Protocolled Redefinition of the Therapeutic Range for Unfractionated Heparin: Lost in Translation?

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Abstract

Background: Protocolled treatment with unfractionated heparin (UFH) is a subject of ongoing debate. Even though international guidelines prescribe calibration of the activated partial thromboplastin time (aPTT) to 0.3 to 0.7 U/mL anti-Xa activity to establish an UFH therapeutic range, evidence for this approach remains scarce. In this study, we evaluated different strategies to delineate the UFH therapeutic range and analyzed the effects on patient therapeutic classification. Methods: In 109 patient samples, the aPTT was measured with 2 different reagents, both of which used mechanical clot detection. The UFH therapeutic range was determined using 3 previously described methods: calibration of the aPTT to 0.3 to 0.7 U/mL anti-Xa activity, application of 1.5 to 2.5 times the control aPTT, or using 0.3 to 0.7 U/mL anti-Xa activity directly. We also applied the UFH therapeutic range of a second hospital to our patient population. Results: Application of the guideline-prescribed anti-Xa calibration method would result in patients receiving increased UFH dosage in comparison to our previous UFH nomogram. Between-method and between-laboratory variations in aPTT and anti-Xa activity assays are a likely cause of these discrepancies. Additionally, we show that individual patient characteristics, such as weight and UFH treatment duration, likely contribute to the discordance between different strategies to establish an UFH therapeutic range. Conclusion: No consensus is reached between different strategies to define the UFH therapeutic range, which could result in relevant differences in UFH doses applied in patients. Clinicians and laboratory specialists should critically evaluate UFH monitoring protocols and be aware of their shortcomings.

Keywords

unfractionated heparin, therapeutic drug monitoring, activated partial thromboplastin time, anti-Xa activity

Introduction

In current clinical practice, low-molecular-weight heparins (LMWHs) have largely replaced unfractionated heparin (UFH) in the prevention and treatment of thromboembolic disease. However, because of its short half-life, partially nonrenal clearance and the availability of the effective antidote protamine,1 UFH continues to be used for specific inpatient categories, such as the intensive care and pediatric population. The UFH therapy demands close monitoring, as its anticoagulant actions are unpredictable in an individual patient due to its complex pharmacokinetic properties.2 To optimally direct UFH dosage for the prevention of thromboembolic events while not causing bleeding complications, different monitoring strategies have been applied throughout the last decades. For long, the activated partial thromboplastin time (aPTT) has been the cornerstone of UFH-monitoring protocols, where a ratio of 1.5-2.5 times the control aPTT was generally accepted as the therapeutic range, based on an observational study by Basu et al dating back to 1972.3 However, the “1.5-2.5 times control” strategy failed to recognize the wide variability in the sensitivity of aPTT reagents to UFH.4 An explanation for this variability can be sought in the different analytical methods for determining the aPTT (optical vs. mechanical clot detection), but also in batch-to-batch differences in UFH sensitivity of aPTT reagents.5 The 1.5-2.5 times control methods therefore likely introduced variation in clinical decision-making concerning UFH dosage.

With the development of automated assays to directly measure functional heparin activity, such as the anti-Xa assay, an opportunity to decrease interlaboratory variation in UFH monitoring seemed to be at hand. International guidelines from the College of American Pathologists (CAP) and the American College of Chest Physicians (ACCP) incorporated these new functional heparin assays by stating that the aPTT-based...
therapeutic range for UFH monitoring should be calibrated to correspond to 0.3 to 0.7 anti-Xa U/mL, a range based on a study by Levine et al.6-8 These recommendations were adopted by the laboratory field in guidelines from the Clinical and Laboratory Standards Institute (CLSI); however, convincing evidence for the anti-Xa-based therapeutic range of 0.3 to 0.7 U/mL and for calibration of aPTT to anti-Xa was and remains scarce. Though some studies reported benefits of an anti-Xa-guided UFH nomogram, such as shorter time to therapeutic anticoagulation and less dosage adjustments compared to an aPTT-guided protocol,9-12 other studies reported on the large discordance between aPTT- and anti-Xa-based estimation of anticoagulation effects of UFH and failed to detect significant effects of an anti-Xa-guided protocol on clinical outcome.5,13-16

In our own hospital, an aPTT-guided nomogram that incorporates the body weight of an individual patient was applied to monitor UFH therapy. Over the years, UFH use had declined because of preferred anticoagulant therapy with LMWH, but each month approximately 2 to 3 patients still required UFH instead of LMWH. The therapeutic UFH range in our hospital was defined as an aPTT between 70 and 120 seconds, which reflected 1.7 to 2.9 times the control aPTT. Although this protocol resembled the heparin nomogram proposed by Raschke et al.,17 it was no exact match. The evidence for the therapeutic range of 70 to 120 seconds was unclear; however, the physicians who still used the protocol did not report bleeding nor thrombotic complications during UFH therapy. The transition of the hospital laboratory to a different aPTT reagent, which had improved reagent characteristics but was less sensitive to UFH, necessitated adaptation of the UFH nomogram to the novel situation. This setting offered an opportunity to compare anti-Xa-based and aPTT-based derivation of UFH therapeutic range and to evaluate the effects of these different protocols on classification of patient coagulation status.

Materials and Methods

Sample Collection and Analysis of aPTT and Anti-Xa Activity

Two distinct sets of samples were used in this study. The first sample set consisted of 39 anonymized sodium citrate samples (0.109 M, BD Vacutainer, Breda, the Netherlands) from adult patients receiving UFH therapy at either the Catharina Hospital Eindhoven (further referred to as hospital A) or the Máxima Medical Centre Veldhoven (further referred to as hospital B), which were collected over a period of 3 months. Patients were included regardless of the possible concomitant use of other anticoagulants, such as vitamin K antagonists, to reflect the real-time situation in which these patients are also monitored with the UFH nomogram. None of the patients actively disproved the use of their leftover material for validation purposes, according to the Dutch legislation. Samples were centrifuged within 30 minutes after blood draw at 2500 Relative Centrifugal Force (RCF) for 15 minutes and plasma was stored at −20°C. On the day of measurement, all samples were thawed at room temperature and analyzed simultaneously in both participating laboratories. In all 39 samples, the aPTT was analyzed on an automated STA-R platform (Diagnostica Stago, Asnières, France) by a mechanical clot detection method. Hospital A used both the STA aPTT reagent and the Cephascreen aPTT reagent, while hospital B used only the Cephascreen aPTT reagent, although a different batch than hospital A. For activation of the intrinsic coagulation pathway, the STA aPTT reagent contained silica, while the Cephascreen aPTT reagent utilized ellagic acid, resulting in shorter clotting times measured for the Cephascreen aPTT reagent. Both laboratories assessed the anti-Xa activity with the STA liquid anti-Xa reagent, which was calibrated using the MultiHep calibrator (both from Diagnostica Stago) and to which no antithrombin was supplemented. The anti-Xa activity was successfully measured in 32 of 39 samples from the first sample set. The anti-Xa assay was validated for accuracy using samples from healthy individuals spiked with the sixth World Health Organization international UFH standard.

The second sample set contained 70 samples of 8 patients from hospital A, collected over a period of 4 months, for whom UFH therapy was monitored during several days. These samples were only analyzed at hospital A. For these samples, equal conditions and methods were used as for the first sample set, except that samples were measured within 6 hours after blood draw and that a different lot of Cephascreen aPTT reagent was used.

Derivation of UFH Therapeutic Range

Different protocols were used to derive the therapeutic range for UFH therapy, based on either aPTT or anti-Xa activity:

1. Linear regression between anti-Xa activity and Cephascreen aPTT, the aPTT values corresponding to 0.3 to 0.7 U/mL anti-Xa activity were used as the UFH therapeutic range (CAP- and ACCP-guideline-recommended protocol).6-8.
2. Direct application of 0.3 to 0.7 U/mL anti-Xa activity as the UFH therapeutic range.6
3. 1.5 to 2.5 times the Cephascreen upper reference limit as the UFH therapeutic range.3,17
4. Application of a UFH therapeutic range defined by hospital B that already used the Cephascreen aPTT reagent.

The letter coding for these different protocols to define the UFH therapeutic range is applied throughout this manuscript.

Statistical Analysis

Laboratory results were compared by means of linear regression and assessment of the coefficient of determination $R^2$ (Microsoft Excel for Windows, version 14.0; Redmond, Washington). To evaluate within-patient variation in anti-Xa activity during the UFH treatment, we calculated the quartile coefficient of dispersion for individual patients by dividing the interquartile range by the median (IBM SPSS Statistics for Windows, version 23.0; Armonk, New York). The relation...
between within-patient variation in anti-Xa activity and patient body weight was analyzed by second-grade polynomial regression (Microsoft Excel for Windows, version 14.0).

**Results**

**Derivation of UFH Therapeutic Range by Linear Regression Between Anti-Xa Activity and aPTT and Effects on Therapeutic Classification of Patients**

To define the novel therapeutic aPTT range for UFH therapy in hospital A, we initially adopted a strategy based on international guidelines. This strategy comprised derivation of the therapeutic range by measurement of anti-Xa activity, Cephascreen aPTT, and STA aPTT in samples of patients on UFH therapy (sample set 1 as described in Materials and Methods section), followed by linear regression between aPTT and anti-Xa and determination of the aPTT range corresponding to 0.3 to 0.7 U/mL anti-Xa (further referred to as protocol A). However, poor correlation was observed between Cephascreen aPTT and anti-Xa assays, with a coefficient of determination ($R^2$) of 0.2686 (Figure 1). Correlation between STA aPTT and anti-Xa was even poorer, with an $R^2$ of 0.1156. Based on protocol A, 0.3 to 0.7 U/mL anti-Xa activity corresponded to a therapeutic Cephascreen aPTT range of 82 to 112 seconds. Correlation between Cephascreen and STA aPTT results was better, with an $R^2$ of 0.6565 (Figure 2).

We then analyzed agreement in therapeutic classification in anti-Xa activity and patient body weight was analyzed by second-grade polynomial regression (Microsoft Excel for Windows, version 14.0).

![Figure 1. Correlation between aPTT and anti-Xa activity for sample set 1. Black squares represent STA aPTT results (linear correlation $y = 71.905x + 92.728, R^2 = 0.9425$), while gray triangles represent Cephascreen aPTT results (linear correlation $y = 73.503x + 60.213, R^2 = 0.2686$). aPTT indicates activated partial thromboplastin time.;](image1)

![Figure 2. Correlation between STA and Cephascreen aPTT for sample set 1. Linear correlation $y = 0.4833x + 30.239, R^2 = 0.6565$, dashed black lines indicate UFH therapeutic range for STA aPTT-based nomogram. aPTT indicates activated partial thromboplastin time; UFH, unfractionated heparin.;](image2)

applied. Strikingly, 3 (7.7%) cases were originally classified as supratherapeutic, while application of protocol A would have marked them as subtherapeutic. In all discordant cases (66.6% of total), patients would receive an increased UFH dosage if the novel anti-Xa-derived protocol had been followed. There were no cases in which the novel protocol would have led to a decreased UFH dose.

Patient samples from the first sample set were also measured in hospital B. This hospital already used the Cephascreen aPTT reagent and applied a UFH therapeutic range of 50 to 100 seconds. Also, an equal anti-Xa reagent was used in both laboratories. Interlaboratory correlation between anti-Xa assays ($R^2$ of 0.9425) was better than correlation between Cephascreen aPTT assays ($R^2$ of 0.6078; Supplemental Figure 1). We applied protocol A for the derivation of UFH therapeutic range on the results measured in hospital B. Also for this laboratory, modest correlation was observed between Cephascreen aPTT and anti-Xa assays, with an $R^2$ of 0.2095 (Supplemental Figure 2). From subsequent regression analysis, 0.3 to 0.7 U/mL anti-Xa activity translated in a therapeutic Cephascreen aPTT range of 95 to 121 seconds. In hospital B, this calculated range substantially differed from the actually applied range of 50 to 100 seconds, as it was 50% narrower and the lower limit approximated the upper limit of the current range. Therefore, also in the situation of hospital B, adherence to international guidelines for definition of the UFH therapeutic range (protocol A) would lead to substantially higher UFH dosage than in the current situation.

**Alternative Strategies for Derivation of UFH Therapeutic Range and Effects on Therapeutic Classification of Patients**

As we were reluctant to implement an UFH therapeutic range that would lead to substantial increase in patient UFH dosage, we evaluated alternative ways to define the UFH therapeutic range.

![Figure 3. Correlation between STA and Cephascreen aPTT for sample set 1. Linear correlation $y = 0.4833x + 30.239, R^2 = 0.6565$, dashed black lines indicate UFH therapeutic range for STA aPTT-based nomogram. aPTT indicates activated partial thromboplastin time; UFH, unfractionated heparin.;](image3)
range for the Cephascreen aPTT reagent. When therapeutic classification was directly based on 0.3 to 0.7 U/mL anti-Xa activity (protocol B6), bypassing aPTT regression analysis, the agreement could be reached only in 21.9% of cases (columns marked B in Table 1). As expected, most cases were therapeutic regarding the STA aPTT-based protocol but subtherapeutic based on protocol B.

We also evaluated the 1.5-2.5 times control method for the definition of the Cephascreen aPTT therapeutic range for UFH therapy (protocol C3,17). Taking the upper reference limit of 33 seconds into account, protocol C would translate into a therapeutic range of 49.5 to 82.5 seconds. This strategy resulted in improved concordance with the STA aPTT-based nomogram, with equal classification in 69.3% of cases (columns marked C in Table 1). Regarding discordant cases, most (17.9% of total) were found to be supratherapeutic based on protocol C, while therapeutic considering the STA aPTT-based protocol, which would hypothetically lead to a dosage decrease. Only in 3 cases (7.7% of total), patient UFH dosage would be higher when protocol C would be applied.

Finally, we applied the therapeutic range of hospital B (50-100 seconds) directly to the results of hospital A (protocol D). This would lead to an improved agreement in UFH dosage classification with the previous UFH nomogram (71.9% concordance; columns marked D in Table 1). Most discordant samples (20.5% of total) were initially marked as supratherapeutic, while protocol D would classify them as falling within the therapeutic range.

**Between-Patient Variation in Achievement of Therapeutic UFH Dosage**

As the first sample set, which contained patient samples of both hospitals A and B, was anonymized, we could not gain insight into the potential variation in aPTT or anti-Xa activity for individual patients. Such variation could likely be a partial explanation for the poor aPTT/anti-Xa correlation and the large discrepancies in therapeutic categorization between different strategies to calculate UFH therapeutic range that became apparent from our data. For the second part of the validation, we therefore used a second sample set (see section on Materials and Methods) containing several samples of individual patients in which the UFH therapy was monitored in time. We used a different lot of Cephascreen reagent for these measurements and recalculated the UFH therapeutic range using protocol A. From this analysis, we found an aPTT range of 65 to 91 seconds to correspond to 0.3 to 0.7 anti-Xa U/mL ($R^2$ for correlation was .4755). When this range was compared to the previous STA aPTT-based nomogram regarding therapeutic classification, in 45.8% of cases an equal result was found (Supplemental Table 1). Similar to the results from sample set 1, the guideline-prescribed protocol A would lead to an increase in UFH dosage in all discrepant cases for sample set 2 also.

We subsequently assessed the achievement of therapeutic UFH levels within individual patients for which at least 4 measurements were performed (Table 2). In half of the patients,

### Table 1. Comparison of Therapeutic Classification of UFH Dosage Between the STA aPTT-Based Nomogram and Four Different Cephascreen aPTT Nomograms (A-D) for Sample Set 1.

<table>
<thead>
<tr>
<th>STA aPTT-based nomogram</th>
<th>Therapeutic</th>
<th>Supratherapeutic</th>
<th>Subtherapeutic</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;100 seconds</td>
<td>A B C D</td>
<td>Subtherapeutic</td>
<td>3 (7.7%)</td>
</tr>
<tr>
<td>&lt;112 seconds</td>
<td>A B C D</td>
<td>Therapeutic</td>
<td>17 (43.6%)</td>
</tr>
<tr>
<td>&gt;82.5 seconds</td>
<td>A B C D</td>
<td>Supratherapeutic</td>
<td>3 (7.7%)</td>
</tr>
</tbody>
</table>

**Abbreviations:** aPTT, activated partial thromboplastin time; UFH, unfractionated heparin.

**Absolute numbers (percentages) are shown. Protocol A: Cephascreen aPTT nomogram based on 0.3 to 0.7 U/mL anti-Xa activity from linear regression; protocol B: nomogram directly based on 0.3 to 0.7 U/mL anti-Xa activity (measured for 32 of 39 samples); protocol C: nomogram based on 1.5-2.5 times the Cephascreen aPTT upper reference limit; protocol D: application of Cephascreen aPTT UFH therapeutic aPTT range from hospital B. Values in boldface represent concordant results.
The therapeutic UFH dosage would be reached more often based directly on 0.3 to 0.7 U/mL anti-Xa (protocol B) than according to the STA aPTT-based nomogram. However, in the other half of the patients, the situation was vice versa, with 2 patients never even reaching a therapeutic anti-Xa activity of 0.3 U/mL, while the corresponding STA aPTT was therapeutic more than 50% of the time. In order to find a possible explanation for these patient-specific anti-Xa versus aPTT discrepancies, we evaluated patient characteristics. Patient 4 was the only patient with simultaneous use of a vitamin K antagonist. This situation, which occurs when vitamin K antagonists are restarted after a period of bridging anticoagulation with UFH, is known to lead to a disproportionate prolongation of the aPTT in comparison to the effects on anti-Xa activity. The previously quoted guidelines advise exclusion of patients with concomitant vitamin K antagonist therapy to determine the UFH therapeutic range. However, in daily practice, the UFH nomogram is equally applied to monitor these patients after a period of bridging. Patient 2 stood out because of strikingly long duration of UFH therapy (26 days). The extensive time span of UFH use might have led to a consumptive deficiency in antithrombin, which is necessary for the formation of the heparin-Xa complex but which is not supplemented in the anti-Xa assay used in this study. An acquired deficiency in antithrombin would cause the anti-Xa activity to be disproportionally low compared to the increase in the aPTT measured. From the correlation between duration of UFH use and anti-Xa activity (Figure 3), it becomes apparent that no therapeutic anti-Xa level could be reached when UFH therapy lasted for more than 10 days.

To further delineate the differences in the achievement of therapeutic UFH dosage between patients, we also analyzed patient-specific variation in STA and Cephascreen aPTT and in anti-Xa results during UFH therapy (Supplemental Figure 3). From this analysis, it was apparent that substantial differences exist between patients in the range of aPTT and anti-Xa values measured during UFH treatment even though an equal nomogram is followed. Patient 7 stood out because of very high variation in both aPTT and anti-Xa activity during UFH therapy. Upon inspection of patient characteristics, it was apparent that patient 7 had the highest body weight of 140 kg, corresponding to a body mass index of 44. This finding prompted us to evaluate the relation between patient weight and the overall within-patient variation in aPTT and anti-Xa during UFH treatment, expressed as quartile coefficient of dispersion. Although we only had results from 8 patients, this relation showed a second-grade polynomial trend ($R^2$ between 0.8 and 0.9; Figure 4), translating as high aPTT and anti-Xa variation for patients with minimum and maximum body weight and an optimal variation around 75 kg body weight. This finding would implicate that fluctuation in UFH dosage

| Table 2. Percentages of aPTT Results Within Therapeutic Range (STA aPTT Nomogram Versus 0.3-0.7 U/mL Anti-Xa Activity, Protocol B) for Individual Patients in Sample Set 2. |
|-----------------|-----------------|-----------------|
| Patient | Number of Measurements | Percentage of Therapeutic UFH Based on STA aPTT (%) | Percentage of Therapeutic UFH Based on Anti-Xa-Activity (%) |
| 1 | 4 | 75 | 50 |
| 2 | 19 | 58 | 0 |
| 3 | 10 | 50 | 40 |
| 4 | 8 | 75 | 0 |
| 5 | 7 | 43 | 86 |
| 6 | 8 | 63 | 71 |
| 7 | 7 | 46 | 71 |
| 8 | 6 | 33 | 67 |

Abbreviations: aPTT, activated partial thromboplastin time; UFH, unfractionated heparin.

Figure 3. Correlation between anti-Xa activity (U/mL) and cumulative duration of UFH use (days) for sample set 2. Horizontal lines indicate anti-Xa activity target range of 0.3 to 0.7 U/mL. UFH indicates unfractionated heparin.

Figure 4. Correlation of within-patient variation in STA aPTT, Cephascreen aPTT, and anti-Xa activity during UFH treatment for sample set 2, expressed as quartile coefficient of dispersion, to patient body weight. Black squares represent STA aPTT, gray triangles Cephascreen aPTT, and white circles anti-Xa activity. A polynomic trend can be observed for each parameter with an $R^2$ for STA aPTT of 0.89, for Cephascreen aPTT of 0.86, and for anti-Xa activity of 0.80. aPTT indicates activated partial thromboplastin time; UFH, unfractionated heparin.
is dependent on patient weight, despite the weight-corrected dosage as prescribed by the UFH nomogram.

Discussion
The validation of a novel aPTT reagent with different reagent characteristics posed an opportunity to evaluate different strategies available from literature to establish a therapeutic range for therapy with UFH. Based on international guidelines, we correlated aPTT to anti-Xa activity to derive the aPTT therapeutic range corresponding to 0.3 to 0.7 U/mL anti-Xa activity. However, this strategy implicated a substantial shift in therapeutic classification of patients on UFH therapy, resulting in 66.6% of discordant cases between previous and new nomogram. In all discordant cases, patients would receive an increased UFH dosage based on the novel protocol. Although we only had subjective evidence for the clinical validity of the previous STA aPTT-based nomogram, physicians who applied this nomogram for patient monitoring were reluctant to substantially increase the dosage of UFH because of the assumed increase in bleeding risk. Also other previously reported strategies for derivation of an aPTT therapeutic range to monitor UFH treatment resulted in relatively poor concordance with classification according to our previous UFH nomogram. These findings left us “lost in translation” as none of the strategies to translate the UFH monitoring protocol from one aPTT reagent to another rendered a satisfactory outcome.

The issues that we touched upon concerning the establishment of a UFH therapeutic interval are in agreement with a previous study by Cuker et al., who found extensive interlaboratory variation in therapeutic UFH classification when the anti-Xa correlation protocol was applied (protocol A). In our study, application of this protocol also produced 3 substantially different therapeutic UFH ranges for an equal type of aPTT reagent, underscoring the lack of robustness of protocol A. Additionally, as the Cephascreen aPTT reagents tested in this study came from different batches, our findings could also be caused by batch-to-batch differences in UFH sensitivity. In a subsequent publication by Cuker et al., data from external quality assessment surveys (EQAS) were evaluated to gain insight in the analytical variation within and between different aPTT and anti-Xa assays. This study highlighted the occurrence of substantial intermethod and within-method interlaboratory variation for both the aPTT and the anti-Xa assay. Regarding the aPTT assay, mechanical clot detection methods appeared to be more sensitive to UFH than the optical detection methods. Also for the anti-Xa assay, different methodologies exist, as some reagents add exogenous antithrombin to the patient plasma to supplement possible deficiencies, while in other reagents, including the one used in this study, this is not the case. To what extent addition versus omission of exogenous antithrombin influences the anti-Xa assay was not evaluated by Cuker et al. Data from the EQAS in which our own laboratory participates (external quality control of diagnostic assays and tests) highlighted that methods supplementing antithrombin measured anti-Xa activities 15% to 55% higher compared to methods that did not add exogenous antithrombin (data not shown). In our study, we used the Stago Liquid anti-Xa assay without supplementation of exogenous antithrombin. Our analysis of achievement of therapeutic UFH levels within individual patients suggested that when UFH treatment lasts longer than 10 days, anti-Xa activity fails to reach a therapeutic level of ≥0.3 U/mL. This could theoretically be due to an acquired antithrombin deficiency which develops when antithrombin production does not equal antithrombin loss through clearance of the heparin–antithrombin complex. The low anti-Xa activity levels measured upon UFH use exceeding 10 days possibly also reflect reduced physiological activity of UFH in antithrombin-deficient patients. However, we did not analyze antithrombin levels in our small patient cohort. The relation between duration of UFH use, antithrombin deficiency, in vivo UFH efficacy, and anti-Xa activity measured with/without antithrombin supplementation warrants further study in a larger patient cohort. Our data also support the notion that a weight-based UFH nomogram, proposed by Raschke et al in 1993, is not suited for patients whose weight is much lower or higher than the average of 75 kg. The 1 patient who stood out in our study because of extremely large within-patient variation in both aPTT and anti-Xa activity had a body weight of 140 kg. The relation between body weight and within-patient variation showed a second-grade polynomic trend, with minimal variation at a weight of 75 kg, but higher fluctuation in patients with aberrant body weight. These findings suggest that the weight-based nomogram does not optimally correct for extremely low or high body weight, which could be explained by the fact that the mean weight of patients in the Raschke et al study was 80.9 kg. In our small patient cohort, the mean weight was 91.9 kg, likely reflecting the increased incidence of obesity in the last decade, which makes the 1993 Raschke protocol less applicable to the current population. In Dutch clinical practice, a substantial number of hospitals already apply a standard nomogram that is not based on patient weight (personal communication, general member meeting of the Dutch Society for Laboratory Diagnostics in Hematology (Vereniging Hematologische Laboratorium Diagnostiek, VHL), February 16, 2016).

Apart from the effects of analytical and patient-related factors on determination of UFH therapeutic range as we present here, our study design itself could have some limitations that possibly influence our data. By using consecutive samples of a small number of individual patients, a patient-related bias could be introduced in the correlation between aPTT and anti-Xa activity. Also the observed effect of prolonged duration of UFH treatment on anti-Xa activity could be due to patient-specific factors instead of acquired antithrombin deficiency. Although the CAP and ACCP guidelines make no specific statements on the composition of the patient sample set to define the aPTT/anti-Xa correlation, Marlar and Gausman suggested that a maximum of 10% of samples should originate from an individual patient. However, they also address the difficulties that small- to moderate-sized hospitals can experience in obtaining an adequate number of samples from patients
on UFH treatment. Because of this slow sample collection rate, we allowed for 6 hours between centrifugation and analysis so samples taken at night could also be included, even though according to the CLSI guidelines, an interval of 4 hours is optimal. Although we recognize the abovementioned limitations of our study, in real-life laboratory practice our approach likely presents the “next best” option.

In conclusion, our data point out the shortcomings of previously published strategies to define an UFH therapeutic range. Between-method, between-laboratory, and between-laboratory variations in both aPTT and anti-Xa assays likely prevent the standardized definition of a therapeutic range for UFH. Although this study presents results from two hospitals, inquiry among several other laboratories in the Netherlands learns that mostly protocols A and C are used for definition of UFH therapeutic range, while in some hospitals the background of the UFH nomogram is actually unknown (personal communication, general member meeting of the Dutch Society for Laboratory Diagnostics in Hematology (Vereniging Hematologische Laboratorium Diagnostiek, VHL), February 16, 2016). Therefore, in the current situation, between-laboratory variation in UFH monitoring is a reality, resulting in laboratory-dependent patient UFH dosage. Although the clinical implications of these disparities have not been studied, clinicians and laboratory specialists should be aware of the uncertainties in UFH monitoring and should critically evaluate the evidence for UFH nomograms in use as long as UFH is still not completely replaced by LMWH. In case of a change in aPTT reagent such as we present here, we advise a thorough validation of the adaptation of the UFH nomogram to bring to light the possible effects on therapeutic classification.

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