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Thrombin generation to evaluate the complex hemostatic balance of hemophilia A plasma containing direct oral anticoagulant and supplemented by factor VIII

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Thrombin generation to evaluate the complex hemostatic balance of a hemophilia A plasma containing DOAC and supplemented by FVIII.

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Essentials

- Age related thrombotic events requiring anticoagulation increase in hemophilia A patients.
- The hemostatic balance with emicizumab, direct oral anticoagulant (DOAC) and FVIII is not known.
- FVIII can counter DOAC concentration up to 100 ng/ml in presence of emicizumab.
- The combined effect of the hemostatic capacity can guide clinicians to manage urgent events.

Abstract

Background: The incidence of cardiovascular diseases is increasing in patients with hemophilia A (HA). Therefore, anticoagulant therapy based on direct oral anticoagulants (DOAC) may be needed, despite the bleeding risk. In case of surgery or bleeding, such patients may be concomitantly treated with emicizumab (routine prophylaxis), factor (F) VIII products, and DOAC. Their concomitant presence constitutes a hemostatic challenge. Recent international guidelines stated that data are scarce on the hemostatic balance of plasma samples from patients with HA receiving emicizumab and DOAC.

Objectives: The aim of this observational study was to assess the coagulation of FVIII-deficient plasma spiked with DOAC and emicizumab and to evaluate the effects of FVIII addition.

Methods: Prothrombin time, activated partial thromboplastin time, and thrombin generation (TG) using the calibrated automated thrombogram method were evaluated in aliquots of a commercial severe HA plasma supplemented with emicizumab (0, 12.5, 25, 50 and 100 ng/ml), DOAC (0, 50, 100, 200 and 400 ng/ml of apixaban, rivaroxaban, edoxaban, or dabigatran) and FVIII (0, 5, 15, 50 and 100%).

Results: DOAC rapidly induced a TG decrease. Emicizumab could counter this effect only for the lowest DOAC dose. FVIII addition to the FVIII-deficient plasma containing a DOAC and emicizumab improved TG and countered the anticoagulant the effect of DOAC at ≤ 100 ng/ml.

Conclusion: Our findings indicate that FVIII can be safely used with emicizumab to counter the anticoagulant effect of DOAC at ≤ 100 ng/ml. The TG assay is an efficient tool to monitor plasma containing anti-FXa DOAC, but not dabigatran (anti-FIIa).

Keywords

Thrombin, emicizumab, direct oral anticoagulant, hemophilia A, monitoring

1. Introduction

Hemophilia A (HA) is an inherited bleeding disorder characterized by a quantitative or qualitative coagulation factor (F) VIII defect. For several decades, HA management has been based on intravenous FVIII infusions that have significantly improved life expectancy [1–3]. The prophylaxis regimen (2 to 3 intravenous infusions of FVIII per week) reduces bleeding events, but is burdensome for patients, thus causing treatment adherence issues [4, 5]. Recently, non-factor replacement strategies have emerged, such as the antibody emicizumab that mimics FVIII activity, the small interfering RNA fitusiran that targets antithrombin and the antibody concizumab against tissue factor pathway inhibitor [6–8]. Emicizumab is a bispecific monoclonal antibody that ensures the spatial interaction of FIXa with FX, thus mimicking FVIIIa procoagulant activity [9]. The HAVEN studies demonstrated that emicizumab reduces the annual bleeding rate in patients with HA (with or without inhibitors), compared to FVIII products [10–13].

The significant improvement in HA management has led to an increase in quality of life and life expectancy. Consequently, age-related diseases, such as cardiovascular diseases (CVD), are now observed in patients with HA. It has been shown that patients with HA have a similar or higher risk of hypertension, atherosclerosis, atrial fibrillation and myocardial infarction compared with the general population [14–17]. Several risk factors of CVD are particularly frequent in patients with HA, for instance physical inactivity, chronic inflammation and smoking [18]. CVD management in patients with HA requires to fine tune the hemostatic balance to avoid both thrombotic and bleeding events. Indeed, direct oral anticoagulants

(DOAC) can be used in patients with HA and a CVD, but their effect will counteract that of the prophylaxis treatment [19, 20]. Recent international guidelines on the management of CVD in patients with HA recommend antithrombotic treatment for patients with FVIII > 20% [21].

Conversely, data on patients on emicizumab prophylaxis are scarce. Yet, the concomitant use of emicizumab and a DOAC in patients with HA leads to new hemostatic challenges. Emicizumab concentration can be assessed using a modified one-stage assay and an emicizumab calibrator. However, caution is needed to interpret these results because the test is influenced by endogenous FVIII [22]. Emicizumab can interfere with several laboratory tests, such as activated partial thromboplastin time (aPTT) and FVIII dosage, that rely on FVIII and FIX activity. Therefore, they cannot be used to monitor hemostasis in patients on emicizumab-based prophylaxis [23]. Alternative assays, such as chromogenic assays, thrombin generation assays (TGA) and thromboelastography, have been proposed as more reliable methods to monitor coagulation in these patients [24–26]. Decreased thrombin generation has been associated with the bleeding phenotype severity in patients with HA [27]. Moreover, TGA allowed identifying the synergistic effect of activated prothrombin complex concentrates (APCC) and emicizumab *in vitro* [28]. TGA is a promising tool for monitoring patients with HA treated with emicizumab because it allows assessing their hemostasis balance regardless of their inhibitor status [29]. Little is known concerning hemostasis in patients with HA receiving both emicizumab and DOAC because routine coagulation tests cannot be used due to the interference of these drugs. Yet, data on their hemostatic balance are needed and are crucial in case of urgent surgery. To this aim, here, we analyzed the effect on TGA of various concentrations of FVIII added to a HA plasma sample spiked with emicizumab and different DOAC at different concentrations (within the therapeutic range). This analysis will provide data for the management of patients with HA treated with emicizumab and who require an anticoagulant. These data will also be useful for the management of bleeding events or surgery in patients with HA treated with emicizumab and anticoagulant therapy.

2. Methods

2.1 Sample preparation

All plasma samples (n=125) were prepared using a commercial FVIII-deficient plasma sample (Cryopep) in which emicizumab (Roche-Chugai), one of four DOAC (apixaban, rivaroxaban,

edoxaban or dabigatran) (Alsachim) and plasma-derived FVIII (Factane, LFB) were added at various doses within the therapeutic range [30]. Specifically, FVIII was added to obtain a final concentration of 0, 5, 15, 50 and 100% (corresponding to 0, 5, 15, 50 and 100 IU/dL), emicizumab to obtain a final concentration of 0, 12.5, 25, 50 and 100 ng/ml (trough plasma levels between 13.4 and 55.2 ng/ml, depending on the regimen [31]), and DOAC to obtain a final concentration of 0, 50, 100, 200 and 400 ng/ml (50 ng/ml represents a subtherapeutic dose and 400 ng/ml is a suprathreshold dose). For each drug, a dosage has been performed to ensure that the right amount was added using the corresponding test and reagents on STA R Max analyzer: emicizumab control/calibrator (r2 diagnostics), Immuno defVIII (Stago), apixaban/rivaroxaban/edoxaban/dabigatran control/calibrator (Stago). For each aliquot, the volume of each added drug represented 1% of the final volume and therefore, the total added volume did not exceed 3%. The commercial solution of emicizumab has been used, FVIII and DOAC were diluted in sterile water and the control sample was spiked with 3% of sterile water. Supplementary Figure 1 shows the schematic representation of the study design.

2.2 Determination of FVIII-deficient plasma characteristics and measurement of PT and aPTT

Coagulation assays were done on STA-R coagulation analyzer using the relevant reagents (Stago). STA-Neoptimal was used for the measurements of prothrombin time (PT) and some factors (FII, FV, FVII, FX), STA-PTTA for aPTT (STA-CK-PREST when activated with kaolin) and intrinsic factors, and STA Liquid Fib for fibrinogen using the Clauss method. The relevant deficient plasma references were used for factor measurements (Stago): STA-deficient II, STA-deficient V, STA deficient VII, STA deficient X, STA deficient VIII, STA deficient IX, STA ImmunoDef XI, STA ImmunoDef XII. STA-ATIII, STACLOT PC, STACLOT PS, STACLOT APCR were used in functional assays to measure antithrombin, protein C, protein S and resistance to protein C, respectively.

2.3 Measurement of emicizumab and exogenous FVIII activity in plasma

To assess the FVIII-like activity of emicizumab in the FVIII-deficient plasma, a modified one-stage FVIII assay was used as previously described [32]. This assay was calibrated using the emicizumab calibrator from r² Diagnostics on a STA-R max analyzer (Stago). This assay

measures the “global” FVIII-like activity due to emicizumab, endogenous and exogenous FVIII. In addition, a chromogenic FVIII assay and bovine reagents were used to determine the exogenous FVIII activity.

2.4 Thrombin generation assay

TGA was used to assess the coagulation of plasma samples supplemented with emicizumab, DOAC and exogenous FVIII. TGA was performed in duplicate using the calibrated thrombogram (CAT) method (Hemker 2002), a fluorometer (Fluoroscanner Ascent, ThermoLab Systems, Franklin, TN, USA) equipped with a dispenser, PPP reagent [5 pM tissue factor (TF), 4 μ M phospholipids, final concentrations], PPP_{low} reagent (1 pM TF, 4 μ M phospholipids, final concentrations) and FluCa kit (Stago). PPP reagent triggers TG with an intermediate TF concentration while PPP_{low} uses a low TF concentration. Before each assay, plates were incubated at 37°C for 10 min. Raw data were analyzed using the Thrombinoscope software (Stago). Endogenous thrombin potential (ETP) and thrombin peak were the main parameters analyzed. ETP is the area under the thrombin generation curve, reflecting the total amount of thrombin generated throughout the assay. Thrombin peak represents the maximum thrombin concentration reached during the coagulation process. As this was an observational study, no statistical analysis was performed.

3. Results

3.1 Characteristics of the FVIII-deficient plasma

Table 1 summarizes the result of coagulation parameter analysis in the FVIII-deficient plasma. Analysis of the FVIII activity before spiking confirmed that the purchased plasma had a FVIII concentration < 1% (i.e. severe HA) and that all the other coagulation factors were normal or just below the normal levels. In this FVIII-deficient plasma, aPTT (97.1s) and aPTT with kaolin (86.7s) were prolonged, whereas PT (14.4s) and fibrinogen concentration (3.3g/L) were within the reference ranges. ETP and thrombin peak were below the reference range, as expected.

3.2 DOAC effect on FVIII-deficient plasma containing emicizumab

3.2.1 Coagulation parameters

In the presence of emicizumab (0 to 100 ng/ml), PT was not affected upon addition of apixaban or dabigatran (range 14.9 - 19.2s), but was prolonged after addition of rivaroxaban or edoxaban (range 14.3 – 29.9s). Anti-Xa DOAC increased aPTT in a dose-dependent manner (range 74.3 - 116.9s at 50 ng/ml to 119 – 169.2s at 400 ng/ml); aPTT reached 180s with 50 ng/ml of dabigatran. In presence of emicizumab, aPTT was shortened, whatever the dose or DOAC used (**Supplementary Table S1**).

3.2.2 Thrombin generation

In FVIII-deficient plasma spiked with increasing emicizumab concentrations, ETP ranged between 725 and 1025 nM.min and the thrombin peak from 56 to 86 nM when TG was initiated with an intermediate TF concentration. Similarly, ETP was between 203 and 550 nM.min and thrombin peak between 9 and 31 nM when TG was triggered with a low TF concentration (**Figure 1**). Inter-assay CV is 10.65% for ETP and 12.50% for peak thrombin while intra-assay CV is ranging from 0.28% to 13.82% for ETP and from 0.32% to 11.61% for thrombin peak.

Apixaban addition decreased thrombin generation in a dose-dependent manner in plasma with/without emicizumab (**Figure 1**). ETP (TGA with intermediate TF) ranged from 372 to 817 nM.min, 256 to 638 nM.min, 166 to 673 nM.min, and 257 to 531 nM.min after addition of 50, 100, 200 and 400 nM.min apixaban, respectively (**Figure 1A**). Thrombin peak ranged from 15 to 44 nM, 10 to 26 nM, 6 to 16 nM, and 9 to 18 nM after addition of 50, 100, 200 and 400 ng/ml apixaban, respectively (**Figure 1E**). This apixaban-induced effect was only slightly compensated by emicizumab. Thrombin generation triggered with low TF could be measured only at the lowest dose of apixaban (50 ng/ml) and in samples with emicizumab. ETP ranged from 0 to 218 nM.min and thrombin peak from 0 to 10 nM (**Figure 1I and M**).

Rivaroxaban addition gave similar results. In the TGA with PPP, ETP ranged from 496 to 770 nM.min, 300 to 563 nM.min, 180 to 387 nM.min, and 0 to 111 nM.min for 50, 100, 200 and 400 nM.min rivaroxaban, respectively (**Figure 1B**). Thrombin peak ranged from 20 to 35 nM, 11 to 25 nM, 7 to 16 nM, and 0 to 4 nM for 50, 100, 200 and 400 ng/ml, respectively (**Figure 1F**). Thrombin generation triggered with intermediate TF concentration gave ETP comprised

between 0 and 171 nM.min and thrombin peak between 0 and 7 nM (**Figure 1J and N**) with 50 ng/ml rivaroxaban.

Similarly, in the TGA with intermediate TF concentration, ETP ranged from 567 to 957 nM.min, 360 to 753 nM.min, 202 to 402 nM.min, and 86 to 222 nM.min for 50, 100, 200 and 400 nM.min edoxaban respectively (**Figure 1C**). Thrombin peak ranged from 25 to 52 nM, 15 to 37 nM, 8 to 18 nM, and 3 to 9 nM for 50, 100, 200 and 400 ng/ml, respectively (**Figure 1G**). Unlike apixaban and rivaroxaban, thrombin generation triggered with low TF concentration could be measured also in the presence of 100 ng/ml edoxaban. ETP ranged from 0 to 322 nM.min and 0 to 145 nM.min for 50 and 100 ng/ml edoxaban. Thrombin peak ranged from 0 to 7 nM and from 0 to 6 nM at 50 ng/ml and 100 ng/ml edoxaban, respectively (**Figure 1K and O**).

On the other hand, dabigatran supplementation only slightly affected thrombin generation in the TGA with intermediate TF concentration. ETP ranged from 792 to 1125 nM.min, 777 to 1084 nM.min, 643 to 936 nM.min, and 644 to 937 nM.min with 50, 100, 200 and 400 nM.min dabigatran, respectively (**Figure 1D**). Thrombin peak ranged from 53 to 111 nM, 51 to 97 nM, 43 to 94 nM, and 41 to 109 nM in the presence of 50, 100, 200 and 400 ng/ml dabigatran, respectively (**Figure 1H**). Unlike the other tested DOAC (anti-FXa molecules), thrombin generation triggered with low TF concentration could be measured at all dabigatran (anti-FIIa DOAC) doses. ETP ranged from 81 to 735 nM.min, 47 to 581 nM.min, 0 to 556 nM.min, and 0 to 557 nM.min for 50, 100, 200 and 400 ng/ml dabigatran, respectively. Thrombin peak ranged from 4 to 43 nM, 3 to 37 nM, 0 to 35 nM, and 0 to 39 nM at 50, 100, 200 and 400 ng/ml dabigatran, respectively (**Figure 1L and P**).

Thrombin generation seemed to reach a plateau at the therapeutic dose of emicizumab (50 ng/ml). Increasing the DOAC dose decreased ETP and thrombin peak; however, emicizumab (at all doses) still exerted its procoagulant activity.

3.3 Effect of FVIII supplementation on FVIII-deficient plasma containing emicizumab and DOAC

3.3.1 Coagulation parameters

Upon FVIII addition (different doses) to FVIII-deficient plasma spiked with emicizumab and DOAC, PT was not affected whatever the dose used (ranging from 14.3 to 18.5s for the all

range of emicizumab and apixaban used). The aPTT values were not significantly altered by FVIII in the presence of emicizumab, ranging for example from 25.1 to 33.3s for all emicizumab (except 0 ng/ml) and apixaban doses (**Supplementary Table 2**).

3.3.2 Thrombin generation

In the absence of FVIII, ETP (TGA with intermediate TF concentration) increased from 372 to 817, 256 to 408, 166 to 373, and 256 to 531 nM.min and thrombin peak from 15 to 44, 10 to 26, 6 to 16, and 9 to 18 nM in the presence of emicizumab and 50, 100, 200 and 400 ng/ml apixaban, respectively (**Figure 2A-B**). Supplementation with 15% FVIII increased ETP from 523 to 844, 393 to 668, 225 to 402, and 307 to 581 nM.min and thrombin peak from 25 to 49, 16 to 28, 9 to 18, and 10 to 20 nM in plasma samples with 50, 100, 200 and 400 ng/ml apixaban, respectively. As observed for emicizumab, high FVIII doses led to minor differences. Indeed, increasing the FVIII dose (50 – 100 %) in plasma containing emicizumab increased ETP from 769 to 1002, 642 to 961, 382 to 730, and 466 to 823 nM.min and thrombin peak from 45 to 90, 27 to 62, 16 to 36, and 16 to 40 nM in plasma spiked with 50, 100, 200 and 400 ng/ml apixaban, respectively. Data on the time to peak parameter are accessible in Supp Figure 2. When TGA was carried out with low TF concentration, thrombin generation could not be measured in samples spiked with the lowest FVIII doses (5-15%), but gave profiles similar to those obtained with intermediate TF concentration for the highest FVIII dose (**Figure 2C-D**). In samples with 200 ng/ml apixaban, ETP ranged from 0 to 75 nM.min with 15% FVIII and from 109 to 290 nM.min with 50-100% FVIII. Thrombin peak followed the same trend: 0 to 3 nM with 15% FVIII and 5 to 15 nM with 50 - 100% FVIII.

In the absence of FVIII, ETP (TGA with intermediate TF concentration) varied from 496 to 770, 300 to 563, 180 to 387, and 0 to 111 nM.min and thrombin peak from 20 to 35, 11 to 25, 7 to 16, and 0 to 4 nM in plasma with emicizumab and 50, 100, 200 and 400 ng/ml rivaroxaban, respectively (**Figure 2, E-F**). Supplementation with 15% FVIII increased ETP from 695 to 865, 430 to 727, 174 to 374, and 142 to 205 nM.min and thrombin peak from 33 to 42, 18 to 32, 7 to 16, and 6 to 8 nM in plasma with emicizumab and 50, 100, 200 and 400 ng/ml rivaroxaban, respectively. Similar to emicizumab, spiking with high FVIII doses only slightly changed the results. Specifically, increasing the FVIII dose (50 – 100%) increased ETP from 921 to 1071, 685 to 971, 490 to 745, and 227 to 655 nM.min and thrombin peak from 51 to 84, 33 to 57, 20 to 36, and 9 to 29 nM in plasma with emicizumab and 50, 100, 200 and 400 ng/ml rivaroxaban,

respectively. When TGA was carried out with low TF concentration, thrombin generation could not be measured after spiking with the lowest FVIII doses (5-15%), but showed a profile similar to that with intermediate TF concentration for the highest FVIII doses used (**Figure 2G-H**). Indeed, in plasma with emicizumab and 200 ng/ml rivaroxaban, the anticoagulation activity was too strong to generate enough thrombin in the presence of only 15% FVIII, conversely ETP ranged from 100 to 415 nM.min with 50 - 100% FVIII. Thrombin peak followed the same trend and ranged between 4 and 18 nM with 50 - 100% FVIII.

In the absence of FVIII, ETP (TGA with intermediate TF dose) ranged from 567 to 957, 360 to 753, 202 to 402, and 86 to 222 nM.min and thrombin peak from 25 to 52, 15 to 37, 8 to 18, and 3 to 9 nM in plasma with emicizumab and 50, 100, 200 and 400 ng/ml edoxaban, respectively (**Figure 3A-B**). Spiking with 15% FVIII increased the ETP from 688 to 889, 547 to 820, 325 to 455, and 160 to 310 nM.min and thrombin peak from 34 to 50, 25 to 41, 14 to 27, and 6 to 14 nM in plasma with emicizumab and 50, 100, 200 and 400 ng/ml edoxaban, respectively. As observed with emicizumab alone, high FVIII doses did not strongly affect the TGA results. Upon spiking with 50 – 100% FVIII, ETP ranged from 867 to 1152, 749 to 995, 577 to 915, and 291 to 661 nM.min and thrombin peak from 56 to 105, 34 to 74, 27 to 55, and 13 to 36 nM in plasma with emicizumab and 50, 100, 200 and 400 ng/ml edoxaban, respectively. In TGA with low TF concentration, thrombin generation was undetectable upon addition of 5-15% FVIII, but showed a profile similar to that with intermediate TF concentration for the highest FVIII doses (**Figure 3C-D**). In plasma with emicizumab and 200 ng/ml edoxaban, the anticoagulant activity was too strong to generate enough thrombin in the presence of 15% FVIII, whereas ETP ranged from 143 to 387 nM.min with 50 - 100% FVIII. Thrombin peak followed the same trend and ranged between 6 and 18 nM with 50 - 100% FVIII.

In the absence of FVIII, ETP (TGA with intermediate TF concentration) ranged from 792 to 1125, 777 to 1084, 643 to 936, and 644 to 937 nM.min and thrombin peak from 53 to 111, 51 to 97, 43 to 94, and 41 to 109 nM in plasma with emicizumab and 50, 100, 200 and 400 ng/ml dabigatran, respectively (**Figure 3E-F**). Spiking with 15% FVIII increased ETP from 875 to 1060, 878 to 999, 804 to 978, and 717 to 984 nM.min and thrombin peak from 75 to 110, 72 to 105, 71 to 101 and 60 to 121 nM in plasma with emicizumab and 50, 100, 200 and 400 ng/ml dabigatran, respectively. Similar to emicizumab, high FVIII concentrations did not strongly affect the TGA results. Indeed, increasing the FVIII dose (50 – 100%) increased ETP from 867

to 1152, 749 to 995, 577 to 915, and 291 to 661 nM.min and thrombin peak from 56 to 105, 34 to 74, 27 to 55, and 13 to 36 nM in plasma with emicizumab and 50, 100, 200, and 400 ng/ml dabigatran, respectively. When TGA was carried out with low TF concentration, thrombin generation was undetectable after spiking with 5-15% FVIII, but showed a profile similar to TGA with intermediate TF after spiking with the highest FVIII doses (**Figure 3G-H**). For instance, in the plasma with emicizumab and 200 ng/ml dabigatran, the anticoagulant activity was too strong to generate enough thrombin with 15% FVIII, but ETP and thrombin peak ranged from 143 to 387 nM.min and from 6 to 18 nM with 50 - 100% FVIII.

TGA allowed monitoring coagulation in plasma containing drugs that exert contradictory effects (DOAC, emicizumab, FVIII) and showed the procoagulant activity of emicizumab, even in the presence of DOAC. FVIII supplementation had a slight additive effect when used at low dose in combination with emicizumab.

4. Discussion

Our study gives an overview of the hemostatic capacity of FVIII-deficient plasma spiked or not with different concentrations of emicizumab, DOAC and FVIII. Our results could help clinicians to choose the FVIII dose to be used before urgent surgery or during a bleeding event, for patients on emicizumab prophylaxis and taking or not a DOAC for a CVD. Our findings support the use of FVIII products, whatever the emicizumab dose, because emicizumab alone does not allow a sufficient thrombin generation level when a DOAC is concomitantly present in the plasma. Indeed, 100% FVIII completely masked emicizumab effect, as already documented, and did not fully compensate the anticoagulant effect of a DOAC at a concentration >100 ng/ml.

We first assessed coagulation using routine tests and observed an increase in PT with DOAC supplementation and also a superior effect of rivaroxaban compared with apixaban, in agreement with the literature [33]. As expected, aPTT was greatly shortened after spiking with emicizumab. In patients with HA on emicizumab-based prophylaxis and treated with a DOAC, PT and aPTT should not be used to monitor their coagulation. FVIII had a bigger effect on TGA than emicizumab, certainly due to its higher affinity for its substrates [9]. Several studies analyzed supplementation of FVIII or recombinant FVIIa in patients with HA undergoing

surgery and concluded that hemostatic supplementation is crucial for major surgical interventions, while it could be skipped for some minor surgeries [34]. Some studies assessed TGA to manage bypassing agents (APCC or recombinant FVIIa) or FVIII product dose-adjustment in few patients with HA (with or without inhibitors) on emicizumab-based prophylaxis. They found that TGA could be used to efficiently monitor the hemostatic balance of plasma samples containing emicizumab and procoagulant products and allowed the efficient and safe surgical management of such patients [29, 35]. Dargaud *et al.* showed a successful adjustment of the bypassing agent dosage using TGA in one patient with HA and inhibitors on emicizumab and undergoing surgery. They also used TGA to manage arterial bleeding by bypassing agent infusions in the same patient [29]. Our study is in line with the literature and is in favor of FVIII supplementation for patients undergoing surgery to avoid any bleeding event or to treat a bleeding event. FVIII supplementation appears to be safe because it only has a weak additional effect with emicizumab at the lowest doses used and allows approaching the normal thrombin generation range.

Spiking with DOAC produced different effects depending on the factor they target. Anti-FXa DOAC decreased ETP and peak thrombin from 50 ng/ml, but not dabigatran (anti-FIIa DOAC). Despite their similar anticoagulant profile by TGA, DOAC targeting FXa showed some discrepancies. Indeed, at 400 ng/ml, apixaban did not further decrease thrombin generation compared with 200 ng/ml, unlike rivaroxaban and edoxaban. Emicizumab partially reversed the anticoagulant effect of anti-FXa DOAC, as indicated by the continuous decrease of ETP and thrombin peak, reaching low values at the highest doses. Nonetheless, these data suggest that DOAC concentration should be monitored before surgery because doses >100 ng/ml produce a strong anticoagulant effect. In this case, FVIII should be supplemented at high dose, over the emicizumab prophylaxis, to evaluate the DOAC effect and a complementary approach may be considered. Below 100 ng/ml of DOAC, FVIII seems sufficient to counter its procoagulant effect. While in non-hemophilia patients, a surgery performed with a dose of DOAC <50 ng/ml or <100 ng/ml does not result in significant blood loss, this result cannot be extrapolated to HA patients [36, 37]. However, it is interesting to determine a threshold for FVIII supplementation depending on the DOAC, its dosage and the emicizumab dosage in HA patients to help clinicians to manage urgent cases.

As routine coagulation tests cannot be used to monitor complex situations (i.e. plasma samples containing emicizumab), TGA appears as a solid assay to evaluate hemostasis in such samples [22, 38]. In DOAC-free plasma containing FVIII, the ETP values obtained with low TF TGA were similar to those reported by previous studies performed with plasma from patients with mild, moderate and severe HA [39, 40], but were lower than in other studies [41, 42]. TGA is not a standardized test and many different protocols can be found in the literature. We chose to perform the test with two TF concentrations to cover the maximum range of thrombin generation due to the complexity of the tested plasma samples. TGA has been used to monitor the hemostatic effect of molecules that mimic FVIII activity (emicizumab and more recently mim8) with promising results [43, 44]. TGA can be triggered by various components (e.g. ellagic acid, tissue factor, FXIa, FIXa), thus giving different thrombin generation profiles. Among these components, FXIa is interesting for carrying out TGA of plasma samples that contain bispecific antibodies mimicking FVIII activity (e.g. emicizumab). FXIa supplementation allows producing a large amount of FIXa that no longer constitutes a limiting factor for thrombin generation [45]. In our study, in the absence of DOAC, a normal ETP value was not observed even with 100% FVIII. This could be explained by the limited FIXa available for emicizumab. It could be interesting to use TGA with FXIa in the same conditions. Indeed, in TGA, FXIa generates more thrombin and accelerates thrombin generation onset compared with TF [38]. Lund et al. tested the effects of the combination of FVIII and mim8 on thrombin generation triggered by FXIa and found an additive effect of the two drugs when FVIII was used at low dose, which is similar to our observations with emicizumab [46]. In line with the literature, our data also indicate that TGA allows monitoring plasma samples containing anti-FXa DOAC, but not dabigatran (anti-FIIa DOAC) [47]. Although thrombin generation decreased with increasing doses of dabigatran, its effect on ETP and thrombin peak was rather limited and did not reflect the anticoagulant effect of this DOAC. Time to peak and to a lesser extent, lag time were the parameters most influenced by dabigatran (data not shown) and should be monitored in patients with HA on dabigatran [48]. However, we choose to not display them because emicizumab displays a delay kinetic response on thrombin generation traduced by a lengthening in time to peak and lagtime while exerting a procoagulant effect.

Recent recommendations on DOAC treated patients with HA, undergoing cardiac surgery, suggest FVIII peak levels of 80-100% [21]. Our data are in line with these recommendations

and support FVIII supplementation, whether emicizumab is present or not. Furthermore, in patients with HA who take emicizumab and DOAC and who require urgent, unplanned surgery, FVIII supplementation should be $\geq 100\%$, in function of the DOAC dose. As already known, special care should be taken with rivaroxaban because it seems to exert a stronger anticoagulant effect at high doses. Besides HA, emicizumab has been repositioned for von Willebrand disease (VWD) management and showed promising results for reducing bleeding events in patients with type 3 VWD, with/without alloantibodies. It also increases thrombus formation *in vitro* in patients with type 2N VWD [49–51]. Therefore, it would be interesting to test whether its beneficial effect on thrombin generation is still present in patients with VWD receiving a DOAC.

The main limitation of the study is the evaluation of only one FVIII-deficient plasma (severe HA), which is not representative of the clinical routine, but the use of this plasma allows to evaluate a large panel of conditions. Moreover, it could be interesting to perform the same study using plasma samples from patients with mild and moderate HA. In our study, the highest FVIII dose used (100%) had a limited effect in FVIII-deficient plasma spiked with high doses of DOAC. It is important to determine whether 150% FVIII would further increase thrombin generation and to what extent. Repeating this study at other centers could be interesting due to the inherent TGA variability.

To conclude, our study brought insights into the effect on thrombin generation of FVIII supplementation in a FVIII-deficient plasma spiked with emicizumab and with different DOAC (increasing concentrations). Our findings demonstrated the importance of FVIII supplementation in the presence of emicizumab to counter the anticoagulant effect induced by DOAC. FVIII and emicizumab can be safely used together because they do not potentiate each other. Our results bring data on the hemostatic response of complex plasma samples using TGA; however, more studies are needed to evaluate *in vivo* combinations.

Author contributions

S. Lamoine have collected, analyzed the data and wrote the paper, V. Jury and A. Lebreton designed the study, V. Fourneyron collected the data, J. Douxfils, D. Teissandier and L. Talon revised the manuscript and gave critical comments, T. Sinegre and A. Lebreton analyzed the data and revised the manuscript. All authors revised and approved the final version of manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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Bibliography

1. Manco-Johnson MJ, Abshire TC, Shapiro AD, et al (2007) Prophylaxis versus Episodic Treatment to Prevent Joint Disease in Boys with Severe Hemophilia. *N Engl J Med* 357:535–544. <https://doi.org/10.1056/NEJMoa067659>
2. Hassan S, Monahan RC, Mauser-Bunschoten EP, et al (2021) Mortality, life expectancy, and causes of death of persons with hemophilia in the Netherlands 2001–2018. *J Thromb Haemost* 19:645–653. <https://doi.org/10.1111/jth.15182>
3. Franchini M, Mannucci P (2014) The History of Hemophilia. *Semin Thromb Hemost* 40:571–576. <https://doi.org/10.1055/s-0034-1381232>
4. Carcao MD, Aledort L (2004) Prophylactic factor replacement in hemophilia. *Blood Rev* 18:101–113. [https://doi.org/10.1016/S0268-960X\(03\)00038-9](https://doi.org/10.1016/S0268-960X(03)00038-9)
5. Schrijvers LH, Uitslager N, Schuurmans MJ, Fischer K (2013) Barriers and motivators of adherence to prophylactic treatment in haemophilia: a systematic review. *Haemophilia* 19:355–361. <https://doi.org/10.1111/hae.12079>
6. Kitazawa T, Igawa T, Sampei Z, et al (2012) A bispecific antibody to factors IXa and X restores factor VIII hemostatic activity in a hemophilia A model. *Nat Med* 18:1570–1574. <https://doi.org/10.1038/nm.2942>
7. Pasi KJ, Rangarajan S, Georgiev P, et al (2017) Targeting of Antithrombin in Hemophilia A or B with RNAi Therapy. *N Engl J Med* 377:819–828. <https://doi.org/10.1056/NEJMoa1616569>
8. Shapiro AD, Angchaisuksiri P, Astermark J, et al (2019) Subcutaneous concizumab prophylaxis in hemophilia A and hemophilia A/B with inhibitors: phase 2 trial results. *Blood* 134:1973–1982. <https://doi.org/10.1182/blood.2019001542>
9. Lenting PJ, Denis CV, Christophe OD (2017) Emicizumab, a bispecific antibody recognizing coagulation factors IX and X: how does it actually compare to factor VIII? *Blood* 130:2463–2468. <https://doi.org/10.1182/blood-2017-08-801662>
10. Oldenburg J, Mahlangu JN, Kim B, et al (2017) Emicizumab Prophylaxis in Hemophilia A with Inhibitors. *N Engl J Med* 377:809–818. <https://doi.org/10.1056/NEJMoa1703068>
11. Young G, Liesner R, Chang T, et al (2019) A multicenter, open-label phase 3 study of emicizumab prophylaxis in children with hemophilia A with inhibitors. *Blood* 134:2127–2138. <https://doi.org/10.1182/blood.2019001869>
12. Mahlangu J, Oldenburg J, Paz-Priel I, et al (2018) Emicizumab Prophylaxis in Patients Who Have Hemophilia A without Inhibitors. *N Engl J Med* 379:811–822. <https://doi.org/10.1056/NEJMoa1803550>
13. Pipe SW, Shima M, Lehle M, et al (2019) Efficacy, safety, and pharmacokinetics of emicizumab prophylaxis given every 4 weeks in people with haemophilia A (HAVEN 4): a multicentre, open-label, non-randomised phase 3 study. *Lancet Haematol* 6:e295–e305. [https://doi.org/10.1016/S2352-3026\(19\)30054-7](https://doi.org/10.1016/S2352-3026(19)30054-7)
14. Guillet B, Cayla G, Lebreton A, et al (2021) Long-Term Antithrombotic Treatments Prescribed for Cardiovascular Diseases in Patients with Hemophilia: Results from the French Registry. *Thromb Haemost* 121:287–296. <https://doi.org/10.1055/s-0040-1718410>
15. Wang J-D (2016) Comorbidities of cardiovascular disease and cancer in hemophilia patients. *Thromb J* 14:34. <https://doi.org/10.1186/s12959-016-0097-x>

16. Fransen van de Putte DE, Fischer K, Makris M, et al (2013) Unfavourable cardiovascular disease risk profiles in a cohort of Dutch and British haemophilia patients. *Thromb Haemost* 109:16–23. <https://doi.org/10.1160/TH12-05-0332>
17. Berger K, Schopohl D, Lowe G, et al (2016) How to compare cardiovascular disease and risk factors in elderly patients with haemophilia with the general population. *Haemophilia* 22:. <https://doi.org/10.1111/hae.13069>
18. Limjoco J, Thornburg CD (2018) Risk factors for cardiovascular disease in children and young adults with haemophilia. *Haemophilia* 24:747–754. <https://doi.org/10.1111/hae.13585>
19. Schutgens R, Voskuil M, Mauser-Bunschoten E (2017) Management of cardiovascular disease in aging persons with haemophilia. *Hämostaseologie* 37:196–201. <https://doi.org/10.5482/HAMO-16-09-0037>
20. Van Der Valk PR, Mauser-Bunschoten EP, Van Der Heijden JF, Schutgens REG (2019) Catheter Ablation for Atrial Fibrillation in Patients with Hemophilia or von Willebrand Disease. *TH Open* 03:e335–e339. <https://doi.org/10.1055/s-0039-1698756>
21. Schutgens REG, Jimenez-Yuste V, Escobar M, et al (2023) Antithrombotic Treatment in Patients With Hemophilia: an EHA-ISTH-EAHAD-ESO Clinical Practice Guidance. *HemaSphere* 7:e900. <https://doi.org/10.1097/HS9.0000000000000900>
22. Nougier C, Jeanpierre E, Ternisien C, et al (2020) Emicizumab treatment: Impact on coagulation tests and biological monitoring of haemostasis according to clinical situations (BIMHO group proposals). *Eur J Haematol* 105:675–681. <https://doi.org/10.1111/ejh.13490>
23. Adamkewicz JI, Chen DC, Paz-Priel I (2019) Effects and Interferences of Emicizumab, a Humanised Bispecific Antibody Mimicking Activated Factor VIII Cofactor Function, on Coagulation Assays. *Thromb Haemost* 119:1084–1093. <https://doi.org/10.1055/s-0039-1688687>
24. Takeyama M, Furukawa S, Yada K, et al (2021) Ex Vivo Prediction of Comprehensive Coagulation Potential Using Simulated Blood Concentrations of Emicizumab in Patients with Acquired Hemophilia A. *Thromb Haemost* 121:1289–1298. <https://doi.org/10.1055/s-0041-1725009>
25. Yada K, Nogami K, Ogiwara K, et al (2019) Global coagulation function assessed by rotational thromboelastometry predicts coagulation-steady state in individual hemophilia A patients receiving emicizumab prophylaxis. *Int J Hematol* 110:419–430. <https://doi.org/10.1007/s12185-019-02698-8>
26. Barg AA, Avishai E, Budnik I, et al (2019) Emicizumab prophylaxis among infants and toddlers with severe hemophilia A and inhibitors—a single-center cohort. *Pediatr Blood Cancer* 66:e27886. <https://doi.org/10.1002/pbc.27886>
27. Verhagen MJA, van Heerde WL, van der Bom JG, et al (2023) In patients with hemophilia, a decreased thrombin generation profile is associated with a severe bleeding phenotype. *Res Pract Thromb Haemost* 7:100062. <https://doi.org/10.1016/j.rpth.2023.100062>
28. Hartmann R, Feenstra T, Valentino L, et al (2018) In vitro studies show synergistic effects of a procoagulant bispecific antibody and bypassing agents. *J Thromb Haemost* 16:1580–1591. <https://doi.org/10.1111/jth.14203>
29. Dargaud Y, Lienhart A, Janbain M, et al (2018) Use of thrombin generation assay to personalize treatment of breakthrough bleeds in a patient with hemophilia and inhibitors receiving prophylaxis with emicizumab. *Haematologica* 103:e181–e183. <https://doi.org/10.3324/haematol.2017.185330>

30. Douxfils J, Ageno W, Samama C -M., et al (2018) Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. *J Thromb Haemost* 16:209–219. <https://doi.org/10.1111/jth.13912>
31. Shima M, Nogami K, Nagami S, et al (2019) A multicentre, open-label study of emicizumab given every 2 or 4 weeks in children with severe haemophilia A without inhibitors. *Haemophilia* 25:979–987. <https://doi.org/10.1111/hae.13848>
32. Bowyer A, Kitchen S, Maclean R (2020) Effects of emicizumab on APTT, one-stage and chromogenic assays of factor VIII in artificially spiked plasma and in samples from haemophilia A patients with inhibitors. *Haemophilia* 26:536–542. <https://doi.org/10.1111/hae.13990>
33. Ofek F, Bar Chaim S, Kronenfeld N, et al (2017) International Normalized Ratio Is Significantly Elevated With Rivaroxaban and Apixaban Drug Therapies: A Retrospective Study. *Clin Ther* 39:1003–1010. <https://doi.org/10.1016/j.clinthera.2017.04.007>
34. Castaman G, Linari S, Pieri L, et al (2023) Safe and Successful Surgical Outcome in Persons with Hemophilia A with and without Inhibitors Treated with Emicizumab: A Large, Single Center, Real-World Experience. *J Clin Med* 12:2317. <https://doi.org/10.3390/jcm12062317>
35. Lockhart M, Tardy-Poncet B, Montmartin A, et al (2021) Surgery with emicizumab prophylaxis for two paediatric patients with severe haemophilia A with inhibitors. *Pediatr Blood Cancer* 68:e29041. <https://doi.org/10.1002/pbc.29041>
36. Kaserer A, Kiavialaitis GE, Braun J, et al (2020) Impact of rivaroxaban plasma concentration on perioperative red blood cell loss. *Transfusion (Paris)* 60:197–205. <https://doi.org/10.1111/trf.15560>
37. Meihandoest T, Studt J-D, Mendez A, et al (2021) Automated Thrombin Generation Assay for Rivaroxaban, Apixaban, and Edoxaban Measurements. *Front Cardiovasc Med* 8:717939. <https://doi.org/10.3389/fcvm.2021.717939>
38. Ogiwara K, Nogami K, Matsumoto N, et al (2020) A modified thrombin generation assay to evaluate the plasma coagulation potential in the presence of emicizumab, the bispecific antibody to factors IXa/X. *Int J Hematol* 112:621–630. <https://doi.org/10.1007/s12185-020-02959-x>
39. van Veen JJ, Gatt A, Bowyer AE, et al (2009) Calibrated automated thrombin generation and modified thromboelastometry in haemophilia A. *Thromb Res* 123:895–901. <https://doi.org/10.1016/j.thromres.2008.09.011>
40. de Koning MLY, Fischer K, de Laat B, et al (2017) Comparing thrombin generation in patients with hemophilia A and patients on vitamin K antagonists. *J Thromb Haemost* 15:868–875. <https://doi.org/10.1111/jth.13674>
41. Dargaud Y, Béguin S, Lienhart A, et al (2005) Evaluation of thrombin generating capacity in plasma from patients with haemophilia A and B. *Thromb Haemost* 93:475–480. <https://doi.org/10.1160/TH04-10-0706>
42. Gilmore R, Harmon S, Gannon C, et al (2010) Thrombin generation in haemophilia A patients with mutations causing factor VIII assay discrepancy. *Haemophilia* 16:671–674. <https://doi.org/10.1111/j.1365-2516.2009.02190.x>
43. Bowyer AE, Kitchen S, Ezban M (2023) The effect of a next generation factor VIII mimetic bispecific antibody (Mim8) on assays of factor VIII activity and thrombin generation. *J Thromb Haemost* 21:480–487. <https://doi.org/10.1016/j.jtha.2022.12.023>
44. Bertaggia Calderara D, Marchi Cappelletti R, Batista Mesquita Sauvage AP, et al (2023) Pharmacodynamics Monitoring of Emicizumab in Patients with Hemophilia A. *Thromb Haemost*

- 123:955–965. <https://doi.org/10.1055/s-0043-1769788>
45. McIntosh JH, Owens D, Lee CA, et al (2003) A modified thrombin generation test for the measurement of factor VIII concentrates. *J Thromb Haemost* 1:1005–1011. <https://doi.org/10.1046/j.1538-7836.2003.00209.x>
46. Lund J, Jensen K, Burnier L, Ezban M (2023) In vitro effects of combining Mim8 with factor VIII, FVIIa, and activated prothrombin complex concentrates in thrombin generation assays. *J Thromb Haemost* 21:1493–1502. <https://doi.org/10.1016/j.jtha.2023.03.007>
47. Bloemen S, Zwaveling S, Douxfils J, et al (2018) 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* 171:160–166. <https://doi.org/10.1016/j.thromres.2018.10.005>
48. Artang R, Anderson M, Riley P, Nielsen JD (2017) Assessment of the effect of direct oral anticoagulants dabigatran, rivaroxaban, and apixaban in healthy male volunteers using a thrombin generation assay. *Res Pract Thromb Haemost* 1:194–201. <https://doi.org/10.1002/rth2.12044>
49. Thomas VM, Abou-Ismaïl MY, Lim MY (2022) Off-label use of emicizumab in persons with acquired haemophilia A and von Willebrand disease: A scoping review of the literature. *Haemophilia* 28:4–17. <https://doi.org/10.1111/hae.14450>
50. Weyand AC, Flood VH, Shavit JA, Pipe SW (2019) Efficacy of emicizumab in a pediatric patient with type 3 von Willebrand disease and alloantibodies. *Blood Adv* 3:2748–2750. <https://doi.org/10.1182/bloodadvances.2019000656>
51. Yaoi H, Shida Y, Kitazawa T, et al (2021) Emicizumab improves thrombus formation of type 2A von willebrand disease under high shear condition. *Haemophilia* 27:.. <https://doi.org/10.1111/hae.14272>

Tables

Table 1. FVIII-deficient plasma characteristics.

PT (prothrombin time), aPTT (activated partial prothrombin time), AT (antithrombin), APCR (activated protein C resistance), ETP (endogenous thrombin potential), ttPeak (time to peak).
Reference ranges are between brackets.

Coagulation parameters					
PT (s)	14.4 [<15.2]	Factor XII (%)	76 [80-120]	AT (%)	93 [80-120]
INR	1.09 [<1.2]	Factor XI (%)	87 [80-120]	Protein C (%)	123 [80-120]
aPTT (s)	97.1 [<37]	Factor IX (%)	96 [80-120]	Protein S (%)	85 [80-120]
aPTT kaolin (s)	86.7 [<34]	Factor VIII (%)	<1 [80-120]	APCR (s)	207 [>120]
Fibrinogen (g/L)	3.27 [2-4]	Factor VII (%)	93 [80-120]		
		Factor X (%)	78 [80-120]		
		Factor V (%)	67 [80-120]		
		Factor II (%)	91 [80-120]		
Thrombin generation assay					
PPP reagent		PPP _{low} reagent			
ETP (nM.min)	724 [1226-1487]	ETP (nM.min)	203 [837-1196]		
Peak (nM)	56 [209-276]	Peak (nM)	9 [82-144]		
Lag time (min)	2.0 [3.0-3.8]	Lag time (min)	3.0 [4.7-6.79]		
ttPeak (min)	6.5 [5.8-7.3]	ttPeak (min)	12.8 [9.74-11.98]		

Figures

Figure 1. DOAC effect on TGA of a FVIII-deficient plasma spiked with emicizumab. ETP (A-D) and (I-L) and thrombin peak (E-H and M-P) obtained with TGA using the intermediate TF (A-H) and low TF concentrations (I-P) in a FVIII-deficient (FVIII $<1\%$) plasma sample complemented with the indicated doses of emicizumab and after addition of the indicated DOAC (0; 50; 100; 200; 400 ng/ml).

Figure 2. Thrombin generation in a FVIII-deficient plasma containing emicizumab, apixaban or rivaroxaban, and FVIII. The heatmaps show ETP and thrombin peak in the presence of apixaban (A-D) and rivaroxaban (E-H). TGA was carried out with intermediate TF (A, B, E, F) and low TF concentrations (C, D, G, H). The doses of emicizumab (x axes) and of each DOAC (top of each heatmap) in each aliquot are indicated. As such, for a single DOAC dose (ie 0 ng/ml), 5 horizontal

rectangles correspond to each emicizumab concentration (0 to 100 µg/ml from left to right) and each row represents an FVIII concentration. A graphical version of these results is available in Supplementary Figures S2 (apixaban) and S3 (rivaroxaban).

Figure 3. Thrombin generation in a FVIII-deficient plasma containing emicizumab, edoxaban or dabigatran, and FVIII. The heatmaps show ETP and thrombin peak in the presence of edoxaban (A-D) and dabigatran (E-H). TGA was carried out with intermediate TF (A, B, E, F) and low TF concentrations (C, D, G, H). The doses of emicizumab (x axis) and of each DOAC (top of each heatmap) in each aliquot are indicated. As such, for a single DOAC dose (ie 0 ng/ml), 5 horizontal rectangles correspond to each emicizumab concentration (0 to 100 µg/ml from left to right) and each row represents an FVIII concentration. A graphical version of these results is available in Supplementary Figures S4 (edoxaban) and S5 (dabigatran).

Supplementary Figure S1. Schematic representation of the study design. For a single dose of emicizumab, every DOAC at every dose have been spiked as well as every concentration of FVIII. Thrombin generation assays were done with intermediate (PPP reagent) and low (PPP_{low} reagent) tissue factor concentrations.

Supplementary Figure S2. DOAC effect on ttPeak parameter on TGA in a FVIII-deficient plasma spiked with emicizumab. The heatmaps show the time to peak in the presence of apixaban (A, B), rivaroxaban (C, D), edoxaban (E, F) and dabigatran (G, H). TGA was carried out with intermediate TF (A, C, E, G) and low TF concentrations (B, D, F, H). The doses of emicizumab (x axes) and of each DOAC (top of each heatmap) in each aliquot are indicated. As such, for a single DOAC dose (ie 0 ng/ml), 5 horizontal rectangles correspond to each emicizumab concentration (0 to 100 µg/ml from left to right) and each row represents an FVIII concentration. Dark squares represent conditions not producing enough thrombin to measure ttPeak. A different scale has been used for dabigatran measured with PPP_{low} due to higher values (H).

Supplementary Figure S3. Apixaban effect on TGA in a FVIII-deficient plasma spiked with emicizumab. ETP and thrombin peak measurement by TGA in a FVIII-deficient (FVIII <1%) plasma sample after addition, or not, of emicizumab and apixaban at the indicated doses. TGA was triggered with intermediate TF (A-J) and low TF concentrations (K-T).

Supplementary Figure S4. Rivaroxaban effect on TGA in a FVIII-deficient plasma spiked with emicizumab. ETP and thrombin peak measurement by TGA in a FVIII-deficient (FVIII <1%) plasma sample after addition, or not, of emicizumab and rivaroxaban at the indicated doses. TGA was triggered with intermediate TF (A-J) and low TF concentrations (K-T).

623

624 **Supplementary Figure S5. Edoxaban effect on TGA in a FVIII-deficient plasma spiked with**
625 **emicizumab.** ETP and thrombin peak measurement by TGA in a FVIII-deficient (FVIII <1%)
626 plasma sample after addition, or not, of emicizumab and edoxaban at the indicated doses.
627 TGA was triggered with intermediate TF (**A-J**) and low TF concentrations (**K-T**).

628

629 **Supplementary Figure S6. Dabigatran effect on TGA in a FVIII-deficient plasma spiked with**
630 **emicizumab.** ETP and thrombin peak measurement by TGA in a FVIII-deficient (FVIII <1%)
631 plasma sample after addition, or not, of emicizumab and dabigatran at the indicated doses.
632 TGA was triggered with intermediate TF (**A-J**) and low TF concentrations (**K-T**).

633

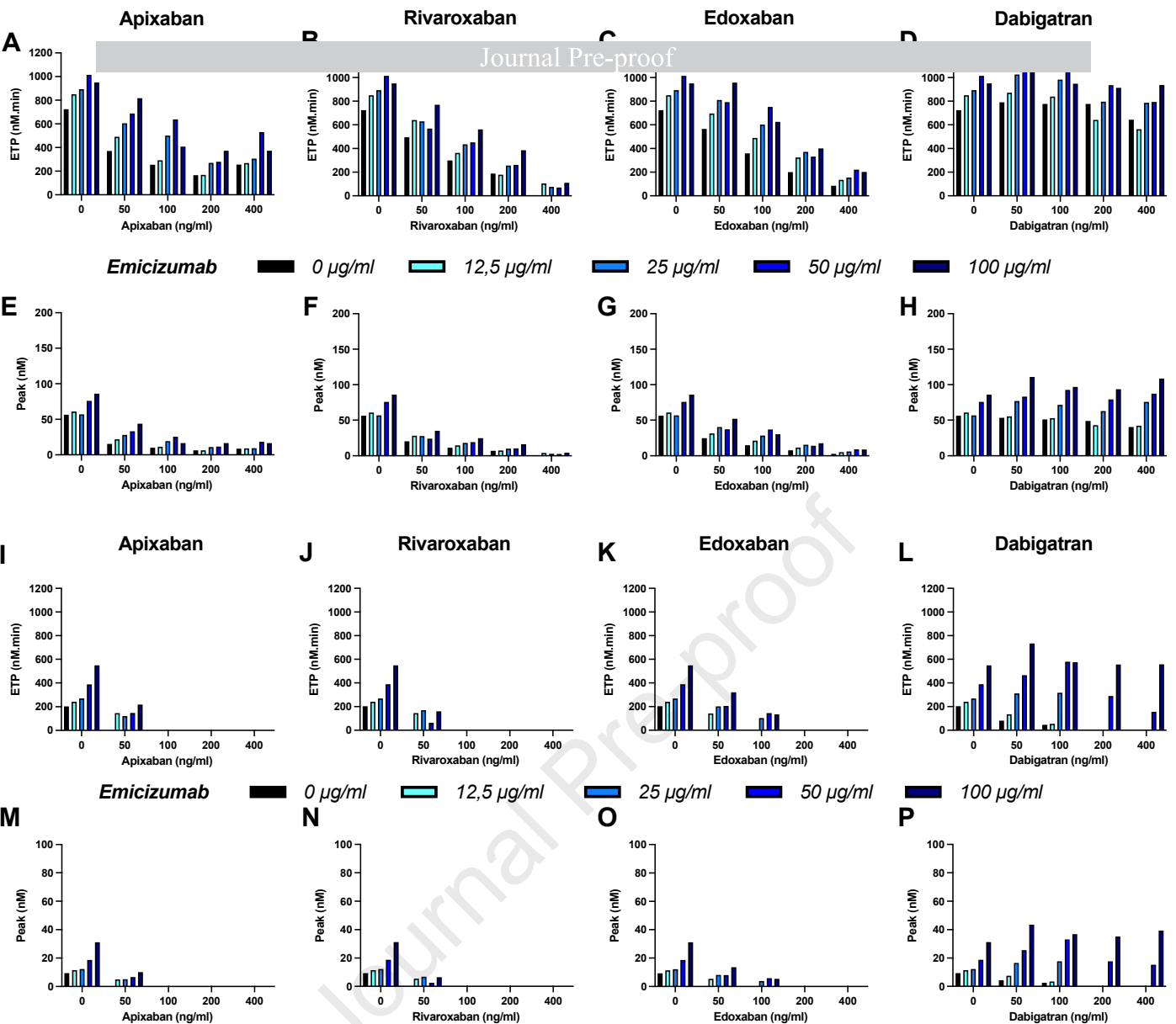


Figure 1.

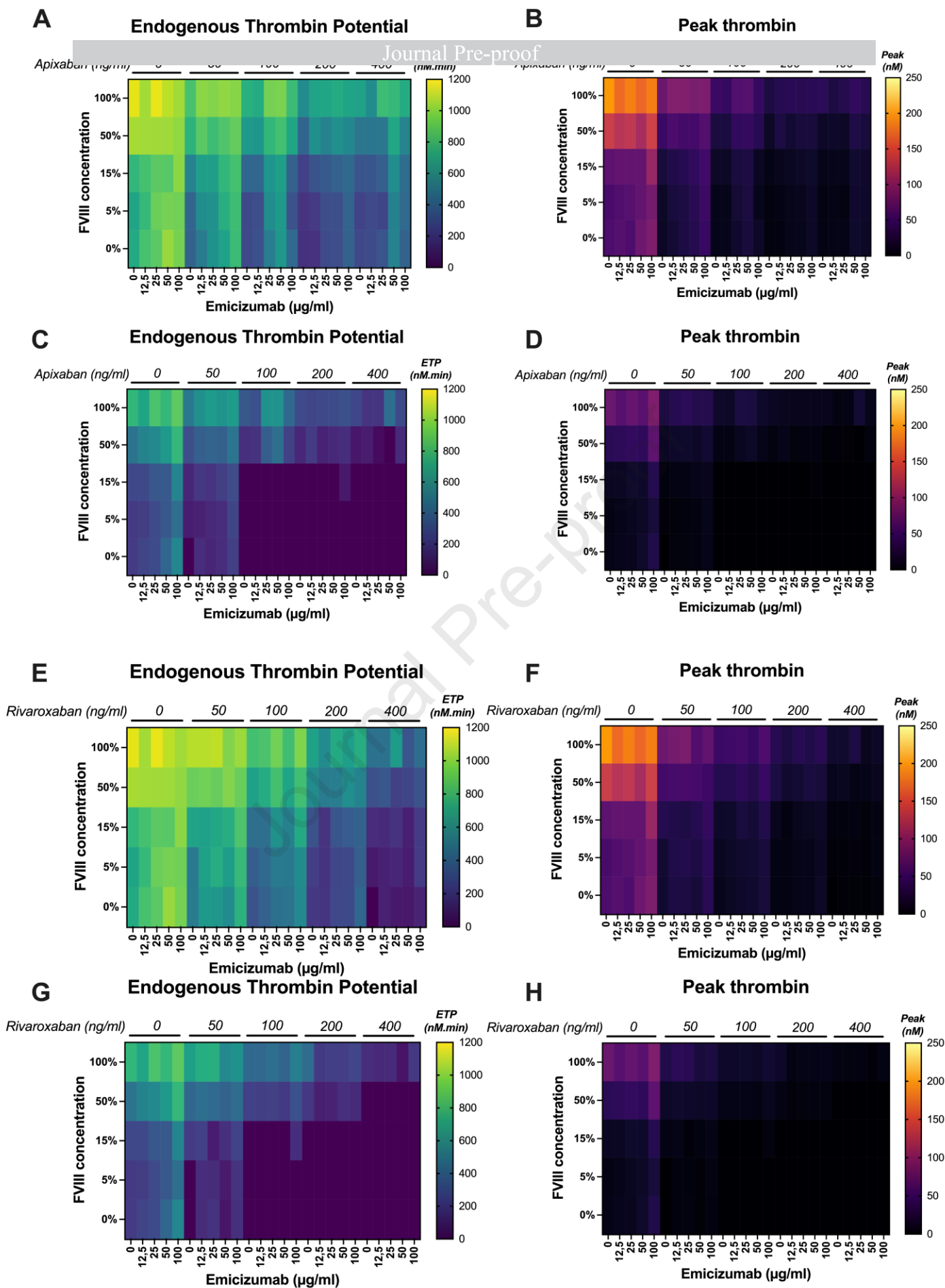


Figure 2.

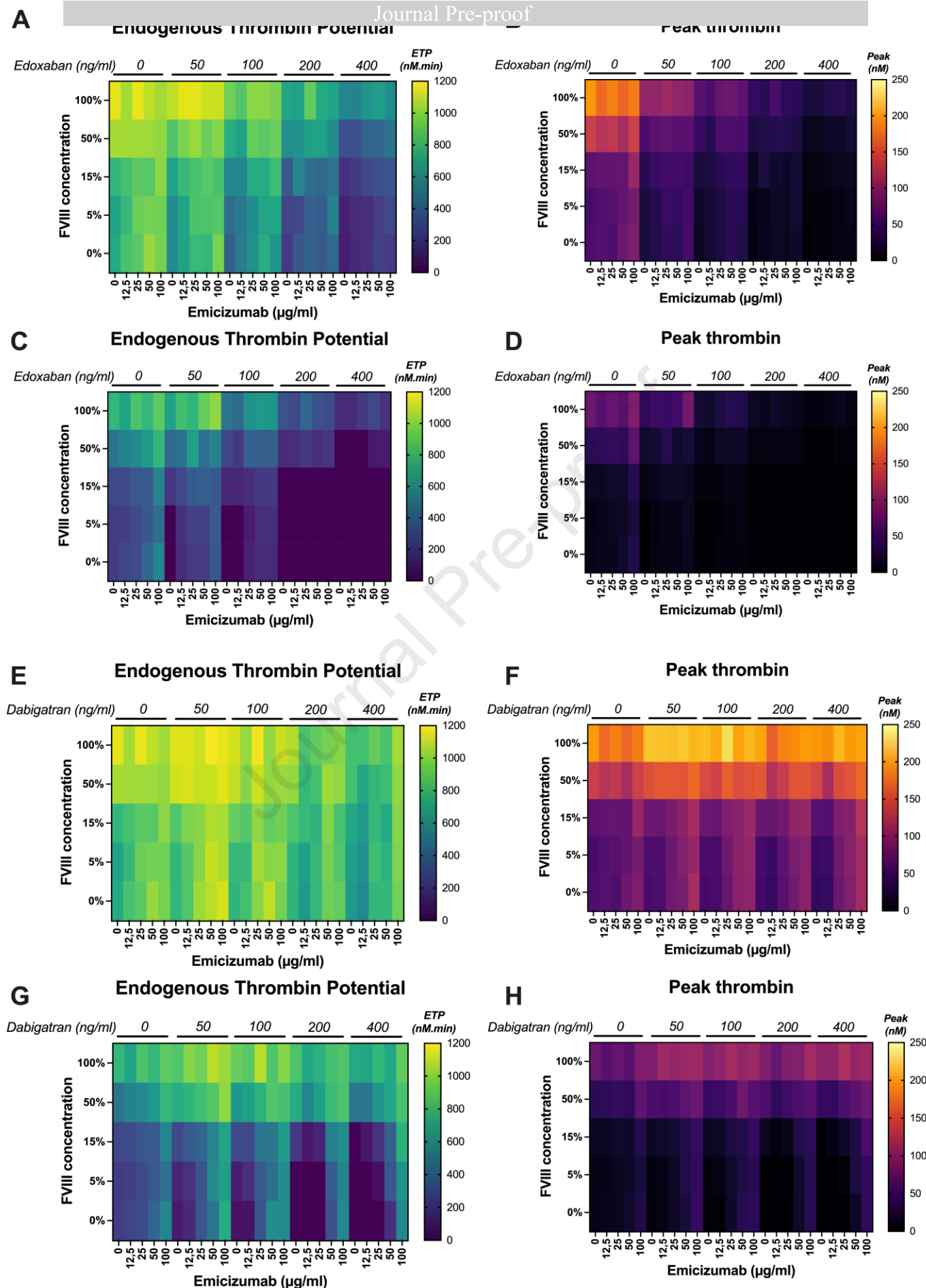


Figure 3.