Acquired alteration in platelets

Valorization

To valorize: to assign value or merit to

Recent years more emphasis has been put on assigning social and/or economical value to scientific findings.[1] However, the direct contribution to society is not as clear-cut for all scientific developments. To be able to improve the patient's quality of life or to ameliorate treatment, first a better understanding of the underlying physiological and pathological mechanisms has to be gained. Here, basic research is essential.

Platelets are an integral part of the hemostatic system. Upon vascular damage, platelets quickly respond by forming a platelet plug, which is stabilized by the formation of fibrin. Aberrant functionality of platelets can result in an imbalance in the hemostatic system and thereby contribute to thrombosis or hemorrhage. Arterial thrombosis underlies the development of cardiovascular disease, the number one cause of death globally, according to the World Health Organization.[2] Here, antiplatelet drugs are an essential part in the treatment of cardiovascular disease.[3] A downfall of antiplatelet therapy is the increased risk of hemorrhage.[4] The high occurrence of bleeding complications as a result of antithrombotic therapy again underlines the delicate hemostatic balance, indicating the need for more insight into the processes of hemostasis and thrombosis to provide optimal treatment.

In this thesis two novel techniques are presented. In *Chapter 3*, we describe a new approach for the detection of juvenile platelets, *i.e.* the oligo-dT staining. The number of juvenile platelets is an indication of the platelet turnover, *i.e.* production of new platelets and degradation/consumption of circulating platelets. In different pathological conditions one or both processes can be affected. Knowledge of merely platelet count is insufficient to distinguish between a defect in platelet production or in degradation/consumption. Here, the measurement of the number of juvenile platelets can be of great use. The current approach to detect juvenile platelets relies on the usage of thiazole orange to label mRNA, which is especially detectable in newly formed platelets.[5] Since thiazole orange shows aspecific staining of the dense granules, results can be confounded by granular content.[6] Although the procedures for oligo-dT staining are more laborious, the oligo-dT staining might be a suitable replacement for thiazole orange due to the higher specificity. The second novel technique is described in *Chapter 4*, in which a new integrative whole blood perfusion assay is presented that allows the simultaneous assessment of both platelet and coagulant functions under flow conditions. Most current assays only study either platelet function or coagulant activity under static conditions. However, platelets promote coagulation by providing a procoagulant surface for the assembly and activation of coagulation factors, which leads to enhanced generation of thrombin. Thrombin in turn is an important platelet activator. Using this novel whole blood perfusion assay, reciprocal effects of thrombus and fibrin formation (coagulation) can be studied, providing a better reflection of the patients' hemostatic potential.

In case immediate improvement of hemostasis is required, such as in conditions with a high risk of bleeding, transfusion of blood components is one of few treatment options available.[7] However, transfusion of blood products entails several severe

side effects such as allergic reactions, infections and incidentally acute lung injury.[7] Furthermore, transfusion of blood products is associated with high costs. Currently, a substantial part of platelet concentrates (67%) is administered to patients with severe thrombocytopenia as a consequence of hematological malignancies and chemotherapy treatment.[8,9] For the prevention of bleeding these patients receive prophylactic platelet transfusions on guidance of the platelet count, i.e. when the platelet count drops below 10 $x10^9$/L.[8] However, in this patient population platelet count shows only a weak correlation with bleeding.[10] In *Chapter 6* we demonstrated that platelets from severely thrombocytopenic cancer patients treated with chemotherapy are highly dysfunctional in multiple aspects. We also showed variability in platelet responsiveness between patients, which was independent of platelet count. The next step would be to study whether platelet responsiveness is linked to the occurrence of bleeding. This may not only lead to better prevention of bleeding but also to a more efficient transfusion practice wherein only those patients will receive platelet concentrates that are likely to benefit. Ultimately this will reduce the number of platelet transfusions and thereby the negative side effects and high costs associated with it.

Patients, who suffer from severe blood loss due to trauma or surgery, may require fluid infusion to maintain electrolyte and fluid homeostasis. However, due to the large volumes of fluid infused, hemostatic factors are diluted as well, resulting in a condition better known as dilutional coagulopathy. In order to correct the dilution of the hemostatic factors, transfusion of blood products is necessary. In *Chapter 4 and 7*, the contribution of multiple blood components to thrombus and fibrin formation was investigated. We demonstrated that the addition of fibrinogen and platelets ameliorated clot formation, while addition of prothrombin complex concentrate improved thrombin generation. The addition of red blood cells only proved to be beneficial for thrombus formation when thrombus formation was studied under flow using the whole blood perfusion assay. This highlights the importance of the incorporation of flow when studying thrombus and fibrin formation. Assays that are currently used in the clinic to monitor clot formation (*e.g.* thromboelastometry) measure platelet function and/or coagulant capacity under static conditions. Where thromboelastometry assesses hemostasis globally[11] and thrombin generation determines the coagulant capacity in the absence or presence of platelets[12], both assays lack the incorporation of shear stress. Here, whole blood perfusion assays such as described in *Chapter 4*, can be of great added value to determine the need and effect of transfusion in a clinical setting.

In order to gain more insight into thrombosis and hemostasis, *in vivo* studies are of great importance. *Chapter 8* presents a synthesis of all published studies on thrombosis and hemostasis in genetically altered mice. By combining the results of all studies, several important observations could be confirmed and made. Firstly, *in vitro* models for thrombus formation correlated well to *in vivo* models for thrombosis. Thereby, *in vitro* whole blood perfusion models can partly replace and refine animal models. Secondly, by constructing a network of all genes, new genes that are highly likely to affect thrombosis and hemostasis, could be detected. Using this network, also new genes could be identified that affected thrombosis but not bleeding and are thereby, possible new targets for antithrombotic therapy.

References

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