The dynamics of thrombin generation

APPENDIX I

IMPACT

SOCIETAL RELEVANCE

Cardiovascular diseases (CVDs) are the leading cause of death worldwide [1]. In 2019, an estimated 17.9 million people died from CVDs, representing 32% of all global deaths, of which 85% were caused by either a heart attack or a stroke. A significant part of CVDs can be prevented by addressing behavioral risk factors such as tobacco use, unhealthy diet, obesity, physical inactivity, and harmful use of alcohol [2]. Changes in coagulation either primary or as a consequence of behavioral risk factors play a major role in this process. Therefore, it is important to detect CVDs as early as possible to enable lifestyle changes or prophylactic treatment with medication to reduce the risk of thrombotic events [3].

OPPORTUNITIES OF THROMBIN GENERATION AND THROMBIN DYNAMICS

Thrombosis is a major complication of CVDs, which can result in myocardial infarction, acute ischemic stroke, or venous thromboembolism (VTE) [4,5]. Therefore, a suitable diagnostic test that can accurately predict the risk of thrombosis and assess the status of the hemostatic system is important in the reduction of cardiovascular events [6,7]. Conventional coagulation assays, such as the prothrombin time and the activated partial thromboplastin time were developed to detect a bleeding tendency, and are not applicable for the prediction of an increased risk for thrombosis [8,9]. The thrombin generation (TG) assay is a tool that gives a comprehensive insight into the coagulation capacity of an individual, and can be used to predict the risk of bleeding or thrombosis [10,11]. Furthermore, TG can be used to monitor patients on anticoagulant and anti-platelet treatment [12,13]. However, due to the global nature of the TG test, it is difficult to pinpoint specific defects by TG alone [9,14,15]. Therefore, by analyzing the pro- and anti-coagulant processes in thrombin dynamics, one can extract prothrombin conversion and thrombin inactivation from the underlying TG that could help clinicians to make treatment decisions for patients [16,17].

We wanted to demonstrate the usefulness of the thrombin dynamics analysis by illustrating it with a clinical example. Patients with an HIV infection are treated with a combination

anti-retroviral therapy (cART) that effectively suppresses the replication of the HIV-virus, thereby greatly reducing morbidity and mortality. The use of cART is associated with a persistently activated coagulation system and an increased risk of CVDs and VTE. Abacavir is one of the nucleoside reverse-transcriptase inhibitors and has been reported to increase the incidence of myocardial infarction [18,19]. The biological mechanism underlying the observed CVD risk associated with abacavir use remains unclear, and possible mechanisms include an abacavir-induced vascular wall inflammation, impairment of endothelial function, and platelet hyper-reactivity. In Chapter 3, we found that the higher prothrombin conversion in abacavir-treated patients contributes to the prothrombotic phenotype, which explains the higher number of thrombotic events observed in these patients. Investigating thrombin dynamics analysis of the underlying TG can be helpful to study the mechanism of cART-related thrombotic risk.

INTRODUCTION INTO THE CLINIC

Thrombin dynamics based on TG data generated by the semi-automated Calibrated Automated Thrombinography (CAT) method has been studied in multiple clinical settings over the past years to analyze the balance between pro- and anticoagulant mechanisms in patients with e.g. liver disease and hemophilia A. It also has been shown to be useful for *in silico* experimentation to investigate how differences in coagulation factor levels affect TG and thrombin dynamics parameters [16,20,21].

Since the TG test has been fully automated on the ST Genesia analyzer, it can also be performed in clinical laboratories. Therefore, we can also investigate thrombin dynamics using TG data obtained with the ST Genesia to detect discrepancies between healthy subjects and patient populations [22,23]. In Chapter 4, we used the ST Genesia to measure TG in plasma of 112 healthy donors and used the data as an input for thrombin dynamics analysis. The analyzed thrombin dynamics data can be used as reference values by other laboratories, that are able to use these data to provide guidance to differentiate between clinically ‘normal’ and ‘abnormal’ thrombin dynamics parameter values. Therefore, the

introduction of the ST Genesia into the clinic is an opportunity to use the thrombin dynamics analysis in specified clinical settings.

SCIENTIFIC IMPACT

Thrombin is the central enzyme of the coagulation system. The formation of thrombin is regulated by platelets, coagulation factors and thrombin inhibitors [11,24]. The study described in Chapter 2 further clarifies the role of FII, FV, FX and antithrombin in the regulation of the coagulation system by measuring prothrombin conversion and thrombin inactivation [25].

Studies of the conversion of prothrombin to thrombin yielded important information concerning clotting abnormalities in various hemorrhagic disorders. On the other side, studies of thrombin inactivation provide an opportunity to study anticoagulant deficiency and therapy in hemostatic disorders, such as targeting the anticoagulant pathway in haemophilia. Chapter 5 gives insight into the effect of the platelet count on plasmatic coagulability and shows that the platelet count can influence prothrombin conversion rather than the inactivation of thrombin [26]. Thrombin dynamics in platelet rich plasma could be a novel tool to study the function of platelets in coagulation on a clinical level. Previous studies have shown that thrombocytopenia, a condition in which a low platelet count can cause mild or severe bleeding events, is associated with lower TG as well [24]. In the future, platelet rich plasma-thrombin dynamics could contribute to the diagnosis and management of patients suspected of suffering from platelet disorders.

CONCLUSION & PROSPECTS

In this thesis, we present novel applications of the thrombin dynamics approach, in both its original research setting, as well as in a clinical setting, using the fully automated TG analyzer ST Genesia. Our results gave us a better understanding of how coagulation factors and platelets influence prothrombin conversion, thrombin inactivation and ultimately TG. In the past, thrombin dynamics analysis had shown its applicability to study the thrombotic/hemostatic disorders in platelet poor plasma. In this thesis, we studied the

influences of thrombin dynamics more thoroughly, and we validated the method for platelet rich plasma. The latter opens new possibilities to study platelet-related disorders and clinical situations in which platelets are affected.

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