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Full Length Article

Edoxaban plasma levels in patients with non-valvular atrial fibrillation: Inter and intra-individual variability, correlation with coagulation screening test and renal function

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A B S T R A C T

Background: High inter-individual variability of the anticoagulant plasma levels of the first three direct oral anticoagulants was previously reported. Aims of the present study were to evaluate edoxaban inter and intra-individual variability in patients with non valvular atrial fibrillation and to assess correlation between edoxaban plasma levels and coagulation screening test and renal function.

Methods: From January 31st 2017 to June 30th 2018, a total of 101 NVAF patients were enrolled: 48 patients were on edoxaban 60 mg and 53 on edoxaban 30 mg, once daily. Blood samples were taken at C-trough and at C-peak within the first month (15–25 days) of treatment and then at C-trough each three months. Prothrombin time (PT), activated partial thromboplastin time (aPTT), specific anti-FXa chromogenic test were performed. Creatinine clearance (CrCl) was calculated using the Cockcroft-Gault formula.

Results: Mean inter-individual variability expressed as overall coefficient of variation (CV%) values was lower at C-peak (CV% = 49) than at C-trough (CV% = 68). Mean CV% intra-individual variability was 26.5. No significant correlation was found between edoxaban plasma levels and CrCl (C-trough $r/r^2 = -0.007/0.000$; C-peak $r/r^2 = 0.129/0.017$). Correlations (r/r^2), at C-trough and C-peak, between edoxaban levels and PT and aPTT, were 0.386/0.149–0.922/0.851 and 0.283/0.080–0.567/0.321, respectively. Significant discrepancies between PT or aPTT and edoxaban levels were found.

Conclusions: This study confirms also for edoxaban a high inter-individual variability in NVAF patients. PT and aPTT are not useful to measure this drug. As for the other two anti-FXa drugs, the absence of a significant correlation between CrCl and edoxaban plasma levels was observed.

1. Introduction

In the last years direct oral anticoagulants (DOACs) have been introduced in clinical practice to prevent stroke and systemic embolism in patients with non valvular atrial fibrillation (NVAF) and to treat and prevent venous thromboembolism [1]. DOACs include two classes of drugs that differ according to their anticoagulant mechanism and their inhibition target: the anti-IIa molecule, of which still at present, dabigatran is the only available drug and the anti-Xa drugs that include rivaroxaban, apixaban, edoxaban and betrixaban [2].

DOACs pharmacological profile is considered predictable, as evaluated in phase II clinical studies [3–6]. The consequent use of fixed dose administration with dosages based only on clinical criteria and the evaluation of creatinine levels has showed efficacy and safety without laboratory control and dose-adjustments in phase III clinical studies [7]. In those trials DOACs fixed doses were used according to clinical indications (non valvular atrial fibrillation or venous thromboembolism), patient characteristics (age, gender, body weight, concomitant administration of potentially interfering drugs) and renal function, assuming that the anticoagulant effect was prevalently controlled by these conditions.

Nevertheless, a significant inter-individual variability has been shown for all DOACs, suggesting a possible relationship between anticoagulant plasma levels and bleeding and thromboembolic complications [8–16]. Besides, a significant increase of plasma level variability from healthy volunteers to patients enrolled in phase III clinical trials up to the post marketing evaluation of real patient populations was shown [17].

In healthy subjects, edoxaban shows a half-life of 10–14 h, it has a rapid absorption (1 − 3 h) and reaches the peak of plasma concentration at nearly 1.5 h. It is substrate of CYP3A4 and of the efflux transporter P-gp; 50% of edoxaban is excreted by kidney [18–20].

Edoxaban is administered once-daily, at fixed doses (60 or 30 mg/day),without the need of laboratory monitoring, according to clinical indications (VTE or NVAF) and patient characteristics including body weight, interfering drugs and renal function [21,22].

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A recent Italian multicenter study on NVAF population treated with dabigatran, rivaroxaban and apixaban, showed a higher DOAC inter-individual variability in comparison with previous results. Mean coefficient of variation of DOAC anticoagulant levels, measured at C-trough, was around 66% for dabigatran, 52% for rivaroxaban and 46% for apixaban in the enrolled population [17].

Currently, DOACs don't require the evaluation of their concentration in routine clinical practice [7], but their measurement is recommended in special clinical conditions such as in case of bleeding and thromboembolic complications, surgery, use of antidotes [23–27]. In these situations, specific laboratory tests are recommended because global coagulation screening tests, such as PT and aPTT, are not correlated with DOAC plasma levels and their results could cause dangerous misinterpretations [24,27–33]. Specific anti-FXa calibrated for edoxaban has shown to be reliable and correlated to drug plasma concentration as measured by mass spectrometry [33,34]. The evaluation of renal function is considered crucial for the prescription of DOACs due to their kidney metabolism, despite being different for the four drugs. A previous study didn't show a relationship, mostly for anti-Xa drugs, between plasma levels and creatinine clearance (CrCl) > 30 ml/min [17].

Aims of the present study were to evaluate edoxaban inter and intra-individual variability in real world NVAF patients and to assess the correlation between edoxaban plasma levels with coagulation screening test and renal function.

2. Design, patients and methods

2.1. Design

This is a prospective, observational study in patients with NVAF treated with edoxaban, performed in a specialized anticoagulation clinic affiliated with the Italian federation of Anticoagulation Clinics (FCSA). It was approved by the ethical committee of the general Hospital of Cremona, conducted in accordance with the Declaration of Helsinki.

2.2. Patients

After giving their informed consent, a total of 101NVAF consecutive patients starting anticoagulation with edoxaban were enrolled in the study from January 31st 2017 to June 30th 2018.

Edoxaban prescription, as recommended by Italian drug regulation agency (AIFA), was based on individual and clinical characteristics (NVAF with one or more of the following risk factors: congestive heart failure, hypertension, age > 75 years, diabetes, previous stroke or transitory ischaemic attack). Edoxaban low dosage was indicated in patients with one or more of the following factors: body weight (≤60 kg), interfering drugs (ciclosporin, ketoconazole, dronedarone, erythromycin), renal function as expressed by creatinine clearance (CrCl 15–50 ml/min).

According to the above mentioned criteria, edoxaban 60 mg was prescribed in 48 patients while 53 patients were treated with edoxaban 30 mg, once daily.

Patients were instructed to take pills regularly at 8.00 a.m.; C-trough samples were taken 24 h after the last dose intake, while C-peak samples 2 h after the controlled administration of treatment. All patients were evaluated at C-trough and C-peak within the first month (15–25 days) of treatment. Then, each three months, clinical evaluation and CrCl measurement were performed in all patients enrolled; C-trough plasma sample for edoxaban measurement was also taken during the three month follow up for two times consecutively in 75 of 101 enrolled patients, and only one time in one patient. Adherence to therapy was assessed by count of pills during the 3-month periodical follow up visits.

2.3. Laboratory tests

Plasma samples were collected in vacuum plastic tubes (Vacutainer, Becton Dickinson, Plymouth, UK), containing 3.2% trisodium citrate (9:1vol/vol, blood/anticoagulant). Blood was centrifuged within 1 h from collection at 2000 ×*g* for 20 min and plasma was quickly frozen and stored at −80 °C until testing.

All coagulation tests were assayed on frozen plasma, using a magneto-mechanical coagulometer (STA-R Stago, France). Prothrombin time (STA- Neoplastin CI Plus, Stago Diagnostica, France), activated tromboplastin time (aPTT- STA-Cephascreen, Stago Diagnostica, France) and specific chromogenic anti-Xa assay calibrated for edoxaban (STA- liquid anti-Xa, STA-edoxaban calibration and control, Stago Diagnostica, France) were performed according to manufacturer's indications. This method has shown an acceptable reproducibility (CV within-run = 4,0 and 9,0% at 60 and 30 ng/ml concentrations, respectively); the limit of detection and quantification were 1.0 and 6.0 ng/ml, respectively. The performance of the test was in line with the results already published [28,32,34].

Serum creatinine was measured at C-trough in all patients enrolled, on the same blood sampling as edoxaban concentration. CrCl was calculated using the Cockcroft-Gault formula.

2.4. Statistical analysis

The inter-individual variability, both at C-trough and C-peak, measured with specific anti-Xa test, was assessed by using mean values, range (min-max) and coefficient of variation (CV%).

The intra-individual variability was assessed on three consecutive determinations and expressed as mean value, range (min-max) and CV %. Measurement of uncertainty (MU) was evaluated by standard error calculation.

The correlation between edoxaban levels versus PT and aPTT, as well as the correlation between edoxaban levels vs CrCl, were assessed by means of the linear regression analysis and the calculation of the r/r^2 values.

Number and percentage of discrepancies between global test and edoxaban anti-FXa activity were evaluated considering PT and aPTT normal range, as expressed in ratio, and edoxaban concentration higher or < 50 ng/ml.

3. Results

Main clinical characteristics of investigated patients are shown in Table 1.

The total number of samples was 353: 101 taken at C-peak, 101

Table 1
Main clinical characteristics of patients. Results are reported as number (n), percentage (%), median and range (min-max).

	Edoxaban n = 101
Age (yr), median (min-max)	80 (61–97)
Weight (kg), median (min-max)	70 (33 – 103)
BMI, median (min-max)	26 (17–35)
Gender (M/F), n	40/61
$CHADS_2$ score, median (min-max)	3.0 (1–6)
Creatinine clearance (ml/min/1.73 m^2), median (min-max)	52.4 (17.8–107.6)
Drug daily dose, n	30 mg (53) 60 mg (48)
Previous stroke or TIA, n (%)	5 (5.9)
Previous AMI, n (%)	1 (1.0)
Interfering drugs (ciclosporin, ketoconazole, dronedarone, erythromycin), n (%)	6 (5.9)
Aspirin, n (%)	18 (17.8)
Diabetes mellitus, n (%)	9 (8.9)
Hypertension, n (%)	57 (56.4)

Table 2
Edoxaban levels determined with specific calibrated anti-FXa chromogenic assay expressed in ng/ml. PT, aPTT are expressed as ratio. Creatinine clearance was calculated using the Cockcroft-Gault formula. The total number of observations was 354.

Pts n°	53	48	101
Drug	Edoxaban 30 mg [mean (min-max); CV %]	Edoxaban 60 mg [mean (min-max); CV %]	Edoxaban 30/60 mg [mean (min-max); CV%]
Anti-Xa C-trough (ng/ml)	38 (7–147); 74.6	39 (13 − 110); 61.4	38 (7–147); 68
Anti-Xa C-peak (ng/ml)	169 (10–400); 47.6	301 (60–569); 36.4	238 (10–569); 49.1
PT C-trough (ratio)	1.15 (1.01–1.53); 8	1.17 (0.96–2.31); 16.3	1.16 (0.96–2.31); 12.9
PT C-peak (ratio)	1.58 (1.01–2.16); 16.9	1.94 (1.25–2.69); 18	1.77 (1.01–2.69); 20.5
aPTT C-trough (ratio)	1.09 (0.88–1.47); 10.6	1.09 (0.82–1.46); 12	1.09 (0.82–1.47); 11.2
aPTT ratio C-peak (ratio)	1.29 (1.02–1.66); 14.3	1.45 (0.96–2.61); 21.6	1.37 (0.96–2.61); 19.7
Creatinine clearance (ml/min/1.73 m^2)	50.3 (17.8–80.7); 27.4	54.6 (17.9–107.6); 35	52.4 (17.8–107.6); 31.8

taken at C-trough and 151 obtained at C-trough, during the follow up. In details: the first blood sampling was taken within the first month (15–25 days) of treatment in all patients enrolled. Then, after the first blood sampling, each three months we performed at C-trough two other measurements in 75 patients; in 1 patient we obtained only one C-trough measurement, after three months from the first blood control.

As expected C-trough were significantly lower as compared with C-peak levels ($p < 0.0001$), showing a significant increase of the anticoagulant effect after 2 h from drug intake (Table 2).

Mean inter-individual variability expressed as overall CV values was 49.1% at C-peak and 68% at C-trough, with a higher, even if not significant, variability in patients taking lower dosage (Table 2).

To assess for the potential of under- or over-anticoagulation we calculated also the percentage of patients outside the 10° and 90° percentile, showing that the cumulative percentage was 10% both at C-trough and C-peak.

Regression lines, equations, correlation coefficients (r values) and coefficients of determination (r^2 values) are summarized in Table 3 and Figs. 1–2.

The correlation between edoxaban (both 30 and 60 mg) and PT at C-trough was $r/r^2 = 0.386/0.149$ while at C-peak was $r/r^2 = 0.922/0.851$. The correlation between edoxaban (both30 and 60 mg) and aPTT at C-trough was $r/r^2 = 0.283/0.080$ and at C-peak was $r/r^2 = 0.567/0.321$. Globally, the coefficient of determination (r^2) showed the highest correlation between edoxaban and PT.

Discrepancies between screening test and edoxaban plasma levels were found, showing the normality of PT and aPTT despite detectable edoxaban plasma levels (> 50 ng/ml) in 16% and 51.4% of cases, respectively. Prolonged PT and aPTT, in absence of significant drug concentration (< 50 ng/ml), was found in 16.6% and 9.1% of cases, respectively (Table 4; Figs. 3–4).

No significant correlation was found between edoxaban plasma levels and CrCl > 17.8 ml/min (value corresponding to the lower level detected in this study population), both at C-peak and at C-trough (Table 3, Fig. 5).

Mean CV% intra-individual variability, calculated on 75 out of 101 patients with three consecutive C-trough edoxaban measurements, was 26.4, without differences among patients treated with edoxaban 30 mg (CV% = 26.7) and edoxaban 60 mg (CV% = 26.5). In addition to intra-individual variability, the measurement of uncertainty (MU) that may have greater clinical significance [35,36] was also calculated. The MU ranged between 1 and 9 ng/ml and between 8 and 13 ng/ml for edoxaban levels around 30 and 50 ng/ml, respectively.

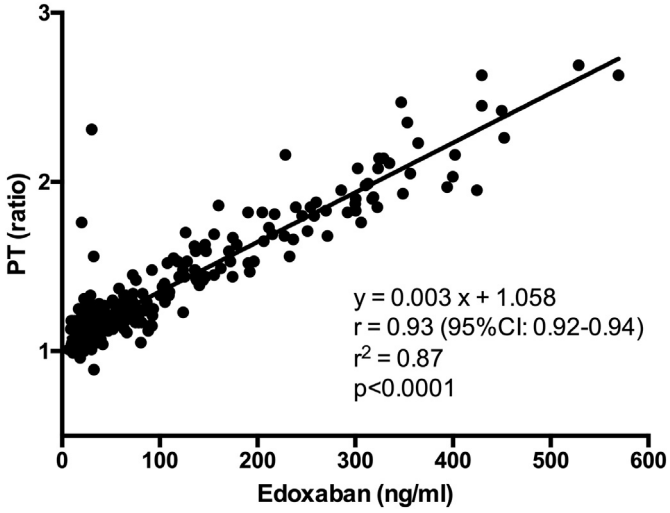

Fig. 1. Edoxaban and PT ratio: linear regression line, equation and correlation coefficients (r/r^2 values).

4. Discussion

Phase II pharmacological studies have demonstrated a sufficiently predictable DOAC anticoagulant effect in standard clinical conditions and in selected patient populations. However, it should be considered that, in general clinical practice, the complexity of patients increases in comparison to patients enrolled in clinical trials because they are older,

Table 3
Correlation (r value), coefficient of determination (r^2) and statistical significance (p) of edoxaban plasma concentrations (at C-peak and C-trough) vs. PT ratio, aPTT ratio and creatinine clearance (ml/min/1.73 m^2).

Anti-FXa (ng/ml) vs lab test	C-trough (r/r^2)	p	C-peak (r/r^2)	p
Edoxaban 30 mg vs PT ratio	0.702/0.493	< 0.0001	0.862/0.743	< 0.0001
Edoxaban 60 mg vs PT ratio	0.262/0.069	ns	0.911/0.830	< 0.0001
All patients vs PT ratio	0.386/0.149	< 0.0001	0.922/0.851	< 0.0001
Edoxaban 30 mg vs aPTT ratio	0.316/0.010	0.025	0.545/0.297	0,0003
Edoxaban 60 mg vs aPTT ratio	0.254/0.065	ns	0.498/0.248	0,0007
All patients vs aPTT ratio	0.283/0.080	0.004	0.567/0.321	< 0.0001
Edoxaban 30 mg vs CrCl (ml/min/1.73 m^2)	− 0.057/0.003	ns	0.184/0.034	ns
Edoxaban 60 mg vs CrCl (ml/min/1.73 m^2)	0.022/0.000	ns	0.000/0.000	ns
All patients vs CrCl (ml/min/1.73 m^2)	− 0.007/0.000	ns	0.129/0.017	ns

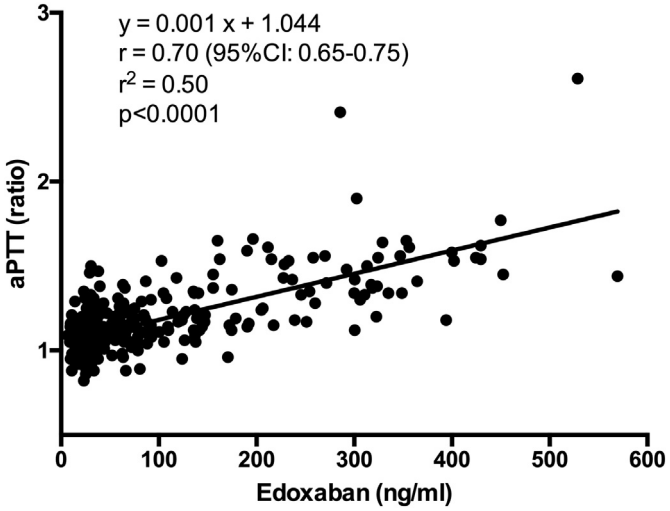

Fig. 2. Edoxaban and aPTT ratio: linear regression line, equation and correlation coefficients (r/r^2 values).

Table 4
Responsiveness of PT or aPTT to edoxaban. Number and percentage of discrepancies between global test and edoxaban anti-Xa activity evaluated considering PT and aPTT normal range, as expressed in ratio, and edoxaban concentration higher or < 50 ng/ml.

Normal PT and edoxaban > 50 ng/ml, n (%)	23/144 (16.0%)
Prolonged PT and edoxaban ≤50 ng/ml, n (%)	35/209 (16.6%)
Normal aPTT and edoxaban > 50 ng/ml, n (%)	74/144 (51.4%)
Prolonged aPTT and edoxaban ≤50 ng/ml, n (%)	19/209 (9.1%)
Normal PT and normal aPTT and edoxaban > 50 ng/ml, n (%)	7/77 (9.1%)
Prolonged PT and prolonged aPTT edoxaban ≤50 ng/ml, n (%)	23/185 (12.4%)

PT: normal = ≤1.20 ratio, abnormal = > 1.20 ratio; aPTT: normal = ≤1.22 ratio, abnormal = > 1.22 ratio.

with multiple comorbidities and treated with many potentially interfering drugs. Probably, because of these reasons, DOACs plasma variability in patients with NVAF treated with dabigatran, rivaroxaban and apixaban was increased if compared with phase II-III clinical studies [3–6,8–14].

This study aimed to investigate intra- and inter-individual variability of edoxaban in NVAF patients, the last anti-Xa drug introduced in Europe for this clinical indication and to assess the correlation between edoxaban plasma levels and coagulation screening test and renal function.

Edoxaban anti-FXa measurements confirmed a high inter-individual variability, as previously reported [8,14], independently from the posology (30 mg and at 60 mg), confirming also for this anti-Xa molecule what observed for both anti-IIa (dabigatran) and anti-Xa drugs (apixaban and rivaroxaban) [9–11,17]. Variability at C-trough was higher than at C-peak, suggesting that metabolism and elimination phases seem to play a more crucial role in the determination of edoxaban plasma levels in comparison to the adsorption phase.

In this study cohort, variability and C-trough edoxaban levels were higher in patients treated with the lowest dose. Previous studies showed that patients treated with the lowest dose of edoxaban had lower anti-FXa activity and had higher rates of bleeding and ischaemic events than patients whose doses were not reduced [14,21]. Probably, the higher variability in patients treated with the lowest dose found in this study cohort is related to the older age, demographic factors and fragility of patients. Our study population had a mean age of 80 years old, with 12 patients older than 90.

Anyhow, a direct comparison with phase III study [21] could be misleading because of the relative small number of the patients enrolled in our study.

Moreover, in this study no correlation between CrCl and edoxaban concentration was found, confirming a previous observation [17] and opening the question on which is the role and how to use the measurement of renal function in the management of DOAC patients in clinical practice. All together these data raise the crucial question of whether DOAC measurement would be more informative than demographic and clinical patient characteristics and, certainly, more studies are needed to better clarify this problem [7].

C-peak and C-trough levels currently available are derived from different studies, in which different methodologies were used to calculate the expected range [31]. However, these plasma levels cannot be considered as therapeutic range because further clinical studies are needed to define the relationship between DOACs plasma levels and bleeding and thromboembolic complications. Therefore, we confirm the high inter-individual variability, waiting for new evidences, other than

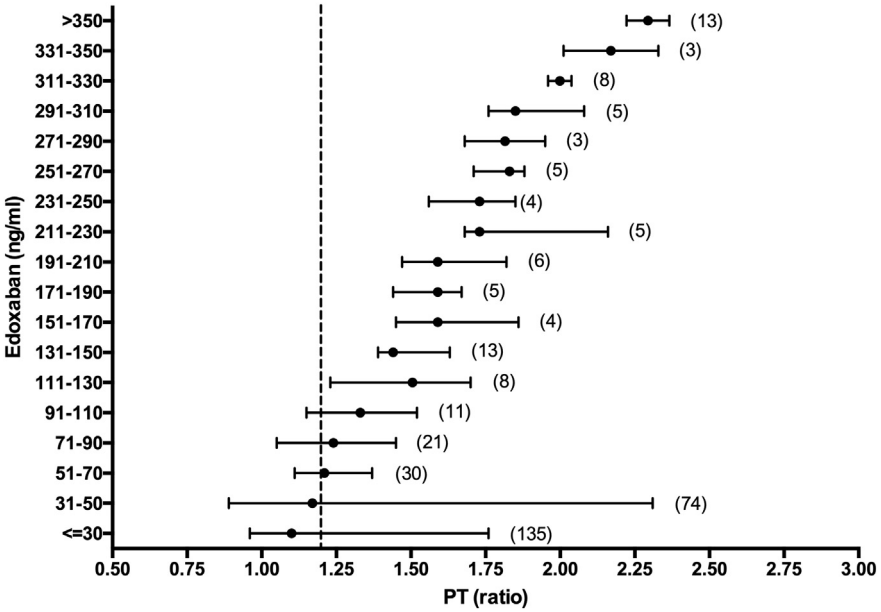

Fig. 3. PT ratio median values and ranges obtained in different arbitrary classes of edoxaban concentrations (number of samples in each class in parenthesis).

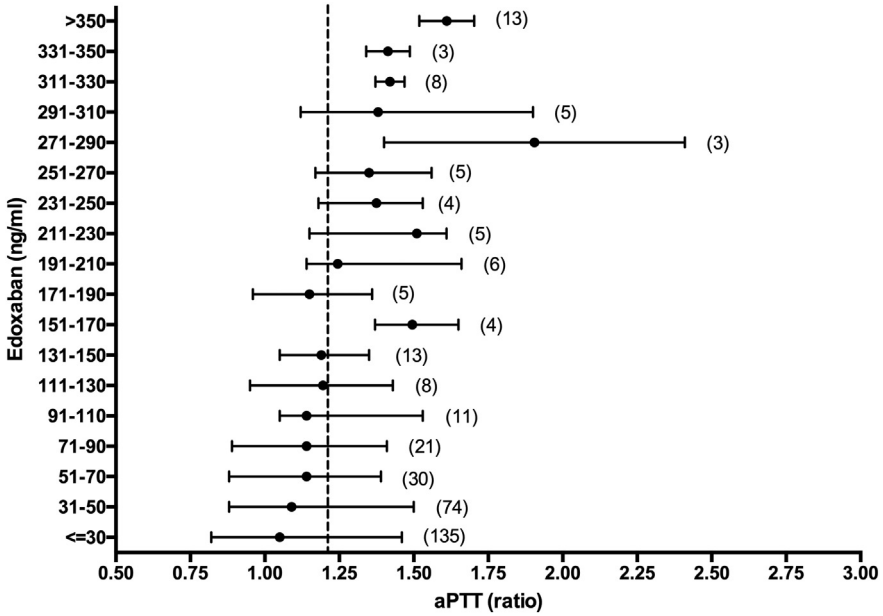

Fig. 4. aPTT median values and ranges obtained in different arbitrary classes of edoxaban concentrations (number of samples in each class in parenthesis).

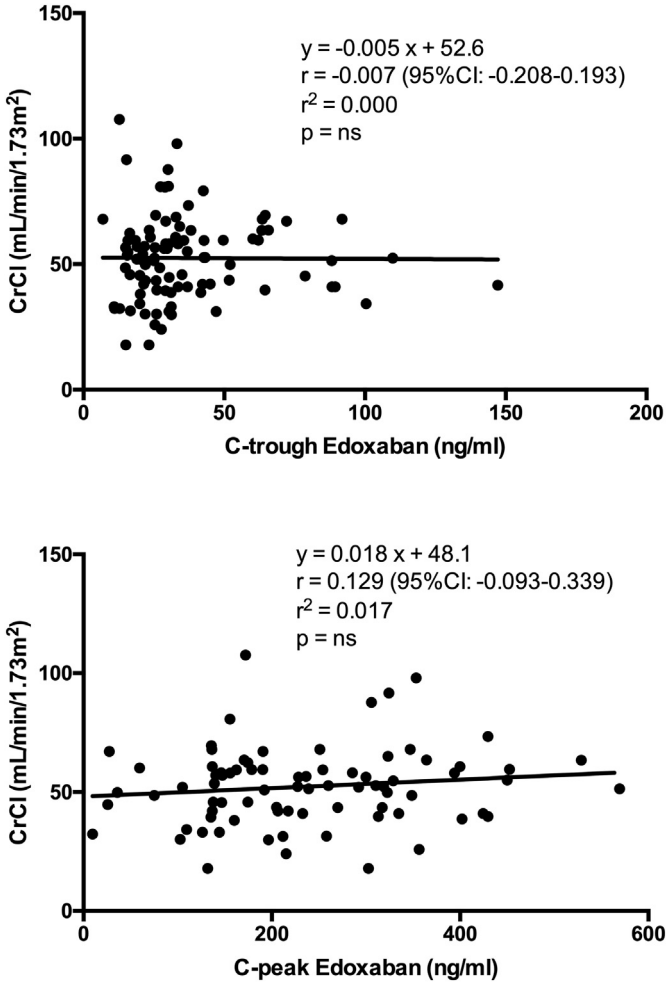

Fig. 5. Edoxaban and CrCl: linear regression line, equation and correlation coefficients (r/r^2 values).

we have at present, indicating DOACs therapeutic ranges, critical values and cut-off, which could be used for improving patient management in daily clinical practice.

In this study population, intra-individual variability was relatively low. This information could have an important clinical implication because, in case of good response to the drug, in absence of intercurrent illnesses, changes of health status or co-therapies, clinicians can be confident on the maintenance of relatively stable anticoagulant levels during time.

Measurement at steady state could represent an important information for clinicians to confirm the presence of drug, to know the individual response and to evaluate possible significant changes in relation to modification of health status, co-therapies or patient adherence to treatment.

Globally, PT shows a better correlation with edoxaban plasma levels in comparison to aPTT, but its responsiveness is inadequate for measuring this drug, as shown in previous studies [28,29,31–33]. As for the other three DOACs, also for edoxaban PT or aPTT can be prolonged in absence or at low edoxaban concentration as well as high drug levels can be associated with PT or aPTT normal range. Therefore, as recommended in more recent guidelines, also this data confirm the indication to measure edoxaban plasma levels with specific test, already commercially available and easy to perform in routine and emergency [25–27,31–33]. Currently, other specific tests are on evaluation and probably they will be introduced in the market in the next future [37] but, still at present, specific chromogenic assay represent the most suitable and accurate test available for measuring edoxaban [28,29,31,33].

In daily clinical practice, DOAC prescription and dose administration are based on results of CrCl, which is also routinely evaluated during the follow up. As known, DOACs are differently cleared by the kidneys and patients with renal impairment are exposed to a risk of drug accumulation equal to 77%, 36%, 21% and 24% for dabigatran, rivaroxaban, apixaban, and edoxaban, respectively [2]. Currently, based on pharmacokinetic characteristics, DOAC plasma levels are considered “a priori” at “normal level” and some aspects, as renal impairment, are considered as single variables that can modify drug exposure. In this study, no correlation was found between edoxaban plasma levels and CrCl, confirming a previous observation on the other three DOACs, in which, at steady state, the correlation between CrCL > 30 ml/min and dabigatran plasma level was very poor, while

for aXa apixaban and rivaroxaban was absent [17]. The clinical value of this observation is highlighted in a recent clinical study conducted on patients on dabigatran, the drug mainly excreted by kidney [38]. Authors showed that CrCl didn't correlated with the risk of drug reappearances after treatment with idarucizumab, while, instead, the risk of rebound effect was significantly correlated with dabigatran levels.

Besides, the absence of a significant correlation between edoxaban plasma levels and renal function (for CrCl > 17.8 ml/min, value corresponding to the lower level detected in this study population),suggest their use also in patients with impaired renal function. These data suggest that edoxaban plasma levels could depend and be determined by many different conditions and metabolic variables other than the evaluation of renal function, currently considered the only laboratory marker used by clinicians to “a priori pre-define” the individual plasma anticoagulant level. Instead, a simple specific measurement, at steady state, could represent the important information that clinicians search through other markers, such as CrCl, to confirm, as an example, the presence of drug, to know the individual response and to evaluate possible significant changes in relation to modification of health status, co-therapies or adherence.

Limit of the present study is mainly represented by the relatively small number of patients enrolled in a single center. On the other hand, the advantages of our observations were the following: 1. a strict follow up of patients that ensured a complete collection of the information required; 2. the evaluation of adherence and persistence of drug intake, obtained through careful clinical monitoring; 3. the accurate timing of blood sampling; 4. the quality of laboratory testing.

In conclusion, this study confirms also for edoxaban a high inter-individual variability in the “real world” NVAF patients. As previously shown also for the other DOACs, PT and aPTT are not useful to measure this drug and a high number of discrepancies have been found. As for the other two anti-FXa drugs, the absence of a significant correlation between CrCl and edoxaban plasma levels was observed. These results confirm our previous observation highlighting that, in patients with creatinine clearance > 17.8 ml/min, the evaluation of renal function cannot be used as indirect expression of drug plasma concentration.

Addendum

ST conceived the study and reviewed the data. ST, OP and CL wrote the manuscript. OP, EC, RM, MT, SZ enrolled patients and supervised laboratory measurement. ST, CD and CL analyzed the data. All the authors revised and accepted the final version of the manuscript.

Disclosure

None to declare.

Conflict of interest

All authors declare no conflict of interest.

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