Innovative assays to detect bleeding and thrombotic tendencies: a focus on thrombin generation and fibrin formation

Valorization

In this thesis we describe the development of two new assays that may help in the detection of bleeding and thrombotic tendencies. Cardiovascular diseases are still the number one cause of death world-wide (1). Therefore there is a need to adequately determine the bleeding or thrombotic tendency of a patient. The calibrated automated thrombinography (CAT) is a well-established method which can successfully distinguish between hypo- and hypercoagulable states (2). This method is frequently used in laboratory settings, but at this moment the implication into clinical practice is not possible due to problems with standardization. Alternatively, viscoelastometry can detect aberrations in the hemostatic system (3,4). However, the lack of agreement on which parameters and initiators to use in which conditions, has led to a poor acceptance of these techniques (3,4). Therefore, we developed two assays which may improve the detection of bleeding and thrombotic tendencies.

The technical and clinical validation of our first new assay which simultaneously detects thrombin generation and fibrin formation under conditions of flow is described in chapter 5. Currently most assays measure under static conditions, while blood is a moving entity that flows through the body. Therefore we implemented flow in our system to be one step closer to physiology. To protect this method a patent was filed and granted (WO2013190071A2, WO2013190071A3, EP2677037A1, US20150118691). Ultimately, we would like to implement our system into clinical practice, but further validation and optimization is necessary. Implementation of our system into clinical practice would lead to less laboratory methods since thrombin generation and fibrin formation are measured simultaneously by the same technique instead of two separate techniques. Consequently, our method would result in lower costs for the hospital as regard to labor and reagents costs.

With the help of our new rheometer-based method and CAT method, we observed that the mechanisms of blood clot formation and structure are different in infants and children compared to adults (chapter 6). In addition, we found that the response of infants and children to thrombolytic therapies is different compared to adults. These findings significantly enhance our understanding of the physiology of thrombosis formation in children, and may have major implications for prophylaxis treatment strategies for this serious condition across all age groups.

Because of the high incidence of the clinical symptoms independent from APS, the diagnosis of APS predominantly relies on the laboratory assays. Therefore, it is of extreme importance that these assays adequately diagnose patients with APS. Numerous publications and reports from the foundation of External Quality Control of Diagnostic Assays and Tests illustrate that the commercially available assays used for the diagnosis of APS produce varying results (5-8). In chapter 8, we provided evidence that these variations partly originate from the way β_2GPI is coated in different commercial available enzyme-linked immunosorbent assays (ELISAs). Our

results demonstrate that the improper coating of β_2GPI results in an underdiagnosis of APS. This misdiagnosis has important consequences for the patients, since they will not receive the proper medical care, such as anti-coagulant treatment to prevent thrombosis. In addition, manufacturers of these ELISAs should be convinced of the importance to properly coat β_2GPI resulting in an adequate diagnosis of APS. In the addendum of chapter 8 we introduce a thrombin generation-based assay for the diagnosis of APS. Currently, APS is diagnosed by clotting time-based assays or ELISAs. Therefore, our method using thrombin generation in combination with a trigger of the intrinsic coagulation cascade and cardiolipin is completely new for the diagnosis of APS. Our assay aims to distinguish APS patients from healthy controls, as well as from patients with other (thrombotic) diseases. Although, the preliminary results look promising, the assay needs to be validated in a large patient population. In case of positive results in a large multicenter study, other assays will be redundant, creating more unity in the diagnosis of APS. We can apply for a patent to acquire the exclusive rights for our method.

In conclusion, we developed two new assays which are innovative and different from the existing methods. We would like to accomplish the implementation of these methods into routine clinical practice, thereby improving the prediction of a bleeding or thrombotic tendency. Ultimately, this will result in an improved patient care of which also hospitals will benefit by a reduction in the patient, labor and reagent costs.

References

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