



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Full Length Article

Apixaban and rivaroxaban anti-Xa level utilization and associated bleeding events within an academic health system



Nicholas Jakowenko^{a,*}, Steffany Nguyen^a, Melanie Ruegger^a, Ashley Dinh^b, Eric Salazar^c, Kevin R. Donahue^a

^a Department of Pharmacy, Houston Methodist Hospital - Texas Medical Center, Houston, TX 77030, United States of America

^b Department of Pharmacy, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States of America

^c Department of Pathology and Genomic Medicine, Houston Methodist Hospital - Texas Medical Center, Houston, TX 77030, United States of America

ARTICLE INFO

Keywords:

Anti-Xa
DOAC
Major bleeding
Reversal
Safety

ABSTRACT

Background: Oral factor Xa inhibitors (FXaI) can be administered in fixed doses without the need for routine laboratory monitoring. Anti-Xa assays can estimate anticoagulant effect for specific FXaI's. The aim of this study was to characterize anti-Xa levels in patients taking apixaban or rivaroxaban with major bleeding events.

Methods: Apixaban and rivaroxaban anti-Xa assays ordered within our hospital system from May 2016 to September 2019 were evaluated. The primary outcome was major bleeding events defined by International Society of Thrombosis and Haemostasis criteria. Median anti-Xa levels for each FXaI were calculated for those with and without major bleeding, as well as those who did and did not receive reversal agents.

Results: A total of 606 anti-Xa levels were analyzed. There were 146 major bleeding events documented, with the most common site being intracranial (63%). Median anti-Xa levels in patients with and without major bleeding were similar, whereas those on apixaban therapy who received reversal agents typically had higher anti-Xa levels (73 ng/mL vs. 153 ng/mL, $p = 0.0019$). Factors significantly associated with increased odds of bleeding were an age > 80 years, inappropriately high dosing regimens, and modest anti-Xa levels (100–300 ng/mL) for rivaroxaban specifically.

Conclusions: Older age and inappropriately high dosing regimens were associated with major bleeding in patients taking apixaban and rivaroxaban. Further investigation into the utility of anti-Xa levels for FXaI is warranted.

1. Introduction

Oral factor-Xa inhibitors (FXaI) have become widely used for both venous thromboembolism (VTE) treatment and prophylaxis, as well as stroke prevention in non-valvular atrial fibrillation. Several large-scale clinical trials have shown that edoxaban, apixaban, and rivaroxaban are as or more effective than warfarin with a more favorable bleeding profile [1–5]. Attributes owing to their widespread use include minimal drug-drug interactions (DDI), tolerability, and lack of routine laboratory monitoring requirements [6]. Liquid chromatography (LC)-mass spectrometry (MS) can accurately quantify FXaI plasma concentrations and is considered the gold standard of assessing FXaI plasma concentrations [7], however not all laboratories have 24-hour access to perform this type of analysis. Commercially available anti-Xa chromogenic assays may be utilized for FXaI levels with appropriate drug calibrators [8,9], and have demonstrated good correlation between

plasma FXaI concentration and anti-factor Xa activity across a range of plasma concentrations [10]. Some researchers have attempted to establish “on therapy” anti-Xa level ranges for apixaban and rivaroxaban in a pragmatic setting [11], whereas other pharmacokinetic studies conducted in healthy volunteers suggest expected plasma levels based on FXaI type, dose, and time since last dose [12,13]. Certain clinical scenarios such as breakthrough thrombosis, bleeding events, and hepatic and renal insufficiency may warrant assessment of physiologically relevant FXaI concentrations [14–18], and there is some evidence correlating plasma concentrations of certain direct oral anticoagulants (DOAC) such as dabigatran and edoxaban with bleeding events [19,20]. However, there is a paucity of data describing the relationship between FXaI anti-Xa levels and clinical outcomes in patients taking apixaban and rivaroxaban in a real-world setting. The objective of this study was to evaluate anti-Xa levels for apixaban and rivaroxaban and their association with major bleeding events.

* Corresponding author at: Department of Pharmacy, Houston Methodist Hospital, 6565 Fannin St., DB1-09, Houston, TX 77030, United States of America.
E-mail address: Nick.jakowenko@gmail.com (N. Jakowenko).

<https://doi.org/10.1016/j.thromres.2020.09.002>

Received 14 June 2020; Received in revised form 30 July 2020; Accepted 1 September 2020

Available online 06 September 2020

0049-3848/ © 2020 Elsevier Ltd. All rights reserved.

2. Methods

2.1. Study design

This retrospective, observational cohort study was approved by the Houston Methodist Research Institute Institutional Review Board (IRB). All anti-Xa levels for apixaban and rivaroxaban ordered at Houston Methodist, a seven-hospital system, from May 2016 to September 2019 were evaluated regardless of whether there was a documented inpatient dose of apixaban or rivaroxaban. Of note, all anti-Xa level tests for FXaI were performed at one laboratory in the Texas Medical Center Hospital. Levels were excluded if any of the following factors were identified: unfractionated heparin (UFH) or low molecular weight heparin (LMWH) product administration within 24 h prior to an anti-Xa level being drawn; the level was deemed to be ordered in error (i.e., incorrect assay ordered based on drug exposure or the patient was not taking a FXaI); or critical information was missing from the electronic health record (EHR). The EHR was also queried for patient demographic information, baseline laboratory values, and all FXaI administrations. Reasons for anti-Xa level ordering and clinical decisions regarding FXaI were chart reviewed by two investigators (NJ and AD). If patients had multiple levels obtained during the same encounter, only initial levels were included in final analyses.

2.2. Study endpoints

The primary endpoint of this study was the prevalence of major bleeding events as defined by the International Society on Thrombosis and Haemostasis (ISTH) which included bleeding that was fatal, located in a critical area or organ (i.e., intracranial, intraspinal, retroperitoneal, intraocular, pericardial, or intramuscular), caused a drop in hemoglobin of ≥ 2 g/dL, or led to the transfusion of two or more units of blood [21]. Major bleeding events were reviewed and adjudicated by two of the authors (NJ and AD) was confirmed either by presence of pertinent laboratory or imaging findings, as well as provider documentation for each patient. Median anti-Xa levels were determined both in patients with and without a major bleeding event. Anti-Xa levels obtained at the time of, or within 24 h after a bleeding event, were considered to be in the context of a major bleed and categorized as such. Secondary endpoints consisted of reversal agent use (four-factor prothrombin complex concentrate (4F-PCC) and andexanet alfa), reasons for obtaining an anti-Xa level, changes in FXaI therapy at hospital discharge, and all anti-Xa levels following a documented inpatient dose. Pre-specified subgroup analyses were planned in order to compare patients with and without a major bleeding event on the basis of age, body mass index (BMI), baseline creatinine clearance (estimated with the Cockcroft-Gault equation), presence of AKI as defined by the Kidney Disease Improving Global Outcomes (KDIGO) criteria [22], use of renal replacement therapy (RRT), dose appropriateness based on approved package labeling [23,24], and concomitant use of antiplatelet drugs or inhibitors of FXaI metabolism.

2.3. Chromogenic anti-Xa assay

Apixaban and rivaroxaban anti-Xa levels were determined using a calibrated STA® - Liquid Anti-Xa Assay that is calibrated with commercial apixaban and rivaroxaban calibrators, and are available 24 h a day, seven days a week at our institution. In this assay, exogenous factor Xa is added to a patient's plasma sample which can then complex with any present FXaI. A substrate that is specific for factor-Xa is then added to the sample, and the factor-Xa-substrate complex then cleaves a chromophore to produce color, with the amount of color produced being inversely proportional to FXaI presence. Expected peak levels (2–4 h post-dose) are provided in the result details as well. The lower limit of the reportable range of our assays for both apixaban and rivaroxaban are < 20 ng/mL, and the upper limit 451 ng/mL and 472 ng/

mL for apixaban and rivaroxaban, respectively. Expected peak levels for apixaban are 16–108 ng/mL and 102–155 ng/mL following a 2.5 mg and 5 mg dose, respectively. Likewise, typically observed peak levels for rivaroxaban are 90–190 ng/mL and 180–340 ng/mL following a 10 mg and 20 mg dose, respectively. These ranges have been adopted from previously conducted pharmacokinetic studies [13,25].

2.4. Statistical analysis

Continuous data were reported as mean and standard deviation or median and interquartile range (IQR) as appropriate. Categorical data were compared with the chi-squared or Fisher's exact test. Differences between two groups were assessed with a student's *t*-test or Mann-Whitney *U* test depending on the data distribution, with a *p*-value of ≤ 0.05 indicating a statistically significant difference at the two-sided level. Binary logistic regression was performed in order to ascertain any factors that predisposed patients to increased odds of experiencing a major bleeding event. A backwards stepwise selection method was implemented utilizing the following variables: age > 80 years, weight < 60 kg, baseline CrCl < 50 mL/min, AKI within 48 h of level, strong CYP3A4 or P-gp inhibitor use, antiplatelet use, inappropriately high dosing regimen, and anti-Xa level stratified into tertiles (< 100 , 100–300, and > 300 ng/mL). Variables with a *p*-value of < 0.2 were included into the final models. Three models were constructed, one combining both apixaban and rivaroxaban, and two for each individual FXaI. Descriptive and comparative analyses were performed with GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA), and binary logistic regression was carried out using STATA version 14.2 (StataCorp, College Station, TX).

3. Results

3.1. Patient characteristics

During the study period, a total of 979 anti-Xa levels for apixaban and rivaroxaban were ordered. After applying inclusion and exclusion criteria, 606 anti-Xa levels remained for full analysis (Fig. 1). Of 412 patients receiving apixaban, 89 patients had more than one level drawn during their hospitalization. Out of the 194 patients receiving rivaroxaban, 35 patients had multiple levels drawn during their hospital stay. Baseline information can be found in Table 1. The majority of patients were elderly, on apixaban, and had an indication of atrial fibrillation for anticoagulation therapy.

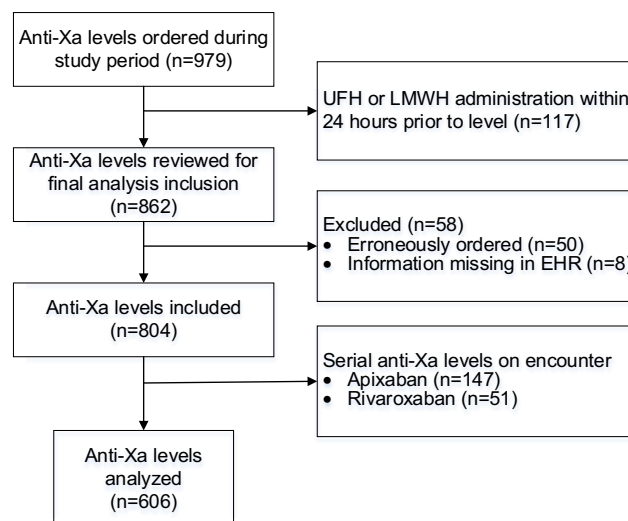


Fig. 1. Flow diagram for anti-Xa level inclusion for final analysis.

Table 1
Baseline characteristics.

	Total (n = 606)	Apixaban (n = 412)	Rivaroxaban (n = 194)
Age in years, median (IQR)	72 (61–81)	73 (60–82)	72 (64–80)
Female gender, n (%)	313 (52)	225 (55)	88 (45)
Weight in kg, median (IQR)	80.7 (67.1–98.4)	79.3 (66.7–94.9)	85.9 (69.2104.7)
Race, n (%)			
Caucasian	424 (70)	277 (67)	147 (76)
African American	149 (25)	111 (27)	38 (19)
Asian	20 (3)	13 (3)	7 (4)
Missing or declined	13 (2)	11 (3)	2 (1)
BMI classification, n (%)			
Underweight (< 18.5 kg/m ²)	20 (3)	15 (4)	5 (3)
Normal (18.5–24.9 kg/m ²)	175 (29)	124 (30)	51 (26)
Overweight (25–29.9 kg/m ²)	187 (31)	132 (32)	55 (28)
Obese (≥ 30–39.9 kg/m ²)	167 (28)	108 (26)	59 (30)
Morbidly obese (≥ 40 kg/m ²)	57 (9)	33 (8)	24 (12)
CrCl in mL/min, median (IQR)			
≥ 50	338 (56)	207 (50)	131 (68)
30–49	149 (24)	98 (24)	51 (26)
< 30	119 (20)	107 (26)	12 (6)
Indication for anticoagulation, n (%)			
Stroke prevention in AF	358 (59)	242 (59)	116 (60)
DVT	109 (18)	76 (18)	33 (17)
PE	41 (7)	21 (5)	20 (10)
DVT/PE	38 (6)	28 (7)	10 (5)
Other	33 (5)	27 (7)	6 (3)
Multiple	27 (3)	18 (4)	9 (5)
Concomitant antiplatelet use, n (%)	183 (30)	133 (32)	50 (26)
Unit where level ordered, n (%)			
Acute care floor	245 (40)	162 (39)	83 (43)
Intensive care unit	182 (30)	123 (30)	59 (30)
Emergency department	179 (30)	127 (31)	52 (27)

BMI, body mass index; CrCl, creatinine clearance; AF, atrial fibrillation; DVT, deep vein thrombosis; PE, pulmonary embolism.

3.2. Major bleeding events

There were 146 confirmed major bleeding events, occurring in 26% (105/412) of patients treated with apixaban and 21% (41/194) with rivaroxaban. Fatal bleeding events occurred in 10 patients on apixaban therapy and seven patients on rivaroxaban therapy. Sixteen of these fatal bleeds were neurological and one was pulmonary. The most common site of bleeding was neurological ($n = 92$), with specific types of intracranial bleeding including: subdural hematoma (SDH; $n = 42$), intracerebral hemorrhage (ICH; $n = 35$), and subarachnoid hemorrhage (SAH; $n = 13$). The second most common major bleeds were gastrointestinal (21%). Other major bleeds consisted of skin and soft tissue bleeding, pericardial effusions, retroperitoneal, and oropharyngeal bleeding. Observed anti-Xa levels in patients with and without major bleed are represented in Fig. 2. The median (10–90th percentile) anti-Xa level for patients taking apixaban with a major bleeding event was 86 ng/mL (< 20–317.5 ng/mL) compared to 105 ng/mL (< 20–323 ng/mL) in those without a major bleeding event ($p = 0.37$). The median anti-Xa level in patients taking rivaroxaban with a major bleed was 110 ng/mL (< 20–432 ng/mL) compared to 60 ng/mL (< 20–387.4 ng/mL) in those without a bleed ($p = 0.078$). In the subgroup analysis, patients with major bleeding displayed a significantly higher proportion of individuals with advanced age and inappropriately high dosing regimens at the time of anti-Xa level obtainment (Table 2). The results

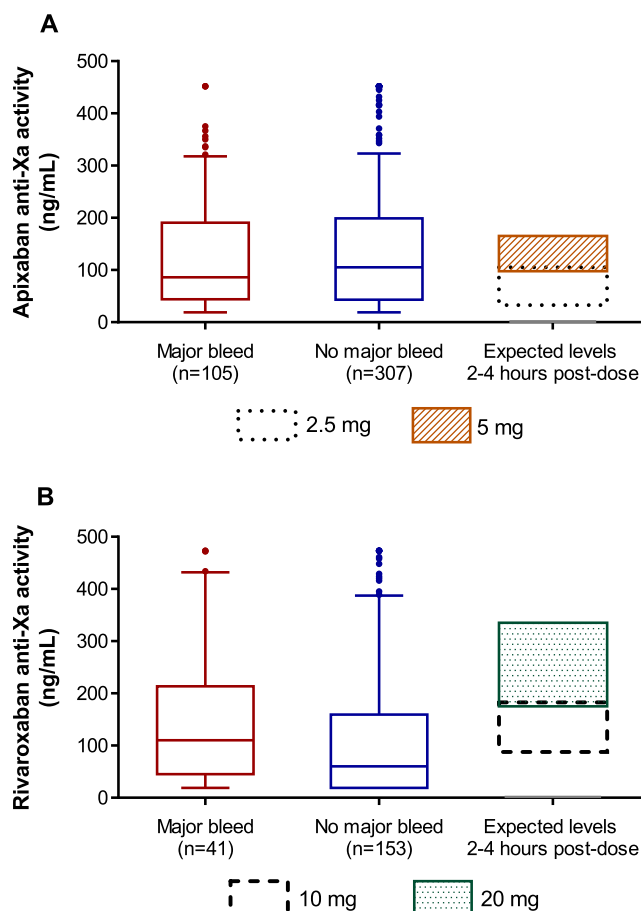


Fig. 2. Distribution of anti-Xa levels in patients with and without major bleeding events for apixaban – A and rivaroxaban – B. The expected levels are for peak measurements [13,25], and the results for certain individual patients may reflect random levels based on the time of last dose or if the time of last dose is unknown.

of binary logistic regression are displayed in Table 3. Only variables with a p -value < 0.2 in univariate analyses are displayed in the final models. In the model with both FXa's, an age of 80 years or greater and inappropriately high dosing regimen were associated with an increase in the odds of experiencing a major bleeding event. Factors associated with an increase in odds for major bleeding were an age of 80 years or greater for apixaban, and inappropriately high dosing regimen and an anti-Xa level of 100–300 ng/mL for rivaroxaban.

3.3. Reversal agent use

A total of 59 patients with a documented major bleeding event received a reversal agent, with 54 patients receiving 4F-PCC, and five patients receiving andexanet alfa. The indications for reversal in patients receiving 4F-PCC for a major bleed were: SDH (31%), ICH (31%), SAH (9%), upper/lower GI bleed (20%), genitourinary bleed (4%), or other. Five patients received andexanet alfa for the following major bleed types: ICH (60%), SDH (20%), and GI bleed (20%). Patients who received 4F-PCC or andexanet alfa had statistically significant higher median anti-Xa levels than those who did not receive a reversal agent for apixaban (73 ng/mL vs. 153 ng/mL, $p = 0.0019$), but not for rivaroxaban (76 ng/mL vs. 131 ng/mL, $p = 0.21$). There were 12 patients who received 4F-PCC ($n = 8$) and andexanet alfa ($n = 4$) outside the context of major bleeding. Three patients who were given 4F-PCC also received fresh frozen plasma (FFP), and six patients received only FFP. In addition, two patients received aminocaproic acid for a major bleed.

Table 2
Subgroup analysis comparing patients with and without a major bleeding event.

	No major bleed (n = 460)	Major bleed (n = 146)	P value
Age in years, median (IQR)	71 (59–80)	77 (66–84)	< 0.001
Dose inappropriately high ^a , n (%)	11 (2)	10 (7)	0.017
BMI in kg/m ² , median (IQR)	28.2 (28.2–33.5)	27.2 (23.7–31.1)	0.17
CrCl in mL/min, median (IQR)	55.9 (32.7–92.7)	51.4 (36.5–69.9)	0.13
AKI within 48 h of level, n (%)	60 (13)	13 (9)	0.19
Renal replacement therapy, n (%)	62 (13)	14 (10)	0.25
Antiplatelet use, n (%)	143 (31)	40 (27)	0.39

CrCl, creatinine clearance; AKI, acute kidney injury; BMI, body mass index.

^a Dose appropriateness was assessed based on the indication for anticoagulation, CrCl for rivaroxaban, and serum creatinine, age, and body weight for apixaban as reported in respective FDA-package approved labeling.

Table 3
Binary logistic regression results for risk factors associated with major bleeding.

Model	Variable	Odds ratio (95% CI)	P value
BOTH FXaI's	Age ≥ 80	1.67 (1.11–2.51)	0.013
	Dose high	2.94 (1.20–7.22)	0.019
APIXABAN	Age ≥ 80	1.92 (1.19–3.11)	0.008
	AKI	0.47 (0.21–1.05)	0.066
RIVAROXABAN	Dose high ^a	18.8 (3.36–105.4)	0.001
	Anti-Xa 100–300 ng/mL	2.73 (1.18–6.28)	0.018

FXaI, factor-Xa inhibitor, AKI, acute kidney injury.

^a Dose appropriateness was assessed based on the indication for anticoagulation, CrCl for rivaroxaban, and serum creatinine, age, and body weight for apixaban as reported in respective FDA-package approved labeling.

3.4. Reasons for obtaining level and changes to therapy

Indications for anti-Xa levels for apixaban and rivaroxaban are shown in Fig. 3. All anti-Xa levels were obtained in the inpatient setting, with the majority being ordered on an acute care floor and a similar distribution between the emergency department and intensive care units (ICU) (Table 1). The most common ordering service or providers were clinical pharmacists (126; 21%), ED physicians (111; 18%), ICU intensivists (90; 15%), and neurosurgery (76; 13%). Other ordering service lines included hematology, neurology, cardiology, and pulmonology. Although there may have been multiple reasons for obtaining a considerable proportion of anti-Xa levels, only the most pertinent reason as ascertained by chart review was categorized as the primary reason (Fig. 3). The most frequently encountered reasons for obtaining anti-Xa levels were confirmed or suspected bleeding, clearance for a procedure, and clearance for heparin initiation or fibrinolytic therapy consideration. Of note, of the 110 anti-Xa levels ordered for surgical clearance, 51%

(56/110) of these were emergent procedures including neurosurgical, spinal, and other time-sensitive procedures. Final changes to FXaI therapy for all patients alive at hospital discharge (n = 562) consisted of: no change (59%), discontinue FXaI (23%), switch to alternative anticoagulant (11%), decrease FXaI dose (4%), or increase FXaI dose (3%). In 145 (24%) patients, there was provider documentation indicating that an anti-Xa level played a role in the final change in therapy. For the patients with a major bleeding event and alive at discharge (n = 129), the final changes to FXaI therapy were as follows: discontinue FXaI (69%), no change (25%), switch to alternative anticoagulant (3%), decrease FXaI dose (2%), or increase FXaI dose (1%).

3.5. Levels following a dose

There were 143 anti-Xa levels following an inpatient dose of apixaban and 15 anti-Xa levels following a dose of rivaroxaban (Fig. 4). There were 9 patients taking apixaban who still had detectable (> 20 ng/mL or greater than the lower limit of assay quantification) anti-Xa levels beyond 30 h (not depicted), all of whom had AKI or were on hemodialysis, but none had a major bleeding event. Median (10–90th percentile) anti-Xa levels observed 2–4 h after a dose of apixaban were as follows: 2.5 mg, 120 ng/mL (76–238 ng/mL); 5 mg, 200 ng/mL (78– > 451 ng/mL). One rivaroxaban level was drawn within 2–4 h after a 15 mg dose (448 ng/mL), and four levels following a 20 mg dose, ranging from 51 ng/mL to > 472 ng/mL. None of the patients with detectable anti-Xa levels for rivaroxaban beyond 4 h had a major bleeding event, although three patients had AKI.

4. Discussion

Several studies have commented on reasons for obtaining anti-Xa levels for apixaban and rivaroxaban in clinical practice [9,17,26,27]. To

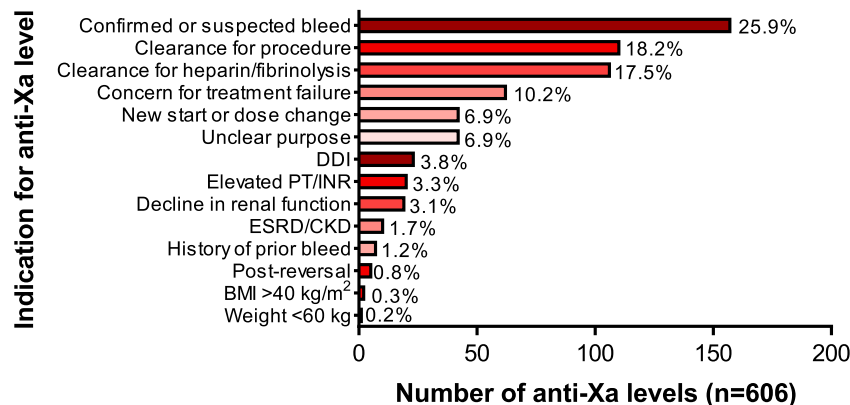


Fig. 3. Indications for ordering an anti-Xa level.

DDI, drug-drug interaction, ESRD, end-stage renal disease, CKD, chronic kidney disease, BMI, body mass index.

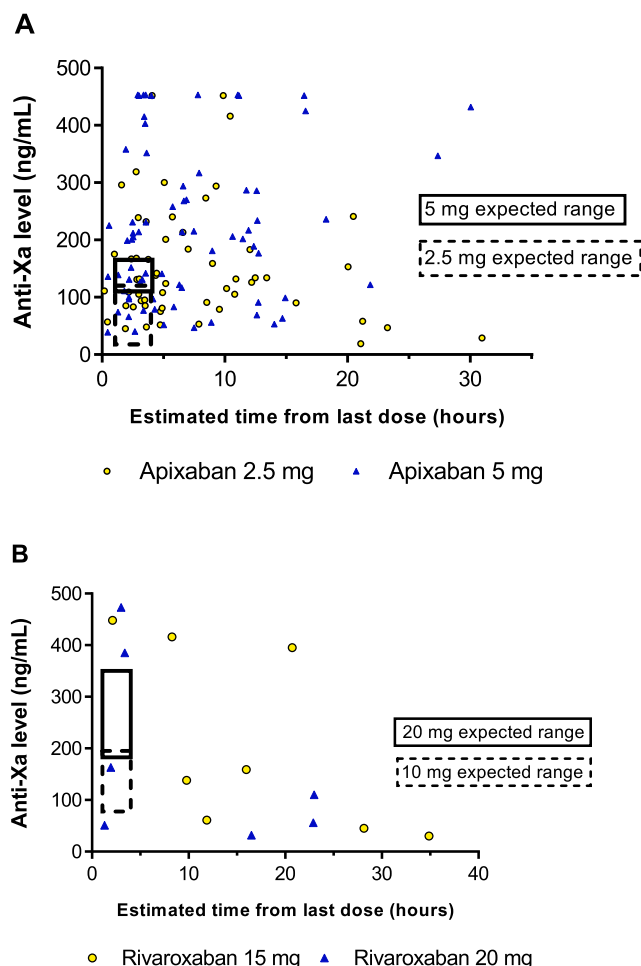


Fig. 4. Anti-Xa levels after a documented dose of apixaban – A and rivaroxaban – B.

The peak anti-Xa levels in the above figure are following a documented dose of apixaban or rivaroxaban. The expected peak levels boxes refer to the expected anti-Xa levels seen 2–4 h after a given dose of FXaI and are adopted from previously conducted studies [13,25].

our knowledge, this is one of the largest cohorts of patients with anti-Xa levels for both apixaban and rivaroxaban available in the context of major bleeding at the time of a bleeding event. Several other reports have attempted to describe the association between anti-Xa levels and clinical outcomes in a real-world setting. One study examined 411 C-peak anti-Xa levels for patients taking apixaban, rivaroxaban, and dabigatran for atrial fibrillation [28]. In a multivariate regression analysis, higher C-peak plasma concentrations were significantly associated with development of bleeding events (OR = 2.7, 95% CI = 1.3–5.4) [28]. Likewise, Japanese patients taking rivaroxaban for atrial fibrillation who experienced a major ($n = 3$) or non-major ($n = 19$) bleed had higher peak anti-Xa levels (2.40 IU/mL vs. 1.85 IU/mL, $p = 0.001$) and lower baseline creatinine clearance (46.2 mL/min vs. 58.8 mL/min, $p = 0.024$) [29]. Within our cohort, subgroup analyses and logistic regression models showed that advanced age was associated with major bleeding, which is in alignment with findings from other analyses of ROCKET-AF and ARISTOTLE [30,31], but did not demonstrate that lower baseline renal function, AKI, or antiplatelet use were associated with increased odds of major bleeding. Given that the timing of last dose was not always known, this may influence the sensitivity of these analyses specific to renal function. Moreover, increasing anti-Xa levels in our study were not necessarily associated with an increased risk of odds for major bleeding, with the exception of modest anti-Xa levels (100–300 ng/mL) for rivaroxaban. This finding also mirrors the anti-Xa

levels seen in patients with acute major bleeding events who received andexanet alfa in the ANNEXA-4 trial [32]. In addition, we identified that patients with inappropriately high-dose regimens of FXaI had increased odds of experiencing major bleeding. This finding is not surprising, and remains in line with evidence-based recommendations to dose adjust based on patient-specific factors for certain indications when clinically indicated [33].

In the ANNEXA-4 trial, the median anti-Xa levels for patients taking apixaban and rivaroxaban were 149.7 ng/mL and 211.8 ng/mL, respectively [32]. Our study found similar median FXaI levels, many of which may be considered as abnormally elevated given that our ranges are based on levels seen 2–4 h after a dose and many patients presented with an unknown last dose of FXaI. Interestingly, the investigators of ANNEXA-4 did not demonstrate a correlation between anti-Xa activity reduction and hemostatic efficacy with receiver operator characteristic (ROC) curve analyses. This is an important consideration, and although anti-Xa activity reduction may not be a strong predictor of clinical response to andexanet alfa, assessment of the extent of residual anticoagulation in patients treated with FXaI's may help guide the use of other reversal strategies. Furthermore, anti-Xa levels may be useful in guiding decisions for reversal of anticoagulation in emergent settings, especially in the absence of a complete history from a patient or when the last dose of drug is unknown and they have risk factors for drug accumulation [9,34]. Given that andexanet alfa is not widely accessible across all institutions, it is vital to consider the role of other reversal strategies and agents including fresh frozen plasma, blood products, and 4F-PCC [35–37]. Some comparative data between andexanet alfa and 4F-PCC are emerging [36], and further studies investigating the utility of anti-Xa levels to guide reversal agent use in the setting of unknown last dose of FXaI are needed [38]. Nonetheless, it may still be appropriate to utilize 4F-PCC for FXaI-associated major bleeding if andexanet alfa is not available, and this is endorsed by a recent consensus statement from the American College of Cardiology [39]. In the absence of a known or estimated last dose, drug specific anti-Xa levels may be invaluable during certain situations such as emergent surgery which may require rapid correction of anticoagulant effect. In our current investigation, obtaining a level for this purpose was common, and facilitated coordination of neurosurgical procedures and use of reversal agents in patients with major bleeding. Nonetheless, it is also important to consider the fact that waiting for an anti-Xa level result should not delay potentially life-saving interventions for the patient [40].

The most common reasons for obtaining an anti-Xa level in our study were similar with reports from other centers [14–16,41] and included new bleeding, prior to a surgical procedure, and prior to heparin or fibrinolytic therapy. One study included 102 patients taking either apixaban or rivaroxaban examined anti-Xa levels prior to cardiac ablation. The investigators found detectable plasma DOAC concentration (> 30 ng/mL) measured 24 h after the last intake in 51.3% of patients [42]. In more severely ill patients, residual anticoagulation resulting from decreased drug clearance may have important implications, especially when transitioning patients to parenteral anticoagulants [43]. Anti-Xa levels were frequently utilized to assess for residual anticoagulation or potential drug accumulation in our study, and we similarly observed some patients with detectable anti-Xa levels beyond 24 h after a dose, most often with concomitant renal impairment. The addition of strong inhibitors of FXaI metabolism may warrant the checking of anti-Xa levels to assess drug exposure. This was evidenced in a study including 12 hospitalized patients being treated for novel coronavirus infection COVID-19, where lopinavir/ritonavir administration resulted in an average 6.14-fold increase in trough plasma concentrations of apixaban and rivaroxaban [44]. Our analysis did not show an association of potential drug interactions and adverse events, however overall exposure for unique drugs was low and impact of different dosing regimens was not assessed. Further investigation is warranted into exploring the impact of drug interactions on adverse

events on a larger scale. Currently no guidelines endorse changes in therapy in response to anti-Xa levels for apixaban and rivaroxaban, however there were instances in our cohort where changes to FXaI therapy were in part due to an anti-Xa level in addition to the overall clinical context. Our results for all anti-Xa levels following a documented dose of FXaI underscores the importance that peak levels have a large inter-patient variability, which may be partly due to differences in anti-Xa assays and reagents used across different laboratories. Despite the fact that the expected level ranges currently utilized at our institution were derived from pharmacokinetic studies in healthy volunteers, growing evidence illustrates that in a real-world setting patient exhibit a wide range of levels after a given dose. For example, in a study of Korean patients, median peak levels for a 5 mg and 2.5 mg dose of apixaban were 202 ng/mL and 151 ng/mL, respectively [18]. The peak anti-Xa levels seen in our patients differed from those studies from which our expected level ranges were derived, highlighting the importance that hospitals may opt to develop and validate institution-specific ranges if anti-Xa levels are utilized on a consistent basis.

This study has several important limitations to consider, a major limitation being that anti-Xa testing was done in the clinical context of the patient, and not by randomization. The decision to assess anti-Xa levels for specific FXaI was therefore patient- and provider-specific, and it is possible that routine anti-Xa testing in unselected patients may yield different results. Second, the inherent limitations of a retrospective cohort study cannot be ignored, and although outcomes and changes to therapy were carefully chart reviewed in an objective manner, there may have been other contributing factors that were not captured. Third, certain risk factors that may predispose patients to develop bleeding (i.e., history of prior bleeding, concomitant comorbidities, and bleeding risk calculator tools such as HAS-BLED scores) were not included in our baseline demographic information. Therefore, it is uncertain how these risk factors may influence the odds of bleeding irrespective of corresponding anti-Xa levels within our patient population. Additionally, the majority of anti-Xa levels obtained in this study would be considered peak or random levels based on the time that they were drawn. Therefore, we cannot make any observations regarding trough levels which have been described in other studies and may display less inter-patient variability. Lastly, the reported upper limit of our institution's anti-Xa assay for apixaban and rivaroxaban is 451 ng/mL and 472 ng/mL, respectively. It is not typical practice at our institution to further dilute samples that exceed this upper limit, therefore the full extent of FXaI exposure may be underestimated in certain patients.

5. Conclusion

In conclusion, this study captured FXaI-specific anti-Xa levels in patients taking apixaban and rivaroxaban who experienced a major bleeding event. Both patients with and without major bleeding events exhibited levels that indicated circulating drug, however higher levels were not necessarily associated with increased odds of major bleeding within our cohort. We did identify advanced age, inappropriately high dosing regimens, and modest peak anti-Xa rivaroxaban levels as being associated with major bleeding. Management of selected patients with major bleeding events included the use of reversal agents such as 4F-PCC and andexanet alfa, and those who received a reversal agent tended to have higher anti-Xa levels than those who did not. The most common reason for obtaining anti-Xa levels seen in our cohort included confirmed or suspected bleeding, heparin or fibrinolysis clearance, and clearance for a procedure. While our study may highlight the utility of anti-Xa levels for apixaban and rivaroxaban in certain clinical situations including confirmed or suspected bleeding, clearance for heparin or fibrinolytic therapy, and prior to a surgical procedure, changes to FXaI therapy should always be made in the context of each patient and take into account other factors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Diane Dreucean and Yung Tran for their contributions.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, nor not-for-profit sectors.

References

- [1] G. Agnelli, H.R. Buller, A. Cohen, et al., Oral apixaban for the treatment of acute venous thromboembolism, *N. Engl. J. Med.* 369 (9) (2013) 799–808.
- [2] EINSTEIN Investigators, R. Bauersachs, S.D. Berkowitz, et al., Oral rivaroxaban for symptomatic venous thromboembolism, *N. Engl. J. Med.* 363 (26) (2010) 2499–2510.
- [3] R.P. Giugliano, C.T. Ruff, E. Braunwald, et al., Edoxaban versus warfarin in patients with atrial fibrillation, *N. Engl. J. Med.* 369 (22) (2013) 2093–2104.
- [4] C.B. Granger, J.H. Alexander, J.J. McMurray, et al., Apixaban versus warfarin in patients with atrial fibrillation, *N. Engl. J. Med.* 365 (11) (2011) 981–992.
- [5] M.R. Patel, K.W. Mahaffey, J. Garg, et al., Rivaroxaban versus warfarin in non-valvular atrial fibrillation, *N. Engl. J. Med.* 365 (10) (2011) 883–891.
- [6] A.M. Pirlog, C.D. Pirlog, M.A. Maghiar, DOACs vs vitamin K antagonists: a comparison of phase III clinical trials and a prescriber support tool, *Open Access Maced. J. Med. Sci.* 7 (7) (2019) 1226–1232.
- [7] E.M. Schmitz, K. Boonen, D.J. van den Heuvel, et al., Determination of dabigatran, rivaroxaban and apixaban by ultra-performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) and coagulation assays for therapy monitoring of novel direct oral anticoagulants, *J. Thromb. Haemost.* 12 (10) (2014) 1636–1646.
- [8] A. Cuker, Laboratory measurement of the non-vitamin K antagonist oral anticoagulants: selecting the optimal assay based on drug, assay availability, and clinical indication, *J. Thromb. Thrombolysis* 41 (2) (2016) 241–247.
- [9] P. Billoir, V. Barbay, L.M. Joly, M. Fresel, M.H. Chretien, V. Le Cam Duchez, Anti-xa oral anticoagulant plasma concentration assay in real life: rivaroxaban and apixaban quantification in emergency with LMWH calibrator, *Ann. Pharmacother.* 53 (4) (2019) 341–347.
- [10] B.T. Samuelson, A. Cuker, D.M. Siegal, M. Crowther, D.A. Garcia, Laboratory assessment of the anticoagulant activity of direct oral anticoagulants: a systematic review, *Chest* 151 (1) (2017) 127–138.
- [11] S.H. Park, Y.H. Seo, P.W. Park, et al., Evaluation of global laboratory methods and establishing on-therapy ranges for monitoring apixaban and rivaroxaban: experience at a single institution, *J. Clin. Lab. Anal.* 33 (5) (2019) e22869.
- [12] H. Ten Cate, Y.M. Henskens, M.D. Lance, Practical guidance on the use of laboratory testing in the management of bleeding in patients receiving direct oral anticoagulants, *Vasc. Health Risk Manag.* 13 (2017) 457–467.
- [13] C. Frost, S. Nepal, J. Wang, et al., Safety, pharmacokinetics and pharmacodynamics of multiple oral doses of apixaban, a factor xa inhibitor, in healthy subjects, *Br. J. Clin. Pharmacol.* 76 (5) (2013) 776–786.
- [14] T.M. Gu, D.A. Garcia, D.E. Sabath, Assessment of direct oral anticoagulant assay use in clinical practice, *J. Thromb. Thrombolysis* 47 (3) (2019) 403–408.
- [15] C. Wright, R. Brown, A. Cuker, Laboratory measurement of the direct oral anticoagulants: indications and impact on management in clinical practice, *Int. J. Lab. Hematol.* 39 (Suppl. 1) (2017) 31–36.
- [16] K. Martin, S. Moll, Direct oral anticoagulant drug level testing in clinical practice: a single institution experience, *Thromb. Res.* 143 (2016) 40–44.
- [17] S. Takatsuki, T. Kimura, K. Sugimoto, et al., Real-world monitoring of direct oral anticoagulants in clinic and hospitalization settings, *SAGE Open Med.* 5 (2017) (2050312117734773).
- [18] H. Shin, M.C. Cho, R.B. Kim, et al., Laboratory measurement of apixaban using anti-factor xa assays in acute ischemic stroke patients with non-valvular atrial fibrillation, *J. Thromb. Thrombolysis* 45 (2) (2018) 250–256.
- [19] P.A. Reilly, T. Lehr, S. Haertter, et al., The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: the RE-LY trial (randomized evaluation of long-term anticoagulation therapy), *J. Am. Coll. Cardiol.* 63 (4) (2014) 321–328.
- [20] C.T. Ruff, R.P. Giugliano, E. Braunwald, et al., Association between edoxaban dose, concentration, anti-factor xa activity, and outcomes: an analysis of data from the randomised, double-blind ENGAGE AF-TIMI 48 trial, *Lancet* 385 (9984) (2015) 2288–2295.
- [21] S. Schulman, C. Kearon, Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis, Definition of major bleeding in clinical investigations

- of antithrombotic medicinal products in non-surgical patients, *J. Thromb. Haemost.* 3 (4) (2005) 692–694.
- [22] A. Khwaja, KDIGO clinical practice guidelines for acute kidney injury, *Nephron. Clin. Pract.* 120 (4) (2012) c179–c184.
- [23] Eliquis [Package Insert], Bristol-Myers Squibb Company, Princeton, NJ, 2019.
- [24] Xarelto [Package Insert], Janssen Pharmaceuticals, Inc, Titusville, NJ, 2020.
- [25] W. Mueck, L.C. Borris, O.E. Dahl, et al., Population pharmacokinetics and pharmacodynamics of once- and twice-daily rivaroxaban for the prevention of venous thromboembolism in patients undergoing total hip replacement, *Thromb. Haemost.* 100 (3) (2008) 453–461.
- [26] V. Dubois, A.S. Dincq, J. Douxfils, et al., Perioperative management of patients on direct oral anticoagulants, *Thromb. J.* 15 (2017) 14–017.
- [27] D.M. Adcock, R.C. Gosselin, The danger of relying on the APTT and PT in patients on DOAC therapy, a potential patient safety issue, *Int. J. Lab. Hematol.* 39 (Suppl. 1) (2017) 37–40.
- [28] S. Testa, C. Legnani, E. Antonucci, et al., Drug levels and bleeding complications in atrial fibrillation patients treated with direct oral anticoagulants, *J. Thromb. Haemost.* 17 (7) (2019) 1064–1072.
- [29] T. Sakaguchi, H. Osanai, Y. Murase, et al., Monitoring of anti-Xa activity and factors related to bleeding events: a study in Japanese patients with nonvalvular atrial fibrillation receiving rivaroxaban, *J. Cardiol.* 70 (3) (2017) 244–249.
- [30] S.G. Goodman, D.M. Wojdyla, J.P. Piccini, et al., Factors associated with major bleeding events: insights from the ROCKET AF trial (rivaroxaban once-daily oral direct factor xa inhibition compared with vitamin K antagonism for prevention of stroke and embolism trial in atrial fibrillation), *J. Am. Coll. Cardiol.* 63 (9) (2014) 891–900.
- [31] E.M. Hylek, C. Held, J.H. Alexander, et al., Major bleeding in patients with atrial fibrillation receiving apixaban or warfarin: the ARISTOTLE trial (apixaban for reduction in stroke and other thromboembolic events in atrial fibrillation): predictors, characteristics, and clinical outcomes, *J. Am. Coll. Cardiol.* 63 (20) (2014) 2141–2147.
- [32] S.J. Connolly, M. Crowther, J.W. Eikelboom, et al., Full study report of andexanet alfa for bleeding associated with factor xa inhibitors, *N. Engl. J. Med.* 380 (14) (2019) 1326–1335.
- [33] S. Moudallel, S. Steurbaut, P. Cornu, A. Dupont, Appropriateness of DOAC prescribing before and during hospital admission and analysis of determinants for inappropriate prescribing, *Front. Pharmacol.* 9 (2018) 1220.
- [34] G.F. Tomaselli, K.W. Mahaffey, A. Cuker, et al., 2017 ACC expert consensus decision pathway on management of bleeding in patients on oral anticoagulants: a report of the American college of cardiology task force on expert consensus decision pathways, *J. Am. Coll. Cardiol.* 70 (24) (2017) 3042–3067.
- [35] N.G. Panos, A.M. Cook, S. John, G.M. Jones, Neurocritical Care Society (NCS) Pharmacy Study Group, Factor xa inhibitor-related intracranial hemorrhage (FiX-ICH): results from a multicenter, observational cohort receiving prothrombin complex concentrates, *Circulation* 141 (21) (2020) 1681–1689.
- [36] M.E. Barra, A.S. Das, B.D. Hayes, et al., Evaluation of andexanet alfa and four-factor prothrombin complex concentrate (4F-PCC) for reversal of rivaroxaban- and apixaban-associated intracranial hemorrhages, *J. Thromb. Haemost.* 18 (7) (2020) 1637–1647.
- [37] M.N. Smith, L. Deloney, C. Carter, K.A. Weant, E.A. Eriksson, Safety, efficacy, and cost of four-factor prothrombin complex concentrate (4F-PCC) in patients with factor xa inhibitor-related bleeding: a retrospective study, *J. Thromb. Thrombolysis* 48 (2) (2019) 250–255.
- [38] K.A. Parsels, R.W. Seabury, W. Darko, L.A. Probst, G.M. Cwikla, C.D. Miller, Is it truly “alpha”? Incidence of thrombotic events with andexanet alfa at a single academic medical center, *Ann. Emerg. Med.* 75 (5) (2020) 675–676.
- [39] 2020 ACC expert consensus decision pathway on management of bleeding in patients on oral anticoagulants: a report of the American College of Cardiology Solution Set Oversight Committee, *J. Am. Coll. Cardiol.* 76 (5) (2020) 594–622.
- [40] J.H. Levy, W. Ageno, N.C. Chan, et al., When and how to use antidotes for the reversal of direct oral anticoagulants: guidance from the SSC of the ISTH, *J. Thromb. Haemost.* 14 (3) (2016) 623–627.
- [41] D.J. Seiffge, C. Traenka, A.A. Polymeris, et al., Intravenous thrombolysis in patients with stroke taking rivaroxaban using drug specific plasma levels: experience with a standard operation procedure in clinical practice, *J. Stroke* 19 (3) (2017) 347–355.
- [42] A. Sairaku, Y. Nakano, Y. Onohara, et al., Residual anticoagulation activity in atrial fibrillation patients with temporary interrupted direct oral anticoagulants: comparisons across 4 drugs, *Thromb. Res.* 183 (2019) 119–123.
- [43] C.N. Lopez, L. Succar, S. Varnado, K.R. Donahue, Direct oral to parenteral anticoagulants: strategies for inpatient transition, *J. Clin. Pharmacol.* (2020), <https://doi.org/10.1002/jcph.1694>. Online ahead of print.
- [44] S. Testa, P. Prandoni, O. Paoletti, et al., Direct oral anticoagulant plasma levels' striking increase in severe COVID-19 respiratory syndrome patients treated with antiviral agents: the Cremona experience, *J. Thromb. Haemost.* 18 (6) (2020) 1320–1323.