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Detection of enoxaparin and argatroban by use of the novel viscoelastic coagulometer ClotPro

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Owing to the simultaneous increase in the risk of thrombosis and bleeding in critically ill patients, point-of-care-available diagnostic tests to guide parenteral anticoagulation are warranted. We evaluated the detection of enoxaparin and argatroban, two commonly used parenteral anticoagulants, using the novel ClotPro viscoelastic coagulometer. For this experimental in vitro study at a tertiary care academic center, blood samples were drawn from twelve (six female, six male) healthy volunteers without intake of antithrombotic medication and no history of hemostatic disorders. Blood samples were spiked with enoxaparin (IU.ml^{-1}) and argatroban (µg.ml^{-1}) at increasing concentrations ranging from 0 to 1. The ClotPro Russell's viper venom (RVV)-test and the ClotPro ecarin (ECA)-test clotting time were performed in parallel with conventional coagulation tests (anti-Xa activity, activated partial thromboplastin time, and diluted thrombin time). We observed a strong correlation between anti-Xa activity and the RVV-test clotting time ($r = 0.88$ (95% confidence interval (CI) 0.8–0.92; $p < 0.001$)). Although clotting time cutoff values of 71 and 145 s provided high sensitivity and specificity for detecting anti-Xa activity of ≤ 0.1 and ≥0.6 IU.ml^{-1}, we found a poor performance at both high and low concentrations. The ECA-test clotting time revealed a very strong correlation with activated partial thromboplastin time ($r = 0.96$ (95% CI 0.93–0.97; $p < 0.001$)) and diluted thrombin time ($r = 0.97$ (95% CI 0.96–0.98; $p < 0.001$)). The clotting time cutoff values of 86 and 298–431 s provided high sensitivity and specificity for detecting diluted thrombin time values ≤ 0.1 and 0.5-1 µg.ml^{-1}. Our results suggest that the RVV test is an unreliable method for monitoring enoxaparin treatment, whereas the ECA-test might be an accurate point-of-care alternative for detecting argatroban concentration with potential advantages over standard coagulation tests in terms of point-of-care applicability and turnaround time.

Keywords Anticoagulants, Coagulation management, Critical care, Point-of-care testing, Viscoelastic haemostatic assays

Abbreviations

aPTT	activated partial thromboplastin time
AUROC	area under the receiver operating curve
CFT	clot formation time
CT	clotting time
DTI	direct thrombin inhibitor
dTT	diluted thrombin time
ECA	ecarin
LMWH	low molecular weight heparin
MCF	maximum clot firmness
POC	point of care
PT	prothrombin time
RVV	Russell's viper venom

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Critically ill patients are at an increased risk of venous thromboembolism, and routine pharmacological thromboprophylaxis forms a part of the standard of care in this patient cohort. In this context, current guidelines recommend the use of low-molecular-weight heparin (LMWHs)[1,2]. In critically ill patients with confirmed or suspected heparin-induced thrombocytopenia, the parenteral direct thrombin inhibitor argatroban is the most commonly used alternative anticoagulant[3].

In addition to an increased risk of thrombosis, critically ill patients typically exhibit a simultaneously increased risk of bleeding[4,5]. To provide reliable monitoring of the balance between thrombosis and bleeding, several laboratory tests are commonly performed. Anti-factor Xa (anti-Xa) activity is a widely used laboratory test to measure LMWH[6]. Target ranges between 0.6 and 1.0 $IU.ml^{-1}$ have been established for therapeutic anticoagulation[7], whereas the utility of anti-Xa measurement in prophylactic anticoagulation remains a matter of debate[8]. For monitoring argatroban, activated partial thromboplastin time (aPTT) represents the most used laboratory test. Target ranges are based on patients' baseline values with a suggested 1.5- to 3-fold increase in aPTT[9]. However, particularly in critically ill patients, the validity of aPTT[10] and its usefulness in argatroban monitoring[11] has been questioned. Diluted thrombin time (dTT) is a promising alternative method that allows quantification of argatroban plasma concentration with suggested target ranges between 0.5 and 1.0 $\mu g.ml^{-1}$.[9,12] However, anti-Xa, aPTT and dTT share the disadvantage of long turnaround times in potentially time-sensitive clinical situations.

Whole blood–based viscoelastic hemostatic assays are available at the point of care (POC) and have been shown to result in shorter turnaround times[13]. They are used in the context of goal-directed, individualized treatment algorithms for bleeding patients, whereas their potential value for the management of anticoagulation remains poorly investigated[14]. ClotPro, a novel viscoelastic coagulometer, offers commercially available assays for measuring the activity of Xa inhibitors (Russell's viper venom test (RVV-test)) and thrombin inhibitors (ecarin-test (ECA-test)). Both tests employ the use of viper venoms that directly activate coagulation factors X and II, respectively, which makes them ideal candidates to detect the activity of anticoagulants targeting these factors, such as LMWH and argatroban.

We aimed to evaluate the feasibility of detecting the two widely used parenteral anticoagulants, enoxaparin and argatroban, at concentrations typically used in critically ill patients using the commercially available ClotPro assays RVV-test and ECA-test performed in whole blood. Additionally, we analyzed the correlations between (i) the RVV-test and plasma-based anti-Xa activity and (ii) the ECA-test and plasma-based dTT- and aPTT-values. We calculated cutoff values for detecting enoxaparin concentrations ≤ 0.1 $IU.ml^{-1}$ and 0.6-1 $IU.ml^{-1}$ as well as argatroban concentrations ≤ 0.1 $\mu g.ml^{-1}$ and 0.5-1 $\mu g.ml^{-1}$.

Methods

This in vitro pilot study of spiked whole blood samples from healthy volunteers was approved by the Ethics Committee of the Medical University of Vienna (EK 1937/2022, 23/02/2023, Dr. Juergen Zezula) and was performed in accordance with the principles of good clinical practice and the Declaration of Helsinki. The study was conducted in the research laboratories of the Medical University of Vienna, Department of Anesthesia, Intensive Care and Pain Medicine, and the Department of Laboratory Medicine. Written informed consent was obtained from all the participants.

Study participants

Blood samples were drawn from 12 adult volunteers (six male and six female). Exclusion criteria were (i) known or identified (during the course of the study) hemostatic disorders, (ii) intake of anticoagulants and/or platelet aggregation therapy 14 days prior to enrolment, (iii) known renal or hepatic impairment, and (iv) current participation in another study. Blood was collected by venipuncture using Venflon™ Pro Safety 18 G in sodium citrate 3.2% blood collection tubes (Greiner Bio-One, Kremsmünster, Austria). No follow-up of volunteers was required.

Sample preparation

Citrated whole blood was spiked with either enoxaparin (Laboratorios Farmaceuticos Rovi SA, Spain) or argatroban (Mitsubishi Tanabe Pharma, Austria) to target the following clinically relevant plasma concentrations:

Enoxaparin: 0, 0.25, 0.5, 0.75, 1.0 anti-Xa $IU.ml^{-1}$.

Argatroban: 0, 0.25, 0.5, 0.75, 1.0 $\mu g.ml^{-1}$.

Spiking solutions with decreasing concentrations of enoxaparin and argatroban were prepared, and 11 µL of each spiking solution was added to 2 ml of citrated whole blood. Spiked citrated whole-blood samples were incubated at 36 °C for 10 min to achieve stable conditions.

Standard laboratory tests and viscoelastic tests

Blood cell counts and standard laboratory coagulation tests were performed as the baseline measurements. Prothrombin time (PT) (Owren), aPTT, thrombin time, and fibrinogen level (Clauss method) were determined in plasma using an STA R Max 2 coagulometer (Diagnostica Stago SAS, Asnieres, France). Antithrombin activity was measured using a heparin cofactor AT assay based on thrombin inhibition (STA-STACHROM ATIII; Diagnostica Stago, Asnieres, France). Complete blood images, including red blood cells, leukocytes, platelet count, and haemoglobin were determined using a Sysmex XN-1500 cell counter (Sysmex, Vienna, Austria).

All viscoelastic measurements were performed in whole blood using the commercially available CE-certified viscoelastic coagulometer ClotPro (Enicor GmbH, Munich, Germany), according to the manufacturer's instructions. The principles of ClotPro measurements have been described previously[15]. Briefly, clotting is activated with different reagents depending on the respective test. Various parameters can be read out from the

typical viscoelastic curve (Supplemental Fig. 1): clotting time (CT [s], defined as the time from the start of the test until an amplitude of 2 mm is reached), clot formation time (CFT [s], defined as the time from reaching 2 mm until an amplitude of 20 mm is measured), amplitude at 5, 10, 20, or 30 min after reaching a 2 mm amplitude (A5, A10, A20, A30 [mm]), and maximum clot firmness (MCF [mm]; maximum amplitude of the clot). EX-Test (coagulation activation by tissue factor) and IN-Test (coagulation activation by ellagic acid) were performed as viscoelastic baseline measurements from native whole blood samples. After spiking the whole blood samples, the RVV-test and ECA-test were performed in duplicate for each of the five enoxaparin-spiked blood samples (0, 0.25, 0.5, 0.75 and 1.0 $IU.ml^{-1}$) and argatroban-spiked blood samples (0, 0.25, 0.5, 0.75 and 1.0 $\mu g.ml^{-1}$), respectively.

The residues of the samples were centrifuged for 15 min at 2500 × g and 15 °C to obtain platelet-poor plasma, which was stored at -80 °C for subsequent measurement of anti-Xa activity (enoxaparin), aPTT, and dTT (argatroban). Anti-Xa activity was determined using STA-liquid anti-Xa calibrated for use with LMWH (Diagnostica Stago, Asnieres, France), and dTT measurements were performed using the Hemoclot DTI assay calibrated for use with argatroban (Hyphen BioMed, Neuville-sur-Oise, France). Measurements of aPTT were performed as previously described.

Statistical analysis

As this study was designed as a pilot study, a formal sample size calculation was not performed. The sample size was determined based on our experience from similar previously conducted experimental studies[16,17] and previously published recommendations for sample size in pilot studies[18].

We performed descriptive statistical analysis, presenting data as either mean with standard deviation or as median with 25th and 75th percentiles, and graphically using boxplots. To assess the association between continuous variables, we drew scatter plots and calculated Pearson's correlation coefficients. We considered values between 0.9 and 1.0 a very strong correlation, 0.7–0.89 a strong correlation, 0.5–0.69 a moderate correlation, 0.3–0.49 a weak correlation and < 0.3 no correlation. Cutoffs for the prediction of pre-specified anti-Xa activity and argatroban concentrations were determined by logistic regression. We considered the optimal cut-off values that maximized Youden's index. We calculated the mean cutoffs, sensitivity, specificity, and the area under the receiver operating curve (AUROC) with standard deviations using bootstrapping with 1000 repetitions. R version 4.2.3, with the *cutpointr* package, was used for statistical analysis.

Results

This study was conducted between February and April 2023. We recruited 12 volunteers who met predefined inclusion and exclusion criteria. The volunteer characteristics and baseline laboratory measurements are shown in Table 1.

	n = 12	Reference range (female/male)
Age (years)	28 (26, 31)	
Sex	F: 6 (50%), M: 6 (50%)	
Weight (kg)	72 (60, 80)	
Height (cm)	178 (170, 183)	
Red blood cells ($t.l^{-1}$)	4.60 (4.10, 5.03)	F: 3.8–5.2 M: 4.4–5.8
Haemoglobin ($g.l^{-1}$)	139.00 (124.75, 145.00)	F: 120–160 M: 135–180
Haematocrit (%)	40.80 (36.93, 42.87)	F: 35–47 M: 40–52
Leukocytes ($g.l^{-1}$)	6.03 (4.58, 6.56)	4–10
Platelet count ($g.l^{-1}$)	263 (238, 303)	150–350
Prothrombin time (s)	27.80 (26.33, 30.10)	24.6–32.7
International normalised ratio (-)	1.00 (1.00, 1.10)	
Activated partial thromboplastin time (s)	34.20 (33.42, 35.50)	27–41
Thrombin time (s)	15.90 (15.47, 16.25)	< 21.0
Clauss' fibrinogen concentration ($g.l^{1}$)	2.46 (2.27, 3.03)	2–4
Antithrombin III activity (%)	112 (100, 114)	80–120
EX-Test clotting time (s)	56 (49, 62)	38–65
EX-Test clot formation time (s)	61 (50, 80)	42–93
EX-Test maximum clot firmness (mm)	58 (56, 61)	53–68
IN-Test clotting time (s)	158 (145, 161)	139–187
IN-Test clot formation time (s)	78 (66, 92)	52–139
IN-Test maximum clot firmness (mm)	55 (54, 60)	49–65

Table 1. Volunteer characteristics and laboratory measurements at baseline. All data given as a median and interquartile range [IQR].

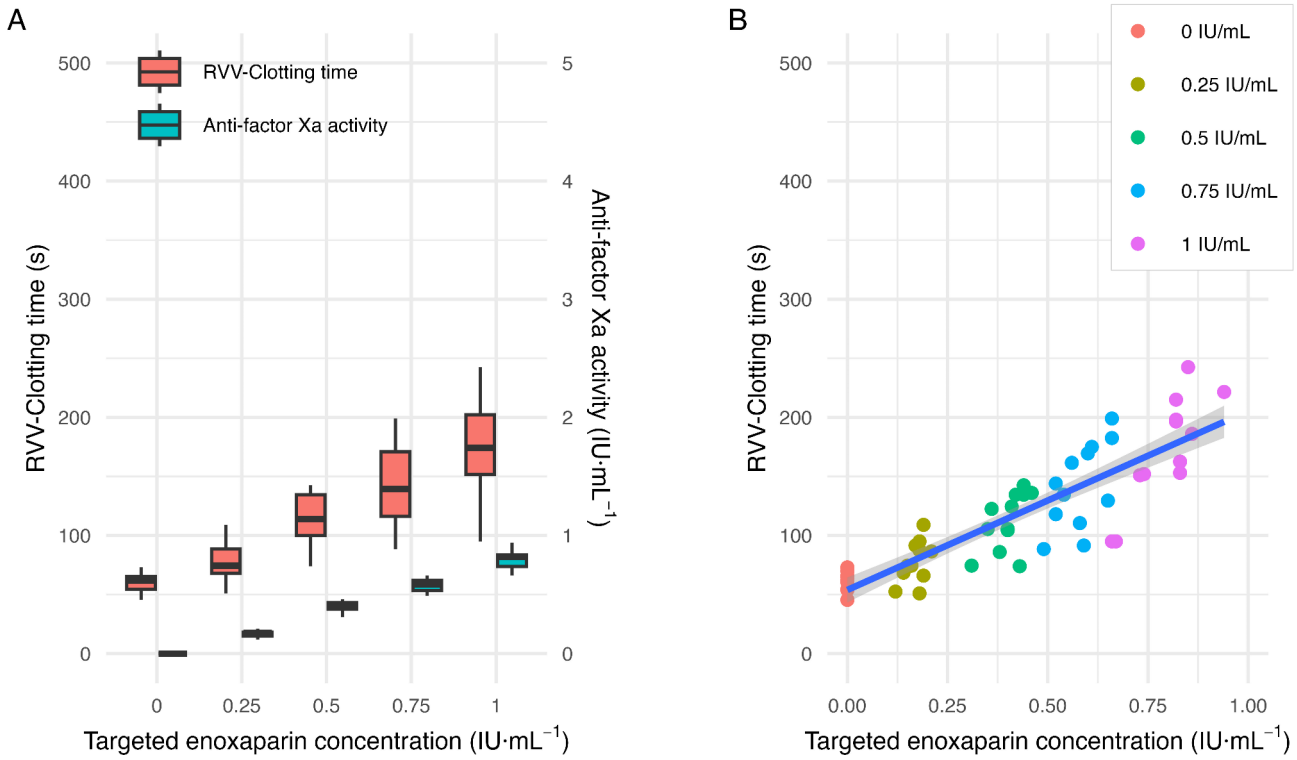

Fig. 1. (**A**) ClotPro RVV-Clotting time and anti-factor Xa activity for samples spiked with enoxaparin. X-axis depicts the targeted enoxaparin concentration in plasma, whereas the right Y-axis depicts the measured anti-factor Xa activity. (**B**) Correlation between anti-factor Xa activity and ClotPro RVV-Clotting time (shaded area depicts 95% CI). (ClotPro was measured using whole blood, whereas anti-factor Xa activity was measured using plasma.)

	Reference range	CON = 0 *N* = 12	CON = 0.25 *N* = 12	CON = 0.5 *N* = 12	CON = 0.75 *N* = 12	CON = 1 *N* = 12	*r*
Anti-factor Xa activity (IU.ml^{-1})	< 0.1	< 0.1 (< 0.1-< 0.1)	0.18 (0.15–0.18)	0.41 (0.38–0.43)	0.59 (0.54–0.62)	0.82 (0.74–0.84)	-
Clotting time (s)	48–77	61 ± 8	77 ± 17	112 ± 24	142 ± 36	172 ± 47	0.88 (95% CI 0.8–0.92; $p < 0.001$)
Clot formation time (s)		63 ± 14	65 ± 16	68 ± 18	72 ± 18	75 ± 18	0.32 (95% CI 0.08–0.53; $p = 0.01$)
Amplitude after 5 min (mm)	38–55	45.3 ± 4.9	45.2 ± 5.2	44.8 ± 5.1	44.2 ± 5.0	43.3 ± 5.2	-0.22 (95% CI -0.45-0.03; $p = 0.09$)
Amplitude after 10 min (mm)	47–63	53.4 ± 4.5	53.3 ± 4.7	53.2 ± 4.6	52.6 ± 4.6	51.8 ± 4.7	-0.2 (95% CI -0.43-0.05; $p = 0.12$)
Amplitude after 20 min (mm)	53–67	57.6 ± 4.2	57.2 ± 4.5	57.3 ± 4.2	56.9 ± 4.2	56.6 ± 4.1	-0.16 (95% CI -0.39-0.1; $p = 0.24$)
Amplitude after 30 min (mm)		57.9 ± 4.2	57.0 ± 4.7	57.5 ± 4.1	57.0 ± 4.2	57.0 ± 3.9	-0.14 (95% CI -0.38-0.12; $p = 0.29$)
Maximum clot firmness (mm)	54–68	58.3 ± 4.2	57.6 ± 4.4	58.0 ± 3.9	57.4 ± 3.9	57.4 ± 3.8	-0.15 (95% CI -0.39-0.11; $p = 0.27$)

Table 2. Anti-factor xa activity and ClotPro values for samples spiked with low molecular weight heparin. Anti-factor Xa activity is given as a median and interquartile range [IQR]. All other variables are given as means and standard deviations (± SD). CON, targeted concentration; r: Pearson's correlation coefficient for variable with anti-factor Xa activity.

Enoxaparin

In samples spiked with enoxaparin, we observed a dose-dependent increase in anti-Xa activity and dose-dependent prolongation of ClotPro RVV-test clotting times (CT) (Fig. 1A). The ClotPro RVV-test CT displayed high variance, particularly in samples with increased enoxaparin concentrations, whereas the anti-Xa activity showed a narrow distribution (Fig. 1A and B; Table 2). We found a strong correlation between anti-Xa activity and the ClotPro RVV-test CT (Fig. 1B; Table 2). In contrast, we observed a weak correlation between anti-Xa activity and the ClotPro RVV-test clot formation time (CFT). Furthermore, we observed no correlation between

anti-Xa activity and ClotPro RVV-test amplitude after 5, 10, 20, or 30 min or between anti-Xa activity and ClotPro RVV-test maximum clot firmness (Table 2).

A ClotPro RVV-test CT cutoff of 71 ± 3 s predicted anti-Xa activity ≤ 0.1 IU.ml^{-1} with a sensitivity of 100 ± 1% and a specificity of 91 ± 4% (AUROC 0.95 ± 0.03), whereas a cutoff of 145 ± 16 s predicted an anti-Xa activity ≥ of 0.6 IU.ml^{-1} with a sensitivity of 86 ± 9% and a specificity of 95 ± 7% (AUROC 0.94 ± 0.03) (Supplemental Fig. 2). Due to missing values that exceeded anti-Xa 1.0 IU.ml^{-1}, we were unable to calculate a respective ClotPro RVV-test CT cutoff.

Argatroban

In samples spiked with argatroban, we observed a dose-dependent prolongation of the dTT, aPTT, and ClotPro ECA-test CT (Figs. 2A and 3A).

We observed a very strong correlation between the dTT values and ClotPro ECA-test CT, as well as between the aPTT values and ClotPro ECA-test CT (Figs. 2B and 3C; Table 3). All three monitoring methods displayed higher variances at increased argatroban concentrations, with aPTT showing the highest variance across all drug concentrations (Figs. 2 and 3; Table 3). In addition, we found a strong correlation between the dTT values and the ClotPro ECA-test CFT as well as between the aPTT values and the ClotPro ECA-test CFT (Table 3). In contrast, we observed no correlation between dTT values and ClotPro ECA-test amplitudes after 5, 10, 20, or 30 min, and no correlation between aPTT values and ClotPro ECA-test amplitudes after 5, 10, 20, or 30 min. Furthermore, we found no correlation between dTT values and the ClotPro ECA-test MCF or between aPTT and the ClotPro ECA-test MCF.

A ClotPro ECA-test CT cutoff of 86 ± 1 s predicted dTT values ≤ 0.1 µg.ml^{-1} with a sensitivity of 100 ± 0% and a specificity of 100 ± 0% (AUROC 1.0 ± 0), whereas a cutoff of 298 ± 9 s predicted dTT values ≥ 0.5 µg.ml^{-1} with a sensitivity of 100 ± 0% and a specificity of 99 ± 2% (AUROC 1.0 ± 0), and 431 ± 12 s predicted dTT values ≤ 1 µg.ml^{-1} with a sensitivity of 98 ± 1% and a specificity of 94 ± 4% (AUROC 0.98 ± 0.01) (Supplemental Fig. 3).

Discussion

In this prospective in vitro pilot study, we investigated the feasibility of detecting two commonly used parenteral anticoagulants (i) enoxaparin and (ii) argatroban, using the ClotPro RVV-test and ECA-test. We demonstrated that depending on their concentrations, prolonged clotting times occurred in the (i) ClotPro RVV-test and in the (ii) ClotPro ECA-test. Additionally, we found strong correlations between (i) anti-Xa activity and ClotPro RVV-

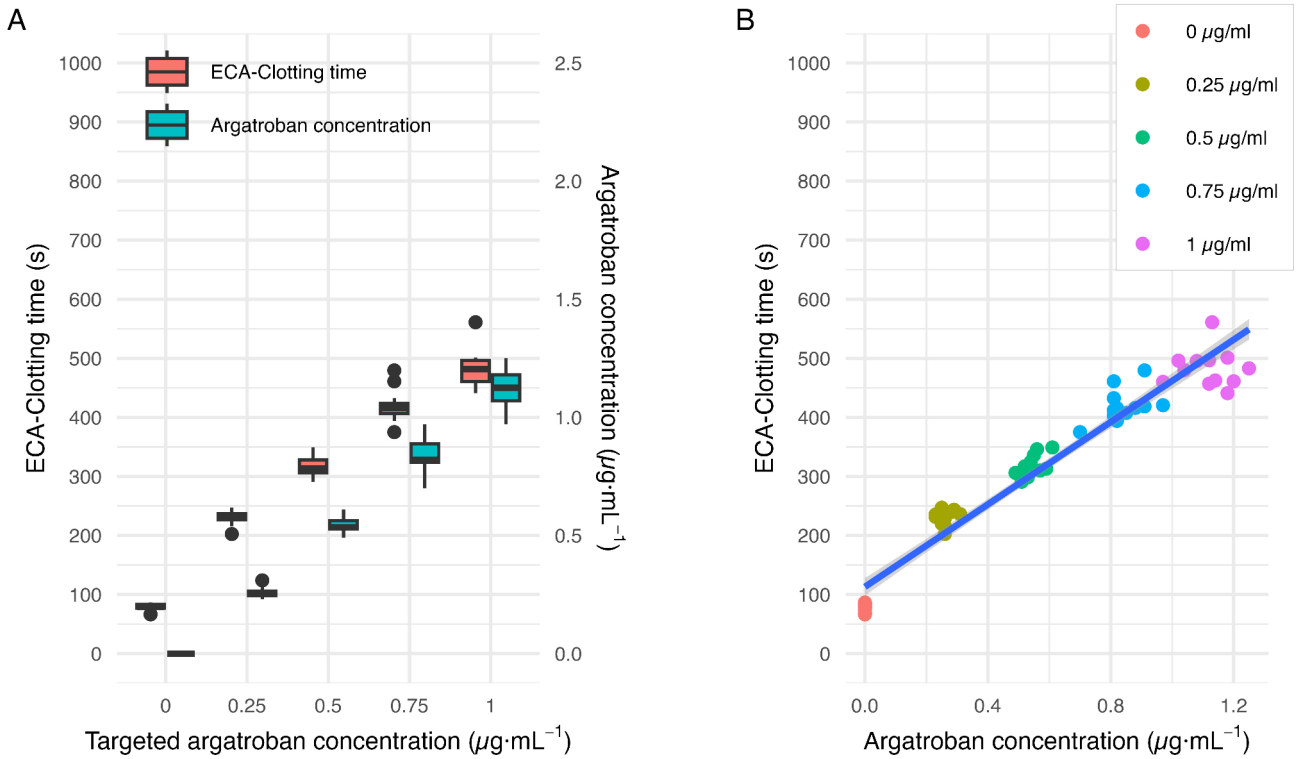

Fig. 2. (**A**) ClotPro ECA-Clotting time and diluted thrombin time for samples spiked with argatroban. X-axis depicts the targeted argatroban concentration in plasma, whereas the right Y-axis depicts the argatroban concentration measured by diluted thrombin time. (**B**) Correlation between diluted thrombin time and ClotPro ECA-Clotting time (shaded area depicts 95% CI). (ClotPro was measured using whole blood, whereas diluted thrombin time was measured using plasma.)

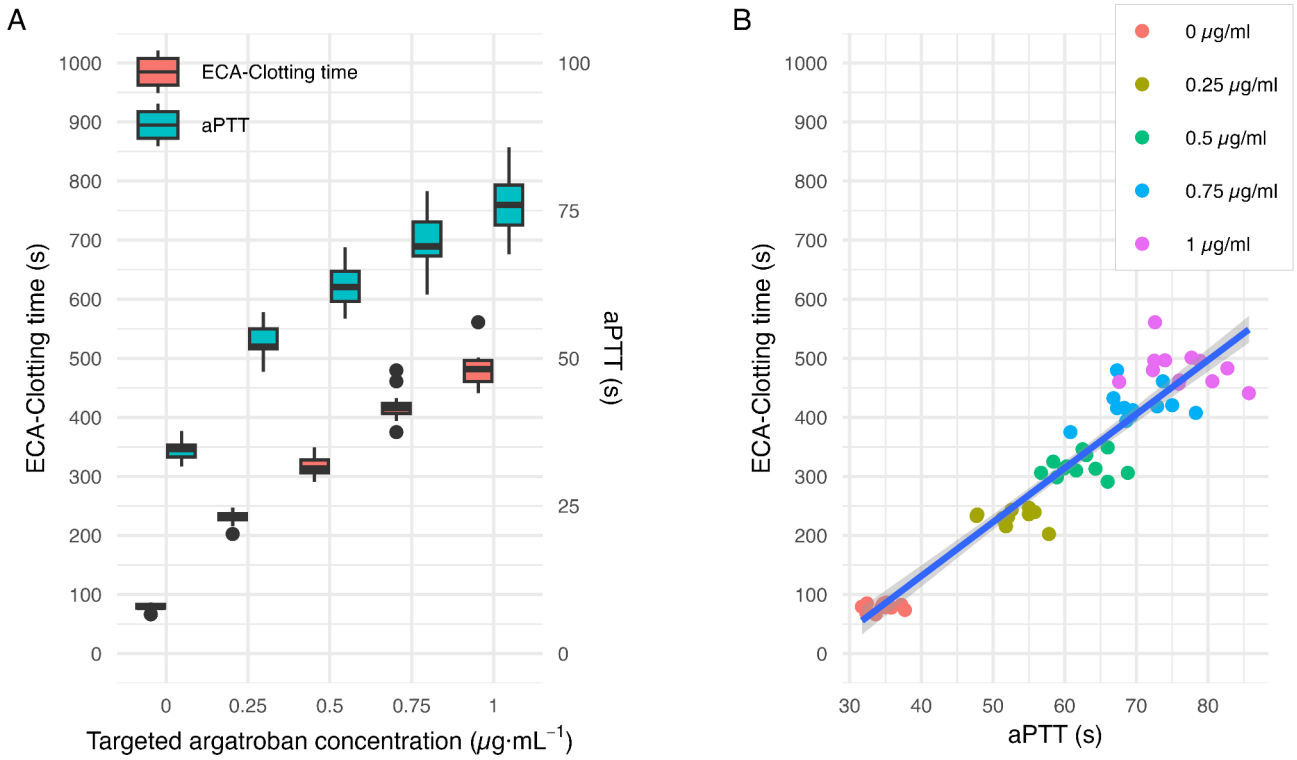

Fig. 3. (**A**) ClotPro ECA-Clotting time and aPTT for samples spiked with argatroban. X-axis depicts the targeted argatroban concentration in plasma, whereas the right Y-axis depicts the aPTT (shaded area depicts 95% CI). (**B**) Correlation between aPTT and ClotPro ECA-Clotting time. (ClotPro was measured using whole blood, whereas aPTT was measured using plasma.)

	Reference range	CON = 0 *N* = 12	CON = 0.25 *N* = 12	CON = 0.5 *N* = 12	CON = 0.75 *N* = 12	CON = 1 *N* = 12	r1	r2
Diluted thrombin time (s)	< 0.1	0.00 (0.00–0.00)	0.26 (0.25–0.27)	0.54 (0.53–0.56)	0.82 (0.81–0.89)	1.13 (1.07–1.18)	-	-
Activated partial thromboplastin time (s)	27–41	35 ± 2	53 ± 3	62 ± 4	70 ± 5	76 ± 5	-	-
Clotting time (s)	68–100	79 ± 6	230 ± 12	318 ± 18	420 ± 28	483 ± 31	0.97 (95% CI 0.96–0.98; $p < 0.001$)	0.96 (95% CI 0.93–0.97; $p < 0.001$)
Clot formation time (s)		74 ± 8	102 ± 16	133 ± 25	147 ± 16	170 ± 28	0.84 (95% CI 0.75–0.9; $p < 0.001$)	0.8 (95% CI 0.68–0.87; $p < 0.001$)
Amplitude after 5 min (mm)	45–48	48.9 ± 4.7	47.6 ± 4.5	43.8 ± 4.7	42.9 ± 3.4	39.0 ± 4.4	-0.65 (95% CI -0.77–0.47; $p < 0.001$)	-0.59 (95% CI -0.73–0.39; $p < 0.001$)
Amplitude after 10 min (mm)	54–66	56.7 ± 4.2	56.5 ± 4.4	55.2 ± 5.2	56.0 ± 4.2	55.0 ± 3.5	-0.17 (95% CI -0.41-0.08; $p = 0.19$)	-0.17 (95% CI -0.4-0.09; $p = 0.21$)
Amplitude after 20 min (mm)	58- -70	61.4 ± 3.5	61.1 ± 3.6	60.2 ± 4.7	61.0 ± 3.7	61.0 ± 3.8	-0.07 (95% CI -0.32-0.19; $p = 0.6$)	-0.08 (95% CI -0.33-0.18; $p = 0.56$)
Amplitude after 30 min (mm)		62.8 ± 3.3	62.5 ± 3.3	61.1 ± 4.3	62.3 ± 3.6	62.1 ± 3.9	-0.1 (95% CI -0.36-0.17; $p = 0.46$)	-0.12 (95% CI -0.37-0.15; $p = 0.39$)
Maximum clot firmness (mm)	61–72	63.2 ± 3.2	62.8 ± 3.1	61.9 ± 4.1	62.6 ± 3.3	62.5 ± 3.5	-0.1 (95% CI -0.34-0.16; $p = 0.45$)	-0.11 (95% CI -0.36-0.14; $p = 0.39$)

Table 3. Diluted thrombin time, activated partial thromboplastin time and ClotPro values for samples spiked with argatroban. Diluted thrombin time is given as a median and interquartile range [IQR]. All other variables are given as means and standard deviations (± SD). CON, targeted concentration; r1: Pearson's correlation coefficient for variable with diluted thrombin time; r2: Pearson's correlation coefficient for variable with activated partial thromboplastin time.

test CT, and very strong correlations between (ii) dTT values and ClotPro ECA-test CT, as well as between aPTT values and ClotPro ECA-test CT. We found no correlation between clot firmness parameters (i.e. amplitude after 5, 10, 20, and 30 min as well as MCF) and anticoagulant concentrations or standard coagulation tests.

Owing to their POC applicability and the rapid availability of diagnostic results, viscoelastic hemostatic assays are recommended by clinical guidelines for the management of bleeding patients[19,20]. In contrast, the capability of viscoelastic devices to detect and guide anticoagulation remains poorly investigated. In the context of bleeding trauma patients, Oberladstätter et al. reported the feasibility of detecting clinically relevant plasma drug levels of direct oral anticoagulants using the ClotPro RVV-test and ECA-test[15].

In line with their results, which showed strong correlations between plasma drug concentrations and ClotPro RVV-test CT for patients receiving direct oral factor Xa inhibitors, we found strong correlations between anti-Xa activity and ClotPro RVV-test CT after spiking blood samples with enoxaparin. The correlation between anti-Xa activity and ClotPro RVV-test CT has previously been investigated in patients receiving LMWH[21,22]. Bösch et al. found only a weak correlation between anti-Xa measurements and ClotPro RVV-test CT for critically ill patients who received LMWH. Similarly, Groene et al. observed moderate correlations between the two parameters in ten patients receiving LMWH.

Although we could identify ClotPro RVV-test CT cutoffs for detecting both anti-Xa activity ≤ 0.1 IU.ml^{-1} and ≥ 0.6 IU.ml^{-1}, we found a wide variance of ClotPro RVV-test CT results at higher enoxaparin concentrations along with a rather poor detection of enoxaparin presence at low concentrations. The mean ClotPro RVV-test CT for both native blood samples and blood samples with a targeted concentration of 0.25 IU.ml^{-1} LMWH were within the manufacturer's reference range. These results are in line with Groene and colleagues, who found no difference in ClotPro RVV-test CT between patients receiving LMWH and a control group[21]. Strikingly, according to our results, an RVV-test CT of 100 s can occur at any enoxaparin concentration between 0.25 and 1.0 IU.ml^{-1}. Thus, we question the conclusions drawn by Groene et al. and argue that the results of our study, together with their findings, suggest the limited usefulness of the ClotPro-RVV test for monitoring LMWH.

When examining the correlation between the results obtained by the novel RVV-test and anti-Xa measurements, it is important to note the general lack of clarity regarding the relationship between anti-Xa activity and thromboembolic events (i.e., the clinical outcome of interest)[8,23,24]. Anti-Xa activity depicts plasma drug concentrations, and their association with the prevention of thromboembolic events, particularly in doses typically used for pharmacological thromboprophylaxis, remains pending[8]. For this reason, future studies employing the ClotPro RVV-test should include clinically relevant endpoints, such as thromboembolic events, instead of mere correlations with anti-Xa measurements.

The ClotPro ECA-test CT has been found to show strong correlations with plasma drug levels in patients taking the oral direct thrombin inhibitor dabigatran[15]. However, to the best of our knowledge, the capability of the ClotPro ECA-test to detect concentrations of the parenteral anticoagulant argatroban has not yet been investigated. In line with the results of Oberladstätter et al., we found very strong correlations between dTT results, aPTT results, and ClotPro ECA-test CT.

Despite being the most widely used laboratory method for guiding argatroban treatment, the accuracy of aPTT measurements has repeatedly been questioned, particularly in critically ill patients. Several factors, such as liver disease-related coagulopathy[11,25], lupus inhibitors[25] and elevated factor VIII[26] have been shown to interfere with a reliable interpretation of aPTT results. Against this background, dTT has been advocated as an alternative, more accurate method for guiding argatroban treatment[27,28]. The results of our study suggest that the ClotPro ECA-test CT might be a valid alternative, with the advantage of rapid availability of diagnostic results at the POC. This is supported by the fact that, compared to the ClotPro RVV-test CT, the ClotPro ECA-test CT results exhibited a narrower range of variability for each concentration, allowing for clearer distinguishability between the different investigated argatroban concentrations. Furthermore, we could identify ClotPro ECA-test CT cutoff values that provided high sensitivity and specificity for detecting clinically relevant dTT ranges of ≤ 0.1 µg.ml^{-1} and 0.5-1 µg.ml^{-1}.

Our study has several limitations. First, we presented the results of an in vitro pilot study with a small sample size. Although the study design does not permit direct translation of our findings into clinical practice, our results suggest the feasibility of guiding argatroban treatment using the ClotPro ECA-test CT, whereas our data do not support the use of the ClotPro RVV-test CT for the guidance of enoxaparin treatment. Second, although these questions might be of particular interest to critically ill patients, it should be stressed that we recruited healthy volunteers for this study, which hinders extrapolation of our results to real-life patient populations. Finally, we correlated the results of the novel diagnostic assays with monitoring methods that, although functionally depicting drug concentrations, have been poorly associated with relevant outcomes of interest, such as thromboembolic events.

Conclusions

We investigated the capability of two novel commercially available ClotPro assays to measure the activity of two parenteral anticoagulants, enoxaparin and argatroban, in vitro. Although we observed a strong correlation between the ClotPro RVV-test CT and anti-Xa activity, the wide variance in RVV-test CT measurements suggests that it is an unreliable method for monitoring enoxaparin treatment. In contrast, our findings indicate that ECA-test CT might be an accurate alternative to established laboratory tests for monitoring argatroban treatment, with advantages in terms of POC availability and turnaround time. Further diagnostic studies incorporating clinically relevant outcomes are warranted to confirm our in vitro results.

Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

JG: Study design, analysis and interpretation of data, manuscript draft and revision. SU: Data collection and analysis, manuscript draft and revision. FS: Data collection and manuscript revision. SK: Data collection and manuscript revision. CD: Analysis and interpretation of data, manuscript revision. MW: Interpretation of data and manuscript revision. PQ: Data collection and manuscript revision. ES: Study design, interpretation of data and manuscript revision.

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Declarations

Competing interests

JG received honoraria, research funding, and travel reimbursement from Alexion, Boehringer Ingelheim, CSL Behring, Instrumentation Laboratory, Johnson & Johnson, Mitsubishi Tanabe Pharma, Octapharma, Portola, and Takeda. SU received speaking fees from CSL Behring, Astra Zeneca, Arjo, Biomedica, Ekomed, NovoNordisk, and Roche. CD received speaking fees from CSL Behring, Astra Zeneca, Arjo, Biomedica, Ekomed, NovoNordisk, and Roche. ES received speaking fees from CSL Behring, Astra Zeneca, Arjo, Biomedica, Ekomed, NovoNordisk, Roche, B. Braun, and Bristol Myers Squibb. FS, SK, MW, and PQ declare that they have no competing interests.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Medical University of Vienna (EK 1937/2022, 23/02/2023) and was performed in accordance with the principles of good clinical practice and the Declaration of Helsinki. Written informed consent was obtained from all the participants.

Additional information

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