



Influence of pre-analytic variables on thrombin generation

L. Wiens¹, K. Wuttig², G. Lutze¹, C. Luley¹



¹Institut für Klinische Chemie und Pathobiochemie, ²Institut für Transfusionsmedizin und Immunhämatologie der Otto-von-Guericke-Universität Magdeburg

Introduction

The determination of thrombin concentration (thrombin generation) can be regarded as a global assay, which represents the current state of the hemostatic system. This assay is performed in citrated plasma after addition of tissue factor, phospholipids and calcium ions. There are a number of parameters which could have an influence on thrombin generation in vitro as well as in vivo.

Such parameters are activation of platelets, of contact phase or others as lipaemia or hyperbilirubinaemia.

Our aim was to determine the influence of avoidable factors on thrombin generation.

Methods

For measuring thrombin generation (Technothrombin® TGA, Technoclone) we used platelet free plasma from 8 healthy subjects who didn't present with any hemostatic failures. Thrombin generation was measured using all three reagents (RB, RC low und RC high) of the TGA test kit. These reagents contain different concentrations of tissue factor and phospholipids. The following pre-analytic variables were analysed:

1. Whole blood samples were transported by letter shoot either immediately after blood taking or 2 or 4 hours later. They were centrifuged after this transport.
2. Centrifugation was done 2 or 4 hours after blood taking, without any transportation or shaking of the samples
3. Prior to centrifugation whole blood samples were shaken for 2 or 4 hours after blood taking.
4. Samples were centrifuged immediately after blood taking and then left standing for 2 or 4 hours.
5. Triglyceride concentrations were varied from 1 to 5.4 mmol/L.

Samples were measured without any prior freezing. In each series the thrombin concentration measured after immediate centrifugation was taken as starting value. The influence of lipaemia was determined by addition of increasing amounts of a defined chylomicron suspension (isolated by ultracentrifugation) to plasma of one test person.

Results

Figure 1: Effect of whole blood sample transport by letter shoot

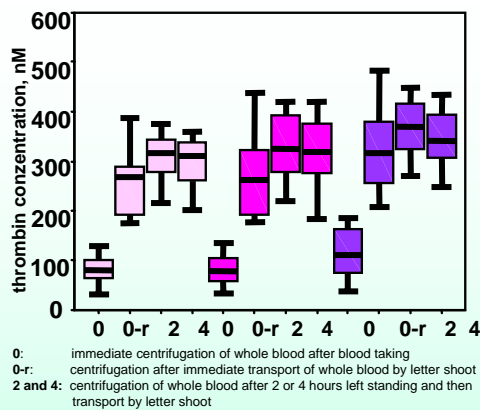


Figure 2: Effect of plasma samples left standing before measurement

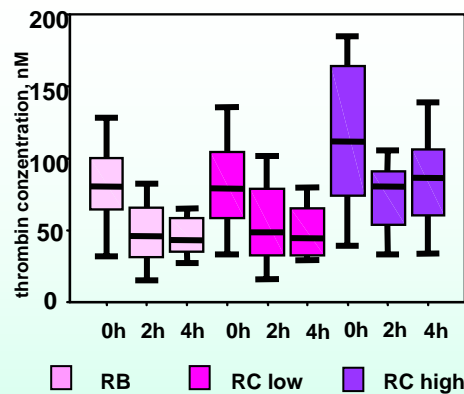
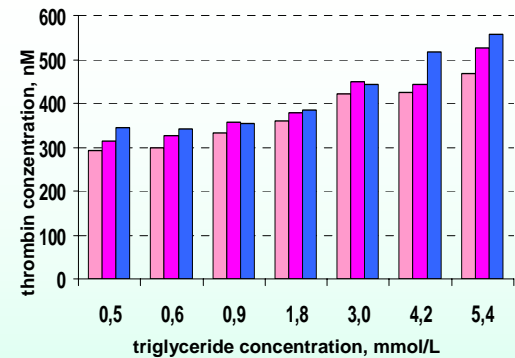


Figure 3: Influence of lipaemia on thrombin generation



All parameters investigated resulted in significant differences in thrombin generation. All three reagents (RB, RC low, RC high) of the TGA test kit (Technoclone) produced the following results:

1. Immediate transport of whole blood samples by letter shoot as well as transport after 2 or 4 hours left standing resulted in a 2- to 4-fold increase in thrombin generation ($p=0.012$) (Fig. 1).
2. Shaking of whole blood for 2 or 4 hours resulted in a significant increase of thrombin generation ($p < 0.05$, data not shown).
3. Leaving whole blood standing for 2 hours resulted in an increased but not significant thrombin generation. After 4 hours incubation the increase in thrombin generation was significant (data not shown).
4. Plasma obtained after immediate centrifugation and left standing for 2 or 4 hours resulted in a significant decrease of thrombin generation (Fig. 2)
5. Addition of increasing amounts of triglycerides (chylomicrons) to plasma of one test person resulted in increased thrombin generation (Fig. 3).

Conclusions

For reproducible measurement of thrombin generation several pre-analytical variables have to be standardized:

1. Whole blood has to be centrifuged immediately after blood taking and plasma has to be transported immediately as well. Alternatively, standardized transport and incubation times of whole blood samples have to be organized.
2. For establishing reference ranges for thrombin generation the local types of transport and times of whole blood left standing until transport have to be considered.
3. The influence of postprandial lipaemia on thrombin generation has to be taken into account.