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Comparison of analytical performances between clot waveform analysis and FibWave in edoxaban-treated patients and healthy controls

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Abstract

Introduction: The activated partial thromboplastin time (aPTT) and the prothrombin time (PT) are widely available coagulation parameters which are however poor predictors of the anticoagulant effect of direct oral anticoagulants (DOACs). Some coagulometers use the clot waveform analysis (CWA) to assess the clotting time but mainly based on a unique parameter. The improvement of these methodologies and the evaluation of the other waveform parameters may increase the sensitivity to DOACs.

Objectives: To assess the performance of an improved clot waveform an method (i.e. FibWave) to detect the impact of edoxaban on the coagulation and the fibrinolytic systems.

Methods: Seventy-one samples from patients treated with edoxaban collected at minimum concentration (C_{TROUGH}) and/or maximum concentration (C_{MAX}), and 45 control samples were included. The aPTT- and PT-based CWA as well as the FibIn, FibEx, and FibLysis methodologies of the FibWave were implemented and performed on an ACL-TOP 700.

Results: PT and FibEx clotting time were strongly correlated to edoxaban concentration (Pearson $r = 0.80$ and 0.89, respectively). The FibEx clotting time allowed a better discrimination for samples with 30 and 50 ng/ml of edoxaban compared to PT (cutoffs of 96.5 and 114.2 s for the FibEx versus a unique cutoff of 13.1 s for the PT). The fibrinolytic process was impaired in the presence of edoxaban in a dose-dependent manner.

Conclusion: FibEx is more sensitive than aPTT- and PT-based CWA for the detection of the clinically relevant anticoagulant level of edoxaban.

Essentials

- The correlation of activated partial thromboplastin time (aPTT)- and prothrombin time (PT)-based clot waveform analysis (CWA) with edoxaban is not known.
- The FibWave was developed to be more sensitive than the CWA.
- The PT-CWA and the FibWave parameters were better correlated with edoxaban levels than aPTT-CWA.
- The FibWave was more sensitive to edoxaban levels compared to the aPTT and PT-based CWA.

1 | INTRODUCTION

Edoxaban is indicated for stroke prevention in patients with non-valvular atrial fibrillation and for the prevention and treatment of venous thromboembolism and pulmonary embolism.[1,2] The monitoring of its anticoagulant activity is generally not required, but the measurement of its plasma levels can be necessary in some clinical situations including bleeding or thromboembolic events in patients on anticoagulation.[3–6] As for the other direct oral anticoagulants (DOACs), edoxaban is also known to impact coagulation assays[3,7–9] and enhance factor X (FX)-dependent fibrinolysis and plasmin generation.[10–12]

The activated partial thromboplastin time (aPTT) and prothrombin time (PT) are coagulation assays widely implemented in clinical laboratories that are poor predictors of the anticoagulant effect of DOACs.[13–16] Furthermore, aPTT and PT provide only qualitative information (i.e., reading end point: clotting time) about the effect of DOACs.[13,14,17–19] Recently, it was reported that aPTT reagents, including high phospholipid content and contact activator, cannot sufficiently reflect the activation by the extrinsic tenase complex and the effect of platelets on physiological clotting.[20,21] Thus, standard coagulation tests have several limitations that are due to the poor level of information extracted from complex interactions between several effectors of the coagulation process and their inhibitors.[22–25]

Clot waveform analysis (CWA) allows in-depth evaluation of the fibrin clot formation from aPTT and PT.[26] Optical end-point coagulation analyzers are able to visualize the kinetics of fibrin clot formation and to provide waveform parameters for exploration of the complex interactions occurring during the coagulation process.[27] Abnormalities in clot waveforms have already been used for the diagnosis and the prognosis of different coagulopathies.[20,21,28–31]

The FibWave is a coagulation test that is also based on the analysis of clot formation kinetics. Compared to CWA, FibWave was developed to be more sensitive and to allow evaluation of the overall coagulation process by measuring the turbidity changes created during the clot formation process.[12,25,27,32] The suitability of FibWave for evaluating the impact of DOACs on clot formation and fibrinolysis and for the detection of hormone-induced changes in coagulation proteins has already been reported.[12,25,32] These investigations were performed in normal pooled plasma supplemented with DOACs and not in patients treated with DOACs.

In the present study, we aimed to confirm our first in vitro experiments and to compare the clinical performances of CWA to FibWave in a cohort of patients treated with edoxaban.

2 | MATERIALS AND METHODS

2.1 | Study population

The study population consisted of 57 patients treated with edoxaban representing 71 plasma samples and 45 healthy volunteers representing 45 control samples. For some patients, plasma samples were collected at C_{TROUGH} and C_{MAX}. Among the 57 patients included, 3 (5.3%) were treated with edoxaban for stroke prevention and 54 (94.7%) for the treatment and the secondary prevention of venous thromboembolic events. The mean age (±standard deviation) was 59.6 (±15.7) years, and the mean BMI (±standard deviation) was 28.0 (±4.9) kg.m^{-2} and 30 were female (52.6%). Among the 45 healthy volunteers, 23 were men (age±standard deviation: 25.3±4.7 years; BMI±standard deviation: 22.9±2.4 kg.m^{-2}) and 22 were women (age: 23.4±3.7 years; BMI: 22.3±2.8 kg.m^{-2}).

2.2 | Healthy volunteers

Healthy volunteers were recruited at the University of Namur (Namur, Belgium) in August 2019. They were students or employees of the University. The exclusion criteria for healthy volunteers were history of thrombotic and/or hemorrhagic events, treatment by antiplatelets or anticoagulant medication, pregnancy, or carriers of factor V Leiden or prothrombin G20210A mutations. Detection of factor V Leiden and prothrombin G20210A mutations was performed by reverse transcriptase polymerase chain reaction. Blood was taken by venipuncture in the antecubital vein with a 21-gauge needle and collected into 0.109 M sodium citrate tubes (9:1 v/v) (Vacuette, Greiner bio-one). Platelet poor plasma was obtained from the supernatant fraction of blood tubes after double centrifugation for 15 min at 2500×g at room temperature. The aliquots of individual plasma were then frozen in liquid nitrogen before being stored at −70°C or less until the

performance of the analyses. Frozen plasma samples were thawed at 37°C for 5 min and mixed gently before the experiment.

2.3 | Patients treated with edoxaban

Patients treated with edoxaban (Lixiana) were recruited from the Cliniques Universitaires Saint-Luc between March 2018 and November 2018. Eligibility criteria included a treatment with edoxaban for at least 2 weeks and the obtention of the patient's informed consent. The exclusion criteria were age less than 18 years, an estimated glomerular filtration rate less than 30 ml/min, and patients geographically inaccessible for follow-up. Plasma samples were collected at C_{TROUGH} (i.e., 12 h after the last drug intake) and/or C_{MAX} (i.e., approximately 3 h after drug intake) according to the patient's willingness. Blood was taken by venipuncture in the antecubital vein and collected into 0.109 M sodium citrate (9:1 v/v) tubes (SARSTEDT Monovette®) using a 21-gauge needle. Once collected, the clinical blood samples

TABLE 1 Demographic characteristics of healthy subjects and edoxaban-treated patients

Characteristic	Healthy subjects (n = 45)	Edoxaban-treated patients (n = 57)
Age, years (mean ± SD)	24.4 ± 5.9	59.6 ± 15.7
Body mass index (mean ± SD)	22.6 ± 4.0 kg.m^2	28.0 ± 4.9 kg.m^2
Female, n (%)	22 (48.9)	30 (52.6)
Dates of recruitment	August 2019	March 2018–November 2018
Edoxaban indication		
Stroke prevention, n (%)	NA	3 (5.3)
Venous thromboembolism, n (%)	NA	54 (94.7)
Glomerular filtration rate <60 ml/min	Not investigated	2 (3.6)
Number of samples collected	45	71
Samples collected at C_{TROUGH}, n (%)	NA	35 (49.3)
Samples collected at C_{MAX}, n (%)	NA	36 (50.7)
Hereditary thrombophilia, n (%)	0 (0.0)	6 (10.5)
Antiphospholipid syndrome, n (%)	0 (0.0)	1 (1.8)

Abbreviations: C_{MAX}, maximum concentration; C_{TROUGH}, minimum concentration; NA, not applicable.

TABLE 2 Summary of clot waveform analyses and FibWave parameters in healthy subjects and edoxaban-treated patients

	Healthy subjects (n = 45)	Edoxaban C_{TROUGH} (n = 35)	Edoxaban C_{MAX} (N = 36)
Clot Waveform Analysis: Median (10th–90th percentile)			
PT–time to Max1 (clotting time, seconds)	10.5 (10.9 to 12.2)	12.9 (11.7 to 17.1)	18.4 (14.6 to 23.5)
PT–Max1 (dmAbs/dt)	301.7 (248.7 to −417.6)	427.9 (265.3 to −702.8)	370.1 (192.6 to −653.7)
aPTT–time to Max2 (clotting time, seconds)	29.3 (27.3 to 32.7)	32.0 (28.4 to 34.8)	37.4 (32.5 to 43.9)
aPTT–Max1 (dmAbs/dt)	198.7 (165.7 to −270.0)	290.7 (189.1 to −472.0)	269.5 (154.4 to −477.1)
aPTT–Max2 (dmAbs/dt^2)	804.0 (665.9 to −1047)	1035 (692.5 to −1640)	900.0 (553.1 to −1547)
aPTT–Min2 (dmAbs/dt^2)	−431.7 (−526.4 to − 352.5)	−461.3 (−707.9 to − 342.7)	−407.4 (−643.9 to − 291.8)
FibWave: Median (10th–90th percentile)			
FibEx–time to Max2 (clotting time, seconds)	69.3 (60.4 to 82.6)	97.2 (75.1 to 122.2)	196.8 (118.5 to 242.4)
FibEx–time to Max1 (time to peak, seconds)	90.1 (76.6 to 107.8)	128.1 (94.5 to 183.2)	252.0 (142.3 to 332.5)
FibEx – Max1 (dmAbs/dt)	77.5 (59.7 to 100.7)	75.5 (58.3 to 116.0)	46.9 (24.0 to 74.0)
FibEx – Max2 (dmAbs/dt^2)	25.6 (19.6 to 41.6)	19.9 (14.9 to 30.0)	9.7 (4.5 to 20.1)
FibEx – Min2 (dmAbs/dt^2)	−16.0 (−21.6 to −6.8)	−10.2 (−19.4 to −4.8)	−3.7 (−9.9 to −2.1)
FibIn–time to Max2 (clotting time, seconds)	78.3 (64.8 to 97.0)	71.9 (54.6 to 89.5)	98.9 (79.5 to 140.3)
FibIn–time to Max1 (time to peak, seconds)	83.2 (69.4 to 104.0)	76.2 (58.7 to 95.6)	104.0 (84.1 to 151.3)
FibIn–Max1 (dmAbs/dt)	203.8 (155.8 to 259.9)	322.9 (192.5 to 600.8)	270.0 (115.3 to 496.8)
FibIn–Max2 (dmAbs/dt^2)	204.7 (145.5 to 308.1)	404.6 (218.3 to 698.3)	285.3 (82.9 to 511.0)
FibIn–Min2 (dmAbs/dt^2)	−199.7 (−266.2 to −141.2)	−306.1 (−660.2 to −207.5)	−240.1 (−428.5 to −81.8)

Abbreviations: aPTT, activated partial thromboplastin time; C_{MAX}, maximum concentration; C_{TROUGH}, minimum concentration; PT, prothrombin time.

(A) CWA - prothrombin time
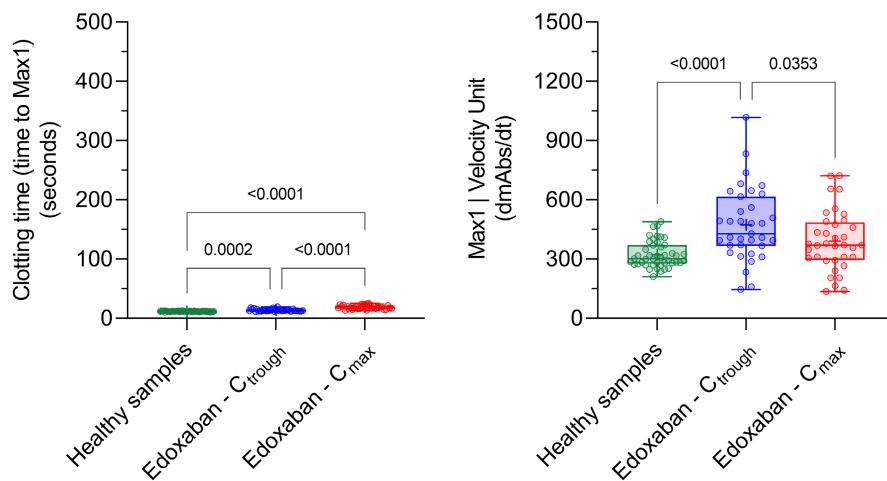
(B) FibWave - FibEx
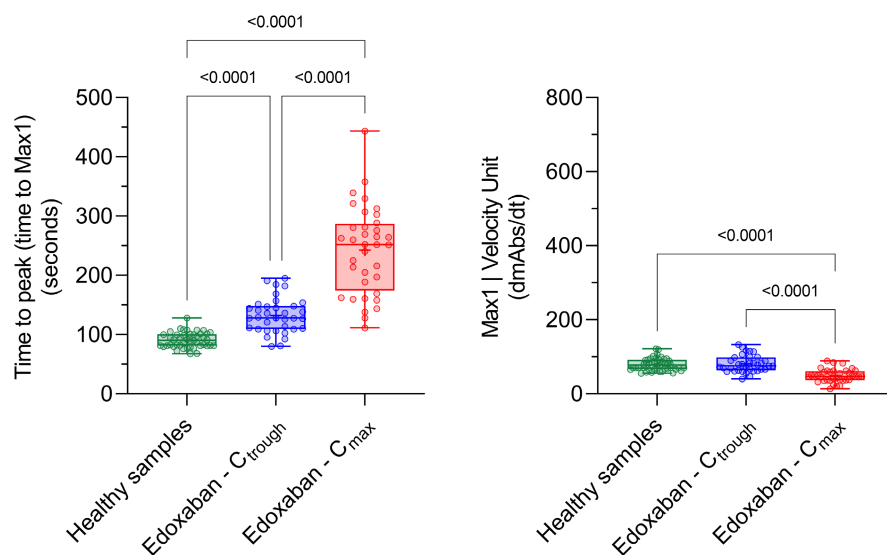
were processed in the same manner as for the samples collected in healthy volunteers. Samples were analyzed within 2 h after thawing according to the procedure established in the laboratory. The aim was to reduce the risk of bias due to the stability of edoxaban in plasma after thawing. Recent data showed that the coagulation parameters' stability in samples containing apixaban or rivaroxaban, at room temperature after one freeze/thaw cycle, was 3 and 2 h, respectively.[33] To our knowledge, no data are available for edoxaban in the same context, but we took this precaution as a conservative approach.

## 2.4 | Methods and parameters

2.4.1 | CWA: aPTT and PT

The aPTT and PT were performed on an ACL TOP 700 CTS (Werfen), and CWA was done using the integrated software. The aPTT was performed using the SynthasIL reagent (Werfen), and the PT was performed with the ReadiPlasTin reagent (Werfen). Plasma

samples were mixed with the aPTT reagent, and the reaction was triggered with the addition of a solution of 20 mM $CaCl_2$, or they were mixed with the PT reagent, according to the manufacturer's recommendations.

For the PT, the software was set up by the manufacturer to provide the time to Max1 (considered as the PT clotting time by the instrument and reported in seconds) and the Max1 (delta milliabsorbance [dmAbs]/dt). For aPTT, the software was set up to provide the Max1 (dmAbs/dt), the Max2 (dmAbs/dt^2), the time to Max2 (considered as the aPTT clotting time by the instrument and reported in seconds) and the Min2 (dmAbs/dt^2) (Figure S1).

2.4.2 | FibWave: FibIn, FibEx, and FibLysis

The different methodologies of the FibWave were implemented on an ACL TOP 700 CTS (Werfen), and the setup of the methods allowed extraction of the following parameters from the integrated software: Max1, time to Max1 (time to peak), Max2, time to Max2

(A) CWA - Activated partial thromboplastin time
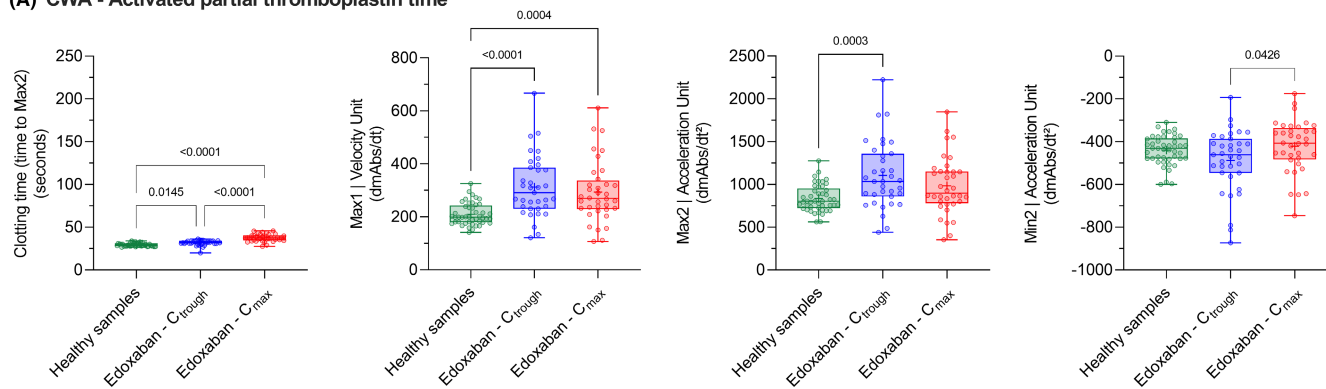
(B) FibWave - FibIn
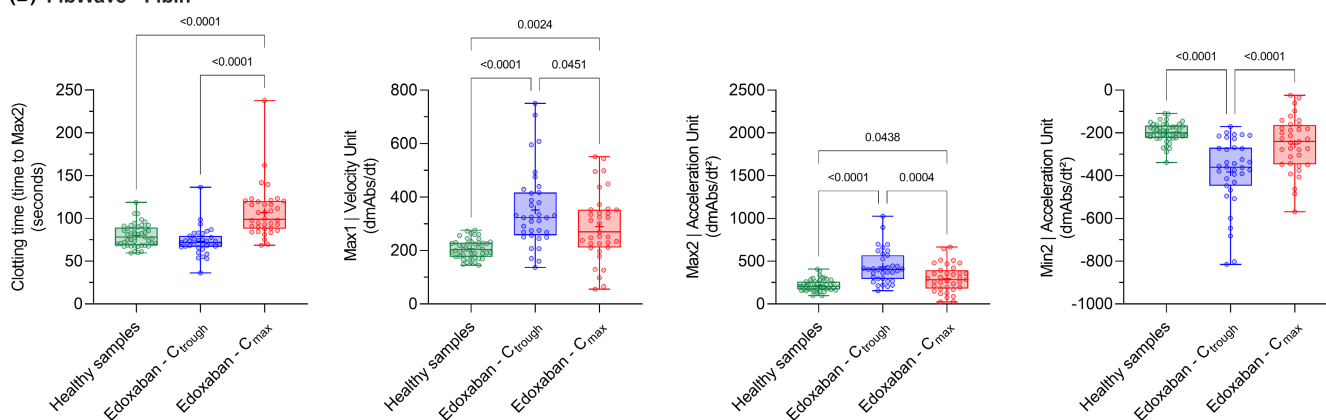
FIGURE 2 Population comparison of activated partial thromboplastin time (A) with the FibIn (B). Only the common parameters of the two tests are presented. C_{MAX}, maximum concentration; C_{TROUGH}, minimum concentration; CWA, clot waveform analysis

(clotting time), and Min2 (Figure S1). The exploration of the intrinsic pathway, that is, FibIn, was realized using a solution of micronized silica with phospholipids, while the extrinsic pathway, that is, FibEx, was assessed using a mixture of phospholipids and tissue factor.

The fibrinolysis pathway, that is, FibLysis, was assessed using a mixture of tissue factor, phospholipids, and tissue plasminogen activator at near physiological concentrations, as described previously.[12] The extracted parameters from the FibLysis were the maximum fibrinolysis velocity (|Min1| in dmAbs/dt[1]), the fibrinolysis time (TFib in seconds) and the difference between time to lyse and time to clot (TFib-Ttpeak in seconds) (Figure S1). Briefly, 100 μl of plasma samples were mixed with 25 μl of the corresponding reagent. The coagulation and fibrinolysis processes were triggered by the addition of 25 μl of a 100 mM $CaCl_2$ solution.

2.4.3 | Determination of edoxaban plasma concentration

The plasma concentrations of edoxaban were measured using a calibrated chromogenic anti-Xa assay (Biophen Direct Anti-Xa Inhibitor, Hyphen BioMed) according to the manufacturer's recommendations on a STA-R Max coagulometer (Diagnostica Stago).

2.5 | Statistical analysis

Statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software). Descriptive statistics were used to analyze the data, and the reference ranges were defined as the 10th–90th percentile of the healthy population. The variability, expressed as coefficient of variations, of the FibWave and the aPTT- and PT-based CWA parameters were assessed for the different groups (healthy subjects, edoxaban C_{TROUGH}, and edoxaban C_{MAX}). Between-group comparisons were done using a one-way analysis of variance with Tukey's multiple comparison test. In the edoxaban groups, the correlations between the different kinetic parameters, that are, time to Max2 (clotting time), time to Max1 (time to peak), Max1 (velocity), Max2 (acceleration) and Min2 (deceleration) and the plasma concentrations, were determined by linear models. Pearson's chi-squared test was performed. The threshold for significance has been set at 0.05. Receiver operating characteristic (ROC) curves were performed at relevant thresholds for clinical decisions, that is, 30 and 50 ng/ml for the most sensitive parameters. Results were reported as the area under the curve with its 95% confidence interval (CI). The Youden's index was determined using MedCalc software (MedCalc Software Ltd) for all analyzed parameters, and sensitivities and specificities at these cutoff indexes were reported with their 95% CIs.

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3 | RESULTS

The demographic characteristics of the study population are reported in Table 1.

3.1 | APTT- and PT-based CWA, FibWave parameters, and plasma concentration of edoxaban results

The median (and 10th–90th percentile) aPTT- and PT-based CWA and the FibEx and FibIn parameters of the healthy population and the population of patients on edoxaban are reported in Table 2.

Among the edoxaban cohort, 35 samples were obtained at C_{TROUGH}, and 36 samples were obtained at C_{MAX}. The median (and 10th–90th percentile) plasma concentration at C_{TROUGH} was 27.0 ng/ml (17.0–83.4 ng/ml) and 232.5 ng/ml (95.8–377.4 ng/ml) at C_{MAX} (p <0.05; Figure S2). Fifty-one and 41 samples were above 30 and 50 ng/ml, respectively.

3.2 | Comparison between edoxaban-treated patients and controls

3.2.1 | CWA—Aptt and PT

Comparisons between edoxaban C_{MAX}, edoxaban C_{TROUGH}, and healthy subjects for the different aPTT and PT CWA parameters are reported in Figures 1 and 2.

Significant correlations between the aPTT or the PT clotting time and the edoxaban concentration were observed (Pearson $r = 0.65$ and 0.80, respectively; Figure 3). There was no significant correlation between the aPTT or the PT Max1 parameter and the edoxaban concentration ($p = 0.96$ and 0.20 for aPTT and PT, respectively).

3.2.2 | FibWave analysis—FibEx and FibIn

Comparisons between edoxaban C_{MAX}, edoxaban C_{TROUGH}, and healthy subjects for the different FibEx and FibIn parameters are reported in Figures 1 and 4.

Significant correlations between the FibEx clotting time ($r = 0.89$), FibEx time to peak ($r = 0.81$), FibEx Max1 ($r = -0.64$), FibEx Max2 ($r = -0.79$), FibEx Min2 ($r = 0.62$), and edoxaban concentration were observed (Figure 5). Correlations between the FibIn clotting time ($r = 0.60$), FibIn time to peak ($r = 0.60$), FibIn Max2 ($r = -0.41$), FibIn Min2 ($r = 0.39$), and edoxaban concentration were also significant (Figure 5). There was no significant correlation between FibIn Max1 and edoxaban concentration ($p = 0.08$).
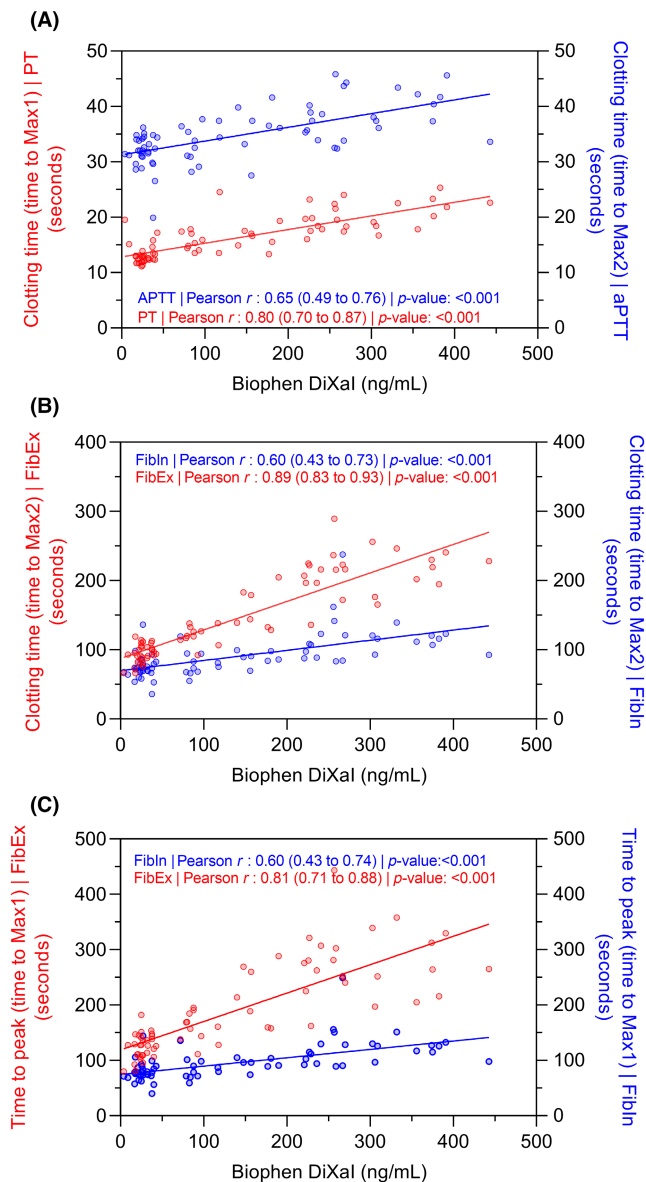
FIGURE 3 Correlation between clot waveform analysis and FibWave parameters with plasma edoxaban concentrations. (A) represents the "clotting time" as reported by the PT and aPTT methodologies on the ACL-TOP analyzer, which correspond to the time to Max1 for the PT and the time to Max2 for the aPTT. (B) represents the time to Max2 for the FibEx and the FibIn. Panel (C) represents the time to Max1 for the FibEx and the FibIn. aPTT, activated partial thromboplastin time; PT, prothrombin time

3.2.3 | ROC curve analyses

ROC curves were performed for the FibEx and the PT-based CWA for the clinical decision thresholds of 30 and 50 ng/ml. The results at 30 and 50 ng/ml are summarized in Table 3 for the clotting time, time to peak, Max1, Max2 and Min2 for the FibEx and for the clotting time and Max1 for the PT. The different ROC curves are available in Figure S3.
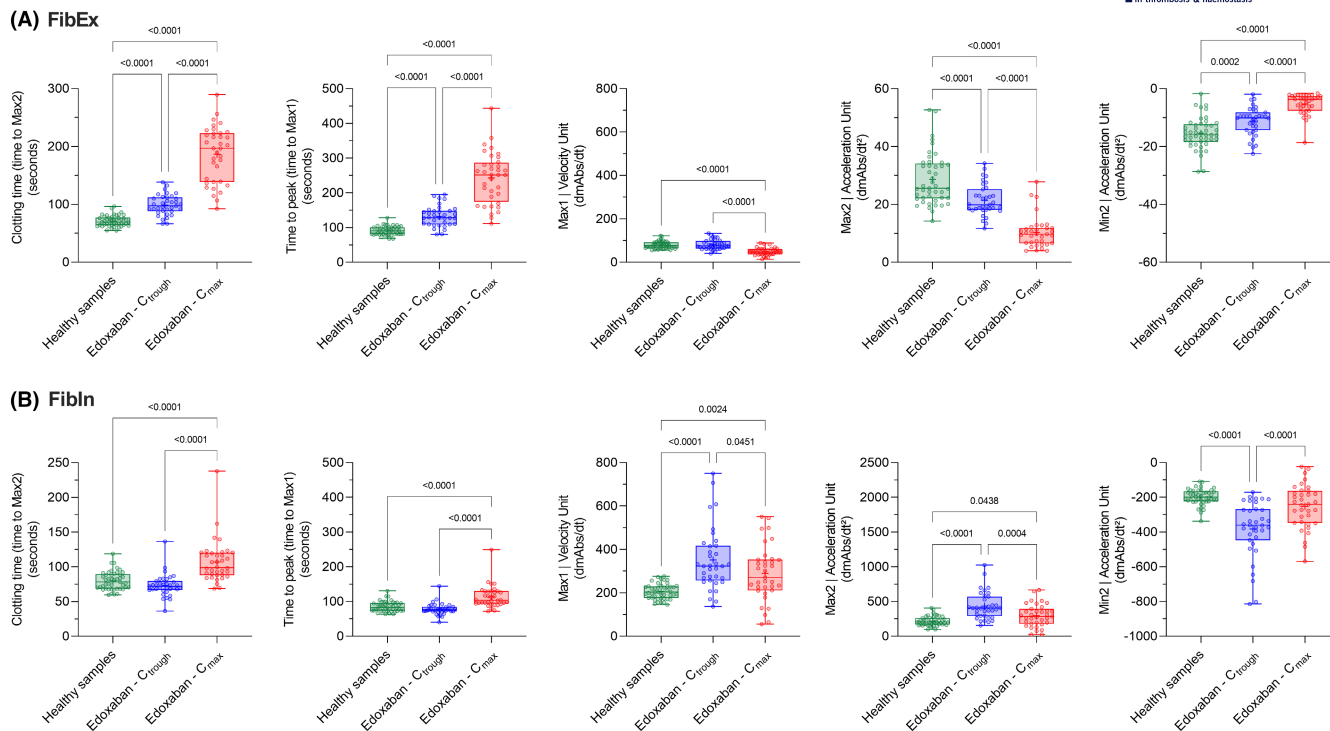
FIGURE 4 Comparison of the FibEx (A) and the FibIn (B) parameters in healthy subjects and in edoxaban-treated patients at C_{TROUGH} and C_{MAX}. C_{MAX}, maximum concentration; C_{TROUGH}, minimum concentration

3.3 | FibWave analysis of FibLysis

Patients treated with edoxaban at either C_{TROUGH} or at C_{MAX} had a prolonged time to lyse and a higher fibrinolysis velocity than the healthy population (Figure 6).

The addition of tissue-type plasminogen activator into the FibEx reagent did not impact the FibEx Max1 nor the FibEx time to peak, the only parameters of the coagulation phase that were used in the calculation of the FibLysis parameters.

4 | DISCUSSION

We demonstrated that FibEx, exploring the extrinsic pathway of the coagulation, was more sensitive than the aPTT- and PT-based CWA for assessing the anticoagulation status of edoxaban-treated patients. All temporal parameters, reflected by the time to Max1 and the time to Max2 as well as velocity (Max1), acceleration (Max2), and deceleration (Min2) markers were better discriminated with the FibEx than the aPTT- and PT-based CWA. With the FibWave, edoxaban prolonged the time to Max1 and the time to Max2 with both the FibEx and FibIn assays, while the velocity, acceleration, and deceleration of fibrin formation were dose-dependently reduced. This is in line with our in vitro experiments.[25]

Currently, the aPTT and PT are standard clotting assays that are widely used for the screening of coagulation abnormalities. These common tests were shown to be relatively insensitive to the effects of DOACs, are reagent- and method-dependent, have significant variability at high concentrations, and do not provide an entire footprint of the intensity of anticoagulation compared to assays that evaluate coagulation in detail like thrombin generation tests and viscoelastic tests.[6,16,19,34–36] On some coagulometers, the definition of the clotting time for the aPTT and the PT are determined using an analysis of the turbidimetry generated over time, a process called clot waveform analysis.[37] For several decades, a particular attention has been focused on additional parameters extracted from aPTT- and PT-CWA, allowing to consider this latter as a global coagulation assay. This technique is of interest for several coagulation abnormalities.[28,29,38,39] Recently, our group developed a new series of tests with improved sensitivity compared to traditional CWA, namely, the FibWave. In vitro results showed encouraging performances of this methodology and reveals its high sensitivity toward prothrombotic tendencies and anticoagulant status.[12,25,40]

Tests triggering coagulation via the extrinsic pathway or the prothrombinase complex have been shown to be more sensitive to the effect of direct factor Xa inhibitors than tests initiating coagulation via the intrinsic pathway.[25,41,42] It was therefore interesting to compare the performance of the traditional PT-based CWA with FibEx. While the time to Max1 showed similar correlations for both PT and FibEx ($r = 0.80$ [95% CI, 70%–87%] and 0.81 [95% CI, 71%–88%]), the time to Max2 (FibEx) reported the best correlation with edoxaban concentration ($r = 0.89$ [95% CI, 83%–93%]). This suggests that a simple parameter like the FibEx–time to Max2 can reliably reflect the intensity of edoxaban without the need for expensive
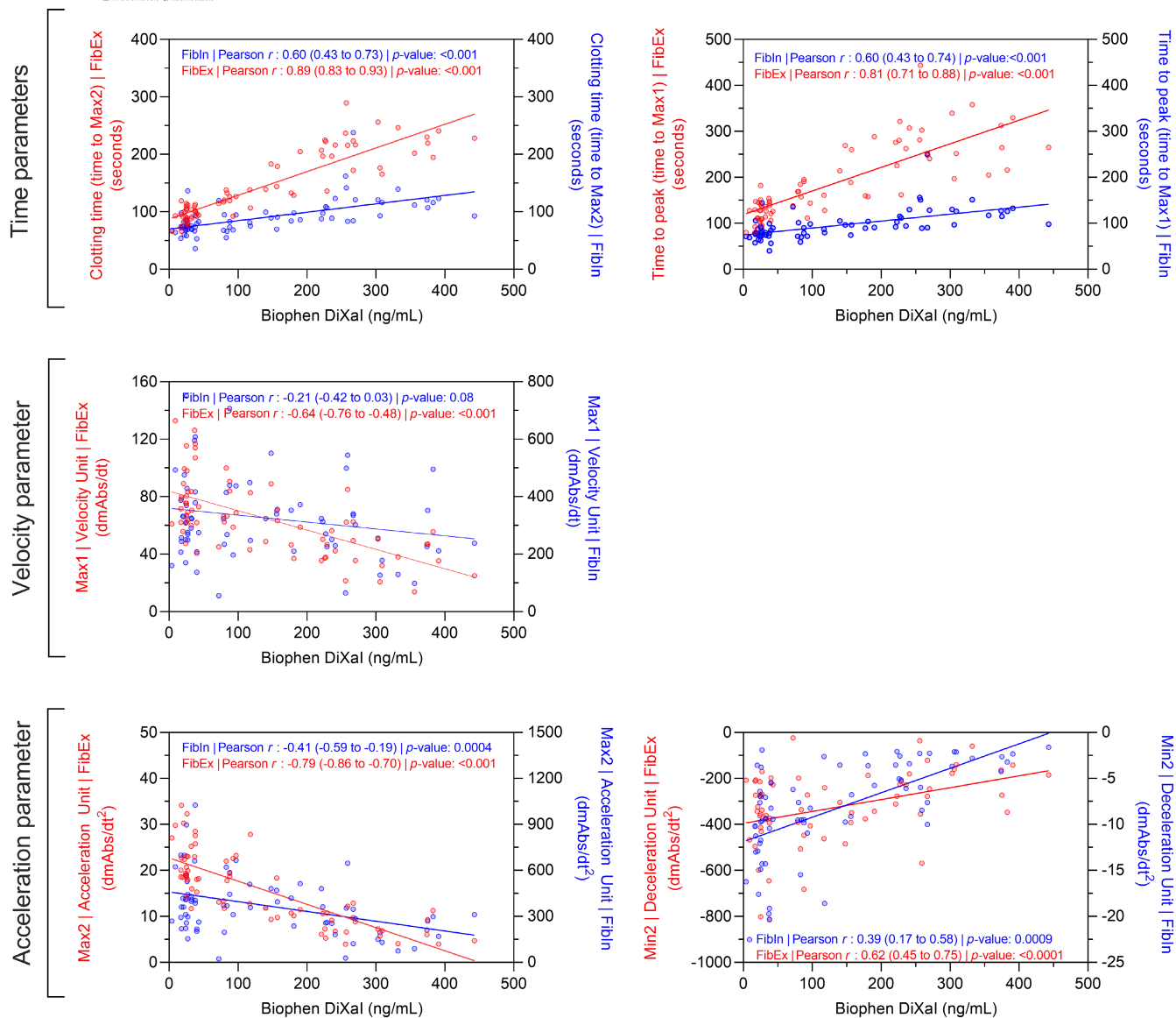
FIGURE 5 Correlation of the different FibWave parameters with the plasma concentration of edoxaban

techniques like chromogenic assays specifically calibrated against the drug of interest.

In addition, other parameters like the Max2 and the Min2 were also informative on the degree of anticoagulation and deserve further investigations, especially in patients with coagulation abnormalities or experiencing clinical events, since the plasma concentration of edoxaban is certainly not the only determinant in the advent of a clinical event.[43,44] For the PT and FibEx, only the Max1 and the time to Max1 were compared because these were the only parameters that were extracted from the ACL-TOP analyzer for the PT procedure. Nevertheless, the FibWave assay demonstrated that the Max2 parameter was also relevant, as it is sensitive to the anticoagulation intensity. The time to Max1 showed the best performance for discriminating samples with plasma concentration above 30 and 50 ng/ml with significant cutoffs. The Youden indexes revealed that FibEx performs better than the PT-based CWA at these

low concentrations. Although these cutoffs need to be challenged in a larger cohort of patients treated with edoxaban and should also be investigated with the other DOACs, these preliminary data are already very promising.

The FibWave and CWA are similar to thrombin generation assay, except that the end point is not the generation of thrombin but rather the formation of fibrin. Recently, relevant concentrations of different DOACs (i.e., 10, 30, 50, and 100 ng/ml) were evaluated in thrombin generation to assess whether the same concentration of different DOACs provides the same degree of anticoagulation.[43] Interestingly, the thrombin generation profiles from the different DOACs significantly differed, suggesting that assessing the ponderal concentration is probably not the best approach to assess the intensity of anticoagulation. These in vitro results are supported by the study of Metze et al.,[44] suggesting that global coagulation assays like thrombin generation, viscoelastometric assays, or FibWave can be of

TABLE 3 ROC curve analysis at relevant clinical decision thresholds

	ROC AUC (95%CI)	Youden index cutoff (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Cutoff = 30 ng/ml				
CWA PT–clotting time (time to Max1, seconds)	0.967 (0.917 to 0.991)	13.1 (13.0 to 15.1)	0.902 (0.790 to 0.957)	0.954 (0.873 to 0.987)
CWA PT–Max1 (dmAbs/dt^{-1})	0.610 (0.515 to 0.699)	331.9 (164.0 to 408.2)	0.686 (0.550 to 0.797)	0.554 (0.433 to 0.668)
FibEx–clotting time (time to Max2, seconds)	0.972 (0.923 to 0.994)	96.5 (86.4 to 119.3)	0.902 (0.790 to 0.957)	0.892 (0.794 to 0.947)
FibEx–time to Peak (time to Max1, seconds)	0.965 (0.913 to 0.990)	110.8 (105.3 to 136.2)	0.980 (0.897 to 0.999)	0.815 (0.705 to 0.891)
FibEx–Max1 (dmAbs/dt^{-1})	0.763 (0.675 to 0.837)	63.2 (56.3 to 70.6)	0.667 (0.530 to 0.780)	0.815 (0.705 to 0.891)
FibEx–Max2 (dmAbs/dt^{-2})	0.895 (0.824 to 0.944)	18.4 (16.0 to 19.4)	0.780 (0.648 to 0.873)	0.938 (0.852 to 0.976)
FibEx–Min2 (dmAbs/dt^{-2})	0.822 (0.739 to 0.884)	−10.2 (−9.6 to −11.7)	0.840 (0.715 to 0.917)	0.785 (0.670 to 0.867)
Cutoff = 50 ng/ml				
CWA PT–clotting time (time to Max1, seconds)	0.978 (0.931 to 0.996)	13.1 (12.8 to 13.9)	1.000 (0.914 to 1.000)	0.893 (0.803 to 0.945)
CWA PT–Max1 (dmAbs/dt^{-1})	0.565 (0.469 to 0.656)	331.9 (204.8 to 466.6)	0.659 (0.506 to 0.784)	0.507 (0.396 to 0.617)
FibEx – Clotting time (time to Max2, seconds)	0.992 (0.953 to 1.000)	114.2 (111.8 to 119.3)	0.951 (0.839 to 0.991)	0.987 (0.928 to 0.999)
FibEx–time to peak (time to Max1, seconds)	0.982 (0.938 to 0.998)	154.1 (151.6 to 182.2)	0.902 (0.775 to 0.961)	0.987 (0.928 to 0.999)
FibEx–Max1 (dmAbs/dt^{-1})	0.837 (0.757 to 0.899)	58.7 (49.4 to 68.7)	0.659 (0.506 to 0.784)	0.907 (0.820 to 0.954)
FibEx–Max2 (dmAbs/dt^{-2})	0.935 (0.873 to 0.973)	13.4 (12.9 to 19.4)	0.800 (0.652 to 0.895)	0.987 (0.928 to 0.999)
FibEx–Min2 (dmAbs/dt^{-2})	0.872 (0.797 to 0.927)	−10.2 (−9.6 to −12.0)	0.925 (0.801 to 0.974)	0.747 (0.638 to 0.831)

Note: ROC curves were performed at the cutoffs of 30 and 50 ng/ml. The AUC and its 95% CI have been calculated. The Youden index was used to determine the best cutoff for the corresponding parameter. Based on this cutoff, the sensitivity and the specificity were then calculated.

Abbreviations: AUC, area under the curve; CI, confidence interval; CWA, clot waveform analysis; dmABS, delta milliabsorbance; PT, prothrombin time; ROC, receptor operating characteristics.
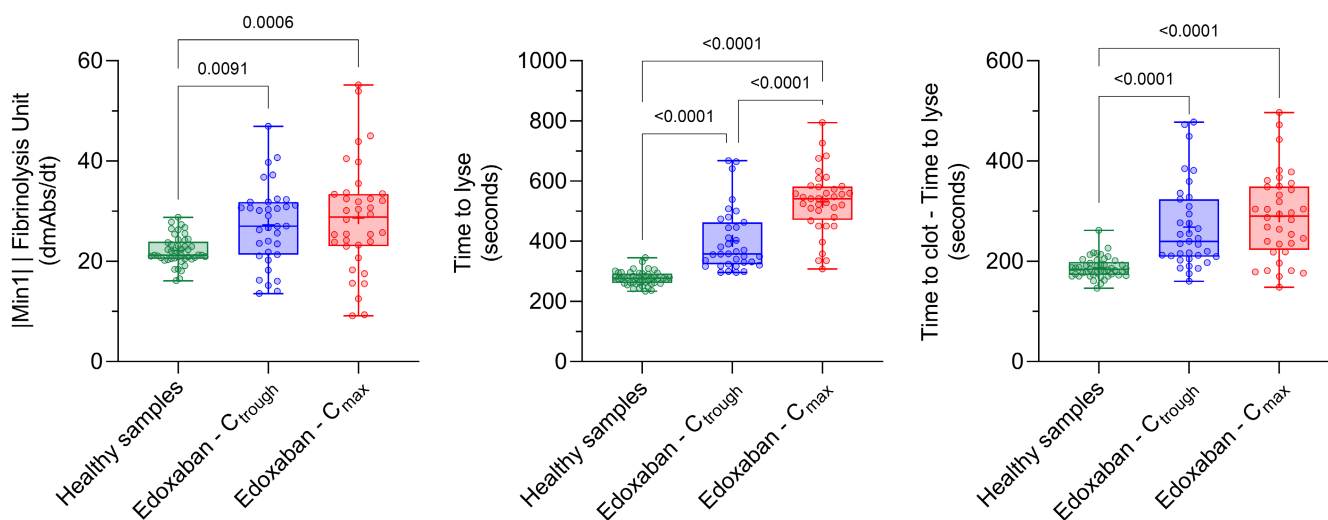
FIGURE 6 FibLysis parameters in healthy subjects and in samples from patients on edoxaban. Stratifications have been made between healthy subjects, edoxaban C_{MAX}, and edoxaban C_{TROUGH}. C_{MAX}, maximum concentration; C_{TROUGH}, minimum concentration

interest if they demonstrate sufficient performance at relevant clinical decision-making thresholds. The sensitivity of the time to Max2 and the time to Max1 to predict edoxaban levels less than 30 ng/ml were at least equal to the one of thrombin generation parameters. Indeed, according to a study of Pfrepper et al.,[45] thrombin generation

parameters showed a sensitivity of 90.5%, which was similar to the sensitivity of 98% (95% CI, 90%–100%) of FibEx–time to Max1. The sensitivity of FibEx to predict edoxaban levels less than 50 ng/ml was also at least equivalent to the performance of thrombin generation, as the sensitivity of FibEx–time to Max2 at this threshold

was above 95% (95% CI, 84%–99%) compared to a sensitivity ranging from 82.6% to 87.0% of thrombin generation. According to our results, the specificity of FibEx–time to Max2, –time to Max1 and Max2, for discriminating edoxaban samples from healthy samples, were comparable to PT-clotting time and thrombin generation parameters. Obviously, based on the principle of the FibWave, this test is not able to differentiate patients on edoxaban from patients on other DOACs. However, the fact that the plasma drug concentration is really the main determinant of the anticoagulation intensity has been questioned.[43]

Similar to the clot-fibrinolysis waveform analysis, the FibWave also allows the investigation of the fibrinolytic pathway.[11,12,46] Interestingly, we showed that the fibrinolytic process was impaired by the presence of edoxaban in a dose-dependent manner. This suggests that edoxaban has either an indirect impact on fibrinolytic proteins or that the structure of the clot differs in the presence of edoxaban.[47] It has been reported that rivaroxaban and apixaban are able to enhance fibrinolysis due to accumulation of FXaβ, which catalyzes the activity of tissue plasminogen activator on plasminogen.[48] The consequent accumulation of FXaβ in the plasma of patients treated with edoxaban could result in persistent FXa-derived fibrinolytic activity. Because our test does not include thrombomodulin, the fibrinolytic activity cannot be explained by a reduction in the activation of thrombin-activatable fibrinolysis inhibitor.[49] In addition, the thinner fibrin fibers and larger pores in clots can also be responsible for the accelerated fibrinolysis in edoxaban samples.[10] The analysis of fibrinolysis activity between C_{MAX} and C_{TROUGH} conditions showed that C_{MAX} and C_{TROUGH} samples had higher fibrinolysis velocity than healthy volunteers, supporting the positive effect of edoxaban on fibrinolysis velocity as described for other direct activated FX inhibitors.[48] The prolonged time to lyse can be explained by the impact of edoxaban on the time to Max1 in the coagulation phase, which was also prolonged compared to healthy subjects. This therefore delays the temporal parameters of the fibrinolysis.

This study presents some limitations. The number of subjects included is small, and the age of the controls does not match those of the edoxaban group. The effect of age on the FibWave parameters deserves further investigations. The information on race/ethnicity or socioeconomic status (income, education, smoking history, etc.) of participants were not collected. Therefore, our data must be interpreted with caution, since this lack of information can represent an interindividual variability. A complete evaluation of the interlaboratory variability will also be needed. Nevertheless, these results are already promising in light of the current limitations of routine coagulation tests like aPTT and PT, which are not sensitive enough to identify low concentrations of edoxaban.[16] Finally, our results were generated in patients treated with edoxaban and cannot be extrapolated to other DOACs. Despite these limitations, our results were consistent with the literature concerning edoxaban and are in line with the expectations supporting the concept that FibWave could be used as a global coagulation assay for the evaluation of the anticoagulant status, at least in patients treated with edoxaban.

5 | CONCLUSION

FibWave, and especially the FibEx methodology, was more sensitive and specific to the presence of edoxaban compared to the aPTT- and PT-based CWA. It also allowed a better discrimination between healthy subjects and patients treated with edoxaban. The performance of FibEx–time to Max1 (i.e., time to peak) to discriminate samples at very low plasma concentrations, that is, 30 and 50 ng/ml, could make this test useful for clinical decision-making. Thanks to its capacity to assess the coagulation and fibrinolysis, FibWave could be used as a global coagulation assay. Further validations and experiments are needed to confirm these promising results in the clinical setting.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.