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BRIEF REPORT

# The PiCT® test is a reliable alternative to the activated partial thromboplastin time in unfractionated heparin therapy management: results from a multicenter study

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## Essentials

- Activated partial thromboplastin time (APTT) or anti-Xa tests are used to monitor heparin.
- Prothrombinase-induced Clotting Time (PiCT) was compared to APTT in a clinical study.
- PiCT shows higher correlation to anti-Xa than APTT does and is more comparable between centers.
- PiCT demonstrates significantly higher accuracy and reliability than APTT in heparin monitoring.

**Summary.** *Background:* Unfractionated heparin (UFH) is still a commonly used anticoagulant for prevention and treatment of thromboembolism in a variety of situations. Increasingly, chromogenic anti-Xa assays are used for UFH monitoring given the high variability of the activated partial thromboplastin time (APTT) in this setting. On the other hand, and despite the known variability, the APTT test remains the most frequently used monitoring tool in UFH therapy because of its broad availability, lower costs and wide acceptance. Various guidelines continue to recommend the use of the APTT as an anti-Xa surrogate, but this approach remains controversial. *Objective:* To assess the prothrombinase-induced clotting time (PiCT®) test, reported in seconds, as an alternative to the APTT in the management of UFH-mediated anticoagulation. *Methods:* Plasma samples from patients receiving UFH were obtained in three different centers in the USA and Europe. Samples were analyzed for PiCT, APTT and anti-Xa activities with conditions set to allow comparability. Target-ranges in seconds for PiCT and APTT were established for a UFH concentration of 0.3–0.7 IU $mL^{-1}$, derived from anti-Xa results as suggested by the ACCP guidelines. *Results:* PiCT demonstrated better correlation with anti-Xa IU $mL^{-1}$ than APTT, higher ability to identify samples within target range and, importantly, comparable target-ranges between different centers. *Conclusion:* Accuracy and reliability of PiCT are significantly better than those of APTT in monitoring UFH for anticoagulant therapy.

**Keywords**: activated partial thromboplastin time; blood coagulation tests; clinical trial; diagnostic; reagent kits; unfractionated heparin.

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## Introduction

Unfractionated heparin (UFH) has been continuously replaced over the past years for many uses by low-molecular-weight heparins (LMWHs) because of a predictable pharmacokinetic profile and ease of use. Nevertheless, UFH is still a commonly used anticoagulant drug for several indications, mainly because of its short half-life and the possibility of completely reversing its effect [1]. A key drawback of UFH is its variable interindividual response. Failure to achieve adequate UFH doses can impair its effective and safe use [2], making monitoring of its anticoagulant action mandatory [3].

The most commonly used parameters to monitor UFH therapy are the activated partial thromboplastin time (APTT) and, to a lesser extent, heparin concentration measured by anti-factor Xa activity (anti-Xa) [4]. The APTT is known to be influenced by the presence of lupus anticoagulants and various coagulation factor deficiencies

(e.g. most prominently FXII, prekallikrein or high-molecular-weight kininogen), as well as elevated levels of FVIII and fibrinogen [5] and high leukocyte counts with release of lactoferrin [6]. Significant reagent and analyzer variability are leading to high intra-laboratory variability; this all results in a need for frequent monitoring and dose adaptation. On the other hand, anti-Xa tests directly quantify residual FXa activity, therefore exhibiting minimal influence by biological factors [7,8]. Despite these advantages of anti-Xa assays, APTT continues to be the methodology of choice for many laboratories monitoring UFH therapy, mainly because of automation, round the clock availability, no need for calibration, and reduced costs [4].

To improve result coherence when assessing APTT-based monitoring of UFH therapy, the American College of Chest Physicians (ACCP) and the College of American Pathologists (CAP) recommend that individual laboratories compare APTT clotting times with UFH levels measured as anti-Xa activity [7,8]. In brief, APTT results from a minimum of 20 patients are plotted against corresponding heparin concentration levels obtained from the same samples based on anti-Xa testing. After regression analysis, the APTT clotting times spanning a UFH concentration between 0.3 and 0.7 U $mL^{-1}$ are considered to reflect the local target-range for APTT monitoring [3], using the APTT as a surrogate for anti-Xa activity [4]. Unfortunately, the correlation of APTT clotting times with anti-Xa activity is sometimes found to be inadequate. Correlation coefficients and agreement factors with the target-range as low as $r = 0.4$ [9] and 54%, respectively, have been reported [10].

The PiCT test is a clotting time-based assay initiating the coagulation cascade at the level of the prothrombinase complex, thus bypassing most interferences associated with the intrinsic pathway-related variability seen with APTT [11]. The PiCT test is responsive to both FXa and thrombin activities. Previous reports have shown that PiCT displays a statistically significantly higher level of correlation with anti-Xa than APTT, suggesting that it could represent a promising solution to resolve issues in the monitoring and management of patients undergoing UFH therapy [12].

In this study, we evaluated the use of PiCT as an alternative to the APTT in the monitoring of UFH therapy in a multicenter setting. APTT and PiCT values were compared with anti-Xa levels, in order to analyze between-center consistency (target-range) as well as differences in categorizing samples as being inside or outside of the target-range.

## Methods

The study was registered at www.ClinicalTrials.gov as #NCT02052544 and performed after local Institutional Review Board/Ethics Committee approval. A total of 377 samples from three centers (Switzerland, Germany and the USA) were obtained from patients receiving UFH therapy who gave informed consent ($n = 121$; 55% male, 45% female). Specimens were collected in Becton Dickinson tubes (containing 3.2% citrate for German and US centers) or Becton Dickinson Vacutainer tubes (containing 3.8% citrate in the Swiss center). Tubes were used according to the recommendation of the manufacturer. Each sample was analyzed by APTT (HemosIL aPTT SP, Instrumentation Laboratory, Bedford, MA, USA), anti-Xa activity (Biophen Heparin 6, Hyphen BioMed, Neuville sur Oise, France) and Pefakit® PiCT (DSM Pentapharm, Basel, Switzerland) with ACL TOP instruments (Instrumentation Laboratory). Plasma samples were prepared according to Heparin 6's instructions for use. Blood samples were centrifuged within 1 h after blood collection for 20 min at 3000 *g* at 18 °C or below. Plasma was immediately decanted into plastic tubes using a plastic pipette. Clotting time-based tests were performed within 2 h of sample collection and anti-Xa tests were performed in batches using frozen samples. Plasma samples from subjects who were treated with anticoagulants other than UFH, who received fibrinolytic therapy during the previous 4 weeks, who had known coagulation factor deficiencies or were known to have an unexplained APTT prolongation before receiving UFH were excluded from the study. Dose–response curves were obtained by plotting APTT or PiCT clotting times values vs. UFH concentration as determined by anti-Xa activity, for each sample. APTT and PiCT target-ranges were determined by identifying the clotting time spanning a concentration of 0.3–0.7 U $mL^{-1}$, derived from regression analyses of PiCT or APTT vs. anti-Xa activity, according to ACCP and CAP guidelines [7,8]. Local standard procedures were followed for prophylactic or therapeutic anticoagulant management with UFH.

Statistical analysis was performed using Microsoft Excel (Redmond, WA, USA) and MedCalc (Ostend, Belgium). Regression analysis was performed to compare PiCT and APTT in seconds with anti-Xa units in the three centers individually and overall. The Bland-Altman method [13] was used to analyze the agreement between PiCT or APTT and anti-Xa for each individual center and for all of them together. The resulting graphs are scatter plots in which the *y* axis corresponds to the difference between PiCT or APTT in IU $mL^{-1}$ and anti-Xa in IU $mL^{-1}$; the *x* axis corresponds to the anti-Xa in IU $mL^{-1}$, considered as the reference method in this study. To quantify agreement between methods, the mean and the standard deviations of the differences between the PiCT or APTT and anti-Xa have been plotted on the graphs.

The percentages of PiCT and APTT results found within and outside the local therapeutic range, defined according to local anti-Xa levels, were calculated. Z-scores, where $Z = (x-\mu)/\sigma$, where x is the calculated

UFH concentration, and μ and σ are the mean and standard deviation of all the calculated UFH concentrations for the assay, were calculated to assess (i) the difference between correlation coefficients calculated for PiCT and APTT against anti-Xa and (ii) the difference between the proportions of samples correctly assigned to the target-range by PiCT and APTT with reference to anti-Xa.

## Results and discussion

Results are displayed in Figs 1–3 and Table 1. Figure 1 depicts scatter plots obtained comparing APTT or PiCT with UFH anti-Xa levels for each sample per center and overall. Table 1 summarizes the key outcomes of this analysis. First, PiCT demonstrates a higher level of correlation with anti-Xa than APTT (0.88 vs. 0.76; $P < 0.0001$). This increased correlation corresponds with a significant improvement in classification of samples as being within or out of the target-range (84% PiCT vs. 74% APTT; $P = 0.007$). From a qualitative standpoint, PiCT target-ranges are highly comparable between centers. This is especially true for centers two and three, which display nearly identical ranges (77–107 s and 74–111 s, respectively), whereas the target-range for center 1 (83–134 s) is remarkably consistent with previously reported ranges for this same center (86–143 s) [12]. Center 1 has a higher target-range, which is probably explained by the citrate concentration used. This demonstrates the need for each laboratory to establish its own target-range. A clearly higher degree of variability in therapeutic ranges between the different centers is observed for APTT.

In order to quantitatively evaluate the impact of the differences in correlation with anti-Xa between PiCT and APTT, the clotting times from respective analyses of samples with these two tests were transformed to IU $mL^{-1}$. To this end, equations obtained from regression analyses (not shown) were used to calculate UFH concentration as IU $mL^{-1}$ from clotting times. Figure 2 depicts an analysis of agreement in UFH content per sample, expressed as IU/ml, between each clotting time test and the anti-Xa test in the form of a Bland-Altman plot.

It can be observed that calculated UFH concentrations based on PiCT consistently demonstrate a smaller deviation from UFH concentrations measured by anti-Xa assays than those calculated concentrations based on APTT. Whereas PiCT-based UFH concentrations show a consistent small deviation throughout the whole concentration range, APTT results show an increasing level of deviation with increasing UFH concentrations, especially within the therapeutic dose target-range of 0.3–0.7 IU $mL^{-1}$. This increasing APTT variability at higher concentrations seems to be the cause of the lower overall variability in classifying samples as within or out of target-range in center 1, as UFH levels were overall lower in center 1. This phenomenon has an effect on the comparability of results between the centers. To further study this phenomenon, samples within therapeutic target-range (0.3–0.7 IU $mL^{-1}$) according to the anti-Xa test were analyzed separately. From these samples, target-ranges of 77–110 s for PiCT and 67–127 s for APTT were obtained (Table 1). Thereafter, all samples were evaluated and were found to lie within a level of agreement with anti-Xa of 73 ± 4% (69%, 77% and 72% for centers 1, 2 and 3, respectively) for PiCT, as compared with 38 ± 10% for APTT (43%, 28% and 48% for centers 1, 2 and 3, respectively).

To further illustrate the differences between the two clotting time-based assays and the three centers, a distribution analysis was performed with this same set of samples (i.e. samples within therapeutic target-range according to anti-Xa test, Fig. 3). PiCT results demonstrated Gaussian distribution (D'Agostino-Pearson test, $P = 0.3011$ overall). Close and nearly identical medians and arithmetic means reflect the limited number of outliers (94 ± 3 s and 95 ± 1 s, respectively), as well as a similar between-center response in seconds to different levels of UFH within the studied range. In the case of APTT, distributions are non-Gaussian (D'Agostino-Pearson test, $P < 0.0001$ overall), with a higher difference between medians and arithmetic means (69 ± 7 s and 85 ± 12 s, respectively). Finally, a significantly lower standard deviation is found for PiCT (16 s) compared with APTT (50 s, variance ratio $F = 9.8603$, $P < 0.001$ overall).

A potential limitation of the study is the (relatively) small number of patients; however, the results were consistent within each of the three centers as well as between the three centers overall.

On the other hand, the study limits the comparison to one APTT test (i.e. HemosIL aPTT SP), and results cannot be in principle generalized to all APTT tests on the market as there are many factors known to influence the responsiveness to UFH, including variations of lipid composition and total phospholipid concentration. HemosIL APTT SP was used as the reference of choice given its high responsiveness to UFH and its lot-to-lot consistency of results.

For the future, it seems important to recognize that the APTT response is mainly a reflection of anti-IIa activity mediated by longer UFH chains [9], and PiCT responds to both anti-Xa and anti-IIa activities [11], probably accounting for the observed higher levels of correlation of PiCT with anti-Xa. It is therefore tempting to speculate that PiCT might make it possible to reach therapeutic ranges for UFH earlier during the course of therapy as compared with the use of APTT. In this sense, PiCT would provide improved management of UFH therapy, but further studies are needed to test this hypothesis. Finally, given its ability to respond to the activity of both FIIa and FXa, PiCT could become a versatile tool to follow-up patients with new oral anticoagulant therapies

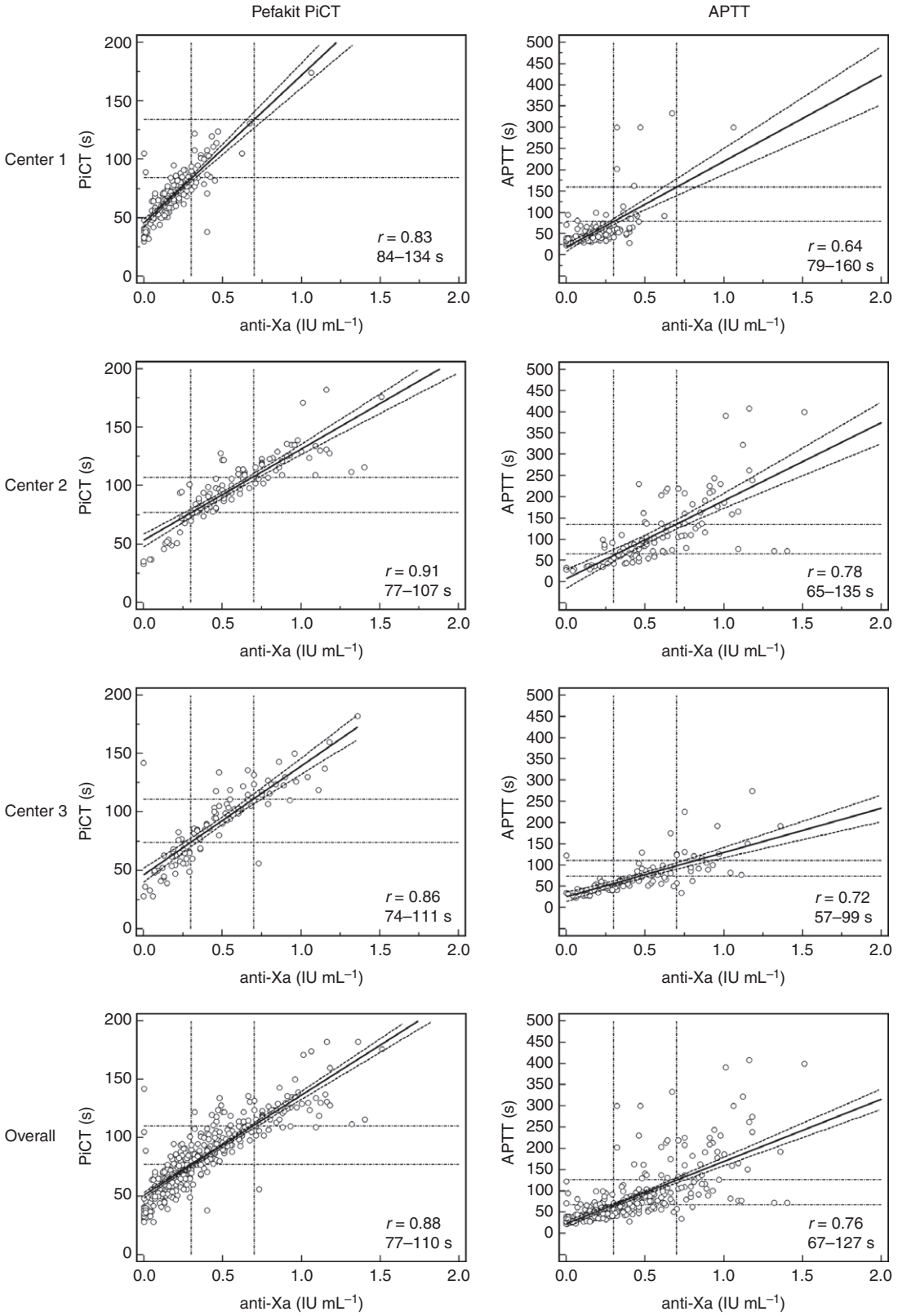


**Fig. 1.** Scatter plots (including regression lines and 95% confidence intervals) describing the relationship between prothrombinase-induced clotting time (PiCT) and activated partial thromboplastin time (APTT) in seconds and anti-Xa units during treatment with unfractionated heparin (UFH) in each of the three different centers and overall. Correlation coefficients ($r$) and deduced therapeutic ranges (in seconds) are displayed in the bottom-right corner of each scatter plot. Vertical dotted lines delimit the 0.3–0.7 IU $mL^{-1}$ therapeutic range defined by anti-Xa tests. Horizontal dotted lines delimit respective therapeutic ranges in clotting time.

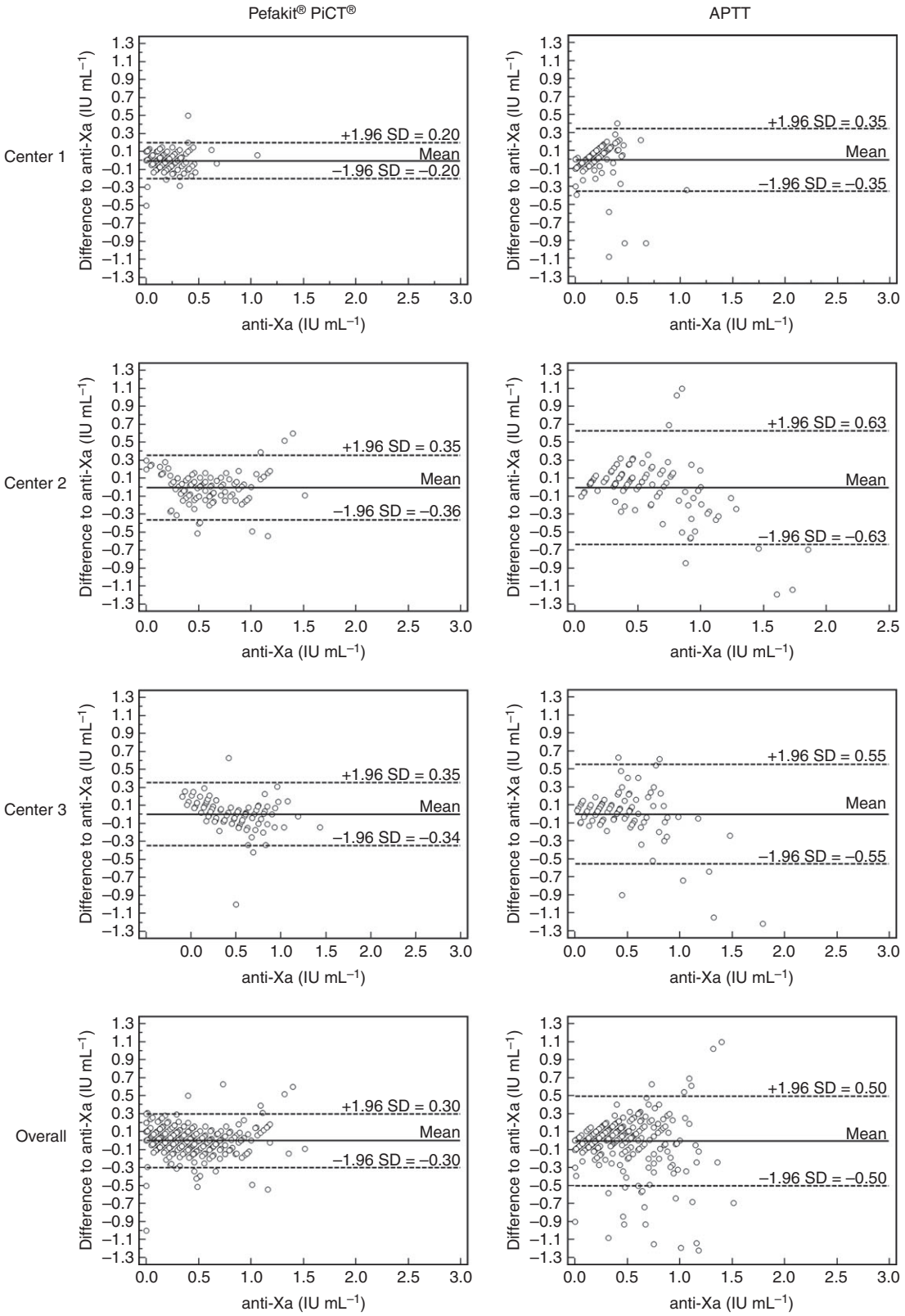


**Fig. 2.** Bland-Altman plot depicting per-sample deviations between unfractionated heparin (UFH) plasma concentration as measured by anti-Xa assay and concentration calculated from clotting time in seconds based on formulas obtained from the respective regression analysis in Fig. 1. A difference from zero on the *y*-axis is indicative of absolute quantitative agreement of a given result in clotting time with its corresponding anti-Xa activity measured in the same sample: the higher the positive or negative difference, the higher the error of the clotting time-based test taking anti-Xa as reference. SD, standard deviation.

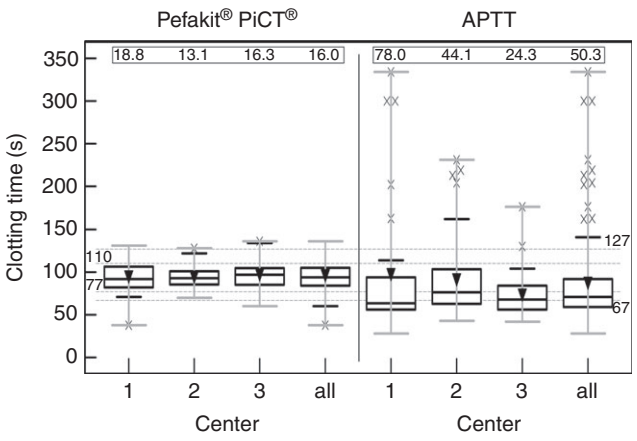


**Fig. 3.** Distribution of clotting time values as box and whisker plots obtained from measurements performed with either prothrombinase-induced clotting time (PiCT) or activated partial thromboplastin time (APTT), per center and overall. Only samples containing unfractionated heparin (UFH) levels between 0.3 and–0.7 IU $mL^{-1}$ are considered in the analysis. Central boxes represent values from the lower to upper quartile (25–75 percentile). The middle line represents the median and inverted black triangles the arithmetic mean. Horizontal short black lines delimit minimum to maximum values excluding outliers (indicated as × symbols) and horizontal larger grey lines delimit respective ranges spanned by all samples. Dotted horizontal lines indicate the lower and upper limits of the overall therapeutic range in seconds for PiCT (77–110 s) and APTT (67–127 s), respectively. Inserts include respective standard deviations calculated per center and overall.

(including rivaroxaban [14] and edoxaban [15]) if needed. But again, more studies are needed to test these hypotheses.

In summary, our study provides evidence that using PiCT for monitoring UFH therapy results in a higher degree of correlation with anti-Xa activity when compared with APTT, and a better overall agreement in sample classification when compared with the anti-Xa target-range recommended by ACCP and CAP guidelines. PiCT-based target-ranges between centers were more consistent than those of APTT, with an overall target-range of 70–110 s (and a median of 94 s), corresponding to UFH plasma levels of 0.3–0.7 IU $mL^{-1}$. This suggests that PiCT is better suited for clinically monitoring UFH therapy than APTT when clotting-based assays are used for this purpose.

## Addendum

A. C. Brisset prepared the figures and wrote the paper. A. Ferrández designed and reviewed the study protocols, analyzed the data and wrote the paper. M. Krause, S. Rathbun and W. Korte enrolled the patients. M. Krause, R. Marlar and W. Korte conducted measurements. W. Korte designed and reviewed the study protocol. All authors reviewed and made critical comments on the manuscript and approved the final manuscript. The

**Table 1** Correlation coefficients of best fit linear regression analyses from scatter plots in Fig. 1 with 95% confidence intervals

| | Pefakit PiCT | | | | APTT | | | |
|---|---|---|---|---|---|---|---|---|
| Centers | 1 | 2 | 3 | All | 1 | 2 | 3 | All |
| *r* (95% CI) | 0.83 (0.78–0.87) | 0.91 (0.87–0.94) | 0.85 (0.79–0.90) | 0.88 (0.86–0.90) | 0.64 (0.54–0.72) | 0.78 (0.70–0.85) | 0.72 (0.61–0.80) | 0.76 (0.71–0.80) |
| Target-range (95% CI) | 84–134 (77–146) | 77–107 (70–117) | 74–111 (65–125) | 77–110 (73–115) | 79–160 (59–195) | 65–135 (48–171) | 57–99 (40–124) | 67–127 (56–142) |
| Agreement with anti-Xa % (95% CI) | 87 (81–91) | 83 (75–89) | 81 (72–88) | 84 (76–88) | 83 (76–88) | 63 (54–71) | 71 (62–79) | 74 (68–77) |

Prothrombinase-induced clotting time (PiCT) and activated partial thromboplastin time (APTT) are both compared with anti-Xa levels. Therapeutic ranges in seconds are calculated as the clotting-time range spanning unfractionated heparin (UFH) concentrations between 0.3 and 0.7 IU mL−1 and confidence intervals are included. Agreement with anti-Xa is defined as the percentage of samples for which assignment as within or out of target-range based on clotting-time data correlates with that of anti-Xa.

measurements were carried out in St Gallen, Wiesbaden and Oklahoma.

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## Disclosure of Conflict of Interests

A. C. Brisset and A. Ferrández were employees of DSM Pentapharm at the time the study was performed. Pefakit® and PiCT® are registered names of DSM Pentapharm's products. W. Korte was an advisor to DSM Pentapharm. S. Rathbun reports grants from Pentapharm during the conduct of the study, and grants from Diagnostica Stago, outside the submitted work. R. Marlar reports grants from DSM Pentapharm during the conduct of the study.

## References

1 Eikelboom JW, Hirsh J. Monitoring unfractionated heparin with the aPTT: time for a fresh look. *Thromb Haemost* 2006; **96**: 547–52.
2 Hirsh J, Anand SS, Halperin JL, Fuster V. Guide to anticoagulant therapy: heparin: a statement for healthcare professionals from the American Heart Association. *Circulation* 2001; **103**: 2994–3018.
3 Marlar RA, Gausman J. The optimum number and types of plasma samples necessary for an accurate activated partial thromboplastin time-based heparin therapeutic range. *Arch Pathol Lab Med* 2013; **137**: 77–82.
4 Guervil DJ, Rosenberg AF, Winterstein AG, Harris NS, Johns TE, Zumberg MS. Activated partial thromboplastin time versus antifactor Xa heparin assay in monitoring unfractionated heparin by continuous intravenous infusion. *Ann Pharmacother* 2011; **45**: 861–8.
5 Bussey HI. Problems with monitoring heparin anticoagulation. *Pharmacotherapy* 1999; **19**: 2–5.
6 Wu HF, Lundblad RL, Church FC. Neutralization of heparin activity by neutrophil lactoferrin. *Blood* 1995; **85**: 421–8.
7 Garcia DA, Baglin TP, Weitz JI, Samama MM. Parenteral anticoagulants: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012; **141**: e24S–43S.
8 Olson JD, Arkin CF, Brandt JT, Cunningham MT, Giles A, Koepke JA, Witte DL. College of American Pathologists Conference I on laboratory monitoring of anticoagulant therapy: laboratory monitoring of unfractionated heparin therapy. *Arch Pathol Lab Med* 1998; **122**: 782–98.
9 Takemoto CM, Streiff MB, Shermock KM, Kraus PS, Chen J, Jani J, Kickler T. Activated partial thromboplastin time and anti-xa measurements in heparin monitoring: biochemical basis for discordance. *Am J Clin Pathol* 2013; **139**: 450–6.
10 Cuker A, Ptashkin B, Konkle BA, Pipe SW, Whinna HC, Zheng XL, Cines DB, Pollak ES. Interlaboratory agreement in the monitoring of unfractionated heparin using the anti-factor Xa-correlated activated partial thromboplastin time. *J Thromb Haemost* 2009; **7**: 80–6.
11 Calatzis A, Spannagl M, Gempeler-Messina P, Kolde HJ, Schramm W, Haas S. The prothrombinase-induced clotting test: a new technique for the monitoring of anticoagulants. *Pathophysiol Haemost Thromb* 2000; **30**(Suppl. 2): 172–4.
12 Korte W, Jovic R, Hollenstein M, Degiacomi P, Gautschi M, Ferrandez A. The uncalibrated prothrombinase-induced clotting time test. Equally convenient but more precise than the aPTT for monitoring of unfractionated heparin. *Hamostaseologie* 2010; **30**: 212–6.
13 Altman DG, Bland JM. Measurement in medicine: the analysis of method comparison studies. *The Statistician*. 1983; **32**: 307–17.
14 Graff J, von Hentig N, Misselwitz F, Kubitza D, Becka M, Breddin HK, Harder S. Effects of the oral, direct factor xa inhibitor rivaroxaban on platelet-induced thrombin generation and prothrombinase activity. *J Clin Pharmacol* 2007; **47**: 1398–407.
15 Mendell J, Noveck RJ, Shi M. A randomized trial of the safety, pharmacokinetics and pharmacodynamics of edoxaban, an oral factor Xa inhibitor, following a switch from warfarin. *Br J Clin Pharmacol* 2012; **75**: 966–78.