Rotation thromboelastometry (ROTEM®) stability and reproducibility over time

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Rotation Thrombelastometry (ROTEM®) stability and reproducibility over time

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Abstract

Background: Thrombelastometry is a whole blood assay to evaluate the viscoelastic properties during blood clot formation and lysis. Rotation thrombelastography (ROTEM®, Pentapharm GmbH, Munich, Germany) has overcome some of the limitations of classic thrombelastography. So far no clinical validation on reproducibility (inter and intra assay variability) and sample stability over time has been published.

Methods: To evaluate pre-analytic aspects sample stability over time was assessed in 48 patients in 8 age groups. Citrated blood was stored at room temperature. Tests measured every 30 minutes from T 0 minutes up to T 120 minutes on two ROTEM® devices were INTEM (ellagic acid activated intrinsic pathway), EXTEM (tissue factor triggered extrinsic pathway) and FIBTEM (with platelet inhibitor (cytochalasin D) evaluating the contribution of fibrinogen to clot formation). In 10 volunteers precision by intra and inter assay variability was evaluated at two points of time. Finally reference intervals and effect of age and sex were evaluated.

Results: Blood was stable over 120 minutes and no significant differences in ROTEM® results were found. Maximum clot firmness measurements had a coefficient of variation of < 3% for EXTEM, < 5% for INTEM and < 6% for FIBTEM. For clot formation time the coefficient of variation was <4% for EXTEM < 3% for INTEM. Coefficient of variation for angle alpha was <3% for EXTEM and < 6% for INTEM. The coefficient of variation for clotting time was < 15% for both EXTEM and INTEM. Small but significant differences between ROTEM® devices were found for maximum clot firmness in FIBTEM and INTEM as well as clot formation time and alpha angle in INTEM.

Conclusions: ROTEM® yields stable results over 120 minutes with a minimal variability on the same ROTEM® device. However, small but significant differences between ROTEM®
devices were observed. Analysis should be performed on the same ROTEM® device if small differences are of importance for treatment.

Key words: Thrombelastometry, Blood coagulation, hemostasis, thrombelastography
Introduction

Rotation thromboelastometry (ROTEM®) (ROTEM® delta, Pentapharm GmbH, München, Germany), a methodology based on thromboelastography originally described by Hartert more than 50 years ago [1], is frequently used today to rapidly assess the viscoelastic properties of the developing clot in cardiac and transplant surgery as well as following trauma. [2-5] ROTEM® documents the interaction of platelets with the coagulation factors from initial platelet-fibrin interaction, through platelet aggregation, clot strengthening, and fibrin cross-linking, to eventual clot lysis. Within 30 minutes, a ROTEM® tracing provides information on clotting factor activity, platelet function, and any clinically significant fibrinolysis. [6, 7]

The goal of this study was to assess stability and reproducibility over time of ROTEM®. The lack of such a study was already mentioned by Dunning et al. [8] in 2008 and by Samama et al. [9] in 2003. Possible changes due to age and sex were also of interest. In thrombelastography (TEG® Haemoscope Corporation, Skokie, IL, USA), stability of results over time is only achieved following a thirty minute waiting time after blood draw and before analysis.[10] Immediate sample analysis however is desirable in the setting of an acute bleeding event. We thus assessed stability over time for ROTEM®.
Material and Methods

This clinical trial was performed after obtaining authorization by the local ethic committee (Kantonale Ethikkommission, Kanton Zürich, Switzerland, Study number StV 27-2007).

Sample size (n=48) was chosen based on statistical considerations and on reported general practice. Horn et al. showed that a minimum of 39 patients is needed for establishing a 95% reference interval by power analysis. [11] In addition, Friedberg et al. reported in 2007 [12], that 50% of laboratories establish enrol 21-50 subjects when establishing reference intervals. As a possible age or gender effect was of interest, three male and three female patients in each of the following eight age categories were included: Below 20 years; 20 – 30 years; 30 – 40 years; 40 – 50 years; 50 – 60 years; 60 – 70 years; 70 – 80 years and above 80 years.

Inclusion criteria were: Scheduled for a non-emergent operation and signed written informed consent. Exclusion criteria were: Known malignancy or immunosuppression, known coagulation disorders, anticoagulation in any form, current treatment with heparin (other than routine preoperative thromboembolic prophylaxis with 3,000 IU low-molecular-weight heparin administered subcutaneously the evening prior to the operation), use of acetyl salicylic acid within the past 5 days, use of nonsteroidal anti-inflammatory agents within the past 24 h, known renal diseases or plasma concentration of creatinine more than 120 mM and liver diseases or increased plasma concentration of aspartate aminotransferase (> 50 U/l) or alanine aminotransferase (> 50 U/l) as well as patients not capable of understanding the German language.

The study’s main objectives were to validate ROTEM® by investigating 1) pre-analytic aspects (sample stability), 2) reproducibility and precision of ROTEM® (intra and inter assay variability) and 3) reference intervals and effect of age and sex.
Several predefined tests were assessed: INTEM (ellagic acid activated intrinsic pathway), EXTEM (tissue factor triggered extrinsic pathway) and FIBTEM (with platelet inhibitor (cytochalasin D) evaluating the contribution of fibrinogen to clot formation). These three assays are performed in citrated samples and represent counterparts of routine tests of plasmatic coagulation: fibrinogen, prothrombin time, activated partial thromboplastin time. The two main differences between thromboelastometric tests and plasmatic coagulation tests are i) the former is performed on whole blood, the latter on plasma and ii) the former measures processes involving thrombin generation, clot formation and clot lysis, the latter processes leading up and until the initial generation of thrombin. By utilising inhibitors of platelet function the developers of the assay have derived a functional test of fibrinogen, called FIBTEM. This can be correlated with functional fibrinogen tests in plasma. By comparing the EXTEM, the thrombelastometric test that utilizes tissue factor (comparable to the prothrombin time test) as an activator, with the FIBTEM, one can deduce relevant information regarding platelet function and factor XIII function, as both of these contribute to the EXTEM measurement.

In EXTEM the extrinsic pathway is activated by thromboplastin from rabbit brain to assess clot formation and fibrinolysis. In INTEM the intrinsic pathway is activated by a contact activator to assess the clot formation and fibrin polymerization. In FIBTEM the extrinsic pathway is activated by tissue factor in presence of a platelet inhibitor to assessment the functional fibrinogen level.

A number of ten volunteers were sufficient to investigate precision and reproducibility according to power analysis with 95% confidence interval (CI). To investigate precision and reproducibility of ROTEM® analysis, for inter-assay (on one single ROTEM® device) reproducibility testing was performed in 10 volunteers, after having obtained written informed consent, withdrawing 3 tubes (Vacutainer Brand, Belliver Industrial Estate, Plymouth, UK,
4.5 ml, 9 NC 0.129 M, a total of 18 ml per volunteer) of citrated blood, the first tube (Vacutainer Brand, Belliver Industrial Estate, Plymouth, UK, 4.5 ml, 9 NC 0.129 M) drawn was discarded to exclude coagulation activation due to vein puncture and blood withdrawal. Inter-device variability was tested using the same samples on a second ROTEM® device. Intra-assay variability was tested by performing duplicate measurements at the time of the first blood draw on the same device using different channels and calculating the coefficient of variation. At a second point of time, i.e. one week later, another 3 tubes (Vacutainer Brand, Belliver Industrial Estate, Plymouth, UK, 4.5 ml, 9 NC 0.129 M, a total of 18 ml per volunteer) of citrated blood were again withdrawn from the same 10 volunteers to assess the reproducibility of the two ROTEM® devices at the second point of time (week 2) as compared with the first week. The 10 volunteers were nurses or physicians from the Institute of Anaesthesiology, of the University Hospital of Zurich, and tests ran for 30 minutes.

After obtaining written informed consent by the patients, 8 tubes (Vacutainer Brand, Belliver Industrial Estate, Plymouth, UK, 4.5 ml, 9 NC 0.129 M) of citrated blood were withdrawn, a total of 36 ml of blood per patient. The first tube (Vacutainer Brand, Belliver Industrial Estate, Plymouth, UK, 4.5 ml, 9 NC 0.129 M) drawn was discarded to exclude coagulation activation due to vein puncture and blood withdrawal. In the 48 patients INTEM, EXTEM, FIBTEM were performed at T 1 = 0 min, T 2 = 30 min, T 3 = 60 min, T 4 = 90 min and T 5 = 120 min. The blood was stored at room temperature. Tests were performed at 37° C. Measurements for this test series ran for 60 minutes.

**Parameters of Rotation Thromboelastometry**

ROTEM® defines various parameters to describe the dynamics, the size and the firmness of clot during clot formation and lysis (Fig. 1). The clotting time (CT) is the period from the start of the analysis until the start of clot formation, normally until the 2 mm amplitude is reached.
The clot formation time (CFT) is defined as the period until an amplitude of 20 mm is reached. The angle alpha is given by the angle between the centre line and a tangent to the curve through the 2 mm amplitude point. The maximum amplitude of the curve is defined as the maximum clot firmness (MCF). The amplitude at different points of time is described by A5 till A30, whereby the number refers to the time since the start of the test. The clot lysis index at 30 and 60 min (CLI30, CLI60) describes the ratio between the maximum clot firmness and the amplitude 30 and 60 min after clotting time, and gives information about the fibrinolysis. The maximum lysis (ML) represents the maximum fibrinolysis detected during the measurement.

The parameters measured in this study were: Clotting time, clot formation time, maximum clot firmness, angle alpha, and amplitude after 5, 10, 15, 20, 25 and 30 minutes.

Test procedure

All ROTEM® devices used in this study were new and set up by a representative of the local distributor. The tests were performed using the automated pipette programs according to the instructions of the manufacturer. For each measurement a new pin was positioned on the axis of the measurement channel and a new cup was put into the special cup holder of the device. According to the pipetting program, 20 µl re-calcification reagent (200 mmol/l calcium chloride solution) and 20 µl of the respective activation reagent (FIBTEM: Cytochalasin D, EXTEM: Thromboplastin from rabbit brain, INTEM: Partial thromboplastin phospholipid made of rabbit brain (chloroform extract), ellagic acid) were added into the pre-warmed cup. Then 300 µl of citrated whole blood was added to the cup and, after a semi-automated mixing step, the cup holder was placed to the measuring position of the ROTEM® device. The measurement started automatically when blood was added to the cup and was stopped after 30
min or 60 min according to the protocol. (ex-TEM lot 41194401, in-TEM lot 41166301, fib-TEM lot 41147601, star-TEM lot 41166101)

**Statistical Analyses**

A trial database within Excel (Microsoft Office 2003, Microsoft Corporation Redmond, WA, US) was used to store study data (transferred from the ROTEM® devices). The statistical analyses were performed using SPSS® (version 13, SPSS Inc. Chicago, Illinois, USA). Continuous variables are summarized as mean ± SD and median with confidence interval (CI) where appropriate. ANOVA for repeated measures was used to analyze all parameters with post hoc comparison and Bonferroni correction. For age and gender regression analyses were used. P-values of 0.05 or less are considered significant.

**Results**

*Baseline information:*

The 48 patients had a mean age of 50±22 years, ranging from 17 to 87 years. For the 24 women the mean age was 50±22 years, ranging from 17 to 85 years. For the 24 men the mean age was 50±22 years, ranging from 17 to 87 years. The 10 volunteers were 5 men and 5 women, the overall mean age was of 37±14 years, ranging from 20 to 63 years.

*Pre-analytic aspects stability over time:* Over the time of 120 minutes there was no significant difference between any parameter in EXTEM, INTEM and FIBTEM, indicating that ROTEM® measurements are stable over two hours at room temperature. (Table 1)

*Reproducibility and precision of ROTEM® devices (intra and inter assay variability):* The reproducibility of results on 2 ROTEM® devices was tested with 10 volunteers. At two points
of time with one week in between tests were performed and an overall analysis was performed between the two points of time (week 1 vs. week 2) and the 2 ROTEM® devices.

The reproducibility of maximum clot firmness in the 10 volunteers at the two points of time in the 2 ROTEM® devices showed no significant difference between the two points of time (p>0.200) and no influence by the ROTEM® device (p>0.200) in the overall effect for EXTEM (Mean 63.0±5.5 mm, reference range 53 to 72 mm, mean difference -0.1 mm, 95% CI -0.8 to 0.7 mm, maximum difference 1.3 %).

For the maximum clot firmness of FIBTEM (Mean 11.9±3.7 mm, reference range 9 to 25 mm, mean difference 0.6 mm, 95% CI 0.2 to 1.0 mm, maximum difference 8.4 %) and INTEM (Mean 61.6±4.3 mm, reference range 53 to 72 mm, mean difference 1.7 mm, 95% CI 1.2 to 2.2 mm, maximum difference 3.6 %) a significant difference between devices (FIBTEM p=0.005, INTEM p<0.001) but not for the points of time (FIBTEM p>0.200, INTEM p=0.187) was found in the overall analysis (Table 2, Figure 2).

For EXTEM and FIBTEM no significant difference between devices or time points was found for clot formation time, clotting time and angle alpha (Table 2). The clot formation time (p=0.001, Mean 74.5±20.1 sec, reference range 35 to 110 sec, mean difference -7.1 sec, 95% CI -10.4 to -3.7 sec, maximum difference 14.0%) and angle alpha (p=0.013, Mean 75.2±3.7 °, reference range 70 to 83°, mean difference 1.2°, 95% CI 0.3 to 2.1°, maximum difference 2.8%) were significantly different between devices in INTEM, but no difference for the two points of time was found. For the two points of time a significant difference was only found for the clotting time (Mean week one 156.4±14.9 sec, Mean week two 162.2±15.7 sec, reference range 100 to 240 sec) in INTEM (p=0.046). For FIBTEM the only analysis being of importance is maximum clot firmness, all other values are not to be used in the interpretation of FIBTEM.
Reproducibility of results for maximum clot firmness was of 97% in EXTEM, 95% in INTEM and 94% in FIBTEM. The coefficient of variation was calculated in the first point of time (week 1) on the same device. Coefficient of variation for maximum clot firmness in EXTEM was < 3%, INTEM < 5% and FIBTEM < 6%. For clot formation time, the coefficient of variation was in EXTEM < 4% and in INTEM < 3%. Coefficient of variation for the angle alpha was for EXTEM < 3% and INTEM < 6%. Clotting time had a coefficient of variation for EXTEM and INTEM < 15%.

Early prediction of maximum clot firmness: As tests for the 48 patients ran over 60 minutes, we calculated for each point of time the percentage of the final maximum clot firmness, showing that in an overall analysis after 10 minutes of running time maximum clot firmness in EXTEM, INTEM and FIBTEM reached at least 98% of the final maximum clot firmness value.

Reference intervals: Reference intervals were calculated according to international guidelines by means and adding one standard deviation on each side. We also calculated reference intervals by gender. (Table 3) We were able to show that reference intervals calculated in this study were closer together then by the manufacturer. A gender specific influence is also to be seen.

Effect of age and gender: With advancing age maximum clot firmness increased significantly in all tests (p<0.001) by a mean of 0.1 mm per year of age over 20 years. Angle alpha also increased significantly for all tests with a mean of 0.07° per year of age over 20 years in EXTEM (p=0.009), 0.14° in FIBTEM (p=0.007) and 0.04° in INTEM (p=0.019). Clot formation time decreased significantly in EXTEM (p=0.008) and INTEM (p=0.014), with a mean of -0.4 sec per year of age over 20 years in EXTEM and -0.2 sec per year of age over 20 years in INTEM. The age had no influence on clotting time in all tests.
Maximum clot firmness (p<0.001 for EXTEM and INTEM) and angle alpha (p=0.009 in EXTEM, p=0.019 in INTEM) were significantly higher and clot formation time lower (p=0.008 in EXTEM, p=0.014 in INTEM) in EXTEM and INTEM in women than in men indicating a somewhat greater coagulability in women. Similar changes in FIBTEM (towards hypercoagulability) did not reach statistical significance. Clotting time was similar in all age groups and in both sexes. (Table 4)

Discussion

Our analysis of pre-analytic aspects showed that ROTEM® tests yield stable results from citrated blood that was re-calcified during the first 120 minutes. This is of high practical relevance since the exact delay from blood drawing to testing would be difficult to standardize, particularly in major trauma. This is in contrast to the thrombelastography where a previous report showed that parameters are unstable in the first 30 minutes after blood draw. [10]

Evaluation of aim 2 (reproducibility: inter assay same device) showed a good reproducibility of results on the same ROTEM® device as well as over a time period of 120 min. Statistically significant differences on different ROTEM® devices occurred for the maximum clot firmness of FIBTEM and INTEM as well as the clot formation time and alpha angle of INTEM as mentioned above. For the interpretation of INTEM these results are statistically significant but not of any clinical relevance because the values are largely within the reference values and would not implement a treatment. Reference intervals calculated for our study and for gender were found to be within a narrower interval then those provided by the manufacturer. According to the manufacturer the company’s reference values are for orientation purposes and should be validated individually, as they may vary from lab to lab, depending on blood sampling technology and other pre-analytical factors.
Evaluation of aim 3 (precision: inter device) demonstrated reproducibility of ROTEM® measures over time and when the blood sample is analyzed on the same ROTEM® device sequentially. In addition, citrated blood samples analyzed after 0 to 120 minutes yield similar results in all ROTEM® tests assessed. However, when a blood sample is analyzed on different ROTEM® devices simultaneously, statistically significant differences (p<0.050) were detected in the overall comparison for the maximum clot firmness in FIBTEM (maximum difference 8.4 %) and in INTEM (maximum difference 3.6 %) as well as for clot formation time (maximum difference 14%) and angle alpha (maximum difference 2.8%) in INTEM. In clinical use, when using algorithms a difference in maximum clot firmness of 1-2 mm in FIBTEM may result in a premature or delayed treatment of the patient. Therefore, when working with exact algorithms, the blood of one individual patient should be measured on one single ROTEM® device.

Compared with the results of Lang et al. [13] we have found a somewhat lower variability for the maximum clot firmness in the FIBTEM test (coefficient of variability for maximum clot firmness < 6%). This may be due to the fact that in our study only three persons performed all ROTEM® tests, which is impossible when pooling data from different centres. Performing ROTEM® tests by persons being really familiar the ROTEM® technology thus appears to reduce the variability.

A progressive change of the ROTEM® parameters towards hypercoagulability with advancing age as observed in this study has also been described by Ng et al. [14] for thrombelastography (TEG® Haemoscope Corporation, Skokie, IL, USA), and is in keeping with previous reports describing hypercoagulability in elderly people [14,15] Since the ROTEM® parameters measured in the current study were still within the reference ranges provided by the manufacturer an age specific adaptation of the reference ranges may not be
required for ROTEM®. Nevertheless the trend of increased clot firmness at advanced age should be kept in mind in the clinical interpretation of ROTEM® results.

We found differences between men and women in ROTEM® parameters which suggest a faster development of the clot and greater clot strength in women as compared with men (table 4). Unfortunately, no measures of fibrinogen according to Clauss were performed in this study. Nevertheless, this may represent a factor predisposing women to thrombotic complications. [16]

A limitation of this study is that we did not take into account the effective temperature in patients since it is known to impair results and interpretation of ROTEM® [17]. We think that this point is of limited importance since the ROTEM® devices work at a temperature of 37 °C and the 300 µL of blood are at this temperature and patient were not hypothermic at the moment when blood was drawn.

ROTEM® results cannot give exact recommendations on the amount of blood products or coagulation factors to be administered. However, ROTEM® can guide the clinician which type of treatment may be most helpful to treat coagulopathy during surgery or in trauma. In 2007 Rugeri et al. showed in trauma patients that ROTEM® can rapidly detect trauma related coagulopathy and might be helpful to guide treatment [18]. Theusinger et al. [19] published a ROTEM® based transfusion algorithm to guide individual goal directed transfusions. A retrospective analysis by Anderson et al. [20] in 990 patients showed that the use of ROTEM® significantly decreases the use of red blood cells and other blood products after cardiac surgery. This is particularly important given the fact that red blood cell transfusion in cardiac surgery is consistently associated with significant morbidity an increased mortality. [21-24] Last but not least, Spalding et al. have demonstrated that a ROTEM® based coagulation algorithm decreased total transfusion costs in cardiac surgery [25].
Conclusion

ROTEM® measurements of EXTEM, INTEM and FIBTEM are reproducible and stable over time regardless of delay form blood draw to analysis (range 0 to 120 minutes after blood withdrawal). There is a high reproducibility with the coefficient of variation < 6% in all assays. Coefficient of variation for maximum clot firmness in EXTEM was < 3%, INTEM < 5% and FIBTEM <6% are small but statistically significant. With age there is a tendency of hypercoagulability and gender (sex) women seem to coagulate better then men but within range. To avoid problems in treating coagulopathies when working with an algorithm or to interpret the evolution of ROTEM® parameters over time ROTEM® tests should preferably be performed on the same ROTEM® device since in our study one ROTEM® device yielded slightly but statistically significantly different results for maximum clot firmness and clot formation time.
Tables:

Table 1. Sample stability over the time, mean values and standard deviation; MCF=maximum clot firmness, CFT=clot formation time, CT=clotting time
Table 2.

<table>
<thead>
<tr>
<th>Test</th>
<th>Week</th>
<th>Device</th>
<th>N</th>
<th>MCF in mm Mean ± SD</th>
<th>CFT in sec Mean ± SD</th>
<th>CT in sec Mean ± SD</th>
<th>Angle Alpha Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXTEM</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>63.3 ± 5.5</td>
<td>104.5 ± 29.1</td>
<td>56.7 ±13.0</td>
<td>69.4 ± 5.4</td>
</tr>
<tr>
<td>EXTEM</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>63.1 ± 5.8</td>
<td>98.3 ± 31.1</td>
<td>61.0 ±11.5</td>
<td>70.5 ± 5.8</td>
</tr>
<tr>
<td>EXTEM</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>62.7 ± 5.5</td>
<td>99.3 ± 24.6</td>
<td>59.0 ± 6.5</td>
<td>70.3 ± 4.7</td>
</tr>
<tr>
<td>EXTEM</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>62.8 ± 5.3</td>
<td>96.8 ± 25.1</td>
<td>58.9 ± 4.5</td>
<td>70.6 ± 4.9</td>
</tr>
<tr>
<td>FIBTEM</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>11.4 ± 4.0*</td>
<td>n.a.</td>
<td>57.9 ± 10.4</td>
<td>n.a.</td>
</tr>
<tr>
<td>FIBTEM</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>12.2 ± 4.2*</td>
<td>n.a.</td>
<td>57.3 ± 7.3</td>
<td>n.a.</td>
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<tr>
<td>FIBTEM</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>11.7 ± 3.2*</td>
<td>n.a.</td>
<td>54.6 ± 7.2</td>
<td>n.a.</td>
</tr>
<tr>
<td>FIBTEM</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>12.1 ± 3.2*</td>
<td>n.a.</td>
<td>54.1 ± 5.2</td>
<td>n.a.</td>
</tr>
<tr>
<td>INTEM</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>60.9 ± 4.8*</td>
<td>77.2 ± 22.5*</td>
<td>155.7 ± 17.7**</td>
<td>74.8 ± 4.0*</td>
</tr>
<tr>
<td>INTEM</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>62.9 ± 4.3*</td>
<td>68.8 ± 21.1*</td>
<td>157.0± 12.0**</td>
<td>76.1 ± 4.0*</td>
</tr>
<tr>
<td>INTEM</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>60.5 ± 4.6*</td>
<td>78.8± 18.6*</td>
<td>164.1 ± 12.6**</td>
<td>74.4 ± 3.4*</td>
</tr>
<tr>
<td>INTEM</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>61.9 ± 4.0*</td>
<td>73.1 ± 18.0*</td>
<td>160.3 ± 18.7**</td>
<td>75.5 ± 3.4*</td>
</tr>
</tbody>
</table>

Table 2. MCF=maximum clot firmness, CFT=clot formation time, CT=clotting time and angle alpha changes between 2 ROTEM® devices and weeks and 2, Mean values and standard deviations, *p<0.050 for ROTEM® devices, **p<0.050 for the weeks
Table 3. Reference values by the manufacture, reference values calculated in this study and calculated by gender in this study; MCF=maximum clot firmness, CFT=clot formation time, CT=clotting time

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Overall</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXTEM MCF mm</td>
<td>50-70</td>
<td>61-72</td>
<td>59-72</td>
</tr>
<tr>
<td>INTEM MCF mm</td>
<td>50-72</td>
<td>59-68</td>
<td>56-68</td>
</tr>
<tr>
<td>FIBTEM MCF mm</td>
<td>9-25</td>
<td>10-22</td>
<td>8-22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Overall</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXTEM CFT sec</td>
<td>34-159</td>
<td>61-97</td>
<td>62-108</td>
</tr>
<tr>
<td>INTEM CFT sec</td>
<td>30-110</td>
<td>49-77</td>
<td>51-86</td>
</tr>
<tr>
<td>FIBTEM CFT sec</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Overall</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXTEM CT sec</td>
<td>38-79</td>
<td>48-63</td>
<td>50-65</td>
</tr>
<tr>
<td>INTEM CT sec</td>
<td>100-240</td>
<td>133-177</td>
<td>131-182</td>
</tr>
<tr>
<td>FIBTEM CT sec</td>
<td>43-75</td>
<td>48-58</td>
<td>51-59</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Overall</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXTEM angle alpha °</td>
<td>63-83</td>
<td>70-78</td>
<td>68-78</td>
</tr>
<tr>
<td>INTEM angle alpha °</td>
<td>70-83</td>
<td>75-80</td>
<td>73-80</td>
</tr>
<tr>
<td>FIBTEM angle alpha °</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
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</table>
Table 4.

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>CT (sec)</th>
<th>CFT (sec)</th>
<th>Alpha Angle (°)</th>
<th>MCF (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INTEM</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>females (n=24)</td>
<td>50.8 ± 21.9</td>
<td>152.5 ± 18.1</td>
<td>57.7 ± 10.5*</td>
<td>78.4 ± 2.1*</td>
<td>64.8 ± 3.8*</td>
</tr>
<tr>
<td>males(n=24)</td>
<td>52.0 ± 22.1</td>
<td>156.8 ± 25.5</td>
<td>68.1 ± 17.5*</td>
<td>76.5 ± 3.2*</td>
<td>62.2 ± 6.1*</td>
</tr>
<tr>
<td><strong>EXTEM</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>females (n=24)</td>
<td>50.8 ± 21.9</td>
<td>53.9 ± 7.8</td>
<td>72.6 ± 13.3*</td>
<td>75.3 ± 2.7*</td>
<td>68.1 ± 4.2*</td>
</tr>
<tr>
<td>males(n=24)</td>
<td>52.0 ± 22.1</td>
<td>57.7 ± 7.3</td>
<td>85.2 ± 23.2*</td>
<td>72.9 ± 4.6*</td>
<td>65.3 ± 6.4*</td>
</tr>
<tr>
<td><strong>FIBTEM</strong></td>
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<td></td>
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<tr>
<td>females (n=24)</td>
<td>50.8 ± 21.9</td>
<td>51.1 ± 5.0</td>
<td>n.d.</td>
<td>71.0 ± 5.6</td>
<td>17.3 ± 4.1</td>
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<tr>
<td>males(n=24)</td>
<td>52.0 ± 22.1</td>
<td>55.0 ± 4.2</td>
<td>n.d.</td>
<td>70.4 ± 6.5</td>
<td>15.0 ± 7.2</td>
</tr>
</tbody>
</table>

Table 4. Sex related differences in the main parameters of ROTEM®, Mean values and standard deviations, *p<0.050 comparison male vs. female; MCF=maximum clot firmness, CFT=clot formation time, CT=clotting time
**Figure legends:**

**Figure 1.** (modified, with authorization of Pentapharm GmbH, Munich, Germany). CT (clotting time): time from the start of measurement until initiation of clotting – initiation of clotting, thrombin formation, start of clot polymerization. CFT (clot formation time): time from initiation of clotting until a clot firmness of 20 mm is detected – fibrinpolymerisation, stabilization of the clot with thrombocytes and F XIII. MCF (maximum clot firmness): firmness of the clot – increased stability of the clot by the polymerized fibrin, thrombocytes as well as F XIII. Lysis: reduction of the clot firmness after MCF in relation to MCF – stability of the clot. A maximum lysis (ML) < 15 % is considered normal), a ML > 15 % within 1h is indicative of exaggerated fibrinolysis (hyperfibrinolysis).

**Figure 2.** Boxplot of MCF=maximum clot firmness for all tests, representing median by device 1 and 2 and point of time 1 and 2, * p<0.050 for differences between ROTEM® devices


\[ \alpha \text{- angle (°)} \]

**A5** = Clot Firmness (mm)  
5 minutes after CT

**AX** = Clot Firmness (mm)  
x minutes after CT

**CT** = Clotting Time (sec)

**MCF** = Maximum Clot Firmness (mm)

**CFT** = Clot Formation Time (sec)

**Lysis (%)**