Received: 20 July 2023 | Revised: 20 September 2023 | Accepted: 18 October 2023

https://doi.org/10.1016/j.rpth.2023.102257

ORIGINAL ARTICLE

Reassessment of dextran sulfate in anti-Xa assay for unfractionated heparin laboratory monitoring

Michael Hardy[1,2,3] | Julien Cabo[1] | Antoine Deliège[1] | Jonathan Douxfils[4,5] | Isabelle Gouin-Thibault[6] | Thomas Lecompte[4,6,7] | François Mullier[1,3,4]

[1]CHU UCL Namur, Namur Thrombosis and Hemostasis Center, Namur Research Institute for Life Sciences, Hematology Laboratory, Yvoir, Belgium

[2]CHU UCL Namur, Namur Thrombosis and Hemostasis Center, Namur Research Institute for Life Sciences, Department of Anesthesiology, Yvoir, Belgium

[3]Université catholique de Louvain, Institut de Recherche Expérimentale et Clinique, Pôle Mont, Yvoir, Belgium

[4]University of Namur, Department of Pharmacy, Namur Thrombosis and Hemostasis Center, Namur Research Institute for Life Sciences, Namur, Belgium

[5]QUALIblood s.a., Namur, Belgium

[6]CHU of Rennes, Department of Laboratory Hematology, IRSET-INSERM-1085, Rennes, France

[7]University of Lorraine, CHRU of Nancy, Nancy, France

Correspondence
François Mullier, Department of Laboratory Medicine, CHU UCL Namur–Site de Godinne, Avenue Docteur G. Thérasse, 1, 5530 Yvoir, Belgium.
Email: francois.mullier@chuuclnamur.uclouvain.be

Handling Editor: Dr Johnny Mahlangu

Abstract

Background: Anti-Xa assays are used for unfractionated heparin (UFH) monitoring. Dextran sulfate (DS) is used in some assays to overcome the artifactual preanalytical release of platelet factor 4. However, the practical implications of this test modification have not been studied extensively.

Objectives: To investigate the impact of the presence of DS in the anti-Xa assay for UFH laboratory monitoring.

Methods: We studied factor Xa inhibition, using an assay without DS (Stago Liquid Anti-Xa), in normal pool plasma spiked with various concentrations of UFH (up to 1 IU/mL) in the presence of increasing concentrations of DS (up to 2560 μg/mL). We also investigated the effect of DS on FXa inhibition measured after the addition of UFH and heparin antagonists (protamine and Polybrene; Sigma Aldrich). Eventually, we compared the anti-Xa levels measured using the assay without DS to those measured with an assay containing DS (BIOPHEN Heparin LRT, Hyphen BioMed).

Results: DS per se had a detectable anti-Xa effect. FXa inhibition in UFH-spiked plasma linearly increased with increasing concentrations of added DS, with a plateau at approximately 160 μg/mL DS, at which the apparent anti-Xa level had almost doubled. In the presence of heparin antagonists, the addition of DS increased anti-Xa levels, corresponding to the dissociation of the UFH-antagonists complexes *in vitro*. With the anti-Xa assay containing DS, UFH inhibition was not detected.

Conclusion: In the presence of high concentrations of DS, FXa inhibition was much higher than that predicted from added UFH amounts, presumably related to the greater availability of UFH for interaction with antithrombin. While the relevance of measuring this "masked" heparin has not been demonstrated, the presence of DS renders the result inaccurate in the presence of protamine or Polybrene.

KEYWORDS
anti-Xa, dextran, heparin, polybrene, protamine, unfractionated heparin

Michael Hardy, Julien Cabo, Thomas Lecompte, and François Mullier contributed equally to this study.

Essentials

- Low concentrations of dextran are used in some anti-Xa reagents to monitor heparin.
- Only high concentrations of dextran can recover all mobilizable unfractionated heparin.
- Even low concentrations of dextran dissociate heparin-protamine complexes.
- The use of dextran in anti-Xa tests is therefore highly questionable.

1 | INTRODUCTION

Chromogenic anti-Xa assays tend to be advocated over activated partial thromboplastin time to monitor unfractionated heparin (UFH) therapy in the clinical laboratory [1–3]. A large interassay variability has been described between the different kits available, especially for low UFH concentrations [4–11]. One substantial difference between the assays is the presence or the absence of dextran sulfate (DS). The addition of DS to these reagents aims at recovering "masked heparin," defined as heparin bound to proteins or cells and not available for antithrombin (AT) binding. Indeed, UFH binds aspecifically to a variety of proteins and membrane-binding proteins (eg, histidine-rich glycoprotein, vitronectin, fibroblast growth factors, annexins, etc.) in the blood and is then biologically inactive [12–19]. In addition, platelet activation following tedious blood collection procedure can result in the release of platelet factor 4 (PF4), which is further capable of binding UFH and thus decreasing the apparent anti-Xa level [20]. To address these 2 issues, Lyon et al. [21] proposed, in 1987, the addition of DS in the anti-Xa assay to recover all the so-called "masked heparin" among others by the artifactual release of PF4 in the preanalytical step and thus assess the total amount of heparin present in the sample. In 2003, Mouton et al. [5] were the first to highlight the potential adverse effect of adding DS in anti-Xa assays in the context of cardiac surgery. They demonstrated that when UFH was neutralized by protamine sulfate *in vivo* or after supplementation *in vitro*, the anti-Xa level measured with a dextran-containing assay was higher than that measured using a dextran-free assay. They hypothesized that DS was capable of dissociating protamine/heparin complexes, thus recovering the total heparin anti-Xa effect.

In clinical laboratories, hexadimethrine bromide (Polybrene) can be used to neutralize UFH in clinical samples [22–24]. As the mechanism of action is like that of protamine sulfate, we hypothesized that the presence of DS could also dissociate hexadimethrine bromide-UFH complexes.

It is still extensively debated today if the presence of DS provides a more reliable surrogate for UFH activity *in vivo* [8,25,26]. Lack of standardization leads to discrepancies in anti-Xa measurements that might have important implications for clinical decision making [9]. The standardization of these assays is mandatory, and the question on the relevance of adding DS in the composition of anti-Xa assays must be fixed. Therefore, we studied the ability of increasing DS concentrations to recover the entire UFH level present in plasma samples. We further investigated the impact of the presence of DS in the anti-Xa assay when protamine sulfate and hexadimethrine bromide, 2 UFH inhibitors, are present.

2 | MATERIALS AND METHOD

2.1 | Materials

This *in vitro* study was performed using commercial normal pool plasma (NPP) (CRYOcheck, Precision BioLogic). UFH and protamine sulfate were purchased from LEO Pharma (; Heparin LEO and Protamine LEO; Heparin Leo is sourced from porcine intestinal mucosa) and DS sodium salt and hexadimethrine bromide (Polybrene) from Sigma-Aldrich. According to the manufacturer, the molecular mass of DS was approximately 8000 Da and the sulfur content was 17% to 20%, which is equivalent to 2.3 sulfate groups per glucosyl residue on average [27].

All intermediate dilutions of the reagents were made in water for injection (Aqua ad Iniectabilia B. Braun). To reflect clinical use, protamine sulfate was used in a ratio of 1U of protamine to 1U of UFH in the sample. Conversely, in accordance with its usual laboratory use, hexadimethrine bromide was used at a constant concentration of 25 µg/mL [22,28–31]. The NPP used in this study was frozen platelet-poor plasma (PPP) constituted by plasmapheresis from a minimum of 20 healthy donors mixed in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (CRYOcheck) [32]. The same batch (A1301) was used for all the study.

Anti-Xa levels were measured with a STA-R Max 2 instrument (Diagnostica Stago) using a chromogenic assay (STA-Liquid Anti-Xa, Diagnostica Stago; final dilution of plasma 1:12). The absorbance signal was reported on a calibration curve to provide an anti-Xa level (STA Multihep calibrator, Diagnostica Stago). The calibration curves range from 0.10 IU/mL (lower limit of quantification [LLOQ]) to 2.00 IU/mL (upper limit of quantification). We also compared the STA-Liquid Anti-Xa assay, which does not contain exogenous DS, to BIOPHEN Heparin LRT (Hyphen BioMed), which does. The calibration of the BIOPHEN Heparin LRT was realized using the BIOPHEN UFH Calibrator [Hyphen BioMed] from 0 to 1.5 IU/mL). The assay contains low-molecular-weight DS (8000 Da; from 6500 to 10,000) at a concentration between 15 and 18 µg/mL in the R2 reagent (which represents 5/12 of the final volume for 1/12 volume of plasma), corresponding to 75 to 90 mcg/mL of DS if DS was added to the plasma (as in our study) instead of to the reagent, and a sulfur content of 16% to 20%, according

to the manufacturer. The LLOQ of this method is 0.05 IU/mL. The tests measure factor Xa inhibition, which is expressed as heparin anti-Xa levels according to the UFH calibration performed.

2.2 | Methods

The study was conducted at the hematology laboratory of the CHU UCL Namur. Each test condition was performed in quintuplicate.

First, FXa inhibition was measured in plasma after addition of UFH concentrations of 0.0, 0.1, 0.3, 0.5, 0.7, and 1.0 IU/mL and increasing concentrations of DS (from 0.1 μg/mL to 2560 μg/mL). This range of concentrations was experimentally designed to define the floor and ceiling concentrations of DS. The floor concentration was defined as the DS concentration below which no significant increase in anti-Xa level is observed, compared with a sample without DS. The ceiling concentration was defined as the DS concentration above which the anti-Xa level does not increase anymore even with increased DS concentrations.

The second part of the study aimed at studying the effect of DS in NPP spiked with both UFH (0, 0.1, 0.5, and 1.0 IU/mL) and heparin inhibitors (protamine sulfate and hexadimethrine bromide). Based on the results of the first part of the study, we used DS concentrations up to 640 μg/mL.

We then measured the FXa inhibition of NPP spiked with UFH in the presence and in the absence of inhibitors (ie, hexadimethrine bromide and protamine sulfate, respectively), comparing an anti-Xa kit that does not contain dextran (ie, STA®-Liquid Anti-Xa) with a kit that does (BIOPHEN Heparin LRT). Finally, we performed cross-calibration between Stago's and Hyphen's assays and calibrators to assess the impact of the calibration on the differences observed between the 2 assays: we measured FXa inhibition in NPP with added UFH concentration from 0 to 1.0 IU/mL with Liquid Anti-Xa (no DS) and BIOPHEN LRT (with DS) assays; each assay was calibrated with Stago's (STA Multihep calibrator) or with Hyphen's (BIOPHEN UFH calibrator) calibrator. FXa inhibition (anti-Xa activity) was compared between both reagents and calibration.

On the day of the experiment, NPP aliquots were thawed at 37 °C in a water bath for 5 minutes and were gently inverted before use. The spiking sequence was UFH first, followed when applicable by the heparin inhibitor (protamine sulfate or hexadimethrine bromide) and finally DS after incubation of 5 minutes. Samples were gently inverted for homogenization between each reagent added in the reactional mixture. The dilution rate of the NPP for addition of the spiked component was calculated to remain constant and was fixed at 1/20, that is, 1 part of spiked solution for 19 parts of NPP. This permits to avoid a dilution effect of the plasma. All samples were analyzed within 30 minutes after spiking to avoid any UFH degradation.

Results are presented as mean ± SD of the quintuplicate. DS concentrations were compared using Friedman tests. Alpha was set a 0.05, and all tests were 2-sided. Data analyses were performed using R (version 4.1.0) [33].

3 | RESULTS

3.1 | Effect of DS in UFH samples

The effect of DS on apparent anti-Xa heparin levels is represented in Figure 1 and in Supplementary Table S1.

In the presence of UFH, DS had no effect on FXa inhibition until 10 μg/mL ($P > .05$). From that DS concentration, the measured FXa inhibition increased progressively with DS concentration and was higher than predicted from UFH amounts added. For higher UFH concentrations (≥0.5 IU/mL), an anti-Xa plateau was observed from 160 to 640 μg/mL DS, depending on the UFH concentration added. At those DS concentrations, the apparent anti-Xa level was roughly twice that observed in the absence of DS. For the lowest UFH concentrations (≤0.3), no plateau was reached even at the highest DS concentration tested (ie, 2560 μg/mL).

In the absence of UFH, FXa inhibition increased progressively with increasing DS concentration from 640 μg/mL ($P = .025$). At the maximal DS concentration tested (ie, 2560 μg/mL), the median apparent anti-Xa level was 0.53 IU/mL (IQR, 0.48-0.55).

3.2 | Effect of DS in UFH-spiked samples in the presence of hexadimethrine bromide (Polybrene)

The effect of increasing DS concentrations on apparent anti-Xa heparin levels measured in plasma samples containing UFH and hexadimethrine bromide 25 μg/mL is presented in Figure 2 and Supplementary Table S2.

In the absence of DS, UFH was fully neutralized by hexadimethrine bromide, as demonstrated by anti-Xa levels below the LLOQ (ie, 0.1 IU/mL). When DS was added in the samples from a concentration of 40 to 80 μg/mL, the apparent anti-Xa level increased ($P = .025$). At the highest DS concentration tested (ie, 640 μg/mL), the apparent anti-Xa level was similar to that observed in the absence of hexadimethrine bromide.

3.3 | Effect of DS in UFH-spiked samples in the presence of protamine sulfate

The effect of increasing DS concentrations on apparent anti-Xa heparin levels investigated in samples containing UFH and protamine sulfate (in a 1:1 ratio) at different concentrations is presented in Figure 3 and in Supplementary Table S3.

In the absence of DS, UFH was fully neutralized by protamine sulfate, as demonstrated by anti-Xa levels below the LLOQ (ie, 0.1 IU/

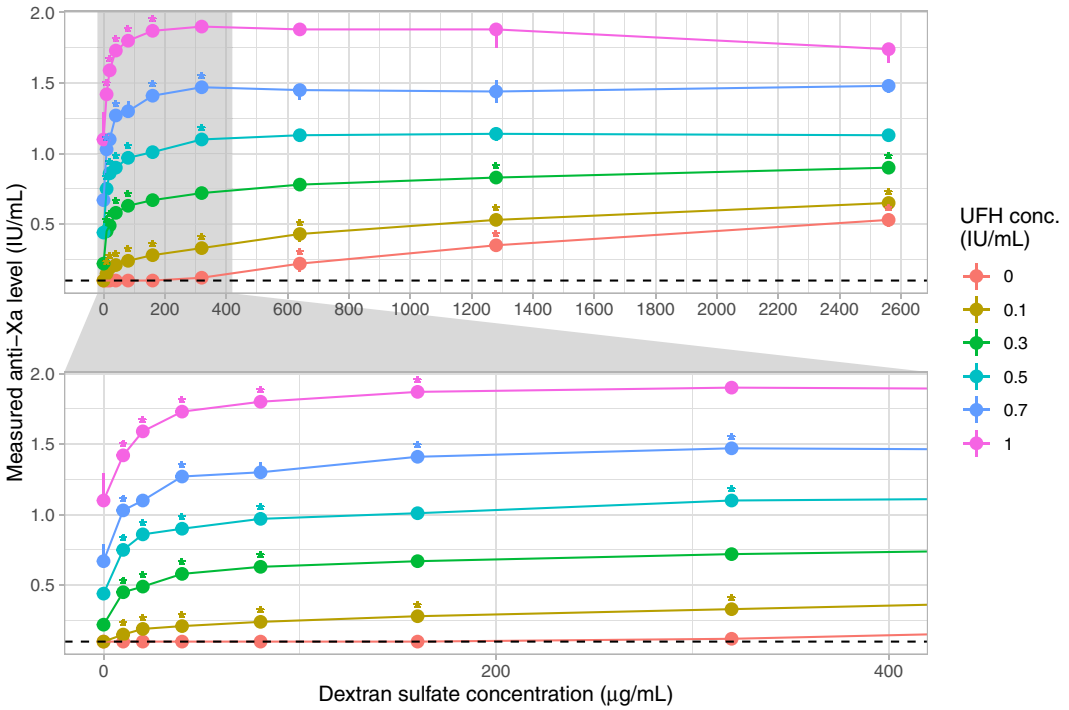

FIGURE 1 Apparent anti-Xa heparin levels according to the unfractionated heparin (UFH) calibration at increasing concentrations of dextran sulfate (DS) in normal plasma spiked with various concentrations of UFH. Upper panel: anti-Xa level (median, IQR of the 5 replicates) was measured using Liquid Anti-Xa reagent (Stago, no DS) in normal plasma samples spiked with UFH from 0 to 1.0 IU/mL and increasing concentrations of DS. Lower panel: enlargement of the upper panel focused on low DS concentrations. Asterisks represent statistically significant increases in apparent anti-Xa levels relative to the next lower DS concentration. DS concentrations lower than 10 µg/mL did not modify the measured anti-Xa levels and are not shown. On the opposite, high concentrations of DS resulted in higher apparent anti-Xa levels, compared to UFH-spiked samples without DS. For UFH concentrations of 0.5 to 1.0 IU/mL, the DS plateau was reached at a DS concentration between 160 and 640 µg/mL. For UFH concentrations of 0.3, 0.1, and 0 IU/mL, the plateau has never been reached despite very high DS concentrations. The dashed line represents the lower limit of quantification of the assay (ie, 0.10 IU/mL).

mL). When DS was added in the samples from a concentration of 10 µg/mL, the apparent anti-Xa level increased (P = .025). At the highest DS concentration tested (ie, 640 µg/mL), the apparent anti-Xa level was similar to that observed in the absence of protamine sulfate.

3.4 | Comparison of 2 anti-Xa assays, with or without DS

In the absence of a neutralizing agent (ie, hexadimethrine bromide or protamine sulfate), the measured anti-Xa levels were always higher when measured using the assay containing DS (Figure 4). When hexadimethrine bromide or protamine sulfate was added to UFH samples, the measured anti-Xa level was always below the LLOQ with the assay that does not contain DS. With the DS-containing test, inhibition of UFH by protamine and Polybrene was incorrectly assessed: anti-Xa levels above the LLOQ were measured while all UFH should have been neutralized.

The cross-calibration procedure confirmed a higher measured anti-Xa level with the Hyphen kit (Hyphen calibration), compared to the Stago kit (Stago calibration). The cross-calibration (Hyphen kit calibrated with Stago calibrator and Stago kit calibrated with Hyphen calibrator) yielded intermediate anti-Xa levels compared to those measured previously (Figure 5).

4 | DISCUSSION

This study demonstrated that the apparent anti-Xa level stabilized at its maximum value only at high concentrations of DS (ie, from 160 µg/mL). However, these high DS concentrations were associated with significant FXa inhibition when there was no heparin added. Furthermore, even low concentrations of DS (ie, 10-40 µg/mL) were able to displace UFH from protamine sulfate and hexadimethrine bromide, resulting in an overestimation of the biologically active UFH level. All in all, these results question the use of DS in anti-Xa assays.

In the presence of UFH, the addition of DS was associated with a concentration-dependent increase in the apparent anti-Xa activity, far beyond what was predicted from the amounts of UFH added. At the plateau of the effect (ie, around 160 µg/mL of added DS), the apparent anti-Xa level almost doubled, which is consistent with previous work performed using low-affinity heparin lacking anti-Xa activity [16]. At lower UFH concentrations, no plateau was achieved at the maximal

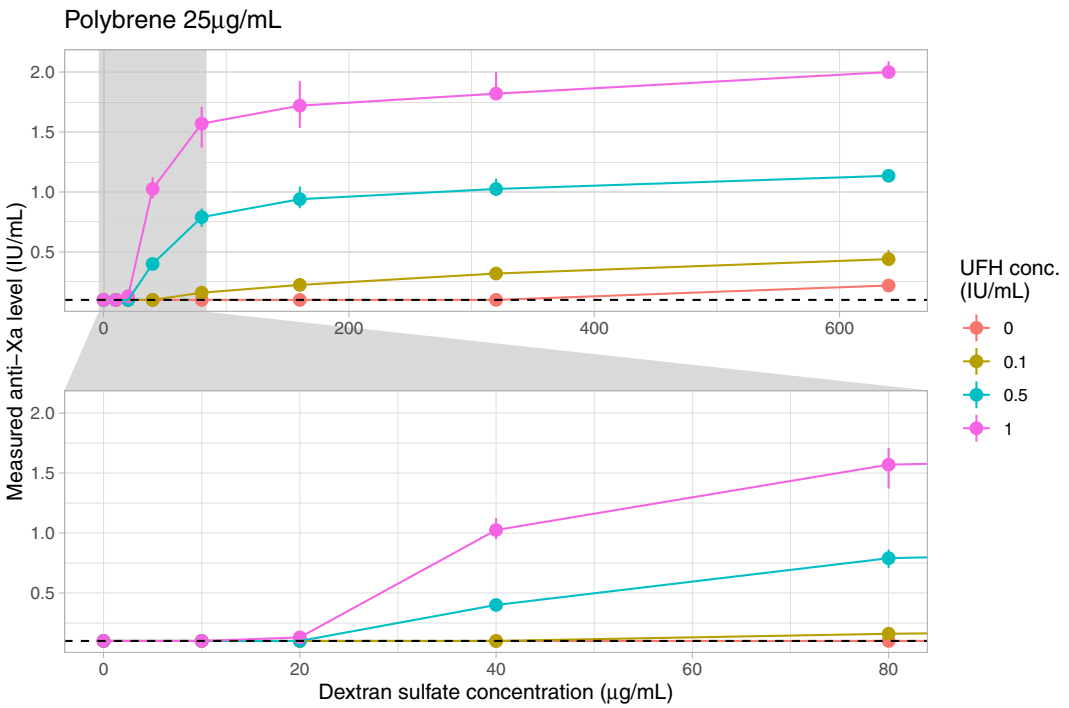

FIGURE 2 Apparent anti-Xa heparin levels according to the unfractionated heparin (UFH) calibration at increasing concentrations of dextran sulfate (DS) in normal pool plasma spiked with various concentrations of UFH and hexadimethrine bromine 25 µg/L (Polybrene). Upper panel: apparent anti-Xa level (median, IQR) according to the added DS concentration in samples (n = 5 per condition) containing increasing UFH concentrations (0, 0.1, 0.5, and 1.0 IU/mL) and hexadimethrine bromide 25 µg/mL. The dashed line represents the lower limit of quantification of the assay (ie, 0.1 IU/mL). Lower panel: enlargement of the upper panel focused on low DS concentrations.

DS concentration tested. The increased FXa inhibition observed after addition of DS could be related to the displacement of UFH by DS (or other molecules displaying an anti-Xa activity) from its so-called aspecific, non-AT binding (ie, its interactome) [16], sometimes called "mobilizable" or "masked" heparin. In blood, UFH is bound reversibly by electrostatic interactions to many plasma proteins and blood cells surfaces [12–19] (see [19] for a review). This would imply that almost half of the UFH molecules present in a normal plasma sample are bound to the interactome and not available for AT binding. The proportion of UFH bound to its interactome, biologically inactive, is variable from one individual to another, depends on the clinical context (eg, increased during the acute phase reaction [34]), and contributes to the complexity of UFH pharmacokinetics [35]. However, it remains to be determined to what extent this non-AT bound UFH could contribute to the overall anticoagulant effect *in vivo*. The additional factor Xa inhibition measured after the addition of DS could also be related, at least in part, to the inhibition of FXa by DS (directly or via AT) and/or to the potentiation of FXa inhibition by the UFH-AT complex by DS, even if to our best knowledge, there are no published data to date supporting this hypothesis.

In the absence of UFH, we observed a concentration-dependent FXa inhibition increasing linearly with the concentration of added DS. This could either be the result of an effect of DS on FXa (directly or via AT) and/or the unmasking of the anti-Xa activity of endogenous substances, such as circulating endogenous glycosaminoglycans [36]; indeed, glycosaminoglycans such as heparan sulfate, dermatan sulfate, or chondroitin sulfate are long sulfated polysaccharide chains that can inhibit FXa in an AT-dependent mechanism and, similar to UFH, are able to bind by ionic interactions to a range of plasma proteins, reducing their bioavailability [37–39].

A substantial variability has been reported between different anti-Xa assays, with potential clinical impact in the management of UFH administration [4–11]. The variability was partly attributed to differences in the composition of the reagents (eg, addition or not of DS and of exogenous AT, the type of FXa and chromogenic substrate used, as well as the buffer used to perform the dilution of the sample) and could also be related to differences in calibrators (Figure 5). However, when DS is present in the reagent, both calibration and sample measurement are performed in the presence of DS. Differences between kits with and without DS are therefore only expected if the size or properties of the interactome differ between calibrator and sample plasma. For patient samples, this could be the case in the presence of a preanalytical artifact (eg, PF4 release), but recent studies have relativized its magnitude on UFH level measurement. Indeed, small differences in anti-Xa levels have been found between samples collected in citrate tubes and those collected in citrate, theophylline, adenosine, and dipyridamole (CTAD) tubes, with the latter used in order to minimize platelet activation and ensuing PF4 release during blood collection and processing [40–42]. It would therefore appear that, provided that the blood sample was carefully drawn, the use of DS in the anti-Xa reagent is not essential for a reliable measurement of anti-Xa levels.

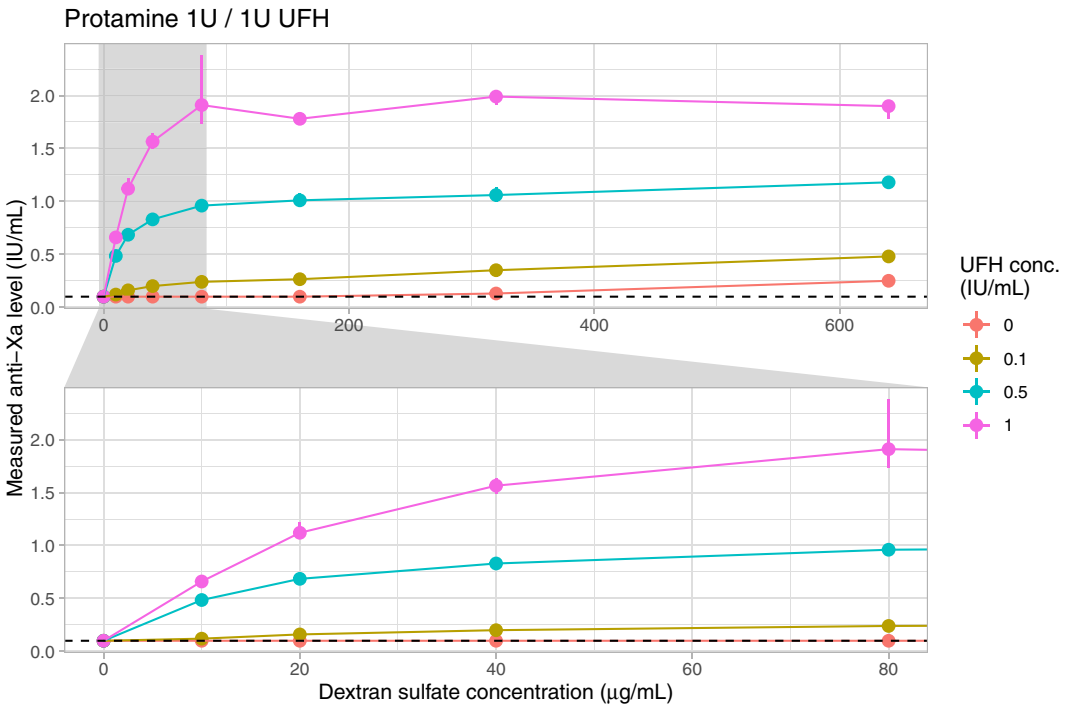

FIGURE 3 Apparent anti-Xa heparin levels according to the unfractionated heparin (UFH) calibration at increasing concentrations of dextran sulfate (DS) in normal pool plasma spiked with various concentrations of UFH and protamine sulfate. Upper panel: apparent anti-Xa level (median, IQR) according to the added DS concentration in samples (n = 5 per condition) containing increasing UFH concentrations (0, 0.1, 0.5, and 1.0 IU/mL) and protamine sulfate (1 unit of protamine sulfate for 1 unit of UFH). The dashed line represents the lower anti-Xa limit of quantification of the assay (ie, 0.1 IU/mL). Lower panel: enlargement of the upper panel focused on low DS concentrations.

Finally, we demonstrated that when measuring UFH anti-Xa level in a sample containing protamine sulfate or hexadimethrine bromide, the measure was inaccurate if the assay contained DS. Indeed, DS can easily displace UFH bound to protamine sulfate or hexadimethrine bromide, thus erroneously increasing the apparent anti-Xa level. This effect had already been identified for protamine sulfate by Mouton et al. [5] and more recently in the DEXHEP trial [43], but the effect of the added concentration of DS had never been studied so far.

One limitation of this study is its *in vitro* design. However, previous studies performed using *in vivo* samples of patients receiving UFH also identified increased FXa inhibition after the addition of low DS concentration [5,43]. Second, we used commercial NPP produced by plasmapheresis, which could behave differently from PPP obtained by direct blood collection and centrifugation. However, during the design phase of the study, we compared commercial NPP to fresh PPP and observed that, despite slightly greater UFH interactome in commercial NPP (lower anti-Xa level recovery after UFH spiking), the effect of added DS was similar (not shown). The results could also be different if patients' samples were used in which the proportion of UFH interactome could vary, for example, during acute phase reaction. Third, we evaluated only 1 type of DS and 1 type of UFH. It is likely that the type of dextran used influences its affinity to the interactome. Indeed, DS interacts with UFH-binding proteins through aspecific electrostatic interactions via its sulfate groups. It is therefore likely that the effectiveness of DS depends on the length of the chains (molecular mass) and the degree of sulfation, which may vary according to the manufacturer [44,45]. However, most manufacturers do not disclose the characteristics (eg, mean molecular mass, concentration, and sulfur content) of the dextran used while it could contribute to the lack of standardization between different anti-Xa reagents. Furthermore, *in vivo*, UFH metabolism is influenced by chain lengths, with longer chains being eliminated more rapidly [46]. The effect of DS could therefore be less in patient samples than in *in vitro* spiked ones, since the longer the chains, the more their binding to heparin interactome. However, the DEXHEP trial [43] demonstrated that in samples obtained from patients who underwent cardiac surgery, the presence of DS could lead to a different clinical decision based on the measurement of residual heparin levels after protamine administration, confirming the effect also when UFH is administered *in vivo*. Finally, this *in vitro* study was not designed to evaluate clinical outcomes and therefore cannot provide an answer as to whether the performance of the anti-Xa assay in presence of added DS has any clinical impact. However, the exclusive use of dextran-free assays would de facto improve interlaboratory reproducibility, which remains an issue with anti-Xa assays.

In conclusion, in order to compensate for the artifactual release of PF4 in the preanalytical step, which is probably of little clinical relevance in most cases, DS should be present in excess in the reagent relative to the physiological interactome. However, such high concentrations of DS greatly increase the apparent anti-Xa activity, which

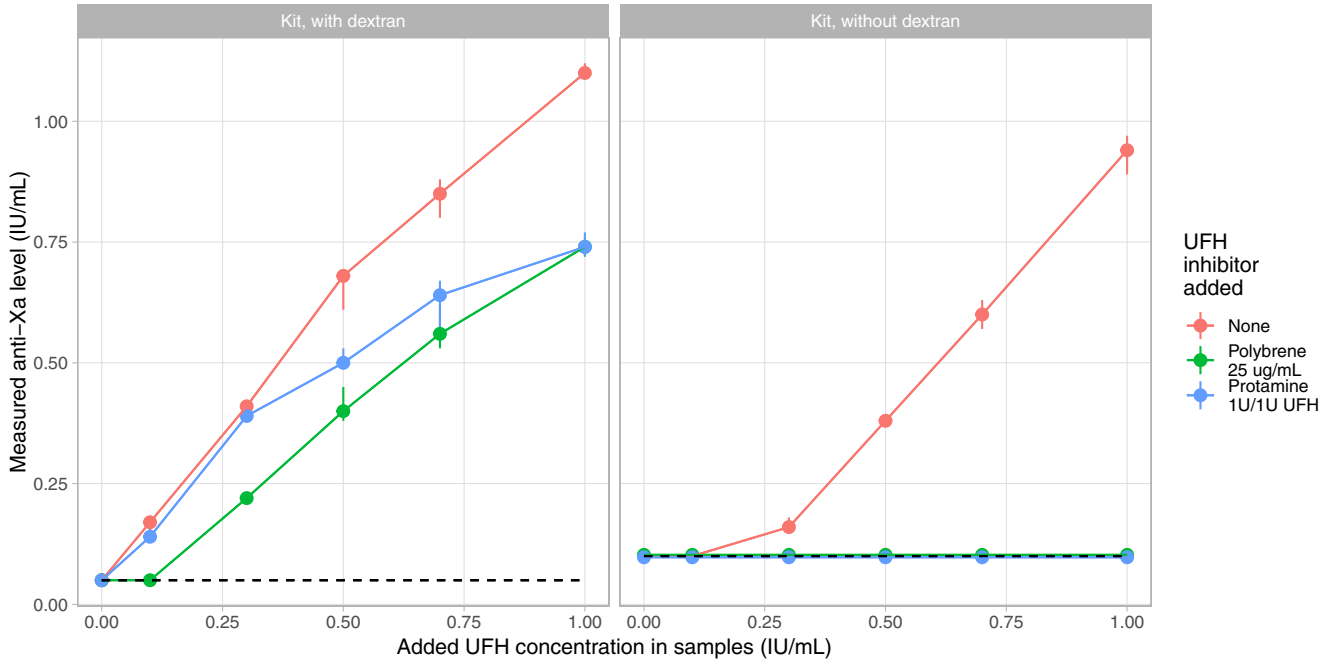

FIGURE 4 Comparison of apparent anti-Xa level according to the unfractionated heparin (UFH) calibration using a kit that contains dextran sulfate (DS) (BIOPHEN Heparin LRT [Hyphen BioMed]; left panel) and a kit that does not (Liquid anti-Xa [Stago]; right panel). Factor Xa inhibition was measured in normal pool plasma (n = 5 per condition) after addition of increasing UFH concentrations (up to 1.0 IU/mL), and heparin antagonists hexadimethrine bromide (25 μg/mL) or protamine sulfate (in a 1U/1U ratio to UFH). Using the kit with DS, the apparent anti-Xa level measured was higher than that in the absence of DS. The presence of DS abolished *in vitro* the effect of heparin antagonists (ie, hexadimethrine bromide and protamine sulfate). Dashed lines represent the lower limit of quantification of the assay (ie, 0.05 IU/mL for BIOPHEN Heparin LRT [left panel] and 0.1 IU/mL for Liquid Anti-Xa [right panel]).

questions the clinical relevance of what is then being measured. Furthermore, the use of DS is inappropriate when heparin has been neutralized by protamine sulfate or hexadimethrine bromide. Therefore, we suggest the use of dextran-free anti-Xa assays, provided that blood collection is performed carefully and the first tube is discarded, thus limiting the amount of PF4 produced artifactually.

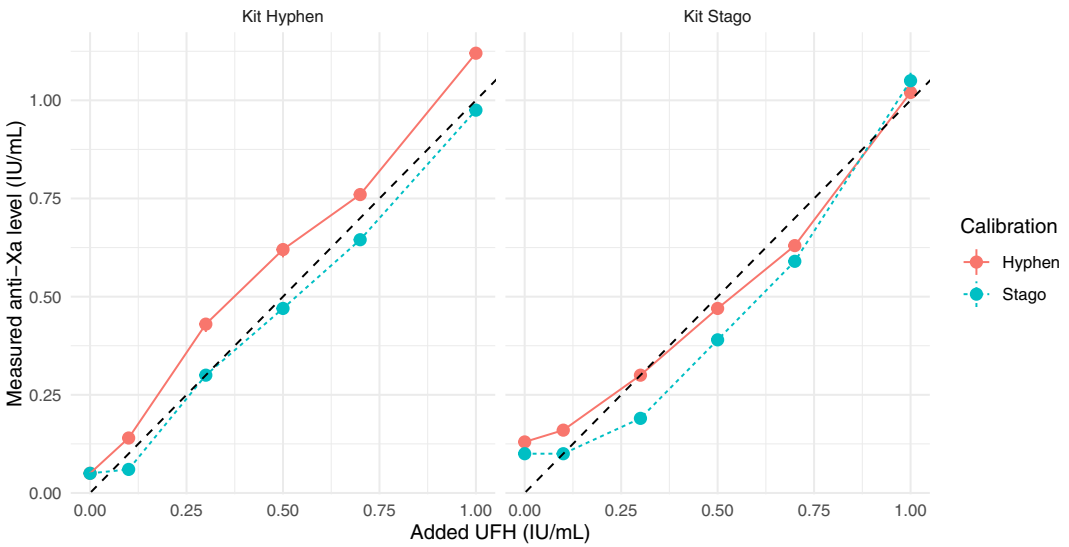

FIGURE 5 Cross-calibration between Stago and Hyphen kits and calibrators. Heparin anti-Xa levels were measured with BIOPHEN Heparin LRT (Hyphen BioMed) or Liquid Anti-Xa (Stago) kits in normal plasma containing increasing concentrations of unfractionated heparin (UFH; 0, 0.1, 0.3, 0.5, 0.7, and 1.0 IU/mL). Both kits were calibrated with Stago's calibrator (STA Multihep Calibrator) or Hyphen's calibrator (BIOPHEN UFH Calibrator, Hyphen BioMed). Each sample was analyzed using both reagents with either calibration. Results are presented as median (IQR) of the 3 replicates.

FUNDING

This work was supported by the Fondation Mont-Godinne.

AUTHOR CONTRIBUTIONS

Conceptualization: M.H., J.C., T.L., F.M. Investigation: M.H., A.D. Formal analysis: M.H., J.C., T.L., F.M. Writing—original draft: M.H., J.C. Writing—review and editing: M.H., J.C., A.D., J.D., I.G.-T., T.L., F.M. Supervision: F.M. All authors have approved the final manuscript.

RELATIONSHIP DISCLOSURE

M.H., A.D., J.C., I.G.-T., and T.L. declare no conflict of interest. J.D. is the Chief Executive Officer and founder of QUALIblood s.a., a contract research organization manufacturing the DP-Filter; is a coinventor of the DP-Filter (patent application number: PCT/ET2019/052903); and reports personal fees from Daiichi-Sankyo, Mithra Pharmaceuticals, Stago, Roche, and Roche Diagnostics, outside the submitted work. F.M. reports institutional fees from Stago, Werfen, Nodia, Roche Sysmex, and Bayer and grant or contract from the National Fund for Scientific Research, Belgium. He also reports speaker fees from Boehringer-Ingelheim, Bayer Healthcare, Bristol-Myers Squibb-Pfizer, Stago, Sysmex, and Aspen, all outside the submitted work.

TWITTER

Jonathan Douxfils @douxfilj

REFERENCES

[1] Samuel S, Allison TA, Sharaf S, Yau G, Ranjbar G, McKaig N, et al. Antifactor Xa levels vs. activated partial thromboplastin time for monitoring unfractionated heparin. A pilot study. *J Clin Pharm Ther*. 2016;41:499–502.

[2] Moores LK, Tritschler T, Brosnahan S, Carrier M, Collen JF, Doerschug K, et al. Prevention, diagnosis, and treatment of VTE in patients with coronavirus disease 2019: CHEST Guideline and Expert Panel Report. *Chest*. 2020;158:1143–63.

[3] Baluwala I, Favaloro EJ, Pasalic L. Therapeutic monitoring of unfractionated heparin - trials and tribulations. *Expert Rev Hematol*. 2017;10:595–605.

[4] Hollestelle MJ, van der Meer FJM, Meijer P. Quality performance for indirect Xa inhibitor monitoring in patients using international external quality data. *Clin Chem Lab Med*. 2020;58:1921–30.

[5] Mouton C, Calderon J, Janvier G, Vergnes MC. Dextran sulfate included in factor Xa assay reagent overestimates heparin activity in patients after heparin reversal by protamine. *Thromb Res*. 2003;111:273–9.

[6] Kitchen S, Theaker J, Preston FE. Monitoring unfractionated heparin therapy: relationship between eight anti-Xa assays and a protamine titration assay. *Blood Coagul Fibrinolysis*. 2000;11:137–44.

[7] Kovacs MJ, Keeney M, MacKinnon K, Boyle E. Three different chromogenic methods do not give equivalent anti-Xa levels for patients on therapeutic low molecular weight heparin (dalteparin) or unfractionated heparin. *Clin Lab Haematol*. 1999;21:55–60.

[8] Hardy M, Douxfils J, Bareille M, Lessire S, Gouin-Thibault I, Fontana P, et al. Studies on hemostasis in COVID-19 deserve careful reporting of the laboratory methods, their significance, and their limitations. *J Thromb Haemost*. 2020;18:3121–4.

[9] Smahi M, De Pooter N, Hollestelle MJ, Toulon P. Monitoring unfractionated heparin therapy: lack of standardization of anti-Xa activity reagents. *J Thromb Haemost*. 2020;18:2613–21.

[10] Cuker A, Raby A, Moffat KA, Flynn G, Crowther MA. Interlaboratory variation in heparin monitoring: lessons from the Quality Management Program of Ontario coagulation surveys. *Thromb Haemost*. 2010;104:837–44.

[11] Toulon P, Smahi M, De Pooter N. APTT therapeutic range for monitoring unfractionated heparin therapy. Significant impact of the anti-Xa reagent used for correlation. *J Thromb Haemost*. 2021;19:2002, 6.

[12] Lijnen HR, Hoylaerts M, Collen D. Neutralization of heparin activity by binding to human histidine-rich glycoprotein. *Thromb Res*. 1983;29:443–6.

[13] Leung LL, Nachman RL, Harpel PC. Complex formation of platelet thrombospondin with histidine-rich glycoprotein. *J Clin Invest*. 1984;73:5–12.

[14] Lijnen HR, Van Hoef B, Collen D. Histidine-rich glycoprotein modulates the anticoagulant activity of heparin in human plasma. *Thromb Haemost*. 1984;51:266–8.

[15] Ruggeri ZM, Zimmerman TS. Von Willebrand factor and von Willebrand disease. *Blood*. 1987;70:895–904.

[16] Young E, Cosmi B, Weitz J, Hirsh J. Comparison of the non-specific binding of unfractionated heparin and low molecular weight heparin (enoxaparin) to plasma proteins. *Thromb Haemost*. 1993;70:625–30.

[17] Schvartz I, Seger D, Shaltiel S. *Vitronectin. Int J Biochem Cell Biol*. 1999;31:539–44.

[18] Jones AL, Hulett MD, Parish CR. Histidine-rich glycoprotein binds to cell-surface heparan sulfate via its N-terminal domain following Zn2 + chelation. *J Biol Chem*. 2004;279:30114–22.

[19] Capila I, Linhardt RJ. Heparin-protein interactions. *Angew Chem Int Ed Engl*. 2002;41:391–412.

[20] Levine SP, Sorenson RR, Harris MA, Knieriem LK. The effect of platelet factor 4 (PF4) on assays of plasma heparin. *Br J Haematol*. 1984;57:585–96.

[21] Lyon SG, Lasser EC, Stein R. Modification of an amidolytic heparin assay to express protein-bound heparin and to correct for the effect of antithrombin III concentration. *Thromb Haemost*. 1987;58:884–7.

[22] Tientadakul P, Kongkan C, Chinswangwatanakul W. Use of an automated coagulation analyzer to perform heparin neutralization with polybrene in blood samples for routine coagulation testing: practical, rapid, and inexpensive. *Arch Pathol Lab Med*. 2013;137:1641–7.

[23] Jacobsen EM, Trettenes EJ, Wisløff F, Abildgaard U. Detection and quantification of lupus anticoagulants in plasma from heparin treated patients, using addition of polybrene. *Thromb J*. 2006;4:3.

[24] Jakimczuk A, Kalaska B, Kamiński K, Miklosz J, Yusa SI, Pawlak D, et al. Monitoring of anticoagulant activity of dabigatran and rivaroxaban in the presence of heparins. *J Clin Med*. 2022;11:2236.

[25] Amiral J, Amiral C, Dunois C. Optimization of heparin monitoring with anti-FXa assays and the impact of dextran sulfate for measuring all drug activity. *Biomedicines*. 2021;9:700.

[26] Ignjatovic V, Summerhayes R, Gan A, Than J, Chan A, Cochrane A, et al. Monitoring unfractionated heparin (UFH) therapy: which anti-factor Xa assay is appropriate? *Thromb Res*. 2007;120:347–51.

[27] Sigma-Aldrich. Dextran sulfate. https://www.sigmaaldrich.com/BE/en/technical-documents/protocol/protein-biology/protein-pulldown/dextran-sulfates. [accessed January 17, 2023].

[28] Rotteveel RC, Roozendaal KJ, Weijers RN, Eijsman L. Influence of heparin, protamine and polybrene on the time integral of thrombin generation (endogenous thrombin potential). *Haemostasis*. 1996;26:1–10.

[29] Carlo A, Arnaud E, Woodhams BJ. The use of polybrene for heparin neutralization in the thrombin generation test. Poster PP-WE-164

presented at: ISTH XXII Congress; July 11–16, 2009; Boston, Massachusetts, USA.

[30] Hardy M, Douxfils J, Morimont L, Didembourg M, Carlo A, de Maistre E, et al. Study of in vitro thrombin generation after neutralization of heparin. *Int J Lab Hematol*. 2022;44:168–76.

[31] van den Boom BP, Bodewes SB, Lascaris B, Adelmeijer J, Porte RJ, de Meijer VE, et al. The international normalised ratio to monitor coagulation factor production during normothermic machine perfusion of human donor livers. *Thromb Res*. 2023;228:64–71.

[32] Cabo J, Morimont L, Baudar J, Guldenpfennig M, Jacqmin H, Soleimani R, et al. Variability among commercial batches of normal pooled plasma in lupus anticoagulant testing. *Int J Lab Hematol*. 2023;45:126–36.

[33] R Core Team. *R*: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2023. https://www.R-project.org/. [accessed November 29, 2023].

[34] Young E, Podor TJ, Venner T, Hirsh J. Induction of the acute-phase reaction increases heparin-binding proteins in plasma. *Arterioscler Thromb Vasc Biol*. 1997;17:1568–74.

[35] 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.

[36] Sie P, Ofosu F, Fernandez F, Buchanan MR, Petitou M, Boneu B. Respective role of antithrombin III and heparin cofactor II in the in vitro anticoagulant effect of heparin and of various sulphated polysaccharides. *Br J Haematol*. 1986;64:707–14.

[37] Staprans I, Felts JM. Isolation and characterization of glycosaminoglycans in human plasma. *J Clin Invest*. 1985;76:1984–91.

[38] Kjellén L, Lindahl U. Specificity of glycosaminoglycan-protein interactions. *Curr Opin Struct Biol*. 2018;50:101–8.

[39] Cosmi B, Fredenburgh JC, Rischke J, Hirsh J, Young E, Weitz JI. Effect of nonspecific binding to plasma proteins on the antithrombin activities of unfractionated heparin, low-molecular-weight heparin, and dermatan sulfate. *Circulation*. 1997;95:118–24.

[40] Gremillet M, Talon L, Lebreton A, Sinegre T. Monitoring heparin therapy: stability of two different anti-Xa assays using blood samples collected in citrate-containing and CTAD tubes. *Thromb J*. 2023;21:21.

[41] Toulon P, Appert-Flory A, Fischer F, Buvat S, Jambou D, Mahagne MH. Monitoring unfractionated heparin therapy. 4 hour-stability of anti-Xa activity in unspun citrated tubes. *Thromb Res*. 2020;186:7–12.

[42] Billoir P, Clavier T, Guilbert A, Barbay V, Chrétien MH, Fresel M, et al. Is citrate theophylline adenosine dipyridamole (CTAD) better than citrate to survey unfractionated heparin treatment? Has delayed centrifugation a real impact on this survey? *J Thromb Thrombolysis*. 2019;48:277–83.

[43] Lasne D, Toussaint-Hacquard M, Delassasseigne C, Bauters A, Flaujac C, Savard P, et al. Factors influencing anti-Xa assays: a multicenter prospective study in critically ill and noncritically ill patients receiving unfractionated heparin. *Thromb Haemost. Published online June*. 2023;15. https://doi.org/10.1055/s-0043-1770096

[44] Ricketts CR. Dextran sulphate—a synthetic analogue of heparin. *Biochem J*. 1952;51:129–33.

[45] Ricketts CR. The blood anticoagulant effect of short chain-length dextran sulphates. *Br J Pharmacol Chemother*. 1954;9:224–8.

[46] Hirsh J, Anand SS, Halperin JL, Fuster V, American Heart Association. Guide to anticoagulant therapy: heparin: a statement for healthcare professionals from the American Heart Association. *Circulation*. 2001;103:2994–3018.

SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at https://doi.org/10.1016/j.rpth.2023.102257.