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LETTER TO THE EDITOR

Thromboelastometry detects enhancement of coagulation in blood by emicizumab via intrinsic pathway

Novel haemophilia non-factor therapy with emicizumab (emi) bridges FIXa with FX to trigger tenase activity.[1] Emi has revolutionized the care in both inhibitor-positive and inhibitor-negative haemophilia A patients.[2,3] It is the first subcutaneous regimen, resolving venous access problems in these patients. Currently, emi's safety profile is supported by clinical studies. However, thrombosis has occurred in some patients, even without co-administration of activated prothrombin complex concentrate, aPCC. Also, breakthrough bleeds may occur.

Practical means to measure the effects of emi are limited. In the intrinsic pathway-based tests, emi overly shortens clotting times and enhances all single factor activities based on APTT, including the one-stage FVIII:C.[2] Our aim was with thromboelastometry assay to monitor the treatment in haemophilia patients during the loading and the steady states of emi. During emi treatment, we serially analysed the specific contribution of the intrinsic pathway by utilizing corn trypsin inhibitor (CTI) upon blood collection. The FXa generation *in vivo* was observed with circulating prothrombin fragments (F1+2).

Three inhibitor-positive (titre >0.6 BU/ml) haemophilia patients (patients 1–3) were treated with emi and followed for several months as part of the STASEY trial protocol. At emi initiation, mean age was 34 years, range being 21–53 years. Historical titre ranged at 5 –109 BU/ml (median 40) and at emi start from 2.3 to 10 (median 5.5). Historical ABR was between 1–10 (median 8).

Four additional patients were recruited (patients 4–7) outside STASEY: all had an inhibitor history, but the current titre was <0.6 BU/ml with a short FVIII half-life (5–8 h) of extended half-life (EHL) replacement therapy. At emi initiation, mean age was 37 years, range being 20–49 years. Historical titre ranged at 18 –718 BU/ml (median 67.5), and historical ABR at 0–3 (median 2).

The ethical permits (HUCH, Project code U1018X2724) allowed us to collect local plasma samples and assess the clinically relevant coagulation safety markers. All patients consented to take part in the study. The patients started emi after a 72-h washout period from the previous regular haemophilia treatment. The follow-up was of 1–15 months. The emi regimens were as follows: 1) inhibitor-positive patients: loading doses at 180–258 mg (median 230 mg; 3 mg/kg in all) and standard doses at 90–120 mg (median 123 mg; 1.5 mg/kg in all), and 2) non-inhibitor patients: loading doses at 210–270 mg (median 232.5 mg; 3 mg/kg in three patients and 2.5 mg/kg in patient 4), and the standard doses at 105 mg (1.0– 1.3 mg/kg).

We used ROTEM (Sigma; Werfen IVD International, Bedford, MA, USA) and non-activated ROTEM (NaTEM; Delta) to screen the blood coagulation at baseline and longitudinally during the emi treatment at loading (LP; weeks 1–4) and maintenance phases (MP; months 3–12). To study the role of the contact pathway, we collected blood to citrate both with and without CTI (50 µg/ml CTI, 11 mM citrate; SCAT-27-4.5/5, Hematologic Technologies Inc., Vermont, USA). We applied the experimental conditions of NaTEM and the intrinsic pathway activator of InTEM. NaTEM was performed at 30 min after blood sampling in all patients. Clotting time (CT, s), clot formation time (CFT, s), alpha angle (α, °) and maximum clot firmness (MCF, mm) were recorded.[4] Circulating prothrombin fragments, F1+2, was measured in the patient plasma with Enzygnost® ELISA (Siemens).

Thromboelastometric traces of NaTEM were practically absent at baseline, except for patient 6, in whom the carry-over effect of EHL was still detected after 72-h washout (Figure 1). NaTEM assesses the native contact pathway, by blood contacting with the cuvette material.[5] Indeed, the CTI- samples dissociated in their NaTEM profiles by the emi treatment. Following emi, all variables of NaTEM (CT, CFT, MCF) improved in citrated samples already at the loading (mean CT 1222 s, CFT 454 s, MCF 46 mm), while CTI eliminated the emi effect (Figure 1). The observations among the inhibitor-positive versus inhibitor-negative patients were similar during loading phase (Figure 1A,B). In patient 4, emi at reduced dose was effective already at week 1 (Figure 1C). Fibrin generation of this patient was delayed with the dose that was 40% of the on-label dose. This lowered dose was selected due to non-alcoholic fatty liver disease, hypertriglyceridemia and loss of active muscle tissues (wheelchair). Interestingly, with the 30% reduced maintenance dose his responses paralleled with the other patients, both in NaTEM and InTEM (Figures 1 and 2). We concluded that NaTEM enabled us to pick up the dose-dependent effects during emi treatment. CTI eliminated these responses, suggesting that the intrinsic contact activation of coagulation contributes to emi-improved haemostasis.

Emi in InTEM shortened both CT and CFT (Figure 2), whereas MCF and the other variables remained normal in citrated blood. In CTI-citrated blood, emi treatment also improved MCF from the baseline level (Figure 2). In citrated samples, the mean CT shortened from baseline already during the loading phase (666 s vs. 194 s; Figure 2). During the follow-up (≥3 months), the mean baseline CT further shortened during the maintenance phase and reached the level of the healthy controls (1842 s vs 1824 s in controls; Figure 2).

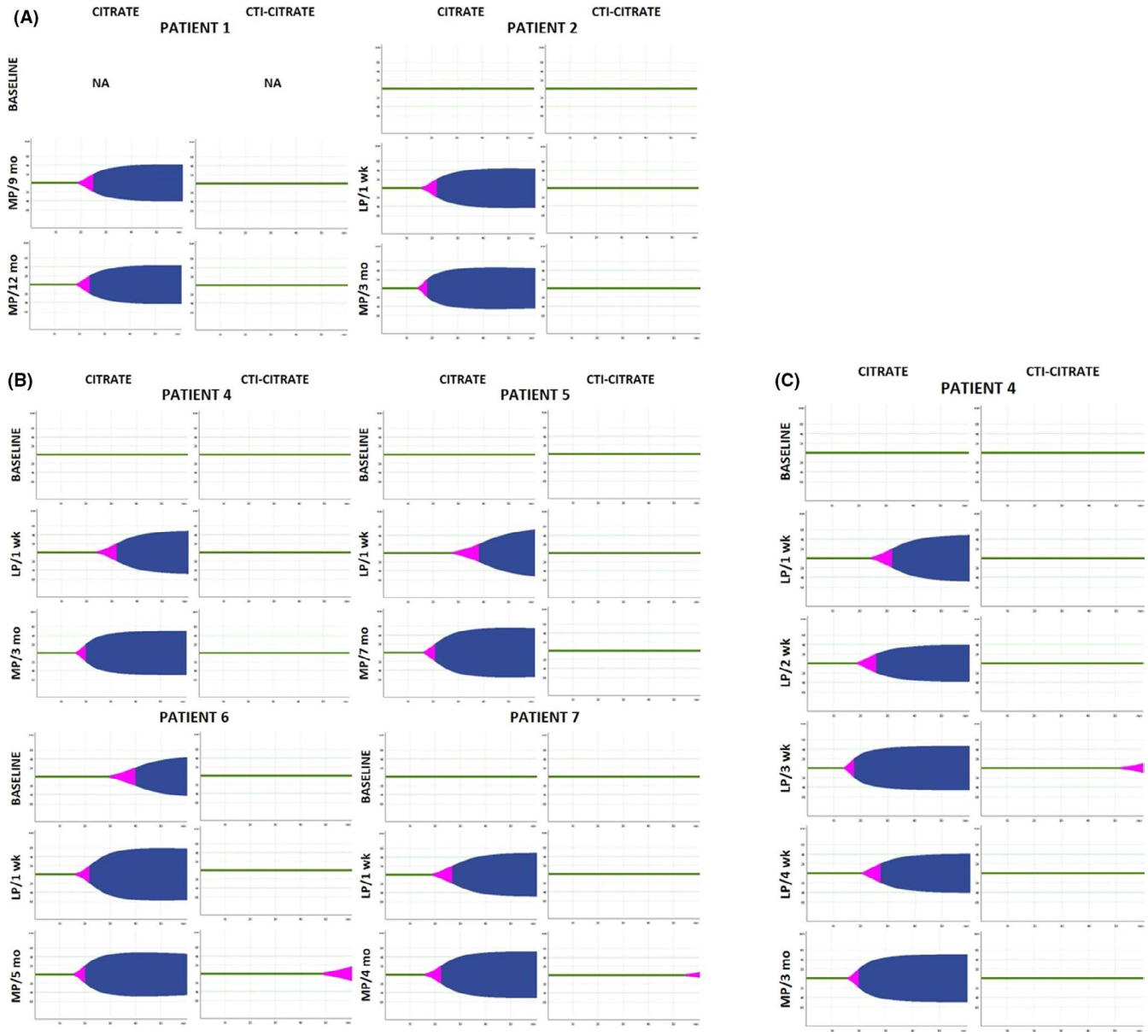

FIGURE 1 Longitudinal results of thromboelastometry by NaTEM during the emi treatment. (A) two inhibitor-positive haemophilia patients (1 and 2), (B) four inhibitor-negative haemophilia patients (4–7) and (C) patient 4 either at baseline, at loading phase (LP) or at maintenance phase (MP) of emi treatment are shown. Blood coagulation was triggered with calcium supplementation alone (NaTEM) and detected from the blood without and with corn trypsin inhibitor (CTI) to illustrate the contribution of the intrinsic pathway of coagulation. The selected doses were lower in patient 4 (2.5 mg/kg for weeks 1–3 and 1.0 mg/kg thereafter) due to the specific thrombosis risk profile of the patient na, not available; wk, week; mo, month

Mean CFT was also shortened by 2-fold (162 s vs. 81 s). Upon inactivation of the contact pathway by CTI, at baseline the CT prolonged significantly more in patients than in controls (2142 s patients vs. controls 295 s; Figure 2). This finding reveals the contribution of lacking FVIII in the InTEM analysis, which under routine citrated conditions of ROTEM did not clearly depict haemophilia (MCF), even in the presence of inhibitors. Emi markedly shortened CT during the first four weeks of emi (mean 369 s; Figure 2). Already, the first dose of emi shortened CT by 5.4-fold. Also, CFT was similarly shortened (579 s vs 103 s), while emi enforced MCF (38 mm vs. 58 mm). Moreover, the alpha angle augmented, also referring to the contribution of platelets in the clot formation by emi.

Overall, the responses in NaTEM especially, and also somewhat in InTEM, improved after the first doses of emi, and the responses progressed to reach a plateau at the maintenance phase. According to inhibition of contact activation by CTI, in comparison with baseline emi clearly enhanced the NaTEM and InTEM effects. In this easily available global assay, the enhanced clot formation indicates that emi seems to carry an additional role via activation of the contact system.

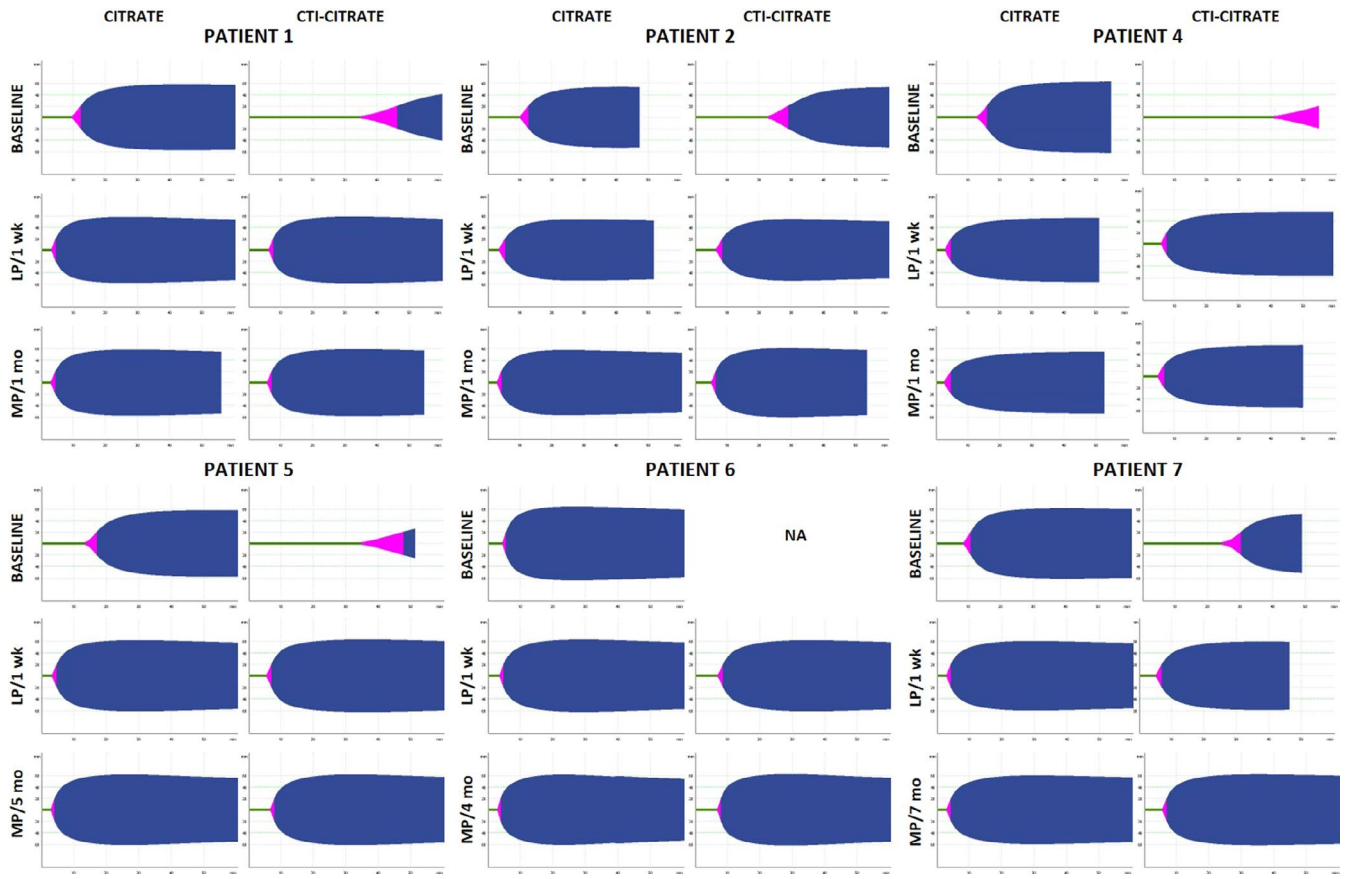

FIGURE 2 Thromboelastographs of InTEM throughout the longitudinal administrations of emi. Results of two inhibitor-positive (1 and 2) and four inhibitor-negative (4–7) haemophilia A patients are shown at baseline, loading phase (LP) at 1 week and maintenance phase (MP) at 1–7 months of emi treatment. The contribution of the intrinsic pathway of coagulation (InTEM) was detected in the absence and presence of corn trypsin inhibitor (CTI)

We followed the circulating biomarkers of F1+2 in patients 1–4. These longitudinal results stayed within the reference ranges.

Our main observation is the suitability of the thromboelastometry for the monitoring of clinical emi effects in whole blood of haemophilia A patients both with historical and current inhibitors. ROTEM, especially its NaTEM format, could trace the stepwise impact of the four-weekly loading and the maintenance doses of emi on clot formation. The emi sensitivity of NaTEM assay has been reported earlier as well.[6] Moreover, CTI practically eliminated this response,[5] suggesting the influence of emi on contact activation. The circulating F1+2 did not increase during the 1-year emi therapy, but remained within the normal references.[6]

Emi's novel mechanism of action on coagulation provides challenges at clinical or practical laboratory level.[5] This uncertainty may prevail under aberrant haemostatic responses, especially under major injury, surgery and severe infection, including sepsis, increasing the risks of these patients for either bleeding or thrombotic complications.[7–9] We confirmed that NaTEM was the best method to demonstrate the effect of the emi treatment.[6] In our whole blood study, the role of platelets and red cells in clot formation during emi treatment was evidenced by the strengthened clot firmness. We used CTI-citrated blood to show that emi may trigger the FIX and FX activation via engaging FXII and FXI in the coagulating blood of both inhibitor-positive and inhibitor-negative patients. In NATEM, the coagulation is triggered with calcium and occurs at the cuvette surface contact. The visual effects of emi could be evidenced both in NaTEM and InTEM (with high-dose ellagic acid) upon CTI-treatment. Indeed, CTI eliminated the effect of emi in NaTEM. In InTEM, CTI revealed the poor response of haemophilia A blood at baseline, whereas emi overruled this CTI effect during the follow-up possibly due to the contact activation-dependence of InTEM. These findings align with the observation that emi dose-dependently improves thrombin generation in FXI-deficient blood.[10] Further studies are needed to confirm the contribution of the intrinsic pathway in haemostatic emi responses.

Since emi significantly supports haemostasis, it may also suit for patients having haemophilia A of moderate/mild severity, with likely reduced doses. We want to highlight that a lower than on-label emi dose managed to control the bleeding tendency and enhanced the ROTEM profiles of the patient 4 with a thrombogenic clinical propensity. He seemed to reach the same levels of coagulation activity with the biomarkers and ROTEM as the other patients with the routine dosing scheme.

In conclusion, we provide evidence that ROTEM and especially NaTEM enable the laboratory assessment of emi effects. These tools are clinically relevant in the operating theatre and emergency settings and may solve the incidental dilemmas of abnormal coagulation during emi therapy.

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DISCLOSURE

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AUTHOR CONTRIBUTIONS

R. Lassila. and T. Szanto designed the study. T. Szanto, I. Vaide and A. Jouppila performed the studies and analysed the data. M. Lemponen performed part of the routine coagulation tests and the spiking studies. All authors contributed to the interpretation of the results. The first draft was written by T. Szanto, I. Vaide and R. Lassila, and all authors contributed to and edited all subsequent drafts.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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