Thrombin generation before and after multicomponent blood collection

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Summary
The development of apheresis technology has increased efficiency in donor blood use by collecting specific blood components in several combinations. The question of donor safety raised by the contact of donor blood with foreign, only in part biocompatible surfaces remains. The aim of this study was to estimate the effect of multicomponent blood collection on thrombin generation performing an overall function test of coagulation. Donors, methods: 26 blood donors were included. Per apheresis two units of platelets and one unit of RBCs were collected by two cell separators (Amicus® and Trima Accel®). Each donor underwent the procedure on both apheresis systems. Samples were collected before, immediately after, and 48 hours after apheresis. Thrombin generation was measured by means of calibrated automated thrombography (CAT). Results: CAT-data changed only slightly and no significant changes were seen before, immediately after, and 48 hours after apheresis. The parameters did not differ significantly between the two different apheresis devices. Conclusion: No change in parameters of continuous thrombin generation occurred, suggesting that apheresis did not lead to severe alterations in the haemostatic system.

The use of new technologies in the field of apheresis has been rapidly growing over the last few years. Especially the development of automated collection systems for specific blood components from a single donor has found widespread routine application. These new procedures are known as multicomponent blood collections and offer increased efficiency in donor blood use, allowing the collection of platelets (PLTs), red blood cells (RBCs) and plasma in various combinations. The possible consequences of blood contact with foreign surfaces during extracorporeal circulation nevertheless are discussed controversially. Possible blood-biomaterial interactions involve the adsorption of proteins, adhesion of leucocytes and erythrocytes, and complement activation. The question if apheresis influences the hemostatic system still remains (5, 7).

Reviewing existing literature on this topic one can conclude that data are barely comparable because of different study settings, numerous apheresis devices and various investigated parameters. Kobayashi observed a marked increase of prothrombin fragment F1 + F2, thrombin antithrombin complex (TAT), and a small increase of fibrinopeptide A (FPA) by using a continuous-flow automated blood cell separator and concluded that these alterations are linked with a hypercoagulable state (12). Other investigators found no significant association between extracorporeal circulation and a procoagulatory state (18, 19).

The elevation of activation markers such as F1+F2 or TAT observed in some studies show that activation may occur but do not allow any definite conclusion whether this activation may lead to a hypercoagulable state or, to the contrary, may render a patient prone to bleeding due to excessive consumption of procoagulatory factors.

The effect of plateletpheresis on routinely determined parameters of hemostasis showed a prolonged prothrombin time (PT), whereas the activated partial thromboplastin (PTT) and thrombin times were not changed significantly (1). However, conventional clotting assays such as the determination of single prothrombotic factors, PT or PTT display only a small part of the complex hemostatic system and cannot replace an overall function test (9).

Measurements of the thrombin generation (TG) represent a method to estimate the individual bleeding as well as thrombotic risk by assessing the coagulability of blood, and reflect the simultaneous independent effect of all plasmatic pro- and
anticoagulant factors (10). The aim of our study was to investigate the possible effect of multicomponent blood collection on TG performing the Calibrated Automated Thrombography (CAT) comparing two types of cell separators.

**Donors, methods**

After approval of the study by the local Ethics Committee (No: 16–153 ex 04/05) 26 healthy donors (22 men, 4 women) aged between 20 and 58 (mean: 40 ± 10.3) years were included. Two units of PLTs (6 × 10^11) and one unit of RBCs (250 mL, Hct 80%) were collected per apheresis by two types of cell separators routinely used at the Department of Transfusion Medicine at the Medical University of Graz (Trima Accel®, Gambro BCT Inc., Colorado; Amicus®, Baxter Healthcare Corp., Illinois). Each donor underwent the procedure on both apheresis systems with at least 8 weeks in between. Blood samples were taken before, immediately after, and 48 hours after apheresis into precipitated S-Monovette tubes from Sarstedt (Nümbrecht, Germany), containing 300 µL 0.106 mol/l citrate. Plasma was separated by centrifugation at 2000 x g per minute for 10 minutes at room temperature, pooled and stored at –70°C.

**Reagents and devices**

Fluobuffer contained 20 mmol/l HEPES and 60 mg/mL bovine serum albumin, both purchased from Sigma, St. Louis, Mo., USA. Working buffer consisted of 140 mmol/l NaCl, purchased from Merck, Darmstadt, Germany, 20 mmol/l HEPES, and 5 mg/mL human serum albumin, purchased from Sigma, St. Louis, Mo., USA. The fluorogenic substrate Z-Gly-Gly-Arg-amino-methyl-coumarin (Bachem, Bubendorf, Switzerland) was solubilized in pure DMSO, which was purchased from Sigma, St. Louis, Mo., USA. Calcium chloride was purchased from Merck, Darmstadt, Germany. The platelet-poor plasma (PPP) reagent with a content of 5 pmol/l tissue factor (TF) and 4 µmol/l phospholipids, and the thrombin calibrator were purchased from Thrombinscope BV, Maastricht, the Netherlands.

**Measurement of the TG**

Automated fluorogenic measurement of the TG under standard conditions was performed using the CAT (11). For each experiment a mixture of 2625 µL fluobuffer and 300 µL 1 M CaCl_2 solution was prepared and incubated for 5 minutes at 37°C. After 5 minutes 75 µL of the Flu-DMSO-solution were added, mixed and incubated for 5 minutes.

![Fig. 1](image-url)  Characteristics of a typical curve: The time until the onset of the TG during the curve remains flat is referred to as lag time. After a steep increase the thrombin generation curve arrives at its peak, the maximum concentration of thrombin. The area under curve (ETP) represents the total amount of thrombin built over the time during the whole process of TG and stands for a real time monitoring of the coagulability of blood.
Results

A typical CAT curve is shown in Figure 1. CAT parameters prior to the start of the procedure showed great interindividual variability (Tab. 1). Parameters of TG changed only slightly and no significant changes were seen before, immediately after, and 48 hours after apheresis. The parameters did not differ significantly between the two different apheresis devices.

Discussion

In this study we investigated the influence of multicomponent collection by two different cell separators on TG. Using the CAT we found no significant alterations of TG parameters at any time and independent of the apheresis system used.

At this time, TG seems to be the best global test of hemostasis available. Indeed, TG is not only sensitive to various bleeding disorders but also reflects congenital and acquired defects making a patient prone to thrombosis (2). As thrombin plays a pivotal role in coagulation, because there are no pathways bypassing it, measurement of TG reflects much of the thrombotic-hemostatic function of the blood (10, 11, 16). The area under the curve of generated thrombin represents the so-called endogenous thrombin potential (ETP) and has been shown to correlate with plasma–based hypo- as well as hypercoagulable states (11, 20). In a general population, ETP as surface under the curve is rather stable from day to day, but shows a large interindividual variation. This is also reflected in our results showing a great variability between persons prior to the procedure but virtually no change in one person prior to and after apheresis.

In contrast to various conventional clotting tests, such as the PTT or the PT, the TG assay is sensitive to hypercoagulable changes in the plasma (11). Hypercoagulability found in AT-, protein C-, and S deficiency, prothrombin hyperexpression, factor V Leiden, and the use of oral contraceptives is linked with an increase of TG (9, 17).

In a recent study we have demonstrated a steady increase of ETP with age that might help to explain the increased incidence of thrombembolic disease observed with higher age (8). Even alterations of hemostasis in obese children are reflected by CAT: We have shown that overweight children after a significantly prolonged lag time generate significantly higher amounts of thrombin in comparison to age matched, normal weight, healthy controls (4). CAT has also been applied in hypocoagulable states such as the control of heparin therapy, to detect the influence of various anticoagulants in- cluding direct thrombin inhibitors, and to monitor hemophilia treatment (3, 6, 9, 13–15). All together, there is a lot of evidence that CAT is very sensitive to many changes in the hemostatic system leading to hypo- as well as hypercoagulability.

Multicomponent collection in this special setting seems to be a rather safe and suitable method to collect a double unit of PLTs and 1 unit of RBCs in one procedure. Extracorporal circulation and a longer donation time seem to have no influence on donor’s TG. However, definite proof that a procedure is safe requires selection of suitable donors, long-term donor surveillance and clinical observation of a large number of probands and can not be obtained by means of a laboratory investigation alone.

Conflic of interest

All authors declare, that there is no conflict of interest.

Tab. 1 The influence of the apheresis procedure performed on Amicus© and Trima Accel© on TG parameters before, immediately after, and 48 hours after donation. Results expressed as ± SD mean.

<table>
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<tr>
<th>cell separator</th>
<th>parameter</th>
<th>before</th>
<th>after</th>
<th>48 h after</th>
<th>significance</th>
</tr>
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<tr>
<td>Amicus</td>
<td>lag time (min)</td>
<td>1.9 ± 0.27</td>
<td>1.86 ± 0.25</td>
<td>1.87 ± 0.27</td>
<td>NS</td>
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<td>peak (nmol/L)</td>
<td>451.0 ± 96.6</td>
<td>419.1 ± 101.5</td>
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<tr>
<td></td>
<td>ETP (nmol/L*min)</td>
<td>1689.7 ± 479.9</td>
<td>1697.4 ± 532.3</td>
<td>1670.7 ± 481.5</td>
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<td>Trima Accel</td>
<td>lag time (min)</td>
<td>1.91 ± 0.24</td>
<td>1.87 ± 0.25</td>
<td>1.81 ± 0.21</td>
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<tr>
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<td>peak (nmol/L)</td>
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<td>ETP (nmol/L*min)</td>
<td>1806.3 ± 498.3</td>
<td>1738.7 ± 529.8</td>
<td>1682.8 ± 515.2</td>
<td></td>
</tr>
</tbody>
</table>

Results expressed as ± SD mean.