# A Novel Laboratory Assay to Monitor Unfractionated Heparin Dosing in Patients Taking Apixaban Prior to Hospital Admission

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**Introduction:** When monitoring heparin, anti-Xa assays are susceptible to interference from apixaban taken before admission and can result in inappropriate dose adjustments that can negatively affect patient care.

**Methods:** We derived a novel assay, termed *corrected heparin* (CH), using quantified values from a chromogenic anti-Xa assay with heparin calibrators before and after heparinase treatment to eliminate any interference from apixaban within the patient sample. We retrospectively assessed 469 specimens from 72 patients at our institution who had their unfractionated heparin infusion monitored using the CH assay because of known apixaban use. These patients were included in the study if they had detectable apixaban levels (>0.1 IU/mL by anti-Xa).

**Results:** The analytical performance of the assay was evaluated, and precision was found to be 8.8% within 1 day and 13.3% over multiple days, with acceptable linearity ($R^2 = 0.997$). Evaluation of clinical performance was compared with the partial thromboplastin time (PTT), showing a lack of correlation similar to comparisons between the PTT and anti-Xa assay (*Blood Coagul Fibrinolysis* 1993;4:635–8). The mean time to a therapeutic result in this cohort was 10 hours and 10 minutes. The CH assay was used to determine how long the apixaban was detected by the anti-Xa assay. The majority of patients (80%) still had measurable anti-Xa assay interference from apixaban at 24 hours after the last apixaban dose.

**Conclusions:** We have developed and evaluated an assay capable of quantifying heparin in the presence of apixaban. This assay showed acceptable performance in both analytical and clinical performance.

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## IMPACT STATEMENT

This study provides a valuable tool for clinicians to monitor heparin dosing in patients admitted to the hospital after a previous apixaban dose was administered. The corrected heparin procedure presented in this study allows clinicians and pharmacists to accurately dose heparin by removing the interference of lingering apixaban in the anti-Xa activity assay. This study demonstrates that even small amounts of apixaban can falsely elevate anti-Xa values, and this interference lasts up to 48 hours past the guideline-recommended time point indicating that apixaban will no longer interfere with laboratory coagulation assays.

## INTRODUCTION

As conditions like nonvalvular atrial fibrillation and venous thromboembolism have become more prevalent in the United States *(1, 2)*, the use of direct oral anticoagulants (DOACs) in the outpatient setting has increased *(3, 4)*. DOACs, such as apixaban and rivaroxaban, are appealing for several reasons. Unlike warfarin, which has been in use since the 1950s *(5)*, DOACs currently do not require routine patient monitoring, and they have fewer interferences from drugs and foods *(3, 5)*. But DOACs, which were introduced in 2010, have limited clinical outcome-based studies when used in conjunction with heparin in hospitalized patients *(3)*. Although some initial studies *(6–8)* have been done to describe diagnostic testing for DOACs, few studies have examined the complexity of measuring DOAC and heparin activity simultaneously.

Patients who receive DOACs as outpatients and present to the hospital with thromboembolic events represent a challenge for monitoring of therapy with unfractionated heparin (UFH). Given UFH's narrow therapeutic window, accurate monitoring of UFH concentrations is required. Use of anti-Xa assays for monitoring of UFH infusions is supported by multiple studies *(6–10)* and leads to better patient outcomes than does use of other coagulation tests. Both UFH and DOACs, however, inhibit the activity of factor Xa, making the anti-Xa assay inappropriate as a test to accurately determine UFH concentrations.

Previous studies have attempted to remove UFH from plasma samples to improve international normalized ratios and activated partial thromboplastin time (PTT) estimations in plasma samples containing both UFH and additional anticoagulants *(9, 10)*. In the present study, we set out to examine the potential of the UFH removal technique to make the anti-Xa assay useful as a tool for determination of UFH concentrations in patients already receiving DOACs. In this report, we refer to the resulting procedure as a *corrected heparin* (CH) assay.

## METHODS

### Patient Sample Characteristics and Criteria for Inclusion

A total of 469 patient specimens (sodium citrate blood vacutainers) from 72 patients containing both apixaban and UFH were collected between August 24, 2016, and January 31, 2017, with the approval of the University of Virginia Institutional Review Board for Health Science Research (number 11666). On admission, patients were asked if any other anticoagulation medications had been taken within the past 24 hours, before starting UFH treatment. If a patient had taken apixaban

within the preceding 24 hours, clinicians ordered a CH assay. Samples that did not contain detectable apixaban (<0.1 IU/mL by the CH assay) were excluded from this study. Inclusion of a patient in the clearance studies required at least 3 CH-reported values with samples extending to at least 72 hours after their last apixaban dose and with a CH value of <0.1 IU/mL before 72 hours (42 patients met these criterion).

### Coagulation Studies

PTT was quantified using a clot-based assay on the ACL TOP instruments with HemosIL reagents (PTT: SythASil and calcium chloride). The anti-Xa assay was also performed on the ACL TOP instruments with HemosIL reagents (HemosIL Liquid Anti-Xa Kit). The concentration of UFH within the sample is inversely proportional to the activity of factor Xa because the heparin-antithrombin complex inhibits the activity of factor Xa. The activity of factor Xa is measured by the conversion of S-2732 (chromogenic substrate) to paranitroaniline, which is monitored kinetically at 405 nm. The CH assay requires samples to be treated with Heparinase *1* (Dade Hepzyme), an enzyme that cleaves UFH at multiple sites and renders it inactive, for 15 minutes at room temperature (20–25 °C). This preparation of heparinase and the protocol are designed to neutralize 2 united states pharmacopeia units of UFH in 1 mL of citrated plasma.

### Regression analysis and statistical analysis

Linear regression was used to evaluate the dilutional linearity of apixaban in patient samples containing UFH and to quantify the $R^2$ value for the relationship between PTT and CH assays. For the precision study, plasma was pooled and aliquoted, and these aliquots were then used to perform the entire process. The within-day precision study consisted of the analysis of 5 aliquots run within a single day, while the between-day experiment was performed with a single experiment over 8 separate days. Normalization of the data was completed using the following equations: normalized result = (result − mean of therapeutic range) / (mean of therapeutic range − lower limit of the therapeutic range), where the therapeutic range for the PTT was 60–90 seconds and the anti-Xa was 0.3–0.7 IU/mL. Values falling within the therapeutic range were between 1 and −1. Deming regression was used to evaluate the correlation between PTT and CH assays on 469 patient samples containing both apixaban and UFH. Two-tailed *t* test analysis was used to determine statistical significance between means.

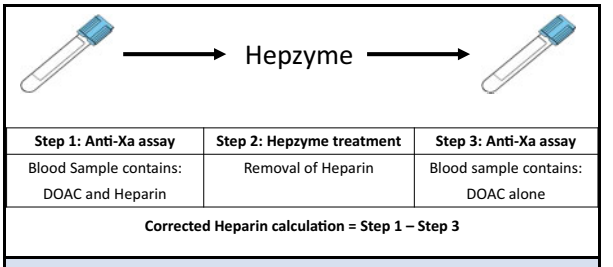


**Fig. 1. Description of the CH assay. CH uses 2 direct factor X inhibitors of patient plasma. In step 1, the anti-Xa assay result reflects contributions from both apixaban and UFH. In step 2, the sample is treated with heparinase to remove heparin. In step 3, the anti-Xa assay indicates only the contribution of apixaban. The reported CH is the difference between the first and second anti-Xa measurements.**

## RESULTS

### Description of the CH Assay

The CH assay uses 2 anti-Xa quantifications in combination with a heparinase treatment (Fig. 1). Step 1 involves an initial anti-Xa assay of the sample, which quantifies the inhibitory contributions on factor X from both UFH and apixaban. A heparinase treatment is then performed to remove UFH from the sample, followed by a second anti-Xa measurement, which quantifies the inhibitory

action of only the apixaban remaining in the sample. The difference between the results of the 2 anti-Xa assays represents the contribution from the UFH alone.

### Analytical Performance of the CH Assay

To address the validity of using the CH assay to monitor UFH in the presence of apixaban, we performed a linearity study with patient samples containing apixaban and apixaban plus UFH. Patient samples previously measured by the anti-Xa assay containing apixaban and apixaban plus UFH were diluted with anticoagulant-free plasma and displayed good correlation ($R^2$ = 0.997 and $R^2$ = 0.999, respectively) when plotted against the expected values (plot not shown). These results indicated that apixaban quantifies linearly when measured by the anti-Xa assay (range, 0–2.6 IU/mL). Precision was also characterized by performing both within- and between-day studies. The precision for the entire process was determined to be 8.8% when performed within a single day and 13.3% when performed over multiple days.

To ensure that the heparinase activity is preserved in the presence of apixaban, UFH was added to apixaban-free plasma samples and to apixaban-containing plasma from patients who were receiving apixaban. Figure 2A shows the baseline PTT of both samples before spiking in UFH, with the PTT for each sample being similar. The PTT was measured in both samples after 2.0 IU/mL was spiked into the samples, showing comparable prolongation of the PTT. These samples were then treated with heparinase and their PTT was measured again, where it returned to the baseline PTT indicating removal of the added heparin. The same experiment was performed using the anti-Xa assay and is shown in Fig. 2B. In both studies, the addition of UFH increased the difference from baseline and treatment with heparinase returned it back to baseline. These results confirm that UFH is being removed completely from both normal and apixaban plasma.

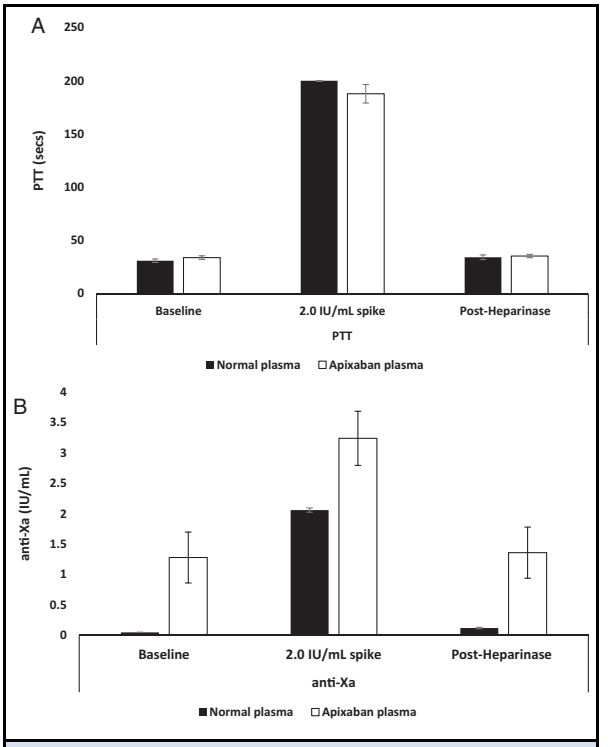


**Fig. 2. Removal of UFH interference in PTT (A) and anti-Xa assays (B) by heparinase. Plasma samples with and without apixaban were analyzed by the PTT assay and by the anti-Xa assay at baseline, after addition of 2.0 IU/mL of heparin (solid bars), and after treatment with heparinase (open bars); n = 3.**

PTT has been suggested as a method that can be used to monitor UFH in patients who also have apixaban present, as apixaban does not affect PTT *(11, 12)*. In this study, the CH assay was used clinically to monitor and adjust UFH dosing in 469 patient samples. In each sample, a PTT was also measured but was unavailable to the providers. The results from each assay were normalized as described in the Methods section and then compared for accuracy (Fig. 3). Deming regression analysis showed that the CH assay was poorly correlated with the reported PTT values.

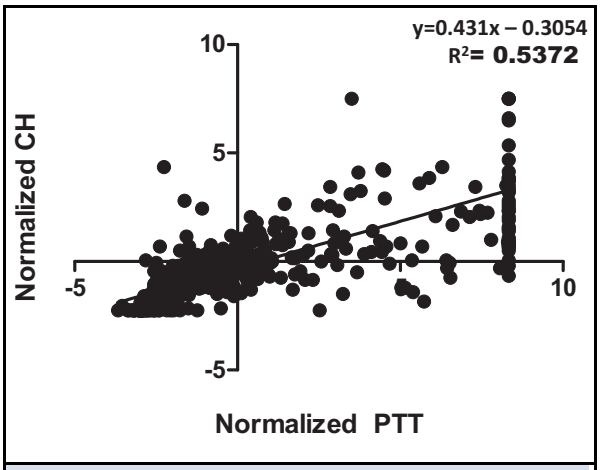


**Fig. 3. Relation of normalized PTT and CH in patients receiving both apixaban and unfractionated heparin. Regression analysis showed a proportional bias between the PTT and CH assays ($y = 0.43x - 0.31$; n = 469) and $R^2 = 0.53$ in samples from patients receiving both apixaban and UFH. Reported values were normalized using the following equations based on reference intervals for each assay: normalized CH = (CH − 0.5) / 0.2 (reference interval, 0.3–0.7 IU/mL) and normalized PTT = (preheparinase PTT − 75) / 15 (reference interval, 60–90 s).**

Both PTT and anti-Xa are commonly used to make dosing adjustments to heparin therapy. In an effort to understand the discrepancy between the CH assay and PTT for monitoring UFH, we hypothesized that the patient's baseline PTT influenced the ability of the PTT to monitor UFH. A comparison was performed in which we categorized patients based on their baseline PTT (UFH removed) and then compared the results of the 2 different assays, therapeutic PTT or CH, to determine whether either assay was influenced by the baseline PTT. The therapeutic PTT in this study refers to samples that have heparin present, and the PTT is used to monitor their heparin with the therapeutic range of 60–90 seconds. Patients were categorized based on their baseline PTT (after UFH removal) into 3 groups: short PTT (<27 s; n = 23), normal PTT (27–37 s; n = 320), and prolonged PTT (>37 s; n = 126) (Fig. 4A). Figure 4B showed the comparison of the therapeutic PTT values for each of the 3 groups. When comparing the therapeutic PTTs, the groups were different, consistent with an influence of the baseline PTT on the therapeutic PTT. Figure 4C showed the results of the CH for the 3 groups, and they were similar, suggesting that the baseline PTT did not influence the CH.

### Clinical Performance of the CH Assay

The mean time to a CH result in the therapeutic range (0.3–0.7 IU/mL) was 10 hours and 10 minutes (SD = 13 h and 2 min) from the time the first sample was drawn (n = 72 patients), and by 36 hours, 86% of patients had reached the therapeutic window (Fig. 5). Of the 10% who did not reach the therapeutic window, they also did not reach the therapeutic window by PTT (60–90 s).

The CH assay allows for the evaluation of the clearance of apixaban over time in the presence of UFH. Forty-two of the total patients included were monitored for at least 72 hours by the CH method, and the values for apixaban were analyzed to assess its clearance. At 24 hours after the last apixaban dose, 81% of patients still had apixaban levels >0.1 IU/mL (Fig. 6), indicating that inhibition from residual apixaban could still occur in the chromogenic anti-Xa assays at this time point. At 48 and 72 hours after the last dose of apixaban, 45% and 19% of patients, respectively, still had detectable apixaban levels (>0.1 IU/mL).

## DISCUSSION

It has been suggested that PTT assays can be used to monitor UFH levels in patients, but others' data *(13, 14)* suggest that direct factor Xa inhibitors could interfere in this assay. PTT results are poorly correlated with the results of other assays,

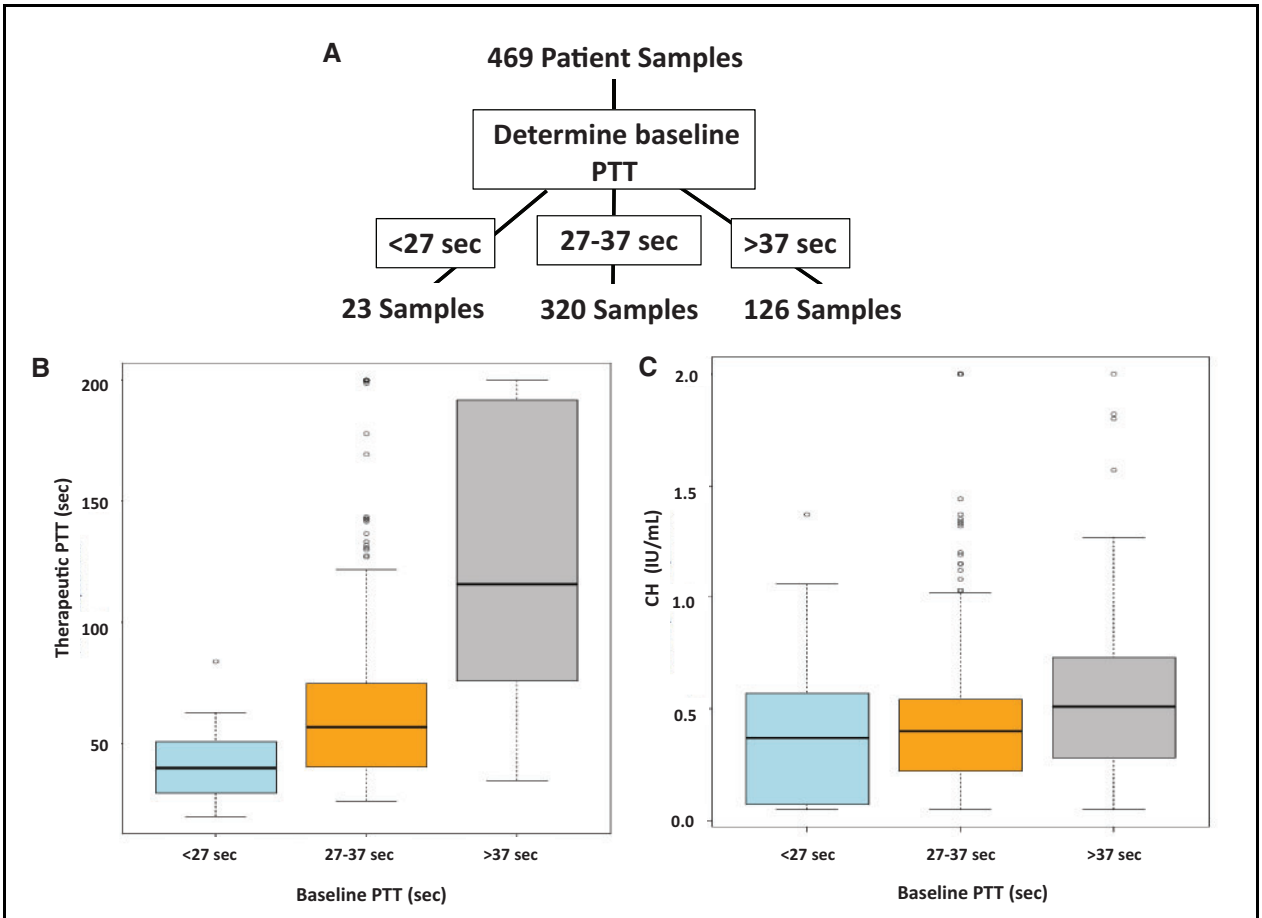


**Fig. 4. Baseline PTT determines therapeutic PTT but not CH. (A), Baseline PTT values (after heparinase treatment) from 469 patient samples (72 patients) collected between August 24, 2016, and January 31, 2017, were grouped into 3 categories—short PTT (<27 s), normal PTT (27–37 s), and prolonged PTT (>37 s)—and then compared with the therapeutic PTT (before heparinase treatment). (B), Baseline PTT influences the range of therapeutic PTT. Baseline PTT values were grouped into 3 categories, as described in (A), and then compared with the therapeutic PTT. (C), Baseline PTT dose did not influence the range of the CH. Baseline PTT values were grouped into 3 categories, as described in part (A), and then compared with the CH.**

including the anti-Xa assay, for monitoring UFH *(13)*. Moreover, the interference of Xa inhibitors on PTT is dependent on both the PTT reagent used and the specific anticoagulant present in the sample, making it an inadequate assay for monitoring UFH in these patients. Rivaroxaban was reported to affect PTT and prothrombin time to varying degrees based on the reagent used, whereas the effect from apixaban was noted to be less significant *(15)*. In addition, patients presenting to the hospital who are placed on a UFH drip often have comorbidities that influence the PTT, including inflammation, thrombosis, consumptive processes (hyperfibrinolysis or consumption), liver disease, and/or vitamin K deficiency (due to antibiotic use or malnutrition). These factors prolong the PTT

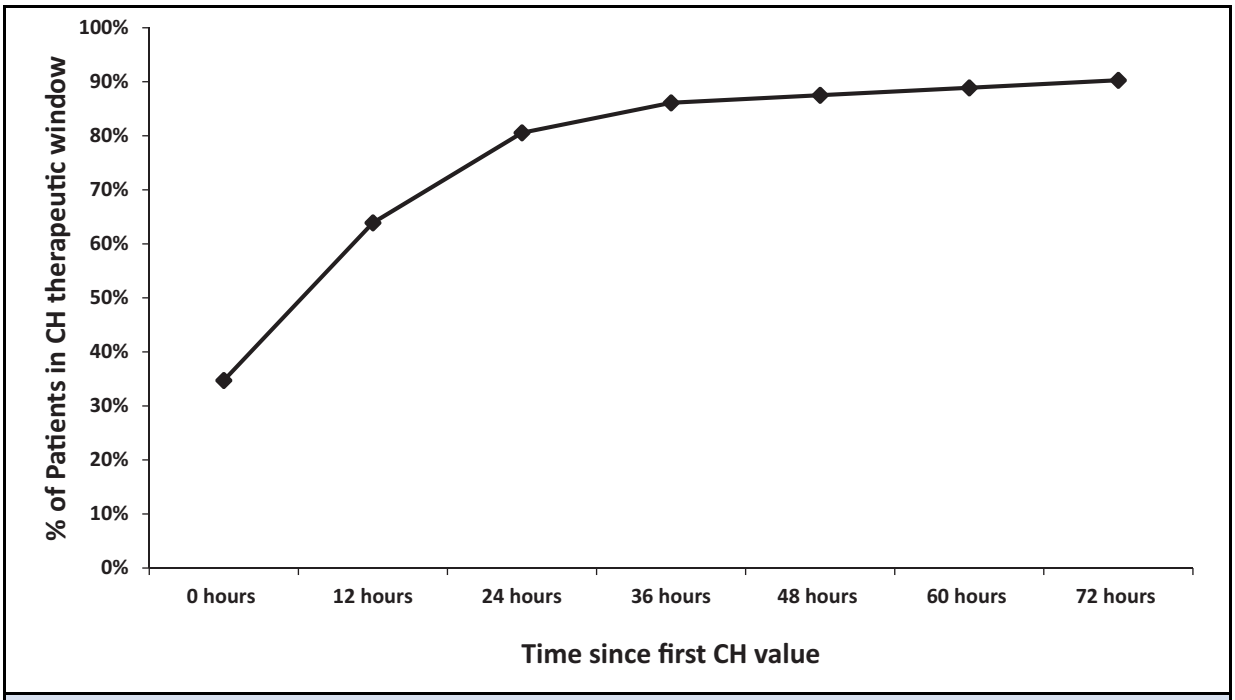


**Fig. 5. Time to reach the therapeutic window during monitoring with the CH assay. Patients were followed from the time of their initial CH assay until they reached the therapeutic window (0.3–0.7 IU/mL). The percentage of patients reaching the therapeutic window at each time is shown (n = 72 patients, 469 samples).**

and may explain the 27% (126/469) of patient samples in this study that had a prolonged baseline PTT (>37 s). Finally, as shown in Fig. 4B, our data suggest that a prolonged baseline PTT affects the ability of this test to accurately monitor heparin concentrations.

Unlike PTT, CH is not influenced by the aforementioned factors and thus is a better monitor for UFH dosing when patient samples contain DOACs. Similar to previous reports of discordance between PTT and anti-Xa assays *(13)*, we showed that CH and PTT are not correlated (Fig. 3), suggesting that each assay is influenced by different biological factors. We also showed that the baseline PTT (influenced by biological processes like inflammation and consumption) does not influence the CH value (Fig. 4B). The CH assay quantifies an anti-Xa level despite the presence of comorbidities that would typically interfere with appropriate UFH dosing. This allows more rapid attainment of therapeutic concentrations of heparin *(13)* (Fig. 5), thereby decreasing negative patient outcomes, such as additional clotting events. No patients in this study experienced clotting or bleeding events during their hospital stay while being monitored using the CH assay. A limitation of this study is that no direct comparisons were made between CH- and PTT-guided dose adjustments for patient outcomes including time to therapeutic or bleeding or clotting events, as all patients' UFH dose adjustments were made according to CH.

Current recommendations on monitoring of UFH dosing in patients who have received previous apixaban doses indicate that clinicians should wait 24 hours from the last dose (approximately 2 half-lives) and then resume normal testing

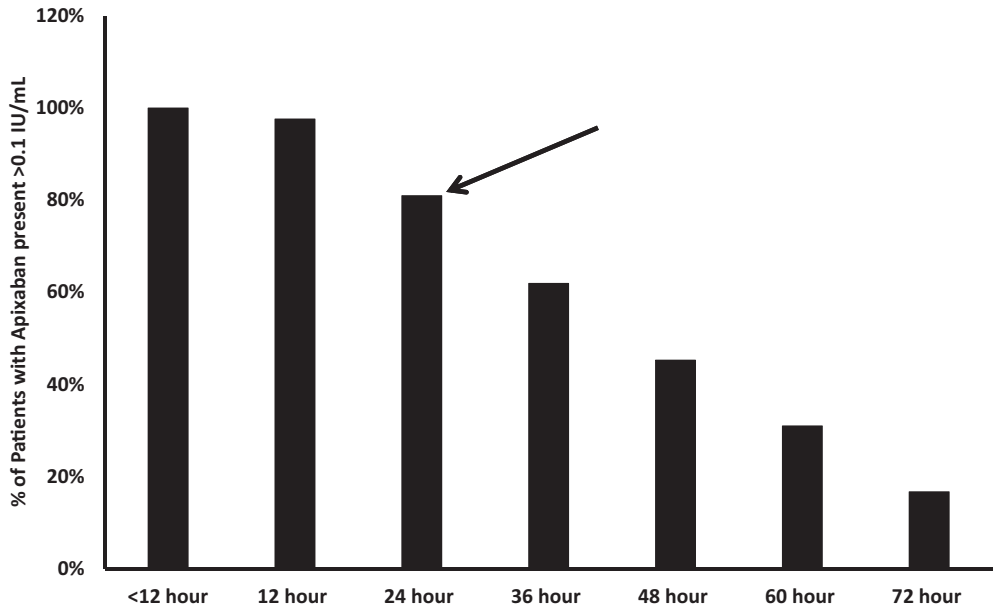


**Fig. 6. Proportion of patients with apixaban >0.1 IU/mL at time points after discontinuation of drug. Data are for 469 patient samples from 42 patients for whom at least 3 CH values were reported and whose CH reached <0.1 IU/mL by 72 hours. At 24 hours, 8 patients had CH values <0.1 IU/mL, whereas 34 patients had CH values >0.1 IU/mL. The ratios for the next 4 time points are 36 hours (16 patients at <0.1 IU/mL and 26 patients at <0.1 IU/mL), 48 hours (23 patients at <0.1 IU/mL and 19 patients at <0.1 IU/mL), 60 hours (29 patients at <0.1 IU/mL and 13 patients at <0.1 IU/mL), and 72 hours (35 patients at <0.1 IU/mL and 7 patients at <0.1 IU/mL). The arrow indicates the guideline-recommended 24-hour time point after which the usual testing pattern can be resumed without interference from apixaban.**

because the apixaban should no longer interfere (*16*, *17*). Our data showed that at 24 hours, 81% of patients still had detectable apixaban levels as measured by the CH method, and 19% still had detectable apixaban levels at 72 hours after the last dose (Fig. 6). It remains unclear if the anticoagulation function of apixaban is active in vivo at these times points, but it does show that interference in in vitro assays may not have been eliminated after only 24 hours, which could lead to incorrect UFH dose adjustments if returning to the anti-Xa assay for monitoring.

The CH method has strengths and limitations. The CH assay (1) reports more nearly accurate UFH levels by negating the contribution of apixaban, (2) shows less interference from biological factors that influence PTT, (3) has an average time to therapeutic value of <12 hours, and (4) is achievable in any laboratory that currently offers an anti-Xa assay. If these results are confirmed in other laboratories, this approach may have broad clinical applicability. One caveat to this assay is an increased analytical time in comparison to a PTT or a standard anti-Xa assay, due to completing 2 anti-Xa assays and a heparinase treatment (approximately 30 additional minutes). This additional time requirement appears warranted to achieve the benefits of more accurate monitoring of UFH therapy.

**Nonstandard Abbreviations:** DOAC, direct oral anticoagulant; UFH, unfractionated heparin; PTT, partial thromboplastin time; CH, corrected heparin.

**Author Contributions:** *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

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