Effect of PCC on Thrombin Generation among Patients on Factor Xa Inhibitors with Major Bleeding or Needing Urgent Surgery (GAUGE): Design and Rationale

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Abstract

Background Direct factor Xa inhibitors (FXaIs) account for most oral anticoagulant use and FXaI-associated bleeding events are common. Clinicians have variable national and regional access to specific FXaI reversal agents such as andexanet alfa. Many centers have adopted the use of prothrombin complex concentrates (PCCs) as hemostatic therapy for FXaI-associated major bleeding events. PCC does not impact circulating FXaI levels and its mechanism of action to achieve hemostasis in FXaI-associated bleeding is uncertain. While PCC increases quantitative thrombin generation assay (TGA) parameters, it does not correct FXaI-altered thrombin generation kinetics, nor does it normalize thrombin generation. Clinical data supporting the use of PCC are based on cohort studies reporting clinical hemostatic efficacy, which is difficult to measure. The benefits of PCC for FXaI-associated bleeding beyond supportive care are uncertain.

Objective GAUGE is a prospective observational study designed to measure the effects of four-factor PCC administration (Octaplex) on TGA parameters among patients with FXaI-associated bleeding or needing urgent surgery.

Methods Laboratory outcomes will include the mean paired change in TGA parameters from pre- to post-PCC administration and the proportion of participants whose post-PCC TGA values fall within a defined reference range. Clinical outcomes will include hemostatic efficacy, thromboembolic complications, and all-cause death at 30 days post-PCC.

Conclusion Development of a viable and universally accessible FXaI bleed management strategy is crucial. GAUGE will provide in vivo data on the effects of PCC among patients with FXaI-associated bleeding.

Keywords
- hemostasis
- factor Xa inhibitors
- dabigatran
- anticoagulation reversal

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Introduction

Direct oral anticoagulants (DOACs) such as direct factor Xa inhibitors (FXaIs; rivaroxaban, apixaban, edoxaban) and direct thrombin inhibitors (dabigatran) have revolutionized anticoagulation therapy over the past decade and now account for most oral anticoagulant use.[1] DOACs are associated with less bleeding as compared to vitamin K antagonists (VKAs).[2] Despite a lower risk of bleeding complications, DOAC-associated bleeding events are commonly encountered in clinical practice. Estimates indicate that 2 to 4% of DOAC-treated patients experience major bleeding per year.[3] Oral anticoagulant-associated bleeding events are associated with significant morbidity and mortality, with case fatality rates on the order of 8 to 15% and as high as 30 to 50% among patients with anticoagulation-associated intracranial hemorrhage (ICH).[4] The management of anticoagulant-associated bleeding was identified as a key research priority through a priority setting partnership between the James Lind Alliance and the Canadian Venous Thromboembolism Research Network (CanVECTOR).[5]

Several hemostatic strategies are used to manage DOAC-associated bleeding events, including nonspecific hemostatic therapies such as prothrombin complex concentrates (PCCs) and specific reversal agents such as andexanet alfa for FXaI-associated bleeding.[6] Prospective single-arm studies have evaluated both andexanet alfa and PCCs using clinical hemostatic efficacy as a primary outcome.[6–8] These cohort studies focused on clinical hemostasis and lacked control groups for comparison (►**Table 1**).[9] Adjudication of clinical hemostasis is complex, necessitates intensive data collection, and frequently relies on surrogate measures of ongoing bleeding such as hemoglobin measurements. It is unclear whether the clinical criteria used to assess "effective" hemostasis reliably detect an impact from anticoagulation reversal.[10] An impact from anticoagulation reversal can be difficult to ascertain, as major bleeding can be multifactorial (e.g., anticoagulation, vascular/tissue disruption, platelet dysfunction, etc.) and reversal of anticoagulation is often only one of several therapeutic measures required to stop bleeding.[11] These clinical criteria employ surrogate clinical findings when bleeding cannot be directly visualized to determine whether hemostasis has been achieved (e.g., hemoglobin measurements, neurological deterioration for ICH).[9,12] These measurements are not solely impacted by ongoing bleeding, nor are they specific to effective hemostasis and may show deterioration irrespective of whether bleeding has stopped. PCC cohort studies that lack a comparator arm and focus solely on clinical hemostasis are of limited utility without first establishing a biologically plausible mechanism of action and obtaining laboratory evidence of an impact on hemostasis in bleeding patients. A phase IV randomized controlled trial (ANNEXa-I) comparing andexanet alfa to usual care (including PCC) is ongoing among patients with ICH (NCT03661528).

Coagulation assays such as the prothrombin time (PT) and activated partial thromboplastin time (aPTT) have limited utility for monitoring the anticoagulant effects of FXaIs.[13] Conventional clotting assays measure specific clotting pathways rather than the overall contribution of the coagulation cascade to hemostasis and do not fully reflect in vivo hemostasis. Moreover, they have not been shown to adequately capture the hemostatic effects of PCC in patients on FXaIs.[10] Most studies evaluating the effect of PCCs on PT among patients on FXaIs have only shown partial corrections.[10] Thrombin generation assays (TGAs) continuously measure the production of thrombin through the cleavage of a fluorogenic substrate and provide information on both kinetic and quantitative parameters of thrombin generation (►**Fig. 1**).[14] Kinetic parameters include the lag time (LT; time for thrombin generation to first occur in minutes) and the time to peak (TTP; start to maximal thrombin generation in minutes), whereas quantitative measures include the peak height ("peak"; maximal thrombin generation, in nanomolar; nM) and the endogenous thrombin potential (ETP; area under the thrombin generation curve, in nM•min). The mean velocity rate index (mVRI) is a composite kinetic/quantitative parameter and is derived by dividing peak height by the difference between TTP and LT (i.e. [peak/TTP − LT], in nM/min). TGAs might be useful to measure hemostatic responses following administration of specific DOAC reversal agents or nonspecific hemostatic therapies.[15] Studies have used TGAs to measure the prohemostatic effects of PCCs in animals and in healthy volunteers treated with DOACs.[10] Two studies evaluated hemostasis among healthy volunteers administered DOACs using a standardized skin punch biopsy and concurrently measured changes in TGA parameters induced by PCC treatment (►**Table 2**).[16,17] One study investigated the ability of four-factor PCC (4FPCC) to correct ETP and bleed duration (BD)/bleed volume (BV) among healthy volunteers administered oral rivaroxaban 20 mg twice daily for 4 days.[16] This study showed that 4FPCC significantly increased the ETP to values that surpassed prestudy baseline levels within 30 minutes of administration, but there was no measurable effect on BD or BV.[16] A second study similarly investigated the effects of 4FPCC on ETP and BD/BV among volunteers given a single 60 mg dose of edoxaban.[17] In this study, LT was significantly correlated with BD ($p = 0.04$); ETP demonstrated a trend toward a significant correlation with BD ($p = 0.07$). This study reported an effect of PCC on ETP and demonstrated study-defined normalization of BD and BV following administration of PCC (95% confidence interval (CI) for a BD posttreatment ratio over baseline to be within a 70 to 143% limit). Effects on LT were not reported. The discrepant findings between these two studies with respect to bleeding might relate to differing DOAC regimens and resulting differences in DOAC levels. PCC effects on TGA parameters might depend on the DOAC level at the time of administration. 4FPCC was previously shown to normalize ETP and peak thrombin generation only when FXaI concentrations were below 75 and 37.5 ng/mL, respectively.[18]

There are limited data evaluating the effects of PCC on TGA parameters among patients with FXaI-associated bleeding. TGA parameters may provide a surrogate measure to assess the hemostatic impact of PCCs among patients with DOAC-associated bleeding. As TGA parameters have been correlated with measures of blood loss in controlled experiments,

Table 1 Prospective studies evaluating PCC effects on hemostasis among patients with FXaI-associated bleeding

Study information	Study design	*N*	PCC formulation		PCC dosing		DOACs	Distribution of bleed sites, *n* (%)		TGA (parameters reported)	Follow-up	Clinical hemostasis definition and assessment/ adjudication	Key results	
Majeed et al (2017)[7] UPRATE Cohort *Blood*	Multicenter, prospective, observational study (25 hospitals; Sweden)	84	Octaplex ($n=39$)	Confidex/ Beriplex ($n=44$)	1,500 IU (weight <65 kg)	2,000 IU (weight >65 kg)	Apixaban ($n=39$) Rivaroxaban ($n=45$)	ICH	59 (70.2)	Not measured	30 days	ISTH definition of hemostatic effectiveness. Assessed independently and in duplicate by two coagulation specialists.	ISTH hemostasis effective = 58/84 (69.1%)	
								GI	13 (15.5)				Thromboembolic events = 2/84 (2.4%)	
								Other	12 (14.3)				Death = 27/84 (32.1%)	
Schulman et al (2018)[8] UPRATE Cohort *Thromb Haemost*	Multicenter, prospective, observational study (9 hospitals; Canada)	66	Octaplex	Confidex/ Beriplex	2,000 IU		Apixaban ($n=29$) Rivaroxaban ($n=37$)	ICH	36 (54.5)	Not measured	30 ± 2 days	Modified criteria from Sarode et al.[12] As assessed by the treating emergency physician or most responsible physician.	Hemostasis good/moderate = 56/66 (84.9%)	
								GI	16 (24.2)				Thromboembolic events = 5/66 (7.6%)	
								Other	14 (21.2)				Death = 9/66 (13.6%)	
Bavalia et al (2020)[54] *Res Pract Thromb Haemost*	Multicenter, prospective, observational (5 hospitals; Netherlands)	76 FXaI-associated bleeding (51 received PCC)	Not specified		Median total dose = 3,500 IU [IQR = 3,000–4,000 IU); corresponding to a median weight-based dose = 50 IU/kg [46–50 IU/kg]		Apixaban ($n=16$ overall) Rivaroxaban ($n=54$ overall) Edoxaban ($n=6$ overall) Note: numbers for each FXaI receiving PCC not reported.	Not reported for subgroup with FXaI-associated bleeding receiving PCC		CAT/ thrombinoscope (lag time, peak, ETP)	30 days	ISTH definition of hemostatic effectiveness for ICH; criteria from Sarode et al[12] for non-ICH bleeding. Hemostasis was assessed by the local study coordinator; re-evaluated by the national study coordinator.	ISTH hemostasis effective = 32/51 (62.7%)	
													Thromboembolic events = 1/51 (2.0%)	
													Death = 9/51 (17.6%)	
													TGA results available for 19/51 patients with FXaI-associated bleeding treated with PCC	Mean ETP increased from 1,108 nM·min to 2,275 nM·min ($p=0.001$)
														Mean peak thrombin generation increased by 113% ($p=0.001$)
														Change in lag time induced by PCC not reported

Abbreviations: CAT, calibrated automated thrombography; ETP, endogenous thrombin potential; GI, gastrointestinal; ICH, intracranial hemorrhage; PCC, prothrombin complex concentrate; TGA, thrombin generation assay.

Note: If studies reported data on both major bleeding and urgent intervention, or data on both FXaI and dabigatran, only data pertaining to FXaI-associated bleeding are shown.

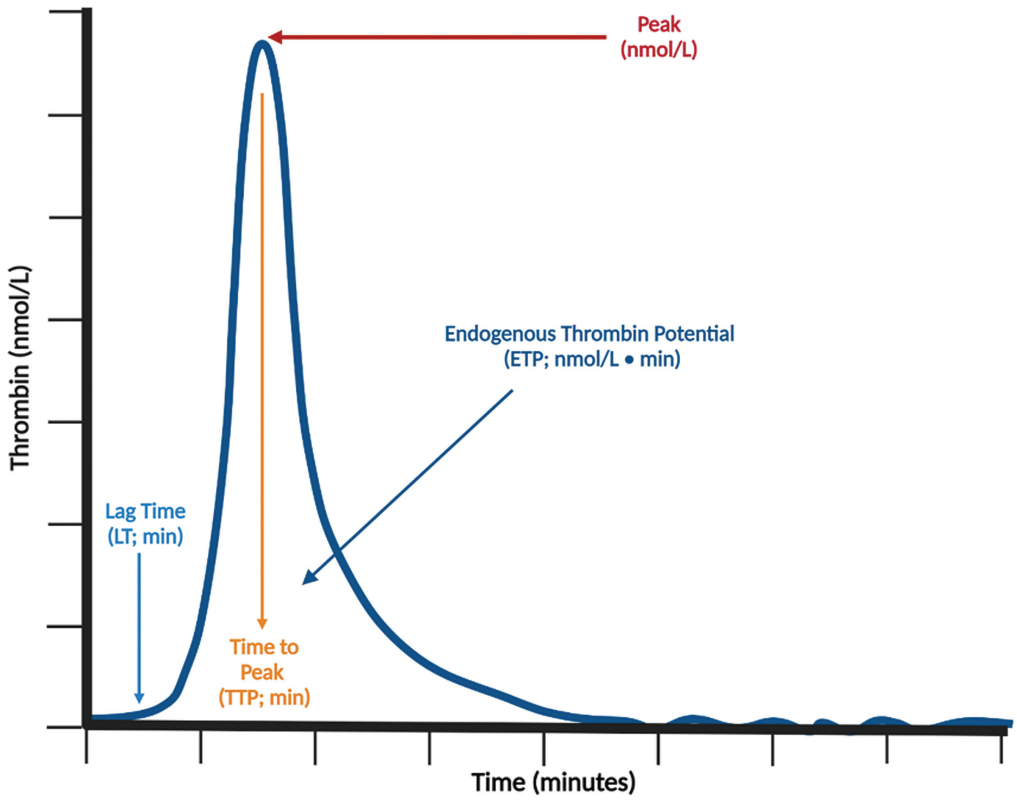

Fig. 1 The thrombogram. Components of a typical thrombin generation curve generated using the calibrated automated thrombography (CAT) assay. ETP, endogenous thrombin potential; LT, lag time; TTP, time to peak. Reproduced with permission from Shaw et al[15].

demonstrating correction of these TGA parameters in vivo among bleeding patients may offer a convincing alternative for illustrating the utility of PCCs in the management of FXaI-associated bleeding.

Study Methods

Study Design and Objectives

GAUGE is a single center, prospective observational cohort study evaluating the effects of 4FPCC on laboratory indices of anticoagulant effect as measured by TGA in patients presenting with FXaI-associated major bleeding or needing urgent surgery (►**Fig. 2**). A surgery/procedure will be considered urgent if it cannot be delayed to allow for FXaI clearance according to current periprocedural anticoagulation management guidelines, due to the urgency of the surgical indication, and for which PCC is felt to be indicated by the operating surgeon to optimize hemostasis.[19] The objectives of this study are threefold: (1) characterize TGA responses following administration of PCC to FXaI-treated patients, (2) assess whether administration of PCC to FXaI-treated patients leads to a measurable effect on kinetic TGA parameters, and (3) collect data on clinical hemostatic efficacy and thromboembolic complications following the administration of PCC to FXaI-treated patients.

Patient Population and Recruitment Procedures

Adult patients (≥18 years of age) will be eligible for recruitment if they are prescribed a FXaI (rivaroxaban, apixaban, or edoxaban) for any indication and present with major or life-threatening bleeding, or need urgent surgery, and require PCC for optimization of hemostasis as deemed by the treating physician/surgeon. Patients with a reduced hemoglobin without overt bleeding and patients who received activated PCC (aPCC) or recombinant factor VIIa (rFVIIa) prior to receipt of PCC will be excluded. FXaI-treated patients presenting with major bleeding or requiring urgent surgery will receive 50 IU/kg of 4FPCC (Octaplex; Octapharma) weight-based dosing as per our institutional protocol, with no maximum dose. PCC dosing will be at the discretion of the treating physician. Written informed consent will be obtained from eligible participants or substitute decision makers (SDMs) to use remaining stored citrated plasma samples drawn per routine care before and after PCC administration for the laboratory analyses outlined below.

Laboratory and Clinical Outcomes

The primary outcome for the study will be the mean paired difference in LT before and after PCC administration. LT was selected as the primary outcome measure given that it demonstrates good correlation with FXaI levels and has been associated with both FXaI-associated bleeding events and hemostasis (see "Sample Size" below).[15,17,20–22] Secondary laboratory outcomes will include the mean paired difference for the remaining TGA parameters (TTP, peak, ETP, mVRI) before and after PCC administration, as well as the percentage of patients with normalization of each TGA parameter (LT, TTP, peak, ETP, mVRI) following PCC administration. Normalization

Table 2 Phase I studies evaluating PCC effects on hemostasis among healthy volunteers

Study information	Study design	DOAC regimen	Hemostatic challenge	*N*	PCC formulation	PCC dosing (IU/kg)	TGA (parameters reported)	Key results
Zahir et al (2015)[17] *Circulation*	Phase 1 double-blind randomized placebo-controlled 2-sequence, 2-period crossover study	Edoxaban 60 mg single dose	Skin punch biopsy	93	Beriplex (4FPCC)	10, 25, 50	Technothrombin (ETP)	*Bleed duration (BD)*: 4FPCC 50 IU/kg produced an ~21% decrease in baseline-normalized postdose BD values. Mean ratio 4FPCC versus placebo was 79.2 [95% CI: 61.5–101.9; $p = 0.0680$]. 4FPCC 50 IU/kg resulted in complete reversal of edoxaban on BD based on a ratio of postdose to baseline least square mean values for BD near unity (96.3; 95% CI: 75.9–122.1; $p = 0.7451$).
								Bleed volume (BV): 4FPCC 50 IU/kg produced an ~24% decrease in baseline-normalized postdose BV values. Mean ratio 4FPCC versus placebo was 76.3 [95% CI: 56.8–102.5; $p = 0.0703$]. 4FPCC 50 IU/kg resulted in complete reversal of the effects of edoxaban on BV based on a ratio of postdose to baseline least square mean values for BV near unity 94.2 [95% CI: 63.9–139.0; $p = 0.7553$].
								ETP: 4FPCC 50 IU/kg resulted in complete reversal of the effects of edoxaban on ETP; 25 IU/kg produced partial reversal and 10 IU/kg did not reverse effects on ETP.
Levy et al (2018)[16] *J Thromb Haemost*	Phase 1 double-blind parallel group	Rivaroxaban 20 mg po BID × 4 days	Skin punch biopsy	147	Not specified (4FPCC)	50	Technothrombin (ETP)	*Bleed duration (BD)*: no differences in baseline-adjusted BD were observed when comparing 4FPCC to saline control. Prestudy baseline mean BD value was 10.0 minutes (range: 3.8–15.0 minutes) compared to 17.7 minutes (range: 6.0–25.0 minutes) following 4 days of rivaroxaban and infusion of 4FPCC.
								Bleed volume (BV): no differences in baseline-adjusted BV were observed when comparing 4FPCC to saline control. Prestudy baseline mean BV value was 2.7 mL minutes (range: 0.7–13.4 mL) compared to 4.1 mL (range: 0.1–14.3 mL) following 4 days of rivaroxaban and infusion of 4FPCC.
								ETP: ETP values after 4FPCC administration rapidly increased compared to saline control and surpassed the mean prestudy baseline values within 30 minutes postdose. ETP values continued to increase until 24 hours postdose and decreased toward prestudy baseline values up to 168 hours postdose.

Abbreviations: BD, bleeding duration; BV, bleed volume; ETP, endogenous thrombin potential; PCC, prothrombin complex concentrate; TGA, thrombin generation assay.
Note: Studies evaluating the effects of 4FPCC on hemostasis among healthy volunteers following FXaI administration and skin punch biopsy.

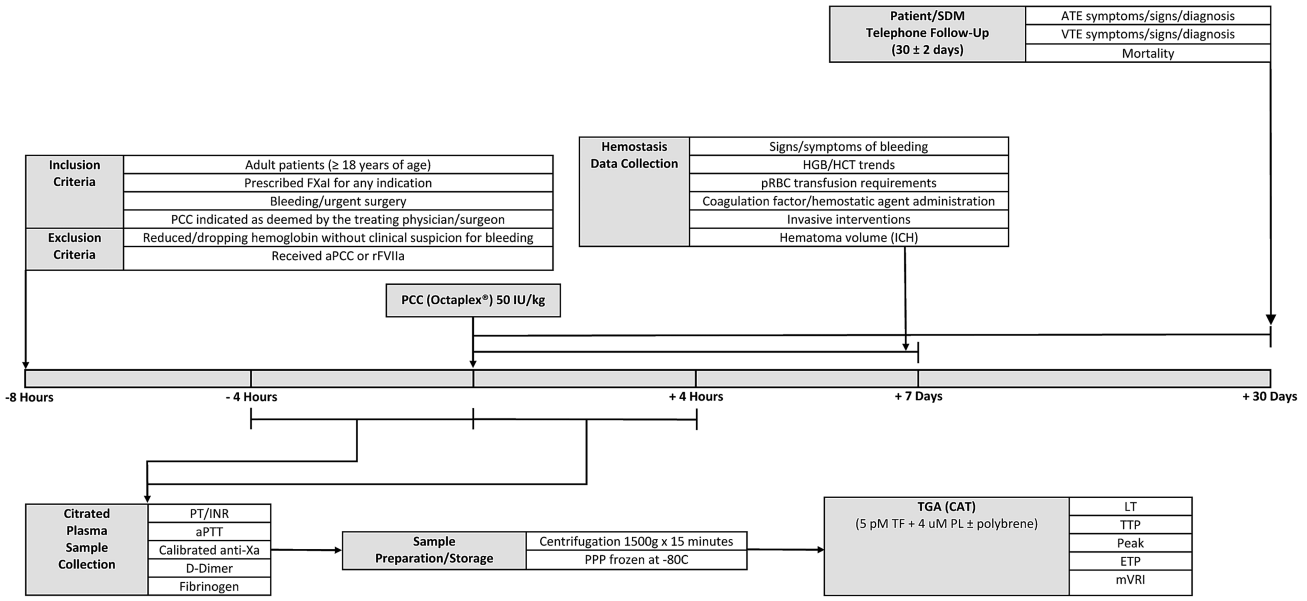

Fig. 2 **GAUGE study flow diagram.** Flow chart demonstrating typical study timeframes, based on time before and after PCC administration. aPCC, activated prothrombin complex concentrate; ATE, arterial thromboembolism; CAT, calibrated automated thrombography; ETP, endogenous thrombin potential; FXaI, direct factor Xa inhibitor; HCT, hematocrit; HGB, hemoglobin; ICH, intracranial hemorrhage; INR, international normalized ratio; LT, lag time; mVRI, mean velocity rate index; PCC, prothrombin complex concentrate; PPP, platelet poor plasma; pRBC, packed red blood cell; PT, prothrombin time; rFVIIa, recombinant factor VIIa; SDM, substitute decision maker; TGA, thrombin generation assay; TF, tissue factor; TTP, time to peak; VTE, venous thromboembolic event.

will be defined based on whether each participant's TGA parameters fall within a nonparametric 95% reference range generated using 30 healthy volunteers (aged 24–55 years, 51.6% female) without a history of bleeding or prior thromboembolic events (data provided by Diagnostica Stago, France). The corresponding reference intervals are 2.67 to 3.33 minutes for LT, 4.50 to 6.00 minutes for TTP, 219.6 to 393.9 nM for peak, 980.6 to 1849.8 nM•min for ETP and 94.2 to 205.3 nM/min for mVRI.

Secondary clinical outcomes will include clinical hemostatic efficacy, the risk of thromboembolic events, and the risk of all-cause mortality within 30 days of PCC administration. For patients with major bleeding, clinical hemostatic efficacy will be evaluated in duplicate by independent adjudicators according to two sets of criteria: (1) as effective versus ineffective as per International Society on Thrombosis and Haemostasis (ISTH) criteria, and (2) good/moderate versus poor as per criteria from Sarode and colleagues (►**Supplementary Appendices A** and **B**).[9,12] Surgical hemostasis will be assessed by the treating surgeon using a binary normal/abnormal rating scale. Thromboembolic events will include deep vein thrombosis, pulmonary embolism, cerebrovascular accidents, transient ischemic attacks, systemic arterial emboli, myocardial infarction, and heart valve/cardiac chamber thrombosis and will be adjudicated in duplicate using standard criteria (►**Supplementary Appendix C**). Thromboembolic events will be adjudicated as definitely, likely, possibly, or unlikely related to PCC administration. Timing of anticoagulation resumption at therapeutic doses relative to thromboembolic events will be documented. Fatal events will be adjudicated as being related to fatal bleeding, a fatal thromboembolic event, or death from another cause.

Data Collection and Follow-Up

Data will be collected on demographics, anticoagulant therapy and concomitant antiplatelet therapy, indication for PCC administration, other transfused blood products, bleeding site, and whether the presenting bleeding event met the ISTH definitions for major bleeding, as well as the type and details of any planned surgical procedures, if applicable.[23,24] Patients presenting with major bleeding and require an urgent procedural intervention for bleed management (e.g., craniotomy for ICH) will be counted as having received PCC for major bleeding. Results of conventional coagulation assays (PT and aPTT), FXaI levels (by calibrated anti-Xa activity assay), and D-dimer and fibrinogen levels will be recorded, when available. ICH hematoma volume will be quantified using Quantomo software. Detailed hemostatic data will be collected and summarized. Hemostatic data will include hemoglobin/hematocrit trends, packed red blood cell transfusion requirements, requirements for additional coagulation factor concentrates or hemostatic agents, need for invasive interventions, hematoma volume (where applicable), and associated signs and symptoms from bleeding.[9,12] A review of medical records, laboratory tests, imaging results, and participant/SDM interview will be carried out at 30 ± 2 days post-PCC administration to determine whether a clinical outcome event had occurred. All suspected outcome events will be independently adjudicated.

Plasma Sample Collection and Laboratory Analyses

An electronic PCC order set was developed by clinical care leaders to standardize PCC dosing for FXaI-associated bleeding. This order set includes recommended timeframes for laboratory testing within 4 hours pre-PCC/post-PCC administration,

at the discretion of the treating physician. Citrated plasma samples will be collected by venipuncture using plastic 2.7 mL 3.2% citrated Vacutainer tubes (0.109 M sodium citrate; Beckton Dickinson). All coagulation analyses will be carried out within 4 hours from sample collection, except for thrombin generation. The following reagents will be used for coagulation assays: Thromborel S for PT/international normalized ratio testing (reference range: 10.0–14.0 seconds; Siemens Healthcare Diagnostics, Marburg, Germany), Actin FS for the aPTT testing (reference range 19.0–28.0 seconds; Siemens Healthcare Diagnostics, Marburg, Germany), and STA-Rivaroxaban and STA-Apixaban calibrators (Diagnostica Stago, Asnière-sur-Seine, France). Rivaroxaban-calibrated levels (STA-Rivaroxaban) will be used to estimate edoxaban levels (when clinically indicated), given similarities between the calibration curves.[25] D-dimer testing will be performed using the Innovance D-dimer kit (reference range $<$ 500 µg/L; Siemens Healthcare Diagnostics) and fibrinogen will be measured using the Clauss method with Dade Thrombin Reagent (reference range: 1.9–4.5 g/L; Siemens Healthcare Diagnostics, Marburg, Germany). Samples will undergo centrifugation at 1,500 g for 15 minutes to generate platelet-poor plasma (PPP) and will be aliquoted and frozen at −80C for up to 6 months prior to thrombin generation testing.

Methods for sample acquisition, plasma processing/handling, sample storage, reagent selection, assay conditions, and result interpretation with respect to thrombin generation testing will be consistent with guidance by the ISTH Scientific and Standardization Committee.[26] Thrombin generation will be measured using calibrated automated thrombography (CAT)/Thrombinoscope on PPP samples by a central research laboratory (Diagnostica Stago, Gennevilliers, France). Fluorescence will be measured using a Fluoroskan Ascent fluorometer (ThermoFischer Scientific, Helsinki, Finland). TGA will be completed using 5 pM tissue factor (PPP Reagent; Diagnostica Stago) and 4 µM phospholipids. Reactions will be initiated by adding 20 µL of FluCa buffer (Diagnostica Stago) to wells containing 80 µL of PPP. Reactions will be carried out in duplicate and averaged values from duplicate runs will be recorded. TGA analyses will be completed in the absence of added corn trypsin inhibitor or exogenous thrombomodulin. TGA parameters to be measured and recorded will include the LT (in minutes), TTP (in minutes), peak thrombin generation (peak; in nM), ETP (in nM•min), and mVRI (in nM/min).

Sample Size

The pharmacologic activity of FXaIs is best reflected through their effects on coagulation initiation and propagation.[27] Further, FXaI levels appear to correlate best with TGA kinetic parameters. PCCs appear to most consistently normalize quantitative parameters of thrombin generation (peak, ETP).[28,29] Few studies have evaluated the impact of PCCs on kinetic TGA parameters, and among those that did, several studies showed only partial correction or no effect at all.[15,28,29] Some anticoagulants appear to exclusively exert their effects on the kinetics of thrombin generation (e.g. hirudin, bivalirudin).[30] Anticoagulation reversal strategies with established mechanisms of action, such as the use of PCC for VKA reversal or andexanet alfa for FXaI reversal, correct both kinetic and quantitative TGA parameters. These reversal strategies normalize the thrombin generation curve (►**Fig. 3**). Moreover, kinetic TGA parameters demonstrate better discrimination with respect to minor bleeding events among rivaroxaban-treated patients.[20] LT reflects the initiation phase of coagulation and may be important with respect to anticoagulation-associated bleeding and hemostasis. Maag and colleagues recently hypothesized that the extent to which factor V activation by factor Xa contributes to initial thrombin generation and subsequent clot formation could form the basis of major bleeding observed during anticoagulant therapy. Their analysis using samples collected from participants of the BLEEDS study[31] found a significant association between anticoagulation-associated major bleeding and the LT (hazard ratio: 2.10; 95% CI: 1.32–3.31). They concluded that factor Xa-mediated activation of factor V represents a specific thrombin generation activation pathway during coagulation initiation, a process that may be particularly important among anticoagulated patients with major bleeding.[32] Taken together, these findings suggest that correcting kinetic TGA parameters, in addition to quantitative parameters, may be relevant with respect to predicting a meaningful hemostatic effect. The mean LT in plasma from healthy volunteers ranges from 1.9 to 2.3 minutes when measured using CAT.[28,29] Addition of FXaIs (rivaroxaban, apixaban) to plasma from healthy volunteers prolongs the mean LT to a range of 5.6 to 5.9 minutes.[28,29] Using a two-sided paired *t*-test with alpha of 0.05 and a conservatively estimated standard deviation of the difference of 2.0 minutes,[28,29] a sample size of 100 paired TGA measurements would provide 90% power to measure a detectable alternative in LT of $\pm$ 0.65 minutes.

Statistical Analysis Plan

Continuous variables will be summarized as mean and standard deviation or median and interquartile range; categorical variables will be presented as frequencies. Normality of distributions will be evaluated using the Shapiro–Wilk test. We will evaluate the correlation between DOAC levels and each TGA parameter using Pearson's/Spearman's correlation coefficients, depending on the underlying distribution of the data. Differences between pre-PCC and post-PCC laboratory measurements will be analyzed using a two-sided paired *t*-test with alpha of 0.05 for normally distributed data, or the Wilcoxon signed rank test for nonnormally distributed data. The proportion of patients with LT/TTP values below the upper limit of normal (ULN) and peak/ETP/mVRI values above the lower limit of normal (LLN) will be reported both pre- and post-PCC based on the reference ranges outlined above. We will compare the proportion of patients post-PCC versus pre-PCC that fall below the ULN for LT/TTP and above the LLN for peak/ETP/mVRI using McNemar's test (asymptotic) with p-values $<$0.05 considered statistically significant. Given the exploratory nature of the analyses, no adjustment for multiple testing will be carried out. Hemostatic efficacy, thromboembolic events, and mortality will be reported as categorical variables with 95% CIs. All statistical

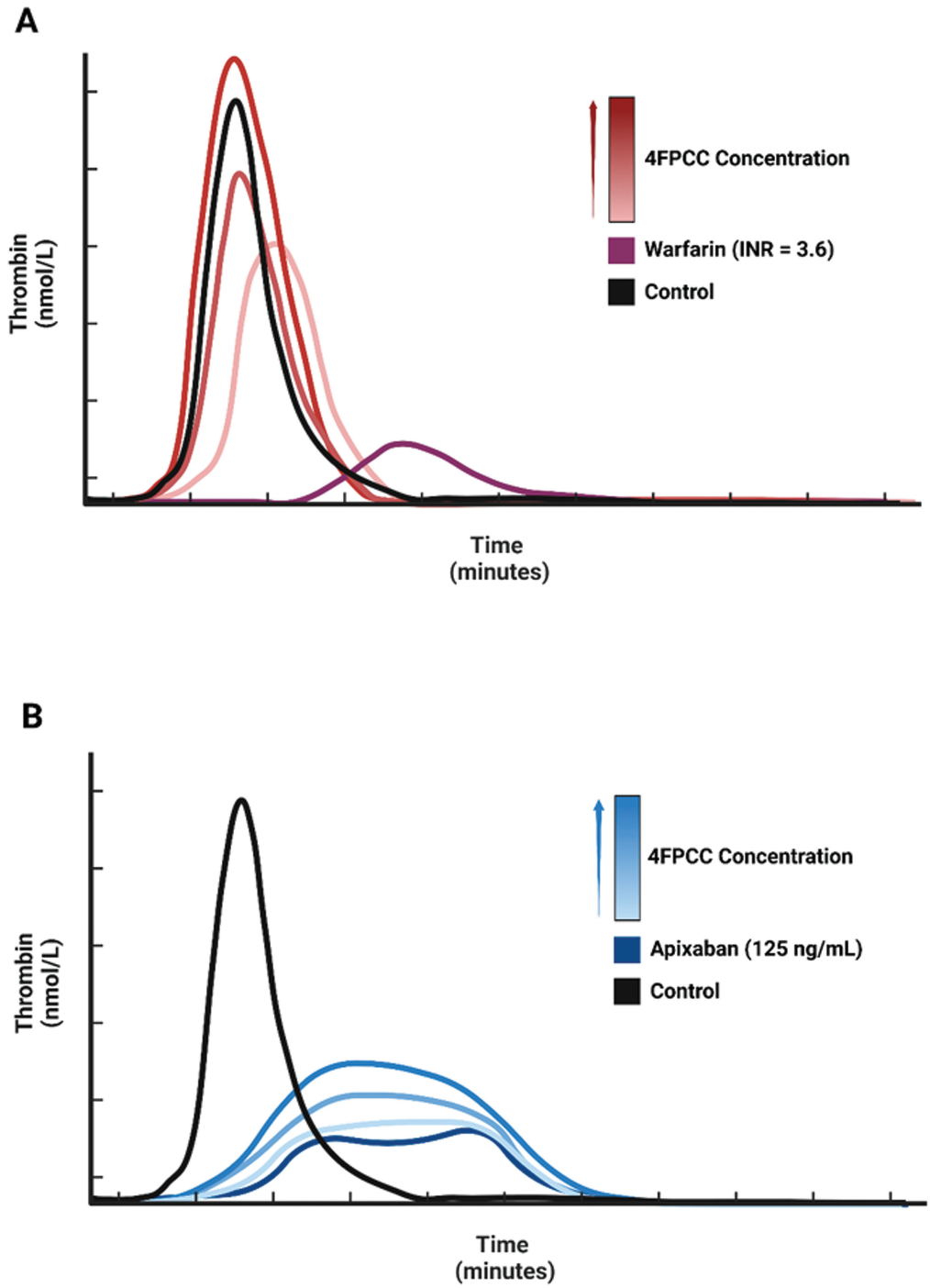

Fig. 3 Representative effects of 4FPCC on the thrombin generation curve in anticoagulated plasma. Approximated representative TGA patterns; the black curves represent reference thrombograms in the absence of anticoagulant. Red (**A**) and blue (**B**) lines represent typical changes to the thrombin generation curve following the in vitro addition of increasing concentrations of 4FPCC to (A) warfarin- and (B) apixaban-anticoagulated plasma using calibrated automated thrombography. Reproduced with permission from Shaw et al[15].

analyses will be conducted with SAS Studio Version 3.81 (Copyright 2012-2020, SAS Institute Inc., Cary, North Carolina, United States).

Exploratory Analyses

First, we will conduct exploratory analyses to evaluate the relationship between TGA measurements and effective hemostasis among patients receiving PCC for major bleeding. We will compare (1) pre-PCC TGA parameters, (2) post-PCC TGA parameters, and (3) the change in each TGA parameter from pre-PCC to post-PCC measurements among patients with effective versus ineffective hemostasis by ISTH criteria and good/moderate versus poor hemostasis by Sarode criteria. Second, invasive procedures or surgery can impact thrombin generation measurements.[33] We will undertake a sensitivity analysis whereby all analyses surrounding TGA

parameters outlined above will be repeated but will exclude patients who underwent an intervening invasive procedure between pre/post-PCC TGA measurements. Octaplex contains a relatively high heparin concentration in comparison to other PCC formulations.[34] TGA measurements are sensitive to the presence of heparin.[35] Polybrene is a polycationic molecule that binds to and neutralizes heparin. We will carry out an exploratory analysis whereby TGA analyses will be repeated for both pre- and post-PCC samples in the presence of polybrene. This will be accomplished by using a polybrene solution with a concentration of 0.025 mg/mL to reconstitute the PPP Reagent for these exploratory TGA analyses.[35] Given the effects of PCC on thrombin generation in the presence of a FXaI might differ depending on the FXaI level, we will also conduct an exploratory stratified analysis of the effects of PCC on TGA parameters among patients with pre-PCC FXaI levels $\geq$75 ng/mL versus $<$75 ng/mL.[18] Lastly, a limitation to the observational design of this study is that we will have no direct control over the time between the pre-PCC and post-PCC sample collection. As a sensitivity analysis, we will conduct a regression analysis estimating the change for a given TGA parameter while adjusting for the time between pre-PCC and post-PCC sample collections. This will enable us to determine whether controlling for time substantively changes our conclusions surrounding the effect of PCC on TGA parameters. If we find that the effect of time is statistically significant, we will also use the regression model to predict the effect of PCC on TGA parameters, independent of time.

Discussion

Andexanet alfa is a recombinant modified human factor Xa decoy protein and is approved by the FDA (United States Food and Drug Administration) and the European Union for use as a specific reversal agent for the FXaIs rivaroxaban and apixaban.[6] Andexanet alfa is not approved for use in all countries, can be costly, and the need for reconstitution from multiple vials could result in delays in administration.[36,37] In the absence of specific reversal therapy, nonspecific treatments, such as PCC, are often given, a practice that is supported by expert consensus guidance.[38,39] Phase II clinical trials evaluating the biochemical effect of reversal agents, such as andexanet alfa, have demonstrated complete reversal of the anticoagulant effects of FXaIs.[40] Andexanet alfa reduces anti-Xa levels to near baseline levels, reduces unbound Xa inhibitor concentrations to near zero, and has been shown to effectively restore thrombin generation to baseline levels.[6,40] This mechanistic evidence does not exist for nonspecific agents such as PCC. The mechanisms by which PCC may improve hemostasis in the setting of FXaI use are unclear but it has been hypothesized to involve a bypassing mechanism in which anticoagulant effects are overcome with a supply of coagulation factors.[41] Some investigators contend that this mechanism is not plausible based on the coagulation factor levels achieved following infusion of PCC, which would be insufficient to overcome anything other than relatively low FXaI levels.[18] Administration of PCC does not decrease circulating FXaI levels.[42]

FXaI-associated bleeding events are commonly encountered in clinical practice and PCCs are increasingly used as a hemostatic therapy in this setting. PCC has, until recently, been referred to throughout the literature as a reversal agent for FXaIs.[17,38,43] The notion that PCCs can reverse direct oral anticoagulation was first proposed by Eerenberg and colleagues, and was based on the effect of PCC on thrombin generation in DOAC-treated healthy volunteers.[43] These effects were captured using ETP for rivaroxaban-treated volunteers and LT for dabigatran-treated volunteers, as these respective parameters were, at the time, felt to be the best measures of anticoagulant effect for rivaroxaban and dabigatran, respectively. Since this pivotal work, a large body of in vitro and animal model research has accumulated surrounding the effects of hemostatic therapies on TGA parameters in the context of direct oral anticoagulation.[15] PCCs consistently improve the ETP across studies, but inconsistently correct kinetic TGA parameters. PCC does not normalize the thrombin generation curve.[15] These results differ from proven anticoagulation reversal strategies. PCC has been shown to normalize the thrombin generation curve in patients on VKAs, with complete correction of both kinetic and quantitative TGA parameters.[44] Similarly, andexanet alfa was reported to completely reverse the effects of FXaIs on LT, peak thrombin generation, and ETP.[45] Moreover, the effects of PCC on thrombin generation among FXaI-treated patients differ from those observed following the administration of clinically recognized bypassing agents (e.g., aPCC; FEIBA or rFVIIa) to patients with hemophilia and inhibitors, a biologically analogous scenario to FXaI-associated bleeding. Although ETP seems to be the TGA parameter of interest, the addition of aPCC in vitro to plasma from patients with hemophilia A and acquired inhibitors appears to correct both kinetic and quantitative TGA parameters and normalizes the thrombin generation curve.[46–48] Importantly, the scientific rationale for using thrombin generation as a pharmacodynamic measure among patients with hemophilia and inhibitors treated with bypassing therapies also applies to evaluating hemostatic therapies among patients with FXaI-associated bleeding.[46] PCCs and aPCCs are multicomponent fractionated products and conventional pharmacokinetic methodologies using absorption, distribution, metabolism, and elimination are not feasible due to their complex mechanisms of action.[46] These mechanisms are poorly understood despite decades of use and their effects are incompletely captured using conventional coagulation assays.[48,49]

While PCC might confer a beneficial hemostatic effect, it does not seem to function as a FXaI reversal agent or bypassing therapy.[15] Furthermore, clinical data supporting the use of PCC for FXaI-associated bleeding are based on single-arm cohort studies with clinical hemostatic efficacy as the primary outcome, precluding conclusions as to whether hemostatic benefit seen in these studies was attributable to PCC. Ideally, the aim of hemostatic therapy should be clinical hemostasis. However, hemostatic efficacy is challenging to measure. Current definitions of clinical hemostasis have important limitations and lack validation. Furthermore, improvements in clinical hemostatic efficacy using current

definitions have not been shown to correlate with mortality. There is mounting evidence in support of thrombin generation as a clinically relevant endpoint, both with respect to thrombosis and bleeding/hemostasis.[20,50–52] Collecting data on the change in thrombin generation profile induced by PCC within each treated patient would provide evidence in support of or against a credible bypassing/hemostatic effect.

The optimal PCC dosing strategy for the management of FXaI-associated bleeding remains uncertain (►**Table 1**).[53] Prior cohort studies have employed either fixed PCC dosing (1,500–2,000 IU)[7,8] or weight-based dosing (50 IU/kg).[54] The underlying biological rationale underpinning fixed PCC dosing for FXaI-associated bleeding is unclear. The pivotal study by Eerenberg and colleagues demonstrating an effect of PCCs on thrombin generation parameters in the context of FXaI anticoagulation employed a weight-based PCC dose of 50 IU/kg.[43] Numerous in vitro analyses have demonstrated more consistent correction of thrombin generation with PCC concentrations approximating in vivo doses of 50 IU/kg.[15] A recent in vivo study of edoxaban-treated healthy volunteers documented normalization of both the ETP and bleeding parameters using weight-based PCC doses of 50 IU/kg, whereas doses <50 IU/kg resulted in incomplete correction.[17] These factors led to the decision to adopt weight-based PCC dosing of 50 IU/kg for FXaI-associated bleeding or prior to urgent surgery at our institution.

The lack of a biologically plausible reversal mechanism and absence of comparative anticoagulation reversal research calls into question the practice of using PCC as a hemostatic therapy for FXaI-associated bleeding. FXaI-associated bleeding events will be increasingly encountered by clinicians in the context of an aging population and growing prevalence of already common conditions (e.g., atrial fibrillation, venous thromboembolic disease), for which oral FXaIs are often prescribed. These bleeding events are costly to health care systems and are associated with a high risk of mortality. Without current or anticipated consistent future access to a specific reversal agent such as andexanet alfa, patients around the world will be left without a viable management strategy for FXaI-associated bleeding events. Demonstrating the effect (or lack thereof) of PCC on a clinically relevant surrogate outcome among bleeding patients could shed light on the suitability of its ongoing use as a FXaI hemostatic therapy. GAUGE will provide valuable in vivo data on the effects of PCC. Development of a viable and universally accessible FXaI bleed management strategy is crucial.

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Conflicts of Interest

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