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CLINICAL PHARMACOLOGY (L BRUNETTI, SECTION EDITOR)

Overview and Practical Application of Coagulation Assays in Managing Anticoagulation with Direct Oral Anticoagulants (DOACs)

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Abstract

Purpose of Review The introduction of newer anticoagulants requires clinicians to fully appreciate, interpret, and correctly apply the use of coagulation assays, such as the prothrombin time (PT), activated partial thromboplastin time (APTT), and anti-factor Xa assays. For oral vitamin K antagonists, the international normalized ratio (INR) is a predictor of anticoagulation intensity. However, for direct oral anticoagulants (DOACs), the PT or INR and APTT are unable to quantify the level of anticoagulation intensity as there is a poor correlation between plasma concentration of DOAC with these routine coagulation assays and that significant anticoagulant effect may still be present despite normal or near normal results for these routine assays.

Recent Findings In the USA, there are 5 DOACs available including dabigatran, a direct thrombin inhibitor and 4 direct factor Xa inhibitors (FXaI), each with varying indications, doses, pharmacodynamic, and pharmacokinetic characteristics. A thorough understanding of these properties aids in the management of the periprocedural or bleeding patient.

Summary In this first section of this manuscript, we will review the laboratory tests that are commonly performed for assessing a patient's coagulation status with known DOAC exposure. The second section will describe 3 real-world challenging case studies in DOAC-treated patients, with a focus on presenting clinical queries, interpretation of baseline laboratory tests with interpretations, followed by the case discussion to combine interpretation of appropriate laboratory tests with clinical patient considerations in an effort to guide clinical decision-making.

Keywords Direct oral anticoagulants · Laboratory testing · Dabigatran · Rivaroxaban · Apixaban · Edoxaban · Betrixaban · Ecarin · Anti-FXa · Thrombin generation · Andexanet · Dilute thrombin time · Thrombin time

Background

The introduction of the direct oral anticoagulants (DOACs), recombinant concentrated factor replacement therapies, and targeted reversal agents requires clinicians to fully appreciate, interpret, and correctly apply coagulation assays, such as the prothrombin time (PT), activated partial thromboplastin time (APTT), and anti-factor Xa assays in patients receiving these newer agents. Unlike VKA's where the international normalized ratio (INR) is a predictor of anticoagulation intensity, general coagulation assays, such as the PT or INR and APTT, are unable to quantify level of anticoagulation intensity for those on DOACs, as there is a poor correlation between plasma concentration of DOAC with these routine coagulation assays and that significant anticoagulant effects may still be encountered in patients despite normal or near normal routine assays. Additionally, they are not designed nor

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standardized to assess the inhibition of one specific coagulation factor. This is a paradigm shift of laboratory testing for the assessment of anticoagulated and bleeding patients. The DOACs available in the USA include a direct thrombin inhibitor and 4 direct factor Xa inhibitors (FXaI) with varying indications, doses, pharmacodynamic, and pharmacokinetic characteristics [1, 2] (Table 1). A thorough understanding of these properties aids in the management of the periprocedural or bleeding patient.

The PT and APTT have been the primary coagulation tests utilized to (1) screen patients for factor deficiencies, (2) monitor anticoagulation of vitamin K antagonists or heparins, or (3) assess the efficacy of factor replacement therapy (e.g., fresh frozen plasma, or factor VIII). As such, these tests are commonly performed in clinical laboratories worldwide. With an increase in availability of automated coagulation analyzers, the testing panel to assess a patient's coagulation status has also evolved to provide a rapid assessment of more specific tests including factor levels (e.g., fibrinogen, factor VIII), markers of fibrin degradation (e.g., D-dimer), and other assays. These assays directly or indirectly measure various components of the coagulation pathway (Fig. 1).

In this manuscript, we will (1) review the laboratory tests that are commonly performed for assessing a patient's coagulation status with known DOAC exposure, and then (2) present common, yet challenging, case studies in DOAC-treated patients, with a focus on how to combine interpretation of appropriate laboratory tests with clinical patient considerations to guide clinical decision-making. The use of point-of-care or viscoelastic platforms will not be addressed, as these methods are currently not widely available in all clinical laboratories or settings.

Prothrombin Time

In 1935, Armand Quick developed a test to estimate the prothrombin concentration, using dehydrated human brain as the tissue thromboplastin source which was subsequently used for diagnosing liver disease and monitoring vitamin K and dicoumarol anticoagulation [3, 4]. Later studies and with the identification of additional coagulation factors determined that the quick prothrombin time (PT) also measures fibrinogen, V, VII, and X, in addition to prothrombin (factor II) [5]. Soon after the quick method was implemented, it was noted that variations in the thromboplastin source, including use of rabbit brain in lieu of human brains, would lead to differences in PT clotting times and thus standardization of the PT seemed prudent [4, 5].

The PT reagent consists of calcium and tissue thromboplastin sources derived from animal or human brains, human placenta, cell cultures, or recombinant material. The sensitivity of PT reagents to factor deficiencies is predominantly based on the concentration of two primary reagent phospholipids (PL), phosphatidyl serine (PS) and phosphatidyl choline (PC), and less influenced by the concentration of the test activator, tissue factor (TF) [6, 7]. There is a decrease of reagent sensitivity to factor deficiencies with increasing PS concentration, whereas increasing PC concentration will have higher sensitivity to factor deficiencies [6, 7]. The differences between PT reagents have thus been shown to be dependent of the type and concentration of activator (e.g., rabbit brain, placenta, synthetic) and PC:PS ratios, which has since been confirmed by the differences in clotting times between laboratory reagent platforms for abnormal samples.

Table 1 DOAC characteristics

	Apixaban	Betrixaban	Dabigatran	Edoxaban	Rivaroxaban
Trade name	Eliquis®	Bevyxxa®	Pradaxa®	Savaysa®	Xarelto®
Mechanism of action	Binds free and bound FXa	Binds free and bound FXa	Binds free and bound thrombin	Binds free and bound FXa	Binds free and bound FXa
Bioavailability	50%	34%	3–7%	62%	80–100%
Protein binding	87%	60%	35%	55%	92–95%
Primary clearance	56% fecal	85% fecal	80% renal	50% renal	67% renal
Time to peak concentration	3–4 h	3–4 h	1.5–3 h	1–2 h	2–3 h
Half-life[§]	12 h	19–27 h	12–14 h	10–14 h	5–13 h
NVAF peak (ng/mL)	91–321	N/I	117–275	12–245	184–343
NVAF trough (ng/mL)	41–230	N/I	61–143	19–62	12–137
VTE treatment peak (ng/mL)	59–302	N/I	117–275	N/A	22–535
VTE treatment trough (ng/mL)	22–177	N/I	61–143	N/A	6–239

[§] Renal function dependent

NVAF, non-valvular atrial fibrillation; *VTE*, venous thromboembolism, *N/A*, not available, *N/I*, drug not approved for this indication

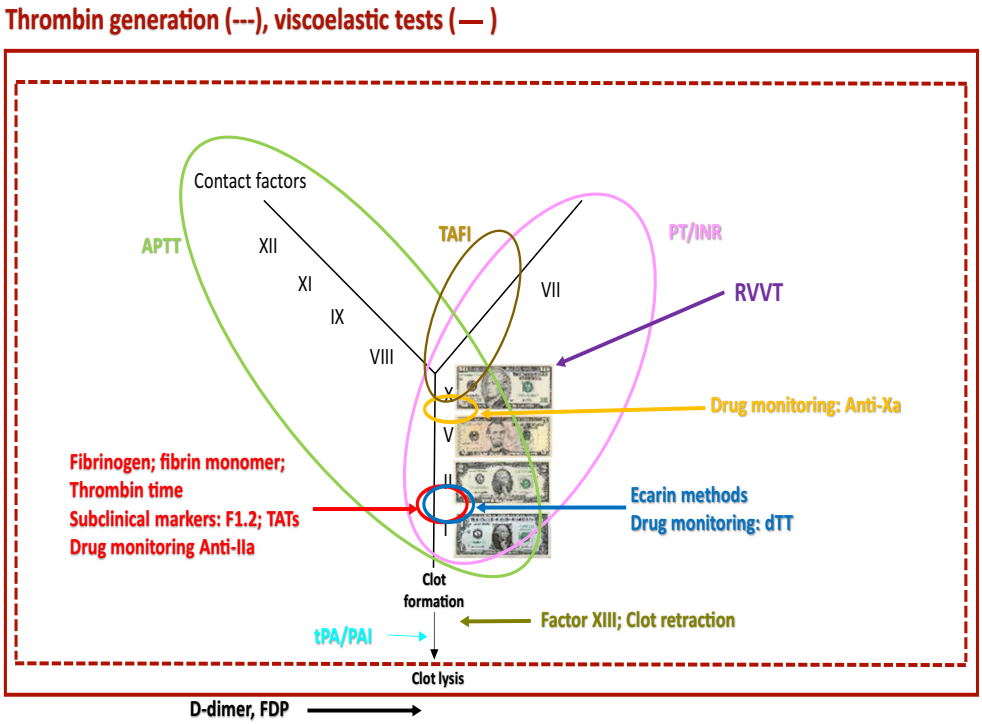

Fig. 1 Laboratory testing of the coagulation cascade. XII, XI, etc.: Factors XII, XI, etc.; APTT, activated partial thromboplastin time; TAFI, tissue factor pathway inhibitor; PT, prothrombin time; INR, international normalized ratio; RVVT, Russell's Viper Venom time; F1.2, prothrombin fragment 1+2; TAT, thrombin-antithrombin complexes; dTT, dilute thrombin time; tPA, tissue plasminogen activator; PAI, plasminogen activator inhibitor; FDP, fibrin(ogen) degradation products. Viscoelastic tests would include thromboelastogram (TEG), rotational thromboelastometry (ROTEM), and equivalent devices

International Normalized Ratio

The PT differences observed between laboratories that used rabbit brain thromboplastins (common in the USA) and human sources (common in Europe) have been reduced by a method aiming of standardizing the PT for warfarin-treated patients. This standardization was proposed by the World Health Organization (WHO) in 1983 [8, 9], and is considered to be standard of practice for monitoring warfarin-treated patients today, although it has been reported that some variability still exists [10].

The international normalized ratio (INR) is a calculated parameter based on the patient's PT result, the mean normal PT (MNPT), and the international sensitivity index (ISI) using the following equation: $[PT/MNPT]^{ISI}$. The ISI reflects the reagent sensitivity to the WHO reagent standard, is instrument specific, and provided with reagent instructions for use (IFU). The MNPT represents the mean of the normal population, which should be locally determined or confirmed. Initial recommendations suggested no less than 30 normal donors of equal gender representation was acceptable for MNPT determination [11], but more recent recommendations suggest 120 ostensibly healthy normal donors [12].

Activated Partial Thromboplastin Time

First described in the early 1950s by Dr. Brinkhaus' group from the University of North Carolina [13], and then later modified by the Rapaport et al. using kaolin activation [14], the partial thromboplastin time (PTT) was used in the diagnosis of hemophilia. With the addition of activators (primarily silica and ellagic acid in the US reagents), the PTT is now described as an activated PTT (APTT). Today, the APTT is commonly used to assess contact factors (high molecular weight kininogen, prekallikrein, factor XII), other intrinsic (factors VIII, IX, XI), and common pathway factors. In addition, the APTT is used for monitoring unfractionated heparin and parenteral direct thrombin inhibitors, monitoring recombinant factor replacement therapies for hemophilia patients, bypassing agent therapies, and is also used in the diagnosis of lupus anticoagulant. While synthetic processes for PL sources and activators have improved the relationship between APTT reagent lots, there has been no standardization of the APTT to date between reagent manufacturers or the detection methods (optical versus mechanical clot detection). As with PT reagents, the activator (e.g., kaolin, celite, silica) and PL type and concentration will be major determinants for factor deficiency sensitivity, heparin response, and lupus anticoagulant detection [15–17]. Prolonged APTT in drug-naïve patients can be secondary to a host of conditions, including but not limited to presence of lupus anticoagulants, hemophilia, contact factor (e.g., factor XII) deficiencies, and von Willebrand disease (when associated with decreased factor VIII). Other limitations of the APTT can be either (or both) analytic and biologic variables such as citrate concentration, time from sample processing to collection, temperature, and significant variation in results due to both instrumentation and reagent variability. Biologic variables include the interference

from elevated concentrations of factor VIII (FVIII) or FBG (potential "resistance" or failure to achieve therapeutic target) or the presence of lupus anticoagulant or clotting factor deficiencies (elevated baseline before initiation of therapy) [18, 19].

Fibrinogen or Thrombin Time Test

Both the fibrinogen (FBG) and thrombin time (TT) are based on the addition of thrombin to a plasma sample. For FBG, the patient sample is typically diluted in saline or buffer, and the thrombin concentration in the reagent is high (e.g., 35–50 UNIH/mL). The FBG test is reported as a concentration based on the clotting times and extrapolated from a calibration curve. Fibrinogen estimation has also been reported from the clot kinetics derived from PT testing, although this method has not been readily embraced as a means for reporting FBG concentration [20]. The FBG has been used in the assessment of fibrinogen abnormalities (e.g., dysfibrinogenemia), consumptive coagulopathies such as disseminated intravascular coagulation (DIC), surgical patients, or other indications.

Because the FBG uses a diluted patient sample and high thrombin concentration, only high concentrations of thrombin inhibitors will interfere with FBG [21, 22]. Nevertheless, some FBG reagents use less thrombin and may be affected by higher concentrations of direct thrombin inhibitors like dabigatran [21, 23].

The TT uses a neat or minimally diluted plasma sample with a reagent containing low concentrations of thrombin (e.g., ~ 1–10 UNIH/mL). The TT is expressed in seconds but can be normalized and expressed as a ratio (result/mean of reference range). The TT has been used for assessing DIC and heparin anticoagulation (uncommon use unless there is an APTT interference). It is now also recommended for the exclusion of the presence of direct thrombin inhibitors like dabigatran [1].

Because the FBG uses a diluted patient sample and high thrombin concentration, and the thrombin time uses an undiluted patient sample and low thrombin concentration, these tests are variably affected by thrombin inhibitors (oral and parenteral). Parenteral DTIs (e.g., bivalirudin or argatroban) impact the TT in a linear fashion [24], but the TT is highly sensitive to dabigatran exposure. In patients treated with dabigatran, TT is used essentially for excluding the presence of the drug if the result is in the normal range [22, 25].

Dilute Thrombin Time

The dilute thrombin time (dTT) was first described as a means of monitoring parenteral direct thrombin inhibitors [26]. The salient differences between a dTT and the aforementioned TT are that for the dTT, the plasma sample is diluted (usually 1:8 or 1:4) in normal pooled plasma (NPP) and the test reagent consists of a low concentration of thrombin (~ 10 UNIH/mL) making the test much less variable to quantitative and qualitative defects in fibrinogen. The dTT is a clot-based assay using either optical or mechanical endpoints with the results reported in seconds and converted in respective concentration units via a calibration. Calibrations are available for the direct thrombin inhibitors (oral or parenteral) but the test itself does not differentiate between thrombin inhibitors.

Ecarin-Based Assays

Ecarin is derived from the saw-scaled viper *Echis carinatus* which will activate prothrombin (factor II) creating meizothrombin, which will then convert fibrinogen to fibrin. The initial uses for ecarin testing were for assessing patients with vitamin K deficiency [27] or for use to determine the presence of a lupus anticoagulant [28], using either chromogenic or clot-based methods. Despite the simple principle, the variations of testing include sample dilution (yes or no), degree of dilution (ratio of sample to diluent), diluent type (saline or buffer), whether preincubation of sample or reagents at 37 °C, and ecarin concentration [21, 29, 30]. As with ecarin clotting time (ECT), the use of ecarin chromogenic assay (ECA) has been described for decades [31–33]. The principle of chromogenic testing is simple with differences noted between sample preparation, addition of a prothrombin buffer, and chromogenic substrate for meizothrombin used [30]. Like dTT, calibrations are available for direct thrombin inhibitors (oral or parenteral) but the test is also unable to differentiate between thrombin inhibitors. One of the advantages of ecarin-based assays is they are not influenced by heparins (heparin does not inhibit meizothrombin [24]) making these tests probably more suitable to assess the dabigatran concentration in patients bridged with heparins. A potential limiting factor for widespread use in the USA is the lack of FDA-approved ecarin-based methods, so any introduction of these methods into the laboratory would constitute a laboratory developed assay.

Anti-FXa Assays

The anti-FXa test is a functional assay that has been available for decades as a means of monitoring low molecular weight or unfractionated heparins or fondaparinux. It can be calibrated using either drug specific calibrators (e.g., UFH, LMWH, direct FXa, or fondaparinux) or a hybrid calibration scheme for UFH or LMWH heparin monitoring. It is important to note that one cannot distinguish the drug type when using any calibrated anti-Xa test, as the method does not differentiate

the factor Xa inhibition as is the case for dTT or ecarin-based assays for direct thrombin inhibitors (Fig. 2).

There are several commercial kits which vary in their methodologies including pipetting schemes, sample dilution, FXa source, and concentration, and different substrate sources. One key feature of the anti-Xa assay, unlike the PT and APTT with its multiple uses (screen, diagnose, monitor), the anti-Xa is only used for drug monitoring or screening. As such, the specificity of this test is high for the inhibition of factor Xa and with few exceptions (interference due to lipemia or icterus), the presence of anti-Xa activity indicates presence of FXa inhibitor drug, including heparins and derivatives, and oral direct FXa [34–36].

Anti-FIIa Assays

Similar to Anti-FXa test principles, the anti-FIIa can be used to measure thrombin inhibitors [29]. With the anti-FIIa tests use a substrate specific for thrombin in a neat (undiluted) or diluted plasma sample. A thrombin reagent is added resulting in cleavage of the specific substrate whose release of chromogenic compound read either kinetically or after a specific time period. These commercial kits may contain a heparin neutralizing agent that can be used in patients who are on transitional therapy. Several commercial anti-FIIa kits are available for quantifying thrombin inhibitors; however, none are approved for use in the USA.

Thrombin Generation Assay

As 95% of thrombin generation occurs after the initial clot formation [37], the thrombin generating capacity of a patient or sample is not readily assessed using screening tests, such as the PT or APTT. The thrombin generation assay (TGA) measures the thrombin generating capacity of a plasma sample after exposure to tissue factor (with or without thrombomodulin) and phospholipids. The thrombin generation is analyzed using a chromogenic or fluorometric substrate over an extended read time (~ 15–20 min). The derivative of the thrombin generation curves results in a thrombogram consisting of a lag time (time for test initiation to start of thrombin generation), time to peak (or propagation indicating the time to reach maximal thrombin generation), peak height (maximal thrombin generated in sample), and the area under the curve (AUC) indicating thrombin generation or often referred as endogenous thrombin potential (ETP) [37] (Fig. 3).

Deviations of the TGA may suggest bleeding (increased lag time, increased time to peak, decreased peak and ETP) or thrombotic (increased ETP) risk [37]. There are few commercial options for TGA, including an automated platform, Genesia ST (Diagnostica Stago, Parsippany, NJ) [38]. However, in the USA, there are no FDA-approved TGA methods, as thus these tests are often used in research capacity.

Utilizing Laboratory Testing to Assess DOAC Exposure

The use of the hemostasis laboratory in assessing DOAC-treated patients is an evolving practice. Historically, the PT and/or APTT were used to estimate a patients exposure to anticoagulation, which included warfarin, heparins, and thrombin inhibitors. In the age of DOACs, the utility of these tests for assessing anticoagulant exposure is greatly limited, as normal PT or APTT do not assure absence of drug presence [34, 35, 39, 40]. A majority of coagulation tests are not standardized, thus making absolute values (e.g., clotting times) diffult to interpret in patients with known or unknown DOAC exposure. However, based on published studies, the aformeniontes tests and their relative sensitivity and/or range of quantitation in relation to DOAC concentration can be estimated.(Fig. 4).

PT and APTT

Early clinical and laboratory society recommendations suggested usage of PT and APTT for estimating DOAC exposure, especially in emergent situations [40–42]. Some of these recommendations still exist, albeit with more prudence and caveats, with a general shift away from utilitizing these tests. While studies using contrived (drug enriched normal plasma) samples demonstrated differences between PT and APTT reagent platforms and DOAC concentrations [2, 21, 22, 29, 43–47], similarities in studies evaluating real world patient samples were not readily apparent [36]. The suggestion of

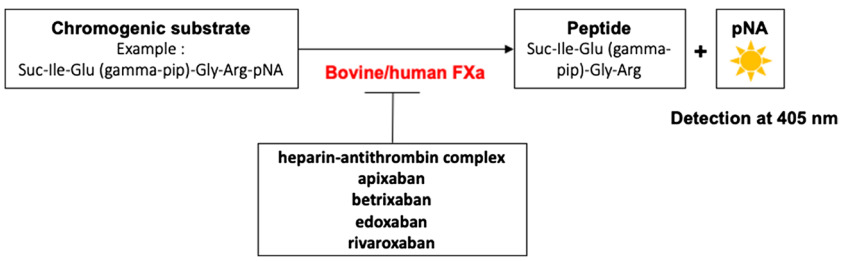

Fig. 2 Schematic representation of anti-FXa testing

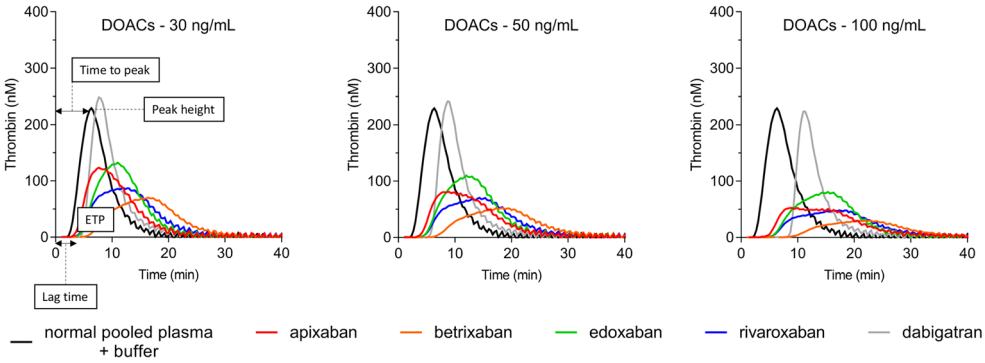

Fig. 3 The thrombogram parameters from thrombin generation test and representative changes in varying concentrations of DOACs

using drug calibrators or related material to gauge reagent concentration was subsequently regarded as questionable, as this practice may overestimate the reagent sensitivity to DOAC concentration, especially with drug calibrators used for specific test calibration, i.e., dTT, anti-FXa, anti-FIIa, or ecarin [47–49]. In addition, most clinicians are unaware of the reagent platform used at their institution [50], and often categorize PTs and APTTs to be as equivelent between labs as a potassium level. Recent recommendations from the International Council for Standardization in Hematology (ICSH) suggest that the PT and APTT not be used for assessing DOAC exposure, as these methods are not suitable or reliable for estimating concentration or, when within the normal reference range, suitable for excluding significant DOAC levels [35]. However, bleeding patients should have PT and APTT assessed, as the results may offer supplemental information about the patient's baseline coagulation status, especially if unexpected prolongation is reported. For example, a FBG test may be useful in a bleeding patient to assess consumptive coagulopathies (e.g., DIC) or other conditions.

TT and dTT

The traditional TT is suitable for excluding significant levels of dabigatran, as a multicenter study indicated that at low concentrations of dabigatran (i.e., 25 ng/mL), the clotting time was 2–3 times baseline [21–23]. As such, this method is useful for detecting dabigatran presence, with a normal TT excluding significant levels of this drug. For dabigatran quantification, using either a drug-calibrated dTT, ECT, ECA, or anti-FIIa method is suitable [35], and provides rapid results within 10 min (once collected blood sample is processed for testing) due to their automation.

Anti-Xa

As the number of patients receiving direct FXaIs increases, there is considerable interest in identifying the utility of using a heparin calibrated or LMWH calibrated anti-Xa assay to evaluate anticoagulation intensity. However, the majority of hospitals do not have these assays calibrated to the specific direct FXaI. Currently, approximately 1000 US-based laboratories perform this test; however, the test results may not be readily available 24/7 [51]. As such, there is considerable interest in identifying the value of using a heparin or LMWH calibrated anti-Xa assay to assess FXaI anticoagulation.

Several publications suggest the utilization of heparin calibrated anti-FXa measurements in the absence of drug-calibrated tests [36, 52–60]. There are essentially two different anti-FXa methods, one with antithrombin supplementation, and one without. The current recommendations for assessing direct FXaI is to utilize methods that are not supplemented with AT [35] as AT supplemented FXa methods tend to overestimate drug concentration [55, 56]. What is unclear from these publications is whether estimated direct FXaI and FXa levels are equivalent between other laboratory anti-Xa methods and reagents. From our published data [57, 58], there is some equivalence between direct FXaI with UFH calibrated Coamatic (Chromogenix) and Berichrom (Siemens) anti-FXa methods. The Stachrom Heparin (Diagnostica Stago) is highly sensitive to direct FXaI as compared to the other anti-FXa methods (the anti-Xa level corresponds with a lower direct factor Xa inhibitor concentration as compared to other test systems) (Table 2).

The applicability of heparin calibrated anti-Xa testing to direct FXaIs has been evaluated and demonstrated a linear correlation with heparin anti-Xa and direct FXaI concentrations [52, 53, 59], but judicous use and cautions are recommended [60]. The lower limit of quantitation (LLOQ) varies between reagent platforms and ranges between 0.04 and 0.10 U/mL, and therefore, the lowest corresponding direct FXaI level detected will be dependent on the platform used (Table 1). Depending on the instutions platform used, one may consider a patient with an anti-Xa level of 0.10 U/mL having neglible direct FXaI effect.

Whether DOAC measurements are useful for reversal management remains controversial. If readily available, DOAC levels could potentially guide reversal agent dosage based upon on drug exposure similar to the use of the INR to determine the 4-factor prothrombin complex

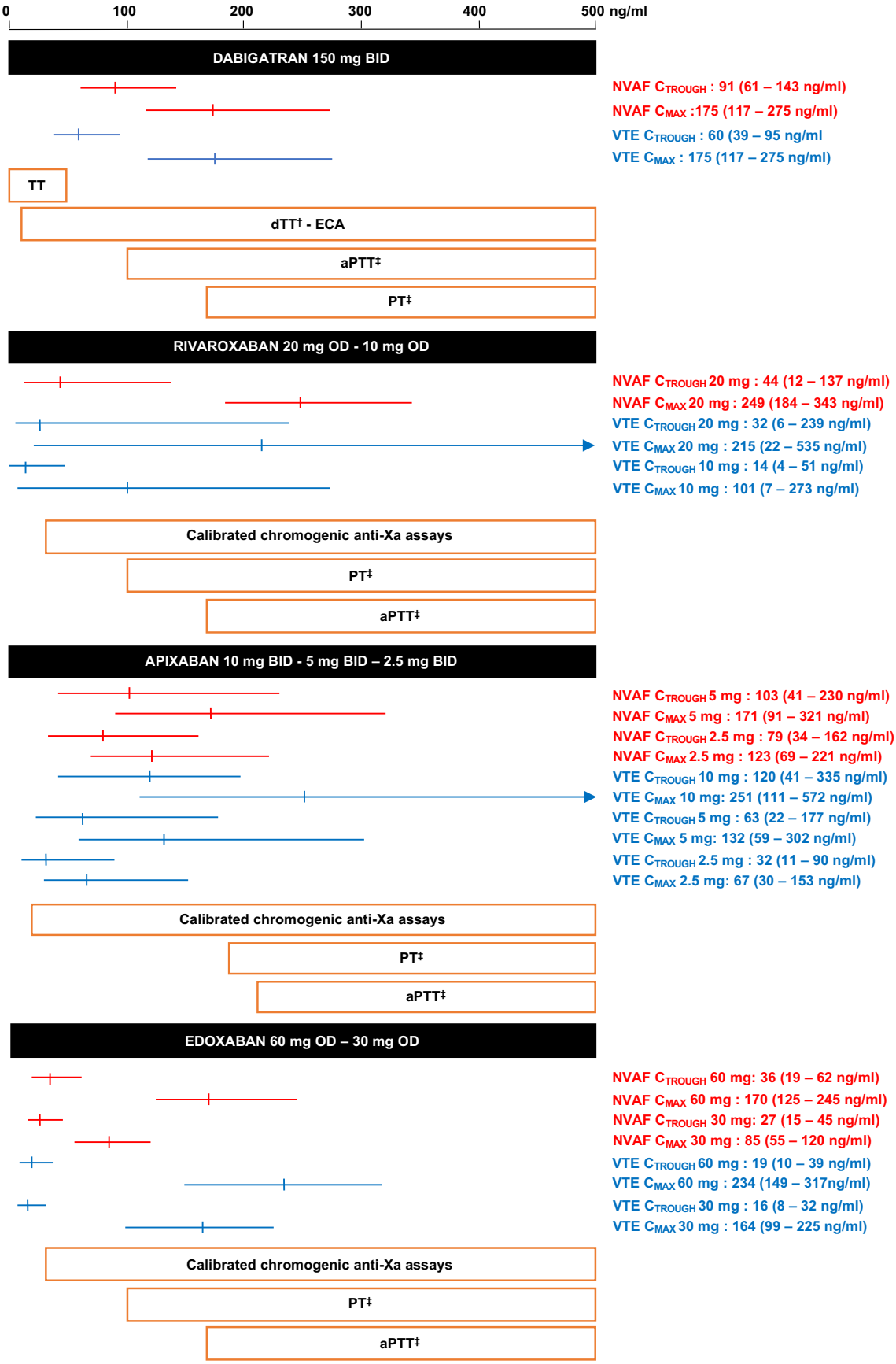

Fig. 4 Relative sensitivity and utility of coagulation assays and estimated DOAC exposure

Table 2 Differences between anti-Xa kits and apixaban, edoxaban, and rivaroxaban concentrations corresponding to 0.10 IU/mL, 0.5 IU/mL, and 1.0 IU/mL low molecular weight heparin (LMWH) anti-Xa activity [56]

Heparin kit	LMWH level	Apixaban (ng/mL)	Edoxaban (ng/mL)	Rivaroxaban (ng/mL)
Coamatic®[1]	0.10 IU/mL	16	26	12
Berichrom®[2]		24	35	21
STA®-Liquid Anti-Xa		1	7	4
Coamatic®[1]	0.50 IU/mL	80	96	70
Berichrom®[2]		90	118	82
STA®-Liquid Anti-Xa		36	36	22
Coamatic®[1]	1.00 IU/mL	168	183	143
Berichrom®[2]		172	223	159
STA®-Liquid Anti-Xa		80	72	45

[1] Coamatic® Heparin kit, Chromogenix/Instrumentation Laboratory, Bedford, MA

[2] Berichrome® Heparin kit; Siemens Healthcare Diagnostics, Deerfield, IL

[3] STA®-Liquid Anti-Xa heparin kit, Diagnostica Stago, Parsippany, NJ

This table should serve as guidance only, as variations on method calibrations will impact direct FXaI estimation of levels. Each laboratory should assess their own direct FXaI estimation using DOAC calibrator material (assigned drug concentrations) diluted appropriately to achieve similar concentrations

concentrate (PCC) dose for VKA reversal. For example, dosing and effect of the two available targeted reversal agents come with caveats. A set dose of Idarucizumab (Praxbind®) 5 g is recommended for dabigatran reversal, and will neutralize up to 1000 ng/mL of dabigatran, with the possibility to observe a "rebound" in therapeutic levels at 18–24 h after cessation of infusion [60, 61]. On the other hand, andexanet alfa (Andexxa®) dosing is based on the last dose of direct FXaI ingested and the time from this last dose. If the dose or time is unknown, the higher dose of 960 mg bolus with 800 mg maintenance infusion is recommended based on pharmacokinetic analyses [62]. In early publications, a "rebound" or return to placebo level was observed at 2 h post-infusion of andexanet alfa suggesting a complete and sustained reversal may not be observed. However, Portola Pharmacetucials (i.e., the marketing authorization holder of Andexxa®) subsequently reported in 2019 that some commercial anti-FXa assays may generate falsely high (residual) anti-FXa levels due to high sample dilutions which results in interference of the binding kinetics of andexanet alfa in the test cuvette [63]. In addition, in the FDA summary approval for andexanet alfa, it was noted that patient outcomes were not related to pre-treatment levels of direct FXaI exposure, and thus the FDA did not recommend the use of anti-FXa measurements as a surrogate for achieving reversal (ANNEXA-4) [64]. However, controversy persists amongst clinicians on the utility and applicability of drug levels in guiding decision-making for adminstering agents such as andexanet alfa or alternative agents.

TGAs

Several in vitro and ex vivo studies have demonstrated the potential of the thrombin generation for the assessment of the effect of DOACs effect and reversal therapy efficacy therapies [2, 22, 43, 44, 65–67]. Preliminary observations demonstrated that thrombin generation testing is affected by all anticoagulant drugs and therefore it could be a viable assay [1, 68, 69]. However, TGAs lack specificity to DOACs, as thrombogram parameters can be altered with pathophysiological changes in the hemostatic pathway in the absence of these drugs [1, 37]. Usually, factor Xa inhibitors reduce the peak and prolonged the time to peak with less effect on the lag time. On the other hand, dabigatran will displace the entire curve to the right (increased lag time) with less effect on the peak. It has been reported that dabigatran, at low dose, may increase the peak but this has been found to be an artifact. Both drugs impact the ETP but only at higher doses. The TGA may also better represent the inter-individual response to DOAC pharmacodynamics than plasma concentrations alone [38, 70]. TGAs may also explore in greater detail the impact of DOACs on the coagulation process, as depending on the type of drug, several studies have confirmed the PD differences between DOACs [2, 22, 43, 44, 64–67, 70, 71]. This is of particular importance as bleeding or thrombosis have been reported within the "on-therapy" range demonstrating that the drug level alone may not be sufficient to identify patients who are more at risk [72]. However, further investigation in patients who bleed or who have recurrent thrombosis while on a fixed dose of anticoagulants is needed to show the benefit of in vitro thrombin generation testing and provide cut-offs for

bleeding and thrombotic complications. While the TGA has also been reported to be an informative tool to document on antidote administration in polytrauma models with direct implication for patient care [73], and TGAs may help in adjusting the dose of reversal products [74, 75], the relationship of TGA parameters with clinical outcomes remains elusive.

Case Studies—Applications of Knowledge About Laboratory Testing and DOAC Exposure

Case 1

HT is a 65-year-old male (5′10″, 82 kg) who presents to the ED with symptoms of acute left sided weakness starting 30 min ago. A head computed tomogorahpy (CT) scan shows a hyperdense appearance in the right middle cerebral artery and without evidence of hemorrhage. The patient's past medical history is positive for hypertension and non-valvular atrial fibrillation for which he takes metoprolol 50 mg twice daily and apixaban 5 mg twice daily. His last apixaban dose was 10 h ago. Admitting relevant laboratory values include a platelet count of 250 × 10 [9]/L, serum creatinine of 1.0 (0.6–1.3 mg/dL), estimated creatinine clearnace 66 mL/min, PT 12.9 (9.2–13.5 s), aPTT 32 (25–37 s), and INR of 1.0. Admitting CT: The patient meets eligibility criteria for alteplase with the exception of the recent administration of apixaban.

Clinical Query

- The emergency room physician is asking the pharmacist for input and recommendations on the potential use of alteplase in this patient.

Laboratory Interpretation and Additional Recommendations

- In a patient with apixaban exposure, a normal PT or APTT does not exclude signficant levels of drug.
- The pharmacist recommends to also assess an anti-Xa (calibrated to UFH/LMWH) to gauge whether apixaban exposure (measured or estimated) is safe to provide thrombolysis.
- The pharmacist recommends a fibrinogen level to have a pre- recombinant tissue plasminogen activator (Rec-tPA) baseline value, and exclude any potential risk for bleeding diathesis.

Case 1 Discussion

As use of DOACs continues to expand, it is not surprising that there is an increase in patients presenting with acute ischemic strokes (AIS) where eligibility for thrombolysis is considered. The American Heart Association (AHA) identifies numerous contraindications for alteplase administration for AIS including the recent use of direct FXaIs or thrombin inhibitors [76, 77]. They recommend the avoidance of alteplase unless laboratory tests such as the aPTT, platelet count, INR, ECT, TT, or "appropriate direct FXa activity assays" are normal, or more than 48 h has passed since the last direct FXaI dose in those with normal renal function (Class III: Harm LoE C-EO) [76, 77]. The coagulation tests cited are not aligned with the class of antithrombotic (direct thrombin inhibitor versus direct FXa inhibitor) and the meaning of "appropriate" direct FXa activity assays is not clearly stated.

Clinical practice experience using thrombolysis in patients on DOACs is limited. There is controversy whether measuring DOAC levels provides added value in the clinical decision process [78–80]. When measurement is undertaken, the direct Xa concentration at which a safe invasive procedure can be performed has been suggested at both < 30 ng/ml and < 50 ng/ml with consensus lacking [78–81]. In the PAUSE study, perioperative DOAC hold times were evaluated in those with low and high bleeding risk. For apxiaban and rivaroxaban patients undergoing a high bleed risk procedure, therapy was held for 2 days corresonding to a 60- to 68-h hold time [82]. Direct Xa inhibitor concentrations of above 50 ng/ml occurred in 2.1% of apixaban patients and 0.6% of rivaroxaban patients while concentrations of above 30 ng/ml occurred in 6.9% of apixaban and 14.7% of rivaroxaban patients. The 30-day rate of major bleeding in all high bleed risk patients was approximately 3% with both apixaban and with rivaroxaban.

Seiffge et al. reported on the use of a calibrated rivaroxaban anti-Xa assay to assess rivaroxaban-treated patients with AIS for thrombolytic therapy. Thombolytics were recommended with a rivaroxaban level < 20 ng/ml and could be considered for levels of 20–100 ng/ml but should be avoided for rivaroxaban levels above 100 ng/ml as this represented efficient anticoaugulation [83]. In their study, sixty-three patients had rivaroxaban levels with a median level of 96ngl/ml [IQR 18–259] with the median time of last dose of 11 h [IQR 4.5–18.5]. Fifteen patients received thrombolysis without adverse bleeding events. This structured approach demonstrated the use of a calibrated anti-FXa assay in expanding the eligility of thrombolytic therapy for AIS in rivaroxaban-treated patients [83]. A consensus paper from France suggests thrombolysis in AIS can be administered when indicated if a drug specific anti-FXa assay result is < 50 ng/ml and further recommends that thrombolysis should be used with caution with levels of 50–100 ng/ml as experience within this latter group is limited [84].

Billoir et al. assessed direct Xa inhibitor anticoagulation in emergency situations using a liquid chromogenic LMWH

anti-Xa assay (Diagnostica Stago, Asnieres sur Seine, France) using LMWH in 37 FXa DOAC-treated patients [52]. The authors proposed that a LMWH < 0.5 IU/mL could exclude the presence of an apixaban or rivaroxaban concentration of above 30 ng/ml which in their study corresponded to an LMWH Anti-FXa of 0.46 IU/mL and 0.40 IU/mL for rivaroxaban and apixaban respectively [52].

Cappellari et al. reported on the use of thrombolysis for AIS in 27 patients treated with a DOAC in whom the last dose within 48 h [85]. Eleven patients were on direct FXaI, including 10 rivaroxaban with a time of last dose ranging from 6 to 26 h and one apixaban patient with a time of last dose of 8.5 h. Four rivaroxaban patients had a rivaroxaban calibrated anti-FXa level with results of 0 ng/nL, 10 ng/ml, 25 ng/mL, and 67 ng/mL. No patients developed hemorrhagic transformation postthrombolysis. The authors recommended that thrombolysis be reserved for patients in whom the time of last dose indicates at least 2 half-lives have elapsed [85]. Although this report indicates that thrombolysis may be safe in patients with a time of last DOAC dose within 48 h without specific anticoagulation tests, one must be cautious of publication bias.

Xian et al. reported on the largest experience with the use of thrombolysis for AIS in DOAC patients developing an acute ischemic stroke [86]. Using data from the AHA Get With the Guidelines-Stroke Registry, the outcomes of thrombolytic therapy were compared in patients on DOACs ($n = 251$ including 129 rivaroxaban and 35 apixaban patients), warfarin ($n = 1500$) and without anticoagulation ($n = 41{,}136$). Alteplase was administered within 4.5 h of presentation. There was no difference in symptomatic intracranial hemorrhage (ICH) or the risk of life-threatening or serious/systemic hemorrhage. Limitations to these data include the absence of information on time of last dose, coagulation study results, and the use of reversal agents, as well as a median INR of 1.2 in the warfarin group [86].

Jin et al. conducted a systematic review of DOAC pateints receiving thrombolysis for AIS [87]. A total of 492 patients were identified in 55 studies. Overall, 55.2% of patients (80/145) presented within 12 h of the last direct FXAi dose and 33.9% (43/127) were presented within 13–24 h of the last dose. Forty-three direct FXaI patients had an anti-FXa level performed, with the highest level observed was 67 ng/mL. The rate of symptomatic ICH, mortality, and favorable outcomes were 4.3% (20/462), 11.3% (48/123), and 43.7% (164/375) respectively. The authors concluded alteplase may be reasonable to administer in select patients within 48 h of the last dose [87].

In returning to our patient case, the patient's last dose was within one half-life of the drug suggesting concentrations have not yet fallen 50% from steady state. If an apixaban calibrated anti-FXa assay is not available, a heparin or LMWH anti-FXa level (without AT) should be obtained. The LLOQ of apixaban and rivaroxaban using an anti-FXa assays is approximately 30 ng/mL (Table 2). It is important to recognize the existence of variability in anti-FXa assay systems such that extrapolation across assay systems should be avoided and each laboratory should perform their own assessment [35]. Generally, most data indicate a heparin or LMWH anti-Xa level of < 0.1 U/mL would suggest the apixaban concentration is negligible or absent and alteplase can be administered. If the local laboratoy has established that a heparin or LMWH anti-FXa level between 0.1 and 0.5 U/mL excludes a direct Xa inhibitor concentration above 30 ng/mL as shown by Billoir, then alteplase can be considered [52]. Alteplase should be avoided with a heparin or LMWH anti-Xa level above 0.5 U/mL ml [52, 84]. If an apixaban calibrated anti-Xa assay result was available, alteplase may be considered based upon an individual risk benefit assessment with a concentration less than 20 ng/mL while concentrations between 50 ng/mL as Seiffge demonstrated the safety of this approach [83]. Concentrations between 21 ng/mL and 100 ng/mL warrant an individualized risk assessment. It should be noted that a previous recommendation suggested safe thrombolysis would be indicated in when apixaban concentrations were < 10 ng/mL [88], but those recommendations have not been replicated elsewhere and additional safety data are needed here. Alteplase should be avoided until further data are available with concentrations above 100 ng/mL [83, 84].

In summary, by applying the AHA criteria for this patient, it remains unclear if he is eligible for alteplase. Thus, even with the AHA guidance, we must apply what we know about direct FXaI and their impact on coagulation test results to provide recommendations. Although literature contains cases of apixaban patients safely receiving alteplase within 48 h of last dose, until additional data are available, we recommend using an anti-Xa level to guide the decision to administer alteplase depending on availability at your institution. If our patient did not have an anti-FXa (UFH or LMWH) level, he would not be eligible for alteplase despite a normal PT, aPTT, and INR as only 10 h have elasped since the last dose and a minimum of 24 h is recommended.

Summary of considerations and recommendations:

- Obtain or estimate the time since the last dose of apixaban
- Assess the patient's baseline and current renal function
- Consider any potential drug-drug interactions (e.g., specifically Pg-p and/or CYP3A4 inhibitors)
- Recommend checking a stat anti-FXa level to guide clinical decision-making.

– If acceptable turn-around-time of anti-FXa levels (i.e., ~ 1 h) is not available, we recommend a minimum time of at least two half-lives to have lapsed since time of last dose before consideration of alteplase administration depending on clinical scenario (e.g., baseline renal function, heart failure exacerbation, interacting medications, overdosescenarios).

- If the anti-FXa level calibrated to heparin/LMWH resulted < 0.10 U/mL, based on presented data, it appears to represent negligible drug concentration
- If the anti-FXa level is calibrated to apixaban and resulted ≤ 20 ng/mL, it is recommended to consider administration. If the level ranges 21–100 ng/mL, this warrants further risk assessment based on available data.

  In the absence of drug-specific calibrated anti-FXa tests, the laboratory should provide estimates of apixaban levels using their locally heparin calibrated (UFH or LWMH or hybrid) results.

Note: Estimation of direct FXaI levels is not equivalent between different anti-FXa methods and heparin calibrations (Table 2).

Case 2

A 73-year-old male (weight 79 kg and CrCl 88 mL/min) with a history most notable for atrial fibrillation on apixaban 5 mg twice daily presenting after mechanical fall with head strike at 05:00 (it is currently 13:30). Subsequently, he became unresponsive at 11:00 and was transferred to your hospital for neurosurgical evaluation. He took his last dose of apixaban 5 mg the evening prior, unknown what exact time. A CT head from 13:40 demonstrated an acute left holohemispheric subdural hematoma (SDH) with layering on the left tentorium associated with 1.3-cm midline shift. The approximate time of the last apixaban dose was ± 18 h prior from presentation. A stat LMWH anti-FXa activity was sent to the laboratory, with a reported result of 2.31 U/mL. The neurosurgical team would like to take the patient to the operating room for evacuation of left SDH and requests andexanet alfa (Andexxa®) for reversal of direct FXaI.

Clinical Query

- How would you proceed knowing the anti-FXa UFH/LMWH level, as well as the need for surgery per request of the neurosurgical team?
- Should the anti-FXa result guide andexanet alfa administration and dose?
- Should the pharmacist recommend obtaining a post-andexanet alfa anti-FXa measurement?

Laboratory Interpretation and Additional Recommendations

- The anti-FXa (LMWH) test suggests apixaban exposure with a result of 2.31 U/mL.
- In the absence of a specific drug-calibrated anti-FXa testing, the laboratory should provide estimates of apixaban levels using heparin calibrated (UFH or LWMH or hybrid) results.

  Note: Estimation of direct FXaI levels is not equivalent between different anti-Xa methods and heparin calibrations (Table 2).

Case 2 Discussion

Although events of life-threatening major bleeding occur less frequently with the direct FXaI compared to warfarin, events still occur. Unlike warfarin where an INR can quickly be drawn to evaluate anticoagulation intensity for reversal, the direct FXaI have a short half-life and laboratory monitoring is not standardized in emergency scenarios. As discussed in case 1, drug specific qualitative tests are preferred; however, due to the availability of this test, the anti-FXa UFH/LMWH/hybrid may be used as an alternative to assess for direct FXaI presence. It is critical for practitioners to remember the variability in ant-Xa assay systems such that extrapolation across assay systems should be done with caution [35].

In acute ICH, priority or statim lab tests are recommended to rule out the presence of direct FXaI given their short half-life and to guide clinical decision-making if reversal agents are warranted. This patient's anti-FXa result of 2.31 IU/mL can be suggestive of levels that are within the expected therapeutic target for trough exposure (Table 1) using STA-Liquid Anti-Xa (Diagnostica Stago) or supratherapeutic on-therapy using Comatic (Chromogenix) or Berichrom (Siemens Healthcare Diagnostics) methods. If heparin calibration anti-FXa testing is provided, as they did for this patient case, the laboratory should assess the estimation of direct FXaI exposure across the analytical measurement range (the reportable range from the instrument and calibration without diluting a sample). This can be achieved using either previously measured patient samples or commercially available direct FXaI calibrators or control materials that have assigned values determined or traceable to the gold standard method using mass spectrometry. Because of the variability between UFH/LMWH calibrated anti-FXa methods, reported equivalent DOAC concentrations and differences between the direct FXaI using those calibrated methods, it is difficult to provide universal guidance regarding numeric results reported by the clinical laboratory and reversal dosing using andexanet alfa or any other reversal strategy (e.g., 4F-PCC). Most published studies correlating FXa DOACs to UFH or LMWH use STA-Liquid Anti-Xa (Diagnostica Stago), although other studies have published findings for apixaban and rivaroxaban, and rarely for edoxaban, using other anti-FXa methods (Table 3).

Clinicians may consider repeat laboratory testing of the anti-Xa to assess a "rebound" or if reversal was achieved with andexanet alfa, given the initials reports of returning to placebo levels 2 h post-infusion. As previously stated, there are two

Table 3 Representative published studies comparing heparin anti-Xa to FXaI levels

Author	Sample type(s)	Laboratory FXa method (calibrator)	Relevant findings
Billior et al [52]	Apixaban and Rivaroxaban-treated patients	Diagnostica Stago (Stago LMWH)	Apixaban ~ 50 ng/mL: 0.79 IU/mL Anti-FXa Apixaban ~ 30 ng/mL: 0.40 IU/mL Anti-FXa Rivaroxaban ~ 50 ng/mL: 0.89 IU/mL Anti-FXa Rivaroxaban ~ 30 ng/mL: 0.46 IU/mL Anti-FXa
Gosselin et al [53]	Rivaroxaban-treated patients	Coamatic, Berichrom, STA-Liquid anti-Xa (UFH and LMWH)	LLOQ UFH Coamatic 0.03: ~ 15 ng/mL LLOQ LMWH Coamatic 0.03 U/mL: ~ 35 ng/mL LLOQ Berichrom UFH 0.03 U/mL: ~10 ng/mL LLOQ STA-Liquid Anti-Xa LMWH/UFH 0 U/mL: < 5 ng/mL
Beyer et al [59]	Contrived and apixaban and rivaroxaban-treated patients	STA-Liquid Anti-Xa (Stago Hybrid calibrator)	Apixaban peak: 1.8–2.2 IU/mL Apixaban trough: 0.7–1.1 IU/mL Rivaroxaban peak: 3.8–6.2 IU/mL Rivaroxaban trough: 0.6–1.0 IU/mL
Gouin-Thibault et al [89]	Contrived, French laboratories	Primarily Diagnostica Stago, (Stago LMWH)	Rivaroxaban 40 ng/mL: LMWH from 0.23–1.74 IU/mL Apixaban 37 ng/mL: LMWH from 0.17–0.55 ng/mL
Godier et al [90]	FXa DOAC-treated patients	Diagnostica Stago and Hyphen Biomedical (unknown LMWH)	< 0.10 IU/mL = < 30 ng/mL PPV 100% (95% CI 97–100) Sensitivity: 54% (95% CI 46–62%)
Maier et al [91]	Apixaban and Rivaroxaban-treated patients	HemosIL Liquid anti-Xa (HemosIL Heparin calibrator)	Apixaban > 50 ng/mL: > 0.33 IU/mL Anti-FXa Apixaban > 30 ng/mL: > 0.16 IU/mL Anti-FXa Rivaroxaban > 50 ng/mL: > 0.37 IU/mL Anti-FXa Rivaroxaban > 30 ng/mL: > 0.21 IU/mL Anti-FXa
Helin et al [92]	Contrived 80 ng/mL apixaban EQA in Europe	Various ("heparin" calibrated)	HemosIL Liquid Anti-Xa: 0.5 U/mL (SD: 0.14) Berichrome Heparin: 1.1 U/mL (SD:0.1) Stago Liquid anti-Xa: 1.2 U/mL (SD:0.07) Coamatic Heparin: 0.7 U/mL (SD:0)
Sabor et al [93] 2017	Contrived apixaban, edoxaban, rivaroxaban and patient apixaban and rivaroxaban samples	STA-Liquid anti-Xa; Hyphen Heparin LRT, and HemosIL Liquid Anti-Xa	Contrived 50 ng/mL: > LLOQ for all methods, all FXa DOACs Contrived 30 ng/mL > 0.10 IU/mL: STA-Liquid anti-Xa & Hyphen Heparin LRT for rivaroxaban only. Difference in sensitivity between contrived and patient samples: patient samples 0.10 IU/mL rule out concentrations up to 30 ng/mL

points to address if these clinical queries occur: (1) The results from the ANNEXA -4 trial demonstrated a "modest prediction" of anti-FXa levels and efficacy of treatment in ICH, and that anti-FXa levels were not overall predictive of hemostatic efficacy [64, 94] and (2) using methods with high pre-test sample dilutions (e.g., 1:33 as noted with Stago method) may result in an in vitro dissociation of andexanet alfa with direct FXaI, causing a falsely elevated anti-FXa result, suggesting a rebound effect [63]. Anti-FXa testing that utilizes lower sample dilutions (e.g., Coamatic) will provide a more accurate assessment of post-andexanet alfa efficacy. As previously indicated, the use of PT and/or APTT to estimate drug efficacy is not recommended, as these tests are not sufficiently sensitive to direct FXaI exposure to warrant their use for this purpose [35].

Thus, in the absence of a life-threatening bleeding event or urgency of direct FXaI reversal, drug levels, whether calibrated to direct FXaI or heparin/LMWH, should be interpreted based on the clinical scenario and if agents such as four factor prothrombin complex concentrates (4F-PCC) and/or andexanet alfa was administered. Although andexanet alfa has not yet been evaluated in a large-scale analysis for bleeding patients requiring surgery,

requests for this indication occur. Until results from the ANNEXA-S Trial (Trial of Andexanet in Patients Receiving an Oral FXa Inhibitor Who Require Urgent Surgery (Annexa-S). NCT04233073; www.clinicaltrials.gov), we have to rely on publications from small series or case studies [95–98]. Given the concern for a short half and possible rebound to placebo levels (ANNEXA 4 Trial), surgeons should be aware of the drug kinetics of andexanet alfa, especially during a prolonged procedure in order to assess for increased bleeding that may not be related to the surgery.

Given the cost expense of andexanet alfa and two dose regimen (low and high), it may be prudent to have drug measurement or estimation, especially if the pre-treatment values exceed the reportable limit and are reported as ">" a numeric value, as these measurements are higher than the analytical measurement range (AMR), and repeat testing (drug concentration) postreversal may be warranted in select cases (e.g., severe renal dysfunction, drug-drug interactions, overdoses). Conversely, in those patients with UFH/LMWH anti-FXa results of $\leq$ 0.10 U/mL, there may be some consideration for not giving andexanet alfa, given the low level of direct FXaI present.

In summary, this patient's anti-FXa level of 2.31 U/mL demonstrates FXaI effect (Table 2), and therapeutic anticoagulation likely persists requiring reversal with andexanet alfa for ICH, or another preferred agent. Discussion with the surgical team should include the duration of reversal effect observed in ANNEXA-4, limited published data administering andexanet alfa in those requiring procedures and the time-length of those procedures, possible rebound to placebo levels, risk of thrombosis, and increased bleeding that is not thought to be from the procedure. Postreversal monitoring should include hematoma expansion on CT and other clinical signs and symptoms of worsening bleeding. Institutions with chromogenic FxaI levels may consider monitoring drug level in conjunction with re-bleeding events to determine clinical decision-making.

Summary of Considerations and Recommendations

- The anti-FXa (LMWH) test suggests apixaban exposure with a result of 2.31 IU/mL. At this time based on data presented in Table 2, this value is suggestive of on-therapy/supratherapeutic apixaban levels and requires reversal in the setting of life-threatening major bleeding and to proceed with surgery.

  Note: For some LMWH platforms, the analytical measurement range may in insufficient for estimating high direct FXaI concentrations. If samples are > AMR, then they could be diluted 1:3 and 1:5 in normal plasma and retested, with the final result multiplied by 3 and 5 respectively, and results averaged for final report. Dilutions may vary depending on expected drug concentration.

  Example:

  - Sample tested and result reported is > 1.5 IU/mL
  - Sample tested at 1:3 = 0.85 IU/mL × 3 = 2.55 IU/mL
  - Sample tested at 1:5 = 0.55 IU/mL × 5 = 2.75 IU/mL

  Average result = 2.65 IU/mL
- Andexanet alfa would be administered for the life-threatening major bleeding event, and the interventionalist should be educated on the kinetics of andexanet alfa and the limited published experience of its use during surgery.
- Anti-FXa values can guide the clinician to rule out therapeutic direct FXaI concentrations based on the data presented in Table 2. Moreover, if institutions have anti-FXa calibrated to the direct FXaI, levels < 50 ng/mL are generally accepted for not utilizing reversal agents.
- A post-andexanet alfa anti-FXa (UFH/LMWH) should not be recommended, but the clinical team should be advised to monitor for new signs and symptoms of bleeding based on the presenting case.
- Institutions in conjunction with their special coagulation laboratory should implement guidelines on how to approach the bleeding patient with recent direct FXaI use, as the associated reversal agents are expensive and come with possible risk, such as thrombosis and rebound to placebo levels.

Case 3

A 78-year-old male (5′10″, 74 kg) on rivaroxaban 20 mg daily for a provoked venous thromboembolism (VTE) 6 weeks ago following a prolonged hospital stay for heart failure. He is admitted to the hospital for community acquired pneumonia. On hospital day 3, the patient's condition deteriorates and is transferred to the ICU and is now in acute kidney injury (AKI). Relevant morning labs at 06:00 return, serum creatinine 1.8 mg/dL, estimated creatinine clearance 35 mL/min, PT: 15 s (normal 10.0–12.5 s), aPTT 43 s (normal 28.0–36.5 s). The time of last rivaroxaban dose was 18:00, the prior evening. The patient is being transitioned to continuous infusion (CI) UFH as a result of AKI and ICU transfer. The hospital uses heparin anti-FXa level monitoring to adjust CI UFH therapy.

Clinical Query

How should be the patient be transitioned from rivaroxaban to unfractionated heparin and what are the considerations?

Laboratory Interpretation and Additional Recommendations

- It has been recommended that the prolongation of a PT and/or APTT in a patient with known DOAC exposure should be considered secondary to DOAC effect until

proven otherwise, but these tests should not be used to estimate DOAC concentration [35].

- With direct FXaI presence (rivaroxaban), the additive effect to UFH anti-FXa testing to bear in mind. As such, consider (prior to UFH infusion)

– Baseline anti-FXa to gauge pre-treatment UFH anti-FXa
– Baseline APTT (and thrombin time)

Case 3 Discussion

The aPTT is the most commonly employed coagulation test for heparin monitoring. However, due to a multitude of limitations described previously and the requirement of re-establishing therapeutic ranges each time a new reagent is introduced, many institutions have transitioned from APTT monitoring to chromogenic heparin anti-FXa level monitoring. An additional challenge for UFH monitoring with the aPTT is the heparin therapeutic range requires assessing with each new lot of APTT reagent (typically every 12–18 months), with the possibility of establishing new therapeutic ranges within an institution for a new lot of APTT reagents [99].

As previously described, direct FXaI have shown a linear dose response curve with chromogenic anti-FXa assays calibrated to UFH/LMWH or specific drug calibrators. The chromogenic anti-FXa assay may be more sensitive to the presence of direct FXaI, with expected on-therapy level exceeding the test reportable range. In those cases, sample dilutions are required to estimate the level of the direct FXaI. Beyer et al. performed anti-FXa analysis using a hybrid heparin/LMWH calibration curve and serial dilutions to identify steady-state rivaroxaban and apixaban concentrations in patients with non-valvular atrial fibrillation [59]. Rivaroxaban peak and trough anti-FXa concentrations ranged from 3.8 to 6.2 IU/mL and 0.6 to 1.0 IU/ml respectively while apixaban peak and trough concentrations ranged from 1.8 to 2.2 IU/mL and 0.7 to 1.1 IU/mL for peak and trough collections respectively [59]. These concentrations are well above the accepted heparin therapeutic range of 0.3 to 0.7 U/mL, and thus, the direct FXaI are expected to provide considerable "interference" with heparin monitoring when using anti-FXa methods.

Macedo et al. measured heparin anti-FXa levels (STA-Multi-Hep Calibrator with STA Liquid Anti-Xa hybrid reagent, Diagnostica Stago) in patients receiving rivaroxaban or apixaban within the past 72 h [100]. When anti-FXa levels were drawn within the dosing interval for apixaban and rivaroxaban, levels were > 1 U/mL in 71% of apixaban patients and 55% of rivaroxaban patients. One rivaroxaban patient with AKI (rivaroxaban 33% renal elimination) had an anti-FXa level of 0.86 IU/mL 63 h postdose. Although this level is within a cited trough anti-FXa range by Beyer et al., it is above the upper limit of the heparin therapeutic range [101]. In a subset of apixaban or rivaroxaban patients transitioned to UFH, supratherapeutic initial anti-Xa levels occurred in 69% of patients [100]. Wendte et al. reported on challenges of transitioning an apixaban patient (NVAF CHA_2DS_2-VASc 4 receiving 5 mg BID) who developed AKI requiring dialysis to UFH therapy [102]. Heparin was initiated (with a bolus dose) 36 h after the last apixaban dose. The anti-FXa level 6 h after heparin initiation was 4.4 IU/mL while an aPTT at that time was 54 s. The heparin infusion was discontinued and anti-FXa levels remained elevated into the fourth day of therapy. On hospital day 5, the heparin anti-FXa level was 0.6 U/ml and the aPTT was 38 s. Despite the 36-h delay to heparin initiation, the prolonged apixaban effect with ESRD (apixaban 27% renal elimination) in addition to the initial heparin bolus administration (uncertain indication with no acute clot) placed the patient at risk for over-anticoagulation. This patient's anti-FXa level returned twice the reported upper level of the peak apixaban concentration range [102].

The optimal management approach for patients transitioning from apixaban or rivaroxaban to CI UFH at institutions which use heparin anti-FXa level monitoring is uncertain. Patients can potentially be at risk for both over-anticoagulation (residual oral FXa inhibitor anticoagulation added to heparin anticoagulation especially in the presence of a bolus) and under-anticoagulation resulting from initial dose reductions in the UFH infusion rate due to elevated anti-FXa levels from direct FXaI interference. The relative risks of over and under-anticoagulation within the transition period will vary with individual patient characteristics including the presence of an acute clot and renal impairment. One must recall that therapeutic levels of direct FXaI produce anti-FXa levels about the upper limit of detection thus requiring sample dilution for quantitation.

Several management options have been suggested. Identifying the time of last dose will be an important consideration. Faust et al. recommended beginning the CI UFH 2 h prior to the next scheduled oral FXai dose and a change to aPTT monitoring for the first 48–72 h until the interference dissipates (longer times may be needed with renal impairment) [103]. If at any time thereafter two consecutive anti-Xa levels returned > 1.1 U/mL, a return to aPTT monitoring was recommended [103]. Switching to aPTT monitoring for the first 72 h has been adopted by institutions who typically use anti-FXa levels analysis for UFH monitoring unless the baseline aPTT is abnormal. This switch will likely require the use of an empiric heparin aPTT therapeutic range of 1.5 to 2.5 times normal. The thrombin time can be also be used to monitoring UFH, as there is a linear response to UFH anticoagulation, but this method is rarely used in routine clinical practice.

For patients with atrial fibrillation without an acute thrombus, an alternative approach may be to draw a baseline heparin anti-FXa level at the expected time of trough concentration

and use the result to guide time to initiation of heparin therapy. In the PAUSE trial, a 1-day interruption in rivaroxaban or apixaban therapy (equivalent to ± 36 h hold) resulted in concentrations < 50 ng/mL in 87.2% of patients on apixaban and 95% on rivaroxaban [82, 104]. The relationship between a LMWH anti-FXa level for different anti-FXa assay systems and direct FXaI concentrations is shown in (Table 2). For patients with an acute thrombus, heparin initiation prior to full dissipation of direct FXaI effect would be desired; however, the anti-FXa level at which this should occur is uncertain. Zochert et al. reported using a rivaroxaban specific anti-FXa level to guide transition from a direct FXaI to UFH in the presence of AKI [105]. In this same study, they also described one atrial fibrillation patient taking apixaban where CI UFH was initiated when the drug concentration was < 50 ng/mL and in 2 VTE patients, CI UFH was initiated when the concentration was < 100 ng/mL for both apixaban and rivaroxaban [106]. Thus, limited data suggest CI UFH may be initiated when the anti-FXa level approximated a direct FXaI concentration of 100 ng/mL (Table 2).

Other options currently lacking published data include the use of the thrombin time or anti-IIa assay for CI UFH monitoring during the transition to guide the adequacy/intensity. Using either of these approaches still requires a careful assessment of when to initiate CI UFH therapy and whether to administer a bolus dose. Use of the TT or anti-IIa assay would require the laboratory to create calibration curves and the pharmacy to develop a heparin dose adjustment based upon results of the calibration curve.

Emerging publications using DOAC neutralizing agents including activated charcoal or filters to eliminate DOAC effect on coagulation testing appear promising [107–112]. The use of Hepzyme® (Siemens Healthcare) to in vitro neutralize heparin then back-calculate the UFH anti-Xa results may be acceptable (Reported U/mL – Hepzyme treated U/mL = FXaI U/mL; Reported U/mL - FXaI U/m = UFH U/mL); however, there are no published data to suggest that Hepzyme does not interfere with DOAC presence.

Summary Points

- Identify time of last dose, estimate drug half-life based upon renal function
- Assess individual patient's risk/concern for thrombosis versus bleeding in conjunction with estimated half-life to inform decision on when to initiate CI UFH
- Draw baseline aPTT
- Consider baseline heparin anti-FXa level in those with renal impairment prior to heparin initiation if data are available with the institution's anti-FXa assay system to estimate rivaroxaban or apixaban concentration (Table 2)
- Heparin infusion initiation +/− bolus: Options include initiation at time of trough direct Xa inhibitor concentration or after 2 half-lives have elapsed or when the heparin/LMWH level suggests direct Xa inhibitor concentration is at a trough
- If baseline aPTT is elevated, check Hepzyme aPTT after 12 h of CI UFH to assess if sustained impact of direct FXaI persists
- Adjust CI UFH therapy using aPTT for 72 h then switch to anti-factor Xa levels if preferred per institution

Conclusion

Patients on oral direct FXaI requiring administration of reversal agents or transitions to alternative anticoagulants present unique challenges to clinicians. Despite over almost a decade of experience with the direct FXaI, methods of assessing coagulation status in these patients have shifted from traditional coagulation assays to more specific assays, such as drug-calibrated tests. Although drug-calibrated direct FXaI assays are just as easy to perform as UFH/LMWH assays, the lack of FDA approval for direct FXaI specific calibrators and controls seems to be a mitigating factor in their widespread use in the USA. Given the limited availability across the country for the DOAC calibrated tests, clinicians are faced with utilizing imperfect assays and interpreting their results with caution based on available data. The anti-FXa (UFH/LMWH) test seen commonly may be perceived in practice as an alternative for providing direct FXaI exposure estimation. However, until data has been generated for each UFH/LMWH anti-FXa reagent platform to estimate direct FXaI levels, the interpretations and applications of these results remain controversial, particularly in cases requiring targeted reversal agents, and should be used with judicious caution.

Compliance with Ethical Guidelines

Conflict of Interest Mr. Gosselin reports personal fees from Expert testimony for rivaroxaban and dabigatran testing, other from Diagnostica Stago, personal fees from Diagnostica Grifols, personal fees from UniQure, other from BioMarin, personal fees from Machaon Diagnostic Laboratory, other from Siemens Healthcare Diagnostics, outside the submitted work; Dr. Douxfils reports personal fees from QUALIblood , personal fees from Stago Diagnostica, personal fees from Daiichi Sankyo, personal fees from Roche, personal fees from Roche Diagnostics, personal fees from Portola, outside the submitted work; In addition, Dr. Douxfils has a patent PCT/EP2019/052903 pending.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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