# Factors Influencing anti-Xa assays: A Multicenter Prospective Study in Critically Ill and Noncritically Ill Patients Receiving Unfractionated Heparin

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## Abstract

**Background** The presence of dextran sulfate (DS) in reagents and the type of blood collection tube (citrate/citrated-theophylline-adenosine-dipyridamole [CTAD]) can lead to discrepancies between unfractionated heparin (UFH) anti-Xa levels.

**Objectives** To evaluate the extent of the effect (1) of different reagents containing or not containing DS and (2) of the blood collection tubes, on UFH anti-Xa levels, in various clinical situations (NCT04700670).

**Methods** We prospectively included patients from eight centers: group (G)1, cardiopulmonary bypass (CPB) after heparin neutralization ($n = 39$); G2, cardiothoracic intensive

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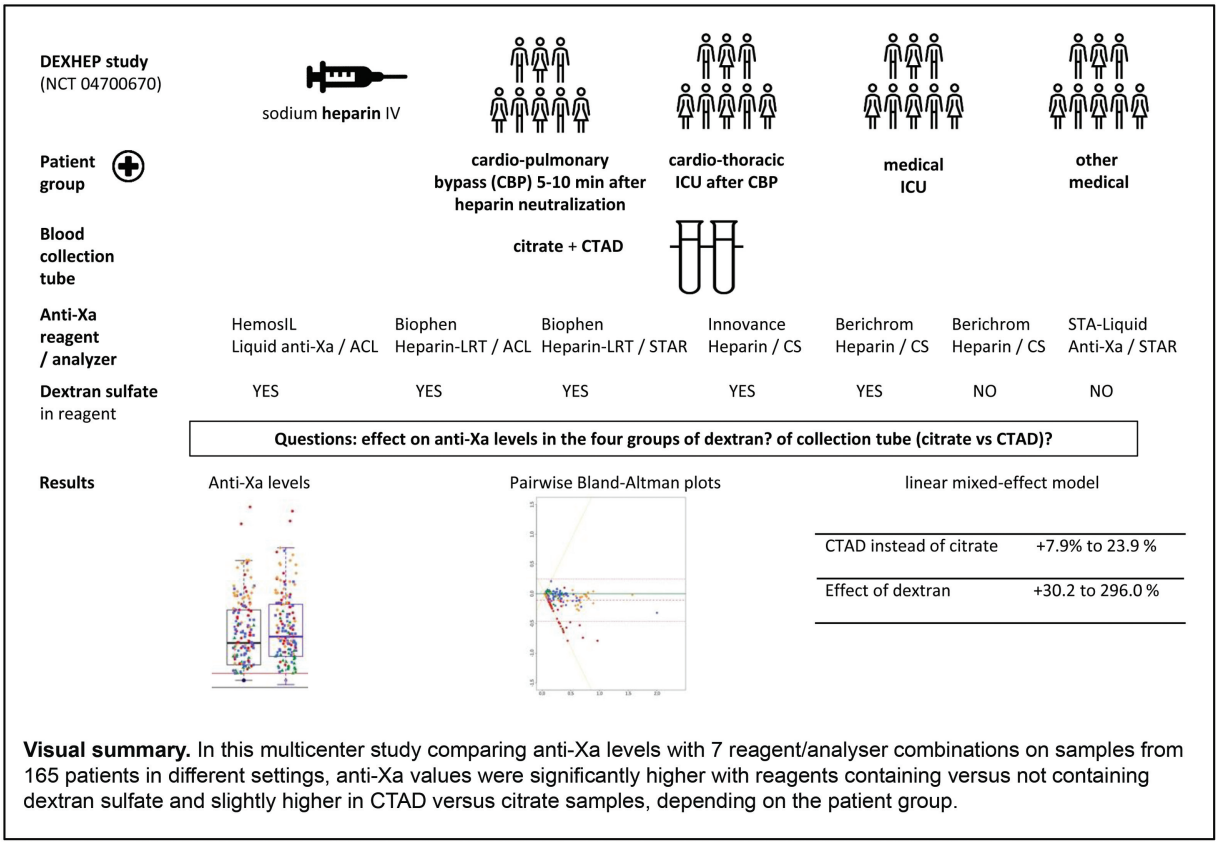


**Visual summary.** In this multicenter study comparing anti-Xa levels with 7 reagent/analyser combinations on samples from 165 patients in different settings, anti-Xa values were significantly higher with reagents containing versus not containing dextran sulfate and slightly higher in CTAD versus citrate samples, depending on the patient group.

care unit (ICU) after CPB ($n = 35$); G3, medical ICU ($n = 53$); G4, other medical inpatients ($n = 38$). Blood was collected into citrated and CTAD tubes. Chromogenic anti-Xa assays were centrally performed, using seven reagent/analyzer combinations including two without DS. The association between anti-Xa levels and covariates was tested using a linear mixed-effects model.

**Results** We analyzed 4,546 anti-Xa values from 165 patients. Median anti-Xa levels were systematically higher with reagents containing DS, whatever the patient group, with the greatest effect observed in G1 (0.32 vs. 0.05 IU/mL). Anti-Xa levels were slightly higher in CTAD than in citrate samples, irrespective of the assay. The model showed: (1) a significant dextran–patient group interaction ($p < 0.0001$), the effect of DS on anti-Xa levels varying from 30.9% in G4 to 296% in G1, and (2) a significant effect of CTAD, varying between patient groups ($p = 0.0302$).

**Conclusion** The variability of anti-Xa levels with a great overestimation of the values, using a reagent containing DS, can lead to different treatment decisions, especially after heparin neutralization by protamine. Clinical consequences of these differences remain to be demonstrated.

## Introduction

Unfractionated heparin (UFH) is the anticoagulant of choice in cardiac surgery and in critically ill patients. Indeed, UFH has a short half-life; it is cleared through a combination of two mechanisms, mainly a saturable mechanism due to the binding to proteins, endothelial cells, and macrophages, but also a non-saturable renal mechanism at high doses. One major advantage of UFH is that intravenous (IV) protamine sulfate can rapidly reverse its anticoagulant effects. However, the anticoagulant response to UFH at therapeutic dose is highly variable among patients and close laboratory monitoring is required for dose adjustments.[1] Different assays may be used to monitor UFH. The older assays are activated partial thromboplastin time (aPTT) and activated

 

clotting time (ACT), the latter to monitor very high doses of UFH, especially in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB). However, such tests suffer from lack of specificity when monitoring UFH. Indeed, aPTT prolongation is frequently encountered especially in critically ill patients, notably due to factor deficiency, presence of lupus anticoagulant, or interference with C-reactive protein with varying sensitivity depending on reagents.[1–5] In contrast, chromogenic anti-Xa assays have the advantage of being specific of anti-Xa inhibitors.[6] However, discrepancies between ex vivo anti-Xa levels according to assays have been pointed out for many years.[7] Recent external quality assessment programs such as External quality Control of diagnostic Assays and Tests (ECAT) using lyophilized samples have shown a substantial inter-laboratory variability of anti-Xa levels according to reagents, especially for the low range of values.[8–10] Different parameters might potentially contribute to the heterogeneity of anti-Xa levels, among which are the type of blood collection tube, the presence or absence of dextran sulfate in reagents, the addition of exogenous antithrombin, the calibrator, and the calibration curve mathematical processing. Some manufacturers chose to add dextran in reagents in order to displace UFH from proteins released ex vivo after blood sampling, notably platelet factor 4 (PF4) following platelet activation; thus, UFH released from those neutralizing proteins recovers its anti-Xa level.[11] In the case of heparin neutralization by protamine sulfate, the risk is to dissociate the heparin/protamine complexes and to overestimate anti-Xa levels. Indeed, significant differences in anti-Xa levels potentially related to the presence of dextran in reagents have been evidenced,[9,10,12] especially in patients with CPB just after UFH neutralization by protamine sulfate.[13]

Another way to prevent the influence of ex vivo platelet activation and release of heparin neutralization proteins is to collect blood into tubes containing citrated-theophylline-adenosine-dipyridamole (CTAD) solution instead of sodium citrated solution.[14,15]

Today, the extent of the effect of dextran sulfate and of blood collection (CTAD vs. citrate) on anti-Xa levels in various clinical situations is not well known. Therefore, we conducted a multicenter study in four predefined groups of hospitalized patients receiving IV UFH from different settings, including post-CBP patients who received protamine sulfate for UFH neutralization. We sought to evaluate the effect on UFH anti-Xa levels assessed using seven reagent/analyzer combinations (1) of different reagents containing or not containing dextran sulfate and (2) of the anticoagulant solution contained in the tubes (containing citrate or CTAD solution).

## Patients and Methods

### Patients

We conducted a prospective noninterventional study in intensive care units (ICUs) or medical wards of eight French hospital centers (Bordeaux, Dijon, Lille, Nancy, AP-HP-Lariboisière, AP-HP-Necker, Rennes, and Versailles) between January 2020 and November 2021. Patients were eligible if they were 18 years or older and were receiving IV infusion of UFH with laboratory monitoring. The UFH dose was at the discretion of the physician. The study protocol was approved by the ethics committee (Comité Consultatif de Protection des Personnes number 19.03.28.40218, NCT04700670). All participants or their relatives gave their informed consent to participate in the study. We predefined four groups of patients, namely:

(1) Group 1, patients undergoing cardiac surgery with CPB, with sampling in the operating room 5 to 10 minutes after neutralization of heparin by protamine.
(2) Group 2, patients in cardiothoracic ICU, 1 to 5 days after cardiac surgery with CPB.
(3) Group 3, patients hospitalized in medical ICU.
(4) Group 4, other medical inpatients (cardiology and internal medicine wards).

### Blood Collection, Processing, and Storage

In addition to samples taken as part of usual care, blood was collected into four tubes: two containing citrate (0.109 M) and two containing CTAD. According to centers, tubes were purchased from Becton-Dickinson (Vacutainer, Ref. 363048 and 364305-citrated, Ref. 367599-CTAD) or from Greiner-Bio-One (Vacuette, Ref. 454327-citrated, Ref. 474304 and 454064-CTAD). All tubes were to be double-centrifuged within 1 hour after blood collection, according to the recommendations.[16] The time between sampling and centrifugation was recorded. Platelet-poor plasma (PPP) was separated in 500 µL aliquots and stored at −70°C. Once all patients were included, one citrated aliquot and one CTAD aliquot per patient were shipped on dry ice to the six centers performing the anti-Xa assays (see below).

### Anti-Xa Assays

Seven reagent/analyzer combinations were used and calibrations were performed using dedicated calibrators and controls, as recommended by the manufacturers:

- HemosIL liquid anti-Xa on ACL-TOP750 (Werfen, Bedford, Massachusetts, United States) performed at Bordeaux using HemosIL Heparin Calibrator (referred below as HemosIL liquid anti-Xa—ACL).
- Biophen heparin LRT (Hyphen BioMed, Neuville sur Oise, France) using Biophen Heparin Calibrator (hybrid curve) on ACL TOP750 performed at Necker-Paris (referred below as Biophen heparin LRT—ACL), and on STAR Max (Diagnostica Stago, Asnières Gennevilliers, France) performed at Lille (referred below as Biophen heparin LRT—STAR).
- Innovance heparin (Siemens Healthcare Diagnostics Products, Marburg, Germany) on Sysmex CS analyzers (Sysmex France, Villepinte, France), performed half at Rennes and half at Nancy using Innovance Heparin calibrator (hybrid curve) (referred below as Innovance heparin—CS).
- Berichrom heparin (Siemens) with and without dextran sulfate on Sysmex CS performed at Rennes and Nancy using Berichrom Heparin UF Calibrator (UFH specific calibration) (referred below as Berichrom heparin—CS).
- STA Liquid anti-Xa (Stago) on STAR Max performed at Lariboisière, Paris using Multihep Calibrator Stago (UFH specific calibration) (referred below as STA Liquid anti-Xa —STAR).

Moreover, for each reagent/analyzer combination, a calibration curve using the sixth World Health Organization

 

International Standard for UFH (purchased from the NIBSC, Potters Bar, United Kingdom) was also obtained. As recommended, the freeze-dried heparin (2,145 UI/ampoule) was reconstituted with 1 mL of distilled water. Then serial dilutions were made to obtain the following final concentrations in normal plasma pool (Cryocheck, Montpellier, France): 0, 0.25, 0.5, 0.75, and 1.0 IU/mL; these calibrators were stored frozen at −80°C a few days before shipping on dry ice to the participating centers.

Among the reagents, four contained dextran sulfate (HemosIL liquid anti-Xa, Biophen heparin LRT, Innovance heparin, Berichrom heparin) and one did not (STA Liquid anti-Xa). One assay, namely Berichrom heparin, was performed with dextran according to manufacturer's recommendations, but also without dextran sulfate: for this purpose, factor Xa reagent was reconstituted either with the manufacturer diluent containing dextran sulfate or with distilled water (adapted assay). Overall, we used five reagents containing dextran and two reagents without dextran. Only one reagent contained exogenous antithrombin (Berichrom heparin).

The anti-Xa levels were centrally determined in the six centers using the two calibration curves (manufacturer's and International Standard), in both citrated and CTAD plasma aliquots after being thawed for 3 to 5 minutes in a 37°C water bath just before testing. When the result was above the upper limit of quantification, patient plasma was diluted in PPP. The lower limit of quantification (LLOQ) was 0.10 IU/mL for all reagents. All values below the LLOQ were referred as 0.05 IU/mL for statistical analysis.

### Statistical Analysis

All statistical analyses were run on R-software (version 4.0.2) using the lme4 package (version 1.1.28).[17,18] Quantitative data were described as median (interquartile range) or mean values (±standard deviation), and minimal and maximal values. The association between anti-Xa levels and covariates (patient group, CTAD vs. citrate, presence or absence of dextran, type of analyzer) was tested using a linear mixed-effects model, with the patient used as a random effect (to account for the measurement of the same sample on all analyzers), fitted using maximum likelihood. The model included interaction terms when possible. Variable significance in the model was tested using nested-models asymptotic likelihood-ratio tests (chi-square distribution); models were also compared using the Akaike Information Criteria (AIC). Confidence intervals (CIs) were determined by profiling. All analyses were done on log-transformed data; model assumptions, including a Gaussian distribution of the logarithm of the anti-Xa activities, were checked graphically (quantile–quantile plots, plots of the residuals and of the random effects). CIs were built with a nominal 95% CI using profiling; tests were done with a type I error of 5%.

Assays were also compared on a pairwise basis using Bland–Altman plots, with the 95% prediction interval of the bias drawn (assuming a constant bias and a Gaussian distribution of the difference).

## Results

### Patients

Overall, 165 patients (51 females and 114 males) were included: 39 in group 1 (patients with CPB, 5–10 minutes after neutralization of heparin by protamine), 35 in group 2 (patients in cardiac ICU, 1–5 days after cardiac surgery with CPB), 53 in group 3 (patients hospitalized in medical ICU), and 38 in group 4 (other medical inpatients). The median (min–max) age was 70 (24–83), 66 (39–81), 68 (27–85), and 70 (36–98) years, in the four groups, respectively; the proportion of females was of 36, 29, 34, and 24%, respectively.

### Anti-Xa Levels Measured with the Different Combinations of Assays in the Four Patient Groups

Overall, 75% of blood samples were centrifuged within 1 hour and 88% within 2 hours. A total of 4,546 anti-Xa values were determined: 2,273 with the manufacturer calibrators and 2,273 with the sixth International Standard for UFH. All tables and figures presented below show the data obtained with the manufacturer calibrators. ►**Fig. 1** displays individual anti-Xa values according to each reagent/analyzer combination and to collection tube (citrate or CTAD). ►**Tables 1** and **2** summarize results according to reagent/analyzers and patient groups, respectively. All results are also displayed on the pairwise Bland–Altman plots (►**Fig. 2**; ►**Supplementary Fig. S1**, available in the online version). Median anti-Xa levels were highly variable between groups.

In citrate samples, median anti-Xa levels were systematically higher with reagents containing dextran sulfate compared to those without, whatever the reagent/analyzer combination and the patient group. Especially, reagents with dextran sulfate led to a lower proportion of values below the LLOQ in all groups with the greatest effect observed in group 1. Indeed, in this group, only 6% of anti-Xa values were below the LLOQ when measured with reagents containing dextran sulfate, while with reagents without dextran sulfate, 77% of the values and the median were below the LLOQ.

The effect of dextran sulfate was comparable in CTAD samples in the four groups of patients (►**Tables 1** and **2**, ►**Fig. 1**, ►**Supplementary Figs. S2** and **S3,** available in the online version). Anti-Xa levels were consistently higher in CTAD samples compared to citrated samples, across patient groups and anti-Xa assays.

In addition, results regarding the effect of dextran were not affected when using the sixth International Standard calibrator instead of the manufacturer's calibrator, both in citrate (►**Supplementary Fig. S4**, available in the online version) and CTAD tubes (►**Supplementary Fig. S5**, available in the online version).

To better delineate the effect of dextran sulfate, we tested the only reagent that could be run with and without dextran sulfate (Berichrom reagent; ►**Fig. 3**). We observed a marked effect of dextran sulfate with higher anti-Xa values than without dextran sulfate especially in group 1.

 

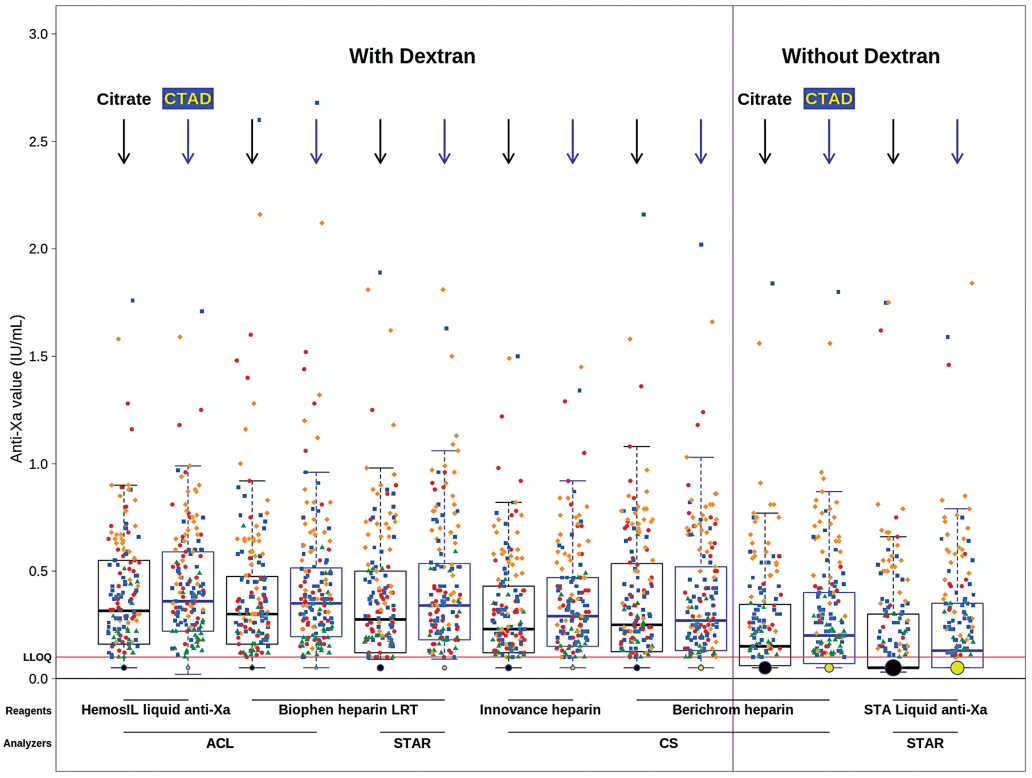


**Fig. 1** Individual anti-Xa levels (IU/mL) ($n = 2{,}310$) according to each reagent/analyzer combination and to the collection tube. The 10 first columns correspond to assays with dextran sulfate and the last 4 columns correspond to assays without; for each combination, citrate and CTAD are indicated with a black and a blue arrow, respectively. Reagents and analyzers are specified at the bottom of the figure. The red horizontal bar indicates the lower limit of quantification (LLOQ) (0.10 IU/mL). By convention, results below the LLOQ ($n = 519$) were referred as 0.05 IU/mL: the area of the black (citrate) and blue (CTAD) circles is proportional to the number of values. Each patient is represented with a symbol: red dots (group 1: CPB after protamine neutralization), green triangles (group 2: cardiothoracic ICU), blue squares (group 3: medical ICU), and orange diamonds (group 4: other medical patients). For each reagent/analyzer combination, box plots represent the median and the interquartile range of anti-Xa levels. Whiskers were obtained by the default algorithm in R. CPB, cardiopulmonary bypass; CTAD, citrated-theophylline-adenosine-dipyridamole; ICU, intensive care unit.

### Modeling the Effect of Dextran Sulfate and CTAD on Anti-Xa Levels (IU/mL)

Modeling using a linear mixed-effects model was first made with a complete model including tube, dextran, and patient group as predictors with all interactions. Both nested model and AIC approaches allowed showing that the triple interaction was not significant ($p = 0.2229$), then that tube–dextran interaction ($p = 0.0904$) was not significant. However, the tube–group interaction was significant ($p = 0.0176$), meaning that the CTAD effect was different between groups, ranging from +8% (CI: −1.6 to +18.3) in group 1 to +24% (CI: +12.5 to +36.5) in group 2 (►**Table 3**). We also showed a significant dextran–group interaction ($p < 0.0001$), meaning that there was an effect of dextran that depended on the patient group. Indeed, the increase in anti-Xa levels varied from 31% (CI: 17.4–44.5) in group 4 to almost 300% [CI: 256–339] in group 1.

Furthermore, we checked that the use of manufacturer vs. the sixth international standard calibrator did not affect anti-Xa results regarding the effect of dextran/type of tube: similar effects of dextran and CTAD were found when anti-Xa levels were determined using either calibrations (►**Supplementary Table S1**, available in the online version>).

## Discussion

To our knowledge, this is the first prospective multicenter study evaluating the extent of the association of dextran sulfate and of blood collection tubes (citrate vs. CTAD) with anti-Xa levels, measured with seven reagent/analyzer combinations. We included a substantial number of adult patients ($n = 165$) receiving intravenously UFH in predefined clinical settings, including post-CPB. We showed a significant increase in anti-Xa levels when reagents containing dextran were used compared to those without dextran, highly varying among patient groups. We also found that blood collection into CTAD-containing tubes was associated with a significant slight increase (in average, 15%) in anti-Xa levels, regardless of the presence of dextran but of variable extent between patient groups.

The results are in line with those of previous studies.[8,10,19,20] However, in those previous studies, data came from external quality assessment programs using lyophilized plasma samples, plasmas spiked with UFH, or left-over plasma samples from patients receiving different doses of UFH or LMWH, making their clinical relevance difficult. The magnitude of the effect of dextran may differ among clinical settings with variable inflammatory status and platelet

 

**Table 1** Anti-FXa values measured using the manufacturer's calibrator according to reagent/analyzer combinations on citrated or CTAD plasma samples

| | | STA-Liquid anti-Xa (Stago) | Berichrom heparin (Siemens) | Berichrom heparin (Siemens) | Biophen heparin LRT (Hyphen Biomed) | Biophen heparin LRT (Hyphen Biomed) | HemosIL liquid anti-Xa (Werfen) | Innovance heparin (Siemens) |
|---|---|---|---|---|---|---|---|---|
| Dextran | | No | No | Yes | Yes | Yes | Yes | Yes |
| Analyzer | | Stago-STAR | Sysmex-CS | Sysmex-CS | Werfen-ACL TOP | Stago-STAR | Werfen - ACL TOP | Sysmex-CS |
| Citrate | *n* | 165 | 164 | 164 | 164 | 162 | 162 | 164 |
| | Median (IU/mL) | 0.05 | 0.15 | 0.25 | 0.30 | 0.28 | 0.32 | 0.23 |
| | Min–max (IU/mL) | 0.05–1.75 | 0.05–1.84 | 0.05–2.16 | 0.05–2.60 | 0.05–1.89 | 0.05–1.76 | 0.05–1.50 |
| CTAD | *n* | 163 | 161 | 161 | 163 | 160 | 159 | 161 |
| | Median (IU/mL) | 0.13 | 0.20 | 0.27 | 0.35 | 0.34 | 0.36 | 0.29 |
| | Min–max (IU/mL) | 0.05–1.84 | 0.05–1.80 | 0.05–2.02 | 0.05–2.68 | 0.05–1.81 | 0.05–1.71 | 0.05–1.45 |

Abbreviation: CTAD, citrated-theophylline-adenosine-dipyridamole.
Note: By convention, all values <0.1 IU/mL were taken as 0.05 IU/mL; *n*: number of samples.

activation. UFH is strongly negatively charged due to its sulfate and carboxylate groups. Thus, UFH chains reversely bind to a number of plasma proteins other than antithrombin, containing a high proportion of positively charged amino-acid residues such as lysyls, including PF4. Overall, this binding of UFH competes with its binding to antithrombin, modulating its anticoagulant effect and contributes to the high inter-individual variability of the anticoagulant response among patients.[1,2]

One strength of our study is that we clearly evidenced a major impact of dextran on anti-Xa levels in patients undergoing cardiac surgery with CPB, 5 to 10 minutes after neutralization of heparin by protamine. In those patients, anti-Xa levels are expected to be low with most of them below the LLOQ. The extent of the effect that we found, namely 6% of anti-Xa levels in citrated plasma samples below the LLOQ when measured with reagents containing dextran, compared to 77% below the LLOQ without dextran, may have a major clinical impact. Indeed, when UFH is monitored with reagents containing dextran, higher levels of anti-Xa could lead to re-administration of protamine in a substantial proportion of patients with potentially deleterious effects.[21] In the context of CPB, it is likely that dextran sulfate dissociates UFH–protamine complexes among UFH–protein complexes, explaining higher anti-Xa levels obtained with these reagents and leading to an overestimation of UFH concentrations as suggested in previous studies.[13] Consequently, guidelines should highlight the risk of anti-Xa overestimation related to the reagent used, and encourage each institution to develop local protocols for management and monitoring of UFH and of its neutralization with protamine. In a study comparing ACT and anti-Xa levels after protamine administration in patients undergoing cardiac surgery with CBP, Galeone et al found elevated anti-Xa levels ($0.32 \pm 0.29$ and $0.19 \pm 0.25$ IU/mL 20 minutes and 3 hours after protamine administration, respectively) while ACT was not prolonged; the authors concluded that ACT was not able to detect incomplete heparin reversal and heparin rebound. Noteworthy, anti-Xa levels were measured with a reagent containing dextran (Coamatic Heparin). The dextran-induced dissociation of UFH–protamine complexes could explain the discrepancies observed between ACT and anti-Xa level activity.[22] This raises questions about the debated issue of the so-called "heparin rebound" after cardiac surgery.

The median anti-Xa levels observed in the other patient groups of our study were as expected: low in patients 1 to 5 days after CPB (group 2); around 0.2–0.3 IU/mL in medical ICU patients (group 3) and within the therapeutic range 0.3–0.7 IU/mL in other medical patients (group 4). In these different settings, with a wide range of anti-Xa levels, a significant effect of dextran was also found, although less marked than in the CPB group. The effect was up to 54% increase in anti-Xa levels, in medical ICU patients (group 3), but the effect must be weighed against their low-level median anti-Xa level activity (0.22 vs. 0.31 IU/mL without and with dextran, respectively). Nevertheless, such differences in anti-Xa values related to the presence or absence of dextran could lead to different treatment decision-making.

 

**Table 2** Anti-Xa values measured with reagents containing or not containing dextran, in plasma samples, containing citrate or CTAD, and proportion of patients below the lower limit of quantification, according to patient groups

| | | | Group 1 (G1) CPB 5–10 minutes after protamine neutralization | Group 2 (G2) Cardiothoracic ICU 1–5 days after CPB | Group 3 (G3) Medical ICU | Group 4 (G4) Other medical patients |
|---|---|---|---|---|---|---|
| Reagents without dextran | Citrate | *n* | 78 | 70 | 105 | 76 |
| | | Median (IU/mL)<br>Min–max<br>Values < LLOQ: *n* (%) | 0.05<br>0.05–1.62<br>60 (77%) | 0.05<br>0.05–0.35<br>45 (64%) | 0.22<br>0.05–1.84<br>29 (28%) | 0.47<br>0.05–1.75<br>20 (26%) |
| | CTAD | *n* | 78 | 70 | 102 | 74 |
| | | Median (IU/mL)<br>Min–max<br>Values < LLOQ: *n* (%) | 0.05<br>0.05–1.46<br>57 (73%) | 0.12<br>0.05–0.42<br>30 (43%) | 0.26<br>0.05–1.80<br>17 (17%) | 0.56<br>0.05–1.84<br>13 (18%) |
| Reagents with dextran | Citrate | *n* | 195 | 175 | 256 | 190 |
| | | Median (IU/mL)<br>Min–max<br>Values < LLOQ: *n* (%) | 0.32<br>0.05–1.60<br>12 (6%) | 0.05<br>0.05–0.71<br>88 (50%) | 0.31<br>0.05–2.60<br>19 (7%) | 0.55<br>0.05–2.16<br>27 (14%) |
| | CTAD | *n* | 195 | 175 | 249 | 185 |
| | | Median (IU/mL)<br>Min–max<br>Values < LLOQ: *n* (%) | 0.34<br>0.05–1.52<br>7 (4%) | 0.13<br>0.05–0.67<br>58 (33%) | 0.34<br>0.05–2.68<br>13 (5%) | 0.59<br>0.05–2.12<br>24 (13%) |

Abbreviations: CPB, cardiopulmonary bypass; CTAD, citrated-theophylline-adenosine-dipyridamole; ICU, intensive care unit; LLOQ, lower limit of quantification (0.1 IU/mL).
Note: By convention, all values <0.1 IU/mL were referred as 0.05 IU/mL.

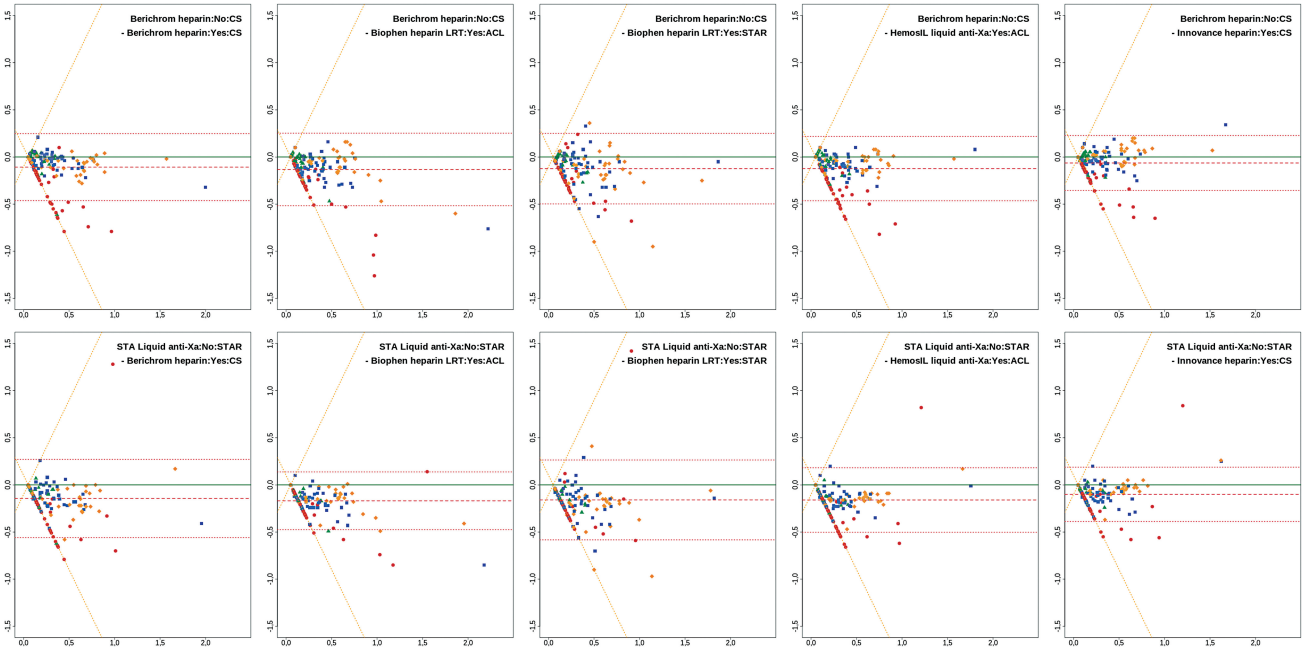


**Fig. 2** Pairwise Bland–Altman plots between anti-Xa assays using reagents without dextran sulfate ("no"—i.e., upper plots: Berichrom heparin; lower plots: STA Liquid anti-Xa) or with ("yes") dextran sulfate in *citrated* samples. Plots were built analyzing the difference of anti-Xa levels (in IU/mL); the two conditions are indicated in each plot (without/with dextran sulfate) (*Y*-axis) as a function of the average (*X*-axis). The horizontal solid green line shows identity between the two conditions. The horizontal dotted lines indicate the mean absolute bias and its 95% prediction interval. The yellow diagonal dotted lines correspond to values ≥0.10 IU/mL (LLOQ) obtained with one method and values below LLOQ obtained with the second method. Each patient is represented with a symbol: red dots (group 1: CPB after protamine neutralization), green triangles (group 2: cardiothoracic ICU), blue squares (group 3: medical ICU), and orange diamonds (group 4: other medical patients). CPB, cardiopulmonary bypass; ICU, intensive care unit; LLOQ, lower limit of quantification.

Noteworthy, the UFH therapeutic ranges were defined using reagents without dextran sulfate.[23] Different local acceptance ranges are probably needed according to the reagent used.

The effect of dextran was specifically illustrated using the same reagent with and without dextran sulfate (Berichrom assay modified as described above). Using a similar approach, significant differences in anti-Xa results were found in samples from CPB patients after administration of protamine or from pediatric patients receiving UFH, run with Rotachrom Heparin and Coamatic assays in presence or absence of dextran sulfate.[13,19]

When the anticoagulant effect of UFH is monitored using the aPTT, another important issue of the discrepancies between anti-Xa levels according to assays is the aPTT range that should be established for each reagent lot/coagulometer against anti-Xa levels, as recommended by the American College of Chest Physicians consensus group.[1]

A few decades ago, CTAD tubes were commercialized to minimize the effect of platelet activation-induced protein release when monitoring heparin.[14,15] Conflicting results have been published regarding the interest of using reagents containing dextran sulfate or of collecting blood into tubes containing CTAD to minimize the effect of heparin binding to plasma proteins on the measurement of anti-Xa levels. Some authors concluded that the use of citrate led to an underestimation of anti-Xa in the absence of dextran sulfate,[10] whereas others showed no difference between citrate and CTAD when anti-Xa was measured with a reagent without dextran after a delayed centrifugation of 4 hours.[24] We found that in all groups of patients, the effect of dextran was found both in citrated and in CTAD plasma samples. Since CTAD is used to prevent in vitro platelet activation and thus partial UFH neutralization, one hypothesis is that dextran also dissociates the in vivo binding of heparin chains to plasma proteins and thus may lead to inappropriate decrease in heparin doses.

In our study, the vast majority of samples were centrifuged within 1 hour after sampling, which should have prevented in vitro UFH neutralization.[16,24] However, we found lower anti-Xa levels in citrated plasma samples compared to CTAD ones. The effect was independent of clinical settings and of dextran effect. The effect, although limited (on average, +15% in CTAD samples), combined with that of dextran may also have clinical relevance for high anti-Xa levels.

The type of calibration may contribute to the inter-assay variability. It has been shown that STA-Liquid anti-Xa assay on STA-R Max could lead to an underestimation of UFH concentrations, especially at low UFH concentrations, when comparing calibration obtained with manufacturer UFH calibrators, UFH-International Standard, or manufacturer hybrid calibrators (UFH/LMWH calibrators).[10] We cannot exclude a contribution of this effect to our results; however, in contrast to Amiral et al's study,[10] we did not use a hybrid calibration. Moreover, we analyzed all samples using the International Standard for UFH and found a similar effect of dextran to manufacturer calibrators.

 

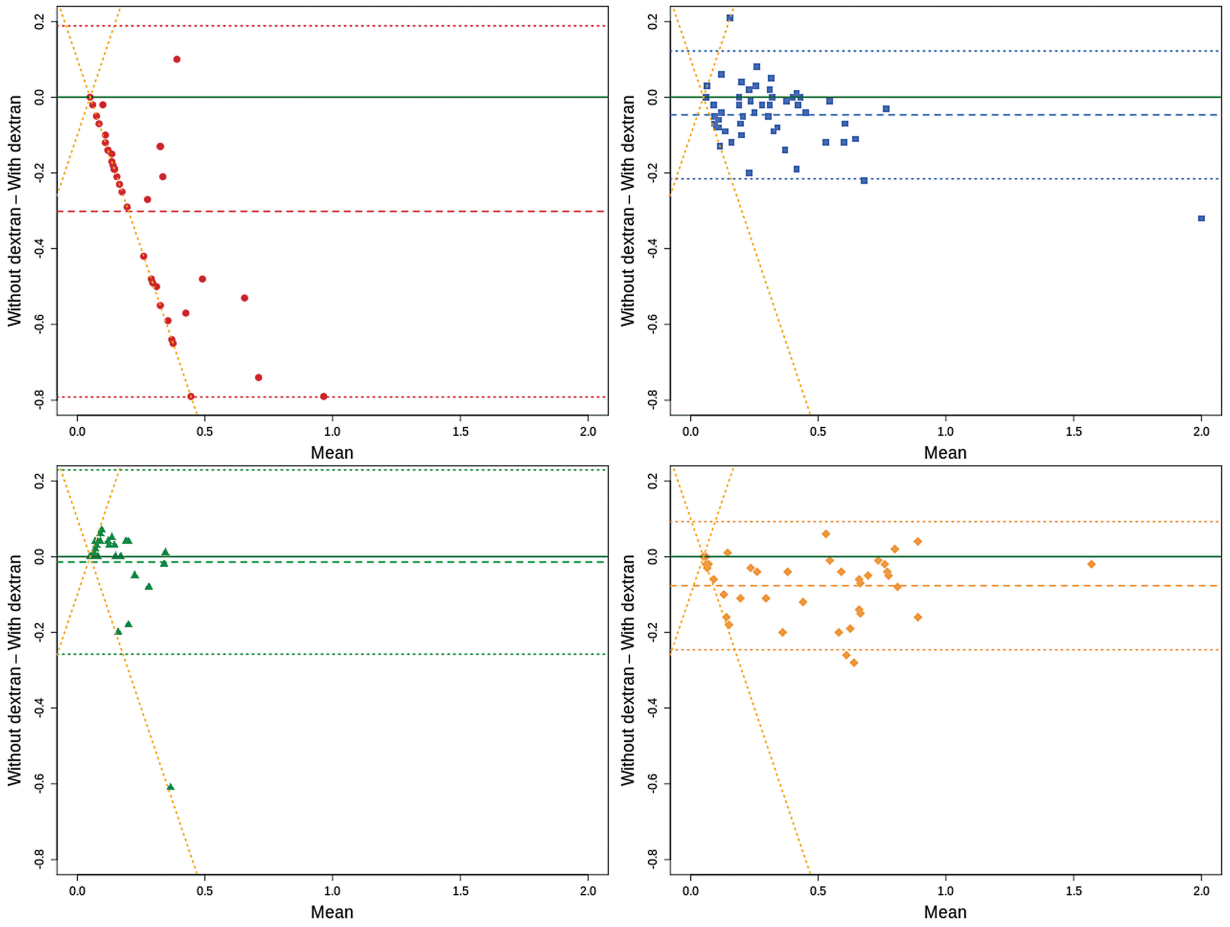


**Fig. 3** Pairwise Bland–Altman plots between anti-Xa assays using Berichrom heparin assay without and with dextran sulfate in *citrated* samples in the four groups of patients. Plots were built analyzing the difference of the two conditions (without/with dextran sulfate in IU/mL) (*Y*-axis) as a function of the average (*X*-axis). The horizontal solid green line shows identity between the two conditions. The horizontal dotted lines indicate the mean absolute bias and its 95% prediction interval. The yellow diagonal dotted lines correspond to values ≥0.10 IU/mL (LLOQ) obtained with one method and values below LLOQ obtained with the second method. Each patient is represented with a symbol: red dots (group 1: CPB after protamine neutralization), green triangles (group 2: cardiothoracic ICU), blue squares (group 3: medical ICU), and orange diamonds (group 4: other medical patients). CPB, cardiopulmonary bypass; ICU, intensive care unit; LLOQ, lower limit of quantification.

**Table 3** Impact of dextran sulfate and CTAD on anti-Xa levels using a linear mixed-effects model

| | Group 1<br>Post-CPB<br>5–10 minutes<br>after protamine<br>neutralization | Group 2<br>Cardiothoracic ICU<br>1–5 days<br>post-CPB | Group 3<br>Medical<br>ICU | Group 4<br>Other medical<br>patients |
|---|---|---|---|---|
| Citrate<br>No dextran (geometric mean anti-Xa level (UI/mL) | 0.077<br>CI: 0.055–0.108 | 0.083<br>CI: 0.058–0.118 | 0.187<br>CI: 0.1410–0.248 | 0.280<br>CI: 0.200–0.391 |
| CTAD<br>instead of citrate | +7.9%<br>CI: −1.6 to +18.3 | +23.9%<br>CI: +12.4 to +36.6 | +13.8%<br>CI: +4.9 to +23.4 | +15.7%<br>CI: +5.2 to +27.1 |
| Effect of dextran | +296.0%<br>CI: 257.7–338.4 | +37.8%<br>CI: 23.7–53.5 | +53.3%<br>CI: 40.3–67.6 | +30.2%<br>CI: 17.4 to 44.5 |

Abbreviations: CI, confidence interval; CPB, cardiopulmonary bypass; CTAD, citrated-theophylline-adenosine-dipyridamole; ICU, Intensive care unit.
Note: Results were obtained using a linear mixed-effects model of the logarithm of the anti-Xa levels, with the patient as a random effect on the intercept, and group, presence of dextran, and type of tube as fixed effects. The model included two interaction terms: between type of tube and group, and between presence of dextran and group. There was no significant other interactions.

 

The study has several limitations. First, we could not design the study to test each reagent with and without dextran; we were able to run only one reagent with and without dextran. Nevertheless, we used a statistical approach allowing modeling the effect of dextran. Second, our study was not designed to determine which assay was the most reliable to monitor patients in terms of risk-to-benefit ratio. A prospective study with primary clinical endpoints only could provide such information, the feasibility of which could be difficult however.

In conclusion, we evidenced an important variability of anti-Xa levels in plasma of patients receiving UFH according to reagents containing or not containing dextran, tubes (citrate vs. CTAD), and patient conditions. Such a variability could lead to different treatment decisions. Especially, after neutralization of heparin by protamine in the context of CPB or heparin overdosing, anti-Xa testing using a reagent containing dextran should be interpreted with much caution since in presence of dextran anti-Xa levels are greatly overestimated. There is a clear need for a better anti-Xa reagent standardization and validation in different patient settings.

## What is known about this topic?

- Discrepancies between anti-Xa levels according to assays have been pointed out for many years.
- Different parameters might potentially contribute to the heterogeneity of anti-Xa levels, among which are the blood collection tube (citrate vs. CTAD) and the presence of dextran sulfate in reagents.

## What does this paper add?

- We evaluated seven reagent/analyzer combinations on samples from 165 patients from different settings including cardiothoracic ICU patients.
- Anti-Xa levels were much higher using reagents containing dextran sulfate and slightly higher in CTAD versus citrate samples, depending on the patient group.
- Anti-Xa testing with a reagent-containing dextran should be used with caution after heparin neutralization by protamine since in presence of dextran anti-Xa levels are greatly overestimated.

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Conflict of Interest
None declared.

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