRP-XL, TRAP6) and to cangrelor was investigated in patients with coronary artery disease (CAD), with or without type II diabetes, using a flow cytometric assay. Cangrelor effectively inhibited platelet responses in all participant groups. The effect of cangrelor was correlated with the initial response to the agonists CRP-XL and TRAP6, suggesting that the responsiveness to a drug might be predictable *in vitro*. Possibly, a very low capacity to respond to agonists before drug

administration, might indicate an increased bleeding risk, however, this should be confirmed with future clinical studies.

In **Chapter 7**, the aim was to assess the role of platelets in the pathogenesis of cerebral small vessel disease (SVD) and the effect of the disease on platelet function. For this purpose, an in-depth characterisation of platelet-related haemostatic and inflammatory responses was performed, using the Maastricht Flow Chamber and a multicolour flow cytometry panel. The platelets from SVD patients and controls showed a comparable haemostatic response, despite the use of antiplatelet therapy in the SVD patients. Clustering analysis of multicolour flow cytometry data pointed to a higher abundancy of a platelet population in SVD patients, in which CD40 ligand and GPVI were shed upon CRP-XL stimulation. Furthermore, platelets of SVD patients demonstrated an elevated inflammatory response, indicated by decreased surface CD40L upon activation and increased (activated) neutrophil-platelet aggregate formation.

Chapter 8 discusses the main findings of this thesis and puts experimental findings in context of relevant literature. The new insights into mechanisms involved in platelet activation through GPVI and PAR1 contribute to a better understanding of the complex platelet activation mechanisms in haemostasis and thrombosis.