

Novel mechanisms of platelet activation and sustained signalling through GPVI and PAR1

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Impact

As scientists, we often question what our research means and what it contributes to the society. The direct clinical relevance of our findings is not always clear at first sight. However, the end goal of our biomedical research is ameliorating patient treatment and hopefully improve the patient's quality of life. To achieve this goal, it is important to increase the understanding of the underlying mechanisms of diseases and therefore, basic research is required.

The role of platelets is to prevent blood loss upon injury of a vessel, by interacting with extracellular matrix components, such as collagen. However, this can become pathologic, when platelets form thrombi which occlude the blood vessel, eventually leading to myocardial infarction (MI) and stroke. In the setting of an acute MI or ischemic stroke, a combination of two antiplatelet agents is administered, for up to 12 months after MI and for a short course of 3 weeks after ischemic stroke. The preferred dual antiplatelet therapy (DAPT) consists of aspirin combined with a potent P2Y₁₂ receptor inhibitor in case of MI, particularly when PCI and stenting is involved.¹ In ischemic stroke aspirin is combined with the less potent P2Y₁₂ inhibitor clopidogrel.² In all cases, administration of DAPT increases the risk of (major) bleeding as compared to single APT. Moreover, recently Olie et al. reported that in frail patients, who underwent PCI within the past 1-2 months and were treated with aspirin combined with a P2Y₁₂ receptor inhibitor, 48.5% reported bleeding events, from which 17.5% suffered from clinically relevant bleeding and nuisance bleeds.³ Thus, there is an unmet clinical need for novel antiplatelet medication, which inhibits thrombosis, without affecting haemostasis. As based on the work presented in this thesis, I propose that GPVI might be an important target, as this receptor is involved in the pathogenesis of arterial thrombosis, but only has minimal roles in haemostasis.⁴ Recently presented data suggest that indeed targeting GPVI might be effective and safe in patients with ischemic stroke, where hemorrhagic transformation of the ischemic area was actually reduced as compared to placebo (unpublished data).

As there is currently an increased interest in targeting GPVI, there is also a need to increase the current understanding on this receptor as much as possible. In **Chapters 3-5** of this thesis, we therefore aimed to increase the knowledge on ligands of GPVI and the working mechanisms.

In **chapter 3**, we provided an overview of the thrombogenicity of several collagen peptides and fibrillar collagens. We showed that in platelet suspension, only collagen peptides with GPVI-activating motif GPO activated platelets, while when immobilised on a surface, thrombi were formed in a Syk-mediated fashion, regardless of the GPO motif. Syk inhibitors, such as fostamatinib, are already approved in refractory immune thrombocytopenia patients.⁵ However, inhibition of Syk affects more than only the GPVI pathway, therefore, there is a need for more precise targeting of the GPVI receptor. Still, existing drugs such as fostamatinib could be of great interest for clinicians, as they could be repurposed as antithrombotics, especially if the treatment is well-tolerated in other patient groups. The microfluidic method employed in this chapter, is the Maastricht Flow Chamber. This tool combines platelet adhesion, activation and thrombus formation under shear. Currently, the microfluidic flow chamber model is only used in laboratory settings due to poor standardization and difficult handling. However, it is a promising tool for the simultaneous assessment of platelet and coagulation activation under flow conditions, and for testing potential novel antithrombotics. Compared to routine haemostasis tests, this assay can offer improved risk prediction of thrombosis and bleeding that enables patient-tailored treatment.

Clinical trials, such as the COMPASS trial, where aspirin was combined with a low dose of rivaroxaban have shown beneficial effects on outcomes, compared to antiplatelet medication alone.⁶ However, this was at the expense of an increased bleeding risk. Future research is required to explain the increased bleeding risk and to investigate how to overcome this. More insights into the platelet-coagulation interplay were gained in **chapter 4**. Here, we found that the coagulation factor (F)XIIIa induces platelet activation through GPVI and the tyrosine kinase Syk, while the anticoagulation factor activated protein C (APC) evokes platelet activation

through PAR1. This chapter expands the current understanding of how platelets are recruited to the site of injury. Then, in **chapter 5**, we mimicked the platelet response of patients who are continuously exposed to activating agents, such as described for cancer patients.⁷ We found that platelets activated through GPVI remain activated for a longer time, compared to platelets activated through the GPCRs PAR1 and P2Y_{1/12}. An important question that future research should answer is to which extent reversibility of platelets is possible, when platelets are exposed to combinations of several agonists. Furthermore, the concept that previously activated platelets can regain their functionality might apply to platelet transfusions, since the process of platelet isolation can cause platelet preactivation.⁸

In **Chapter 6**, it was shown that the initial capacity to respond to an agonist correlated to the reduction in response after cangrelor treatment, by using a novel flow cytometric setup. Future clinical trials should resolve whether this setup might be useful in assessing a patient's response to antiplatelet therapy, leading to more tailored antiplatelet treatments. Also in **Chapter 7**, a flow cytometric setup was used for in-depth characterisation of haemostatic function, followed by a clustering tool to assess differences between platelet populations. The flow cytometric assays used in **chapters 6 and 7** could contribute to a better stratification of patients, which might help in monitoring drug effectiveness and thrombotic or bleeding risks. In addition, identified populations might function as biomarkers for disease severity and progression.

As people nowadays are getting older than ever before, age-related pathologies require some attention. With ageing, the risk for developing malignancies and thrombosis increases. Future research should assess whether differences in platelet populations between ageing and younger individuals can be identified, and possibly might be used as predictive biomarker, for thrombotic risk prediction. Moreover, in **Chapter 2**, where we provided an overview of CHIP mutations linked to altered platelet traits, we also described that CHIP mutations are more common within the ageing population. Therefore, there is a need for novel sensitive tools to

track CHIP mutations, which might make individuals more prone for development of cardiovascular diseases.

Overall, the impact of my thesis is in exploring basic mechanisms of platelet-coagulation interactions as a basis for developing diagnostic and therapeutic tools to improve individual patient antithrombotic management. The ultimate result should be less bleeding complications and in particular those bleeds that require medical attention, including visit to the GP or emergency room, or interventions to stop bleeding.

To make scientific contributions in the society, it is crucial that basic researchers and clinicians exchange knowledge and ideas. One way to do this from a researcher's perspective, is by publishing the state-of-the-art findings in clinical or translational journals. I did this, by publishing the paper 'Glycoprotein VI as target for novel antiplatelet therapy' in *NTVH (Ned Tijdschr Hematol. 2021;18:266-71)*. Here, I summarised the current state of GPVI inhibitors, in laboratory use, but also in clinical trials. Another way to exchange knowledge, is by attending conferences, where scientists share their latest findings. Those events allow researchers and clinicians to network, so that ideas can be shared, which can possibly lead to novel collaborations.

Eventually, it is valuable to inform patients on their disease, in a way that they can understand. If their interest in science grows, then they will be more prone to participate in clinical studies. Patients can learn more about their condition by attending workshops. An event that brings clinicians, researchers and patients together is 'World Thrombosis Day'. Finally, basic researchers should meet patients from time to time, and listen to their story, because they, eventually, are a scientists' motivation to keep going.

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