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ORIGINAL ARTICLE

Emicizumab, the factor VIII mimetic bispecific monoclonal antibody: effects on thrombin generation and thromboelastometry

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Abstract

Background: Emicizumab is used for prophylaxis of patients with hemophilia A. Because it is used at a fixed dose that is not based on laboratory testing, evaluation of its activity is occasionally needed. Global coagulation procedures such as thrombin generation assays (TGAs) or thromboelastometry are obvious candidates for testing. Information on the significance of TGA or thromboelastometry parameters in patients treated with emicizumab is limited.

Objectives: We performed a 2-step study to gain insight into the pattern of variation of TGA or thromboelastometry results in patients treated with emicizumab.

Methods: The first experiment was an *in vitro* investigation of the best conditions in terms of TGA reagent composition needed to show the best dose-response of TGA parameters and emicizumab concentrations. In the second, we evaluated *ex vivo* the correlation of TGA or thromboelastometry parameters vs emicizumab concentrations achieved by patients receiving prophylaxis.

Results: While TGA thrombin peak and endogenous thrombin potential (ETP) showed good dose-response with emicizumab concentrations, lag time and time-to-peak did not. The best TGA condition in terms of reagent composition was 1 pM tissue factor plus 1 μM phospholipids. There was a strong correlation between thrombin peak or ETP and emicizumab concentrations. The correlation was highly significant for thromboelastometry clotting time, but only when the procedure was performed without exogenous triggers. Based on the above correlations, we estimated the value of TGA or thromboelastometry parameters corresponding to the critical values of 40 or 80 μg/mL emicizumab.

Conclusion: TGA thrombin peak or ETP performed at low tissue factor and phospholipid concentrations should be used to evaluate emicizumab activity. Thromboelastometry clotting time is valuable when performed without exogenous triggers.

KEYWORDS
hemophilia, hemorrhage, laboratory testing, monitoring, viscoelastometry

Essentials

- Thrombin generation assay (TGA) and thromboelastometry are candidate assays for emicizumab measurement.
- We aimed to get information on the responsiveness of TGA or thromboelastometry to emicizumab.
- TGA performed with low trigger concentrations is responsive to emicizumab.
- Thromboelastometry clotting time is responsive only when performed without exogenous triggers.

1 | INTRODUCTION

Hemophilia A is an X-linked congenital hemorrhagic disease characterized by low or dysfunctional levels of factor (F)VIII [1]. Spontaneous and posttraumatic bleeding in the joints, but also in soft tissues, are typically observed in patients with severe hemophilia [1]. Furthermore, one of the most severe complications is the development of alloantibodies against FVIII (inhibitors), which challenges replacement therapy [1]. The historical treatment of hemophilia A has been intravenous injection of human-derived or recombinant FVIII concentrates at the time of bleeding or during surgery [2]. More recently, prophylaxis by regular infusion of the missing factor was shown to be more effective than on-demand treatment to prevent arthropathy [3]. However, the presence of high-titer inhibitors to FVIII precludes treatment with factor concentrates [1]. Hence, patients must be treated with FVIII bypassing agents such as activated prothrombin complex concentrates or recombinant activated FVII [4,5].

Recently, emicizumab, a bispecific monoclonal antibody mimicking the function of activated FVIII, was developed and is currently used for prophylaxis of hemophilia A patients. Emicizumab can be given at a fixed dose without dose adjustment based on laboratory testing. Indeed, clinical trials showed that upon administration, patients reach and maintain relatively constant emicizumab trough levels (ie, 40-80 μg/mL) between 2 subsequent injections [6–9].

Since emicizumab is a mimetic FVIII agent, thrombin generation is increased after prophylaxis [9]. Hence, thrombin generation assays (TGAs) might be useful to assess for interindividual variations and/or in special situations to assess the global level of coagulation after emicizumab administration. These include suspicion of poor patient compliance or the presence of antifactor antibodies or, more generally, to understand the procoagulant mechanism mediated by emicizumab. The same considerations apply to the other global assay, thromboelastometry, that is performed on whole blood.

In this study, we aimed to explore some of the key effects of emicizumab infusions in patients with hemophilia A. Although some of the above issues have already been explored, for others, there is still little or no information available. For example, although TGA and thromboelastometry have been widely used in the context of hemophilia treatment with emicizumab, few studies have evaluated its performance based on the various TGA (eg, lag time, time-to-peak, peak thrombin, and endogenous thrombin potential [ETP]) and thromboelastometry (eg, clotting time, clot formation time and maximal clot formation) parameters. Some studies investigating patients receiving emicizumab [10–12] used TGA triggers other than the traditional tissue factor (TF) and negatively charged phospholipids (PL), and other studies used TF and PL at different concentrations. However, measurements of dose-response curves for all the TGA parameters are limited. Furthermore, the use of triggers other than the traditional TF and PL requires modifications of commercial kits, which exceeds the capability of core laboratories where coagulation testing for hemophilia is now centralized.

With this background, we undertook a comprehensive study with 2 different designs. An *in vitro* study was performed in which FVIII-deficient plasma was spiked with graded amounts of emicizumab. The plasmas were then tested in TGAs using low or high reactant concentrations to trigger coagulation. This *in vitro* study is useful to assess the best TGA conditions that should be used to capture the effect of emicizumab.

The second investigation is an *ex vivo* study in which we tested plasma or whole blood of persons with hemophilia receiving emicizumab prophylaxis using TGA or thromboelastometry. The results of this study were used to assess the correlation between TGA and thromboelastometry parameters and emicizumab plasma concentrations and to assess the distribution of these values over time during treatment.

2 | METHODS

2.1 | Emicizumab test plasma

A lyophilized preparation of FVIII-deficient plasma containing a certified concentration of emicizumab was purchased (r^2 Diagnostics) and reconstituted with distilled water. This material was further diluted into FVIII-deficient plasma (Werfen) to obtain 5 test samples with increasing emicizumab concentrations ranging from 0 to 92 μg/mL. Test plasmas were subdivided into aliquots in plastic tubes, frozen by immersion in liquid nitrogen, and stored at −70 °C. These plasmas were used for the *in vitro* study.

2.2 | Patients

Consecutive adult Caucasian patients with severe hemophilia A, followed at the outpatient clinic of the Maggiore Hospital, Milan, Italy, were enrolled in this study after obtaining informed consent.

The study was approved by the local ethics committee (Milano Area 2). Patients were receiving standard prophylaxis with emicizumab (Hemlibra; Roche). According to the European Medicines Agency technical documentation, emicizumab was administered at 3 mg/kg per week from weeks 1 to 4, and at week 5, the dose was reduced to 1.5 mg/kg. Blood was collected at each of the indicated time points and at steady state immediately before any emicizumab subcutaneous injection into 1/10 volumes of 0.105 M trisodium citrate (Vacuette; Greiner). Blood for baseline measurements was collected from patients at the time of entry into the study regardless of whether they were receiving prophylaxis with factor concentrates. Steady state was defined for blood samples collected after the fifth week from the initiation of the prophylaxis when the loading dose (ie, 3 mg/kg once every week) was reduced to 1.5 mg/kg once every week. In case of recent bleeding during follow-up, blood was collected ≥1 week after the last treatment with adjunctive FVIII or bypassing agents. The above specimens were used for the *in vivo* study. One portion of blood was centrifuged at 3000*g* (20 minutes at room temperature). Supernatant plasma was aliquoted into plastic tubes, immersed in liquid nitrogen, and stored at −70 °C. These aliquots were used to test for TGA, activated partial thromboplastin time (aPTT), and emicizumab concentrations. A second blood sample was used for thromboelastometry.

2.3 | Emicizumab concentrations

Emicizumab plasma concentrations for both the *in vitro* and *ex vivo* test samples were determined as previously reported [13] using a modified FVIII one-stage clotting assay with SynthASil, a silica-based aPTT reagent, on an ACL Top instrument (Werfen) and calibrated with a certified emicizumab concentration (r^2 Diagnostics).

2.4 | aPTT

aPTT was measured using SynthASil (Werfen) on an ACL Top, and the results were expressed as clotting time in seconds.

2.5 | TGA

TGA was performed using a homemade method [14] according to Hemker et al. [15]. The procedure is based on the initiation of coagulation in test plasma by means of such triggers as calcium chloride, TF (RecombiPlasTin 2G; Werfen), and synthetic PL (Avanti Polar Lipids) composed of a mixture of dioleoylserine, dioleoylethanolamine, and dioleoylchosphatidylcholine in a 20:20:60 ratio. We recorded the following TGA parameters: (i) lag time, defining the time (minutes) elapsing from the initiation of coagulation to the appearance of the first amounts of thrombin; (ii) time-to-peak, defining the time (minutes) needed for thrombin to reach the peak value; (iii) thrombin peak (nM); and (iv) ETP (nM × minutes), defining the area under the thrombin generation curve and representing the net amount of thrombin that can be produced by the test plasma under the opposing forces driven by pro- and anticoagulants. Thrombin generation was continuously recorded with a dedicated fluorimeter (Fluoroskan Ascent; ThermoLabsystem) with a specific fluorogenic substrate (417 μM) (Bachem). Thrombinoscope software (Diagnostica Stago) was used to calculate the parameters stemming from the thrombin generation curve.

For the *in vitro* experiments, coagulation was triggered by calcium chloride in combination with 1 pM TF plus either 1 μM or 4 μM PL, or with 5 pM TF plus either 1 μM or 4 μM PL. Each measurement was taken in duplicate over 3 independent working sessions using a freshly thawed set of test samples. For the *ex vivo* experiments, coagulation was triggered by calcium chloride in combination with 1 pM TF plus 1 μM PL. Measurements were taken in duplicate.

2.6 | Thromboelastometry

Thromboelastometry parameters were measured by means of rotation thromboelastometry (RoTem; Werfen) with reagents and instructions provided by the manufacturer. We evaluated the following parameters: (i) clotting time (CT), the time (seconds) needed for blood to start clotting; (ii) clot formation time (CFT), the time (seconds) needed for the clot to reach a defined size, which is a measure of the speed of clot formation; and (iii) maximal clot firmness (MCF), which is a measure of the maximal firmness and strength of the clot (mm). The above parameters were recorded using reagents exploring the viscoelastic properties of whole blood triggered through the intrinsic (INTEM) pathway of coagulation as specified by the manufacturer. The native viscoelastic properties of whole blood (no exogenous triggers other than calcium chloride, called NATEM) were also analyzed by mixing 300 μL of whole citrated blood with 20 μL calcium chloride (100 mM). Thromboelastometry according to extrinsic pathway of coagulation (EXTEM) was not investigated because of insufficient volumes of blood samples. However, EXTEM in hemophilia patients should be near (normal) [16].

2.7 | Data analyses

2.7.1 | *In vitro* experiments

The mean values for of each TGA parameter obtained over the 3 independent experiments for *in vitro* test samples were plotted against emicizumab concentrations. Best fit regression lines were drawn through the points.

2.7.2 | *Ex vivo* experiments

TGA or thromboelastometry parameters for each of the investigated patients were plotted against emicizumab plasma concentrations,

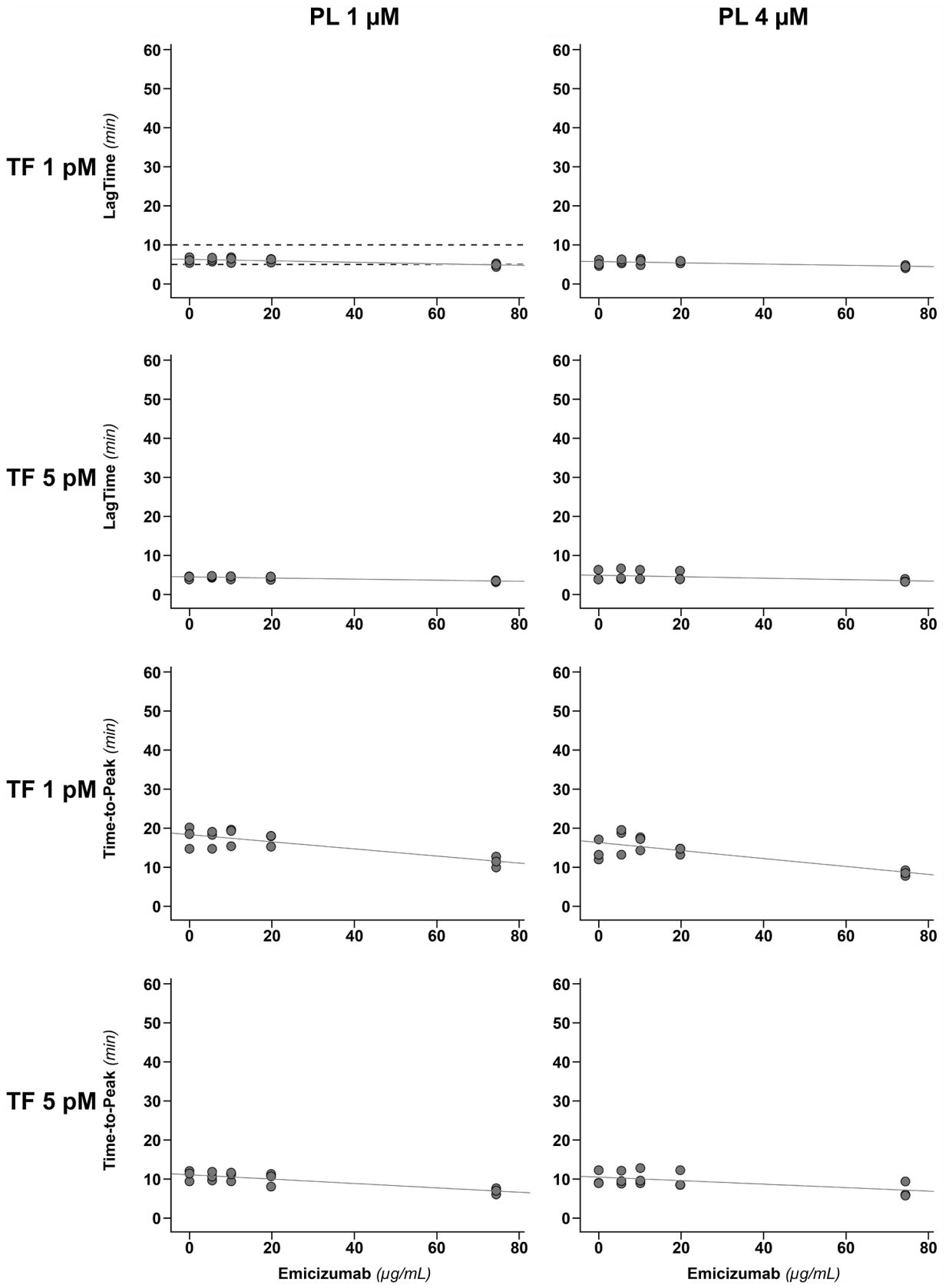

FIGURE 1 *In vitro* experiments. Lag time (upper panels) and time-to-peak (lower panels) for test plasmas added with increasing concentrations of emicizumab. Results of 3 independent experiments are reported for each test plasma. Dotted horizontal lines represent the limits of the normal range. PL, phospholipids; TF, tissue factor.

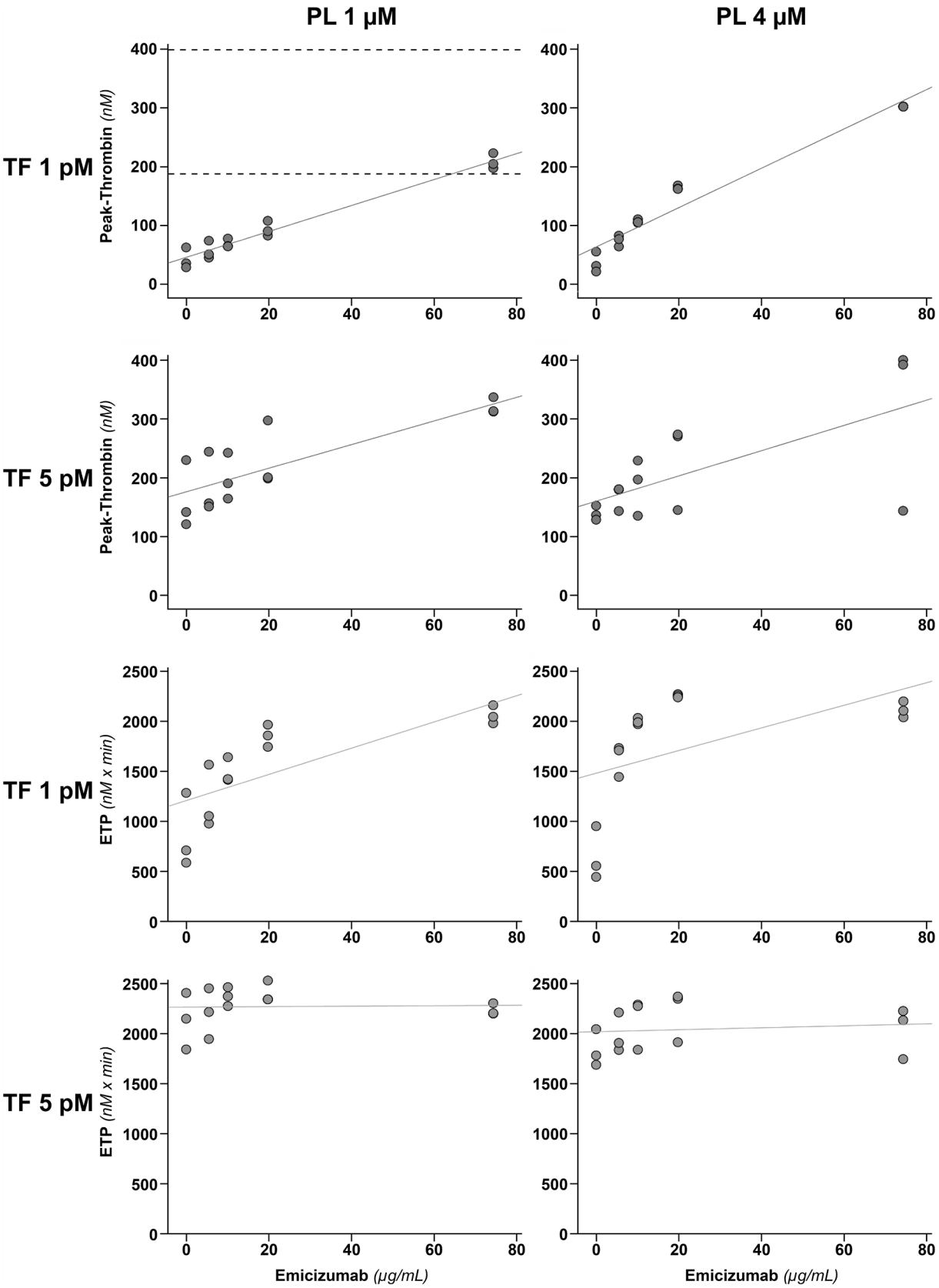

FIGURE 2 *In vitro* experiments. Thrombin peak (upper panels) and endogenous thrombin potential (ETP) (lower panels) for test plasmas added with increasing concentrations of emicizumab. Results of 3 independent experiments are reported for each test plasma. Dotted horizontal lines represent the limits of the normal range. PL, phospholipids; TF, tissue factor.

TABLE 1 Correlation of emicizumab concentrations vs coagulation parameters for patients (number of observations 98) on standard emicizumab prophylaxis.

Parameter	ρ	*P*
Thrombin generation assay		
Lag time	−0.096	.39
Time-to-peak	0.258	<.05
Thrombin peak	0.475	<.001
Endogenous thrombin potential	0.395	<.001
Thromboelastometry-INTEM		
CT	−0.211	.05
CFT	−0.232	<.05
CT + CFT	−0.104	.35
Maximal clot firmness	−0.331	<.01
Thromboelastometry-NATEM		
CT	−0.642	<.001
CFT	−0.228	<.05
CT + CFT	−0.472	<.001
Maximal clot firmness	−0.472	<.001
Activated partial thromboplastin time	−0.652	<.001

Data recorded at all time points were included. INTEM refers to the procedure performed through the activation of the intrinsic pathway of coagulation activated by exogenous triggers. NATEM refers to the procedure performed through the activation of coagulation without addition of exogenous triggers other than calcium chloride.
CFT, clot formation time; CT, clotting time.

and the correlation was assessed using the Spearman rank order correlation coefficient (ρ). For the purposes of this study, we pooled the data obtained at different time points for TGA, thromboelastometry parameters, and aPTT. To account for possible interference of residual prophylactic FVIII concentrates, results of TGA and thromboelastometry for baseline samples for each of the investigated parameters were excluded from the calculation of correlation. ρ and *P* values for correlations were not adjusted for multiple comparisons. Based on the regression lines (TGA or thromboelastometry vs emicizumab concentrations), critical values of TGA or thromboelastometry parameters corresponding to the expected emicizumab concentrations in patients at steady state (ie, 40 or 80 μg/mL) were estimated.

3 | RESULTS

3.1 | *In vitro* experiments

Results of TGA parameters for the *in vitro* experiments are displayed in Figures 1 and 2. These experiments were for evaluating the effect of emicizumab on TGA parameters according to the composition of the triggers.

3.1.1 | Lag time and time-to-peak

Relatively flat dose-response curves were obtained for the lag time and time-to-peak over the entire range of emicizumab concentrations, regardless of the trigger composition (Figure 1, upper panels).

3.1.2 | Thrombin peak

Relatively high steepness of dose-response curves was obtained, regardless of the trigger composition. However, the best linearity was obtained when the trigger composition was 1 pM TF plus 1 μM PL (Figure 2).

3.1.3 | ETP

The dose-response curve of ETP vs emicizumab concentration was curvilinear with a steep increase over the range 0 to 20 μg/mL and then reached a plateau when the trigger was composed of 1 pM TF plus 1 or 4 μM PL (Figure 2). There was a relatively poor dose-response when the trigger composition was 5 pM TF plus 1 or 4 μM PL (Figure 2).

3.2 | *Ex vivo* experiments

The results from 40 individual patients were available for TGA, thromboelastometry parameters, and aPTT, measured at different time points, for a total of 98 individual measurements. The number of patients analyzed at different time points were as follows: 16 (at baseline), 12 (at 5 weeks), 12 (at 10 weeks), 11 (at 20 weeks), and 35 (at steady state).

3.2.1 | Correlation of emicizumab concentrations vs TGA parameters

Based on the preliminary experiments to evaluate the effect of emicizumab on TGA parameters according to trigger composition, the TGA parameters of the patient samples were recorded by using triggers at concentrations of 1 pM TF plus 1 μM PL. Correlations were highly significant for ETP ($\rho = 0.395$, $P < .001$); thrombin peak ($\rho = 0.475$, $P < .001$), but not for lag time ($\rho = -0.096$, $P = .39$). There was a weak correlation for the time-to-peak ($\rho = -0.258$, $P < .05$) (Table 1). Based on correlation lines, we estimated the values of the thrombin peak achieved by critical emicizumab concentrations corresponding to 40 or 80 μg/mL as 62 nM (95% CI, 42-82) or 98 nM (95% CI, 78-118), respectively.

TABLE 2 Critical values (median and 95% CI) of thrombin generation assay parameters (ie, thrombin peak) and NATEM clotting time corresponding to 40 to 80 μg/mL emicizumab based on the linear correlation between thrombin peak or NATEM clotting time vs emicizumab concentrations.

Parameter	Value corresponding to 40 ng/mL	Value corresponding to 80 ng/mL
Thrombin peak (nM)	62 (42-82)	98 (78-118)
NATEM clotting time (s)	929 (801-1057)	721 (593-849)
Activated partial thromboplastin time (s)	24 (23-26)	21 (20-22)

NATEM refers to the procedure performed through the activation of coagulation without addition of exogenous triggers other than calcium chloride.

3.2.2 | Correlation of emicizumab concentrations vs thromboelastometry parameters

There was no correlation for CT-INTEM or (CT + CFT)-INTEM ($\rho = -0.211$, $P = .05$ or $\rho = -0.104$, respectively; $P = .35$), but there were significant correlations for MCF-INTEM ($\rho = -0.331$, $P < .01$). When thromboelastometry parameters were measured as NATEM (ie, without exogenous triggers), correlations were highly significant for all thromboelastometry parameters (Table 1). Based on correlation lines, we estimated the values of the thromboelastometry CT-NATEM achieved by critical emicizumab concentrations corresponding to 40 or 80 μg/mL at 929 seconds (95% CI, 801-1057) or 721 seconds (95% CI, 593-849), respectively (Table 2).

3.2.3 | Correlation of emicizumab concentrations vs aPTT

Correlation was highly significant ($\rho = -0.652$, $P < .001$) (Table 1). Based on correlation lines, we estimated the values of the aPTT achieved by critical emicizumab concentrations corresponding to 40 or 80 μg/mL at 24 seconds (95% CI, 23-26), or 21 seconds (95% CI, 20-22), respectively (Table 2).

3.2.4 | Distribution of aPTT

aPTT was prolonged at baseline and was completely normalized at weeks 5 to 20 and at steady state (Figure 3).

3.2.5 | Distribution of TGA parameters

Median ETP increased from baseline to 5 weeks and then remained stable up to 10 weeks and during steady state (Figure 4).

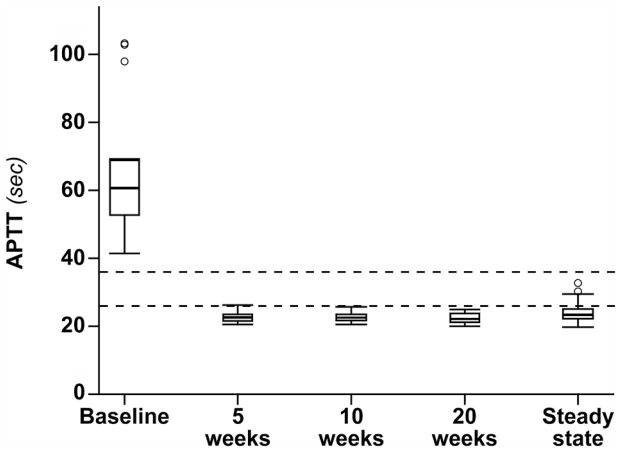

FIGURE 3 Box plots of the distribution of activated partial thromboplastin time (APTT) recorded at different time points for patients on standard prophylaxis. Dotted horizontal lines represent the limits of the reference range.

3.2.6 | Distribution of thromboelastometry parameters

Median CT recorded without exogenous triggers (ie, NATEM) decreased from baseline to 5 weeks and then remained stable and within normal limits up to 10 weeks and during steady state (Figure 5, left panel). The pattern of variation for the combination of CT + CFT was similar (Figure 5, right panel).

4 | DISCUSSION

As an FVIII mimetic agent, emicizumab is expected to modify TGA and/or thromboelastometry of hemophilic plasma (see Supplementary Figures 1 and 2). However, both procedures are characterized by different parameters, ie, lag time and time-to-peak, which are measures of the speed with which thrombin is generated, and ETP and thrombin peak, which represent the quantity of the generated thrombin. Information on how emicizumab modifies the above parameters is relatively limited, even though TGA is occasionally used to test hemophilia patients on prophylaxis with emicizumab. On the other hand, the thromboelastometry parameters CT and CFT pertain to the speed of clot formation, whereas the MCF is is a measure of the maximal clot firmness. Furthermore, the combination of CT + CFT has been reported to be a more sensitive parameter to assess emicizumab than either parameter considered in isolation [16]. Finally, earlier *in vitro* experiments showed that the aPTT of hemophilic plasma is shortened to normal by as little as 5 μg/mL emicizumab and remained constant at higher concentrations [13].

To understand the value that global coagulation measurements may have on monitoring emicizumab prophylaxis, we sought to evaluate the pattern of responsiveness that TGA or thromboelastometry parameters have as a function of emicizumab concentrations. To this end, we performed both *in vitro* and *ex vivo* experiments.

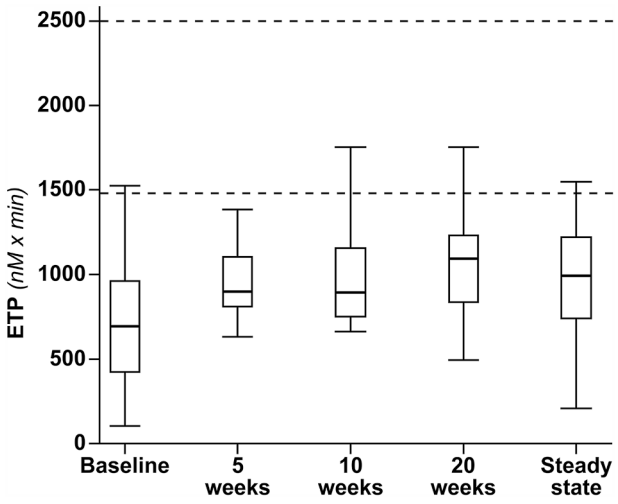

FIGURE 4 Box plots of the distribution of endogenous thrombin potential (ETP) recorded at different time points for patients on standard prophylaxis. Dotted horizontal lines represent the limits of the reference range.

The first set of experiments involved the addition of graded amounts of emicizumab to FVIII-deficient plasma, followed by the measurement of TGA parameters. The results of the *in vitro* study showed that the lag time and time-to-peak were barely affected by emicizumab, regardless of the composition of the triggers. The thrombin peak showed good dose-dependent and linear responses to emicizumab over the entire range of investigated concentrations, regardless of the TF and PL concentrations used in the trigger, although the highest steepness of the dose-response curve for thrombin peak was obtained when triggers were 1 pM TF plus either 1 or 4 μM PL. The ETP showed dose-response curves with steepness similar to those of the thrombin peak. All in all, the best compromise in terms of steepness and linearity of the dose-response curve was achieved when triggers were set at 1 pM TF plus 1 μM PL. Based on the above results, we propose that clinical laboratories should use thrombin peak or ETP (at low trigger concentrations), but not lag time or time-to-peak when performing TGA for patients on emicizumab.

In the *ex vivo* experiments, we aimed to establish the distribution of results of TGA and thromboelastometry parameters recorded at different time points and during steady state treatment. Correlation of TGA or thromboelastometry parameters vs emicizumab concentrations achieved *in vivo* were also evaluated.

The results showed that thrombin peak and ETP are suitable parameters to represent the pattern of expected variation of global coagulation at different time points during treatment. Finally, the study showed a strong correlation of thrombin peak vs emicizumab concentrations. Based on the above correlation, we attempted the estimation of thrombin peak values corresponding to the critical values of emicizumab concentrations achieved *in vivo* (ie, 40 or 80 μg/mL) in patients receiving prophylaxis. These values can be tentatively taken as yardsticks to judge the function of emicizumab and as functional surrogate measures of emicizumab concentration in patients receiving prophylaxis. Indeed, clinical trials showed that emicizumab plasma concentrations equivalent to 40 to 80 μg/mL were achieved in patients receiving prophylaxis who had reached regular steady state [7–9]. However, the thrombin generation may not correlate with clinical efficacy (see below) and therefore, it is still uncertain if it can be used to evaluate the drug's clinical effectiveness [12,17].

Thromboelastometry deserves different considerations. None of the parameters, with the exception of MCF, was correlated with emicizumab concentrations when thromboelastometry was measured in the presence of exogenous triggers, known as INTEM. In contrast, all the thromboelastometry parameters were strongly correlated with emicizumab concentrations when measured without exogenous triggers, known as NATEM. These results support the concept that NATEM is more informative than regular thromboelastometry measured with the addition of exogenous triggers, as shown by Yada et al. [16].

Based on the correlation, we estimated CT-NATEM values corresponding to the critical values of emicizumab concentrations achieved *in vivo* (ie, 40 or 80 μg/mL) in patients receiving prophylaxis. These values can be tentatively taken as functional surrogate measures of emicizumab in patients receiving prophylaxis.

Cumulatively, the information stemming from this report elicits the following considerations. Results from registration clinical trials showed that patients receiving prophylaxis with emicizumab can be treated at fixed doses based on patient characteristics without dose adjustment based on laboratory testing. However, in cases where assessing the emicizumab effect is needed, there are 2 options available. The first concerns the measurement of the plasma emicizumab concentrations by a dedicated method based on the modification of the 1-stage FVIII clotting activity in combination with an emicizumab certified standard [13]. This method, however, measures the concentrations of emicizumab and not the functional activity achieved *in vivo*. The logical alternative is to use global coagulation procedures based on TGA or thromboelastometry, the endpoints of which are thrombin generation or clot formation, respectively. In this respect, the present study shows that both procedures are suitable laboratory tools to be used as surrogate measures of the effect of emicizumab achieved *in vivo* in patients on prophylaxis. Concerning TGA, we showed that the parameters denoting the quantity of thrombin formation (ie, thrombin peak or ETP) may be more useful than the parameters denoting the speed of thrombin formation (ie, lag time or time-to-peak) and that both parameters should be measured by using trigger concentrations corresponding to 1 pM TF plus 1 μM PL.

With regard to thromboelastometry, we confirmed that the parameters measured by NATEM are more useful than those measured by INTEM [16]. The latter, commonly used in most clinical laboratories employing thromboelastometry, should not be used to monitor patients on emicizumab. We also confirmed that the combined value of CT + CFT may be a useful parameter [16], although its correlation with emicizumab concentration is not superior to that of CT (see Table 1).

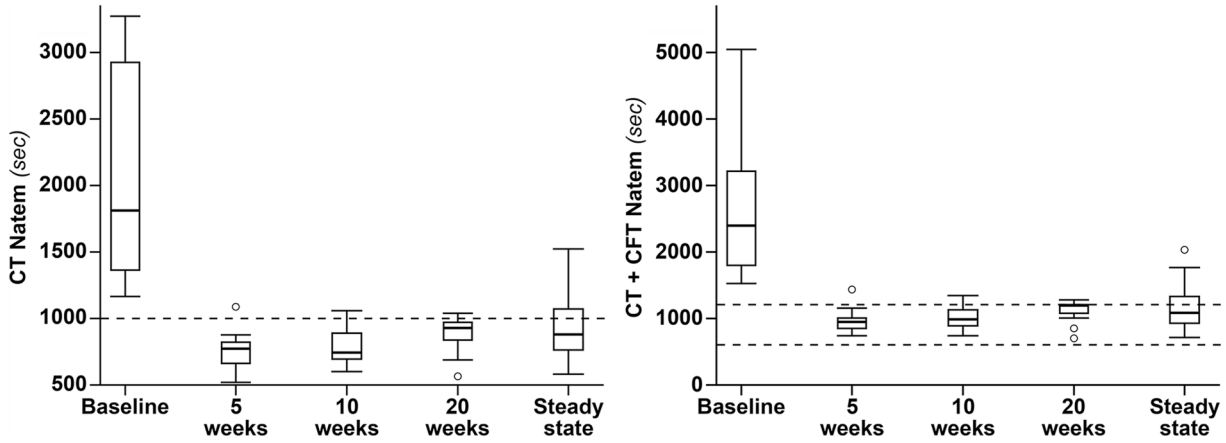

FIGURE 5 Box plots of the distribution of the thromboelastometry parameter clotting time (CT) (left panel) or for the combination of CT + clot formation time (CFT) (right panel), recorded at different time points for patients on standard prophylaxis. Testing was performed in whole blood without addition of exogenous triggers other than calcium chloride (NATEM). Dotted horizontal lines represent the limits of the reference range.

Finally, based on the correlations (TGA or thromboelastometry parameters vs emicizumab concentrations), we estimated TGA thrombin peak or thromboelastometry CT-NATEM values corresponding to 40 or 80 μg/mL emicizumab concentrations. Thus, we propose that the TGA thrombin peak or the thromboelastometry CT-NATEM can be used as surrogate measures of the effect of emicizumab achieved *in vivo* in patients receiving prophylaxis.

It should, however, be acknowledged that the clinical value stemming from the above laboratory parameters has not yet been conclusively confirmed. Data on the association of the above parameters with the clinical outcome in patients receiving prophylaxis with emicizumab are still conflicting. Arcudi et al. [17] reported that TGA or thromboelastometry parameters was not associated with spontaneous joint bleeding in a population of persons with hemophilia receiving emicizumab prophylaxis, although ETP was close to statistical significance. In contrast, Josset et al. [12] reported that ETP of platelet poor plasma, modified to include activation with TF and FXIa, was associated with the clinical response. It should, however, be considered that the study of Josset et al [12] did not use the regular commercially available TGA, and modifications were required that exceed the capacity of core laboratories where coagulation testing for hemophilia is now centralized. Furthermore, it should be considered that numbers of investigated patients are still limited and that bleeding in persons with hemophilia is a multifactorial phenomenon that does not depend entirely and solely on the extent of the effect of emicizumab. Hence, many other variables in addition to emicizumab may play a confounding role. It is, therefore, logical to assume that relatively large numbers of patients followed up for long time should be evaluated before drawing definitive conclusions. Until this information is available, we suggest that, whenever needed to assist clinicians, the laboratory should measure the TGA thrombin peak (or ETP), with triggers set at 1 pM TF plus 1 μM PL or the thromboelastometry CT, measured without exogenous triggers (ie, NATEM) to monitor patients suspected of poor compliance or who are over- or undertreated. Decision on clinical management of patients based on the above results should be taken with caution.

4.1 | Overall conclusions

1. Measurement of emicizumab concentration or activity in persons with hemophilia receiving prophylaxis is not generally needed, but there are occasions (ie, to assess for interindividual variation, to check for compliance, suspicion of anti-drug antibodies, to understand the mechanism of action of the drug, and others) when measurement is useful.
2. On the above occasions, TGA or thromboelastometry can be used to assess for the effect of emicizumab.
3. TGA assessed as thrombin peak or ETP and performed with low trigger concentrations can be used as surrogate measures of the effect achieved by emicizumab.
4. Thromboelastometry assessed as CT without triggers (NATEM) can be used as a surrogate measure of the effect achieved by emicizumab.
5. There are contrasting results on whether global coagulation procedures are reliable predictors of bleeding in patients receiving emicizumab prophylaxis.
6. Further trials with clinical endpoints and large number of persons with hemophilia are needed before TGA or thromboelastometry can be used to predict bleeding in patients receiving prophylaxis with emicizumab.

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AUTHOR CONTRIBUTIONS

A.T. conceived the study, reviewed results, and wrote the manuscript. M.C., E.S., and C.N. performed laboratory testing and data analysis. S. A. and R.G. were responsible for patient management. All authors reviewed and accepted the manuscript.

RELATIONSHIP DISCLOSURE

A.T. received speakers' fees from BioMarin, Stago, and Werfen. R.G. received honoraria for participating as a speaker on advisory boards and seminars organized by Pfizer, Roche, Novo Nordisk, and Takeda, outside the present work. F.P. received honoraria for participating in advisory boards organized by CSL Behring, BioMarin, Roche, Sanofi, and Sobi. The remaining authors declare no competing financial interests.

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SUPPLEMENTARY MATERIAL

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