Platelets in thrombosis and haemostasis

IMPACT

Platelet activation and coagulation are essential parts in hemostasis. Many steps in platelet thrombus formation are closely connected to the different stages of thrombin formation. We explored a novel mechanism for the interplay between platelets and coagulation by studying the FXII-independent initiation of thrombin generation by collagen in vitro. More in detail, platelets activated with GPVI agonists (CRP-XL and convulxin), but not with α2β1 agonists (GFOGER), were able to induce thrombin generation. The FXa generation experiments done with purified coagulation factors and inhibition of an anti-FIX antibody, along with the recovery of TG in FIX deficient plasma with the addition of FVIII, suggested FIXa activity from GPVI activated platelets. Taken together, our findings indicate that apart from the well-known participations of platelets in coagulation (providing an assembly site for coagulation factors by exposed PS, releasing partially activated FV and polyP), activated platelets can contribute to thrombin generation via FIXa activity. The clinical significance of our findings remains to be demonstrated. Our findings show that coagulation can be initiated via tissue factor and contact activation independent triggers. This pathway may be important for certain forms of venous thrombo-embolism (VTE), because in the majority of VTE patients, there are no indications of exposure to TF or contact activation. Furthermore, our findings indicate a potential mechanism to explain why the clinical manifestations in haemophilia B patients are milder compared to the bleeding severity in haemophilia A patients [1], while treatment of haemophilia B or A mice with platelet-targeted FIX or FVIII gene therapy, respectively, reduces bleeding and restores haemostasis [2, 3].

On-treatment multiple myeloma (MM) patients have an increased incidence of venous thromboembolism (VTE), despite a high prevalence of thrombocytopenia and anaemia. We investigated platelet reactivity, platelet activation, plasma thrombin generation and whole blood thrombin generation profiles in MM patients and healthy controls. Compared to controls, plasma thrombin generation was not different in treated MM patients. However, they showed diminished platelet reactivity, delayed initiation but increased thrombin generation potential in whole blood, which were associated with several properties of RBCs and platelets. These findings help to understand the disbalanced haemostasis in treated MM patients with an emphasis on the contributions of blood cells, which may further help providing more specific prevention strategies for VTE in MM patients. Also, the WB-TG test applied in this study may be a promising tool for future research studying blood cells in coagulation and thrombosis related diseases. COPD is a chronic, progressive condition with a high prevalence. The Global Burden of Disease Study 2015 estimated the global prevalence of COPD at about 174 million cases [4]. For COPD patients, the periodic deteriorations called exacerbations pose the highest financial burden due to supplementary treatment and increased hospital

admissions [5]. Also, exacerbations are associated with an increased risk of thrombotic cardiovascular events, which contribute significantly to the overall disease morbidity and mortality [6]. Exacerbations were found to be associated with increased PMCs, but not with changes in platelet reactivity. VWF and TG were significantly higher during AE-COPD compared to convalescence. Furthermore, PMCs, VWF and TG were positively associated with systemic inflammation. Therefore, we concluded that acute exacerbations are associated with an inflammation-associated prothrombotic state. Our findings justify the further investigation on biomarkers of endothelial cell activation, platelet pathophysiology, hyper-coagulation and hypo-fibrinolysis to predict the risk of exacerbation relapse and of cardiovascular events. If large clinical outcome studies would prove that these markers have additional diagnostic value, implementation in clinical chemistry laboratories may be considered.

In the secondary prophylaxis of CVD such as myocardial infarction and stroke, anti-platelet therapy is the cornerstone. High on-treatment residual platelet reactivity (HRPR) is associated with high risk of thrombotic events and thus testing response to anti-platelet therapy may bring value for patients [7]. LTA is the standard technique to monitor HRPR, however, LTA is poorly standardized despite existing guidelines, requires a considerable volume of fresh blood and is time and labor intensive. We found a moderate agreement between MesADP-triggered whole blood platelet activation test (WB-PACT) and ADP-triggered LTA on recognizing HRPR in patients receiving aspirin and clopidogrel. This study indicates that WB-PACT is an optional platelet reactivity test for monitoring anti-platelet therapy. Patients with a high platelet reactivity on clopidogrel/aspirin treatment are suitable for treatment with new antiplatelet agents like prasugrel or ticagrelor.

The prevalence of inherited platelet function disorders (IPFDs) among the general population has not been established, and little is known about the burden of IPFDs, probably because the identification of inherited platelet function defects is challenging due to the complexity of platelet function and consequently, the large diversity of platelet defects. It is relatively straightforward to diagnose severe IPFDs since these patients with distinct clinical and laboratory features can readily be detected with current available diagnostic tools, while for mild IPFDs the diagnosing could be cumbersome due to the heterogeneous phenotype that requires a combination of tests. We investigated the correlation between the flow cytometric WB-PACT and LTA in patients with suspected bleeding diathesis for platelet function defect identification. A moderate correlation was found between LTA and WB-PACT and there was agreement between both tests in 62% of the cases. By comparing to bleeding score, the WB-PACT detected more abnormal responses than LTA in high BS patients. Therefore, with the advantages of less handling steps, suitability for low platelet count samples and neonatal blood samples, WB-PACT

may have added value for the routine diagnostic work-up in platelet function defect recognition. Accurate and timely diagnosis for patients with suspected IPFDs would help them with appropriate treatments, minimize the associated bleeding risk, and therefore improve their quality of life [8].

Lastly, an immunosorbent assay was characterized to directly detect circulating VWF in its active conformation. Current evidence indicated a possible role of active VWF measurements in monitoring TTP patients, while large, clinical outcome studies are still needed for exploring its clinical usefulness [9, 10]. At the moment, the value of active VWF quantification seems to lie in the research field for unraveling the pathophysiological mechanisms of hemostatic complications in a variety of conditions [11-13].

Concluding remark

The research presented in this thesis provides new insights in the initiation of coagulation and in the role of blood cells in coagulation. Both insights may lead to new diagnostic targets to estimate the risk of thrombosis in high-risk patients. Furthermore, intervention in platelet activation induced thrombin generation and in cell dependent thrombin generation may be novel targets for anti-thrombotic treatment. These hypotheses need to be further explored in prospective follow-up studies and, if confirmed, in intervention studies.

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