Full-Blood Thrombin Generation Time: A Blood Coagulation Assay as Alternative for aPTT and PT using Flowing Blood.

Menno L.W. Knetsch, Lieke L.H. Both, Leo H. Koole.
Department of Biomedical Engineering/Biomaterials Science, Faculty Health, Medicine and Life Sciences,
Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands

Introduction

Determination of the potential for blood coagulation is of great importance in patients with haemostatic disorders or patients that carry a blood contacting device. Hyper-coagulability can lead to thrombosis and embolisms, while hypo-coagulability increases the chance of hemorrhage. Contact of the blood with synthetic implants can lead to onset of the blood-coagulation cascade which may result in failure of the blood contacting device.Additionally, the formed thrombus on the implant surface can release into the circulation and lead to distant embolism, e.g. pulmonary embolism. Anti-coagulant or anti-platelet drugs are applied to prevent thrombotic complications. To assess the haemostatic status of blood, aPTT (activated partial thromboplastin time) and PT (prothrombin time) are used in the clinic today.[1,2] However these methods have some serious drawbacks. Most importantly, they make use of plasma under static conditions. Because of this, the effect of anti-platelet drugs like aspirin or clopidogrel (Plavix) can not be assessed. Also the effect of the anti-coagulant drug heparin can only be determined with aPTT, the PT assay is insensitive for heparin. We have developed an assay that measures the thrombin generation time in full, flowing blood, the full blood thrombin generation time (FB-TGT). The device to measure FB-TGT makes use of exactly defined surface coatings to obtain a reproducible generation of thrombin and coagulation. The polymeric coating was designed to result in coagulation between 5-8 minutes. This time-frame ensures the possibility to detect hyper- and hypo-coagulability and a reasonable assay-time. Here we describe the initial evaluation of FB-TGT.

Methods

The scheme of the newly developed device for measuring FB-TGT is depicted in figure 1.

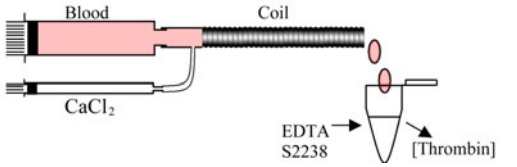

Figure 1. Scheme of the Thrombin Generation Time assay setup.

A syringe with freshly drawn, citrated, blood is connected to one inlet of the mixing chamber. A syringe with $CaCl_2$ is attached to the second inlet. The blood is recalcified in the chamber and guided through a coil covered uniformly with a polymeric coating with defined thrombogenicity.[3] Blood is collected in a buffer containing EDTA and the chromogenic substrate S2238. The conversion of this substrate is used to determine thrombin concentration.

The polymeric surface coating consists of a random copolymer of n-vinyl-pyrrolidinone (NVP) and butyl-methacrylate (BMA).[3] Different ratios of the monomers were tested. Full blood thrombin generation times were determined in blood obtained from healthy donors. To assess the effect of heparin, this anti-coagulant drug was added to blood at concentrations of 0.1-0.3 U/ml, which equals clinically used concentrations. Additionally, donors took aspirin (500 mg total) 2 hours before determining FB-TGT. In parallel, plasma samples were prepared and aPTT and PT were determined by the clinical hematology department of the Maastricht University Medical Centre.

Results

The FB-TGT assay resulted in reproducible results (table 1). The addition of heparin results in a concentration dependent increase in thrombin generation time, which means a slower and reduced blood coagulation response. The effect of heparin could be demonstrated by the aPTT but not the PT method.[4] Aspirin use resulted in an increased thrombin generation time, which could not be detected by both aPTT and PT (table 1).[2]

	Heparin experiment		Aspirin experiment	
	control	+heparin	control	+aspirin
FB-TGT (min)	5.3 ± 0.6	> 30	6.3 ± 1.0	13.0 ± 0.5
aPTT (sec)	28.31	100.57	24.71	25.40
PT (sec)	7.81	8.21	7.92	8.35

Table 1. Results of thrombin generation time assays of blood treated with heparin (0.3 U/ml) and aspirin (500 mg total). Results are compared to aPTT and PT assays.

Conclusions: The new FB-TGT assay may be a good addition to the range of blood analyses in clinical use today. The disadvantage of the currently used aPTT and PT methods is that they use plasma under static conditions, neglecting the influence of blood cells and flow on blood coagulation. The FB-TGT assay may initially be most suited to assess the coagulability of patients with permanent or long-term blood contacting devices, like synthetic vascular grafts or central venous catheters. Further investigations should determine if the FB-TGT assay can be a replacement of the aPTT and PT methods.

References

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