Utility of Thrombin Generation Assays Towards Measuring the Anticoagulant Effects of Direct Oral Anticoagulants and Anticoagulation Reversal

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Preface

Research Approval – The Ottawa Hospital Science Network Research Ethics Board provided all relevant approvals with respect to the research presented herein.

Joseph R. Shaw is the lead investigator for all work presented herein. He led the design of the included studies, sought relevant ethical approvals and trial funding, supervised research support staff, collected the data, performed the analyses, interpreted the data and drafted the manuscripts.

Marc Carrier is the MSc thesis supervisor. He provided supervision and feedback on all steps of the research presented herein. He approves the submission of this thesis and attests to the candidate's contributions.

Lana A. Castellucci is the thesis co-supervisor and a member of the thesis advisory committee. She provided guidance on study design, data analysis, interpretation of results and provided key revisions to the drafted manuscripts.

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Abstract

Direct factor Xa inhibitors (FXaI) account for most oral anticoagulant use. FXaI-associated bleeding events are common and are associated with substantial morbidity and mortality. Nonspecific hemostatic therapies such as prothrombin complex concentrates (PCC) are often administered for FXaI-associated bleeding. The mechanism by which these agents improve hemostasis in the setting of direct oral anticoagulation is unclear. Thrombin generation assays may effectively measure the effect of anticoagulation reversal among FXaI-treated patients when bleeding cessation would otherwise be challenging to measure. To build a research program on the utility of thrombin generation assays to measure both the impact of direct oral anticoagulation and anticoagulation reversal, we completed a review of the literature with narrative synthesis and carried out a pilot study to determine the feasibility of a full scale prospective observational study of TGA responses among patients receiving PCC for FXaI-associated major bleeding or needing urgent surgery.

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List of Abbreviations

3FPCC	3-Factor PCC
4FPCC	4-Factor PCC
AF	Atrial Fibrillation
aPCC	Activated Prothrombin Complex Concentrate
APS/APLAS	Antiphospholipid Antibody Syndrome
aPTT	Activated Partial Thromboplastin Time
AUC	Area Under the Curve
BD	Bleed Duration
BV	Bleed Volume
CAT	Calibrated Automated Thrombography
CrCl	Creatinine Clearance
CTI	Corn Trypsin Inhibitor
DAPT	Dual Antiplatelet Therapy
DOAC	Direct Oral Anticoagulant
DTI	Direct Thrombin Inhibitor
ETP	Endogenous Thrombin Potential
FXaI	Direct Factor Xa Inhibitor
ICH	Intracranial Hemorrhage
INR	International Normalized Ratio
IQR	Interquartile Range
ISTH	International Society on Thrombosis and Haemostasis
LLN	Lower Limit of Normal
LT	Lag Time
mVRI	Mean Velocity Rate Index
PCC	Prothrombin Complex Concentrate
pRBC	Packed Red Blood Cells
Peak	Peak Thrombin Generation
PPP	Platelet Poor Plasma
PRP	Platelet Rich Plasma
PT	Prothrombin Time
RCT	Randomized Controlled Trial
RFU	Relative Fluorescence Units
rFVIIa	Recombinant Factor VIIa
ROTEM	Rotational Thromboelastometry
SDM	Substitute Decision Maker
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TGA	Thrombin Generation Assays
TTP	Time to Peak
ULN	Upper Limit of Normal
VKA	Vitamin K Antagonist
VTE	Venous Thromboembolic Disease

Chapter 1 – Introduction, Objectives and Thesis Outline

1.1 Clinical Impact of Direct Oral Anticoagulant-Associated Bleeding Events

Direct factor Xa inhibitors (FXaIs), a type of direct oral anticoagulant (DOAC), are approved for use as stroke prevention in atrial fibrillation and the treatment/prevention of venous thromboembolic disease (VTE). By the year 2050, it is estimated that the number of patients with atrial fibrillation will increase 2.5-fold, many of whom will be prescribed an oral anticoagulant [1]. Seven million prescriptions for oral anticoagulants were issued across Canada in 2013, of which DOACs make up a majority [2]. Age is a major risk factor for development of both atrial fibrillation and VTE. Furthermore, age is a known risk factor for anticoagulant-associated bleeding events [3]. It follows that FXaI-associated bleeding will be increasingly encountered by physicians from a wide range of specialties [4]. Oral anticoagulant-associated bleeding events are associated with significant morbidity and mortality, with case fatality rates on the order of 8-15% [5, 6]. A secondary analysis of a randomized controlled trial (RCT) evaluating apixaban for patients with atrial fibrillation found that 14.9% of patients who experienced a major bleeding event died within 30 days of the bleeding, and among patients with intracranial hemorrhage (ICH), the 30-day mortality rate was 43.2% [7]. Gastrointestinal bleeding is far more prevalent than ICH and carries a substantial 11% 30-day risk of mortality [6]. Moreover, concern over bleeding risk often pushes physicians to prescribe lower FXaI dosing among patients who would otherwise not qualify for dose reduction, leading to pervasive off-label use of low-dose direct Xa inhibitors [8]. Some physicians may opt to withhold anticoagulation or discontinue anticoagulation altogether after a bleeding event, which in turn places patients at risk of thromboembolic complications [9].

1.2 Lack of Effective Management Options in Canada

Andexanet alfa, a recombinant modified human factor Xa decoy protein, was recently approved by the FDA and the European Union for use as a specific reversal agent for the FXaIs rivaroxaban and apixaban [10]. The clinical utility of andexanet alfa may be limited by its cumbersome administration, need for reconstitution from multiple vials which could result in delays in administration, and high cost [11]. Andexanet alfa is not currently approved for use in Canada as a reversal agent for Xa inhibitors. Due to the lack of an approved specific reversal agent for Xa inhibitors, centers across Canada have adopted the use of non-specific hemostatic agents such as prothrombin complex concentrates (PCCs) [4].

1.3 Laboratory and Clinical Evidence Supporting the Use of PCC for Direct Xa Inhibitor-Associated Bleeding is Limited

Phase II clinical trials evaluating the biochemical effect of reversal agents, such as andexanet alfa, have demonstrated effective and complete reversal of the anticoagulant effects of FXaIs [12]. 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 [10, 12]. This mechanistic evidence does not exist for non-specific agents such as PCC. It has been proposed that infusion of large quantities of coagulation factors may “overwhelm” or “bypass” direct Xa inhibitors and lead to effective hemostasis [13]. This mode of action remains unproven, compounding the uncertainty as to whether they contribute to hemostasis at all. Some

investigators contend that this mechanism is not plausible based on the factor levels achieved following infusion of PCC, which would be insufficient to bypass anything other than relatively low direct Xa inhibitor levels [14]. Some studies have demonstrated partial improvement in several biochemical measures of hemostasis and clot formation, such as clotting times, thrombin generation assay parameters and thromboelastography/ROTEM®, both in animal models [15-17] and in healthy volunteers receiving FXaIs [18-20], whereas other studies were unable to detect an effect of PCCs on these parameters [21]. The use of PCCs in bleeding patients has been evaluated in a few retrospective chart reviews [22-24] and cohort studies [25, 26]. These cohort studies focused solely on clinical hemostasis [26, 27] as an outcome and lacked control groups for comparison. A phase IV RCT comparing PCC to andexanet alfa is ongoing among patients with ICH (NCT03661528).

1.4 Shortcomings of Clinical Hemostasis as an Outcome Measure

It remains unclear whether the clinical criteria used to assess “effective” hemostasis reliably detect an impact from anticoagulation reversal [28]. 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. Criteria used to adjudicate clinical hemostasis were developed by expert consensus and have not been externally validated [27, 29]. They 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). These measurements are not solely impacted by ongoing bleeding and are not specific to effective hemostasis. These measurements may show deterioration irrespective of whether bleeding has stopped. The clinical criteria for effective hemostasis have not been shown to correlate with favorable objective clinical outcomes (e.g., mortality). Furthermore, the criteria for adjudication of effective or “good” hemostasis are modified from study to study and are not applied in a consistent manner [10, 26, 29]. There are no published randomized controlled trials (RCTs) comparing the use of PCCs to best supportive care. The incremental benefit of PCC administration over best supportive care, if any, is unknown [30, 31]. PCC cohort studies that lack a comparator arm and that assess 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.

1.5 Overview of Thrombin Generation Assays

Coagulation assays such as the PT and aPTT have limited utility for monitoring the anticoagulant effects of direct Xa inhibitors [32]. The sensitivity of conventional coagulation assays to direct Xa inhibitor levels is dependent on the reagents used and the direct Xa inhibitor in question. 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. Conventional assays were developed with a specific purpose in mind (e.g., monitoring vitamin K antagonist anticoagulation) rather than measuring overall coagulation or monitoring DOAC therapy. Moreover, they have not been shown to adequately capture the hemostatic effects of PCC in patients on direct

Xa inhibitors [28, 33, 34]. Most studies evaluating the effect of PCCs on PT among patients on direct Xa inhibitors have only shown partial corrections [28]. Modern thrombin generation assays (TGAs) use fluorometric or chromogenic methods to detect thrombin generation, usually through thrombin-mediated cleavage of a substrate, generating a fluorogenic/chromogenic cleavage product that can be measured over time [35]. TGAs capture the total output and efficiency of the coagulation system by measuring the enzymatic activity of thrombin, the final common step of the coagulation cascade. TGAs report both kinetic and quantitative parameters of thrombin generation. Kinetic parameters include the lag time (LT), the time it takes to initiate thrombin generation; time to peak (TTP), the time between the start of the assay and the time point of maximal thrombin generation. Quantitative parameters include the endogenous thrombin potential (ETP), which represents the total amount of thrombin generated throughout the entire assay (measured using the area under the curve; AUC); as well as the peak thrombin generation [35, 36]. The mean velocity rate index (mVRI) incorporates both kinetic and quantitative information into a single composite measure. Thrombin generation has been shown to be one of the best methods to measure the thrombogenic potential of plasma *ex vivo [37]*. Rivaroxaban, apixaban, edoxaban have been shown to affect both kinetic (LT) and quantitative (ETP) TGA parameters [37-40]. Overall, LT seemed to correlate best with DOAC levels, as measured using high-performance liquid chromatography, across all DOAC classes [40]. Furthermore, several studies have identified that ETP correlates poorly with DOAC drug levels [37-40].

1.6 Utility of Thrombin Generation Assays to Measure the Effect of PCCs on Coagulation

Studies have applied TGAs towards measuring the prohemostatic effects of PCCs in animals and in healthy volunteers treated with DOACs [28]. PCCs appear to most consistently normalize quantitative parameters of thrombin generation (peak, ETP) [18, 19]. Despite the fact that DOAC anticoagulant activity appears to correlate best with TGA kinetic parameters [40], 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 [18-20]. Similarly, PCC seemed to exert a variable effect on PT measurements, with some studies showing partial correction, whereas others showed no effect at all [20, 41, 42]. A porcine polytrauma model found that PCC administration increased ETP measurements with concurrent improvements in total blood loss [43]. Similarly, a phase I study among healthy human volunteers treated with edoxaban and subjected to a skin punch biopsy showed concurrent improvements in blood loss, bleeding duration and correction of ETP following PCC administration [44]. 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 [43, 44], 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 direct Xa inhibitor-associated bleeding

1.7 Thesis Research Questions

Primary Research Question – Is a prospective observational study evaluating thrombin generation parameters following administration of PCC for direct Xa inhibitor-associated

major bleeding or prior to urgent surgery feasible with respect to participant recruitment and sample acquisition?

Secondary Research Questions –

1. What is the current state of knowledge surrounding the utility of thrombin generation assays for measuring the effect of PCCs on direct oral anticoagulation and have any studies been able to demonstrate a statistically significant relationship between correction of TGA parameters and clinical hemostatic efficacy?
2. Does PCC administration to patients with direct Xa inhibitor-associated bleeding or needing urgent surgery lead to a statistically significant change in thrombin generation parameters?

1.8 Thesis Objectives and Outline

The thesis was designed and structured to address the following objectives:

Objective 1 - Review and summarize the literature on the utility of TGAs for evaluating the therapeutic effects of PCCs for the management of direct Xa inhibitor-associated bleeding or prior to urgent surgery.

Objective 2 - Conduct a feasibility pilot study evaluating the effect of PCCs on thrombin generation among direct Xa inhibitor-treated patients.

Objective 3 - Measure the effects of PCC on kinetic and quantitative TGA parameters and conventional coagulation assays.

Objective 4 - Evaluate the clinical efficacy and safety of PCC administration to patients for direct Xa inhibitor-associated bleeding or for attempted hemostatic normalization prior to urgent surgery.

Two manuscripts have been prepared for publication in peer-reviewed medical journals to meet the objectives outlined above.

Manuscript 1 - "DOAC-Associated Bleeding, Hemostatic Strategies and Thrombin Generation Assays – A Review of the Literature"

Manuscript 2 - "Effect of Prothrombin Complex Concentrates on Thrombin Generation Parameters Among Patients with Major Bleeding or Needing Urgent Surgery on Factor Xa Inhibitors – A Pilot Study"

Chapter 2 – Literature Review and Narrative Synthesis

DOAC-Associated Bleeding, Hemostatic Strategies and Thrombin Generation Assays – A Review of the Literature

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Abstract

Direct oral anticoagulants (DOACs) account for most oral anticoagulant use. DOAC-associated bleeding events are commonly encountered in clinical practice and are associated with substantial morbidity and mortality. Both specific reversal agents and non-specific hemostatic therapies, such as prothrombin complex concentrates (PCCs), are used in the management of DOAC-associated bleeding. Measuring hemostatic efficacy and demonstrating a clinical impact from these therapies among studies of bleeding patients is challenging. Thrombin generation assays provide information on the total hemostatic potential of plasma and have emerged as a promising modality to both measure the impact of DOACs on coagulation and to evaluate the effects of hemostatic therapies among patients with DOAC-associated bleeding. The mechanisms by which non-specific hemostatic agents impact coagulation and thrombin generation in the context of DOAC therapy are unclear. As a result, we undertook a review of the literature using a systematic search strategy with the goal of summarizing the effects of DOACs on thrombin generation and the effect of both specific reversal agents and non-specific hemostatic therapies on DOAC-altered thrombin generation parameters. We sought to identify clinical studies focusing on whether altered thrombin generation is associated with clinical bleeding and whether correction of altered thrombin generation parameters predicts improvements in clinical hemostasis. Lastly, we sought to outline future directions for the application of thrombin generation assays towards anticoagulation therapies and the question of anticoagulation reversal.

Keywords: Hemostasis, Factor Xa Inhibitors, Dabigatran, Anticoagulation Reversal, Biologic Assay

Introduction

Direct oral anticoagulants (DOACs) such as direct factor Xa inhibitors (FXaIs; rivaroroxaban, apixaban, edoxaban) and direct thrombin inhibitors (DTIs; e.g. dabigatran) have revolutionized oral anticoagulation therapy over the past decade. DOACs now account for the majority of oral anticoagulant use [1]. Despite a lower risk of bleeding complications, DOAC-associated bleeding events are commonly encountered in clinical practice [2-4]. Hemostatic strategies may be used for the management of DOAC-associated bleeding events. Agents that specifically reverse the anticoagulant effect of direct/indirect factor Xa inhibitors (andexanet alfa) and dabigatran (idarucizumab) are currently in clinical use, and a non-specific reversal agent for oral anticoagulants (ciraparantag) is under clinical development. Specific reversal agents are not approved by all regulatory authorities, can be costly, and are not always immediately available for clinical use [5, 6]. As a result, nonspecific hemostatic therapies such as prothrombin complex concentrates (PCC) and activated PCCs (aPCC) are administered for DOAC-associated bleeding [7]. The mechanism through which these agents improve hemostasis in the setting of direct oral anticoagulation is unclear but has been proposed to occur through a bypassing mechanism [8, 9].

Numerous studies have been published on the effects of both reversal agents and nonspecific hemostatic therapies, with respect to clinical hemostasis and impact on laboratory parameters [10-15]. Although criteria have been used to standardize measurements of hemostasis in clinical studies, adjudication of clinical hemostasis is complex, necessitates intensive data collection and frequently relies on surrogate

measures of ongoing bleeding (e.g., hemoglobin concentration) [16]. Conventional coagulation assays (such as the prothrombin time and activated partial thromboplastin time) generally have low sensitivity to DOAC levels [17, 18]. Moreover, administration of nonspecific agents such as PCC does not affect plasma DOAC concentrations [19, 20], and often has little effect on conventional clotting assays [7].

Thrombin generation assays (TGAs) continuously measure the production of thrombin through the cleavage of a fluorogenic substrate [21]. TGAs have been described as a "global coagulation assay", provide substantially more information on hemostatic potential than conventional clotting assays and can be carried out using both platelet-poor plasma (PPP), as well as platelet-rich plasma [22, 23]. They provide information on both kinetic and quantitative parameters of thrombin generation (**Figure 1**) [23]. 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 have been used to measure hemostatic responses in studies evaluating both specific DOAC reversal agents and nonspecific hemostatic therapies [10, 15] and appear to be better able to detect changes in the coagulation system following administration of hemostatic therapies in the setting of DOAC use [7, 24].

TGAs may effectively measure the effects of reversal and/or hemostatic therapies among DOAC-treated patients when bleeding cessation would otherwise be challenging to measure. We conducted a review of the literature with the following objectives in mind:

1. Review the effect of DOACs on TGA parameters.
2. Outline the effects of both non-specific hemostatic therapies and specific reversal agents on TGA parameters in the presence of DOACs.
3. Summarize existing evidence on correlations between DOAC-altered TGA parameters and bleeding outcomes, as well as any improvements in TGA parameters and clinical hemostatic efficacy following administration of hemostatic therapy among DOAC-anticoagulated patients.

Methods

A review of the literature using a comprehensive search strategy (**Supplementary Appendix A**), in collaboration with a trained library information specialist (Risa Shorr, MLIS; The Ottawa Hospital) was performed. Indexed databases (EMBASE, MEDLINE, Web of Science and the Cochrane Central Register of Controlled Trials) were searched from inception to October 2021 using a combination of MeSH terms and keywords. Studies were eligible for inclusion if they evaluated either the effect of DOACs on TGA parameters; or evaluated the therapeutic effect of hemostatic therapies on TGA parameters among DOAC-treated human participants. We included both in vitro and in vivo studies. Eligible DOACs, hemostatic therapies and TGA assays are listed in

Supplementary Appendix A. Study designs eligible for inclusion were case series (reporting results on ≥ 10 patients), case-control studies, cross-sectional studies, prospective or retrospective cohort studies and randomized controlled trials. Studies focusing exclusively on animal subjects were excluded. Abstract and full text eligibility screening was performed using Covidence® software. Reasons for full text exclusion were recorded using Covidence® and are reported using a flow diagram (**Supplementary Figure 1**). Data was abstracted using Excel® databases to record results electronically (**Supplementary Appendices B and C)**. Key characteristics of included studies were narratively summarized according to the three objectives outlined above and presented using color-coded tables and figures.

Results

Literature Overview

The search identified 4,807 abstracts, of which 411 were identified as potentially eligible following initial screening of abstracts, and 282 were excluded after full text review. The remaining 129 full text articles were included in the review (**Supplementary Figure 1**).

Among the 129 included studies (**Supplementary Appendices B and C**), 75 recruited healthy volunteer subjects, and the others evaluated patients receiving DOACs for a variety of indications, usually atrial fibrillation (AF) and/or venous thromboembolism (VTE). Seventy-two studies used in vitro methodology (1 study also used in silico modelling [25]) and the remaining 57 in vivo studies involved volunteers/patients

receiving DOACs. We identified 9 studies that included only patients with AF [26-34], 5 studies that included only patients with VTE [35-39] and 15 studies that included patients with mixed indications for anticoagulation (usually AF and/or VTE) [10, 15, 40-52]. Some articles included participants with coronary artery disease (CAD) or those on dual antiplatelet therapy (DAPT; 3 studies) [53-55], acute stroke (1 study) [56], antiphospholipid antibody syndrome (APS;1 study) [57], advanced age (1 study) [58], liver disease (5 studies) [59-63], mechanical heart valves (3 studies) [64-66], orthopedic surgery (8 studies) [67-74], protein C deficiency (1 study) [75] and solid organ transplant recipients (1 study) [76].

The most frequently used TGA platform was Calibrated Automated Thrombography (CAT)/Thrombinoscope® in 95 studies, whereas Innovance ETP® was used in 7 studies, Technothrombin® in 14 studies, ST-Genesia® in 6 studies and other assays in 7 studies (mix of uncalibrated or manual methods). A wide range of tissue factor (TF) concentrations were used for initiation of thrombin generation (0.15 pM to 850 pM), depending on the objectives of the study. The most consistently used TF concentration was 5 pM (e.g. Stago's PPP Reagent® in 30 studies), which is a physiologically relevant TF concentration [77]. The majority of studies used PPP to carry out thrombin generation testing (117 studies, 90.7%). Studies seeking to evaluate the contribution of platelets to thrombin generation, or the impact of antiplatelet therapy, used PRP [53-55]. Only 7 studies (5.4%) pre-treated samples with corn trypsin inhibitor (CTI) [26, 35, 48, 73, 78]. A total of 13 studies (10.1%) performed TGA with added thrombomodulin, usually with the goal of evaluating the impact of DOACs on the activated protein C

system [32, 59-63, 75, 79-84]. Out of 129 included studies, 126 (97.7%) reported at least one TGA parameter, and 3 studies used non-calibrated methodology and reported results in relative fluorescent units (RFU) [8, 74, 85]. Most studies reported peak height (110 studies, 85.3%), lag time (105 studies, 81.4%) and ETP (98 studies, 76.0%), whereas fewer reported the time to peak (77 studies, 59.7%) and mVRI (43 studies, 33.3%).

Effect of DOACs on TGA Parameters

In vitro and in vivo studies describing the effects of DOACs on TGA parameters included 42 for apixaban, 78 for rivaroxaban, 51 for dabigatran, 23 for edoxaban, 6 for betrixaban and 7 for melagatran (**Figure 2**). The concentrations tested for each DOAC varied across studies but ranged from 0-1000 ng/mL for apixaban, 0-1090 ng/mL for rivaroxaban, 0-5000 ng/mL for dabigatran, 0-1650 ng/mL for edoxaban, 0-1000 ng/mL for betrixaban. All DOACs affected each TGA parameter if tested at a sufficiently high concentration. Relatively low DOAC concentrations (< 30 ng/mL) had a substantial effect on TGA parameters [86], with peak and mVRI IC50 values of < 30 ng/mL [87-89].

Several studies reported statistically significant correlations between DOAC concentrations and TGA parameters [32, 39, 40, 42, 49, 90, 91]. FXaIs tended to affect both kinetic and quantitative TGA parameters. FXaI concentrations correlated well with both kinetic and quantitative parameters, although typically higher concentrations were required to suppress ETP [92-94]. DTIs exerted a greater effect on kinetic parameters at

low concentrations [40, 87-89, 95, 96]. Several studies were unable to demonstrate any statistically significant correlation between either FXaI or DTI levels and ETP [40-42, 46]. These patterns are reflected in representative TGA curves for FXaIs and DTIs, as depicted in **Figure 3**. FXaIs tend to produce a flat, protracted or "camel-back" curve with prolonged kinetic parameters, a suppressed peak but relatively preserved ETP [36, 86, 97, 98]. DTIs, on the other hand, appear to mainly delay onset of thrombin generation with a sharp, right-shifted TGA curve [86, 95]. These patterns may be due to the impact of FXaIs and DTIs on coagulation initiation and propagation [99, 100]. For comparison, warfarin, which exerts its effects on vitamin K dependent coagulation factors, suppresses all phases of thrombin generation and leads to comparatively greater reductions in ETP as compared to DOACs [37, 57].

A number of studies described a paradoxical increase in both peak thrombin generation and ETP in the presence of DTIs, particularly at lower concentrations (≤ 100 ng/mL; **Figures 2 and 3**) [47, 71, 101-103]. This was attributed to several artifactual and physiological factors. Firstly, TGA typically involves use of a thrombin-based calibrator (α2-macroglobulin-thrombin with CAT) [22]. As the calibration is performed using a duplicate patient plasma sample, several groups reported that the presence of DTI within the calibrator well can inhibit the calibrator and lead to falsely elevated quantitative TGA measurements [47, 71, 102, 103]. Second, DTIs have been shown to impact the algorithm that subtracts α2-macroglobulin-thrombin activity from the total measured amidolytic activity, leading to a mathematical artifact and spuriously elevated peak thrombin and ETP values [102, 104]. Others proposed that these paradoxical

changes may represent DTI-induced hypercoagulability, mediated via suppression of the thrombin-thrombomodulin negative feedback system and impaired protein C activation, the clinical significance of which is unclear [82, 105]. These DTI-induced effects on the activated protein C system, however, would only be present among studies performing TGA with added thrombomodulin. It follows that most of the paradoxical changes observed with DTIs are likely artifactual in nature. Studies have attempted to overcome the impact of DTIs on thrombin calibrators using DOAC-Stop® [103], idarucizumab [102], or alternative calibration methodology [45, 106].

Effect of Hemostatic Strategies on DOAC-Altered TGA Parameters

Forty-one studies evaluated the effect of hemostatic therapies among healthy volunteers (30 studies), among patients with AF or VTE (9 studies), or patients in whom the indication for anticoagulation was not reported (2 studies). Twenty-four studies evaluating hemostatic therapies were in vitro studies and 17 were in vivo studies (**Tables 1-4**). Among studies evaluating nonspecific hemostatic therapies, 28 tested PCC [8, 10, 19, 20, 38, 39, 48-50, 52, 105, 107-123], 13 tested aPCC [39, 48-51, 105, 107, 110, 112, 113, 115, 116, 124], and 13 tested recombinant factor VIIa (rFVIIa) [39, 48-50, 105, 107, 110-113, 115, 116, 125]. TGA parameters were inconsistently reported across studies evaluating hemostatic therapies. Several studies selectively reported a single TGA parameter (usually ETP), whereas others selected parameters of interest and did not report the remaining parameters (**Tables 1-4**). TTP was the least frequently reported parameter.

PCC

Studies evaluating PCC are outlined in **Table 1**. Twenty-five of 28 studies evaluating PCC used 4-factor PCC (4FPCC, including Beriplex®/Kcentra®, Kanokad®, Kaskadil®, Octaplex®, Prothromplex® and Cofact®) and 3 studies used 3-factor PCC (3FPCC, including Bebulin® and Prothrombinex®) [20, 50, 122]. Two studies did not specify the type of PCC used [10, 117]. Doses of PCC tested ranged from 10 IU/kg to 50 IU/kg for in vivo studies and ranged from 0-80 IU/kg for in vitro studies. TGA parameters were inconsistently corrected across both in vitro and in vivo studies, and none of the included studies convincingly demonstrated a normalization of all TGA parameters. The most consistently corrected/normalized parameter was the ETP. However, 22 of the 24 studies demonstrating normalization of ETP also showed overcorrection of the ETP, either immediately after infusion of PCC or during the 12-24 hours following PCC administration. Some studies reported a concentration-dependent correction of ETP [20, 105, 109, 110, 119-121]. A number of studies reported serial ETP measurements over the 24 hours following PCC infusion and demonstrated an immediate increase in ETP, followed by a more gradual correction to baseline values and subsequent overcorrection of ETP observed 12-48 hours following PCC infusion [19, 117, 119, 120, 122, 123]. PCC doses < 50 IU/kg appeared to be less likely to normalize ETP immediately following PCC administration [111, 120, 121]. A statistically significant impact of PCC on ETP was not always reported at doses of 25 IU/kg, although doses even as low as 25 IU/kg eventually resulted in overcorrection of ETP at 24 hours post-PCC administration [119]. The impact of PCCs on TGA parameters appeared to be independent of DOAC

concentrations, with a few pharmacokinetic studies demonstrating that PCC had no impact on FXaI levels as compared to placebo [19, 122]. PCCs appeared to be better able to correct quantitative parameters as opposed to kinetic parameters (**Table 1**). Comparatively few studies documented a statistically significant effect on kinetic TGA parameters [39, 50, 105, 108, 113, 118] and several studies specifically reported on the inability of PCC to exert an effect on kinetic parameters [19, 39, 49, 110, 111, 122]. The changes induced by PCC to DOAC-anticoagulated plasma can be contrasted with those observed following the addition of PCC to warfarin-anticoagulated plasma (**Figure 4**) [126]. PCC is used as a VKA reversal agent and its mechanism has been well characterized in this setting. PCC replenishes gamma-carboxylated vitamin K-dependent coagulation factors that are depleted by warfarin therapy [127]. Tanaka and colleagues were able to demonstrate in vitro that the addition of 4FPCC to warfarin-anticoagulated plasma corrects both kinetic and quantitative parameters, resulting in normalization of the TGA curve [126].

An in vitro study that included various 4FPCC formulations based on the heparin content demonstrated differences in TGA responses according to heparin content in rivaroxaban-spiked plasma [109]. PCC formulations containing greater quantities of heparin (Octaplex®, Prothromplex®) demonstrated prolongation of TGA kinetic parameters and suppression of quantitative parameters. A second study evaluating compositional differences across 4FPCC formulations reported similar findings with respect to heparin content [128]. Moreover, several included studies evaluating PCC reported statistically significant prolongations in either the LT or TTP following

administration of heparin-containing PCCs (**Table 1**) [19, 38, 49]. Complete suppression of thrombin generation was observed after spiking rivaroxaban-containing plasma samples with 25-50 IU/kg of Octaplex®, whereas Beriplex® and Kanokad® had prohemostatic effects [38]. The in vivo effects of co-infused heparin would presumably be short due to heparin's short half-life, although might be important in the context of PCC administration to manage an acute DOAC-related bleeding event [109].

Activated PCC (aPCC)

Studies evaluating aPCC are outlined in **Table 2**. aPCC was evaluated at doses ranging from 5-150 IU/kg. Most studies evaluating aPCC recorded statistically significant changes to all measured TGA parameters, including kinetic parameters. aPCC affected all TGA parameters more consistently as compared to PCC. Normalization of ETP was noted in all studies and several studies reported normalization of kinetic parameters [48, 110, 112, 113, 115, 116, 124]. At similar dosing, aPCC tended to produce greater increases in ETP as compared to PCC [39, 48, 105, 107, 116]. As was observed with PCC, most studies reporting normalization of ETP also recorded an overcorrection of ETP.

Recombinant Factor VIIa (rVIIa)

rFVIIa was tested in vitro at doses ranging from 72-270 ug/kg (**Table 3)**. We did not identify any in vivo studies evaluating the effects of rFVIIa on TGA parameters in humans. rFVIIa appeared mainly to affect kinetic parameters.

Specific Reversal Agents (Idarucizumab, Andexanet Alfa and Ciraparantag)

We identified fewer studies that included specific reversal agents and reported TGA measurements as an outcome. TGA parameters were less consistently reported, with most studies focusing on ETP (**Table 4**). Seven studies assessed idarucizumab [10, 102, 107, 129-132] and 6 studies assessed andexanet alfa [8, 15, 85, 133-135]. Specific reversal agents consistently reversed all TGA parameters (**Table 4**), and normalization was noted in all cases when reported. ETP overcorrection was observed in several studies evaluating andexanet alfa. In contrast to PCC, andexanet alfa resulted in an immediate normalization/overcorrection of ETP post-bolus/infusion followed by a rebound in FXaI levels and a drop in ETP once the infusion was completed [15, 135]. One study of volunteers treated with rivaroxaban/apixaban found the ETP to remain elevated as compared to placebo following completion of the andexanet alfa infusion, and this was despite a rebound in DOAC levels [135]. This rebound in rivaroxaban/apixaban levels might relate to redistribution from the extravascular spaces to the vasculature due to the high affinity of andexanet alfa towards FXaIs [136]. Andexanet alfa can bind to tissue factor pathway inhibitor (TFPI), and some investigators have proposed this might contribute to a prothrombotic effect [133]. This might partly explain the persistent elevation in ETP despite a rebound in unbound anticoagulant levels that exceed those seen with placebo infusion.

Ciraparantag is a small cationic molecule that binds to both heparins and DOACs through non-covalent charge interactions. Ciraparantag is under clinical development as a potential DOAC reversal agent. Due to its cationic charge, it binds to anionic

substances used for plasma-based coagulation testing (e.g., sodium citrate) [137]. Citrated plasma testing cannot be carried out among samples containing ciraparantag. As a result, our search did not identify any studies measuring the impact of ciraparantag on TGA parameters.

DOAC-Induced Alterations in TGA Parameters and Association with Clinical Outcomes

This review identified very few clinical studies of DOAC-treated patients associating TGA parameters to clinical outcomes. One study sought to correlate CAT parameters with bleeding events among patients on rivaroxaban/vitamin K antagonists (VKAs) for VTE [35]. Subjects on rivaroxaban with study-defined minor bleeding events had longer LT ($p < 0.001$), longer TTP ($p < 0.001$), lower peak thrombin generation ($p < 0.001$) and lower ETP ($p < 0.001$) as compared to subjects without minor bleeding. ROC analysis revealed that both LT and TTP demonstrated good discrimination with respect to predicting minor bleeding events (AUC = 0.85 [95% CI 0.79-0.92; $p < 0.0001$] for both the LT and TTP), as compared to AUC values of 0.77 [95% CI 0.67-0.87; $p < 0.0001$] and 0.73 [95% CI 0.61-0.84] for peak and ETP, respectively. LT remained a statistically significant predictor of minor bleeding events following multivariable regression adjustment, with an OR of 1.006 [95% CI 1.002-1.010] per 1 second of lag time. Another study, the RAPS trial, was a randomized, open-label, phase 2/3 non-inferiority trial among patients with thrombotic APS [57]. Patients were randomized to either ongoing VKA therapy or rivaroxaban and TGA parameters were measured at 42 days post-enrolment. The primary outcome was the ETP. At day 42, patients randomized to

rivaroxaban had longer LT and TTP values, and higher peak and ETP values as compared to patients on VKA therapy. No thromboembolic events were observed in the RAPS trial. However, other randomized controlled trials among patients with APS have observed increased thromboembolic risk among patients randomized to DOAC therapy as compared to VKAs, suggesting that ETP may potentially correlate with thromboembolic risk among anticoagulated patients [138, 139]. Similar findings with respect to ETP were noted among in vitro studies evaluating DOAC anticoagulation for mechanical cardiac valves, for which DOACs provide inadequate antithrombotic effect compared to VKAs [65, 66, 140].

There were no thromboembolic events reported among studies measuring TGAs before and after PCC treatment in DOAC-treated healthy volunteers [19, 20, 117, 120-122]. Two studies evaluated hemostasis among healthy volunteers administered DOACs using a standardized skin punch biopsy [117, 120]. The study by Levy and colleagues investigated the ability of 4FPCC to correct ETP and bleed duration (BD)/bleed volume (BV) among healthy volunteers administered oral rivaroxaban 20 mg twice daily for 4 days [117]. This study showed that 4FPCC significantly increased the ETP to values that surpassed pre-study baseline levels within 30 minutes of administration, but there was no measurable effect on BD or BV [117]. Zahir and colleagues similarly investigated the effects of 4FPCC on ETP and BD/BV among volunteers given a single 60 mg dose of edoxaban [120]. This study reported a statistically significant impact of PCC on ETP and did demonstrate a statistically significant normalization of BD and BV following administration of PCC. The discrepant findings between these two studies with

respect to bleeding might relate to differing DOAC regimens. The rivaroxaban dosing scheme used in the first study would have resulted in supratherapeutic DOAC levels as compared to the single dose of edoxaban used for the second study. Neither study measured DOAC levels. PCC effects on TGA parameters might depend on the DOAC level at the time of administration. Lu et al. were able to demonstrate in vitro that 4FPCC could only normalize ETP and peak thrombin generation when inhibitor concentrations were below 75 ng/mL and 37.5 ng/mL, respectively [8].

Studies on the use of PCC or andexanet alfa among patients with DOAC-associated bleeding did not formally correlate corrections of TGA parameters to clinical hemostasis [15, 52]. One study reported stratified post-PCC peak/ETP measurements according to effective/ineffective hemostasis and did not observe any differences in either median peak or ETP values [10].

Discussion

Our review highlights recurrent patterns among FXaIs and DTIs with respect to their impact on TGA parameters at different concentrations. Even low DOAC concentrations (< 30 ng/mL) impacted some TGA parameters, suggesting that low DOAC concentrations could exert an anticoagulant effect and potentially contribute to impaired hemostasis among patients with DOAC-associated bleeding or requiring urgent surgery [86-89]. FXaIs and DTIs seem to differentially affect components of the thrombin generation curve. Although some studies did not identify a statistically significant correlation between FXaI levels and ETP [40, 42], FXaI concentrations seem to show

moderate/good correlation with kinetic parameters and peak thrombin generation [40-42, 46]. In contrast, DTIs predominantly affect kinetic parameters, particularly at concentrations that are considered clinically relevant [42]. These findings suggest that both kinetic and quantitative thrombin generation parameters may be relevant with respect to measuring an anticoagulant effect. Indeed, a DTI (r-hirudin) has been shown to result in dramatic prolongations in kinetic parameters without any measurable effect on quantitative parameters [141].

PCCs and aPCCs have differing effects on TGA parameters when added to DOAC-treated plasma. PCCs most consistently corrected the ETP. aPCC, on the other hand, appeared to consistently improve all TGA parameters, both kinetic and quantitative. Irrespective of which TGA parameters are considered, none of the nonspecific hemostatic therapies consistently normalized all TGA parameters. This contrasts with the effects of specific reversal agents, which normalized parameters [15, 135]. Although PCC normalized the ETP in most cases, this normalization did not occur immediately following administration, but rather, occurred gradually over the 6-12 hours following administration, likely partly due to DOAC clearance, and almost universally resulted in eventual overcorrection of the ETP (**Table 1**) [19, 119, 120]. As ETP measurements have been associated with thromboembolic events, an overcorrection of the anticoagulant effects of DOACs could potentially increase the risk of thrombotic complications for these patients [142-144]. Importantly, we failed to identify any studies that demonstrated a measurable effect of either PCC or aPCC on DOAC levels. Although aPCC seems to provide the most consistent effect on all TGA parameters,

little data exists surrounding the use of aPCC in vivo [11, 145] and our review did not identify any in vivo studies measuring TGA parameters following the administration of either aPCC or rFVIIa. Taken together, these findings suggest that PCC and aPCC should not be considered by clinicians as “reversal agents” or “antidotes” and appear unlikely to provide true “anticoagulation reversal”. They may confer a beneficial hemostatic effect when used as part of a multimodal strategy to manage DOAC-associated bleeding. Given limited availability and variable geographic access to specific reversal agents, non-specific therapies will continue to be used by many clinicians when treating FXaI-associated bleeding. More work is needed to characterize the hemostatic effects of non-specific therapies and determine whether they do provide a hemostatic benefit, particularly in situations where DOAC levels are significantly elevated (e.g. ≥ 75 ng/mL).

Our review suggests that ETP is the most frequently studied parameter with respect to reporting the effect of hemostatic therapies on TGA parameters [15, 117, 120, 135]. ETP seems to provide a single comprehensive measure of thrombin generation. In addition, several early studies have demonstrated that increased ETP is associated higher risk of thromboembolic events [142-144]. Further, anecdotal evidence among patients with hemophilia and inhibitors treated with bypassing therapies suggests ETP might improve concurrently with improvements in bleeding episode frequency [146]. While it is true that ETP measures overall thrombin generation, ETP alone fails to provide all information supplied by the thrombogram. DOACs affect all TGA parameters and predominantly kinetic parameters, and some anticoagulants seem to exert their

effect entirely by delaying the onset of thrombin generation. These findings raise questions as to whether normalizing ETP is sufficient to improve hemostasis and whether selectively reporting a single TGA parameter is the most suitable approach to applying TGAs to the question of anticoagulation reversal. The thrombogram is fundamentally a composite measure that integrates information on all aspects of thrombin generation. Administration of PCC to a DOAC-treated patient might normalize the ETP, but that same patient's thrombogram could differ dramatically from that of a normal individual. Some data would suggest that hemostatic agents that correct both kinetic and quantitative parameters (e.g., aPCC) lead to improved restoration of clot structure in DOAC-treated plasma [110]. Although it is reasonable to prioritize a TGA parameter of interest a priori as part of a statistical analysis plan, all TGA parameters should be reported to ensure consistency across studies and avoid selective reporting of results.

We identified relatively little data that analyzed TGA parameters with respect to clinical outcomes. Lag time was found to be an independent predictor of minor bleeding events [35]. Some studies provided indirect evidence that insufficient ETP suppression might correspond to lower anticoagulation efficacy in some patient populations [57, 66]. Studies of PCC among healthy volunteers given a DOAC and subjected to a bleeding challenge are conflicting, with one study demonstrating an improvement in blood loss with concurrent correction in ETP whereas another study did not identify a correction in bleeding parameters despite a significant improvement in ETP [117, 120]. TGAs represent a promising quantitative and reproducible addition to assessments of clinical

hemostatic efficacy, but studies are needed to clarify its role. Further attempts should be undertaken to correlate correction of TGA parameters to clinical outcomes in patients with bleeding, recognizing that normalization of thrombin generation is only one of several factors likely required to achieve a desirable hemostatic outcome. Experiments on human volunteers that make use of a standardized method for inducing bleeding (e.g., punch biopsy) and measuring blood loss may be better suited to isolate a treatment effect from hemostatic therapies as compared to criteria for clinical hemostatic efficacy among bleeding patients due to the substantial risk of unmeasured confounding and clinical heterogeneity in the latter setting. Future studies of attempted anticoagulation reversal should aim to measure and report all TGA parameters in concert with both DOAC levels and clinical outcomes.

Limitations to the implementation of TGA in routine clinical practice include their sensitivity to preanalytical variables [147], lack of standardization and heterogeneity with respect to analytical conditions secondary to differences in reagents and methodology leading to inter-center variability [148], slow turnaround time (on the order of 60-120 minutes) and limited availability. Some progress has been made with respect to surmounting barriers to the clinical implementation of TGAs, with the publication of recommendations on standardized preanalytical and analytical conditions for TGA measurements in hemophilia [149]. As reported by Dargaud and colleagues, TGA assay precision has markedly improved over the past decade with some studies reporting intra- and inter-assay variability of < 10% [148, 149]. The use of TGAs to date has largely been limited to the research laboratory, although efforts are underway to

implement TGAs with greater clinical applicability, throughput, reproducibility and standardization [150]. Our review indicates some degree of consistency with respect to TGA reaction conditions when applied towards the study of DOACs, with a substantial proportion of included studies (40 studies) using a commercially available standard trigger reagent produced by one of the TGA assay manufacturers. Studies evaluating the impact of preanalytical variables (contact activation and use of CTI, venipuncture, sample transportation and storage) have focused on patients with hemophilia, usually with TGAs using low TF concentrations (< 1 pM) [151-153]. These preanalytical factors may have less of an impact when higher TF concentrations (≥ 5 pM) are used [154], as is often the case when evaluating anticoagulant therapy [23]. Limitations to the data presented within this review include the heterogeneity of characteristics across included studies. Studies employed a wide range of different study designs, TGA assays, reagents and reaction conditions, DOAC concentrations, PCC formulations/doses and included diverse patient populations. It is for this reason that we opted for a narrative synthesis of the identified data. Nevertheless, the information included herein should prove useful to translational and clinical scientists with respect to the design of future preclinical and clinical studies.

Thrombin generation represents a promising new pharmacodynamic measure of anticoagulant effect and preliminary evidence indicates some association with both bleeding and thromboembolic events. Further study is needed to determine whether correction of thrombin generation parameters predicts improvements in clinical

hemostasis, and which parameters are of greatest importance with respect to both clinical hemostasis and thrombotic complications.

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Conflicts of Interest

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Figure 1. The Thrombogram

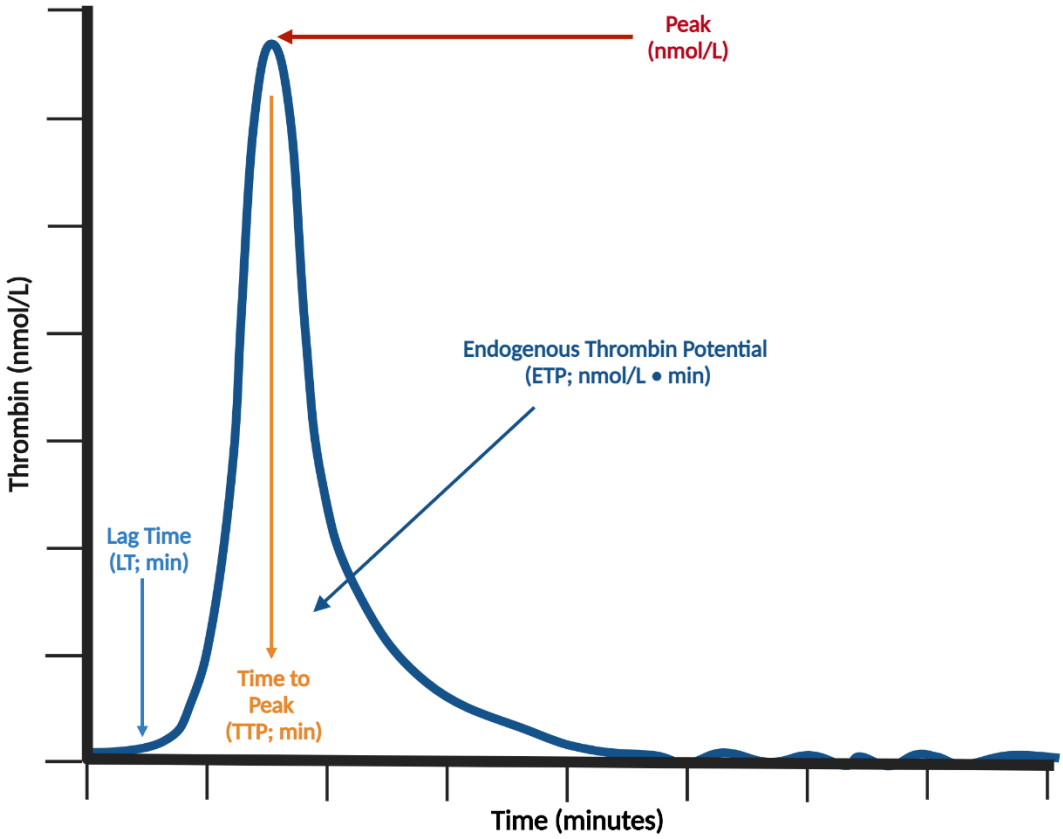


Figure 1 The Thrombogram. Components of a typical thrombin generation curve generated using the Calibrated Automated Thrombography (CAT) assay. LT = lag time; TTP = time to peak; ETP = endogenous thrombin potential.

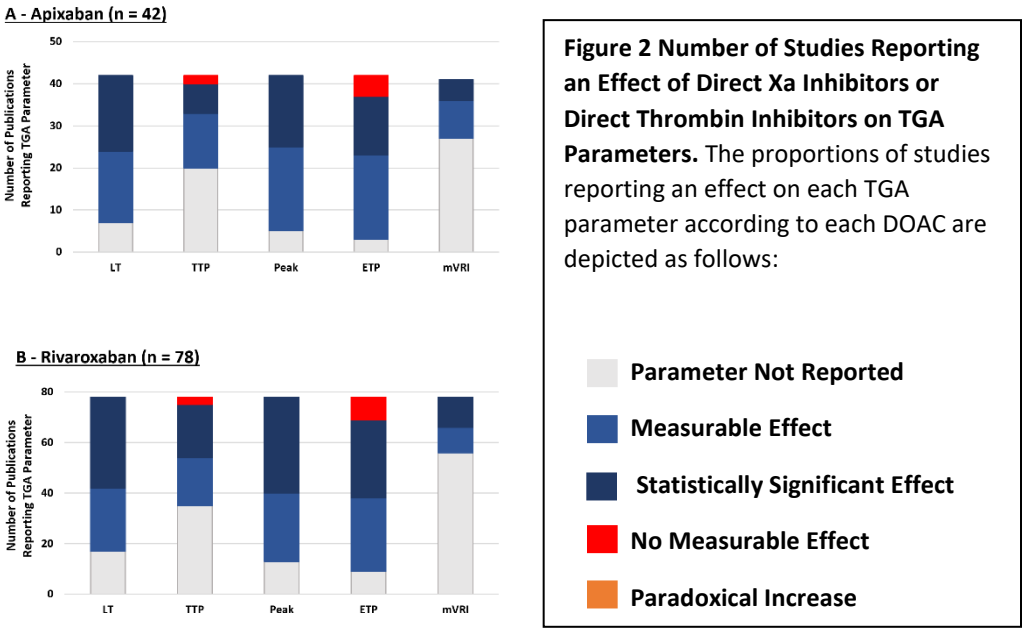


Figure 2 Number of Studies Reporting an Effect of Direct Xa Inhibitors or Direct Thrombin Inhibitors on TGA Parameters. The proportions of studies reporting an effect on each TGA parameter according to each DOAC are depicted as follows:

- **Parameter Not Reported**
- **Measurable Effect**
- **Statistically Significant Effect**
- **No Measurable Effect**
- **Paradoxical Increase**

Figure 3. Representative Changes to the Thrombin Generation Curve with Increasing DOAC Concentrations

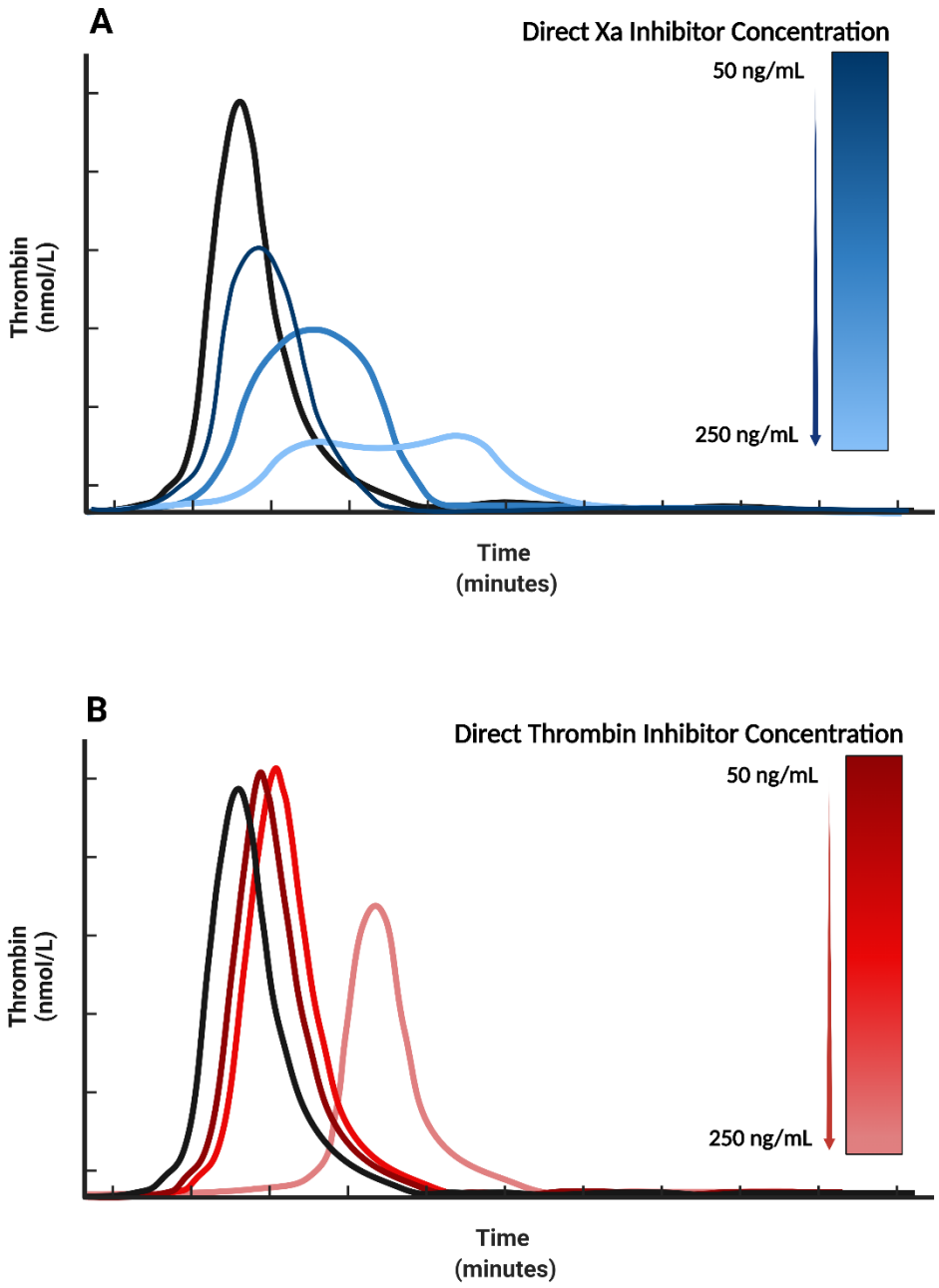


Figure 3 Representative Changes to the Thrombin Generation Curve with Increasing DOAC Concentrations. Approximated representative TGA patterns generated using information from references #35, 36, 38, 88, 89, 95, 96. The black curves represent reference thrombograms in the absence of DOACs. Blue and red lines represent typical changes to the thrombogram in the presence of increasing concentrations of direct Xa inhibitors (A) and direct thrombin inhibitors (B), respectively, as seen using Calibrated Automated Thrombography and 5 pM tissue factor concentrations.

Figure 4. Representative Effects of 4FPCC on the Thrombin Generation Curve in Anticoagulated Plasma

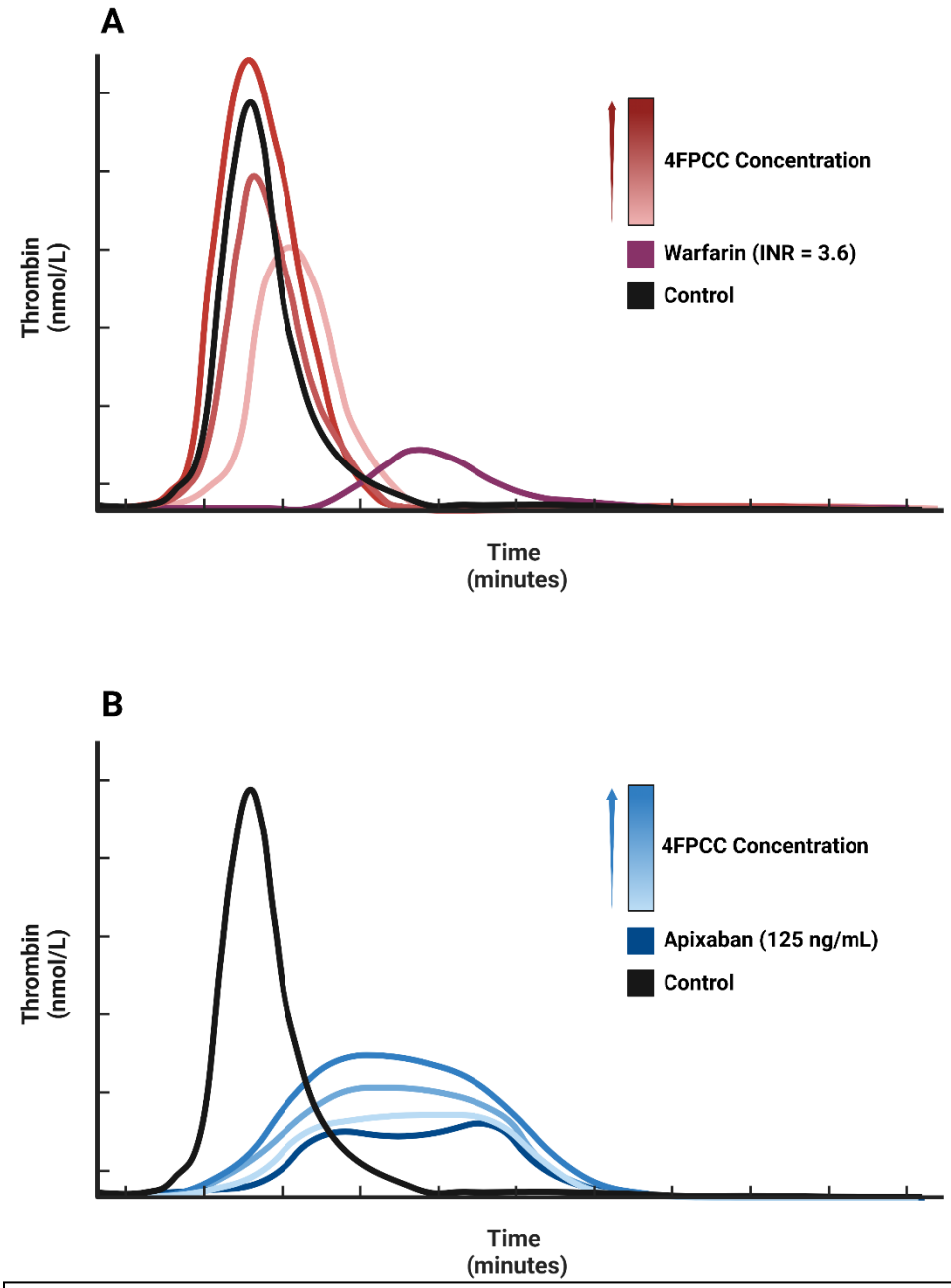


Figure 4 Representative Effects of 4FPCC on the Thrombin Generation Curve in Anticoagulated Plasma. Approximated representative TGA patterns generated using information from references #8, 114, 126. The black curves represent reference thrombograms in the absence of anticoagulant. Red (A) and blue (B) 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.

Table 1. Effect of PCC on TGA Parameters

Abstract ID Author Year [Reference]	Study Population	PCC Formulation	DOACs	PCC Concentrations/ Dosing Tested [Corresponding In Vivo Dosing]	Kinetic TGA Parameter Impact		Quantitative TGA Parameter Impact		Kinetic TGA Parameter Normalization		Quantitative TGA Parameter Normalization		
					LT	TTP	Peak	ETP	LT	TTP	Peak	ETP	ETP Overcorrection
IN VITRO													
#431 Lu et al. 2020 **[132]**	Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Rivaroxaban	0-1.0 IU/mL [1.0 IU/mL = 50 IU/kg]									Yes
			Apixaban										Yes
#1039 Arellano-Rodrigo 2019 **[107]**	Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Dabigatran	0.98 IU/mL [70 IU/kg]									No
#1305 Helin 2018 **[108]**	Healthy Volunteers	**4FPCC** (Cofact)	Rivaroxaban	2 IU/mL [50 IU/kg]									Yes
#2319 Brinkman 2016 **[109]**	Healthy Volunteers	**4FPCC** (Cofact)	Rivaroxaban	0-2.0 IU/mL [0-80 IU/kg]									No
		4FPCC (Beriplex/KCentra)											No
		4FPCC (Octaplex)											No
		4FPCC (Prothromplex)											No
#2573 Martin 2015 **[110]**	Healthy Volunteers	**4FPCC** [Kaskadil]	Apixaban	0.5-1.0 IU/mL [25-50 IU/kg]									Yes
#2655 Hoffman 2015 **[111]**	Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Dabigatran	0-2.0 IU/mL [1.0 IU/mL = 50 IU/kg]									Yes
#2707 Escolar 2015 ***[112]***	Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Rivaroxaban	Not Reported [50 IU/kg]									Not Reported
#2804 Arellano-Rodrigo 2015 **[113]**	Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Rivaroxaban	Not Reported [50 IU/kg]									No
			Dabigatran										Yes
#2912 Perzborn 2014 **[82]**	Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Rivaroxaban	0.4-0.7 IU/mL (25-50 IU/kg)									Yes
#3038 Dinkelaar 2014 **[114]**	Healthy Volunteers	**4FPCC** (Cofact)	Apixaban	0-4.0 IU/mL [Not Reported]									Yes
			Dabigatran										Yes
#3415 Escolar 2013 **[115]**	Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Apixaban	Not Reported [50 IU/kg]									No
#3589 Marlu 2012 **[116]**	Healthy Volunteers	**4FPCC** (Kanokad)	Rivaroxaban	0.25-1.0 IU/mL [0.5 IU/mL = 25 IU/kg]									Yes
			Dabigatran										Yes
#1543 Schultz 2017 **[38]**	AF VTE	**4FPCC** (Cofact)	Rivaroxaban	Not Reported [40 IU/kg]									Yes
#2011 Schenk 2016 **[48]**	AF VTE Healthy Volunteers	**4FPCC** (Beriplex)	Rivaroxaban	0.3-1.0 IU/mL [1000-5000 IU]									Yes
#511 Giffard-Quillon 2020 **[37]**	VTE Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Rivaroxaban	0-1.25 IU/mL [0-50 IU/kg]									Yes
		4FPCC (Octaplex)											No
		4FPCC (Kanokad)											Yes

#1544 Schultz 2017 **[47]**	VTE Healthy Volunteers	**4FPCC** (Cofact)	Apixaban	"80%, 100% and 125% of suggested doses for clinical use" [32-50 IU/kg]									Yes
IN VIVO													
#1229 Levy 2018 **[117]**	Healthy Volunteers	Not reported	Rivaroxaban	50 IU/kg									Yes
#1526 Song 2017 **[20]**	Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Apixaban	50 IU/kg									Yes
		4FPCC (Cofact)											Yes
#2076 Nagalla 2016 **[118]**	Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Apixaban	25 IU/kg									Not Reported
#2349 Barco 2016 **[119]**	Healthy Volunteers	**4FPCC** (Cofact)	Rivaroxaban	25-37.5 IU/kg									Yes
#2403 Zahir 2015 **[120]**	Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Edoxaban	10-50 IU/kg									Yes
#2764 Cheung 2015 **[121]**	Healthy Volunteers	**4FPCC** (Cofact)	Apixaban	25-37.5 IU/kg									Yes
#2775 Brown 2015 **[21]**	Healthy Volunteers	**3FPCC** (Bebulin)	Edoxaban	25-50 IU/kg									Yes
#2969 Levi 2014 **[122]**	Healthy Volunteers	**4FPCC** (Beriplex/KCentra)	Rivaroxaban	50 IU/kg									Yes
		3FPCC (Profilnine)		50 IU/kg									Yes
#3833 Eerenberg 2011 **[123]**	Healthy Volunteers	**4FPCC** (Cofact)	Rivaroxaban	50 IU/kg									Yes
			Dabigatran										Not Reported
#3027 Herrmann 2014 **[49]**	AF VTE	**3FPCC** (Prothrombinex)	Rivaroxaban	0.5 IU/mL [Not Reported]									No
			Dabigatran										Yes
#599 Bavalia 2020 **[13]**	AF VTE Other	Not Reported	Rivaroxaban Apixaban Edoxaban Dabigatran	Median dose 3500 IU (Median 46 IU/kg; IQR = 46-50)									Yes
#1149 Schenk 2018 **[51]**	Not Reported	**4FPCC** (Beriplex/KCentra)	Rivaroxaban	Median dose = 2000 IU (Range 1500-2400 IU)									Yes

Table 1 Effect of PCC on TGA Parameters. Overview of the effect of PCC on DOAC-altered TGA parameters. An effect was deemed to be present if it was observed at any of the tested PCC concentrations. Results were considered to be statistically significant when authors reported them as such in the form of a statistical analysis (e.g. $p < 0.05$). We considered there to be evidence of an effect (without statistical significance) when reported as such by authors or when there was clear evidence of an effect based on review of numerical data and graphical trends. ETP overcorrection was deemed to be present when ETP values exceeded the defined reference range, either immediately after PCC infusion or in the hours following infusion.

- **No effect or normalization**
- **Effect noted, statistical significance not reported**
- **Statistically significant effect**
- **Parameter normalization**
- **Inhibition of thrombin generation (prolonged kinetic parameters, reduced quantitative parameters)**
- **Not reported**

Table 2. Effect of aPCCs on TGA Parameters

Abstract ID Author Year [Reference]	Study Population	DOACs	aPCC Concentrations/ Dosing Tested [Corresponding In Vivo Dosing]	Kinetic TGA Parameter Impact		Quantitative TGA Parameter Impact		Kinetic TGA Parameter Normalization		Quantitative TGA Parameter Normalization		
				LT	TTP	Peak	ETP	LT	TTP	Peak	ETP	ETP Overcorrection
IN VITRO												
#49 Schultz 2021 **[124]**	Healthy Volunteers	Rivaroxaban	0.08-1.60 IU/mL [5-100 IU/kg]									No
		Apixaban										Yes
#1039 Arellano-Rodrigo 2019 **[107]**	Healthy Volunteers	Dabigatran	0.35-1.05 IU/mL [25-75 IU/kg]									Yes
#2573 Martin 2015 **[110]**	Healthy Volunteers	Apixaban	1-2 IU/mL [80-160 IU/kg]									Yes
#2707 Escolar 2015 **[112]**	Healthy Volunteers	Rivaroxaban	Not Reported [75 IU/kg]									Not Reported
#2804 Arellano-Rodrigo 2015 **[113]**	Healthy Volunteers	Rivaroxaban	Not Reported [75 IU/kg]									Yes
		Dabigatran										Yes
#2912 Perzborn 2014 **[82]**	Healthy Volunteers	Rivaroxaban	0.2-1.0 IU/mL [50-100 IU/kg]									Yes
#3415 Escolar 2013 **[115]**	Healthy Volunteers	Apixaban	Not Reported [75 IU/kg]									Not Reported
#3589 Marlu 2012 **[116]**	Healthy Volunteers	Rivaroxaban	0.25-2 IU/mL [1 IU/mL = 80 IU/kg]									Yes
		Dabigatran										Yes
#1543 Schultz 2017 **[38]**	AF VTE	Rivaroxaban	"80%, 100% and 125% of suggested doses for clinical use" [100% = 50 IU/kg]									Yes
#2011 Schenk 2016 **[48]**	AF VTE Healthy Volunteers	Rivaroxaban	1.5-2.25 IU/mL [100-150 IU/kg]									Yes
#3027 Herrmann 2014 **[49]**	AF VTE	Rivaroxaban	0.5 IU/mL [Not Reported]									No
		Dabigatran										No
#3332 Khoo 2013 **[50]**	AF VTE	Dabigatran	1-2 IU/mL [50-100 IU/kg]									Yes
#1544 Schultz 2017 **[47]**	VTE Healthy Volunteers	Apixaban	"80%, 100% and 125% of suggested doses for clinical use" [25-50 IU/kg]									Yes

Table 2 Effect of aPCC on TGA Parameters. Overview of the effect of aPCC on DOAC-altered TGA parameters. An effect was deemed to be present if it was observed at any of the tested aPCC concentrations. Results were considered to be statistically significant when authors reported them as such in the form of a statistical analysis (e.g. $p < 0.05$). We considered there to be evidence of an effect (without statistical significance) when reported as such by authors or when there was clear evidence of an effect based on review of numerical data and graphical trends. ETP overcorrection was deemed to be present when ETP values exceeded the defined reference range, either immediately after aPCC infusion or in the hours following infusion.

- **No effect or normalization**
- **Effect noted, statistical significance not reported**
- **Statistically significant effect**
- **Parameter normalization**
- **Inhibition of thrombin generation (prolonged kinetic parameters/reduced quantitative parameters)**
- **Not reported**

Table 3. Effect of rFVIIa on TGA Parameters

Abstract ID Author Year	Study Population	DOACs	rFVIIa Concentrations/ Dosing Tested [Corresponding In Vivo Dosing]	Kinetic TGA Parameter Impact		Quantitative TGA Parameter Impact		Kinetic TGA Parameter Normalization		Quantitative TGA Parameter Normalization		
				LT	TTP	Peak	ETP	LT	TTP	Peak	ETP	ETP Overcorrection
IN VITRO												
#1039 Arellano-Rodrigo 2019 **[107]**	Healthy Volunteers	Dabigatran	1.68 ug/mL [120 ug/kg]									No
#2573 Martin 2015 **[110]**	Healthy Volunteers	Apixaban	1.8-3.0 ug/mL [90-150 ug/kg]									No
#2655 Hoffman 2015 **[111]**	Healthy Volunteers	Dabigatran	100 nmol/L [Not Reported]									No
#2707 Escolar 2015 **[112]**	Healthy Volunteers	Rivaroxaban	Not Reported [270 ug/kg]									Not reported
#2804 Arellano-Rodrigo 2015 **[113]**	Healthy Volunteers	Rivaroxaban	Not Reported [270 ug/kg]									No
		Dabigatran										No
#2912 Perzborn 2014 **[82]**	Healthy Volunteers	Rivaroxaban	5-50 ug/mL [5 ug/mL = 270 ug/kg]									No
#3169 Wong 2013 **[125]**	Healthy Volunteers	Apixaban	5-50 ug/mL [Not Reported]									No
#3415 Escolar 2013 **[115]**	Healthy Volunteers	Apixaban	Not Reported [270 ug/kg]									Not Reported
#3589 Marlu 2012 **[116]**	Healthy Volunteers	Rivaroxaban	0.5-3.0 ug/mL [3 ug/mL = 120 ug/kg]									No
		Dabigatran										No
#1543 Schultz 2017 **[38]**	AF VTE	Rivaroxaban	"80%, 100% and 125% of suggested doses for clinical use" [100% = 90 ug/kg]									No
#2011 Schenk 2016 **[48]**	AF VTE Healthy Volunteers	Rivaroxaban	1.5-4.05 ug/mL [100-270 ug/kg]									No
#3027 Khoo 2013 **[50]**	Not Reported	Rivaroxaban	1 ug/mL [Not Reported]									No
		Dabigatran										No
#1544 Schultz 2017 **[47]**	VTE Healthy Volunteers	Apixaban	"80%, 100% and 125% of suggested doses for clinical use" [72-112.5 ug/kg]									No

Table 3 Effect of rFVIIa on TGA Parameters. Overview of the effect of rFVIIa on DOAC-altered TGA parameters. An effect was deemed to be present if it was observed at any of the tested rFVIIa concentrations. Results were considered to be statistically significant when authors reported them as such in the form of a statistical analysis (e.g. $p < 0.05$). We considered there to be evidence of an effect (without statistical significance) when reported as such by authors or when there was clear evidence of an effect based on review of numerical data and graphical trends. ETP overcorrection was deemed to be present when ETP values exceeded the defined reference range, either immediately after rFVIIa infusion or in the hours following infusion.

- **No effect or normalization**
- **Effect noted, statistical significance not reported**
- **Statistically significant effect**
- **Parameter normalization**
- **Inhibition of thrombin generation (prolonged kinetic parameters, reduced quantitative parameters)**
- **Not reported**

Table 4. Effect of Idarucizumab and Andexanet Alfa on TGA Parameters

Abstract ID Author Year	Study Population	Reversal Agent	DOACs	Reversal Agent Concentrations/ Dosing Tested [Corresponding In Vivo Dosing]	Kinetic TGA Parameter Impact		Quantitative TGA Parameter Impact		Kinetic TGA Parameter Normalization		Quantitative TGA Parameter Normalization		
					LT	TTP	Peak	ETP	LT	TTP	Peak	ETP	ETP Overcorrection
IN VITRO													
#205 Mijovski 2021 **[127]**	AF	Idarucizumab	Dabigatran	125 ug/mL [Not Reported]									Yes
#297 Takeshita 2020 **[138]**	Healthy Volunteers	Idarucizumab	Dabigatran	1000 ug/mL [Not Reported]									Not Reported
#1039 Arellano-Rodrigo 2019 **[107]**	Healthy Volunteers	Idarucizumab	Dabigatran	0.3-3.0 mg/mL [1.6-15 g]									No
#1412 Bloemen 2018 **[101]**	AF Healthy Volunteers	Idarucizumab	Dabigatran	6 umol/L [Not reported]									No
#431 Lu 2020 **[9]**	Healthy Volunteers	Andexanet Alfa	Rivaroxaban Apixaban	0-4.0 uM [approximating low and high dosing schedules]									Yes
#717 Siddiqui 2019 **[131]**	Healthy Volunteers	Andexanet Alfa	Rivaroxaban Apixaban Edoxaban Betrixaban	100 ug/mL [Not Reported]									Yes
IN VIVO													
#599 Bavalia 2020 * **[13]**	AF VTE Other	Idarucizumab	Dabigatran	Median 5.0 g (IQR 5.0-5.0]									Not Reported
#432 Lu 2020 † **[#132]**	Healthy Volunteers	Andexanet Alfa	Rivaroxaban Edoxaban	210-800 mg bolus 4-8 mg/hour infusion									Not Reported
#1532 Siegal 2017 † **[84]**	Healthy Volunteers	Andexanet Alfa	Apixaban	90-420 mg bolus + various infusion regimens									Not Reported
#2476 Siegal 2015 **[133]**	Healthy Volunteers	Andexanet Alfa	Rivaroxaban	400-800 mg IV bolus + 4-8 mg/min infusion									Yes
			Apixaban										Yes
#994 Connolly 2019 **[12]**	AF VTE Other	Andexanet Alfa	Rivaroxaban Apixaban Edoxaban	400 mg bolus + 480 mg infusion 800 mg bolus + 960 mg infusion									No

Table 4 Effect of Idarucizumab and Andexanet Alfa on TGA Parameters. Overview of the effect of specific reversal agents on DOAC-altered TGA parameters. An effect was deemed to be present if it was observed at any of the tested reversal agent concentrations. Results were considered to be statistically significant when authors reported them as such in the form of a statistical analysis (e.g. $p < 0.05$). We considered there to be evidence of an effect (without statistical significance) when reported as such by authors or when there was clear evidence of an effect based on review of numerical data and graphical trends. ETP overcorrection was deemed to be present when ETP values exceeded the defined reference range, either immediately after idarucizumab/andexanet alfa infusion or in the hours following infusion.

No effect

Effect noted, statistical significance not reported

Statistically significant effect

Parameter normalization

Inhibition of thrombin generation (prolonged kinetic parameters, reduced quantitative parameters)

Not reported

* Reported TGA parameters for cases involving a direct Xa inhibitor – See Table 1
† Reported TGA measurement as relative fluorescent units (RFU).

Supplementary Appendix A - Search Strategy and Eligibility Criteria

Embase: 2898
Medline: 1870
Cochrane: 330
WOS: 2381

Total: 7479

Total after duplicates: 4807

Duplicates: 2672

Embase Classic+Embase <1947 to ~~2021 October 13~~> (September 8, 2021)

1 *blood clotting factor 10a inhibitor/ or *apixaban/ or *betrixaban/ or *edoxaban/ or *rivaroxaban/ 10707
2 *dabigatran/ 4349
3 (apixaban or betrixaban or edoxaban or rivaroxaban or dabigatran).tw. 20704
4 (doac* or noac* or Non-vitamin K antagonist oral anticoagul* or non vitamin k antagonist oral anti coagul*).tw. 11591
5 ((direct oral or novel) adj3 (anticoagul* or anti coagulat*)).tw. 10744
6 or/1-5 31247
7 (thrombin adj2 generation adj5 (assay* or measur* or test*)).tw. 3826
8 *thrombin/ and *assay/ 164
9 Thrombinoscop*.tw. 251
10 (thrombograph* or thrombinograph*).tw. 665
11 (Innovance adj3 ETP).tw. 12
12 *"calibrated automated thrombography"/ or calibrated automated Thromb*.tw. 1496
13 exp *thromboelastography/ 3526
14 thromboelastograph*.tw. 4790
15 Technothrombin.tw. 108
16 exp *fluorometry/ 17411
17 fluorogen*.tw. 9112
18 *chromogenic substrate/ 798
19 chromogen*.tw. 17356
20 tga.tw. 18419
21 Prothrombin Complex Concentrate*.tw. 3539
22 *recombinant blood clotting factor 7a/ 2603
23 (Recombinant factor VIIa or Recombinant factor 7a).tw. 2111
24 *fresh frozen plasma/ 1905
25 Fresh Frozen Plasma.tw. 10848

26 *andexanet alfa/ 247
27 Andexanet.tw. 434
28 *idarucizumab/ 465
29 Idarucizumab.tw. 770
30 Praxbind.tw. 158
31 *activated partial thromboplastin time/ 384
32 (activated PCC or activated partial thromboplastin time).tw. 12206
33 aPCC.tw. 914
34 FEIBA.tw. 1371
35 Factor Eight Inhibitor Bypassing Activity.tw. 85
36 *blood clotting factor 8/ 11144
37 (anti-factor Xa or anti-Xa activity or anti-IIa activity).tw. 2735
38 Endogenous Thrombin Potential.tw. 2017
39 peak thrombin generation.tw. 218
40 lag time.tw. 10255
41 time to peak.tw. 17441
42 *ciraparantag/ or ciraparantag.tw. or aripazine.mp. 102
43 or/7-42 137541
44 6 and 43 3625
45 (exp animal/ or nonhuman/) not exp human/ 7460230
46 case report/ 2767688
47 44 not (45 or 46) 2927
48 ("20210909" or 2021091* or 2021092* or 2021093* or 20211*).dc. 295325
49 47 not 48 2898

Ovid MEDLINE(R) ALL <1946 to ~~October 13, 2021~~> (September 8, 2021)

1 exp Factor Xa Inhibitors/ 8467
2 apixaban.mp. 4496
3 Betrixaban.mp. 204
4 edoxaban.mp. 1852
5 Dabigatran.mp. 6016
6 Rivaroxaban.mp. 6892
7 (doac* or noac* or Non-vitamin K antagonist oral anticoagul* or non vitamin k antagonist oral anti coagul*).tw,kf. 6034
8 ((direct oral or novel) adj3 (anticoagul* or anti coagulat*)).tw. 5988
9 (direct* oral anticoagul* or direct oral anti coagul* or novel oral anticoagul* or novel oral anti coagul*).kw. 1678
10 or/1-9 19974
11 (thrombin adj2 generation adj5 (assay* or measur* or test*)).tw,kf. 1578
12 Thrombinoscop*.tw,kf. 35

13 (thrombograph* or thrombinograph*).tw,kf. 280
14 (Innovance adj3 ETP).tw,kf. 5
15 calibrated automated Thromb*.tw,kf. 483
16 Thrombelastography/ 5671
17 thromboelastograph*.tw,kf. 3125
18 Technothrombin.tw,kf. 17
19 Fluorescent Dyes/ 78790
20 fluorometry/ or spectrometry, fluorescence/ 83974
21 fluorogen*.tw,kf. 7535
22 Chromogenic Compounds/ 2663
23 chromogen*.tw,kf. 11442
24 tga.tw,kf. 15218
25 Prothrombin Complex Concentrate*.tw,kf. 1960
26 Factor VIIa/ 4048
27 Recombinant factor VIIa.tw,kf. 1419
28 Fresh Frozen Plasma.tw,kf. 6278
29 Andexanet.mp. 273
30 Idarucizumab.mp. 542
31 Praxbind.mp. 34
32 activated PCC.tw,kf. 44
33 aPCC.tw,kf. 321
34 FEIBA.tw,kf. 310
35 Factor Eight Inhibitor Bypassing Activity.tw,kf. 52
36 factor viii/ 16864
37 (anti-factor Xa or anti-Xa activity or anti-IIa activity).tw,kf. 1823
38 Endogenous Thrombin Potential.tw,kf. 815
39 peak thrombin.tw,kf. 338
40 lag time.tw,kf. 7135
41 time to peak.tw,kf. 12073
42 (ciraparantag or aripazine).mp. 78
43 or/11-42 235559
44 10 and 43 2254
45 exp animals/ not exp humans/ 4897471
46 case reports.pt. 2216395
47 44 not (45 or 46) 1870

EBM Reviews - Cochrane Central Register of Controlled Trials <~~September 2021~~>
August 2021

1 Factor Xa Inhibitors/ 599
2 apixaban.mp. 1165

3 Betrixaban.mp. 109
4 edoxaban.mp. 699
5 Dabigatran.mp. 1241
6 Rivaroxaban.mp. 2064
7 (doac* or noac* or Non-vitamin K antagonist oral anticoagul* or non vitamin k antagonist oral anti coagul*).tw,kw. 932
8 ((direct oral or novel) adj3 (anticoagul* or anti coagulat*)).tw. 741
9 (direct* oral anticoagul* or direct oral anti coagul* or novel oral anticoagul* or novel oral anti coagul*).kw. 0
10 or/1-9 4717
11 (thrombin adj2 generation adj5 (assay* or measur* or test*)).tw,kw. 213
12 Thrombinoscop*.tw,kw. 1
13 (thrombograph* or thrombinograph*).tw,kw. 32
14 (Innovance adj3 ETP).tw,kw. 0
15 calibrated automated Thromb*.tw,kw. 52
16 Thrombelastography/ 259
17 thromboelastograph*.tw,kw. 482
18 Technothrombin.tw,kw. 2
19 Fluorescent Dyes/ 192
20 fluorometry/ or spectrometry, fluorescence/ 168
21 fluorogen*.tw,kw. 37
22 Chromogenic Compounds/ 22
23 chromogen*.tw,kw. 336
24 tga.tw,kw. 191
25 Prothrombin Complex Concentrate*.tw,kw. 216
26 Factor VIIa/ 153
27 Recombinant factor VIIa.tw,kw. 141
28 Fresh Frozen Plasma.tw,kw. 810
29 Andexanet.mp. 42
30 Idarucizumab.mp. 51
31 Praxbind.mp. 1
32 activated PCC.tw,kw. 3
33 aPCC.tw,kw. 42
34 FEIBA.tw,kw. 61
35 Factor Eight Inhibitor Bypassing Activity.tw,kw. 9
36 factor viii/ 378
37 (anti-factor Xa or anti-Xa activity or anti-IIa activity).tw,kw. 455
38 Endogenous Thrombin Potential.tw,kw. 152
39 peak thrombin.tw,kw. 57
40 lag time.tw,kw. 794
41 time to peak.tw,kw. 1929
42 (ciraparantag or aripazine).tw,kw. 13
43 or/11-42 6445

44 10 and 43 330

Web of Science – Sept. 8, 2021

1. Factor Xa Inhibitors (Topic)

2. TS=(apixaban or betrixaban or edoxaban or rivaroxaban or dabigatran)

3. TS=(doac* or noac* or "Non-vitamin K antagonist oral anticoagul*" or "non vitamin k antagonist oral anti coagul*")

4. ((#1) OR #2) OR #3

5. (TS=(thrombin NEAR/2 generation)) AND TS=((assay* or measur* or test*))

6. (((((((TS=(Thrombinoscop*)) OR TS=(thrombograph* or thrombinograph)) OR TS=(Innovance NEAR/3 ETP)) OR TS=(calibrated automated Thromb*)) OR TS=(thromboelastograph*)) OR TS=(Technothrombin)) OR TS=(fluorogen*)) OR TS=(chromogen*)

7. ((((((TS=(tga)) OR TS=("Prothrombin Complex Concentrate*")) OR TS=("Recombinant factor VIIa" or "Recombinant factor 7a")) OR TS=("Fresh Frozen Plasma")) OR TS=(Andexanet)) OR TS=(Idarucizumab)) OR TS=(Praxbind) OR TS=(aripazine) OR TS=(ciraparantag)

8. (((((((TS=(activated PCC or "activated partial thromboplastin time")) OR TS=(aPCC)) OR TS=(FEIBA)) OR TS=("anti-factor Xa" or "anti-Xa activity" or "anti-IIa activity")) OR TS=("Endogenous Thrombin Potential")) OR TS=("peak thrombin generation")) OR TS=("lag time")) OR TS=("time to peak")

9. (((#5) OR #6) OR #7) OR #8

10. (#4) AND #9

11. TI=(case report)

12. (#10) NOT #11

13. TS=(animal or animals or pisces or fish or fishes or catfish or catfishes or sheatfish or silurus or arius or heteropneustes or clarias or gariepinus or fathead minnow or fathead minnows or pimephales or promelas or cichlidae or trout or trouts or char or

chars or salvelinus or salmo or oncorhynchus or guppy or guppies or millionfish or poecilia or goldfish or goldfishes or carassius or auratus or mullet or mullets or mugil or curema or shark or sharks or cod or cods or gadus or morhua or carp or carps or cyprinus or carpio or killifish or eel or eels or anguilla or zander or sander or lucioperca or stizostedion or turbot or turbots or psetta or flatfish or flatfishes or plaice or pleuronectes or platessa or tilapia or tilapias or oreochromis or sarotherodon or common sole or dover sole or solea or zebrafish or zebrafishes or danio or rerio or seabass or dicentrarchus or labrax or morone or lamprey or lampreys or petromyzon or pumpkinseed or pumpkinseeds or lepomis or gibbosus or herring or clupea or harengus or amphibia or amphibian or amphibians or anura or salientia or frog or frogs or rana or toad or toads or bufo or xenopus or laevis or bombina or epidalea or calamita or salamander or salamanders or newt or newts or triturus or reptilia or reptile or reptiles or bearded dragon or pogona or vitticeps or iguana or iguanas or lizard or lizards or anguis fragilis or turtle or turtles or snakes or snake or aves or bird or birds or quail or quails or coturnix or bobwhite or colinus or virginianus or poultry or poultries or fowl or fowls or chicken or chickens or gallus or zebra finch or taeniopygia or guttata or canary or canaries or serinus or canaria or parakeet or parakeets or grasskeet or parrot or parrots or psittacine or psittacines or shelduck or tadorna or goose or geese or branta or leucopsis or woodlark or lullula or flycatcher or ficedula or hypoleuca or dove or doves or geopelia or cuneata or duck or ducks or greylag or graylag or anser or harrier or circus pygargus or red knot or great knot or calidris or canutus or godwit or limosa or lapponica or meleagris or gallopavo or jackdaw or corvus or monedula or ruff or philomachus or pugnax or lapwing or peewit or plover or vanellus or swan or cygnus or columbianus or bewickii or gull or chroicocephalus or ridibundus or albifrons or great tit or parus or aythya or fuligula or streptopelia or risoria or spoonbill or platalea or leucorodia or blackbird or turdus or merula or blue tit or cyanistes or pigeon or pigeons or columba or pintail or anas or starling or sturnus or owl or athene noctua or pochard or ferina or cockatiel or nymphicus or hollandicus or skylark or alauda or tern or sterna or teal or crecca or oystercatcher or haematopus or ostralegus or shrew or shrews or sorex or araneus or crocidura or russula or european mole or talpa or chiroptera or bat or bats or eptesicus or serotinus or myotis or dasycneme or daubentonii or pipistrelle or pipistrellus or cat or cats or felis or catus or feline or dog or dogs or canis or canine or canines or otter or otters or lutra or badger or badgers or meles or fitchew or fitch or foumart or foulmart or ferrets or ferret or polecat or polecats or mustela or putorius or weasel or weasels or fox or foxes or vulpes or common seal or phoca or vitulina or grey seal or halichoerus or horse or horses or equus or equine or equidae or donkey or donkeys or mule or mules or pig or pigs or swine or swines or hog or hogs or boar or boars or porcine or piglet or piglets or sus or scrofa or llama or llamas or lama or glama or deer or deers or cervus or elaphus or cow or cows or bos taurus or bos indicus or bovine or bull or bulls or cattle or bison or bisons or sheep or sheeps or ovis aries or ovine or lamb or lambs or mouflon or mouflons or goat or goats or capra or caprine or chamois or rupicapra or leporidae or lagomorpha or lagomorph or rabbit or rabbits or oryctolagus or cuniculus or laprine or hares or lepus or rodentia or rodent or rodents or murinae or mouse or mice or mus or musculus or murine or woodmouse or apodemus or rat or rats or rattus or norvegicus or guinea pig or guinea pigs or cavia or porcellus or hamster or hamsters or mesocricetus or cricetulus or cricetus or gerbil or gerbils or jird

or jirds or meriones or unguiculatus or jerboa or jerboas or jaculus or chinchilla or chinchillas or beaver or beavers or castor fiber or castor canadensis or sciuridae or squirrel or squirrels or sciurus or chipmunk or chipmunks or marmot or marmots or marmota or suslik or susliks or spermophilus or cynomys or cottonrat or cottonrats or sigmodon or vole or voles or microtus or myodes or glareolus or primate or primates or prosimian or prosimians or lemur or lemurs or lemuridae or loris or bush baby or bush babies or bushbaby or bushbabies or galago or galagos or anthropoidea or anthropoids or simian or simians or monkey or monkeys or marmoset or marmosets or callithrix or cebuella or tamarin or tamarins or saguinus or leontopithecus or squirrel monkey or squirrel monkeys or saimiri or night monkey or night monkeys or owl monkey or owl monkeys or douroucoulis or aotus or spider monkey or spider monkeys or ateles or baboon or baboons or papio or rhesus monkey or macaque or macaca or mulatta or cynomolgus or fascicularis or green monkey or green monkeys or chlorocebus or vervet or vervets or pygerythrus or hominoidea or ape or apes or hylobatidae or gibbon or gibbons or siamang or siamangs or nomascus or symphalangus or hominidae or orangutan or orangutans or pongo or chimpanzee or chimpanzees or pan troglodytes or bonobo or bonobos or pan paniscus or gorilla or gorillas or troglodytes)

14. (#12) NOT #13 **2381**

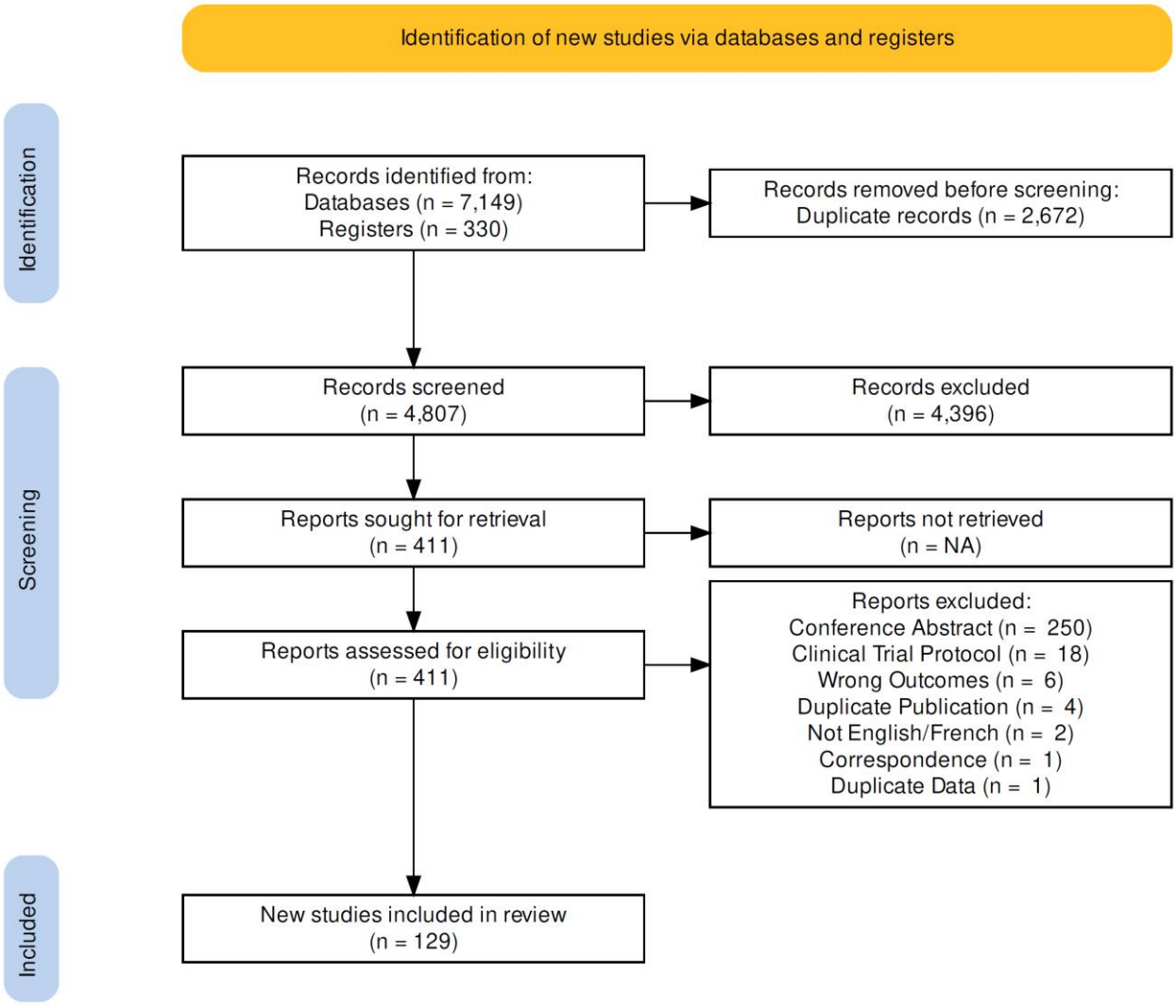
Eligible DOACs, Hemostatic Therapies and Thrombin Generation Assays

Direct Oral Anticoagulant	DOAC Type
Dabigatran	Direct Thrombin Inhibitor
Ximelagatran	Direct Thrombin Inhibitor
Melagatran	Direct Thrombin Inhibitor
Rivaroxaban	Direct Xa Inhibitor
Apixaban	Direct Xa Inhibitor
Edoxaban	Direct Xa Inhibitor
Betrixaban	Direct Xa Inhibitor

Hemostatic Agent	Types
Prothrombin Complex Concentrate (PCC)	3-factor PCC (e.g. Bebulin®) 4-factor PCC (e.g. Octaplex®)
Activated Prothrombin Complex Concentrate (aPCC)	aPCC (e.g. FEIBA®)
Recombinant factor VIIa (rFVIIa)	rFVIIa (e.g. Novoseven®)
Fresh Frozen Plasma (FFP)	---
Andexanet alfa	---
Idarucizumab (Praxbind®)	---
Ciraparantag (aripazine)	---

Thrombin Generation Assay
Thrombinoscope/Calibrated Automated Thrombography (Stago)
ST Genesia (Stago)
Innovance ETP (Siemens)
Technothrombin (Technoclone)

Supplementary Figure 1. Study Flow Diagram



Chapter 3 – Pilot Observational Study

Our review of the literature and narrative synthesis identified trends with respect to the effects of DOACs on TGA parameters, as well as the effect of hemostatic therapies on DOAC-altered TGA parameters. FXaIs and DTIs lead to seemingly distinct alterations in TGA parameters. Moreover, kinetic TGA parameters appear to be important with respect to predicting an anticoagulant effect.

Hemostatic therapies confer varying degrees of correction with respect to DOAC-altered TGA parameters. PCCs are among the most widely used non-specific hemostatic agents for the management of FXaI-associated major bleeding, particularly in jurisdictions where andexanet alfa is not approved for use or clinically accessible, including Canada. Data collected as part of our literature review would suggest that non-specific hemostatic therapies fail to normalize the thrombin generation curve. PCC inconsistently corrects kinetic TGA parameters and many studies failed to identify any measurable impact with respect to TGA lag time or time to peak. We discussed the fact that some anticoagulants, such as hirudin and bivalirudin, appear to exert their anticoagulant effects primarily by prolonging kinetic TGA parameters and the onset of thrombin generation, suggesting that these kinetic parameters may be important contributors towards predicting an anticoagulant effect. Further, correction of these parameters might be important in terms of predicting a hemostatic effect from therapies used in the context of FXaI-associated bleeding.

To further address the objectives set out in this thesis, we designed and completed a pilot prospective observational study measuring TGA responses among FXaI-treated patients with major bleeding or needing urgent surgery. We opted for an observational study design to ensure adequate recruitment of patients from this critically ill patient population, in addition to optimizing the external validity of our findings by avoiding selection bias that would be inherent to an interventional study design. Furthermore, the use of a self-controlled before/after design would circumvent limitations surrounding interindividual variability in TGA results by allowing participants to function as their own controls and measuring the impact of PCC on TGA parameters within the individual patient.

The goals of the pilot observational study were threefold. First, we sought to evaluate the feasibility of a full-scale prospective observational study, particularly with respect to patient recruitment and sample acquisition. Second, we sought to collect further information on TGA responses among FXaI-treated patients receiving PCC for either major bleeding or needing urgent surgery. Lastly, we aimed to measure clinical hemostatic efficacy and risk of thromboembolic complications among PCC-treated participants.

Effect of Prothrombin Complex Concentrates on Thrombin Generation Parameters Among Patients with Major Bleeding or Needing Urgent Surgery on Factor Xa Inhibitors – A Pilot Study

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Abstract

Non-specific hemostatic agents such as prothrombin complex concentrates (PCCs) are used for factor Xa inhibitor (FXaI)-associated bleeding. Thrombin generation assays (TGAs) may measure the effect of hemostatic therapies among FXaI-treated patients. A single center prospective feasibility pilot study was conducted on the effects of four-factor PCC (PCC) among FXaI-treated patients presenting with major bleeding or needing urgent surgery. Primary feasibility endpoints included the mean number of patients recruited per month over the course of 6 months and the proportion of recruited patients with complete TGA results both pre-PCC and post-PCC administration. TGA parameters were measured on available pre- and post-PCC plasma samples using calibrated automated thrombography (CAT). Secondary clinical outcomes included adjudicated hemostatic efficacy and the 30-day risk of thromboembolic events and death. The average recruitment rate was 3 participants per month. Complete TGA analyses were available on 16 of 18 recruited participants. Thirteen participants received PCC for major bleeding and 5 participants received PCC for urgent procedures. Median PCC dosing was 47.8 IU/kg [IQR 38.7-49.6]. After PCC administration, peak thrombin generation and ETP increased by a mean of 90.2 nM [95% CI 16.4 to 164.0; p = 0.0198] and 903.3 nM•min [95% CI 390.7 to 1415.9; p = 0.0019), respectively. PCC had no significant effect on kinetic TGA parameters. Effective hemostasis occurred in 46.2% [95% CI 19.2-74.9] of participants with major bleeding. No thromboembolic events were recorded. TGAs represent a promising supplement to clinical measures of hemostatic efficacy. Further study is needed to clarify the association between correction of TGA parameters and clinical hemostasis.

Introduction

Direct oral anticoagulants (DOACs) such as direct factor Xa inhibitors (FXaIs; rivaroroxaban, apixaban, edoxaban) and direct thrombin inhibitors (DTIs; e.g., dabigatran) have revolutionized oral anticoagulation therapy over the past decade and now account for most oral anticoagulant use [1, 2]. DOACs are associated with less bleeding as compared to vitamin K antagonists [3]. Despite a lower risk of bleeding complications, DOAC-associated bleeding events are commonly encountered in clinical practice. It is estimated that 2-4% of DOAC-treated patients experience major bleeding per year [4]. Case fatality rates for DOAC-associated major bleeding range from 8-15% and can be as high as 30-50% among patients with anticoagulation-associated intracranial hemorrhage (ICH). Several hemostatic strategies are used to manage DOAC-associated bleeding events, including specific reversal agents such as andexanet alfa for FXaI-associated bleeding [5-7]. Andexanet alfa is not approved for use in all regions, can be costly, and is not always immediately available for clinical use [8, 9]. In the absence of specific reversal therapy, nonspecific treatments, such as prothrombin complex concentrate (PCC) are often given, a practice which is supported by expert consensus guidance [10].

The mechanisms by which PCC may improve hemostasis in the setting of FXaI use are unclear but it has been proposed to occur through a bypassing mechanism in which anticoagulant effect is overcome with a supply of coagulation factors [11].

Administration of PCC has not been shown to impact FXaI levels [12, 13], and has little effect on conventional clotting assays [14]. Prospective studies have evaluated both andexanet alfa and PCCs using clinical hemostatic efficacy as a primary outcome [6, 15-17]. Adjudication of clinical hemostasis is complex, necessitates intensive data collection and frequently relies on surrogate measures of ongoing bleeding such as hemoglobin measurements.

Thrombin generations 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 [18, 19]. 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 could be used to measure hemostatic responses following administration of specific DOAC reversal agents or nonspecific hemostatic therapies [6, 17]. Studies using animal models and healthy volunteers have shown that correction of TGA parameters correlated with reductions in blood loss [20-22]. PCCs have been shown to consistently normalize TGA endogenous thrombin potential (ETP) in the context of DOAC anticoagulation, both in vitro and in healthy volunteer in vivo studies [14]. However, PCCs do not appear to affect kinetic

TGA parameters, which have better discrimination with respect to predicting minor bleeding events among rivaroxaban-treated patients [12, 23, 24]. Further, there are limited data with respect to the effects of PCC on TGA parameters among patients with FXaI-associated bleeding. Demonstrating correction of TGA parameters in vivo among bleeding patients may offer a useful adjunct to clinical hemostatic efficacy for determining the effect of PCCs for the management of patients with FXaI-associated bleeding.

We designed and implemented a pilot study to evaluate the feasibility of a full scale prospective observational study assessing whether treatment with PCC affects TGA parameters among patients with FXaI-associated bleeding or needing urgent surgery. The objective of the full scale prospective observational study will be to characterize TGA responses following administration of PCC to FXaI-treated patients. Second, we aim to assess whether administration of PCC affects kinetic TGA parameters. Lastly, we aim to collect data on clinical hemostatic efficacy and the risk of thromboembolic complications following the administration of PCC to FXaI-treated patients.

Methods

Study Design

A single-center pilot prospective observational study measuring TGA parameters before and after administration of PCC to patients presenting with FXaI-associated major bleeding or needing urgent surgery from May 2021 to November 2021 at The Ottawa Hospital General and Civic campuses was conducted. Patients were followed for 30 days.

The study protocol and informed consent forms were approved by the Ottawa Health Science Network Research Ethics Board.

Study Population and Recruitment Procedures

Adult patients (≥ 18 years of age) prescribed a FXaI (rivaroxaban, apixaban or edoxaban) for any indication, presenting with major or life-threatening bleeding or requiring urgent surgery as deemed by the treating physician/surgeon, and received PCC, were eligible for recruitment. Patients with a reduced hemoglobin without overt bleeding and patients who had already received activated PCC or recombinant factor VIIa prior to receipt of PCC were excluded. FXaI-treated patients presenting with major bleeding or requiring urgent surgery received 50 IU/kg of four-factor PCC (Octaplex®; Octapharma) weight-based dosing as per The Ottawa Hospital's institutional protocol, with no maximum dose. Andexanet alfa is not available and is not approved for use in Canada at the time of this study. PCC dosing was at the discretion of the treating physician. Written informed consent was obtained from eligible participants or substitute decision makers (SDM) to use remaining stored citrated plasma samples drawn per routine care before and after PCC administration for the laboratory analyses outlined below.

Data Collection

Data were 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 definition for major bleeding [25,

26], as well as the type and details of any planned surgical procedures, if applicable. Patients who presented with major bleeding but subsequently underwent an urgent procedural intervention as part of bleed management (e.g. craniotomy for ICH) were counted as having received PCC for major bleeding. Results of conventional coagulation assays (prothrombin time; PT and activated partial thromboplastin time; aPTT), FXaI levels (by calibrated anti-Xa activity assay), and d-dimer and fibrinogen levels, were recorded, when available. Intracranial hemorrhage hematoma volume was quantified using Quantomo® software [27]. Detailed hemostatic data were collected, summarized, and provided to adjudicators. Hemostatic data included hemoglobin/hematocrit trends, packed red blood cell (pRBC) 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 [28, 29]. A review of medical records, laboratory tests, imaging results and participant/SDM interview was conducted up to 30 ± 2 days post-PCC administration to determine whether a clinical outcome event had occurred. Pertinent information was provided to duplicate adjudicators for assessment of suspected outcome events.

Plasma Sample Collection and Laboratory Analyses

An electronic PCC order set at The Ottawa Hospital was developed by clinical care leaders to standardize PCC dosing for FXaI-associated bleeding. This order set included recommended timeframes for laboratory testing within 4 hours pre-PCC/post-PCC administration, at the discretion of the treating physician. Citrated plasma samples were collected by venipuncture using plastic 2.7 mL 3.2% citrated Vacutainer® tubes (0.109M

sodium citrate; Beckton Dickinson). All coagulation analyses were carried out within 4 hours from sample collection, except for thrombin generation. The following reagents were used for the coagulation assays: Thromborel S® for PT/INR 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). Calibrated edoxaban levels are not available for clinical care at The Ottawa Hospital. Use of a rivaroxaban-calibrated level was recommended when an approximate DOAC level was felt to be clinically indicated among edoxaban-treated patients, due to similarities between the calibration curves [30]. D-dimer testing was performed using the Innovance® D-Dimer kit (reference range < 500 ug/L; Siemens Healthcare Diagnostics) and fibrinogen was measured using the Clauss method with Dade® Thrombin Reagent (reference range 1.9-4.5 g/L; Siemens Healthcare Diagnostics, Marburg, Germany). Samples subsequently underwent centrifugation at 1500g for 15 minutes to generate platelet poor plasma (PPP), were aliquoted and were frozen at -80C for up to 6 months prior to shipment and thrombin generation testing.

Thrombin generation was measured using calibrated automated thrombography (CAT)/Thrombinoscope® on PPP samples by a central research laboratory (Diagnostica Stago, Gennevilliers, France). Fluorescence was measured using a Fluoroskan Ascent® fluorometer (ThermoFischer Scientific, Helsinki, Finland). TGA was completed using 5 pM tissue factor (PPP Reagent®; Diagnostica Stago) and 4 uM phospholipids. Reactions were initiated by adding 20 uL of FluCa buffer (Diagnostica Stago) to wells containing 80

uL of PPP. Reactions were carried out in duplicate and averaged values from duplicate runs were recorded. TGA analyses were completed in the absence of added corn trypsin inhibitor or exogenous thrombomodulin. TGA parameters recorded included the lag time (LT; in minutes), time to peak (TTP; in minutes), peak thrombin generation (peak; in nM), endogenous thrombin potential (ETP; in nM•min), mean velocity rate index (mVRI; in nM/min).

Feasibility Endpoints

The primary feasibility endpoints were (i) the mean number of patients recruited per month over 6 months, and (ii) the proportion of recruited patients with complete TGA results both pre-PCC and post-PCC administration. Secondary feasibility outcomes included the proportion of eligible patients successfully consented and the proportion of patients who completed 30-day follow-up.

Clinical and Laboratory Endpoints

Clinical endpoints were clinical hemostatic efficacy and the cumulative incidence of thromboembolic events and all-cause mortality within 30 days of PCC administration. For patients with major bleeding, clinical hemostatic efficacy was evaluated in duplicate by independent adjudicators according to two sets of criteria: (i) as effective vs. ineffective as per the International Society on Thrombosis and Haemostasis (ISTH) criteria [28], and (ii) good/moderate vs poor as per criteria from Sarode et al, a previously published randomized trial of PCC versus plasma for warfarin reversal [29] (**Supplementary Appendices A and B**). Surgical hemostasis was assessed by the

treating surgeon using a binary normal/abnormal rating scale. Thromboembolic events included deep vein thrombosis, pulmonary embolism, cerebrovascular accidents, transient ischemic attacks, systemic arterial emboli, myocardial infarction, and heart valve/cardiac chamber thrombosis and were adjudicated in duplicate using standard criteria (**Supplementary Appendix C**). Thromboembolic events were adjudicated as definitely, likely, possibly or unlikely related to PCC administration. Timing of anticoagulation resumption at therapeutic doses relative to thromboembolic events was documented. Fatal events were adjudicated as being related to fatal bleeding, a fatal thromboembolic event or death from another cause.

Laboratory endpoints included the mean difference for individual TGA parameters before and after PCC administration, as well as the percentage of patients with normalization of each TGA parameter following PCC administration. Normalization was defined based on whether a participant's TGA parameter fell 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). The corresponding reference intervals were 2.67-3.33 minutes for LT, 4.50-6.00 minutes for TTP, 219.6-393.9 nM for peak, 980.6-1849.8 nM•min for ETP and 94.2-205.3 nM/min for mVRI.

Rationale, Feasibility Criteria and Sample Size for the Full-Scale Study

This pilot study was designed to assess feasibility and was not powered to detect differences in clinical or laboratory endpoints, although these were measured. The planned recruitment target for the full-scale study is 100 patients over 3 years. Criteria for the full-scale study to be deemed feasible included: 1) meeting a target mean pilot recruitment rate of 2-3 patients per month, which would allow the full-scale study to be completed within 3 years and 2) 90% of recruited patients would need to have both pre- and post-PCC samples drawn and have TGA analyses successfully completed on these samples. The second feasibility criterion would ensure that a sufficient proportion of recruited participants would have a full collected set of pre-/post-PCC samples and TGA analyses completed on these samples to yield informative results from the full-scale study, as outlined below.

Based on this sample acquisition target of 90% and a recruitment target of 16 patients over 6 months during the pilot study, a Clopper-Pearson exact 95% confidence interval for the proportion of patients with complete TGA results would be 61.7% to 98.5%. The lower bound of this 95% confidence interval would yield, at worst, an estimated 62 recruited patients in the full-scale study with pre- and post-PCC TGA analysis results. The mean LT in plasma from healthy volunteers ranges from 1.9-2.3 minutes when measured using the Stago Thrombinoscope Assay [23, 31]. Addition of FXaIs (rivaroxaban, apixaban) to plasma from healthy volunteers prolongs the mean LT to a range of 5.6-5.9 minutes [23, 31]. 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, an ideal sample size of 100 paired TGA measurements from the full-scale study would provide

90% power to measure a detectable alternative in lag time of ± 0.65 minutes, whereas a lower boundary sample size of 62 paired TGA measurements from the full-scale study would be able to detect an alternative of ± 0.84 minutes [32].

Statistical Analyses

Continuous variables are summarized as mean and standard deviation or median and interquartile range (IQR); categorical variables are presented as frequencies. Normality of distributions was evaluated using the Shapiro-Wilk test. Differences between pre-PCC and post-PCC laboratory measurements were analyzed using a two-sided paired t-test with alpha of 0.05 for normally distributed data, or a Wilcoxon Signed Rank test for non-normally 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 compared the proportion of patients post-PCC vs pre-PCC that fell 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 was conducted. Data surrounding hemostatic efficacy, thromboembolic events and mortality are reported as categorical variables with 95% confidence intervals calculated using exact methods. All statistical analyses were conducted with SAS Studio Version 3.81 (Copyright © 2012-2020, SAS Institute Inc., Cary, NC, USA).

Results

A total of 18 participants were recruited over 6 months for an average recruitment rate of 3 participants per month. Two potentially eligible participants were approached and provided with consent forms but ultimately declined participation in the study, yielding a consent rate of 90% (18/20). All surviving participants completed follow-up to 30 ± 2 days. The median age of participants was 80 years [IQR 69.0-85.0] and a majority (55.6%) were female (**Table 1**). Atrial fibrillation (AF) was the most common indication for anticoagulation (77.8%) and most participants had not experienced a thrombotic event within the preceding 6 months (78%). Participants were receiving apixaban (n = 11; 61.1%), rivaroxaban (n = 6; 33.3%) and edoxaban (n = 1; 5.6%). None of the participants were receiving antiplatelet therapy. Thirteen participants received PCC for major bleeding and 5 participants received PCC prior to 6 urgent procedures (one participant received PCC twice prior to two separate urgent procedures). The most common bleeding site was ICH (7 participants; 53.9%) (**Supplementary Table 1**). All participants presenting with bleeding met the ISTH definition for major bleeding. Among the 13 patients who received PCC for major bleeding, 4 underwent a procedural intervention as part of bleed management (**Supplementary Table 1**).

For participants receiving PCC for major bleeding, the median time between the last DOAC dose and presentation with bleeding was 9.3 hours [IQR 5.3-10.9] and the median time from presentation to PCC administration was 7.3 hours [IQR 4.5-9.0]. Pre-PCC DOAC levels were available for 11 of 18 recruited participants, with a median

value of 80 ng/mL [IQR 73-152; range 52-396]. Median PCC dosing was 3500 IU [IQR 3000-5000], corresponding to a median weight-based dose of 47.8 IU/kg [IQR 38.7-49.6]. For participants receiving PCC for urgent surgery, the median time from preprocedural PCC administration to surgery was 11 minutes [IQR 3-23 minutes].

TGA analyses were completed on 16 of 18 recruited participants. One participant who received PCC twice had two paired sets of plasma samples. The completion rate for collection and analysis was 89.5% [95% CI 66.9-98.7] (17 out of 19 sets of samples). Two participants did not have post-PCC samples available for analysis. One participant with severe liver failure had undetectable thrombin generation both before and after PCC administration and the results were not included, leaving 16/19 paired samples available for TGA analyses.

Among the 13 participants receiving PCC for major bleeding, hemostatic efficacy was adjudicated for all 13 patients using ISTH criteria, and for 12 of 13 participants using the modified Sarode criteria. Effective hemostasis occurred in 6/13 participants by ISTH criteria (46.2% [95% CI 19.2-74.9]), with “excellent clinical outcome” noted in 3 of 5 eligible cases. Hemostasis was rated as “good/moderate” in 8/12 participants (good = 6, moderate = 2) by Sarode criteria (66.6% [95% CI 34.9-90.1]), and “poor” in 4 participants. Among participants receiving PCC prior to urgent surgery, procedural hemostasis was rated as normal in 5 out of 6 procedures (83.3%; 95% CI 35.9-99.6]). No thromboembolic events occurred during the 30-day follow-up (0%; 95% CI 0.0-16.7).

Three participants died, corresponding to a 30-day overall mortality rate of 16% [95% CI 3.6-41.4]. One death was due to fatal bleeding and 2 deaths were due to another cause.

Laboratory measurements before and after PCC administration are reported in **Table 2**. A statistically significant shortening of the PT was seen following PCC administration ($p < 0.0001$; **Figure 1**). There was no significant change in the aPTT ($p = 0.12$), d-dimer or fibrinogen ($p > 0.05$ for both d-dimer and fibrinogen; **Figure 2**). The paired differences in TGA measurements were normally distributed ($p > 0.05$ using the Shapiro-Wilk test). The distribution of TGA parameters before and after PCC administration and corresponding reference boundaries are shown in **Figure 3**. Representative thrombograms from participants with major bleeding receiving PCC or patients undergoing urgent procedural interventions are shown in **Figures 4 and 5**, respectively.

There was no significant effect of PCC on kinetic TGA parameters (LT, TTP) or the mVRI. The mean difference in LT following PCC administration was -0.03 minutes [95% CI -0.48 to 0.42] ($p = 0.88$). There was a mean increase in the TTP of 0.67 minutes [95% CI -0.16 to 1.51] ($p = 0.11$) following PCC administration. The mean change in mVRI was 25.2 nM/min [95% CI -8.52 to 58.9] ($p = 0.13$). There was a statistically significant increase in quantitative TGA parameters after PCC administration (**Figure 3**). The mean increase in peak thrombin generation following PCC administration was 90.2 nM [95% CI 16.4 to 164.0] ($p = 0.0198$) and the mean increase in ETP was 903.3 nM•min [95% CI 390.7 to 1415.9] ($p = 0.0019$).

We compared the proportion of participants with TGA parameters that fell within the defined reference ranges before and after PCC administration (i.e. ≤ ULN for LT/TTP and ≥LLN for peak, ETP and mVRI). There was no statistically significant change in the proportion of patients with kinetic parameters ≤ ULN for either the LT (31.3% pre-PCC to 31.3% post-PCC; p = 1.0) or TTP (25.0% pre-PCC to 12.5% post-PCC; p = 0.32). The proportion of patients with quantitative parameters ≥ LLN increased for both peak thrombin generation from 18.8% pre-PCC to 50.0% post-PCC (p = 0.0588) and ETP from 50.0% to 82.3% (p = 0.0588). The proportion of patients with mVRI ≥ LLN increased from 12.5% pre-PCC to 31.3% post-PCC (p = 0.18). Patients who had an urgent procedure between pre-PCC and post-PCC TGA sampling tended to have larger increases in ETP as compared to participants who did not have an invasive procedure between TGA measurements (**Figures 4 and 5**).

Discussion

We designed and completed a prospective pilot study to determine the feasibility of evaluating the effects of PCC on thrombin generation parameters among FXaI-treated patients presenting with bleeding or needing urgent surgery. We achieved an average enrolment target of 3 participants per month and TGA results were obtained for 89% of participants. These pilot results support the feasibility of a full-scale prospective observational study of 100 patients over 3 years. Although not powered to detect a difference in kinetic parameters, the feasibility estimates obtained demonstrated that a

sufficient proportion of recruited participants in the full-scale study will have TGA data available to provide informative results and test our hypothesis surrounding the effects of PCC on kinetic TGA parameters.

Fewer participants treated for bleeding in our study had effective clinical hemostasis following PCC administration (46.2% by ISTH criteria and 66.6% by Sarode criteria) compared to prior studies (69.1-85.0%) [15, 16]. This could reflect the small sample size of our pilot study and resulting uncertainty surrounding the estimate of clinical hemostatic efficacy, heterogenous populations, differences in bleed location and severity, or differences in management. Unlike our study, the UPRATE cohorts, which evaluated an institutional protocol of PCC (2,000 IU) for bleeding on FXaI, excluded patients with preexisting “do not resuscitate” wishes, potentially leading to the exclusion of participants with poorer prognoses [15, 16]. Hemostasis was rated as normal in all but one participant among those patients in our study treated with PCC prior to urgent surgical procedures, for whom technical complications may have contributed to bleeding. There are little data on the use of PCC prior to urgent interventions [33, 34]. More data are required to further characterize the role of PCC in this patient population.

Our preliminary TGA results are generally consistent with the existing literature. In vitro and healthy volunteer studies have consistently shown an effect of PCCs on ETP, but most studies have not demonstrated an effect of PCCs on kinetic parameters [14]. In this study, PCC increased quantitative TGA parameters (peak, ETP), but did not appear

to shorten kinetic TGA parameters (LT, TTP). Importantly, although we observed an increase in both peak and ETP post-PCC, the thrombin generation profile of most participants remained abnormal post-PCC. Although a statistically significant effect of PCC on peak thrombin generation was demonstrated, only 50% of participants had post-PCC peak thrombin generation values within our defined reference range. Patients with an intervening surgery/procedure between TGA measurements had higher increments in ETP following PCC administration. A study among participants undergoing orthopedic surgery reported a reduction in LT/TTP and an increase in peak/ETP when comparing pre-operative to intraoperative TGA measurements [35]. The effects of surgery itself on thrombin generation will need to be considered when interpreting the results of our full-scale analysis. To accomplish this for the full-scale study, we intend to undertake a sensitivity analysis of the changes in TGA parameters, excluding patients who had a surgery between TGA measurements.

FXaI levels correlate well with kinetic TGA parameters, and some studies have failed to identify a statistically significant effect of FXaIs on ETP at clinically relevant concentrations [36-38]. Our preliminary results are consistent with these findings, given the measured pre-PCC median ETP value from this study fell just below the lower limit of the defined reference range, with considerable overlap. Pre-PCC LT and TTP measurements were prolonged compared to the reference range, reflecting FXaI activity. Fundamentally, normalization of the thrombin generation curve could be considered the benchmark for true anticoagulation reversal based on TGA responses following the administration of reversal agents with proven mechanisms of action, such

as andexanet alfa for FXaI or PCC for vitamin K antagonists. Studies reporting TGA data for these specific reversal agents suggest that they normalize the thrombin generation curve [39-41]. It is not known whether correction of all TGA parameters is necessary to produce a clinically relevant improvement in hemostasis, or whether improvement of only some parameters is sufficient. Our preliminary TGA findings suggest that PCCs might confer a beneficial hemostatic effect, based on an increase in the ETP in the setting of FXaI-associated bleeding, but they do not appear to normalize the thrombin generation curve.

The results of this pilot study carry implications for future studies evaluating anticoagulation reversal. Many studies of hemostatic therapies selectively report a single TGA parameter (usually ETP) rather than reporting effects on all TGA parameters [6, 42-44]. Although it is reasonable to prioritize a TGA parameter of interest a priori, as part of a statistical analysis plan, the results from this pilot study highlight the importance that all TGA parameters should be transparently reported in an exploratory fashion. This will improve consistency across studies, avoid selective reporting of positive results and omission of null findings. Moreover, this approach will further our understanding as to whether correcting a single TGA parameter (such as ETP) is sufficient to impart a clinically significant change to hemostasis, or whether therapies that normalize the thrombin generation curve (e.g. andexanet alfa) are preferable [39].

The pragmatic design of our study is a strength. Our inclusion criteria were broad to reflect the range of patients presenting with FXaI-associated bleeding in routine practice. We did not restrict inclusion to participants with ISTH-defined major bleeding, nor based on the timing of last DOAC intake. The decision to use PCC was at the discretion of the treating physician, as would be the case with usual care. We were able to enroll critically ill participants by using a deferred consent procedure that included substitute decision makers if patients were unable to provide informed consent. Our study also has limitations. First, we were unable to standardize the timing of pre- and post-PCC plasma sampling. A PCC electronic order set was implemented during the pilot study, with suggested timeframes for blood work. Our pilot data indicates that this approach was successful, and that pre- and post-PCC plasma samples could be obtained for most included participants. This order set will continue to be used, optimizing sample acquisition for the full-scale study. Second, it is important to consider that changes to TGA parameters between pre- and post-PCC sampling could have been influenced by factors beyond the effect of PCC itself, such as surgical interventions, concurrent blood products (e.g. plasma) or renal FXaI elimination. The half-lives of FXaIs are on the order of 5-17 hours [45] and the median timeframe between pre- and post-PCC TGA analyses in our study was 5.0 hours [IQR 3.5-6.5]. Use of an electronic order set mitigated large delays between pre- and post-PCC sampling and would have mitigated changes to TGA parameters from FXaI clearance. Third, some participants had relatively low FXaI levels at the time of PCC administration, and the clinical effects of FXaI levels at varying concentration thresholds remains to be established [46]. It could be that some participants had FXaI levels that

were low enough to not exert a meaningful impact on bleeding and hemostasis. Lastly, given the observational design and lack of a control group, we are unable to definitively state whether PCC improved clinical hemostatic efficacy. Given clinical hemostatic efficacy in our study was lower than in prior studies, these results suggest that there is room for improvement with respect to hemostatic therapies administered for FXaI-associated bleeding.

Our results suggest that a full-scale prospective observational study measuring the effect of PCCs on TGA parameters among FXaI-treated patients is feasible. Preliminary TGA results suggest that PCC administration may not lead to measurable effect on kinetic TGA parameters but appears to increase quantitative TGA parameters, findings that need to be confirmed in a larger study. PCC did not normalize the thrombin generation curve in most participants. No thromboembolic events occurred following PCC administration among any of the participants recruited during the 6-month pilot phase. Further research is needed to determine the effect of hemostatic therapies on TGA parameters, and whether correction of TGA parameters results in improvements to clinical hemostasis.

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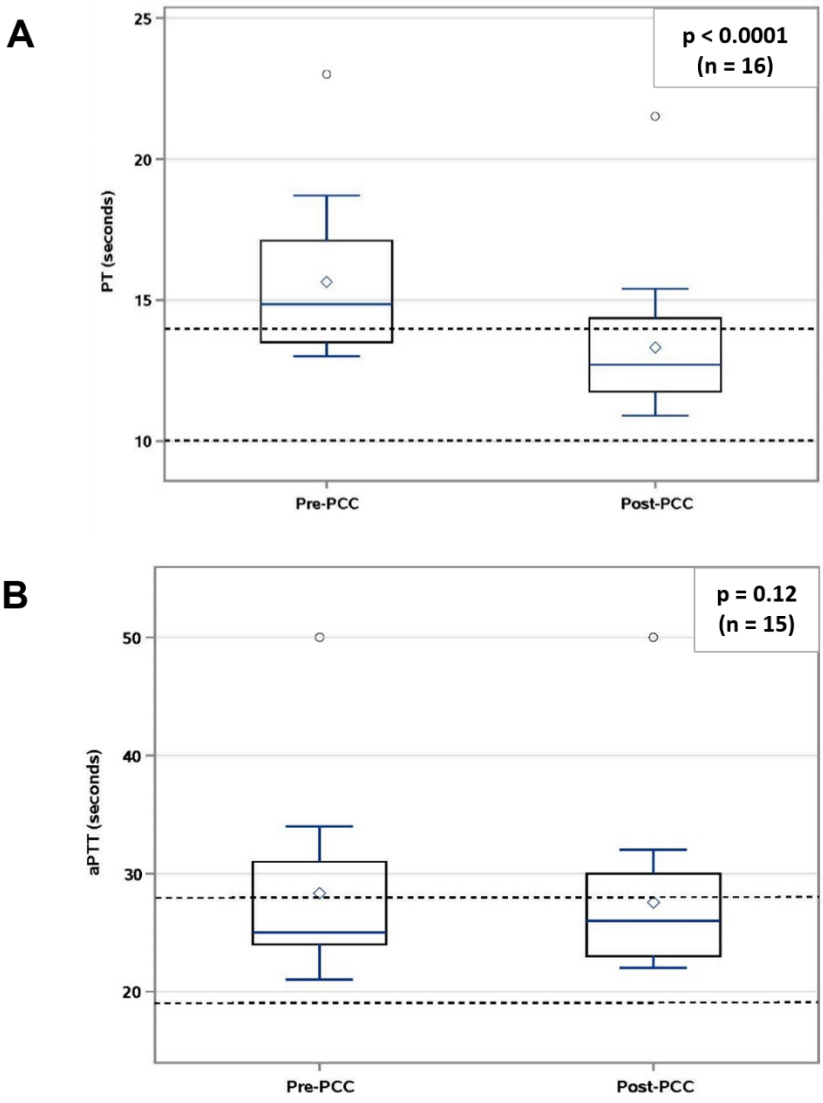


Figure 1 Coagulation Assay Results Pre-PCC and Post-PCC. A. Prothrombin Time (n = 16 ;PT in seconds). B. Activated Partial Thromboplastin Time (n = 15; aPTT in seconds). Reference intervals are indicated with dashed lines (10.0-14.0 seconds for PT and 19.0-28.0 seconds for aPTT). Results are presented only for those patients with paired results available both pre- and post-PCC. Statistical comparison for the difference between pre-PCC and post-PCC using the Wilcoxon Signed Rank test.

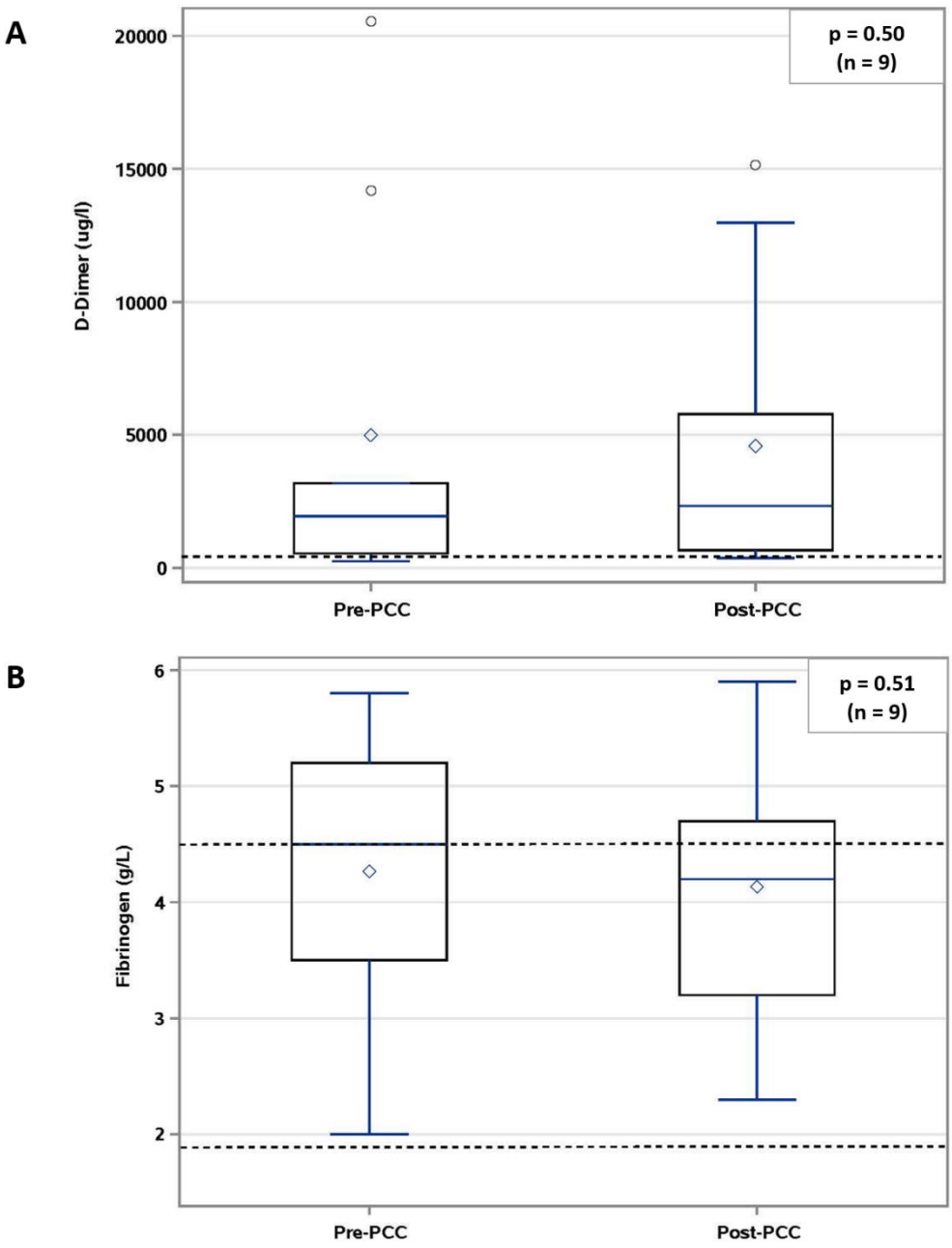


Figure 2 D-Dimer and Fibrinogen Results Pre-PCC and Post-PCC. A. D-Dimer in ug/L B. Fibrinogen in g/L. Reference intervals are indicated with dashed lines (< 500 ug/L for d-dimer and 1.9-4.5 g/L for fibrinogen). Results are presented only for those patients with paired results available both pre- and post-PCC. Statistical comparison for the difference between pre-PCC and post-PCC using the Wilcoxon Signed Rank test.

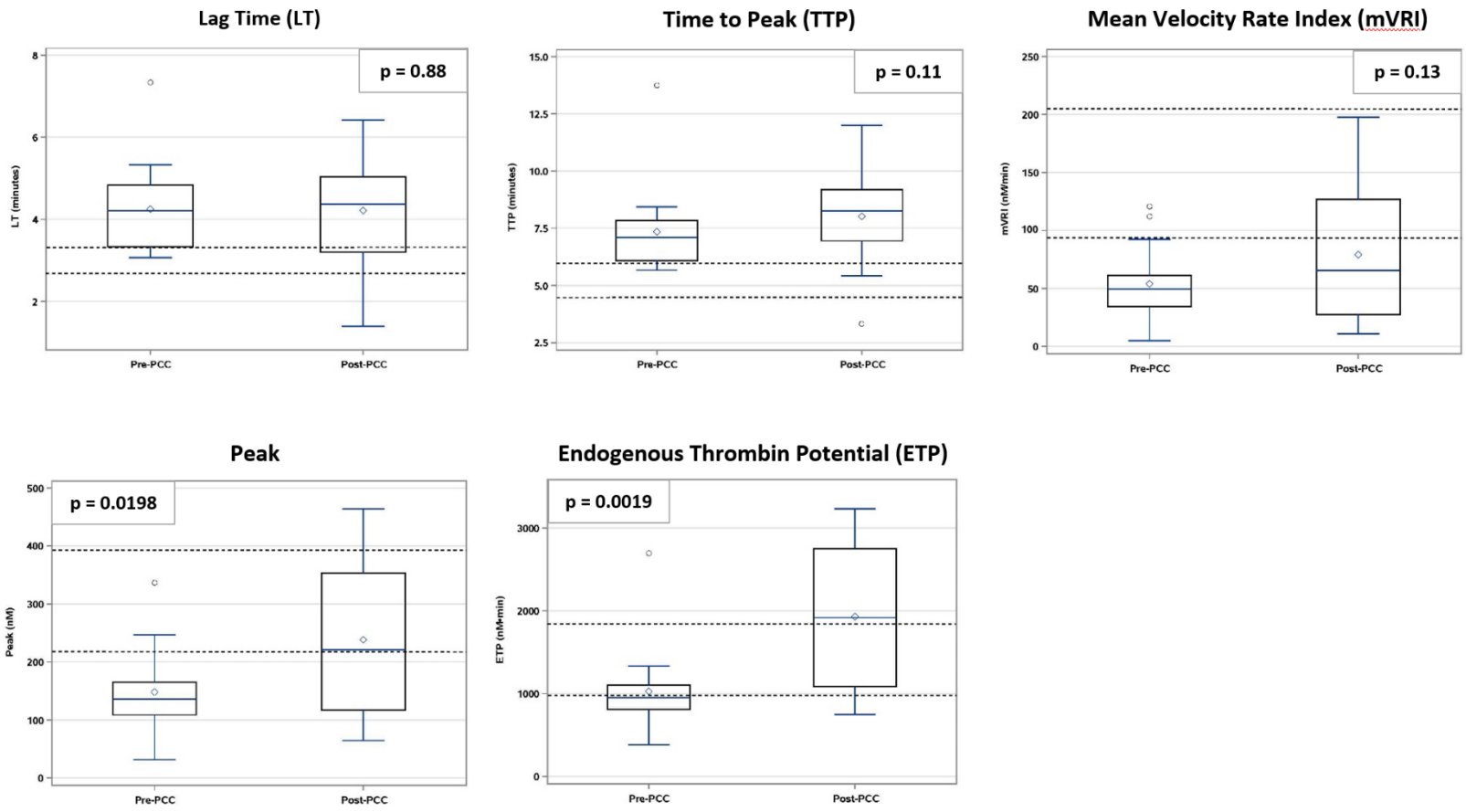


Figure 3 Effect of PCC on TGA Parameters. Reference intervals are indicated with dashed lines. Results are presented only for those patients with paired results available both pre- and post-PCC (n = 16). Statistical comparison for the difference between pre-PCC and post-PCC using a paired two-tailed t-test with results considered significant when $p < 0.05$. PCC = prothrombin complex concentrate; nm = nanomolar.

Figure 4. Representative Thrombograms among Patients with Major Bleeding Treated with Prothrombin Complex Concentrate

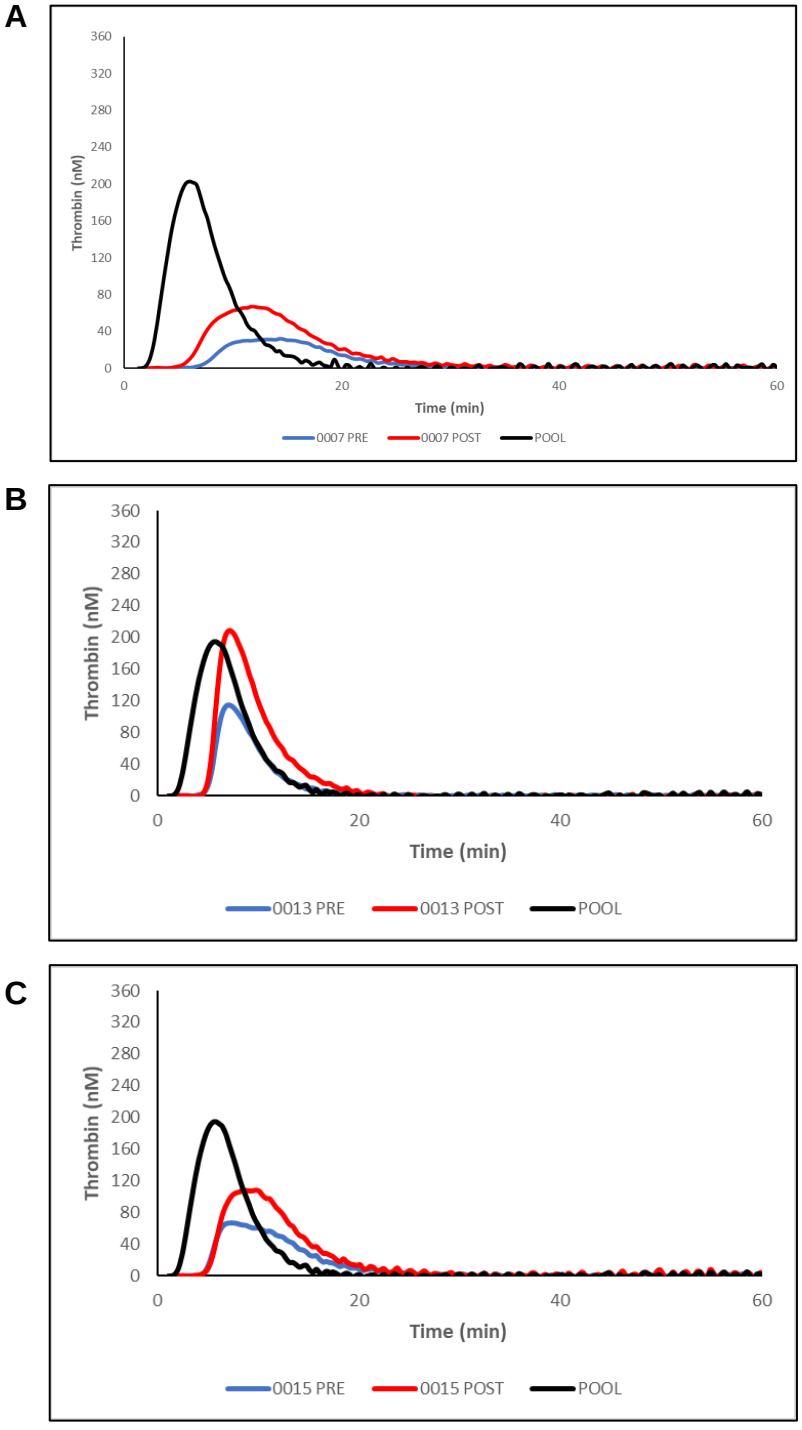
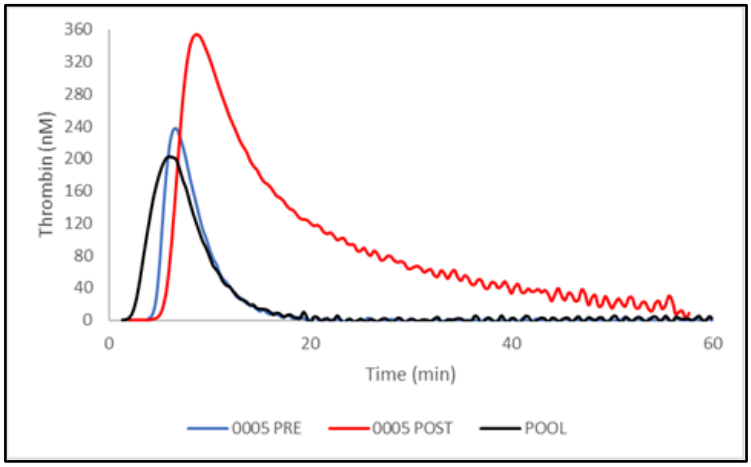


Figure 4 Representative Thrombograms among Patients with Major Bleeding Treated with Prothrombin Complex Concentrate. Pre-PCC thrombograms are in blue, post-PCC thrombograms in red and a representative normal thrombogram generated using normal pooled plasma is shown in black. None of the 3 participants shown underwent a procedure between pre-PCC and post-PCC TGA measurements. A. Participant #0007 (rivaroxaban) received PCC for ICH (SDH); pre-PCC rivaroxaban level was 152 ng/mL, they received 48.4 IU/kg of PCC. B. Participant #0013 (apixaban) received PCC for ICH (SDH); no pre-PCC apixaban level was available, pre-PCC PT was 17.0 seconds, they received 42.0 IU/kg of PCC. C. Participant #0015 (apixaban) received PCC for ICH (SAH); pre-PCC apixaban level 219 ng/mL, they received 50.8 IU/kg of PCC.

Figure 5. Representative Thrombograms among Participants Undergoing an Urgent Procedural Intervention Treated with Prothrombin Complex Concentrate

A



B

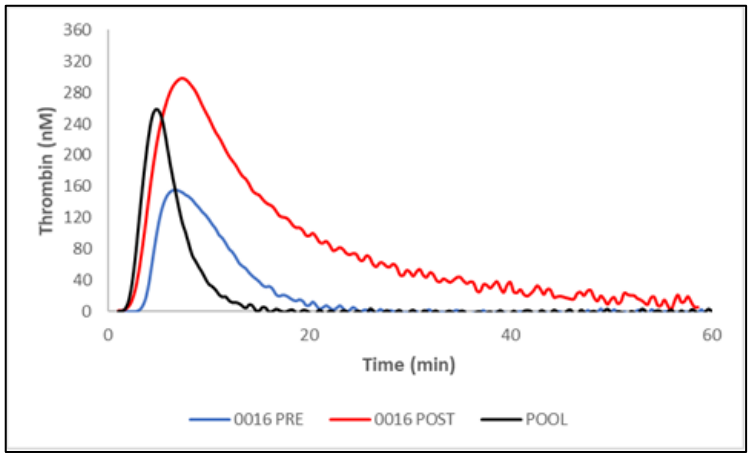


Figure 5 Representative Thrombograms among Participants Undergoing an Urgent Procedural Intervention Treated with Prothrombin Complex Concentrate. Pre-PCC thrombograms are in blue, post-PCC thrombograms in red and a representative normal thrombogram generated using normal pooled plasma is shown in black. Both participants shown underwent a procedure between pre-PCC and post-PCC TGA measurements. A. Participant #0005 (apixaban) received PCC prior to a laparotomy; pre-PCC apixaban level was 108 ng/mL, they received 49.5 IU/kg of PCC. B. Participant #0016 (apixaban) received PCC for major bleeding (lower gastrointestinal bleeding) and underwent an urgent colonoscopy as part of bleed management; pre-PCC apixaban level was 80 ng/mL, they received 45.5 IU/kg of PCC.

Table 1. Participant Characteristics

Baseline Characteristic			N = 18
Age (years) *Median [IQR]*			80.0 [69.0 – 85.0]
Sex *n (% Male)*			8 (44.4)
Race *n (%)*	*White/Caucasian*		17 (94.4)
	Asian		1 (5.6)
Indication for Anticoagulation * *n (%)*	*Atrial Fibrillation*		14 (77.8)
	VTE Treatment/Prevention		5 (27.8)
	ATE Treatment/Prevention		1 (5.6)
CHADS2 Score † *Median [IQR]*			2.0 [1.0-4.0]
DOAC/Dosing *n (%)*	*Apixaban*	*2.5 mg po BID*	4 (22.2)
		5 mg po BID	7 (38.9)
	Edoxaban	*60 mg po OD*	1 (5.6)
	Rivaroxaban	*15 mg po OD*	1 (5.6)
		20 mg po OD	5 (27.8)
Median Time From Last DOAC Dose (hours) *Median [IQR]*			9.3 [5.3 – 10.9]
Median DOAC Level (ng/mL) ‡ *Median [range]*			80 [52-396]
Major Bleeding in Past 6 months *n (%)*			1 (5.6)
Thrombotic Event in Past 6 months *n (%)*			4 (22.2)
Renal Function at Presentation *Median [IQR]*	*Creatinine (umol/L)*		85.5 [74.0 – 177.0]
	CrCl (mL/min)		55.0 [37.0 – 103]
Weight (kg) *Median [IQR]*			81.0 [62.0 – 109.0]
Active Cancer n (%)			5 (27.8)

Table 1 Participant Characteristics and PCC Dosing. * Proportions add up to > 100% as some patients had multiple indications for anticoagulation therapy/bleeding sites. † Only those patients with atrial fibrillation. ‡ Pre-PCC DOAC levels available for 11 patients.

Table 2. Laboratory Parameters Before/After PCC Administration

Laboratory Parameter	Paired Samples (N)	Pre-PCC *Median [IQR]*	Post-PCC *Median [IQR]*	p-value
Coagulation Parameters *				
PT (seconds)	16/19	14.9 [13.5 - 17.1]	12.7 [11.8 – 14.4]	**< 0.001**
aPTT (seconds)	15/19	25.0 [24.0 – 31.0]	26.0 [23.0 – 30.0]	p = 0.12
D-Dimer (ug/mL)	9/19	1947 [542 – 3183]	2325 [661 – 5786]	p = 0.50
Fibrinogen (g/L)	9/19	4.0 [3.5 – 5.2]	4.2 [3.2 – 4.7]	p = 0.51
TGA Parameters †				
LT (minutes)	16/19	4.21 [3.33 – 4.84]	4.37 [3.20 – 5.04]	p = 0.89
TTP (minutes)	16/19	7.11 [6.09 – 7.84]	8.25 [6.95 – 9.18]	p = 0.11
mVRI (nM/min)	16/19	49.4 [34.3 – 61.3]	65.4 [27.6 – 126.8]	p = 0.13
Peak (nM)	16/19	136.1 [108.7 – 165.0]	221.0 [116.7 – 353.4]	**p = 0.0198**
ETP (nM•min)	16/19	950.4 [809.9 – 1102.8]	1918.3 [1084.1 – 2748.6]	**p = 0.0019**

Table 2 Laboratory Parameters Before/After PCC Administration. Median [IQR] timeframes between pre-PCC and post-PCC measurements were 5.0 hours [3.0-6.0] for PT/aPTT, 5.0 hours [4.0-6.0] for d-dimer/fibrinogen and 5.0 hours [3.5-6.5] for TGA parameters. PT = Prothrombin Time; aPTT = activated partial thromboplastin time; TGA = thrombin generation assay; LT = lag time; TTP = time to peak; mVRI = mean velocity rate index; ETP = endogenous thrombin potential.

* Statistical significance using Wilcoxon Signed Rank test.
† Statistical significance using a paired t-test.

Supplementary Table 1. Bleed Sites and Procedural Interventions

Bleed Sites Among Patients Receiving PCC for Major Bleeding (N = 13) *n (%)*	
Intracranial hemorrhage	7 (53.9)
Gastrointestinal	2 (15.4)
Genitourinary	1 (7.7)
Retroperitoneal	1 (7.7)
Musculoskeletal	1 (7.7)
Paraspinal	1 (7.7)
Procedural Interventions Among Patients Receiving PCC for Major Bleeding (N = 4) *n (%)*	
Esophagogastroduodenoscopy (upper gastrointestinal bleeding)	1 (25.0)
Colonoscopy (lower gastrointestinal bleeding)	1 (25.0)
Renal artery pseudoaneurysm embolization	1 (25.0)
Irrigation and debridement (muscular hematoma)	1 (25.0)
Procedural Interventions Among Patients Receiving PCC for Urgent Surgery (N = 6) *n (%)*	
Laparotomy	3 (50.0)
Repair of incisional dehiscence	1 (16.7)
Bilateral percutaneous nephrostomy tubes	1 (16.7)
Dental extractions and incision/drainage of submandibular abscess	1 (16.7)

SUPPLEMENTARY APPENDICES

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A - ISTH Definition for Effective Hemostasis
Effective hemostasis is achieved when:

Non-Visible Bleeding †	***Definition of "Non-Visible Bleeding":*** *All bleeds that do not classify as visible, musculoskeletal, or intracranial bleeds. This includes gastrointestinal (GI) bleeds when the bleeding site is not visible to the naked eye, regardless of visible signs (e.g. melena).*
	Good effectiveness is achieved when all below criteria are met: 1. *At 48 hours after presentation or at discharge (whichever is first) the hemoglobin level has not dropped more than 10% compared to the hemoglobin level measured after completing the **initial management*** (including infusion of red blood cells). (**¶ see below for further clarification**).* 2. *By 48h from the end of the **initial management***, there is no need for further treatment with hemostatic agents or coagulation factors, or transfusion of other blood products.* 3. *Invasive interventions are either avoided or carried out with blood loss not exceeding the expected amount in a patient with normal hemostasis (**§ see below for further clarification**).* 4. *No unscheduled (re-)interventions are needed for bleeding management within 48 hours from **initial management***.*
Visible Bleeding	***Definition of "Visible Bleeding":*** *Bleeding site is visible to the naked eye, e.g. skin bleeding, visible mucosal bleeding (oral, nose, anal), or active bleeding is located in a compartment in which blood cannot be occult (epistaxis, vaginal bleeding).*
	Good effectiveness is achieved when all below criteria are met: 1. *Visible bleeding has ceased within 4 hours from the end of the **initial hemostatic management***.* 2. *By 48 hours from the end of the **initial management***, there is no need for further treatment with hemostatic agents or coagulation factors, or transfusion of other blood products.* 3. *Invasive interventions are either avoided or carried out with blood loss not exceeding the expected amount in a patient with normal hemostasis (**§ see below for further clarification**).* 4. *No unscheduled (re-)interventions are needed for bleeding management within 48 hours from **initial management***.*
Musculoskeletal Bleeding	*Good effectiveness is achieved when all below criteria are met:* 1. *Pain is stable or reduced, and swelling is stable or reduced within 24h from the end of **initial hemostatic management*** or at time of discharge from the acute hospitalization (whichever is first).* 2. *By 48 hours from the end of the **initial management***, there is no need for further treatment with hemostatic agents or coagulation factors, or transfusion of other blood products.* 3. *Fasciotomy is either avoided or carried out with blood loss not exceeding the expected amount in a patient with normal hemostasis.* 4. *No unscheduled (re-)interventions are needed for bleeding management within 48 hours from **initial management***.* **Annotation of effective hemostasis with excellent clinical outcome when also: No neurologic dysfunction or limb loss is present at time of discharge from the acute hospitalization (at discharge can be replaced by "at day = 30", whenever applicable).*
Intracranial Bleeding	*Good effectiveness is achieved when all below criteria are met:*

	1. *The hematoma volume is stable, or increased by < 35% as compared to baseline volume, as assessed by a computed tomography (CT) scan within 12h (time window of 6-24h after the index CT).* 2. *No deterioration of the Glasgow Coma Scale (GCS) as assessed at 24h in comparison with that at presentation.* 3. *By 48 hours from the end of the **initial management***, there is no need for further treatment with hemostatic agents or coagulation factors, or transfusion of other blood products.* 4. *Invasive interventions are either avoided or carried out with blood loss not exceeding the expected amount in a patient with normal hemostasis (**§ see below for further clarification**).* 5. No unscheduled (re-)interventions are needed for bleeding management within 48 hours from **initial management***. **Annotation of effective hemostasis with excellent clinical outcome when: No neurologic deterioration/dysfunction is present at discharge from the acute hospitalization (at discharge can be replaced by "at day = 30", whenever applicable) as assessed with any validated scoring system (e.g. GOS-E) as compared to that at presentation.*

*** "Initial Management" –** The term "initial management" is used to indicate any treatment or strategy that is part of the initial bleeding management, including any studied treatment. This can also include infusion of blood products, hemostatic agents, reversal agents, coagulation factors, and invasive interventions with diagnostic and therapeutic intention.

¶ HGB drop – The two hemoglobin values used to determine the hemoglobin drop should be determined as follows:

- The second hemoglobin (T1) value should be the hemoglobin at 48 hours AFTER presentation to hospital, or as close to 48 hours as possible depending on when HGB values were captured. If the patient is discharged in stable condition prior to the 48 hours mark, then the hemoglobin at discharge is used as the second HGB value.
- The first hemoglobin value should be the first hemoglobin value AFTER "end of initial management" of bleeding. A patient may present and require several units of pRBCs and the hemostatic agent in question (i.e. PCC) prior to "end of initial management" of the bleeding. Once initial management has been completed, the hemoglobin that is closest to this "end of initial management" is used as the initial hemoglobin value (T0) to which the HGB value at 48 hours after presentation (T1) is compared.
- **Example:** A patient presents with GI bleeding and a HGB of 120 g/L at presentation. Within the first 12 hours of presentation, they received 5 units of pRBCs and 50 IU/kg of PCC. HGB at 12 hours is 80 g/L. Endoscopy is attempted at 18 hours, bleeding vessel is clipped. The patient did not require further pRBCs after the endoscopic intervention.
    - The HGB at 24 hours is 75 g/L.
    - HGB value at 36 hours is 74 g/L.
    - HGB value at 48 hours is 72 g/L.
    - In this example, the HGB value chosen following "completing of initial management" was at 24 hours, as the investigator deemed completion of initial management to have occurred following the endoscopic intervention, which was accompanied by hemodynamic stabilization. This HGB = 75 g/L value would be selected for T0.
    - In this example, the HGB drop would be 75 g/L (at 24 hours; T0) – 72 g/L (at 48 hours; T1) = 3 g/L. This would be a 3/75 = 4% drop from the hemoglobin value following initial stabilization.
    - As such, hemostasis would be deemed "effective" in this case.

§ Invasive Interventions –

- If invasive interventions are employed as part of routine bleed management and are an expected component of bleed management (i.e. invasive endoscopy and cautery for management of GI bleeding), then hemostastic adjudication should proceed according to bleeding type as outlined above (e.g. if invasive endoscopy is performed for a GI bleed and blood loss/hemostasis is within expected limits, hemostasis should still be considered effective as outlined above).
- However, if initial medical management is attempted and fails, and the treating team must proceed to an invasive intervention to control bleeding that they were trying to avoid, this should be counted as ineffective hemostasis as the invasive intervention in question was not avoided.
- Similarly, if an invasive intervention is attempted but leads to excessive or unusual blood loss as deemed by the treating team, hemostasis should be adjudicated as ineffective.
- Of note – If a procedure is undertaken after initial bleed management for the purposes of diagnosis rather than intervention (e.g. "second look" diagnostic endoscopy), this should NOT be considered ineffective hemostasis. Invasive interventions that are used to deem that hemostasis was ineffective should be interventional in nature (i.e. team is trying to "stop the bleeding".

† **Incomplete Data:** In the event that data required for adjudication of hemostasis is incomplete, an assessment can still be made with respect to ISTH criteria if the incomplete data is a result of clear clinical deterioration despite maximal medical measures and administration of PCC.

- **Example 1:** 91M, previously documented category 3, presents to ER with a severe headache. CT head on presentation reveals intraparenchymal hematoma. GCS on presentation was 15. Patient receives PCC 50 IU/kg shortly after presentation. At 8 hours post-PCC administration, patient's GCS rapidly deteriorates to GCS = 3. Patient is no longer protecting their airway and has fixed, dilated pupil on right side. MRP team contacts family and discusses GOC, and family decides to changes GOC to comfort measures only and forgoes further investigations. No subsequent CT head is performed. Patient dies 12 hours after neurological deterioration. In this case, despite lack of a follow-up CT head, there was clear neurological deterioration that occurred following PCC administration, with well documented initial normal GCS. In this case, an adjudication of "ineffective" hemostasis could be made purely on clinical grounds since not all of the ISTH criteria for effective hemostasis are met, despite lack of follow-up CT head data.

- **Example 2:** 91M, previously documented category 3, brought in from home after being found on ground. GCS = 3 at scene, unresponsive. CT head on presentation reveals intraparenchymal hematoma. ER MRP decides to give PCC 50 IU/kg. Patient's POA is adamant that patient would not have wanted any aggressive measures. Decision is made shortly after PCC administration to transition to full comfort measures. No further investigations or interventions are performed. Patient dies 12 hours after PCC administration. In this case, insufficient data is available to adjudicate hemostasis. The patient had already experienced clear clinical, catastrophic neurological deterioration PRIOR to PCC administration at onset of ICH. No follow-up CT head data is available. As such, adjudication of hemostasis cannot be conducted reliably in this case and should indicate that hemostasis cannot be adjudicated for ISTH criteria.

B- Sarode Criteria (Modified)

Bleeding Type	Rating	Criteria
Visible Bleeding	Good	*Cessation of bleeding ≤ 1 hour after the end of infusion and no additional coagulation intervention* required*
	Moderate	*Cessation of bleeding > 1 and ≤ 4 hours after end of infusion and no additional coagulation intervention* required*
	Poor	*Cessation of bleeding > 4 hours after the end of the infusion, and/or additional coagulation intervention* required*
Musculoskeletal Bleeding	Good	*Pain relief or no increase in swelling or unequivocal improvement in objective signs of bleeding ≤ 1 hour after the end of infusion; and the condition has not deteriorated during the 24-hour period* *AND* *No additional plasma, blood products (whole blood products not including pRBCs) and/or coagulation factor products required after initial treatment with study drug*
	Moderate	*Pain relief or no increase in swelling or unequivocal improvement in objective signs of bleeding > 1 and ≤ 4 hours after the end of infusion; and the condition has not deteriorated during the 24-hour period* *AND* *No more than 2 additional units of plasma, blood products (whole blood products not including pRBCs) and/or coagulation factor products required after initial treatment with study drug*
	Poor	*No improvement by 4 hours after the end of infusion and/or the condition has deteriorated during the 24-hour period* *OR* *More than 2 additional units of plasma, blood products (whole blood products not including pRBCs) and/or coagulation factor products required after initial treatment with study drug*
Intracranial Bleeding	Good	*≤ 20% increase in hematoma volume compared to baseline on repeat CT scan performed at the 6-24 hour timepoint* *AND* *No additional plasma, blood products (whole blood products not including pRBCs) and/or coagulation factor products required after initial treatment with study drug*
	Moderate	*> 20% but ≤ 35% increase in hematoma volume compared to baseline on a repeat CT scan performed at the 6-24 hour time point* *AND* *No more than 2 additional units of plasma, blood products (whole blood products not including pRBCs) and/or coagulation factor products required after initial treatment with study drug*
	Poor	*> 35% increase in hematoma volume compared to baseline on repeat CT scan performed at the 6-24 hour time point* *OR* *More than 2 additional units of plasma, blood products (whole blood products not including pRBCs) and/or coagulation factor products required after initial treatment with study drug*
Non-Visible Bleeding (e.g. GI bleeding)	Good	*≤ 10% decrease in both HGB/HCT¶ at 24 hours compared to baseline†* *AND* *No additional plasma, blood products (whole blood products not including pRBCs) and/or coagulation factor products required after initial treatment with study drug*
	Moderate	*> 10% but ≤ 20% decrease in both HGB/HCT¶ at 24 hours compared with baseline†* *AND* *No more than 2 additional units of plasma, blood products (whole blood products not including pRBCs) and/or coagulation factor products required after initial treatment with study drug*
	Poor	*> 20% decrease in both HGB/HCT¶ at 24 hours compared to baseline†* *OR* *More than 2 additional units of plasma, blood products (whole blood products not including pRBCs) and/or coagulation factor products required after initial treatment with study drug*

*Coagulation intervention: plasma, whole blood cell pack, or coagulation factor products.

¥ **Incomplete Data:** Contrarily to ISTH criteria, adjudication based on Sarode criteria primarily relies on objective data. As such, if insufficient data are available for adjudication, hemostasis cannot be adjudicated. As an example, if a patient with ICH experiences neurological deterioration following PCC administration and no follow-up CT head was obtained, hemostasis cannot be adjudicated using Sarode criteria, although ISTH criteria might still be applied (see ISTH Hemostasis Assessment Guide).

† For consistency, consider the **baseline HGB to be the HGB measured just prior to PCC administration.** In other words, if the patient had been bleeding for a few hours, received several units of pRBCs, HGB was repeated and then PCC was subsequently administered, the HGB just prior to PCC administration should be used as the "baseline" hemoglobin.

¶ The smallest percentage decrease in HGB or HCT should be used to determine the efficacy rating of good, moderate or poor. An adjustment is made for pRBC administration. The assumption is that HGB would increase by 1 g/dL or 3% increase in HCT for each unit of pRBC. This amount is taken into account when estimating the drop in HGB/HCT over the 24 hour period in question. The adjustment to account for transfused pRBCs within the first 24 hours are added to the **initial hemoglobin value**.

Example 1: A patient presenting with a HGB of 69 g/L who received 1 unit of pRBC over the course of 24 hours with a 24 hour HGB of 65 g/L would be considered to have had a 14 g/L (69 + 10 = 79 – 14 = 65 g/L) HGB drop, i.e. 20.3%. The transfused 1u pRBC is added to the baseline HGB (69 + 10 = 79). This corrected initial hemoglobin of 79 g/L is used as the baseline hemoglobin to calculate the drop at 24 hours (79 – 65 = 14 g/L). The 14 g/L recorded changes takes into account the 4 g/L change between initial HGB measurement and the measurement at 24 hours, and the additional 10 g/L is to account for the transfused 1 unit of pRBCs.

Example 2: A patient presenting with HGB of 57 g/L was transfused 4 units of pRBCs over the course of 24 hours. Measured HGB after initial 24 hours was 94 g/L. The baseline HGB adjusted for the transfused pRBCs would be 57 + (4x (10)) = 97 g/L. The calculated drop over the course of 24 hours would be 97 - 94 = 3 g/L. The % drop over 24 hours would be 3/97 = 3.1%, so this case would be rated to have "Good" hemostasis.

C – Thromboembolic Event Outcome Definitions

1. **DVT** is defined as new non-compressibility or intra-luminal filling defect of lower or upper extremity deep venous segments by compression ultrasonography or venogram respectively.

a. Proximal lower extremity DVT is defined as a thrombus within the popliteal or more proximal vessel.
b. Proximal upper extremity DVT is defined as a thrombus within the axillary or a more proximal vein.

2. **PE** is defined as :

- Pulmonary angiography:
  - Intraluminal defects in two or more views.
  - Sudden contrast cut-off of one or more vessels greater than 2.5mm in diameter.
- V/Q Scan:
  - A high probability V/Q lung scan in patients with non-low clinical probability of PE.
- Computed tomographic pulmonary angiography:
  - Filling defect in a subsegmental or larger vessel.

3. **Fatal PE** is defined as:

- Death with no other explanation other than PE and/or autopsy or radiological confirmation.

4. **Ischemic Cerebrovascular Accident** (CVA):

a. Focal neurological deficit from a non-traumatic cause AND/OR

b. Signs of focal ischemic changes and infarction on computed tomography (CT) or magnetic resonance imaging (MRI), with or without the presence of a vascular cutoff or visible thrombus on CT angiography (CTA)

5. Transient Ischemic Attack (TIA):

a. Focal neurological deficit from a non-traumatic cause, lasting less than 24 hours AND
b. No signs of focal ischemic changes on computed tomography (CT) or magnetic resonance imaging (MRI) and the absence of a vascular cutoff or visible thrombus on CTA.

6. Systemic Embolism:

a. Signs/symptoms of focal ischemia and acute loss of blood flow to a peripheral artery (or arteries) in the affected organ (pain, numbness/paresthesias, pallor, poikilothermia)
b. With or without an elevated lactate/creatine kinase
c. AND imaging findings consistent with systemic embolism (angiography, CTA, magnetic resonance angiography)

7. Myocardial Infarction:

- Presence of acute myocardial injury detected by abnormal cardiac biomarkers (CK, TnI) in the setting of evidence of acute myocardial ischemia (ischemic ECG changes AND/OR clinical symptoms)
- AND deemed to be secondary to acute thrombotic coronary artery occlusion either clinically or angiographically (i.e. type 1 myocardial infarction).

8. Heart valve or cardiac chamber thrombosis as detected by transthoracic echocardiography (TTE) or transesophageal echocardiography (TEE)

Chapter 4 – Discussion

The contents of this thesis endeavoured to address four objectives. First, we sought to survey and summarize the literature on the application of thrombin generation assays towards the study of direct oral anticoagulants and anticoagulation reversal strategies. Second, we aimed to evaluate the feasibility of a prospective observational study measuring thrombin generation parameters before and after PCC administration to FXaI-treated patients with major bleeding or needing urgent surgery. Lastly, we sought to collect preliminary data among FXaI-treated patients with respect to the effects of PCC on TGA parameters and clinical outcomes. The work presented herein will form the basis of a research program geared towards evaluating the pharmacodynamic impact of anticoagulant therapies and attempted anticoagulation reversal on TGA parameters. This research program is necessary to better understand how effects on the coagulation system and thrombin generation parameters might impact and help predict thrombotic/bleeding outcomes. An extensive review of the literature on the utility of TGAs towards measuring the pharmacodynamic effects of DOACs on coagulation, as well as the effects of various hemostatic therapies on reversing anticoagulation was conducted. Second, a pilot study was conducted, demonstrating the feasibility of a prospective observational study measuring TGA responses among FXaI-treated patients requiring optimization of hemostasis in the setting of either major bleeding or the need for an urgent invasive procedure. These findings will inform future studies seeking to apply TGAs towards measuring the pharmacodynamic effects of

anticoagulation therapy in various settings, as well as to inform the design and conduct of a full-scale prospective observational study.

The literature review identified a large body of work focused on measuring the effects anticoagulants on TGA parameters. We highlighted distinct TGA effect patterns for FXaI and DTIs, contrasted these patterns with those observed for VKAs and discussed indirect evidence that these differences may correspond to or predict superior antithrombotic efficacy among specific patient populations with unique prothrombotic mechanisms (e.g., antiphospholipid antibody syndrome; APLAS). Many questions remain unanswered surrounding the effects of different anticoagulant classes on coagulation and their relative antithrombotic potency. Thrombin generation could prove useful as tool for delineating the relative antithrombotic efficacy of various anticoagulant classes across differing patient populations. Our review suggests that TGAs might serve as a universal instrument for measuring relative antithrombotic potency. Many clinical scenarios lead physicians to empirically alter anticoagulation therapy due to perceived differences in the efficacy of anticoagulation. Examples include anticoagulation “failure” and recurrent or breakthrough thrombotic events, differences with respect to perceived bleed risk, as well as empiric differences in antithrombotic efficacy in large clinical studies across patient populations due to unclear mechanisms (e.g., cancer-associated VTE, APLAS, unusual site thrombosis, thrombosis in the setting of artificial surfaces such as left ventricular assist devices or mechanical heart valves). Prior studies indicate wide variability in both baseline thrombin generating capacity and DOAC levels, in addition to variability from the resulting pharmacodynamic impact of anticoagulant levels

[45-48]. TGAs provide an overall measure of prothrombotic and antithrombotic contributions to coagulation, thereby providing an opportunity to circumvent the complexities of the coagulation system by capturing a single comprehensive measure of genetic, age- and sex-related, pathological and pharmacological contributions to an individual's thrombotic and hemostatic potential. In this respect, more work is needed to optimize its application towards the study of anticoagulation therapies. Our review exposed several knowledge gaps that should be the focus of future research. First, further work is needed to develop recommendations on standard TGA reaction conditions when applied towards the study of anticoagulation therapy. Moreover, attempts should be undertaken to further delineate age- and sex-specific TGA reference ranges among healthy volunteers. These steps will facilitate the application of TGAs towards the study of anticoagulation therapy. Second, studies are needed to identify predictors of both excessive and inadequate thrombin generation, at baseline and in the context of anticoagulation therapy. Third, TGAs could be used to explore and delineate the differing effects of anticoagulant classes on thrombin generation and how this might relate to both their underlying mechanism of action and relative antithrombotic efficacy. Importantly, our review unearthed few studies attempting to correlate DOAC-altered TGA parameters to clinical outcomes. Given the wide interindividual variability with respect to baseline thrombin generation, DOAC levels and the resulting pharmacodynamic impact on TGA parameters, thrombin generation might offer better correlation to clinically relevant outcomes as compared to DOAC levels alone. Our review provides the groundwork for future studies seeking to relate alterations in thrombin generation profiles to clinically relevant outcomes among anticoagulated

patients. Moving forward, we aim to apply this information towards the study of existing anticoagulants, as well as anticoagulant classes under clinical development such as the factor XIa inhibitors. Our review also identified a substantial body of research on the effects of hemostatic therapies and reversal agents on DOAC-altered TGA parameters. The findings from this review suggest that agents specifically designed for the purpose of reversing anticoagulation do indeed provide true anticoagulation reversal, with resulting normalization of thrombin generation, whereas off-label use of hemostatic therapies such as PCC fails to normalize coagulation and often results in overcorrection of the ETP.

The results from our pilot study support the feasibility of a full-scale prospective observational study. We were able to meet our average monthly recruitment and sample acquisition targets. Moreover, the preliminary results from our pilot observational study support the findings from our literature review. PCC administration to FXaI-treated patients led to a statistically significant improvement in peak and ETP but had no effect on either the LT or TTP. Importantly, the thrombin generation curve remained abnormal in most participants following PCC administration, suggesting that PCC did not normalize coagulation. The proportion of patients with effective clinical hemostasis was lower in our pilot study as compared to prior studies [25, 26]. This finding relates in part to differences in underlying bleed severity and differences in inclusion/exclusion criteria across studies. On the other hand, more than half of patients with major bleeding in our pilot study were identified as having experienced ineffective hemostasis by ISTH criteria. Our findings suggest that there is room for improvement

with respect to attempted anticoagulation reversal and supportive care in this patient population, particularly among patients with severe bleeding (e.g., ICH). Reassuringly, we did not identify any thromboembolic complications within 30 days of PCC administration. There is ongoing debate surrounding the potential thromboembolic risk associated with the administration of both nonspecific prohemostatic agents and andexanet alfa [49-52]. Overcorrection of the ETP was observed in several participants following administration of PCC (**Chapter 3**, **Figure 3**). It is not known whether overcorrection of the ETP following attempted anticoagulation reversal portends increased thromboembolic risk, although indirect evidence supports the notion that ETP provides an indirect measure of thrombotic risk [53]. Our full-scale study will provide further information on the thromboembolic risk associated with PCC administration in a representative patient population.

More comparative research is needed to determine whether sole correction of the ETP is sufficient or whether specific reversal agents that normalize the thrombin generation curve lead to improved clinical hemostatic outcomes. Similarly, we discussed the potential significance of kinetic versus quantitative thrombin generation parameters, and the validity of focusing on a single TGA parameter as opposed to normalization of the thrombin generation curve. Given the ongoing limited availability and high cost of andexanet alfa, non-specific hemostatic agents such as PCC will continue to be used in some regions. More research is warranted to determine whether correction of all TGA parameters is necessary to produce a clinically significant improvement in hemostasis. A randomized controlled trial comparing andexanet alfa to best supportive care among

patients with ICH is ongoing (NCT03661528), although it is unclear whether thrombin generation will be measured as a secondary outcome in this study.

Conclusion and Future Directions

A research program focused on correlating thrombin generation measurements to clinical outcomes has the potential to improve our understanding surrounding the relative efficacies of various antithrombotic therapies and help with the development of improved anticoagulation reversal strategies. The results of our pilot study indicate that a full scale prospective observational study of the effects of PCC on thrombin generation parameters among patients with FXaI-associated bleeding is feasible. PCC is likely to provide a hemostatic benefit, but preliminary thrombin generation results suggest that it falls short of providing complete anticoagulation reversal.

Enrolment into the full-scale prospective observational study continues with anticipated completion by early 2024. The results of a full-scale observational study will provide valuable data on the clinical hemostatic efficacy of PCC, its effects on thrombin generation parameters and will provide further information on its associated thromboembolic risk.

An additional area of study would potentially include a trial comparing aPCC versus PCC for the management of FXaI-associated major bleeding, with measurement of

thrombin generation as a surrogate outcome. The findings from our review suggest that aPCC might provide better correction of thrombin generation parameters, including kinetic parameters, and preclinical data suggests it may lead to superior restoration of clot structure as compared to PCC [19]. Little data exists on the use of aPCC in vivo [54], although one prospective observational study suggested that aPCC is being used at some centers for the management of FXaI-associated bleeding and showed a trend towards superior clinical hemostasis among patients with ICH as compared to PCC [55]. A pilot study comparing aPCC to PCC would provide valuable data on the relative thromboembolic risks associated with these two management strategies.

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