MONITORING UNFRACTIONATED HEPARIN IN CHILDREN - A PARALLEL-COHORT RANDOMIZED CONTROLLED TRIAL COMPARING TWO DOSE PROTOCOLS

Short title: HEARTCAT Study

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Key points:

- Anti-Xa, aPTT and ACT discriminate well between different heparin dose protocols but the assays are poorly correlated with each other.
- The heparin effect was lower in younger children. This influence of age was dose-dependent and more pronounced at low vs. high dose heparin.
Abstract

Monitoring unfractionated heparin (UFH) is crucial to prevent over- or under-anticoagulation. However, the optimal parameters for monitoring UFH in children are not well established. The study objectives were to investigate i) the relationship between UFH dose and its anticoagulant effect as assessed by anti-Xa, APTT and ACT, ii) other factors influencing UFH effect, iii) the agreement between the assays, and iv) the association between UFH effect and clinical outcome. HEARTCAT was a parallel-cohort randomized controlled trial comparing high-dose (100 units/kg bolus followed by age-based continuous infusion in randomized children) versus low-dose UFH (50 units/kg bolus) during cardiac catheterization in children. Blood samples were drawn before and after UFH administration at 30, 60 and 90 minutes.

Four-hundred-two samples of 149 patients were evaluable. Anti-Xa, APTT and ACT all showed good discrimination between UFH doses. Regression models demonstrated the following determinants of UFH effect: UFH dose, age, baseline antithrombin (for anti-Xa), baseline levels of APTT and ACT, respectively. UFH effects were lower in infants compared to older children, which was more pronounced at low-dose than at high-dose UFH. Agreement between the three assays was poor. Most APTT values were above therapeutic range or beyond measuring limit, thus of limited value for UFH monitoring. No association of UFH dose or effect with clinical outcome could be observed.

In conclusion, all assays reflected a significant UFH dose-effect relationship, however with poor agreement between the respective tests. The age-dependency of UFH effect was confirmed. Notably, the influence of age on UFH effect was dose-dependent.
INTRODUCTION

Unfractionated heparin (UFH) is the most commonly used anticoagulant administered for primary prophylaxis of thrombotic events (TE) in children. Up to 15% of inpatients at tertiary care pediatric centers are regularly exposed to UFH. The pharmacodynamic and pharmacokinetic properties of UFH are complex, leading to significant inter-individual variation of the anticoagulant response to the same weight-adapted UFH dose. This variation is especially profound in infants and children, whose haemostatic system is still developing. Therefore, laboratory monitoring of the UFH effect is crucial to prevent both under- and over-anticoagulation. For children receiving therapeutic UFH, an aPTT range correlating to an anti-Xa level of 0.35 to 0.7 units/ml is recommended. However, these recommendations are extrapolated from studies in adults, and their appropriateness for children is increasingly questioned.

Several pediatric studies demonstrated poor correlation between UFH dose and its anticoagulant effect as assessed by various laboratory methods and poor agreement between the respective tests. However, only three studies prospectively investigated the effect of a defined intravenous single bolus of UFH in children, and none of them compared different UFH dosage protocols or evaluated the clinical outcome regarding thrombotic or bleeding events. As – given its short half life and reversibility - UFH will continue to be a preferred anticoagulant for children, systematic studies on monitoring UFH in children are needed.

HEARTCAT (Heparin Anticoagulation Randomized Trial in Cardiac Catheterization) was a parallel-cohort randomized controlled trial comparing two UFH dose protocols for primary prevention of TE in children undergoing cardiac catheterization. The present manuscript reports the results of the laboratory substudy investigating the monitoring of UFH in children. The specific study objectives were i) to investigate the relationship between UFH dose and UFH anticoagulant effect as assessed by various assays, ii) to assess other factors influencing the UFH effect, iii) to determine the agreement between these assays, and iv) to describe the association between UFH effect and clinical outcome.
METHODS

Study design
The study design was a single-centre, double-blinded parallel-cohort randomized (RCT) of consecutive children undergoing cardiac catheterization comparing a high-dose (100 units/kg body weight bolus followed by continuous infusion of 20 units/kg/h for children older than one year or 28 units/kg/h for infants) versus a low-dose (50 units/kg bolus) UFH protocol. Patients with no consent for randomization received UFH as per standard-of-care and were followed in a parallel cohort (50 units/kg for venous diagnostic catheterization, 100 units/kg for arterial diagnostic any interventional catheterization). Patients in the cohort study consented to clinical outcome assessment and laboratory testing. All patients were treated with UFH (“Immuno”, 1000 international units/ml, EBEWE Pharma, Austria) intravenously during cardiac catheterization. The study protocol was approved by the ethics committee of the Medical University of Vienna and registered as a clinical trial in EudraCT, registration number 2005-004150-27 (https://clinicaltrialsregister.eu). The study design has previously been described in detail.

Study population
The study population consisted of patients, 0 to 18 years of age, requiring diagnostic or interventional cardiac catheterization at the Division of Paediatric Cardiology, Medical University of Vienna. Written informed consent was obtained from parents and patients of appropriate age, in accordance with the Declaration of Helsinki. Exclusion criteria were pre-existing anticoagulation or antiplatelet therapy.

Study outcomes
To monitor UFH during cardiac catheterization, blood samples were taken to measure anti-factor-Xa (anti-Xa), activated partial thromboplastin time (aPTT), activated clotting time (ACT), anti-factor-IIa (anti-IIa), protamine titration, and antithrombin levels. The results of anti-IIa, protamine titration and thrombin generation will be reported in a separate manuscript.
Clinical efficacy outcome was a thromboembolic event at puncture site diagnosed by vascular ultrasonography, safety outcome was bleeding at the puncture site or other locations as previously described in detail.²⁶

**Blood sample acquisition**

Blood samples were drawn directly from cardiac catheters or vascular sheaths. To avoid UFH contamination, UFH was strictly administered via an additional peripheral venous line. A baseline sample was obtained immediately after the insertion of the femoral venous or arterial sheath and before the administration of UFH. Thereafter, blood samples were taken during the procedure at predefined time points (30 minutes, 60 minutes and 90 minutes after UFH administration, and at the end of the procedure. The ACT was measured immediately, the rest of the blood samples were collected in tubes containing 3.8% sodium citrate in a proportion of 9:1. Platelet poor plasma was prepared by centrifugation at 1300g for 10 minutes, aliquoted and frozen at -80°C for batch analysis.

**Anti-Factor-Xa assay**

The anti-Xa assay was performed on the STA Compact Coagulation Analyzer (Diagnostica STAGO) according to manufacturer instructions and using the commercially available test kit STA Rotachrom Heparin, Diagnostica STAGO, which does not contain antithrombin supplementation. The therapeutic range used for anti-Xa was 0.35 to 0.7 units/ml.

**Activated partial thromboplastin time**

APTT values were measured using the commercially available test kit STA APTT, Diagnostica Stago (STA Compact Coagulation Analyzer, Diagnostica STAGO) according to manufacturer instructions. For the purpose of this study, the upper limit of time measurement was set at 300 seconds, and un-recordable high values were set at 301 seconds. The therapeutic range used for the APTT was 60-85 seconds.

**Activated clotting time**

The ACT was measured using the ACTester™ (QUEST Medical Inc., Allentown Parkway, Allen, Texas 75002, USA). Immediately after acquiring each blood sample,
0.6ml whole blood were added into a cartridge containing a coagulation activator (6 – 20 mg bruised diatomaceous earth) and swung 20 times. Then, the cartridge was put into the ACTester™ and the time measurement was started. Once a clot was detected by photometric methods, the time measurement stopped and the ACT was shown on the screen of the ACTester™. For the purpose of this study, un-recordable high results were set at 500 seconds. The therapeutic range used for the ACT was 150-250 seconds, representing the therapeutic range used for children on ECMO at our institution.

**Statistical analysis**

Statistical analysis was performed using SPSS 22.0 for Windows (Chicago, Ill). Continuous variables are presented as median, minimum and maximum values, categorical variables as absolute frequencies and percentages. Figures display medians with 95% confidence intervals. Linear mixed regression modelling was performed to assess the influence of UFH dose, body weight, age, sex and baseline antithrombin level on the UFH anticoagulant response assessed by anti-Xa, aPTT and ACT, respectively. Various models with UFH dose or age either as continuous variable or as categorical variable (low dose versus high dose group; infants versus children) were calculated and the best fitting model according to AIC (Akaike information criterion) values was chosen as the final model which is presented in the results section. No significant differences in UFH levels over time could be observed between the high-dose group of the RCT (receiving a UFH bolus followed by continuous infusion) and the high dose group of the cohort study (high dose UFH bolus only). Therefore, the results of the RCT and the cohort study were combined. To quantify the agreement between the results of different UFH monitoring assays, Cohen’s Kappa were calculated. A Kappa value of <0.4 was considered a poor agreement. To test whether UFH effects were associated with clinical outcomes, logistic regression modelling was performed with TE or bleeding complications as dependent variables and anti-Xa, aPTT, and ACT results (30 min post-UFH samples), respectively, as independent variables, without and with inclusion of co-variables such as dose and age.
RESULTS

Study population
The flow of participants in the HEARTCAT study has previously been described in detail in the publication reporting clinical outcomes. Of 227 children enrolled in the overall study, 163 had blood samples taken for UFH monitoring. The blood samples of 14 patients could not be evaluated for the study for several, mostly technical, reasons (e.g. clotted sample, no heparin administered). Therefore, the final cohort of the laboratory study consisted of 149 patients (402 blood samples including baseline samples prior to UFH administration).

Patient demographics
Median age was 5.5 years (minimum 0.01; maximum 18) and median body weight was 17 kg (3.1; 90.5). There were 29 (19%) infants, 50 (34%) children 1-5 years, 30 (20%) 6-10 years, and 40 (27%) 11-18 years of age. Eighty-five (57%) patients were female. Overall, 78 (52%) patients were in the low-dose and 71 (48%) in the high-dose UFH group; 94 (63%) patients were enrolled in the RCT (47 (50%) low-dose, 47 (50%) high-dose UFH), and 55 (37%) patients in the parallel cohort receiving UFH as per standard-of-care (31 (56%) low-dose, 24 (44%) high-dose UFH).

Timing of blood samples
Samples obtained during cardiac catheterization were taken at the predefined time points of 30, 60, 90 minutes after UFH administration. However, the final samples at completion of catheterization were taken at various times as the duration of the procedure varied considerably, sometimes ending even before 30 minutes. As a result, the actual timing of sampling was somewhat heterogeneous. For the purpose of analysis, samples obtained within defined time bands were assigned to the predefined time points (20-44=30min, n=149; 45-74=60min, n=42; 75-104=90min, n=41). Because only few (n=21) samples were collected beyond 104 minutes after UFH administration, these were not used for the analysis of UFH time course and age effect, but were evaluated for the comparison of UFH assays.

Time course of UFH effect
Figure 1 shows the time course of median anti-Xa, aPTT and ACT values after administration of UFH. All parameters showed peak levels at 30 minutes and then decreased quite rapidly, except for aPTT values in the high dose group that remained above measurement limit. For all parameters, there was a clear discrimination between high and low dose over all times points. Anti-Xa levels in the high-dose group were above therapeutic range until 60 minutes after UFH administration, while in the low-dose group anti-Xa levels were within therapeutic range only at 30 minutes and dropped below the lower limit at 60 minutes. APTT values were above therapeutic range at all time points in both UFH dose groups. ACT values in the high-dose group were within therapeutic range at all time points, while in the low-dose group they were below therapeutic range at all times.

Factors influencing UFH effect

Table 1 shows the results of the linear mixed models analysing the influence of various parameters on anti-Xa, aPTT, or ACT levels over time following UFH administration. The best fitting models are presented including only significant determinants (p<0.05).

Anti-Xa levels were significantly associated with UFH dose group, age, baseline antithrombin level, sample time point, and there was interaction between dose group and sample time point. For example, anti-Xa levels increased by an average of 0.02 unit/ml for every year of age, and by 0.06 unit/ml per 0.1 unit/ml higher baseline antithrombin levels. In the overall study cohort, median antithrombin at baseline was 0.9 units/ml (minimum 52; maximum 116), and 24% of patients had antithrombin levels below respective age-specific reference values. Anti-Xa levels decreased over time after UFH administration and decreased more rapidly in the high-dose group (figure 1).

APTT levels showed a significant interaction between UFH-dose group and sample time point since (table 1), an artificial phenomenon since levels in the high-dose dose group remained beyond measurement limits while the low-dose group declined steeply over time (figure 1). In addition, post-UFH APTT levels were positively associated with age, female sex, and baseline aPTT level.

ACT levels significantly increased with UFH dose group, baseline ACT level, and decreased over sample time points, with an interaction between dose group and sample
time point, i.e. a more rapid decrease in the high-dose group (figure 1). Age was not significantly associated with the UFH effect on ACT levels.

**Influence of age on UFH effect**

Figure 2 shows median anti-Xa, aPTT and ACT values after UFH administration by age groups, separate for dose groups but independent of time point (30, 60 and 90 minutes). All parameters were lowest in infants and, in most instances, steadily increased over age groups 1-5 years, 6-10 years, and 11-18 years. Exceptions were aPTT levels in the high UFH dose group that were all beyond measurement limits, and ACT values that were not increasing with age in the high-dose group and only slightly increased over age groups in the low-dose group.

In the overall regression models including all time points, there was no significant interaction of age and dose, i.e. the differences between age groups was not dependent on dose. However, when evaluating anti-Xa levels at peak (30 minutes after UFH administration), infants had significantly lower levels compared to older children in the low-dose group but not in the high dose group (figure 3; interaction dose group * age group, p=0.001). Thus, while infants had a significantly decreased response to low-dose UFH compared to older children, this influence of age on the UFH effect diminished at high-dose UFH. Similar trends were seen for the APTT and ACT but were not statistically significant.

**Agreement between assays**

Figure 4 shows the results of anti-Xa, aPTT, and ACT plotted against each other for all samples after UFH administration. Shaded areas represent the respective target therapeutic ranges as defined. Table 2 shows agreement between the respective assays, the shading indicating concordant cells for results below, within, and above therapeutic ranges. APTT results showed very poor agreement with both anti-Xa and ACT results. There was zero concordance of results within therapeutic range. Values within the therapeutic range for anti-Xa and ACT were all above therapeutic range for APTT and mostly beyond measuring limit (median 301 seconds (minimum 96; maximum 301). For samples with anti-Xa values below therapeutic range, APTT results ranged from 38-300 seconds. Agreement was better between anti-Xa and ACT with more concordant results below, between and above therapeutic ranges, respectively (in total
57%). However, there were relevant proportions of values within therapeutic range for anti-Xa but below for ACT (13%) and above therapeutic range for anti-Xa but within for ACT (23%). Cohens Kappa as measure of agreement between assays was K = 0.37 for ACT versus anti-Xa, K = 0.21 for aPTT versus anti-Xa, and K = 0.04 for aPTT versus ACT).

**Association of UFH effect with clinical outcome**

The previous HEARTCAT manuscript reported that there was no significant relationship between UFH dose group and the incidence of TE and bleeding complications during and after cardiac catheterization. Infants had an increased incidence of TE and bleeding independent of dose. To test whether the UFH effects actually achieved in plasma were associated with clinical outcomes, logistic regression was performed, without and with inclusion of the co-variables UFH dose group, age, antithrombin at baseline and sex. The results of logistic regression analysis revealed no association of UFH effect as measured by any of the assays with TE or bleeding complications.

**DISCUSSION**

HEARTCAT was a large randomized controlled trial comparing high-dose versus low-dose UFH during cardiac catheterization in children that evaluated UFH effect in patients’ plasma monitored by various laboratory assays and determined clinical outcomes using objective assessment. Thus, HEARTCAT was well designed for the objectives to investigate i) the relationship between UFH dose and UFH effect, ii) to identify other determinants of UFH effect, iii) to compare various laboratory assays to monitor UFH, and iv) to determine whether UFH dose or effect are associated with clinical outcome. Thereby, the study aimed to critically assess the optimal UFH dose to prevent TE or bleeding during cardiac catheterization.

Previous studies on UFH monitoring in children had several limitations. Some studies were performed only *in vitro* using plasma from children, others included only small numbers of patients, focused exclusively on older children, or on critically ill children during intensive care who received different UFH doses for various indications. Only three studies prospectively investigated the anticoagulant
response to a defined intravenous single UFH bolus in children, but none compared
different UFH doses and none evaluated clinical outcome regarding thrombotic or
bleeding complications.21-23,25

HEARTCAT investigated primary UFH prophylaxis in clinically stable children who
mostly had elective catheterization. Another design feature was the clear separation of
UFH administration via a peripheral vein and UFH sampling via large bore cardiac
catheters. Thus, HEARTCAT represents a homogeneous patient population studied with
well controlled methodology. The results can be taken as model situation, however, one
needs to keep in mind that considerations for dose and therapeutic range cannot fully be
extrapolated to other clinical situations. A limitation of the current study is that most
patients received a single UFH bolus and only a proportion of patients received a
continuous UFH infusion (RCT high dose). As there was no difference in UFH levels
between those with and without continuous infusion, likely the UFH effect over the first
90 minutes was primarily affected by the UFH bolus. Therefore, the data do not reflect
steady state heparinization.

In HEARTCAT, anti-Xa, aPTT, and ACT discriminated well between the high-dose and
low-dose UFH protocol studied. The clear dose-response relationship observed in the
current study differs from the results of a previous study by Kuhle et al. who reported a
poor correlation between UFH dose administered and the anticoagulant response as
assessed by anti-Xa and APTT.17 At least three facts may explain these differences:
First, in this randomized comparison there was a more clear-cut separation of doses and
probably less analytical variability. Second, Kuhle et al. investigated the UFH response
in critically ill children requiring intensive care. In clinically unstable patients there may be
more factors influencing the UFH effect, e.g. variable plasma and cell binding of UFH,
variable antithrombin levels, and others. Third, the UFH dose administered was lower
(20-40 units/kg) than both the low and the high dose in the current study.

Patients’ age had a significant influence on the UFH effect as measured by anti-Xa and
aPTT. Infants demonstrated reduced UFH effect compared to older children. Age-
dependency of UFH effect was most evident when measured by the anti-Xa followed by
the aPTT, in spite of its high variability and the artificial ceiling effect. The ACT also
showed a trend to increase with age. These findings are consistent with previous reports
on the age-dependency of the UFH effect in children. An *in vitro* study by Ignjatovic et al. showed that aPTT values corresponding to a therapeutic anti-Xa level (0.35–0.7 units/ml) were significantly higher in plasma from infants and young children than in older children. They also investigated the *in vivo* effect of UFH administered for various indications on a paediatric intensive care unit and found that younger children had lower anti-Xa and compared to older children. Newall et al. investigated the UFH effect in children undergoing cardiac catheterization and found a trend towards increasing anti-Xa levels with age, suggesting that older children were more sensitive to UFH. HEARTCAT confirms the age-dependency of the UFH effect in a large cohort and adds important information regarding UFH dose. Interestingly, the difference in UFH effect between age groups was more pronounced at low UFH dose and almost inexistent at high-dose UFH. This age-dose interaction was significant only for anti-Xa and when comparing infants versus all older age groups but there were similar trends for the other UFH assays and across increasing age groups. Thus, the influence of age on UFH effect appears to be dose-dependent.

Other determinants of UFH effect were baseline antithrombin levels for anti-Xa and baseline levels of APTT and ACT for their respective post-UFH levels. The anti-Xa assay employed in this study was not supplemented with excess antithrombin, hence patients’ endogenous antithrombin level did affect the UFH effect. The results support the view that non-antithrombin-supplemented anti-Xa assays better reflect the physiological dependence of UFH on in-vivo antithrombin levels. Antithrombin was an independent determinant of anti-Xa in addition to the influence of age in multiple regression analysis. Thus, lower antithrombin levels in infants as well as pathologically decreased antithrombin levels influenced the UFH effect. On the other hand, the decreased response to UFH at young age apparently is not solely mediated through lower antithrombin levels.

There was poor agreement between the three assays investigated. For samples within therapeutic anti-Xa range, APTT values were all high and mostly beyond measuring limit but still showed large variability. These results are similar to previous studies who also found highly increased and partially unrecordable APTT values at therapeutic anti-Xa range. However, in contrast to Newall et al. who found 28% of samples within anti-Xa and aPTT therapeutic range the present study observed no concordance within
therapeutic range. There was better agreement between anti-Xa and ACT assays but there were relevant proportions of discordant values. The therapeutic range applied for the ACT is used for children on extracorporal circulation, as there is no target range specific for cardiac catheterization reported in the literature.

The best discrimination between doses was achieved by the anti-Xa. Because of its test principle, the anti-Xa is most specific for the effect of UFH and is less sensitive to other variables in the coagulation system. Moreover, the anti-Xa test seems to have the least analytical variability. Both ACT and APTT are influenced by other variables in the coagulation system, thereby may be better markers of the overall coagulability of blood. Interestingly, however, the anti-Xa is influenced by physiological factors as it showed the most distinct age-dependent differences of UFH effect. In summary, the study results favour anti-Xa over APTT for UFH monitoring in children. We conclude that the APTT used was simply too sensitive for monitoring UFH in children in the setting of cardiac catheterization. Moreover, it is well known that the APTT is affected by numerous pre-analytic and analytic variables that cause its unspecific variability. The ACT proved a comparably reliable assay for UFH monitoring and is a good alternative where a bedside test is more appropriate.

Frequencies of TE and bleeding were low and not associated with UFH dose or effect. Therefore, the study did not allow validating the optimal dose and appropriate therapeutic range based on clinical outcomes. One may conclude that neither the high nor low UFH dose was associated with a relevant risk of over- or under-anticoagulation in cardiac catheterization. Interestingly, the incidence of both thrombosis and bleeding was increased in infants compared to older children but this was also not dependent on UFH dose or UFH effect. Thus, the lower UFH effects observed in infants did not translate into different risks of clinical outcomes. Rather, the higher frequency of complications in infants may be attributed to anatomical differences and the technical challenges of catheterization of smaller vessels. In a study of infants less than 6 months of age who were treated for DVT with continuous UFH targeting therapeutic anti-Xa levels, many infants remained subtherapeutic in spite of increased UFH doses. The majority of infants had thrombus regression and no recurrence but there was 11% risk of major bleeding. Based on these data, infants may not require increased UFH doses in spite of decreased UFH response based on standard laboratory tests.
Clinical outcomes were not informative to establish the optimal UFH dose for children undergoing cardiac catheterization. Besides, there is no established anticoagulant target level for the prevention of cardiac catheterization-associated thrombotic complications in children. As a proxy, one may speculate based on UFH effects achieved in plasma and using the anti-Xa and its therapeutic range of 0.35-0.7 units/ml, as used for treatment of TE. The 100 units/kg UFH bolus achieved mostly supratherapeutic anti-Xa levels while 50 units/kg resulted in marginal/subtherapeutic levels. For infants, the UFH effect of 50 units/kg was much too low but 100 units/kg still induced supra-therapeutic anti-Xa levels (figure 4). Thus, a 75 units/kg bolus dose appears equally appropriate for children and infants during cardiac catheterization, as age-differences of UFH effect were small at high dose.

In conclusion, HEARTCAT demonstrated that all three assays discriminated well between high-dose and low-dose UFH, however with poor agreement between assays. Infants showed lower UFH effects in plasma compared to older children. This age-dependency of UFH effect was more pronounced at low UFH dose than at high dose. The APTT showed limited value for UFH monitoring. There was no association of UFH dose or UFH effect achieved in plasma with clinical outcome. Concluding from the UFH effects observed, an UFH bolus dose of 75 units/kg appears most appropriate for the setting of cardiac catheterization, both for older children and infants.
AUTHORSHIP CONTRIBUTION

A. Hanslik designed the study, analysed data and wrote the manuscript. E. Kitzmüller and K. Thom collected data and contributed substantially in writing the manuscript. H. Karapetian, N. Prutsch and J. Voitl collected blood samples and data and performed laboratory analyses. U. Tran performed statistical analyses and critically revised the manuscript. I. Michel-Behnke provided valuable support for performing the study and critically revised the manuscript. F. Newall contributed to data analysis and critically revised the manuscript. C. Male designed the study, participated in data analysis and contributed substantially in writing the manuscript.

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DISCLOSURE OF CONFLICTS OF INTEREST

All authors declare no competing financial interests. The study was supported by an institutional research fund. There was no relationship with industry. There are no conflicts of interest to be declared.
REFERENCES


Table 1. Linear mixed regression models of factors influencing the effect of UFH (anti-Xa, aPTT, ACT).

b, unstandardized partial correlation coefficient; CI, confidence interval.

<table>
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<tr>
<th>UFH assay</th>
<th>Determinant</th>
<th>b</th>
<th>95% CI</th>
<th>p</th>
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<tbody>
<tr>
<td>Anti-Xa</td>
<td>UFH dose group (high versus low)</td>
<td>0.66</td>
<td>0.42; 0.89</td>
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<td>(units/ml)</td>
<td>Age (per year)</td>
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<td>Baseline antithrombin (per 0.1 units/ml)</td>
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<td>Sample time point (30, 60, 90 minutes)</td>
<td>-0.16</td>
<td>-0.21; -0.10</td>
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<td>-0.08</td>
<td>-0.01; -0.15</td>
<td>0.019</td>
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<td>APTT</td>
<td>Interaction UFH dose group * sample time point</td>
<td>42</td>
<td>17; 68</td>
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<td>(seconds)</td>
<td>Age (per year)</td>
<td>3</td>
<td>1; 5</td>
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<td>Sex (female)</td>
<td>25</td>
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<td>Baseline aPTT (per second)</td>
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<td>1.8; 4</td>
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<td>UFH dose group (high versus low)</td>
<td>190</td>
<td>117; 263</td>
<td>&lt;0.001</td>
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<td>(seconds)</td>
<td>Baseline ACT (per second)</td>
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<td>0; 2</td>
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Table 2. Agreement between anti-Xa, APTT and ACT assays for results below, within, and above therapeutic ranges for samples after administration of UFH. Cells show absolute numbers (% of total number of samples; n=246 for anti-Xa vs APTT; n=224 for anti-Xa vs ACT and APTT vs ACT). Concordant cells are shaded. Abbreviations: s, seconds; u/ml, units/millilitre.

<table>
<thead>
<tr>
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<th>anti-Xa</th>
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<th>ACT</th>
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<td>&lt; 0.35 u/ml</td>
<td>0.35-0.7 u/ml</td>
<td>&gt; 0.7 u/ml</td>
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<td>APTT &gt; 85 s</td>
<td>38 (16%)</td>
<td>82 (33%)</td>
<td>97 (39%)</td>
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<td>60-85 s</td>
<td>15 (6%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>&lt; 60 s</td>
<td>14 (6%)</td>
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<tr>
<td>APTT &gt; 85 s</td>
<td>38 (16%)</td>
<td>82 (33%)</td>
<td>97 (39%)</td>
</tr>
<tr>
<td>60-85 s</td>
<td>15 (6%)</td>
<td>0 (0%)</td>
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<tr>
<td>&lt; 60 s</td>
<td>14 (6%)</td>
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<td>0 (0%)</td>
</tr>
<tr>
<td>ACT &gt; 250 s</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>34 (15%)</td>
</tr>
<tr>
<td>150–250 s</td>
<td>7 (3%)</td>
<td>41 (18%)</td>
<td>51 (23%)</td>
</tr>
<tr>
<td>&lt; 150 s</td>
<td>53 (24%)</td>
<td>29 (13%)</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>ACT &gt; 250 s</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>34 (15%)</td>
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<td>150–250 s</td>
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<td>&lt; 150 s</td>
<td>53 (24%)</td>
<td>29 (13%)</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>APTT &gt; 85 s</td>
<td>62 (28%)</td>
<td>98 (44%)</td>
<td>37 (17%)</td>
</tr>
<tr>
<td>60-85 s</td>
<td>14 (6%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>&lt; 60 s</td>
<td>13 (6%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>
Figure 1. Time course of anti-Xa, aPTT and ACT values (median, 95% confidence interval) comparing the high-dose versus the low-dose UFH group.

Figure 2. Stratification by age groups of anti-Xa, aPTT and ACT values (median, 95% confidence interval) in samples after UFH administration comparing dose groups (high-dose versus low-dose).

Figure 3. Time course of anti-Xa values (median, 95% confidence interval) comparing age groups (infants versus older children) and dose groups (high-dose versus low-dose). Numbers indicate the number of samples available per time point. Significant interaction of age group * dose group at time point 30 minutes, p=0.001.

Figure 4. Agreement between anti-Xa, APTT and ACT results for individual samples after UFH administration plotted against each other. Shaded areas represent the therapeutic ranges for the respective test. Black dots represent low dose, white dots high dose group.
Figure 2.
Figure 3.
Figure 4.
Monitoring unfractionated heparin in children - a parallel-cohort randomized controlled trial comparing two dose protocols

Andreas Hanslik, Erwin Kitzmüller, Ulrich S. Tran, Katharina Thom, Hratsch Karapetian, Nicole Prutsch, Jasmin Voitl, Ina Michel-Behnke, Fiona Newall and Christoph Male