Delicate interactions between plasma factors and blood cells affect thrombin generation

IMPACT

Cardiovascular disease (CVD) represents the number one cause of death globally, claiming an estimated 17·8 million deaths in 2017 [1]. Especially, the threat of thrombosis is increasing as the global population is aging. Dysfunction of the blood coagulation system is one of the major causes of CVD. New developments in coagulation tests will give better insights in the involvement of coagulation in CVD[2].

Thrombin generation (TG) is a test to measure the capacity of blood to form thrombin, the key enzyme in the blood coagulation system. TG gives a global overview of the coagulation capacity of an individual, thus may give insight in the risk of bleeding/thrombosis. Furthermore, TG may be a tool to guide patients on anticoagulant treatment. Although TG is the most complete plasma based coagulation assay [3], it does not involve the interplay between coagulation and blood cells [4]. The main goal of this thesis was to innovate/improve whole blood (WB) -TG assays to study the contribution of blood cells to the regulation of TG.

Key contributions of this thesis:

We developed a novel fluorogenic WB-TG assay with good reproducibility. This assay is a major step forward compared to previous WB-TG assays that had a poor reproducibility [5], or were prone to contact activation induced by the filter paper in the assay [6]. Platelet and erythrocyte counts, as well as platelet (in)activation were found to be crucial determinants of WB-TG, supporting our hypothesis that our WB-TG assay rightfully includes the influence of blood cells on coagulation. A strong enhancing effect of erythrocytes on the velocity of TG was observed, even in the presence of high platelet numbers. We also explored the performance of this assay in a healthy population and studied it in relation to age, gender, oral contraceptive use and blood cell count, which could serve as useful references for future studies.

We studied WB-TG profiles of cirrhotic patients with an optimized near patient assay and found that the TG capacity of these patients was comparable to that of normal people, suggesting a normal coagulability, in line with the widely accepted concept of rebalanced hemostasis in these patients [7]. Interestingly, the WB-TG velocity was slower in cirrhotic patients despite an intact WB-TG capacity. This observation might be explained by our previous results in reconstituted blood that counts of erythrocytes and platelets impact the TG velocity and capacity differently, thus a mild decrease of platelet and erythrocyte counts in cirrhosis only impaired the velocity of WB-TG but not the total capacity. The balance of the coagulation system in cirrhotic patients is very fragile and may easily tip towards a bleeding or a thrombosis phenotype. By using WB-TG a likely better representation of the in vivo situation is established, and WB-TG might be the assay of choice to study influences of therapy on fragile equilibria such as with liver cirrhosis and to predict their bleeding or thrombotic risk.

We found an inhibiting role of erythrocytes on the anticoagulant function of activated protein C in WB-TG. The protein C system is an important anticoagulant pathway and impairment of this

system is a common cause of thrombosis [8]. Higher erythrocyte count was related with a reduced anticoagulant effect of active protein C and thrombomodulin in WB-TG, both in a healthy population and in reconstituted blood samples. This effect was not dependent on platelets and was likely related with the phospholipid composition of erythrocytes. The inhibiting role of erythrocytes on APC function, combined with the observation of the enhancing effect of erythrocytes on WB-TG velocity, might provide a possible explanation for the increased thrombotic risk related with increased erythrocyte counts, for example polycythemia and erythrocyte infusion [9, 10].

We studied the plasmatic coagulability of HIV-infected individuals on combined antiretroviral therapy and found that the plasma TG capacity of these individuals was lower than healthy controls. This observation, together with their reduced prothrombin levels and increased markers of inflammation and endothelial activation, suggest that the increased thrombotic risk of these individuals was not due to hypercoagulability and was most likely related to increased stimulation of coagulation by endothelial activation and inflammation during HIV infection. We also found that abacavir-use was associated with a prothrombotic TG profile compared to non-abacavir regimens, irrespective of age, sex and inflammation, thus providing new data for the debated thrombotic effect of abacavir [11].

We used TG as an intermediate coagulation phenotype in a genome wide association study and discovered that the *KLKB1* gene was related to the anticoagulant function of the protein C system. Functional experiments showed that in vitro supplementation of kallikrein augments the anticoagulant function of TM and APC in TG. This provides a possible mechanism for the previously observed association between the *KLKB1* gene and thrombosis [12, 13]. This also reinforces that the TM-modified TG assay could serve as a tool to discover novel mutations related to the protein C pathway.

Conclusion &prospects

In this thesis we present new tools for improved WB-TG measurements and data on the utility of TG assays in both fundamental and clinical research. Our data support the view that blood cells play important roles in the regulation of coagulation and suggest that WB tests provide additional insights into plasma tests. WB-TG is still in its infancy and additional research is needed to improve its standardization and establish its clinical utility.

WB-TG does not require a plasma preparation step, thus the research described in this thesis could aid to the development of a point-of-care (POC) TG test for coagulation assessment in places without a specialized hematology laboratory. Such a POC-TG test could allow people to have a more detailed and timely overview of their coagulation system, reaching an important step towards personalized medicine that may improve the management of bleeding or thrombosis. Furthermore, POC-TG would be especially beneficial for under-developed communities that suffer the most from the lack of laboratory resources.

In conclusion, the current thesis is a major step forward to a more comprehensive and timely assessment of blood to improve the management of coagulation disorders.

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