ANESTHESIOLOGY

Monitoring of Argatroban in Critically Ill Patients: A Prospective Study Comparing Activated Partial Thromboplastin Time, Point-of-Care Viscoelastic Testing with Ecarin Clotting Time and Diluted Thrombin Time to Mass Spectrometry

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ABSTRACT

Background: The direct thrombin inhibitor argatroban is indicated for the treatment of heparin-induced thrombocytopenia II, but it is also used off-label to treat critically ill patients presenting with heparin resistance, severe antithrombin deficiency, or hypercoagulability. Direct drug monitoring is not routinely available, and argatroban dosing is mainly based on global coagulation assays such as activated partial thromboplastin time (PTT) or diluted thrombin time (TT), both of which have limitations in patients with hypercoagulability.

Methods: Blood samples were obtained from critically ill patients treated with argatroban. Activated PTT and diluted TT were measured with a STA R Max3 analyzer (STAGO Deutschland GmbH, Germany) using an argatroban-calibrated kit. Ecarin clotting time was measured using a point-of-care viscoelastic test device. Liquid chromatography with tandem mass spectrometry was performed using a reversed-phase column, a solvent gradient, and an API4000 mass spectrometer with electrospray. Correlation was described using Pearson correlation coefficient r and Bayesian multilevel regression to estimate relationships between outcomes and covariates.

Results: From June 2021 to March 2022, 205 blood samples from 22 patients were analyzed, allowing for 195 activated PTT–liquid chromatography with tandem mass spectrometry comparisons, 153 ecarin clotting time–liquid chromatography with tandem mass spectrometry comparison, and 105 diluted TT–liquid chromatography with tandem mass spectrometry comparisons. Compared to liquid chromatography with tandem mass spectrometry, performance of argatroban quantification was best for diluted TT ($r = 0.91$), followed by ecarin clotting time ($r = 0.58$) and activated PTT ($r = 0.48$). Regression analysis revealed that patients with sepsis were more prone to argatroban overdosing (coefficient, 4.194; 95% credible interval, 2.220 to 6.792).

Conclusions: Although activated PTT monitoring of argatroban is the most commonly used test, in critically ill patients, diluted TT provides more precise measurements. Alternately, point-of-care viscoelastic ecarin clotting time also provides guidance for argatroban dosing to identify overdosing if available. The data also suggested that patients with sepsis are at greater risk for argatroban overdosing.

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Intravenous direct thrombin inhibitors, bivalirudin and argatroban, are indicated for treating heparin-induced thrombocytopenia and used off-label with heparin resistance, extracorporeal membrane oxygenation, and hypercoagulability
- Unlike heparin, direct drug monitoring is not routinely available, and dosing is based on activated partial thromboplastin time or diluted thrombin time

What This Article Tells Us That Is New

- Although activated partial thromboplastin time monitoring of argatroban is the most commonly used test, diluted thrombin time and point-of-care viscoelastic ecarin clotting times provide improved guidance for optimal dosing and identifying overdosing

Heparin–induced thrombocytopenia type II is a severe immune–mediated disease that is characterized by massive thrombin overload, induced by the activation of platelets by platelet factor-4–heparin complexes.[1,2] The resulting prothrombotic state leads to a consumption

of platelets (causing thrombocytopenia), to micro- and macrovascular thrombosis and high mortality.[1,3] Direct thrombin inhibitors such as argatroban are used to treat heparin–induced thrombocytopenia type II and to reduce thrombin overload.[4] Additionally, argatroban is used for anticoagulation during extracorporeal membrane oxygenation (ECMO)[5,6] and in patients with heparin resistance.[7] Other prothrombotic states, such as severe sepsis, may also be caused by thrombin overload and high plasma levels of fibrinogen, and argatroban has been used off-label in such cases for more than a decade.[8–11]

Bleeding is one of the most common complications of argatroban therapy due to its anticoagulatory properties and its predominant use in critically ill patients who may present with organ failure including impaired liver metabolism of argatroban. Therefore, precise dosing of argatroban is important and depends on monitoring its anticoagulatory effect. Argatroban is routinely measured using the activated partial thromboplastin time (PTT) with an estimated target range of 1.5- to 3-fold increase from baseline values (both of which are manufacturer and patient dependent) with a maximum of 100s (table 1). However, values of activated PTT must be considered with caution in patients with elevated FVIII levels due to hypercoagulable or proinflammatory states. In this situation, activated PTT sensitivity for anticoagulatory effects is reduced, an *in vitro* effect that may not necessarily reflect the clinical coagulation status in these patients and that can lead to an underestimation of the anticoagulation effects of argatroban or heparin.[12–14] On the other hand, septic or ECMO patients frequently present with decreased FXII values, resulting in a prolonged activated PTT, which does not correlate with increased bleeding risk but may lead to an overestimation of the anticoagulation effects of argatroban or heparin.[15] As an alternative to activated PTT testing, diluted thrombin time (TT)[16] or ecarin-dependent assays[17] can be used to monitor direct thrombin inhibitors including argatroban.

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Both diluted TT and ecarin-based tests are currently not recommended for monitoring of argatroban in the summary of product characteristics but may offer some advantages compared to activated PTT (table 1).[18] For diluted TT, citrated plasma samples are diluted with pooled plasma, and coagulation is started with added thrombin. In contrast to activated PTT, heparin, lupus anticoagulants, or fibrin(ogen) degradation products have little impact on diluted TT assays.[19]

The direct influence of argatroban on thrombin may also be assessed in ecarin-based test assays. For example, the ecarin clotting time can be performed as point-of-care viscoelastic testing, which allows for rapid anticoagulation monitoring at the bedside.

The aim of this study was to compare established monitoring tests for argatroban (activated PTT and diluted TT) with a point-of-care viscoelastic ecarin test and to validate the performance of these assays against the gold standard of argatroban plasma concentrations measured by liquid chromatography with tandem mass spectrometry.

Materials and Methods

Study Design

This was a single-center, prospective observational clinical study performed in the anesthesiologic intensive care unit (ICU) at the University Hospital "Carl Gustav Carus" of the Technical University of Dresden (Germany). From June 2021 to March 2022, all patients anticoagulated with argatroban were consecutively enrolled. Argatroban was used as first line therapy in patients diagnosed with heparin-induced thrombocytopenia type II (confirmed by enzyme-linked immunosorbent assay and heparin-induced platelet activation tests). Additionally, argatroban was also used outside of label for patients with assumed heparin resistance or hypercoagulability, *e.g.*, due to COVID-19–related thrombin overload. According to our institutional standard operational procedure, heparin resistance was defined as (1) subtherapeutic activated PTT (less than 60s) and anti–Xa activity levels (less than 0.5 IE/ml for therapeutic anticoagulation) despite at least 48h of unfractionated heparin administration of more than 35,000 IU/day[20] or (2) otherwise unexplained progression of venous thromboembolism despite therapeutic heparin anticoagulation with anti–Xa activity in the target range (0.5 to 0.7 aXa units/ml at peak level).

Anticoagulation monitoring was initially performed according to local guidelines,, using activated PTT targets of 60 to 80s. Considering the high proportion of COVID-19 patients with heparin resistance or hypercoagulability due to increased FVIII levels, the institutional procedure for the monitoring of argatroban was changed from measuring activated PTT to diluted TT, using an argatroban-calibrated assay (argatroban target range, 500 to 1,000 ng/ml)

Table 1. Overview of Three Coagulation Tests and Some Characteristics

Test	Principle of Test	Range with Good Correlation	Target Range for Therapeutic Argatroban	Turnaround Time	Availability	Influences	
						Reasons for "Falsely" Elevated Results*	Reasons for "Falsely" Decreased Results*
Activated PTT	▪ Contact pathway activation in platelet poor plasma ▪ Optical or mechanical assay	▪ Less than 1,000 ng/ml ▪ Less correlation with increasing plasma levels (cutoff is manufacturer dependent)	▪ 1.5- to 3-fold baseline ▪ 100 s or less	▪ 30 to 60 min (plus transport to lab)	▪ 24/7 (mostly)	▪ XII ↓ ▪ XI ↓ ▪ X ↓↓ ▪ IX ↓ ▪ VIII ↓ ▪ V ↓↓ ▪ Prothrombin ↓↓ ▪ Lupus anticoagulants ▪ Fibrin degradation products ↑	▪ VIII ↑ ▪ VII ↑ ▪ Fibrinogen ↑
Diluted TT	▪ Dilution with pooled plasma ▪ Optical or mechanical assay	▪ Low and high ranges	▪ 50 to 90 s	▪ 30 to 60 min (plus transport to lab)	▪ Depends on institution	▪ Fibrin degradation products ↑	▪ Fibrinogen ↑ ▪ Bilirubinemia ↑ (optical interference)
Point-of-care ecarin essay	▪ Whole blood ▪ Mechanical assay	▪ Unknown	▪ 350 to 500 s	▪ Less than 15 min (point of care)	▪ 24/7	▪ Fibrinogen ↓ ▪ Prothrombin ↓	

*Results are "false" because they are correct from a laboratory perspective, but due to the mentioned influences they do not reflect the coagulation system in the patient. ↓decreased. ↓↓significantly decreased. ↑increased.
PTT, partial thromboplastin time; TT, thrombin time.

in October 2021. At the same time, point-of-care viscoelastic testing including ecarin tests with a therapeutic target of 350 to 500 s was established bedside as an additional part of the clinical routine care in patients with heparin resistance.

Laboratory Analysis

Standard laboratory analyses were performed at least once a day as part of the clinical routine and included prothrombin time, activated PTT, fibrinogen, fibrin monomers, and D-dimer. Analyses were performed on STA R Max3 analyzers (STAGO Deutschland GmbH, Germany). Starting October 2021, routine sampling was extended to diluted TT, which was analyzed using the BIOPHEN direct thrombin inhibitor kit with argatroban calibration (CoaChrom Diagnostica GmbH, Austria) on STA R Max3 analyzers (STAGO Deutschland GmbH). Ecarin tests were performed according to the manufacturer's instructions using ClotPro (Haemonetics, USA).

ClotPro is a viscoelastic testing system that uses a cup and a pin to measure clot formation, with the cup rotating *via* an elastic element and the pin functioning as a stationary counterpart.[21] The basic principle is similar to the original technique described by Hartert in 1951.[22] However, ClotPro is mechanically different from other viscoelastic testing methods like rotational thromboelastometry and thromboelastogram, which use a fixed cup and a spinning pin. In ClotPro, the mechanical deceleration of the cup rotation is detected and translated into a viscoelastometric amplitude. Viscoelastic testing provides various parameters such as clotting time (period of time from start to a 2-mm thickness of clot amplitude), clot formation time (time needed for clot amplitude from 2 to 20 mm), A5 (thickness of clot amplitude 5 min after clotting time), A10 (thickness of clot amplitude 10 min after clotting time), maximum clot firmness (overall maximum thickness of the clot amplitude), maximum lysis (percentage of lysis in relation to maximum clot firmness during the overall time of measurement), and lysis time (period of time from clotting time until 50% lysis of clot formation). ClotPro is mainly used in the ICU, operating room, or emergency department. The pipette tips contain the test-specific reagents and are ready to use. All measurements were performed following the manufacturer's guidelines using the test-specific syringe for pipetting 340 µl of citrated patient blood per test and releasing it into the cups. The samples were processed for 60 min of operating time under standardized conditions at 37°C within a maximum of 2 h after arterial blood sampling. For the current study, we used the ecarin test, which contains the snake venom metalloprotease from *Echis carinatus*,[23] Polybrene (a heparin inhibitor), and $CaCl_2$ for recalcification. Ecarin activates prothrombin to the thrombin intermediate meizothrombin, which is inhibited by direct IIa inhibitors leading to a prolongation of the clotting time.[24–26] The idea of

using a chromogenic thrombin substrate was developed in 1980s,[27] and subsequently, various ecarin-based anti-IIa assays were developed and used for laboratory testing.[25,28]

Blood samples for both conventional laboratory and ecarin testing were drawn from each patient at the same time. After performing routine laboratory tests, the remaining citrated blood samples were centrifuged, and plasma was frozen at −20°C and used for liquid chromatography with tandem mass spectrometry analyses.

For liquid chromatography with tandem mass spectrometry, 100 µl of serum was thawed, and 20 µl (2 ng) of the internal standard caffeine $^{13}C_3$ was added. The samples were diluted with 200 µl of acetonitrile, vortexed, and centrifuged for 10 min (16,500g). Then 20 µl of the clear supernatants were injected. Liquid chromatography with tandem mass spectrometry was performed using an UltiMate3000 pump and autosampler (Dionex, Thermo Fisher Scientific, USA), a reversed-phase column, a solvent gradient, and an API4000 mass spectrometer with electrospray.[29] Argatroban and caffeine $^{13}C_3$ were measured using the multiple reaction monitoring mode. Quantification was performed by the peak area ratio method. This method was suitable for the quantification of argatroban in serum over the range of 2.0 to 500 ng/ml. Samples with higher concentrations were diluted.[29]

Statistical Analysis

Statistical analyses were performed using the SPSS Statistics 27 software (IBM, Inc., USA) and R version 3.2.4. All categorical variables were described by absolute and relative frequencies; comparisons between groups were done using Fisher's exact test. Continuous variables were presented as median and interquartile range, and group comparisons were based on the Mann–Whitney U test. The significance level was set at 0.05.

We applied Bayesian multilevel regression to estimate relationships between deviations of activated PTT, ecarin clotting time test), and diluted TT from liquid chromatography with tandem mass spectrometrymeasurement results and selected covariates. To define the outcome, we classified the adequacy of the measurement results using activated PTT, ecarin-clotting time test, and diluted TT relative to liquid chromatography with tandem mass spectrometry as lower, concordant, or higher (fig. 2 and 3 and tables 5 and 6 in the Supplemental Digital Content, https://links.lww.com/ALN/D327). Because of the ordinal nature of this outcome, we used a cumulative model for ordinal response variables with logit link.[30] This model included a normally distributed random intercept at the patient level to account for correlation of outcome values measured for the same patient over time. Due to the small number of patients included in the analysis, frequentist approaches to estimation of multilevel models that rely on asymptotic properties of estimators may yield inaccurate results. Simulation studies

demonstrated that Bayesian estimation approaches for multilevel models provide more accurate results in the presence of only few clusters (patients).[31,32] Hence, we applied Bayesian estimation based on the Markov chain Monte Carlo method. For each model, we ran three Markov chains, each with 2,000 iterations and a burn-in of 1,000 iterations. Sampling was done using the No-U-Turn sampler.[33]

To minimize the influence of previous specification on estimation results, we chose weakly informative normal priors with expected values of 0 and a standard deviations of 100 for all fixed regression coefficients and half-t distribution priors for variance parameters.[34] The precision of regression coefficient estimates was quantified by 95% credible intervals.

For estimation, missing values in our data were filled by multiple imputation. We used a bootstrapping-based algorithm to generate five imputed data sets.[35] Combined estimates across imputed data sets were derived by combining the sampled Markov chains of all estimations. In an additional analysis, we evaluated predictors of very high liquid chromatography with tandem mass spectrometry values (greater than 1,000 ng/ml) using Bayesian multilevel logistic regression. Candidate parameters were chosen similarly to the models described above.

Ethics

The study was designed in accordance with the Declaration of Helsinki. The Ethics Committee of the Technische Universität Dresden reviewed the study protocol (BO-EK-64022022). The study was registered at the German Clinical Trial Register (DRKS00028689). Study conduct and reporting followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement guidelines.[36]

Results

Characteristics of the Cohort

From June 2021 to March 2022, 22 critically ill patients treated with argatroban were enrolled in this study. All patients presented with acute respiratory distress syndrome due to COVID-19. Of these, 81% (n = 18) presented with sepsis, and 41% (n = 9) presented with septic shock. ECMO therapy was necessary in 59% (n = 13), and 36% of patients (n = 8) needed continuous renal replacement therapy. Indication for anticoagulation therapy with argatroban was confirmed by heparin-induced thrombocytopenia type II in 45% (n = 10) and heparin resistance in 55% (n = 12). The median Sequential Organ Failure Assessment score was 13 points (interquartile range, 12 to 14 points), and the median simplified acute physiology score II was 38 (interquartile range, 32 to 44). The median age was 59 yr (interquartile range, 55 to 63 yr), 36% were

female (n = 8), and the median body mass index was 30 kg/m² (interquartile range, 28 to 35 kg/m²). During ICU stay, none of the patients developed recurrent or new thromboembolic complications during argatroban therapy, and six patients (27%) suffered from bleeding complications, with one fatal bleeding (intracranial hemorrhage). Of all patients, 14 (64%) were discharged from the ICU alive (table 2).

Comparison of Clinical Monitoring Parameters and Argatroban Plasma Concentrations

The 22 patients provided a total of 205 blood samples for liquid chromatography with tandem mass spectrometry analyses, allowing for 195 comparisons of activated PTT *versus* liquid chromatography with tandem mass spectrometry 153 for ecarin *versus* liquid chromatography with tandem mass spectrometry and 105 for diluted TT *versus* liquid chromatography with tandem mass spectrometry. Complete matching was impossible due to missing routine values. Such were caused by high bilirubin values (n = 40 for diluted TT), followed by a lack of diluted TT availability at our institution after 6 PM and technical or logistic problems to obtain ClotPro ecarin–clotting time tests from the same blood sample as used for routine parameters.

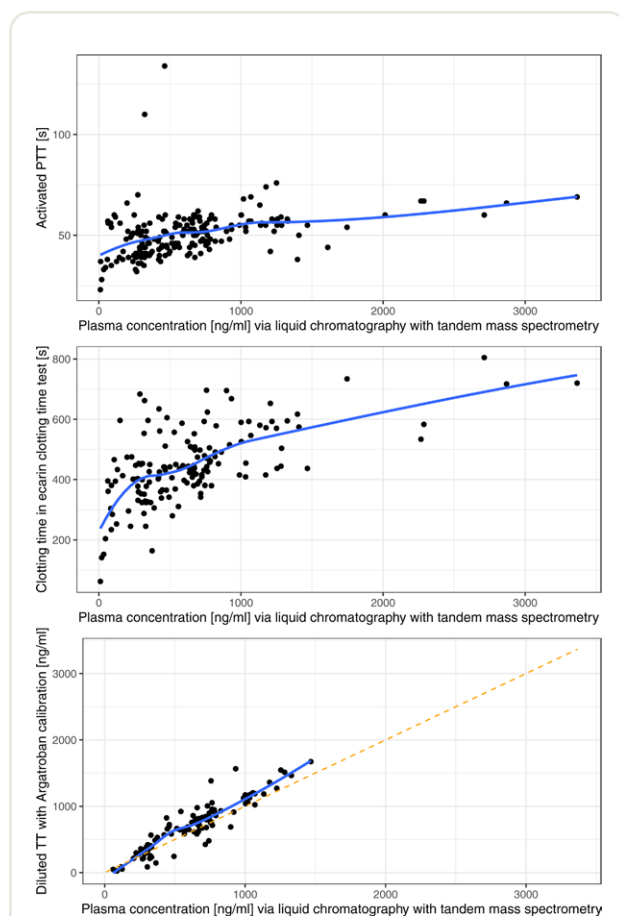For all available measurements, median values were 51 s (interquartile range, 44 to 56 s) for activated PTT, 446 s (interquartile range, 380 to 509 s) for ecarin–clotting time test, 691 ng/ml (interquartile range, 370 to 911 ng/ml) for diluted TT, and 640 ng/ml (interquartile range, 304 to 767 ng/ml) for liquid chromatography with tandem mass spectrometry. Correlation between activated PTT and argatroban plasma concentration ($r = 0.48$; fig. 1) was low. The correlation between ecarin–clotting time test and argatroban plasma concentration ($r = 0.58$; fig. 1) was superior to activated PTT but inferior to diluted TT. Scatter plots demonstrated the best correlation between diluted TT and plasma concentration with a correlation coefficient of 0.91 (fig. 1). In addition, the performance of activated PTT, diluted TT, and ecarin–clotting time test showed differences within clinically established target ranges for argatroban plasma concentrations (fig. 2 and 3), predefined as less than 500 ng/ml (*i.e.*, argatroban underdosed), 500 to 1,000 ng/ml (*i.e.*, argatroban in therapeutic range), and greater than 1,000 ng/ml (*i.e.*, argatroban overdosed). Specifically, of 22 activated PTT values within the clinically established target range of 60 to 80 s, only 4 measurements were found to also have a therapeutic plasma concentration of argatroban (500 to 1,000 ng/ml) in liquid chromatography with tandem mass spectrometry, while 5 were underdosed and 13 were overdosed (argatroban liquid chromatography with tandem mass spectrometry concentrations less than 500 or 1,000 ng/ml, respectively; fig. 3, left panel). Of note, of

Table 2. Demographic and Baseline Characteristics

Characteristic	All Patients	Range
n	22	
Female	8 (36%)	
Age, yr	59 (55 to 63)	38 to 75
Body mass index, kg/m²	30 (28 to 35)	−19 to 54
Simplified acute physiology score II at day of measurement, points	38 (32 to 44)	17 to 69
Sequential Organ Failure Assessment score at day of measurement, points	13 (12 to 14)	3 to 22
Heparin-induced thrombocytopenia type II	10 (45%)	
Heparin resistance	12 (55%)	
Invasive mechanical ventilation	22 (100%)	
Sepsis	18 (81%)	
Septic shock	9 (41%)	
ECMO therapy	14 (64%)	
Continuous renal replacement therapy	8 (36%)	
Bleeding complication during ICU stay after change to argatroban	6 (27%)	
Bleeding Academic Research Consortium 1	0	
Bleeding Academic Research Consortium 2	1 (5%)	
Bleeding Academic Research Consortium 3a	2 (9%)	
Bleeding Academic Research Consortium 3b	2 (9%)	
Bleeding Academic Research Consortium 4	0	
Bleeding Academic Research Consortium 5	1 (5%)	
Thromboembolic event during ICU stay after change to argatroban	0	
ICU survival	14 (64%)	

The data represent the median (interquartile range) or n (%). Bleeding complications were defined according to the Bleeding Academic Research Consortium[37]: type 1, patient does not need medical care due to bleeding; type 2, actionable sign of hemorrhage; type 3, overt bleeding plus hemoglobin drop or requiring transfusion or requiring surgical intervention or intracranial bleeding; type 4, need more than 4 units red packed blood cells within 48 h; and type 5, fatal bleeding.
ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit.

Discrepancy of Monitoring Parameters to Mass Spectrometry in Subgroups

Compared to plasma concentration measured with liquid chromatography with tandem mass spectrometry, activated PTT, diluted TT, and ecarin-clotting time test performed poorly in patients with septic shock (table 3). In particular, values for ecarin-clotting time test (coefficient, 3.527; 95% credible interval, 0.863 to 7.148) and diluted TT (coefficient, 4.948; 95% credible interval, 0.810 to 10.160) overestimated the exact argatroban plasma concentration significantly. Furthermore, diluted TT showed a strong trend to overestimate the argatroban plasma concentration in patients with renal replacement therapy. However, the 95% credible interval included the value of 1 (coefficient, 4.076; 95% credible interval, −3.869 to 13.702). In patients receiving parenteral nutrition, diluted TT underestimated argatroban plasma levels (coefficient, −4.614; 95% credible interval, −8.233 to −1.683), whereas underestimation of activated PTT was less pronounced (coefficient, −1.113; 95% credible interval, −3.303 to 0.877). In contrast, ecarin-clotting time test correlated well with liquid chromatography with tandem mass spectrometry plasma levels (coefficient, 1.005; 95% credible interval, −0.163 to 2.225). Patients presenting with sepsis showed a significantly increased risk for argatroban overdosing (liquid chromatography with tandem mass spectrometry plasma levels greater than 1,000 ng/ml; coefficient, 4.194; 95% credible interval, 2.220 to 6.792; table 4).

Discussion

This prospective study compared three available clinical monitoring tests for anticoagulant therapy with argatroban in critical ill patients with the plasma concentration measured by gold standard liquid chromatography with tandem mass spectrometry. The results of our study suggest that activated PTT may not provide optimal monitoring in critical ill patients. In greater than 50% of the blood samples analyzed, activated PTT did not sufficiently correlate to liquid chromatography with tandem mass spectrometry. In particular, the activated PTT did not detect argatroban overdosing. Further, in 22 of 35 patients (63%) with argatroban levels greater than 1,000 ng/ml, activated PTT values were lower than 60 s (lower boundary of therapeutic target), which would have resulted in a further increases of argatroban dosing. Using activated PTT (as recommended in argatroban summary of product characteristics) in critically ill patients potentially increases the risk for overdosing and bleeding complications, in particular with septic patients, who were at a higher risk for overdosing. We found diluted TT to be more accurate for predicting a correct plasma concentration in greater than 80% of our measurements. Recently, developed point-of-care ecarin-based assays for viscoelastic testing can more reliably detect argatroban overdosing and may offer an appropriate (but less

Fig. 1. Scatter plot diagrams comparing activated partial thromboplastin time (PTT), ecarin clotting time test, and diluted thrombin time (TT) to argatroban plasma concentration measured by liquid chromatography with tandem mass spectrometry. *Blue curves* represent locally estimated scatterplot smoothing estimators for each parameter. The *yellow dotted curve* is a 45º line indicating perfect agreement (only available for diluted TT).

the 35 samples with overdosed argatroban concentrations and activated PTT measurements, none were detected by an activated PTT greater than 80 s.

Using ecarin-clotting time test, a sufficient discrimination between patients within or below the therapeutic range and patients above the therapeutic range was possible if a clotting time target of 350 to 500 s was used as an indicator for argatroban levels within the target range. Of note, of the 25 samples with overdosed argatroban concentrations and ecarin measurements, 19 were detected by an ecarin clotting time greater than 500 s (fig. 3, middle panel).
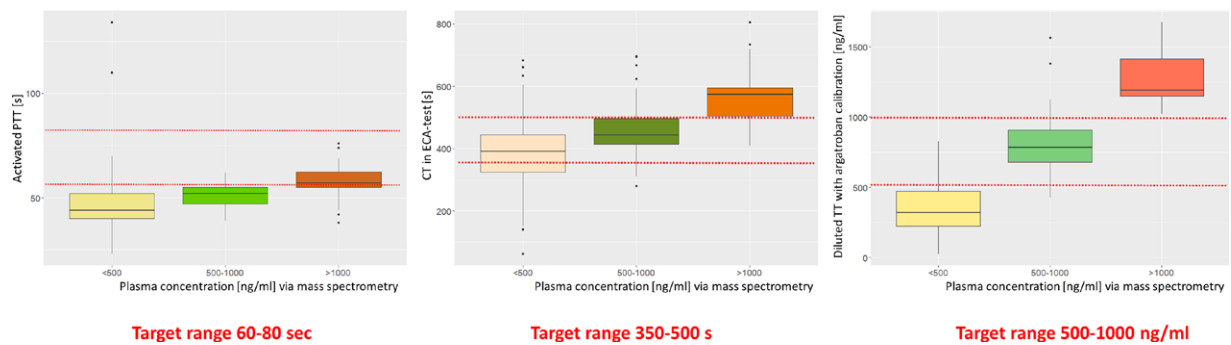
The calibration curve for diluted TT sufficiently discriminated patients in all three ranges compared to drug plasma concentration. As demonstrated in figure 3 (*right panel*), diluted TT detected all 15 patients with argatroban overdosing and diluted TT measurements.

Fig. 2. Box plots comparing diluted thrombin time (TT), activated partial thromboplastin time (PTT), and ecarin clotting time test and recommended cutoffs for argatroban plasma levels. The box plots compare activated PTT, ecarin clotting time test, and diluted TT to different clinically relevant argatroban plasma concentrations (500 to 1,000 ng/ml according to the summary of product characteristics) measured by liquid chromatography with tandem mass spectrometry. The *red lines* indicate therapeutic target range of activated PTT, ecarin-clotting time test, and diluted TT.
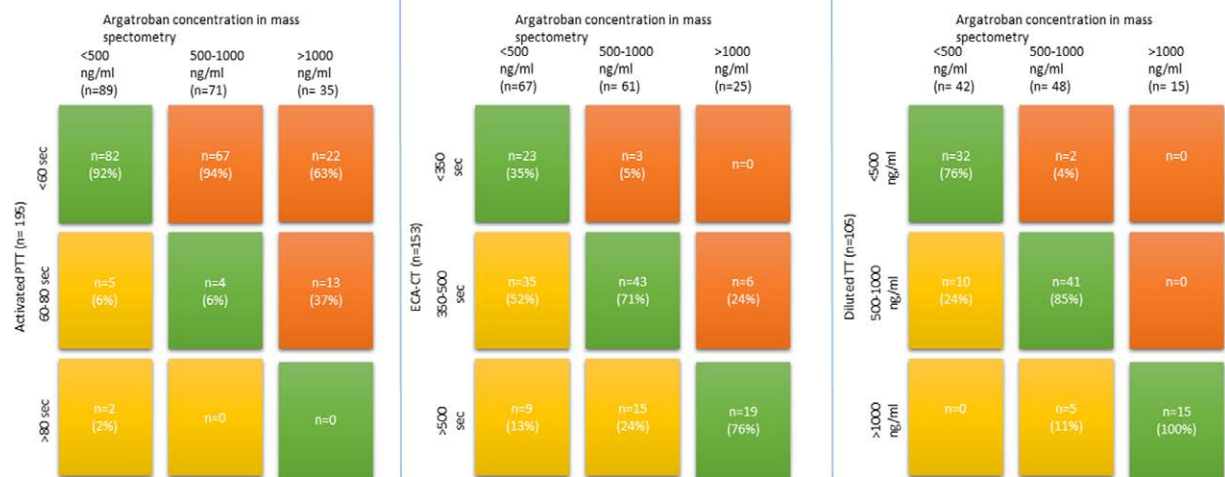
Fig. 3. Prediction of argatroban plasma concentration by lab test. The nine field matrices compare activated partial thromboplastin time (PTT), ecarin clotting time test, and diluted thrombin time (TT) to different clinically relevant argatroban plasma concentrations measured by liquid chromatography with tandem mass spectrometry to evaluate correct prediction and over- and underestimation. *Green*, correct prediction; *yellow*, lab test false high, underestimation; *red*, lab test false low, overestimation.

accurate) alternative, when diluted TT is not available or applicable.

The poor performance of activated PTT for thrombin inhibitor monitoring is not a new finding, since several previous studies found similar results.[38] However, the majority of these studies lacks a reference method for the measurement of argatroban plasma concentrations such as liquid chromatography with tandem mass spectrometry. Hasan *et al.*[38] compared activated PTT and diluted TT to dose response in 45 pediatric patients and reported a better correlation and more stable dose response of diluted TT compared to activated

PTT, but diluted TT was established as the reference method beforehand. Guy *et al.*[39] reported results from 25 samples of eight patients comparing four different activated PTT assays to ecarin-based anti-IIa and hemoclot thrombin inhibitor anti-IIa assay and found that both anti-IIa assays (ecarin and hemoclot thrombin inhibitor) were more suitable than all different activated PTT assays. However, hemoclot thrombin inhibitor was set as reference method. Furthermore, Guy *et al.*[39] reported similar results for the monitoring of argatroban in patients with vaccine-induced thrombocytopenia and thrombosis, concluding that the significant differences

Table 3. Bayesian Multilevel Regression for Deviation of Clinical Monitoring Parameters to Liquid Chromatography with Tandem Mass Spectrometry

Parameter	Activated PTT	Ecarin-Clotting Time Test	Diluted TT
Billirubin values	0.011 (−0.001 to 0.026)	0.017 (−0.002 to 0.043)	−0.219 (−0.375 to −0.100)
Parenteral nutrition	−1.113 (−3.303 to 0.877)	1.005 (−0.163 to 2.225)	−4.614 (−8.233 to −1.683)
Sequential Organ Failure Assessment score	0.012 (−0.329 to 0.326)	−0.039 (−0.284 to 0.210)	0.298 (−0.275 to 0.918)
Simplified acute physiology score II score	0.056 (−0.053 to 0.161)	0.017 (−0.062 to 0.093)	−0.057 (−0.215 to 0.098)
Sepsis	−0.848 (−2.982 to 1.192)	−0.836 (−2.268 to 0.547)	−0.997 (−3.354 to 1.298)
Septic shock	1.276 (−1.061 to 3.771)	3.527 (0.863 to 7.148)	4.948 (0.810 to 10.160)
ECMO	−0.207 (−2.222 to 2.090)	0.811 (−0.586 to 2.269)	0.578 (−3.869 to 13.702)
Continuous renal replacement therapy	0.261 (−3.057 to 3.613)	−0.060 (−2.940 to 2.991)	4.076 (−3.869 to 13.702)

The data are presented as regression coefficient (95% credible interval).
ECMO, extracorporeal membrane oxygenation; PTT, partial thromboplastin time; TT, thrombin time.

Table 4. Bayesian Multilevel Regression for Liquid Chromatography with Tandem Mass Spectrometry Greater than 1,000 ng/ml

Parameter	Coefficient	95% Credible Interval
Billirubin values	0.008	−0.027 to 0.047
Parenteral nutrition	−1.850	−5.190 to 0.646
Sequential Organ Failure Assessment score	0.049	−0.364 to 0.465
Simplified acute physiology score II score	0.047	−0.075 to 0.171
Sepsis	4.194	2.220 to 6.792
Septic shock	−1.506	−5.764 to 2.218
ECMO	0.452	−1.541 to 2.824
Continuous renal replacement therapy	−3.121	−14.039 to 3.953

The data are presented as regression coefficient and 95% credible interval.
ECMO, extracorporeal membrane oxygenation.

between activated PTT and diluted TT could have a relevant clinical impact.[12] Siegmund et al.[40] compared different activated PTT assays to an ecarin-based assay with argatroban-spiked blood from different donors and reported wide differences between the different activated PTT agents, as well as the plateau effect for some activated PTT agents. They concluded that an ecarin-based assay should be preferred over activated PTT. However, this was an *in vitro* study, and the results are not adaptable without caution to *in vivo* conditions. All of the aforementioned studies rely on diluted TT as a reference method for monitoring direct thrombin inhibitor.

Currently, only one study used a somewhat comparable approach to our study, as published by Beyer et al.[41] This group also compared the results of activated PTT, diluted TT, and an ecarin-based assay in 198 blood samples from 101 patients receiving either argatroban or bivalirudin. However, instead of performing objective quantification by liquid chromatography with tandem mass spectrometry in all patients, they limited exact quantification to samples showing discordant activated PTT and diluted TT results.[41] Even with this limitation, the authors also found a high rate of discordance between the different monitoring parameters and demonstrated that diluted TT or ecarin showed a stronger correlation

to liquid chromatography with tandem mass spectrometry results than activated PTT.[41] Notably, the ecarin-based assay was running on conventional laboratory analyzer (STA-ECA II, Diagnostica Stago, France) and is not directly comparable to the ecarin test for point-of-care viscoelastic testing used in our study. Based on all these data, we suggest that activated PTT is not optimal to monitor direct thrombin inhibitor therapy in critically ill patients and that product summaries should recommend diluted TT, or a less accurate alternative such as ecarin clotting time, as the recommended monitoring and dose-adjustment strategy for argatroban-treated patients.

The ecarin-based assay used in this study is a recently developed assay for point-of-care viscoelastic testing, is an important development that may allow for direct thrombin inhibitor monitoring at the bedside, and facilitates timely dosing adjustments of direct thrombin inhibitors including argatroban. However, comparable data on the performance of other tests, including the ClotPro ecarin test in patients with direct thrombin inhibitor, are still scarce. Oberladstätter et al.[26] reported a strong correlation between ecarin-clotting time test and dabigatran plasma concentration, measured with STA-ECA II (Diagnostica Stago) as reference method. In line with these findings, Groene et al.[42] reported a successful detection of clinically relevant

dabigatran concentrations with ecarin test compared to an anti-IIa assay (Biophen Heparin LRT 7.5, Hyphen Biomed, France) as reference method in a small number (10) of patients. However, currently there are no published data available for the performance of the ClotPro ecarin test for argatroban. Considering the results for dabigatran in both available studies, the results for argatroban in our study do not reach the same level of accuracy. The correlation between ecarin test and liquid chromatography with tandem mass spectrometry was higher compared to activated PTT and liquid chromatography with tandem mass spectrometry, but clearly not as good as for diluted TT and liquid chromatography with tandem mass spectrometry. Most cases of ecarin test inaccuracy were related to samples with low argatroban plasma concentrations, for which ecarin–clotting time test overestimated argatroban concentrations. In contrast to this finding, activated PTT generally failed to detect argatroban overdosing, and for activated PTT, this plateau effect has been described[12,40] and could be related to high FVIII levels,[13,43] which was not investigated in our study. In contrast, an overestimation of low argatroban plasma concentrations in the ecarin test has not been described so far. Further research is necessary to evaluate ecarin–clotting time test inaccuracy, before this concept can be considered as a stand–alone method for argatroban therapy monitoring.

Considering the data of our study, patients with sepsis are at a particularly high risk for argatroban overdosing if monitored with activated PTT. Regarding the current lack of evidence by randomized controlled trials, our results support the use of diluted TT if available instead of activated PTT. The point-of-care viscoelastic testing with ecarin test represents a potential alternative to predict argatroban overdosing but requires point-of-care device availability and trained personnel, which is a limitation similar to diluted TT, which is often not available 24/7 in most hospital central laboratories.

Study Limitations

This study is mainly limited by the small number of patients and some missing values for each alternative test method. In particular, the number of available samples measuring diluted TT was much lower compared to samples measuring activated PTT, indicating clinical (elevated bilirubin plasma levels) and logistic challenges. As a consequence, 24/7 availability of diluted TT measurements was since established in our hospital. Another limitation of our study relates to the generalizability of the findings that we generated in critically ill COVID-19 patients. We cannot rule out that activated PTT performance is better in clinically more stable patients. Still, the inaccuracy of activated PTT in the most vulnerable population is concerning and cannot be balanced out by a hypothetically better performance in less sick populations with lower risks from under- or overtreatments. The ultimate goal of drug monitoring is to achieve drug plasma concentrations within the therapeutic window, thus optimizing patient outcome and reducing avoidable harm. Even within the above mentioned limitations, our study demonstrates that activated PTT is not an optimal monitor for argatroban-treated patients. Additional studies including a larger number of data points for underdosed and overdosed patients are needed to better define validity and clinical applicability of diluted TT and ecarin.

Conclusions

Monitoring of argatroban in critical ill patients is challenging. This study demonstrates the lack of accuracy for activated PTT-based argatroban monitoring and dosing and the inability to detect argatroban overdosing. Our data support the use of diluted TT to monitor argatroban whenever possible and, alternately, point-of-care ecarin tests if available instead of activated PTT. Septic patients were at a higher risk for argatroban overdosing, which may necessitate additional monitoring strategies. Further studies are needed to evaluate optimal monitoring of direct thrombin inhibitors in critically ill patients.

Competing Interests

The authors declare no competing interests.

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Supplemental Digital Content

Supplemental tables, https://links.lww.com/ALN/D327

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