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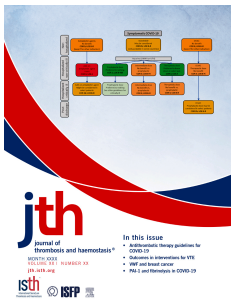

Effect of heparins, DOACs, and FXII/FXI inhibitors on catheter-initiated thrombin generation in PRP: an in vitro study.

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Effect of heparins, DOACs, and FXII/FXI inhibitors on catheter-initiated thrombin generation in PRP: *an in vitro* study.

Short title: Anticoagulants effect on coagulation on catheters.

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Abstract

Background: Invasive endovascular procedures often require anticoagulation to prevent device-associated thrombosis. High doses of unfractionated heparin (UFH) are the gold standard. The efficacy of factor XII(a) and XI(a) inhibitors remains unexplored.

Objectives: We aim to investigate, using a validated *in vitro* model of catheter-initiated thrombin generation (TG) in platelet-rich plasma (PRP), the effects of garadacimab, abelacimab, asundexian, milvexian, and commonly used anticoagulants apixaban, dabigatran, fondaparinux and heparins (enoxaparin, UFH).

Methods: Anticoagulants were added to PRP at six concentrations and effects compared with UFH as a reference. The impact of adding anticoagulants into collection tubes before most preanalytical contact activation was also assessed. Concentrations achieving a 50% reduction in TG peak height (PH) were determined (IC50).

Results: All anticoagulants prolonged lag time (LT) and reduced PH and endogenous thrombin potential, albeit to varying extents. UFH was the only anticoagulant able to suppress TG at clinically achieved concentrations (1 U/mL). The following IC50 were found: milvexian 2,400 ng/mL, apixaban and dabigatran 175 ng/mL, fondaparinux 2.4 µg/mL, enoxaparin 0.5 U/mL and UFH 0.25 U/mL. Asundexian up to 5,000 ng/mL failed to reduce TG PH by 50%. Adding garadacimab and abelacimab to collection tubes resulted in greater efficacy than when added to PRP: LT increased by 89% and 32%, respectively; IC50 were 25 and 12.5 µg/mL, respectively.

Conclusions: Heparins were by far the most effective anticoagulants in our *in vitro* model. Factor XIIa and XI(a) inhibitors did not adequately prevent coagulation on the studied artificial surface. DOACs and fondaparinux showed limited efficacy, aligning with clinical observations.

Keywords: catheter; thrombin generation; anticoagulants; factor XIa inhibitors; heparin; direct oral anticoagulants.

Introduction

Invasive medical procedures such as interventional radiology or cardiology procedures are increasingly widely used because of their better tolerability and faster recovery compared with conventional surgery (1, 2). They involve the introduction of plastic sheaths and metallic instruments via the arterial or venous route to reach the target organ and perform the procedure (e.g., cerebral aneurysm embolization, catheter ablation of atrial fibrillation (AF), vascular stenting…). The presence of foreign bodies in the bloodstream activates hemostasis, with a risk of potentially dramatic thromboembolic events (e.g., strokes) (3).

To prevent this thrombotic risk, antithrombotic drugs, most often an anticoagulant, are administered in high doses for the duration of the procedure. The gold standard for anticoagulation during invasive procedures is unfractionated heparin (UFH). Direct oral anticoagulants have so far failed to get the same efficacy in preventing thromboembolism associated with foreign surfaces in the few contexts clinically studied (mechanical heart valves (MHV)) or in *in vitro* models (extracorporeal circuits, catheters) (4-9). However, the *in vitro* studies were performed with platelet-poor plasma (PPP), where the absence of platelets leads to an overestimation of the effect of UFH, seriously biasing the results (10-13).

The development of a new class of anticoagulants, the inhibitors of (activated) factors XI or XII (FXI(a) or FXII(a)), seems promising in the context of thrombosis associated with foreign surfaces, initiated by the activation of factor XII, which activates in turn factor XI. This class comprises antisense oligonucleotides (degradation of factor XI mRNA (IONIS-FXIRx), small inhibitors that bind to factor XIa active site (asundexian, milvexian) (14-16), and an array of monoclonal antibodies directed against factor XIIa (garadacimab), factor XIa (xisomab, osocimab) or both factors XI and XIa (abelacimab). While factor XII(a) inhibitors have not yet been studied in humans as antithrombotic agents, anticoagulants targeting FXI(a) were evaluated for thromboprophylaxis in nonvalvular AF (17, 18), postoperative thromboprophylaxis after major orthopaedic surgery (19-22), secondary prevention of stroke (23, 24) or acute myocardial infarction (25); their place in the prevention of thrombosis associated with foreign surfaces has not yet been studied in humans or *in vitro* with human blood, except small studies in end-stage renal disease patients undergoing hemodialysis (26-28).

The present study primarily aimed to compare the efficacy of the main classes of anticoagulants used clinically – low molecular weight heparins (LMWH), fondaparinux, direct oral anticoagulants (DOACs) and the new factor XI(a) or XII(a) inhibitors in mitigating *in vitro* thrombin generation in the presence of a medical device, i.e. an interventional cardiology catheter, and to compare their effect with that of UFH, the gold standard, using a previously validated catheter-initiated thrombin generation model (13).

Methods

This study was conducted at the CHU UCL Namur with the approval from the local Ethics Committee (NUB: B03920096633). Healthy volunteers were recruited from the healthcare staff. Exclusion criteria included any systemic disease, drugs, or substances that could interfere with haemostasis, including contraceptive pills.

Garadacimab (supplied in a 100 mM proline-acetate buffer with 20 mM arginine), abelacimab (supplied in 20 mM HEPES buffer with 150 mM NaCl and 240 mM sucrose), asundexian (supplied as powder, diluted to a 1 mg/mL stock solution in DMSO), milvexian (supplied as powder, diluted to a 1 mg/mL stock solution in DMSO), apixaban (supplied as powder, diluted to a 1 mg/mL stock solution in DMSO) and dabigatran (supplied as powder, diluted to a 1 mg/mL solution in DMSO with HCl 0.1 M) were purchased from MedChemExpress (Monmouth Junction, USA) and handled according to manufacturer's recommendations. Stock solutions were aliquoted and frozen at -80°C until use. Aliquots were quickly thawed in a water bath at 37°C for 5 minutes. Further dilutions were performed in polyphosphate-buffered

saline when needed. Fondaparinux (supplied in water for injection with sodium chloride, chlorhydric acid and sodium hydroxyde) was purchased from Viatris (Canonsburg, USA). Enoxaparin (supplied in water for injection) was purchased from Sanofi Belgium (Machelen, Belgium). Unfractionated heparin (supplied in water for injection with benzyl alcohol, methyl parahydroxybenzoate, propyl parahydroxybenzoate, sodium citrate, sodium chloride, and pH adjusted with hydrochloric acid) was purchased from Leo Pharma (Lier, Belgium).

(i) Effect of anticoagulant drugs on catheter- or ellagic acid-initiated thrombin generation.

Blood was collected in the morning from four fasting volunteers who had not previously performed any strenuous physical effort or taken tobacco or coffee. Blood was collected by antecubital venipuncture into 109 mM citrate tubes (Vacuette, Greiner Bio-One, Vilvoorde, Belgium) after a first discarded priming tube. Blood was allowed to rest for 15 minutes before undergoing centrifugation at 200g for 10 minutes at 20°C to prepare the platelet-rich plasma (PRP). PRP was carefully harvested and pooled, left unadjusted regarding platelet count, aliquoted and spiked with a solution containing the anticoagulant drug to be studied (1:19 v:v to minimize plasma dilution). A rest period of 15 minutes was observed after spiking anticoagulant solutions to ensure sufficient plasma incubation of the inhibitors.

Each healthy volunteer was included on two days to assess the effect of several concentrations of a wide range of anticoagulants. On the first day, the effect of drugs acting on factor IIa and/or factor Xa (enoxaparin, fondaparinux, apixaban, dabigatran) was compared with that of UFH. On the second day, the effect of drugs acting on factor XI(a) or factor XIIa (asundexian, milvexian, abelacimab, garadacimab) was compared with that of UFH. Vitamin K antagonists were not studied since mere spiking cannot mimic their effect. The concentrations were chosen as multiples of the average maximum concentration (C_{max}) observed in the thromboembolic prevention of AF (or of venous thromboembolism if not available), or equivalent: 0.5 U/mL for UFH (29), 1.0 U/mL for enoxaparin (30), 1.2 µg/mL (694 nM) for fondaparinux (31, 32), 175 ng/mL (381 and 371 nM) for apixaban and dabigatran (33), 600 ng/mL (1.01 and 0.96 µM) for asundexian and milvexian (34, 35), 50 µg/mL (346 nM) for abelacimab (36); as such data was not available for garadacimab in an antithrombotic setting, we used the same concentration as for abelacimab, i.e. 50 µg/mL (344 nM). Each drug was studied at six concentrations: $6*C_{max}$, $4*C_{max}$, $2*C_{max}$, $1*C_{max}$, $0.5*C_{max}$, $0.25*C_{max}$; e.g., for UFH: 3, 2, 1, 0.5, 0.25, 0.125 U/mL. A condition without anticoagulant (vehicle) was also performed at each run.

Thrombin generation initiated by catheter segments was studied using our previously validated model developed with the Calibrated Automated Thrombogram (CAT, Diagnostica Stago, Asnières-sur-Seine, France) (13). Briefly, interventional cardiology catheter segments (Launcher, Medtronic, Minneapolis, USA) were cut, flattened, and inserted circumferentially at the bottom of the wells in a multi-well plate (Immulon 2 HB flat bottom microtiter plates, Thermofisher, Rochester, USA). In each well, 80 µL PRP was added, with 20 µL phosphate-buffered saline (PBS) or calibrator (Thrombin Calibrator, Diagnostica Stago) in the *ad hoc* wells. After a 10-minute incubation, 20 µL trigger reagent (FluCa, Diagnostica Stago) were added to the wells by the automated dispensing system before an automated stirring. The fluorescence signal was then monitored by a Fluoroskan FL (Thermo Scientific, Illkirch) for 60 minutes and converted into thrombin concentration. Each experimental condition was studied in triplicate. We also verified the effect of the drugs on thrombin generation initiated by a reference activator of the contact pathway, ellagic acid (MedChem Express, Monmouth Junction, USA; 20 µL of 1 µM solution added to the reaction well), as a reference condition.

As primary endpoint, we assessed, for each anticoagulant separately, the ability of increasing concentrations to prolong the lag time (LT), decrease the peak height (PH) and the endogenous thrombin potential (ETP) using Friedman tests, treating each volunteer as a block to account for inter-individual

variability. The effect of the drugs was then compared with the reference anticoagulant, UFH, using linear mixed-effects models (lme4 package version 1.1-32),modelling an exponential PH decrease as drug concentration increased. Random intercepts were modelled to capture within-subject correlation. P-values were calculated according to Satterthwaite's method and residual diagnostics showed no major deviation. Statistical analyses were performed in R (version 4.2.3).

As secondary endpoints, (i) we estimated, among the concentrations studied, the concentration that reduced by minimum 50 % the PH (IC_{50}), except for dabigatran, were it was the concentration that minimum doubled the LT (37). (ii) We explored a higher concentration range with the PRP of a single healthy volunteer for anticoagulants were the IC_{50} could not be determinated (weak TG inhibition). (iii) We calculated the concentration required to suppress thrombin generation at 60 min. (iv) We assessed inter-individual variability in catheter-initiated thrombin generation.

(ii) Assessment of the impact of pre-analytical contact pathway activation in assessing anticoagulant drugs effect in vitro.

We assessed whether contact pathway activation during the pre-analytical phase led to a significant bias in the evaluation of the effect of anticoagulants with our model. To do so, we compared thrombin generation initiated by the catheter after adding the anticoagulant either directly into the tube before blood collection or spiked into the PRP as previously described. Blood was collected and processed as described above from two healthy volunteers (given the low interindividual variability observed during the first part of the study). Sarstedt sodium citrate 109 mM tubes (Nümbrecht, Deutschland) were used in this experiment because they allow the vacuum to be created manually after the anticoagulant solution has been added to the tube.

In the test group, the anticoagulant solution was added to the tubes before blood collection, and water for injection was added to the PRP before analysis (Figure 1). In the control group, the same volume of water for injection was added to the tubes before blood collection and the anticoagulant solution was added to the PRP before analysis. The concentrations of anticoagulant added to the tubes were estimated based on the last known hematocrit of healthy volunteers and assuming no entry into cells (red blood cells), to aim for the same plasma concentration as added above in PRP. For practical reasons, only one concentration, C_{max}, was assessed for each anticoagulant. We measured the effect of garadacimab, abelacimab, asundexian, milvexian, apixaban, dabigatran, enoxaparin, UFH and corn trypsin inhibitor (CTI; 40 µg/mL in PRP) a potent FXIIa inhibitor as a positive control (38). We also performed the experiment without any dedicated activator to measure the effect of the drugs on coagulation that is artefactually initiated during blood collection, in the collecting tube, or on the walls of the wells. Thrombin generation was studied on the same system after initiation by a catheter segment.

To assess the difference between the test group and the control group, we calculated the reference change value (35). Based on the experimental variability observed during previous model validation (analytical coefficient of variation of 3.7% and intra-individual coefficient of variation of 10.4% for PH (13)), we estimate that a difference of more than 21% between the two conditions (i.e. anticoagulant added to the collection tube vs. to the PRP), for each of the two volunteers, would be clinically relevant.

Finally, in case of better inhibition of catheter-initiated TG by the anticoagulant added into the tube compared with plasma, we repeated the experiment, this time spiking the entire concentration range studied into the collection tube.

Results

(i) Effect of anticoagulant drugs on catheter- or ellagic acid-initiated thrombin generation.

Four healthy volunteers (two men and two women, one premenopausal and one postmenopausal) aged 25 to 55 were included. Platelet counts ranged from 167 to 277.10^9/L in (EDTA) whole blood, and ranged from 209 to 345.10^9/L in citrated PRP. Standard coagulation tests (activated partial thromboplastin time, prothrombin time, Clauss fibrinogen) were within local reference ranges for all healthy volunteers. Interindividual variation in catheter-initiated thrombin generation among the four volunteers was 5.9 % for LT, 12.7 % for PH and 8.7 % for ETP (interindividual coefficient of variation) in the absence of added anticoagulant.

Prolongations of LT and reductions of PH and ETP at each concentration studied are reported in Table 1. All anticoagulants mitigated catheter-initiated thrombin generation LT, PH and ETP ($p<0.05$ for all drugs and parameters; Figure 2) and ellagic acid-initiated thrombin generation ($p<0.05$ for all drugs and parameters; Figure S2). All anticoagulants studied were less effective than UFH at mitigating catheter-initiated thrombin generation PH ($p<0.001$ for all), except enoxaparin ($p=0.60$). The IC_{50} (the concentration studied that reduced by minimum 50 % the PH) was 0.5*C_{max} for abelacimab (i.e. 25 µg/mL), UFH (i.e. 0.25 U/mL) and enoxaparin (i.e. 0.5 U/mL), 1*C_{max} for apixaban and dabigatran (i.e. 175 ng/mL, 2*C_{max} for fondaparinux (2.4 µg/mL), and 4*C_{max} for milvexian (2,400 ng/mL). For asundexian, even concentrations as high as 5,000 ng/mL could not decrease thrombin generation (PH) by 50% (Figure S1). With garadacimab, we observed a plateau: above 19 µg/mL, there was no longer any significant reduction in thrombin generation, which remained at the level observed in the absence of catheter segment and anticoagulant (Figure 3). UFH was the only anticoagulant capable of suppressing thrombin generation at concentrations compatible with clinical care (i.e., 1.0 U/mL).

(ii) Analysis of the role of pre-analytical activation in assessing the effect of anticoagulant drugs on thrombin generation in vitro.

Two volunteers, a man and a post-menopausal woman, were included. When CTI, garadacimab and abelacimab were added in the collecting tube, before most of the preanalytical activation of factor XII took place, the inhibition of thrombin generation was greater (LT +63%, +89% and +32%, and PH -37%, -46% and -28%, respectively) than when it was added to the plasma after centrifugation (Table 2, Figure 4); these differences were greater than the predefined threshold of 21% for both healthy volunteers. However, the addition of the anticoagulant to the tube did not alter the ability of asundexian, milvexian, apixaban, dabigatran, fondaparinux, enoxaparin, and UFH to mitigate catheter-initiated thrombin generation, compared to their effect when added directly to the PRP. The results for thrombin generation in the absence of a catheter are shown in Figure S3.

Finally, we repeated the catheter-initiated thrombin generation measurement after addition of garadacimab and abelacimab directly into the collection tube (Figure 5). The IC50 was 0.5*Cmax for garadacimab (25 µg/mL) and less than 0.25*Cmax for abelacimab (< 12.5 µg/mL). However, neither drug was capable of suppressing thrombin generation at the maximum concentration tested (200 µg/mL).

Discussion

To our best knowledge, this is the first study to have compared the ability of such a large panel of anticoagulant drugs to inhibit coagulation activation at a foreign surface. Using our *in vitro* model, we

observed that factor XIIa and XI(a) inhibitors were less effective than heparins (UFH or LMWH) in mitigating catheter-initiated thrombin generation. We could also repeat in an *in vitro* model the clinical observations showing the lack of efficacy of DOACs in the prophylaxis of contact-initiated thrombosis, for example in the MHV setting (4, 5).

UFH and enoxaparin were the most effective in our hands, abolishing catheter-initiated thrombin generation at concentrations of 1.0 U/mL and 2.0 U/mL respectively. In clinical studies, the UFH concentrations measured during interventional cardiology procedures ranged between 1.0 and 2.0 U/mL, which seems entirely consistent with our results (39). The concentrations of enoxaparin required to obtain a similar effect to UFH were just over twice as high. The anti-Xa/IIa activity ratio of enoxaparin is around 4 (40), suggesting, as already demonstrated, that the effect of heparins is mainly driven by their action on thrombin (41, 42). Accordingly, fondaparinux, which does not inhibit thrombin, was unable to suppress catheter-initiated thrombin generation, even at high concentrations (6 times the average peak concentration observed in clinical practice for venous thromboembolism treatment), confirming a previous study performed in the absence of platelets (43). Fondaparinux effect seems closer to that of apixaban, a direct factor Xa inhibitor, than that of UFH or enoxaparin. In accordance, although more effective than heparin in limiting mortality and the occurrence of ischemic complications in acute coronary syndrome, fondaparinux use was associated with an increased incidence of catheter-associated thrombosis in a large randomized trial (44, 45). This suggests less effectiveness than UFH or LMWH in limiting thrombosis associated with medical devices.

Regarding DOACs, apixaban could not suppress catheter-initiated thrombin generation at the concentrations studied (up to 1,000 ng/mL). At this concentration only, dabigatran could suppress thrombin generation in our model. Previous *in vitro* data demonstrated that supratherapeutic concentrations of apixaban and dabigatran were required to blunt MHV-initiated thrombin generation to a similar extent than warfarin (i.e., 210-396 ng/mL for apixaban and 254-488 ng/mL for dabigatran) (46, 47). Another work concluded that 300 ng/mL of dabigatran was ineffective at preventing thrombus formation at the surface of MHV in a whole blood *in vitro* model under flow conditions, whereas 600 ng/mL of dabigatran was as effective as UFH (~0.7 U/mL) and enoxaparin (1 U/mL) (48). Similarly, in *ex vivo* bypass models, very high concentrations of dabigatran (> 5,000 ng/mL) were required to avoid clot formation and fibrin deposition for two hours as effectively as UFH control (3 U/mL). Furthermore, two prospective randomized controlled multicenter trials comparing apixaban and dabigatran to vitamin K antagonists, the gold standard anticoagulant in thromboprophylaxis of MHV, identified a higher incidence of thromboembolic events with the DOACs (4, 5). Despite different models used in those studies, this suggests that, overall, DOACs are less effective at inhibiting thrombin generation initiated by the contact pathway, i.e. by activation of factor XII on foreign surfaces.

The three factor XIa inhibitors studied could delay and reduce catheter-initiated thrombin generation, although only at concentrations higher than those used in current phase III trials for asundexian. The difference observed between asundexian and milvexian is consistent with the pharmacological data from the phase I studies, which measured an almost 10-fold higher inhibition constant (Ki) for asundexian compared with milvexian (Ki ~ 1 nM vs 0.11 nM), as already pointed out (49). Besides, none of these agents could suppress thrombin generation in our model at the concentrations tested, contrary to UFH or LMWH. In the setting of contact activation, three other factor XI(a) inhibitors, osocimab, xisomab and the antisense oligonucleotide IONIS-FXIRx have been studied in patients with end-stage renal disease undergoing haemodialysis. Among these, osocimab reduced dialysis circuit clotting compared to placebo in an exploratory analysis, but the studies performed with xisomab and IONIS-FXIRx were too small to draw any conclusions (26-28). However, it is unlikely that osocimab will perform better than abelacimab, as the former has 1,000 times less affinity for factor XIa than abelacimab (50, 51).

The effect of garadacimab, the only factor XIIa inhibitor studied, is a little more complex to study *in vitro*. *In vitro*, the auto-activation of factor XII on foreign surfaces is independent of calcium ions, as is

the activation of factor XI by factor XIIa (52); these two reactions can therefore take place in a citrated medium. Activation of factor IX and other downstream factors, on the opposite, requires calcium ions (53). During blood collection and tube processing, a small proportion of factor XII can be activated on the sampling system and the walls of the tube, activating a small amount of factor XI. This factor XIa generated during the preanalytic phase can no longer be inhibited by a factor XII(a) or a factor XI (non-activated) inhibitor. This phenomenon, well described for CTI, an inhibitor of factor XIIa (and to a lesser extent factor XIa) (54, 55), only becomes significant when the activating stimulus is weak, which is the case with our catheter model (56, 57). Consequently, the effect of garadacimab, a factor XIIa inhibitor, and to a lesser extent abelacimab, a factor XI (dissociation constant, K_D 1.3 pM) and XIa (K_D 4.7 pM) inhibitor (50), was underestimated in our *in vitro* model. Adding these two drugs to the collection tube, bypassing most of the preanalytical artefact, revealed their true potential, which however remains lower than that of heparins. The clinical implication is that these two anticoagulants will only express their maximum effect if they are administered before the contact of blood with the foreign material.

Targeting the inactive zymogen rather than its activated protease could be a more efficient way to mitigate surface-driven thrombin generation. Abelacimab illustrates this approach: it targets the catalytic domain of both factor XI zymogen and its activated form, locking the protein in an inactive conformation (50). Nevertheless, abelacimab leaves the upstream contact system untouched. Once factor XII auto-activates on a procoagulant surface, it produces plasma kallikrein, which can directly activate factor IX, bypassing FXIa and allowing some thrombin to be generated (58). A more effective blockade of contact activation could therefore require inhibition at the level of FXII zymogen itself, instead of activated factor XII or factor XI.

In this study we were not able to test Ixodes ricinus contact phase inhibitor (Ir-CPI), a protein extracted from the tick *Ixodes Ricinus*, which specifically binds both factor XIIa and factor XIa. This drug has demonstrated a capacity in reducing thrombin generation in a previous model of catheter-induced thrombin generation performed with frozen-thaw PPP (59). A phase IIa trial with Ir-CPI in patients suffering from spontaneous intracerebral hemorrhage is ongoing. We also could not study vitamin K antagonists, as their effect cannot be reproduced *in vitro* by mere spiking. Studying them under our conditions would have meant working with fresh patient samples, implying considerable variability and methodological constraints. However, as UFH is the reference anticoagulant for the perioperative setting, the comparisons made in this study seem entirely sound. Finally, it might be worth investigating whether the combined use of a factor XII(a) or XI(a) inhibitor with heparin could provide effective and safer anticoagulation than with either class of drug alone.

This study has several limitations. First, it is an *in vitro* study using a simplified model. Several factors, such as the absence of endothelium, red blood cells or flow, represent important limitations to draw definitive conclusions. Nevertheless, thrombin generation is the most accurate model for the fine study of coagulation and our results correlate with the findings of large clinical phase III trials (60). Our model incorporates platelets, which enables the correct assessment of heparins (10-13) and pharmacological inhibitors of factors XI(a) and XII(a) effect. Indeed, activated platelets release factor XI and polyphosphates, which promote optimal factor XI activation (61, 62). Moreover, the presence of platelets is necessary for predicting the bleeding phenotype of patients with inherited factor XI deficiency using a thrombin generation assay (63, 64). Although working with platelets increases experimental variability, the platelet counts of all PRPs studied ranged between 209 to 345 10^9/L, which is within the 50 – 400 10^9/L range where thrombin generation is considered independent of platelet count (65). We also studied fresh plasma, avoiding the artefacts associated with plasma freeze-thaw cycles (66). Second, the effect of dabigatran is slightly overestimated when measured with the thrombin generation assay due to the inhibition of the thrombin-alpha2-macroglobulin complex by this drug (and not by UFH or LMWH) (67, 68). However, this should not alter the conclusions of this study, as, despite this, dabigatran failed to suppress thrombin generation at the concentrations used clinically.

In conclusion, using our *in vitro* model in the presence of platelets, factor XIIa and XI(a) inhibitors failed to suppress thrombin generation initiated at the catheter surface, whereas heparins did. However, these findings should be confirmed in clinical trials. In line with clinical observations, DOACs appear limited in mitigating thrombin generation in this setting, at least at concentrations compatible with clinical use. Overall, these results call for caution in using factor XII(a) and XI(a) inhibitors in situations associated with a high risk of thrombosis on foreign surfaces, such as MHV or ECMO.

Authorship Contributions
Conceptualization: MH, JD, TL, SL, FM; Investigation: MH; Formal analysis: MH, FM; Writing - Original Draft: MH, TLE, FM; Writing - Review & Editing: MH, HT, JD, JV, IGT, SL, TL, FM.

Disclosure of Conflicts of Interest
HT, JV, IGT, SL, TL: none.
MH reports research grants from the Belgian National Fund for Scientific Research and from the Fondation Mont-Godinne.
JD reports honoraria from Gedeon Richter Benelux and Mithra Pharmaceuticals outside the submitted work. He is also the CEO and founder of QUALIblood s.a., a contract research organization manufacturing the DP-Filter.
FM reports speaker's fees from Diagnostica Stago (Asnières-sur-Seine, France) outside the submitted work.

Data Sharing Statement
Original data are available upon reasonable request from the first author (michael.hardy@uclouvain.be).

References

1. Dabu-Bondoc S. NonOperating Room Anesthesia: distancing from invasive surgery, embracing the era of interventional medicine. Curr Opin Anaesthesiol. 2017;30(6):639-43.
2. Cheng Y, Li L. Interventional Medicine. J Interv Med. 2018;1(1):1-2.
3. Jaffer IH, Fredenburgh JC, Hirsh J, Weitz JI. Medical device-induced thrombosis: what causes it and how can we prevent it? J Thromb Haemost. 2015;13 Suppl 1:S72-81.
4. Eikelboom JW, Connolly SJ, Brueckmann M, Granger CB, Kappetein AP, Mack MJ, et al. Dabigatran versus warfarin in patients with mechanical heart valves. N Engl J Med. 2013;369(13):1206-14.
5. Wang TY, Svensson LG, Wen J, Vekstein A, Gerdisch M, Rao VU, et al. Apixaban or Warfarin in Patients with an On-X Mechanical Aortic Valve. NEJM Evidence. 2023;2(7):EVIDoa2300067.
6. Nadtochiy SM, Stefanos T, Angona RE, Lebedko N, Baldzizhar A, Feng C, et al. Rivaroxaban Reduces the Dabigatran Dose Required for Anticoagulation During Simulated Cardiopulmonary Bypass. Anesth Analg. 2022;135(1):52-9.
7. Nadtochiy SM, Baldzizhar A, Stefanos T, Feng C, O'Leary KE, Jones-Smith KL, et al. High-Dose Dabigatran Is an Effective Anticoagulant for Simulated Cardiopulmonary Bypass Using Human Blood. Anesth Analg. 2021;132(2):566-74.
8. Yau JW, Liao P, Fredenburgh JC, Roberts RS, Weitz JI. Only high levels of dabigatran attenuate catheter thrombosis in vitro and in rabbits. Thromb Haemost. 2014;112(1):79-86.
9. Engelen MM, Van Laer C, Jacquemin M, Vandenbriele C, Peerlinck K, Verhamme P, et al. Heparin is more effective than apixaban in inhibiting in vitro contact activated coagulation. European Heart Journal. 2020;41(Supplement_2).
10. Gerotziafas GT, Petropoulou AD, Verdy E, Samama MM, Elalamy I. Effect of the anti-factor Xa and anti-factor IIa activities of low-molecular-weight heparins upon the phases of thrombin generation. J Thromb Haemost. 2007;5(5):955-62.
11. Norrheim L, Abildgaard U, Larsen ML, Lindahl AK. Involvement of the extrinsic pathway in the activities of low molecular weight heparins. Thromb Res Suppl. 1991;14:19-27.
12. Hemker HC, Beguin S, Bendetowicz AV, Wielders S. Determination of the levels of unfractionated and low-molecular-weight heparins in plasma: their effect on thrombin-mediated feedback reactions in vivo. Preliminary results after subcutaneous injection. Haemostasis. 1991;21(4):258-72.
13. Hardy M, Douxfils J, Xhaet O, Robaye B, Lessire S, Lecompte T, et al. Development and validation of an in vitro model to study thrombin generation on the surface of catheters in platelet-poor and platelet-rich plasma. Thromb Res. 2024;244:109194.
14. Heitmeier S, Visser M, Tersteegen A, Dietze-Torres J, Glunz J, Gerdes C, et al. Pharmacological profile of asundexian, a novel, orally bioavailable inhibitor of factor XIa. J Thromb Haemost. 2022;20(6):1400-11.
15. Roehrig S, Ackerstaff J, Jimenez Nunez E, Teller H, Ellerbrock P, Meier K, et al. Design and Preclinical Characterization Program toward Asundexian (BAY 2433334), an Oral Factor XIa Inhibitor for the Prevention and Treatment of Thromboembolic Disorders. J Med Chem. 2023;66(17):12203-24.
16. Dilger AK, Pabbisetty KB, Corte JR, De Lucca I, Fang T, Yang W, et al. Discovery of Milvexian, a High-Affinity, Orally Bioavailable Inhibitor of Factor XIa in Clinical Studies for Antithrombotic Therapy. J Med Chem. 2022;65(3):1770-85.
17. Piccini JP, Caso V, Connolly SJ, Fox KAA, Oldgren J, Jones WS, et al. Safety of the oral factor XIa inhibitor asundexian compared with apixaban in patients with atrial fibrillation (PACIFIC-AF): a multicentre, randomised, double-blind, double-dummy, dose-finding phase 2 study. Lancet. 2022;399(10333):1383-90.
18. Ruff CT, Patel SM, Giugliano RP, Morrow DA, Hug B, Kuder JF, et al. Abelacimab versus Rivaroxaban in Patients with Atrial Fibrillation. N Engl J Med. 2025;392(4):361-71.
19. Weitz JI, Strony J, Ageno W, Gailani D, Hylek EM, Lassen MR, et al. Milvexian for the Prevention of Venous Thromboembolism. N Engl J Med. 2021;385(23):2161-72.

20. Weitz JI, Bauersachs R, Becker B, Berkowitz SD, Freitas MCS, Lassen MR, et al. Effect of Osocimab in Preventing Venous Thromboembolism Among Patients Undergoing Knee Arthroplasty: The FOXTROT Randomized Clinical Trial. JAMA. 2020;323(2):130-9.
21. Verhamme P, Yi BA, Segers A, Salter J, Bloomfield D, Buller HR, et al. Abelacimab for Prevention of Venous Thromboembolism. N Engl J Med. 2021;385(7):609-17.
22. Buller HR, Bethune C, Bhanot S, Gailani D, Monia BP, Raskob GE, et al. Factor XI antisense oligonucleotide for prevention of venous thrombosis. N Engl J Med. 2015;372(3):232-40.
23. Shoamanesh A, Mundl H, Smith EE, Masjuan J, Milanov I, Hirano T, et al. Factor XIa inhibition with asundexian after acute non-cardioembolic ischaemic stroke (PACIFIC-Stroke): an international, randomised, double-blind, placebo-controlled, phase 2b trial. Lancet. 2022;400(10357):997-1007.
24. Sharma M, Molina CA, Toyoda K, Bereczki D, Bangdiwala SI, Kasner SE, et al. Safety and efficacy of factor XIa inhibition with milvexian for secondary stroke prevention (AXIOMATIC-SSP): a phase 2, international, randomised, double-blind, placebo-controlled, dose-finding trial. Lancet Neurol. 2024;23(1):46-59.
25. Rao SV, Kirsch B, Bhatt DL, Budaj A, Coppolecchia R, Eikelboom J, et al. A Multicenter, Phase 2, Randomized, Placebo-Controlled, Double-Blind, Parallel-Group, Dose-Finding Trial of the Oral Factor XIa Inhibitor Asundexian to Prevent Adverse Cardiovascular Outcomes After Acute Myocardial Infarction. Circulation. 2022;146(16):1196-206.
26. Weitz JI, Tanko LB, Floege J, Fox KAA, Bhatt DL, Thadhani R, et al. Anticoagulation with osocimab in patients with kidney failure undergoing hemodialysis: a randomized phase 2 trial. Nat Med. 2024;30(2):435-42.
27. Lorentz CU, Tucker EI, Verbout NG, Shatzel JJ, Olson SR, Markway BD, et al. The contact activation inhibitor AB023 in heparin-free hemodialysis: results of a randomized phase 2 clinical trial. Blood. 2021;138(22):2173-84.
28. Walsh M, Bethune C, Smyth A, Tyrwhitt J, Jung SW, Yu RZ, et al. Phase 2 Study of the Factor XI Antisense Inhibitor IONIS-FXI(Rx) in Patients With ESRD. Kidney Int Rep. 2022;7(2):200-9.
29. Garcia DA, Baglin TP, Weitz JI, Samama MM. Parenteral anticoagulants: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e24S-e43S.
30. Sanofi. Lovenox 100 mg/1 mL solution for injection [SmPC]. European Medicines Agency; 2017. Available from: https://www.ema.europa.eu/en/documents/referral/lovenox-article-30-referral-annex-iii_en.pdf.
31. Boneu B, Necciari J, Cariou R, Sie P, Gabaig AM, Kieffer G, et al. Pharmacokinetics and tolerance of the natural pentasaccharide (SR90107/Org31540) with high affinity to antithrombin III in man. Thromb Haemost. 1995;74(6):1468-73.
32. Donat F, Duret JP, Santoni A, Cariou R, Necciari J, Magnani H, et al. The pharmacokinetics of fondaparinux sodium in healthy volunteers. Clin Pharmacokinet. 2002;41 Suppl 2:1-9.
33. Douxfils J, Adcock DM, Bates SM, Favaloro EJ, Gouin-Thibault I, Guillermo C, et al. 2021 Update of the International Council for Standardization in Haematology Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. Thromb Haemost. 2021;121(8):1008-20.
34. Thomas D, Kanefendt F, Schwers S, Unger S, Yassen A, Boxnick S. First evaluation of the safety, pharmacokinetics, and pharmacodynamics of BAY 2433334, a small molecule targeting coagulation factor XIa. J Thromb Haemost. 2021;19(10):2407-16.
35. Perera V, Wang Z, Luettgen J, Li D, DeSouza M, Cerra M, et al. First-in-human study of milvexian, an oral, direct, small molecule factor XIa inhibitor. Clin Transl Sci. 2022;15(2):330-42.
36. Yi BA, Freedholm D, Widener N, Wang X, Simard E, Cullen C, et al. Pharmacokinetics and pharmacodynamics of Abelacimab (MAA868), a novel dual inhibitor of Factor XI and Factor XIa. J Thromb Haemost. 2022;20(2):307-15.

37. Evrard J, Hardy M, Dogne JM, Lessire S, Maloteau V, Mullier F, et al. Are the DOAC plasma level thresholds appropriate for clinical decision-making? A reappraisal using thrombin generation testing. Int J Lab Hematol. 2021;43(1):e48-e51.
38. Spronk HM, Dielis AW, Panova-Noeva M, van Oerle R, Govers-Riemslag JW, Hamulyak K, et al. Monitoring thrombin generation: is addition of corn trypsin inhibitor needed? Thromb Haemost. 2009;101(6):1156-62.
39. Benali K, Verain J, Hammache N, Guenancia C, Hooks D, Magnin-Poull I, et al. Running after Activated Clotting Time Values in Patients Receiving Direct Oral Anticoagulants: A Potentially Dangerous Race. Results from a Prospective Study in Atrial Fibrillation Catheter Ablation Procedures. J Clin Med. 2021;10(18).
40. Frydman AM, Bara L, Le Roux Y, Woler M, Chauliac F, Samama MM. The antithrombotic activity and pharmacokinetics of enoxaparine, a low molecular weight heparin, in humans given single subcutaneous doses of 20 to 80 mg. J Clin Pharmacol. 1988;28(7):609-18.
41. Beguin S, Lindhout T, Hemker HC. The mode of action of heparin in plasma. Thromb Haemost. 1988;60(3):457-62.
42. Colman RW, Scott CF, Pixley RA, de la Cadena RA. Effect of heparin on the inhibition of the contact system enzymes. Ann N Y Acad Sci. 1989;556:95-103.
43. Yau JW, Stafford AR, Liao P, Fredenburgh JC, Roberts R, Weitz JI. Mechanism of catheter thrombosis: comparison of the antithrombotic activities of fondaparinux, enoxaparin, and heparin in vitro and in vivo. Blood. 2011;118(25):6667-74.
44. Fifth Organization to Assess Strategies in Acute Ischemic Syndromes I, Yusuf S, Mehta SR, Chrolavicius S, Afzal R, Pogue J, et al. Comparison of fondaparinux and enoxaparin in acute coronary syndromes. N Engl J Med. 2006;354(14):1464-76.
45. Mehta SR, Boden WE, Eikelboom JW, Flather M, Steg PG, Avezum A, et al. Antithrombotic therapy with fondaparinux in relation to interventional management strategy in patients with ST- and non-ST-segment elevation acute coronary syndromes: an individual patient-level combined analysis of the Fifth and Sixth Organization to Assess Strategies in Ischemic Syndromes (OASIS 5 and 6) randomized trials. Circulation. 2008;118(20):2038-46.
46. Jaffer IH, Stafford AR, Fredenburgh JC, Whitlock RP, Chan NC, Weitz JI. Dabigatran is Less Effective Than Warfarin at Attenuating Mechanical Heart Valve‐Induced Thrombin Generation. Journal of the American Heart Association. 2015;4(8):e002322.
47. Jaffer IH, Fredenburgh JC, Stafford A, Whitlock RP, Weitz JI. Rivaroxaban and Dabigatran for Suppression of Mechanical Heart Valve-Induced Thrombin Generation. Ann Thorac Surg. 2020;110(2):582-90.
48. Maegdefessel L, Linde T, Krapiec F, Hamilton K, Steinseifer U, van Ryn J, et al. In vitro comparison of dabigatran, unfractionated heparin, and low-molecular-weight heparin in preventing thrombus formation on mechanical heart valves. Thromb Res. 2010;126(3):e196-200.
49. Vassart J, Didembourg M, Morimont L, Brisbois C, Jamart L, Lebreton A, et al. Asundexian in atrial fibrillation: Can pharmacodynamic data explain the failure? Thromb Res. 2024;236:236-9.
50. Koch AW, Schiering N, Melkko S, Ewert S, Salter J, Zhang Y, et al. MAA868, a novel FXI antibody with a unique binding mode, shows durable effects on markers of anticoagulation in humans. Blood. 2019;133(13):1507-16.
51. Schaefer M, Buchmueller A, Dittmer F, Strassburger J, Wilmen A. Allosteric Inhibition as a New Mode of Action for BAY 1213790, a Neutralizing Antibody Targeting the Activated Form of Coagulation Factor XI. J Mol Biol. 2019;431(24):4817-33.
52. Ataullakhanov FI, Pohilko AV, Sinauridze EI, Volkova RI. Calcium threshold in human plasma clotting kinetics. Thromb Res. 1994;75(4):383-94.

53. Sinha D, Seaman FS, Walsh PN. Role of calcium ions and the heavy chain of factor XIa in the activation of human coagulation factor IX. Biochemistry. 1987;26(13):3768-75.
54. Hansson KM, Nielsen S, Elg M, Deinum J. The effect of corn trypsin inhibitor and inhibiting antibodies for FXIa and FXIIa on coagulation of plasma and whole blood. J Thromb Haemost. 2014;12(10):1678-86.
55. Butenas S, Mann KG. The effect of corn trypsin inhibitor and inhibiting antibodies for FXIa and FXIIa on coagulation of plasma and whole blood: comment. J Thromb Haemost. 2015;13(3):487-8.
56. Luddington R, Baglin T. Clinical measurement of thrombin generation by calibrated automated thrombography requires contact factor inhibition. J Thromb Haemost. 2004;2(11):1954-9.
57. Van De Berg T, Suylen DPL, Thomassen MGLCD, van Oerle R, Spronk HMH, Apitz R, et al. Novel Thermostable Inhibitor of Contact Activation Tica Effectively Blocks Contact Activation in Low Tissue Factor Thrombin Generation. Blood. 2019;134(Supplement_1):1146-.
58. Kearney KJ, Butler J, Posada OM, Wilson C, Heal S, Ali M, et al. Kallikrein directly interacts with and activates Factor IX, resulting in thrombin generation and fibrin formation independent of Factor XI. Proc Natl Acad Sci U S A. 2021;118(3).
59. Pireaux V, Tassignon J, Demoulin S, Derochette S, Borenstein N, Ente A, et al. Anticoagulation With an Inhibitor of Factors XIa and XIIa During Cardiopulmonary Bypass. J Am Coll Cardiol. 2019;74(17):2178-89.
60. Tripodi A. Thrombin Generation Assay and Its Application in the Clinical Laboratory. Clin Chem. 2016;62(5):699-707.
61. Choi SH, Smith SA, Morrissey JH. Polyphosphate is a cofactor for the activation of factor XI by thrombin. Blood. 2011;118(26):6963-70.
62. Zucker M, Hauschner H, Seligsohn U, Rosenberg N. Platelet factor XI: intracellular localization and mRNA splicing following platelet activation. Blood Cells Mol Dis. 2018;69:30-7.
63. Pike GN, Cumming AM, Hay CR, Bolton-Maggs PH, Burthem J. Sample conditions determine the ability of thrombin generation parameters to identify bleeding phenotype in FXI deficiency. Blood. 2015;126(3):397-405.
64. Rugeri L, Quelin F, Chatard B, De Mazancourt P, Negrier C, Dargaud Y. Thrombin generation in patients with factor XI deficiency and clinical bleeding risk. Haemophilia. 2010;16(5):771-7.
65. Gerotziafas GT, Depasse F, Busson J, Leflem L, Elalamy I, Samama MM. Towards a standardization of thrombin generation assessment: the influence of tissue factor, platelets and phospholipids concentration on the normal values of Thrombogram-Thrombinoscope assay. Thromb J. 2005;3:16.
66. Stief TW, Otto S, Renz H. Intrinsic hemostasis activation by freezing and thawing of plasma. Clin Appl Thromb Hemost. 2009;15(1):59-64.
67. Bloemen S, Zwaveling S, Douxfils J, Roest M, Kremers R, Mullier F. The anticoagulant effect of dabigatran is reflected in the lag time and time-to-peak, but not in the endogenous thrombin potential or peak, of thrombin generation. Thromb Res. 2018;171:160-6.
68. Wagenvoord RJ, Deinum J, Elg M, Hemker HC. The paradoxical stimulation by a reversible thrombin inhibitor of thrombin generation in plasma measured with thrombinography is caused by alpha-macroglobulin-thrombin. J Thromb Haemost. 2010;8(6):1281-9.

Figure captions

Figure 1: Experimental design of the second part of the study comparing two time-points for addition of anticoagulants. In the control condition (left), a phosphate saline buffer (PBS) solution was added in the collection tube before blood sampling and the anticoagulant was added to platelet-rich plasma (PRP) after centrifugation (200g, 15 min, 20°C). In the test condition (right), the anticoagulant was added in the collection tube before blood sampling, and PBS was added to PRP. Thrombin generation (TG) was measured after initiation with a catheter segment or in the absence of any added activator (= TG initiated during the preanalytical phase or by the wells' wall).

Figure 2: Effect on catheter-initiated thrombin generation of the nine anticoagulants. Thrombin generation (TG) was studied in triplicate with platelet-rich plasma (PRP) from four healthy volunteers. The tracings represent the mean thrombin generated over time from four volunteers. The black curve represents TG in the absence of anticoagulants, while the blue curves correspond to thrombin generation in the presence of anticoagulants, with lighter shades indicating higher concentrations. Garadacimab and abelacimab at 50 µg/mL correspond to approximately 345 nM; asundexian and milvexian at 600 ng/mL correspond to approximatively 1 µM; apixaban and dabigatran at 175 ng/mL correspond to approximatively 375 nM; and fondaparinux at 1.2 µg/mL corresponds to approximatively 700 nM. Six concentrations were tested for each anticoagulant.
UFH, unfractionated heparin.

Figure 3: Assessment of the effect of an extended range of garadacimab concentrations on catheter-initiated thrombin generation (TG) in platelet-rich plasma (PRP). The maximum possible concentration (i.e., 900 µg/mL) was added to assess whether there was a plateau in the effect of the drug. A condition without anticoagulant (black line), and a condition without anticoagulant and without catheter (red line) were also used. The blue curves correspond to TG in the presence of garadacimab, with lighter shades indicating higher concentrations. Garadacimab at 50 µg/mL correspond to approximately 345 nM, based on an estimated molecular weight of 145 kDa. TG was studied in triplicate with a single healthy volunteer PRP.

Figure 4: Analysis of the impact of preanalytical activation of the contact pathway on the estimation of the effect of the nine anticoagulants and corn trypsin inhibitor (CTI) on catheter-initiated thrombin generation (TG). Anticoagulants were added either to platelet-rich plasma (PRP) after centrifugation at C_{max} (yellow lines; see Methods), or in the collection tube before blood collection (blue lines), aiming for the same C_{max} in PRP (see Methods). An anticoagulant-free condition was also studied (grey lines). All TG experiments were performed in triplicate and the mean thrombin generated over time for two healthy subjects is shown. Please note that, for the curves that do not return to baseline (CTI, garadacimab, abelacimab, enoxaparin added to collection tubes), thrombin levels at all time points are slightly overestimated due to the impossibility of subtracting by the software the *in vitro* effect of the thrombin-α2-macroglobulin complex which has no activity against fibrinogen *in vivo*.
CTI, corn trypsin inhibitor; UFH, unfractionated heparin.

Figure 5: Effect of garadacimab and abelacimab added in the collection tube on catheter-initiated thrombin generation (TG). The experiment was performed in triplicate with platelet-rich plasma (PRP) from two healthy volunteers. The mean thrombin curves generated in PRP from two volunteers are represented. The black curve represents TG in the absence of anticoagulants, while the blue curves correspond to thrombin generation in the presence of anticoagulants, with lighter shades indicating higher concentrations. Garadacimab and abelacimab at 50 µg/mL correspond to approximately 345 nM, based

on an estimated molecular weight of 145 kDa. Please note that the curves that do not return to baseline (100 and 200 µg/mL for both drugs) are slightly overestimated due to the impossibility of subtracting the artefactual effect of the thrombin-α2-macroglobulin complex by the software.

	6*C_{max}	4*C_{max}	2*C_{max}	1*C_{max}	0.5*C_{max}	0.25*C_{max}	C_0
Lag time (min)							
Apixaban	11.4	10.2	8.2	7.6	7.5	7.2	7.2
Dabigatran	> 25.0	23.8	21.8	16.0	13.1	10.8	7.2
Enoxaparin	-	-	> 32.2	18.3	12.9	9.9	7.2
Fondaparinux	12.4	12.2	10.3	9.7	8.5	7.8	7.2
UFH	-	-	-	29.5	21.5	14.3	7.2
Asundexian	12.0	11.0	9.3	7.7	7.7	7.7	8.0
Milvexian	21.0	17.9	14.7	11.2	9.2	8.4	8.0
Abelacimab	22.5	21.7	19.7	18.4	17.5	16.1	8.0
Garadacimab	14.4	14.3	14.8	14.7	13.4	10.6	8.0
Peak height (nM)							
Apixaban	32	42	50	68	90	115	134
Dabigatran	2	7	57	94	113	121	134
Enoxaparin	0	0	13	31	64	96	134
Fondaparinux	34	40	61	74	102	118	134
UFH	0	0	0	30	69	97	134
Asundexian	97	106	121	132	135	138	146
Milvexian	40	60	76	107	127	132	146
Abelacimab	40	43	50	59	67	78	146
Garadacimab	93	99	100	97	104	118	146
Endogenous thrombin potential (nM*min)							
Apixaban	< 712	< 951	1174	1355	1474	1539	1543
Dabigatran	n.c.	n.c.	582	1077	1411	1626	1543
Enoxaparin	n.c.	n.c.	n.c.	< 574	1037	1330	1543
Fondaparinux	583	666	918	1084	1312	1389	1543
UFH	n.c.	n.c.	n.c.	n.c.	< 1059	1321	1739
Asundexian	1512	1565	1672	1735	1764	1770	1739
Milvexian	917	1176	1333	1508	1694	1658	1739
Abelacimab	< 807	< 836	1015	1128	1219	1341	1739
Garadacimab	1396	1456	1500	1473	1521	1583	1739

Table 1: Mean lag time (LT), peak height (PH) and endogenous thrombin potential (ETP) for catheter-initiated thrombin generation measured in platelet-rich plasma from four healthy volunteers, for nine anticoagulants at six concentrations studied. C_0 is the reference condition without anticoagulant. Estimated C_{max} observed in the thromboembolic prevention of AF (or of venous thromboembolism if not available) are 50 µg/mL (~345 nM) for garadacimab and abelacimab, 600 ng/mL (~1 µM) for asundexian and milvexian, 175 ng/mL (~375 nM) for apixaban and dabigatran, 0.5 U/mL for unfractionated heparin, 1.0 U/mL for enoxaparin, and 1.2 µg/mL (~700 nM) for fondaparinux. The mean thrombin generation parameters across the four volunteers are represented. As experiments with anticoagulants were separated on two different plates on two different days, the reference condition without anticoagulant (C_0) is not the same for all the anticoagulants.

UFH, unfractionated heparin; n.c., not calculable (no return of the thrombin generation tracing to baseline during the measurement period, precluding calculation of the area under the curve; in this setting, PH is likely to be overestimated as well).

Angle brackets are used when LT was > 60 min or when ETP was not calculable for only one or two volunteers. When a LT or an ETP was only available for one volunteer, we did not provide a value in the table.

	LT (min)	PH (nM)	ETP (nM*min)
CTI (40 µg/mL)	22.4 – 36.5 **(+63%)**	46 – 29 **(-37%)**	1143 – n.c.
Garadacimab (50 µg/mL)	22.4 – 42.3 **(+89%)**	50 - 27 **(-46%)**	1144 – n.c.
Abelacimab (50 µg/mL)	27.0 – 35.6 **(+32%)**	22 - 16 **(-28%)**	568 – n.c.
Milvexian (600 ng/mL)	19.6 – 18.9 (-4%)	39 – 44 (+13%)	856 – 870 (+2%)
Asundexian (600 ng/mL)	10.9 – 9.6 (-12%)	100 – 121 (+21%)	1459 – 1441 (-1%)
Apixaban (175 ng/mL)	12.2 – 11.0 (-10%)	59 – 56 (-6%)	1279 – 1329 (+4%)
Fondaparinux (1.2 µg/mL)	16.4 – 15.1 (-8%)	29 – 25 (-14%)	654 – 565 (-14%)
Dabigatran (175 ng/mL)	21.7 – 22.6 (+4%)	52 – 58 (-7%)	831 – 794 (-4%)
Enoxaparin (1.0 U/mL)	25.0 – 28.4 (+14%)	19 – 13 (-28%*)	510 – n.c.
UFH (0.5 U/mL)	28.6 – 24.2 (-15%)	5 – 4 (-20%)	n.c. – n.c.
No anticoagulant	9.4	100	1442

Table 2: Effects of adding the anticoagulant in the collection tube (test condition) vs. to the plasma (control condition). Anticoagulant solutions were added to achieve C_{max} in platelet-rich plasma (PRP), based on the last known hematocrit value of the volunteer and assuming no entry into cells (red blood cells). The catheter-initiated thrombin generation parameters (LT, PH and ETP) are presented as control condition – test condition followed by the difference expressed in percentage of control condition (in brackets). Each parameter represents the mean of results with PRP from two healthy volunteers. Bold numbers represent differences above the predefined threshold (21%; see Methods) for both volunteers. The asterisk indicates differences above the threshold for only one of the two volunteers (enoxaparin: PH from 18 to 16 nM (-11%) for volunteer 1; 19 to 10 nM (-45%) for volunteer 2). Garadacimab and abelacimab at 50 µg/mL correspond to approximately 345 nM; asundexian and milvexian at 600 ng/mL correspond to approximatively 1 µM; apixaban and dabigatran at 175 ng/mL correspond to approximatively 375 nM; and fondaparinux at 1.2 µg/mL corresponds to approximatively 700 nM.
LT, lag time; PH, peak height; ETP, endogenous thrombin potential; n.c., not calculable (no return of the thrombin generation tracing to baseline during the measurement period, precluding calculation of the area under the curve); UFH, unfractionated heparin.