A New Test for the Detection of Direct Oral Anticoagulants (Rivaroxaban and Apixaban) in the Emergency Room Setting

Galit H. Frydman, DVM, ScD[1,2]; Felix Ellett, PhD[2]; Elizabeth M. Van Cott, MD[3]; Douglas Hayden, PhD[4]; Maulik Majmudar, MD[5]; Charles R. Vanderburg, PhD[6]; Haley Dalzell, BS[5]; Divya L. Padmanabhan, BS[5]; Nick Davis, BS[1]; Julianne Jorgensen, BS[2]; Mehmet Toner, PhD[2]; James G. Fox, DVM, MS, DACLAM[1]; Ronald G. Tompkins, MD, ScD[2]

[1]Division of Comparative Medicine, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA.

[2]BioMEMS Resource Center, Department of Surgery, Massachusetts General Hospital, Boston, MA.

[3]Department of Pathology, Massachusetts General Hospital, Boston, MA.

[4]Department of Biostatistics, Massachusetts General Hospital, Boston, MA.

[5]Department of Cardiology, Massachusetts General Hospital, Boston, MA.

[6]Advanced Tissue Resource Center, Harvard NeuroDiscovery Center, MGH, Boston, MA.

Objectives: Determining whether a patient has taken a direct oral anticoagulant (DOAC) is critical during the periprocedural and preoperative period in the emergency department. However, the inaccessibility of complete medical records, along with the generally inconsistent sensitivity of conventional coagulation tests to these drugs, complicates clinical decision making and puts patients at risk of uncontrollable bleeding. In this study, we evaluate the utility of inhibitor-II-X (i-II-X), a novel, microfluidics-based diagnostic assay for the detection and identification of Factor Xa inhibitors (FXa-Is) in an acute care setting.

Design: First-in-human, 91-patient, single-center retrospective pilot study.

Setting: Emergency room.

Patients: Adult patients admitted into the emergency department, which received any clinician-ordered coagulation test requiring a 3.2% buffered sodium citrate blood collection tube.

Interventions: None.

Measurements and Main Results: Plasma samples from patients admitted to the emergency department were screened for the use of FXa-Is, including apixaban and rivaroxaban, within the past 24 hours using our new i-II-X microfluidic test. i-II-X results were then compared with results from conventional coagulation tests, including prothrombin time (PT) and international normalized ratio (INR), which were ordered by treating clinicians, and an anti-Xa assay for rivaroxaban. The i-II-X test detected DOACs in samples collected from the emergency department with 95.20% sensitivity and 100.00% specificity. Unlike PT and INR, i-II-X reliably identified patients who had prolonged clotting times secondary to the presence of a FXa-I.

Conclusions: The i-II-X test overcomes the limitations of currently available coagulation tests and could be a useful tool by which to routinely screen patients for DOACs in emergency and critical care settings. Our new diagnostic approach is particularly relevant in clinical situations where medical records may be unavailable, or where precautions need to be taken prior to invasive interventions, such as specific reversal agent administration.

Direct oral anticoagulants (DOACs) are a popular and effective treatment for venous thromboembolism (VTE) and nonvalvular atrial fibrillation (1–3). These drugs have several advantages over traditional anticoagulants, such as a more rapid onset of action, as compared with warfarin, and oral dosing and longer half-life, as compared with heparin (3, 4). DOAC prescriptions have grown rapidly, and routine monitoring of coagulation in the context of DOAC therapy has not historically been recommended. Adverse events have mounted in recent years as these drugs are prescribed to increasingly complex patient populations, and a lack of U.S. Food and Drug Administration (FDA)-approved diagnostic tests to evaluate patient coagulation status in the context of DOAC therapy may: 1) increase the complexity of the continued adoption of these drugs for complex patient populations (such as those with renal disease, obesity, and polypharmacy) and 2) complicate clinical decision making in emergency and critical care settings (5–15).

Conventional tests for coagulation, such as prothrombin time (PT), activated partial thromboplastin time (aPTT), and activated clotting time are not specific for Factor Xa inhibitor (FXa-I) detection; in addition, these tests lack the sensitivity needed to rule out FXa-I–induced anticoagulation in patients (16). Viscoelastic assays, such as thromboelastoraphy and rotational thromboelastometry, have mixed reports on the sensitivity to all DOACs and appear to be consistently insensitive at lower, although therapeutic, concentrations (17–19). Currently, mass spectrometry and anti-Xa chromogenic assays are the only tests reported to be consistently sensitive and specific to the presence of FXa-I, but these tests are mostly performed in specialized or central laboratories with a 30–120-minute turnaround time, limiting their utility in the emergency setting and in facilities that may not have 24/7 access to this equipment. These tests also require an accurate medical history for drug-specific calibration (20–23). To address these limitations, we developed a microfluidics-based assay, the inhibitor-II-X (i-II-X) test, to detect the presence of FXa-Is in patient samples. We evaluated the i-II-X test for the detection of both apixaban and rivaroxaban in patient blood and whether this test could help clinicians identify patients in which prolonged clotting times (PT/international normalized ratio [INR]) are secondary to the presence of FXa-I.

MATERIALS AND METHODS

Study Design and Sample Collection

We performed a single-center pilot study involving 91 adult patients at the Massachusetts General Hospital (MGH) (Boston, MA, USA) in order to evaluate this new assay approach in emergency department patient samples. All samples and patient information were collected and handled according to MGH and Massachusetts Institute of Technology (Cambridge, MA) Institutional Review Board committee approval (approval numbers: MGH No: 2014P002087; MIT No: 150100681R001); because discarded plasma samples were used, patient consent was not required. Patients admitted to the emergency department were screened and selected for medical histories indicating recent DOAC prescription (**Supplementary Table 1**, Supplemental Digital Content 1, http://links.lww.com/CCX/A66) and had a 3.2% sodium citrate blood tube drawn for clinician-ordered coagulation testing. On days when DOAC patient samples were collected, additional non–anticoagulated emergency department patient samples were also collected to serve as "negative controls." Patients were not screened out for any condition or concurrent medication, with the exception of the recent use of other anticoagulants, such as heparin and warfarin. Platelet-poor plasma was collected from the same tube that clinician-ordered coagulation tests were performed, de-identified and stored at –80°C until analysis. Following i-II-X analysis, we retrospectively examined coagulation test results from patient medical records. Coagulation tests ordered in the emergency department included a combination of PT, INR, and aPTT and D-dimer. Due to the design of this study, PT/INR results were not available for every patient (i.e., the attending clinician did not order a PT/INR for 3/43 of the FXa-I patients and 5/48 of the control patients), and although some patients received aPTT or D-dimer, too few patients had these results to allow statistical analysis. It is important to note that no clinicians ordered an anti-Xa assay or mass spectrometry on any sample. The purpose of this experimental design was to compare the i-II-X test results with what the attending clinician ordered to evaluate the patient's coagulation status. PT/INR was performed on the Destiny Max (Stago Diagostica, Asnieres, France) using the PT HTF reagent (Stago Diagostica). The rivaroxaban anti-Xa chromogenic assay was performed at the MGH Coagulation Laboratory using the STA-Liquid Anti-Xa reagent and Stago rivaroxaban calibrators on the STAR Max Analyzer (Stago, Parsippany, NJ). Apixaban calibrators were not approved for use at MGH at the time of this study; therefore, only rivaroxaban samples were evaluated using the anti-Xa method. Sample volume was insufficient to perform mass spectrometry.

Design and Fabrication of the Microfluidic Devices

The microfluidic devices were manufactured using standard microfabrication techniques. In brief, a single-layer photoresist design (SU-8; MicroChem, Newton, MA), with a 50-μm-thick layer was patterned on one silicon wafer via a photolithography masks and standard processing, according to the manufacturer's protocols. The resulting patterned wafer was then used as a mold to produce polydimethylsiloxane (Thermo Fisher Scientific, Waltham, MA) devices, which were subsequently, irreversibly bonded to glass slides (1 in. × 3 in.; Thermo Fisher Scientific). The microfluidic design included four channels, each with their own inlet and outlet ports, and one common central imaging area (**Supplementary Fig. 1, A** and **B**, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). This configuration allowed for the simultaneous imaging and analysis of multiple conditions (**Supplementary Fig. 1C**, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). The chips were pretreated with a corona plasma gun (Elveflow, Paris, France) prior to sample loading to eliminate the need for fluid pumps.

Coagulation Curve Generation

The patient samples were run blinded. Coagulation curves were calculated by using our i-II-X microfluidic assay to detect the "time to clot" (TtC) in plasma. Briefly, after thawing plasma at 37°C, we added 20 mM calcium (Boston Bio Products, Boston, MA), 488-conjugated fibrinogen (Thermo Fisher Scientific), and various concentrations of "Agonist A" and "Agonist B" to detect and distinguish the presence of FXa-I and Factor IIa inhibitor (FIIa-I) (**Supplementary Fig. 1D**, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). Briefly, Agonist A tests for presence of an inhibitor at or downstream of FXa and Agonist B tests for the presence of an inhibitor at or downstream of FIIa; taken in combination, the test results from Agonists A and B can detect the presence of the FXa-I and FIIa-I. Samples were loaded into the i-II-X microfluidic chip and a fully automated Nikon TiE microscope and NIS Elements Software (Nikon Instruments, Melville, NY) imaged the chip every 15 seconds for up to 10 minutes in order to document the TtC.

Clotting Time Score Generation

Coagulation curves were generated for each sample by plotting TtC for each agonist concentration using GraphPad (GraphPad Software, San Diego, CA) (**Supplementary Fig. 1E**, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). A predictive model for the detection of FXa-I from the clotting curve was then generated using the R Software using the multivariate logistic regression analysis (24). The resulting model was then used to assign a numerical clotting time score (CTS) to each patient. A cutoff score of 0.5 (functional coagulation level [FCL]/milliliter) was selected for the presence of factor inhibition. Briefly, if the CTS was greater than 0.5 FCL/mL, this indicated that there was inhibition at or downstream of the factor being tested; alternatively, if the CTS was less than or equal to 0.5 FCL/mL, this indicated that there was no inhibition of coagulation at or downstream of the factor being tested (Supplementary Fig. 1D, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). Clotting curves and CTSs were generated using commercially available calibrators for apixaban, dabigatran, rivaroxaban, and edoxaban and in-house generated calibrators for betrixaban made with lyophilized normal control plasma (HYPHEN BioMed, Aniara Diagnostica, LLC, West Chester, OH) (Portola Pharmaceuticals, South San Francisco, CA). Warfarin plasma was purchased from George King Bio-Medical (Overland Parks, KS). Edoxaban calibrators were spike with FEIBA (Shire, Lexington, MA).

Statistical Analysis

Both Excel and GraphPad Prism (GraphPad Software, La Jolla, CA) were used for basic descriptive and comparative statistics. To evaluate conventional hospital coagulation tests, a one-way ANOVA ($p < 0.05$ for significance) was used to compare PT/INR results for patients without a documented history of anticoagulant use (control) against results from the same tests for DOAC patients. Receiver operating characteristic (ROC) curves were generated, and sensitivity and specificity were calculated to quantitatively assess the utility of PT/INR and the i-II-X test for the detection of FXa-I in plasma samples. Control samples were further subdivided into "normal" and "abnormal" controls using MGH's reference ranges for conventional coagulation testing: normal PT was defined as greater than 14 seconds and normal INR was defined as greater than 1.2.

Principal component analysis (PCA) was performed to identify patterns in covariance amongst raw clotting curve data for control (C, $n = 48$), apixaban (A, $n = 20$), and rivaroxaban (R, $n = 23$) sample groups across three agonist concentrations. PCA was performed using uncertainty testing to optimize the number of components and the NIPALS algorithm (maximum iterations = 100) of Unscrambler X (CAMO Software, Woodbridge, NJ). The "find outlier" function was used to identify clotting times that caused over-fitting from the control group ($n = 1$), whereas a second application of this function on remaining measurements identified putative influencers of the model. A PCA triplot was made using centered and standardized data on the same measurement scales.

RESULTS

Patient Cohort

From January 2017 through August 2017, a total of 91 patient samples (control, $n = 48$; FXa-I, $n = 43$) were collected from the emergency department at MGH (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). The cause for emergency department admittance varied, with many FXa-I patients having exhibited complications of cardiac disease. Other patients were admitted into the emergency department for acute trauma, infection, pain or fever, neoplasia, and chest pain or respiratory distress. Among control patients, one patient was admitted for a bleeding event and two patients were admitted for a clotting event. Among FXa-I patients, one patient was admitted for a bleeding event and one patient was admitted for a clotting event; both patients were on rivaroxaban.

Detection of FXa-I using PT/INR

To evaluate whether clinician-ordered coagulation testing could detect the presence of FXa-I in patient samples, we evaluated PT/INR results. PT and INR in the MGH laboratory are sensitive to the presence of FXa-I in plasma samples with sensitivities 95.12% and 87.80%, respectively (**Supplementary Fig. 2, A** and **B**, and **Supplementary Table 2**, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). As expected, the specificity of PT and INR for FXa-I was low at 54.55% and 75.00%, respectively. Further subdivision of the control samples into "normal" and "abnormal" cohorts confirmed these findings (**Supplementary Fig. 2C**, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). These results suggest that, although PT/INR may be sensitive to anticoagulant effect DOACs in our laboratory, these tests are not specific for FXa-I–induced anticoagulation.

Comparison of Clotting Times

Clotting curves were produced for each patient sample, and the sensitivity and specificity of the i-II-X assay for the detection of FXa-I were assessed. Control sample i-II-X results had low variability, with higher concentrations of the agonists resulting in

decreased TtC (**Supplementary Fig. 3***A*, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). FXa-I patients' clotting curves showed an increase in TtC (**Supplementary Fig. 3***B–D*, Supplemental Digital Content 1, http://links.lww.com/CCX/A66) with statistically significant differences in TtC relative to control i-II-X results (**Fig. 1***A*). PCA of the TtC results for these three agonist concentrations recapitulated the biphasic behavior between control and FXa-I samples—control i-II-X results (C, *blue* circle) demonstrate much lower covariance and form a distinct cluster relative to FXa-I results (A and R, *pink* circle; **Fig. 1***B*). The comparison between the "normal" and "abnormal" control patient TtCs confirmed no significant effect on the i-II-X results in the face of abnormal PT and INR values (**Supplementary Fig. 3,** *E* and *F*, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). In aggregate, these data underscore the sensitivity and specificity of i-II-X for the detection of FXa-I.
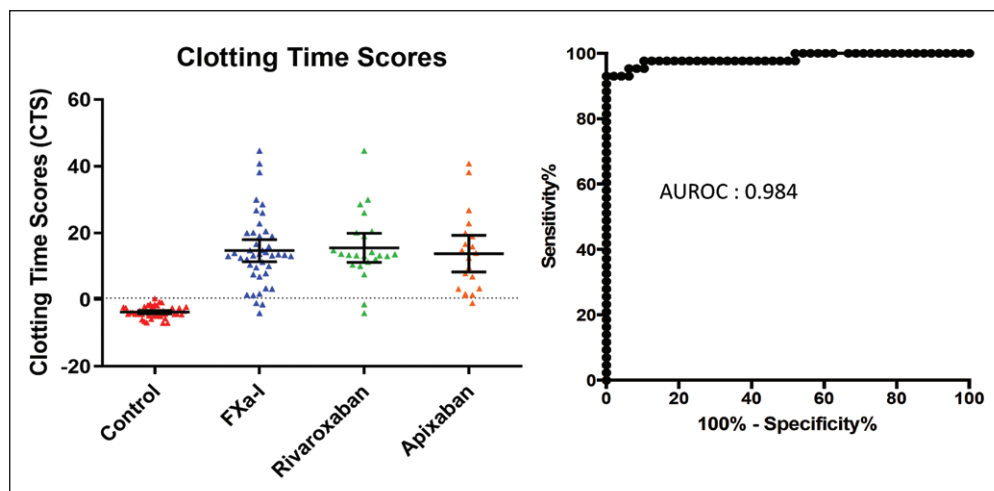
Evaluation of CTS

Each patient was assigned a CTS based on their TtC curves. The CTS is indicative of the functional coagulation level (FCL per milliliter) of each specific factor being tested; specifically, it is indicative of the amount of anticoagulation secondary to inhibition from the FXa-I. A CTS of greater than 0.5 FCL/mL indicated the presence of anticoagulation secondary to FXa-I in a patient sample, and a CTS of less than equal to 0.5 FCL/mL indicated the absence of anticoagulation secondary to FXa-I in a patient sample. Plotting CTS data for control and FXa-I patients highlighted distinct differences between the two sample groups (**Fig. 2***A*; **Supplementary Fig. 4***A*, Supplemental Digital Content 1, http://links.lww.com/CCX/A66), and the ROC curve had an area under the curve of 0.984 (**Fig. 2***B*). Collectively, i-II-X test results demonstrated a sensitivity of 93.02% and a specificity of 100.00% for all FXa-I patients (**Supplementary Fig. 4,** *B* and *C*, Supplemental Digital Content 1, http://links.lww.com/CCX/A66) and the CTS algorithm was highly sensitive and specific to both rivaroxaban (91.7% and 100.00%, respectively) and apixaban (95.0% and 100.00%, respectively).

To evaluate whether the CTS was indicative of the FXa-I concentration, we compared CTS-derived rivaroxaban concentrations to the rivaroxaban-calibrated anti-Xa chromogenic assay-derived concentrations (**Supplementary Fig. 4,** *D* and *E*, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). To calculate the drug concentration based on the CTS score, rivaroxaban calibrators were used to generate a best-fit line equation for the relationship between in vitro drug concentration (nanogram per milliliter) and the i-II-X–generated CTS (Supplementary Fig. 4*D*, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). We then applied this equation to the CTS of each patient and compared the i-II-X drug concentration with the anti-Xa concentration (Supplementary Fig. 4*E*, Supplemental Digital Content 1, http://links.lww.com/CCX/A66),
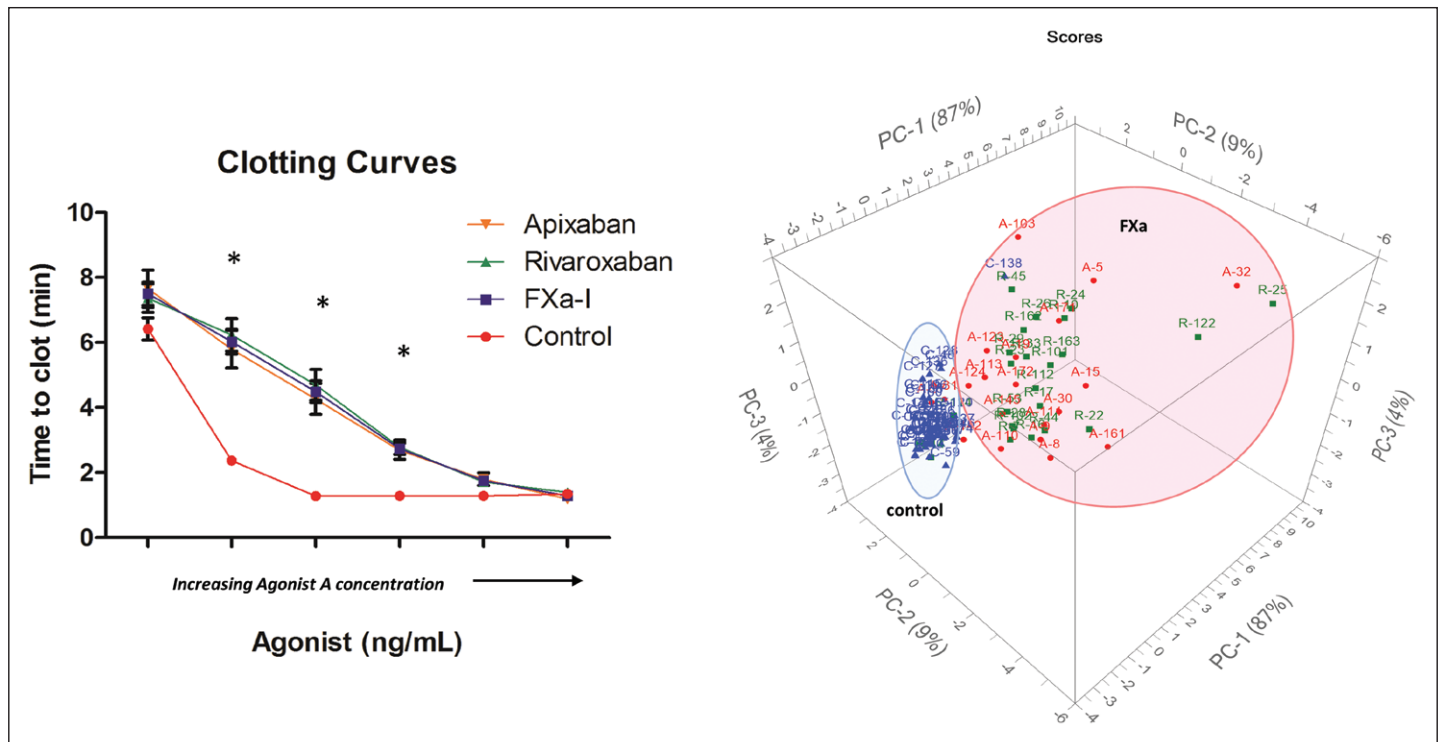
Figure 1. Comparative clotting curves. Clotting times were compared at various agonist concentrations for all the patient groups to construct clotting curves. **A,** Clotting curves with the mean and sᴇ bars of all patient groups are demonstrated on a single graph for comparison. All three FXa-I groups appear subjectively very different from the control group, with there being multiple concentrations where there are significant statistical differences between the controls and the total FXa-I, rivaroxaban, and apixaban groups. **B,** Principal component analysis (PCA) triplot of times to clot for control (*blue*), apixaban (*red*), and rivaroxaban (*green*) patient samples at three separate agonist concentration. Control and FXa groups demonstrate distinct clotting profiles over the agonist concentrations tested, and FXa patient samples demonstrate increased clotting variability relative to control samples. Statistical significance is defined as *p* value less than 0.05 (*asterisk*).

Figure 2. Clotting time score (CTS) analysis. Evaluation of CTS utilization for the detection of FXa-I in patient samples. **A**, Scatter plot with mean and SE bars for CTS comparison between patient groups. *Dashed line* at CTS 0 represents the chosen cutoff for the determination of whether there is FXa inhibition in the patient sample. **B**, Receiver operating characteristic curve of utilizing the CTS to determine whether a patient has an FXa-I in their system. AUROC = area under the receiver operating curve.
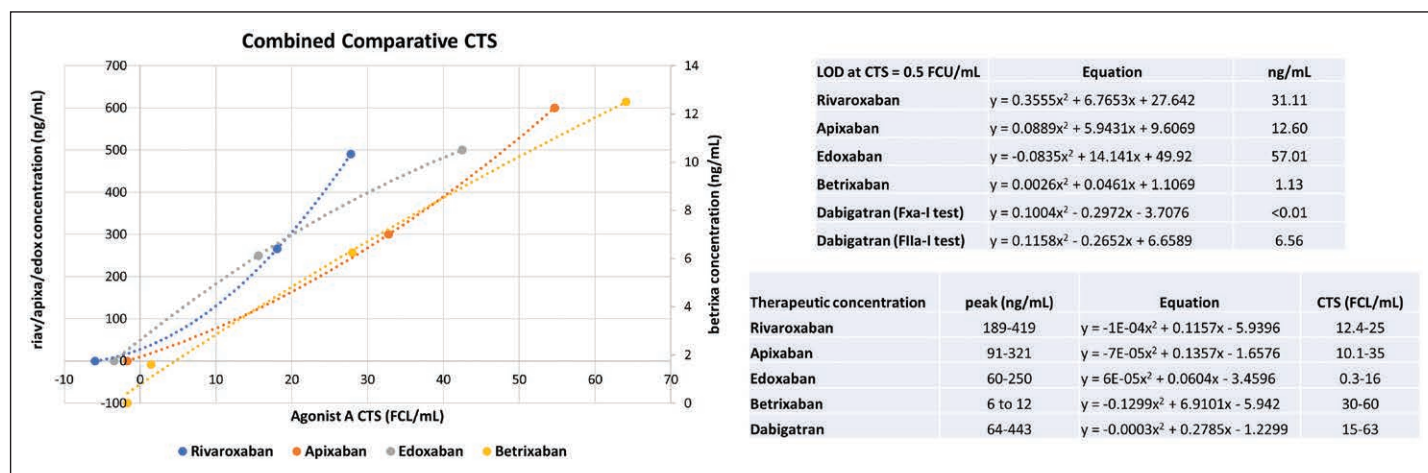
DOAC Detection with the i-II-X Test

Samples containing known amounts of DOACs were evaluated using the i-II-X test. Using the described CTS algorithmic approach, best-fit lines were generated using the CTS and drug concentration (**Supplementary Fig. 5A–F**, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). The CTS curves were generated with both Agonist A and Agonist B, but, importantly, FXa-I samples tested with the Agonist B test indicated no detectable inhibition at FIIa (CTS < 0, data not shown). Dabigatran, a FIIa-I, demonstrated both Agonist A and Agonist B inhibition, consistent with the logic scheme of the i-II-X test (Supplementary Figs. 1D and 5, E and F, Supplemental

which yielded an R^2 of 0.86. Importantly, only 14 of 23 rivaroxaban samples were included in this comparison—largely owing to insufficient sample volumes for the anti-Xa assay or other reasons, including the presence of hemolysis, lipemia, or icterus, which can affect the anti-Xa assay (25). Based on the anti-Xa test results, we eliminated one rivaroxaban false negative, which turned out to be a true negative due to a rivaroxaban concentration result of 2 ng/mL, which is below the detection limit of the chromogenic anti-Xa assay (25 ng/mL). This increased the i-II-X test's overall sensitivity and specificity to 95.20% and a specificity of 100.00% for all FXa-I patients and the rivaroxaban sensitivity and specificity to 95.45% and 100.00%, respectively (Supplementary Table S2, Supplemental Digital Content 1, http://links.lww.com/CCX/A66).

Digital Content 1, http://links.lww.com/CCX/A66). Intriguingly, comparison of the various DOAC CTSs highlighted unique curve shapes for each FXa-I (**Fig. 3A**), suggesting possible differences in each drug's pharmacokinetics, despite their similar target and mechanism of action. We also used these curves to estimate the current limit of detection (LOD), based on a CTS cutoff of 0.5 FCL/mL, and the expected CTS for the therapeutic ranges of each drug (**Fig. 3, B** and **C**).

DISCUSSION

Although the FDA has historically not recommended routine testing and monitoring of DOACs, the rapid adoption and prescription of these drugs has led to a growing need for an FDA-approved DOAC testing system, especially in the emergency setting (5–7, 26).

LOD at CTS = 0.5 FCU/mL	Equation	ng/mL
Rivaroxaban	y = 0.3555x² + 6.7653x + 27.642	31.11
Apixaban	y = 0.0889x² + 5.9431x + 9.6069	12.60
Edoxaban	y = -0.0835x² + 14.141x + 49.92	57.01
Betrixaban	y = 0.0026x² + 0.0461x + 1.1069	1.13
Dabigatran (Fxa-I test)	y = 0.1004x² - 0.2972x - 3.7076	<0.01
Dabigatran (FIIa-I test)	y = 0.1158x² - 0.2652x + 6.6589	6.56

| Therapeutic concentration	peak (ng/mL)	Equation	CTS (FCL/mL)
Rivaroxaban	189-419	y = -1E-04x² + 0.1157x - 5.9396	12.4-25
Apixaban	91-321	y = -7E-05x² + 0.1357x - 1.6576	10.1-35
Edoxaban	60-250	y = 6E-05x² + 0.0604x - 3.4596	0.3-16
Betrixaban	6 to 12	y = -0.1299x² + 6.9101x - 5.942	30-60
Dabigatran	64-443	y = -0.0003x² + 0.2785x - 1.2299	15-63

Figure 3. Clotting time scores (CTS) for factor Xa and factor IIa inhibitors. CTS are plotted for each concentration of various direct FXa inhibitors in response to the FXa test, including rivaroxaban, apixaban, edoxaban, and betrixaban. **A**, A combined CTS plot of all four FXa-I tested. This graph shows the differences in the CTS results for these drugs, despite a reportedly similar mechanism of action (direct FXa inhibition). **B**, A table showing the expected limit of detection (LOD) for each drug tested based on the current CTS equation and setting the negative control reference range less than or equal to 0. **C**, An example of how the CTS curve equation may be used to predict the CTS for each drug at its proposed peak therapeutic plasma concentration. **H** and **I**, The CTS approach can detect the inhibitors tested at their respective therapeutic concentrations. Note: peak concentration varies based on publication evaluated, and values selected are representative.

Importantly, the need for DOAC testing will likely continue to mount as DOAC approval extends into vulnerable patient populations, where pharmacodynamics may vary (10, 13, 27–31). In this single-center pilot trial, patients admitted to the emergency department at MGH were evaluated using the i-II-X microfluidic test for the detection and identification of FXa-Is, rivaroxaban and apixaban. We show that this test is both sensitive and specific to FXa-I, even in the face of variable PT/INR results, suggesting that this test may indicate whether a patient's prolonged PT/INR may be secondary to a DOAC.

Although other studies evaluating DOAC testing are usually done in controlled patient populations, in this study, we include patients admitted into the emergency department with a variety of diagnoses and comorbidities, including diabetes, neurodegenerative disease, cancer, heart disease, COPD, liver dysfunction, and infections (32). By including patients with complex medical histories, we were able to objectively investigate the robustness of the

i-II-X test in real-world patient cohorts. In addition to being more sensitive and specific to the presence of FXa-I than PT/INR, when the PT is prolonged, the i-II-X test can provide valuable information as to whether prolongations may be secondary to an FXa-I. This kind of actionable information is important and timely, especially with the recent approvals of specific DOAC reversal agents (7, 21). Additionally, we have preliminary evidence that the i-II-X test can detect and monitor the reversal of the DOAC's anticoagulant effect in the context of treatments such as anti-inhibitor coagulant complex (FEIBA) (**Supplementary Fig. 6**, Supplemental Digital Content 1, http://links.lww.com/CCX/A66), suggesting that this test may be useful for the dosing/monitoring of reversal agents, a valuable tool, especially in a setting where rapid decision making and administration of high-cost treatments are necessary (33, 34).

There is mounting evidence that there is interpatient variation in the level of anticoagulation secondary to FXa-I administration (35–40). The two i-II-X false negatives had corresponding anti-Xa
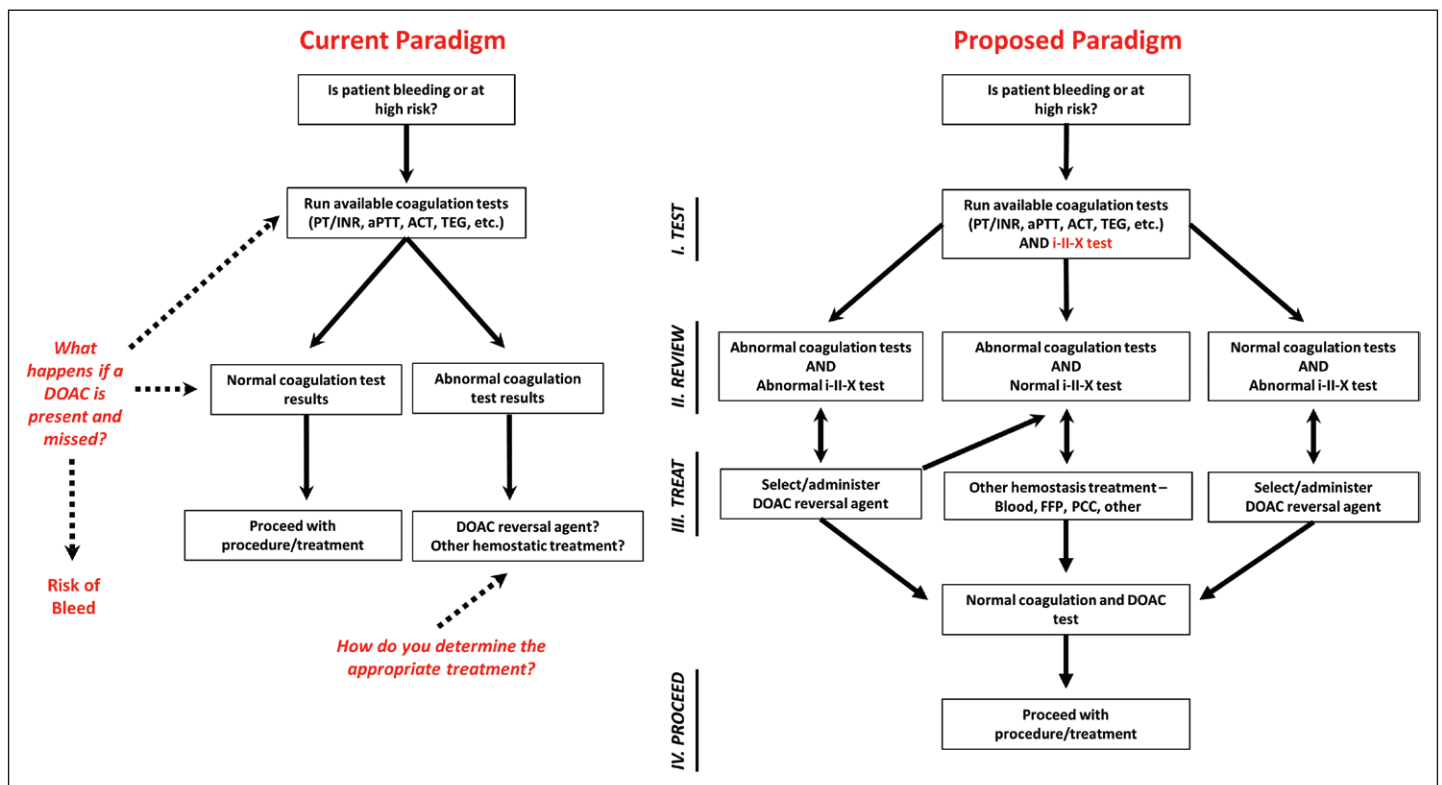
Figure 4. Current and future diagnostic paradigms for patients on direct oral anticoagulants. **A**, Flow summary of existing decision-making framework for coagulation testing in the emergency setting. Clinicians first decide whether to proceed with coagulation testing by determining if a patient is actively bleeding, at heightened risk of bleeding due to pre-existing medical condition, or warrants invasive medical/surgical intervention. The clinician then typically orders coagulation tests that are currently available in the emergency department setting (e.g., prothrombin time [PT], international normalized ratio [INR], activated partial thromboplastic time [aPTT], activated clotting time [ACT], and thromboelastography [TEG]). If these tests demonstrate abnormal clotting profiles or prolonged clotting times, the clinician must next decide appropriate treatment. Pre-existing tests have variable sensitivity and specificity toward direct oral anticoagulants (DOACs), and the choice of appropriate treatments for DOAC patients (e.g., DOAC-specific reversal agent vs plasma vs clotting factors) can be challenging. As many of these tests are insensitive or nonspecific to DOACs, normal clotting results are nonconclusive to patients on these drugs and present risks for false negatives. Without a patient's complete medical record, a clinician may proceed with surgical or medical interventions that pose increased risks of an uncontrollable bleeding. **B**, Flow summary of proposed decision-making framework for coagulation testing in the emergency setting. Conventional tests are performed alongside the i-II-X test (factor IIa- and factor Xa-inhibition tests) in order to detect and quantify the potential presence of these drugs. (i) If conventional tests demonstrate abnormal clotting profiles or prolonged clotting times and the i-II-X test detects the presence of a DOAC, the clinician can then decide whether to administer a DOAC-specific reversal agent. After treating a patient with a DOAC reversal agent, the clinician repeated testing until the patient's clotting times/profiles are normal and the i-II-X test confirms reversal by yielding a negative reading. (ii) Alternatively, if conventional tests return abnormal results, but the i-II-X test is negative, the clinician may choose to administer a different hemostatic therapy. (iii) If conventional tests yield normal values, but the i-II-X test indicates that a DOAC is on board, the clinician can again determine which DOAC reversal agent to administer. Once the patient's coagulation times/profiles revert to normal limits, and the i-II-X test returns a negative for inhibition of factor IIa or factor Xa, the clinician may elect to proceed with their surgical or medical intervention.

drug concentrations of 37 and 2 ng/mL. This finding is important, as current surgical guidelines recommend rivaroxaban concentrations less than or equal to 30 ng/mL to avoid adverse bleeding events (7, 21). Additionally, rivaroxaban 37 ng/mL is known to be a trough level. The estimated i-II-X–based LODs for these drugs (Fig. 3*B*) further support current surgical guidelines, including 30 ng/mL cutoff for surgical intervention and 50 ng/mL cutoff for reversal administration in the case of uncontrolled bleeding (7, 21). These results suggest that the i-II-X CTS may serve as a physiologically relevant predictor of anticoagulation status secondary to DOAC administration (40, 41). Additionally, because the i-II-X test measures a patient's functional coagulation based on the TtC, it may not suffer from the potential challenges of performing chromogenic-based anti-Xa assays (25, 41–46).

Future studies will address some of the inherent limitations of the present clinical study, namely, increasing the number of patients, performing mass spectrometry, having complete coagulation testing data for all patients, and including patients on conventional anticoagulants, that is, warfarin, although preliminary data suggest a lack of interference with warfarin (**Supplementary Fig. 7**, Supplemental Digital Content 1, http://links.lww.com/CCX/A66). We also plan to examine the effects of non-drug–related coagulopathies on the i-II-X test. One of the rivaroxaban patients exhibited concurrent abnormal PT and INR results (17.4 s and 1.4, respectively), along with an FXa-I CTS with a shift in the clotting curve. This may suggest a concurrent coagulopathy, such as dys- or hypofibrinogenemia, resulting in a clotting curve shift. In this regard, using the i-II-X test in conjunction with coagulation tests, such as viscoelastic testing, could provide a powerful solution for managing patients with complex coagulation profiles and medical histories (**Fig. 4**).

CONCLUSIONS

In this proof of concept pilot study, we have demonstrated that the i-II-X test can sensitively and specifically identify whether patients in an emergency department setting are anticoagulated secondary to the presence of an FXa-I. Although these results are promising, further clinical study should be performed to further establish the robustness of this new technology. In this study, the assay was performed in 10 minutes using less than 10 µL of plasma. We are currently developing an automated system to be used as a point-of-care assay on whole blood, providing results in less than 10 minutes in an emergency department setting. Future trials should include samples from multiple hospitals/institutions, strengthening the comparison between the i-II-X, currently available POC tests, and DOAC plasma levels.

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