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Plasma levels of direct oral anticoagulants in real life patients with atrial fibrillation: Results observed in four anticoagulation clinics

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ABSTRACT

Introduction: Direct oral anticoagulant (DOAC) intra- and inter-individual variability was previously reported, but its magnitude is still considered negligible for patient management.
Objective: To evaluate inter- and intra-individual variability in real-world atrial fibrillation patients on dabigatran, rivaroxaban or apixaban in four Italian anticoagulation clinics and to assess the correlation between DOAC plasma concentration and creatinine-clearance (CrCl).
Materials and Methods: A total of 330 consecutive patients were enrolled, of which 160 were on dabigatran (70 and 90 taking 150 mg or 110 mg twice-daily, respectively), 71 on rivaroxaban (37 and 34 taking 20 mg or 15 mg once-daily) and 99 on apixaban (73 and 26 taking 5 mg or 2.5 mg twice-daily). Blood was taken at trough and peak within the first month (15–25 days) of treatment. Diluted-thrombin-time (dTT) calibrated for dabigatran and anti-FXa calibrated for rivaroxaban or apixaban was performed.
Results: Mean inter-individual variability expressed as overall CV values for all drugs was lower at peak (CV = 46%) than at trough (CV = 63%). Mean CV% intra-individual variability was 36.6% at trough and 34.0% at peak. Correlation with CrCl was poor for all drugs and only dabigatran at trough showed a significant correlation.
Conclusion: This multicenter study confirms high DOAC inter-individual variability that cannot be explained by the rate of renal clearance to which the three DOAC were subjected since the correlation with CrCl was relatively poor. This poor correlation suggests caution in using CrCl as the sole laboratory parameter to indirectly evaluate residual circulating DOAC.

1. Introduction

Anticoagulation is recommended for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation. Oral drugs available for short- and long-term treatment are vitamin K antagonists (VKA) or direct oral anticoagulants (DOAC). DOAC include two classes of drugs that differ according to their anticoagulant mechanisms: dabigatran targets thrombin, while rivaroxaban and apixaban act specifically by inhibiting factor Xa. Phase III clinical trials showed DOAC efficacy and safety without laboratory control and dose-adjustment. In those trials fixed doses [1–4] were used according to clinical indications (atrial fibrillation or venous thromboembolism), patient characteristics (age, gender, body weight, concomitant administration of potentially interfering drugs), and renal and liver function. With this in mind it was assumed the anticoagulant effect was prevalently controlled by these conditions. DOAC intra- and inter-individual variability has been previously reported [5–14], but its magnitude is still considered negligible for management. Furthermore, DOAC showed inter-individual variability of plasma concentration at steady state regardless of type of drug and patient characteristic such as renal function and body weight [9]. Results are however scanty and conflicting, leaving room for further investigation.

In this study, we report results on inter-individual variability assessed in patients with atrial fibrillation treated with dabigatran,

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rivaroxaban or apixaban in four Italian anticoagulation clinics. In one clinic we also evaluated intra-individual variability by measuring the DOAC anticoagulant effect over three consecutive time points. Finally, we evaluated the correlation between DOAC anticoagulant levels measured with specific coagulation tests and creatinine clearance.

2. Materials and methods

2.1. Design

This is a prospective observational multicenter study in patients with atrial fibrillation treated with DOAC and was approved by the ethical committee of the general hospital of Cremona. Four large Italian anticoagulation clinics [Bologna (A), Cremona (B), Padua (C) and Florence (D)], affiliated with the Italian Federation of Anticoagulation Clinics (FCSA) and engaged in the Start Register (Survey on anTicoagulated pAtients RegisTer) (www.start-register.org), were asked to join the collaborative study by collecting plasma from patients treated with DOAC.

2.2. Patients

After giving their informed consent, a total of 330 consecutive patients seen at the anticoagulation clinics from Jan. 1st 2014 to Dec. 31st 2014 were enrolled in the study providing they had been treated with DOAC for at least one week and were available to attend the clinic for blood sampling at the specified time points (see below). Before starting anticoagulation, liver function was assessed by means of liver enzymes (aspartate aminotransferase-AST and Alanine aminotransferase-ALT). A total of 160 patients were on dabigatran (70 and 90 taking 150 mg or 110 mg twice-daily, respectively), 71 on rivaroxaban (37 and 34 taking 20 mg or 15 mg once-daily, respectively), and 99 on apixaban (73 and 26 taking 5 mg or 2.5 mg twice-daily, respectively). Patients were evaluated at enrolment and the type and dosage of drug prescribed at the discretion of the attending physician based on clinical characteristics. Patients were followed in the first month (15–25 days) of treatment when trough and peak blood samples were taken. The trough sample was obtained at 12 h from the last dose intake for dabigatran and apixaban, and at 24 h for rivaroxaban. The peak sample was obtained at 2 h from ingestion for all drugs and 2 h after through sample. Concomitant food intake was ensured in patients on rivaroxaban. Plasma samples were collected in vacuum plastic tubes (Vacutainer, Becton Dickinson, Plymouth, UK), containing 3.2% trisodium citrate (9:1 vol/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. In one clinic (B), intra-individual variability was evaluated on 120 patients (20 patients for each drug and posology) at three consecutive controls (within the first 3 months of treatment) at trough and peak.

2.3. Laboratory tests

Diluted thrombin time (dTT) calibrated for dabigatran [15], and anti-Xa assays calibrated for rivaroxaban or apixaban [16] were performed locally in each clinic with appropriate coagulation platforms according to manufacturer's indications. Methods, analytical performances, in-house intra- and inter-assay coefficient of variation (CV%) for these methods are in Table 1. The lower limit for dTT and for the anti-FXa is 20 ng/mL and 15 ng/mL, respectively. Results below the lower limits were interpolated on the calibration curve from the clotting time for dTT and from the optical density for the anti-FXa assay.

Serum creatinine was measured in samples taken at trough and creatinine clearance calculated using the Cockroft-Gault formula for each patient.

2.4. Statistical analysis

Inter- and intra-assay variability have been determined by calculation of the mean, standard deviation and coefficient of variation (CV) according to CLSI recommendations (17), using internal controls at two known drug level concentrations.

The inter-individual variability both at trough and peak was assessed by calculating the following parameters. (i) Mean values and range (min-max) and standard deviations (SD) for each DOAC concentration measured for all the patient population. (ii) Coefficient of variation (CV%) calculated as (Mean/SD) × 100, both as the overall value (all patients from the four clinics) and the value for each clinic. The intra-individual variability was assessed in one clinic (clinic B) by calculating the mean, SD and CV within each patient for whom the DOAC concentration was measured at peak and trough at three time points two weeks apart one from the other. The correlation between DOAC levels at peak and trough vs. creatinine clearance was assessed by means of the linear regression analysis and calculation of the r/r^2 values.

3. Results

The clinical characteristics of investigated patients are in Table 2.

3.1. Inter-individual variability

The inter-individual variability expressed as CV for each drug, dosage and clinic is in Tables 3–5. For dabigatran 110 mg, CV values ranged from 56% to 71% at peak and from 36% to 72% at trough. For dabigatran 150 mg, CV values ranged from 45% to 56% at peak and from 42% to 92% at trough (Table 3). For apixaban 5 mg, CV values ranged from 31% to

Table 1
Methods, analytical performances, in house intra- and inter-assay coefficient of variation (CV%).

	Bologna (A)	Cremona (B)	Padua (C)	Florence (D)
Coagulometer	STA compact (Stago)	STA-R (Stago)	CA7000 (Sysmex)	ACL TOP 700 (Werfen)
Reagents				
Dabigatran	Thrombin Siemens	Thrombin Stago	Hyphen Hemoclot	Hyphen Hemoclot
Rivaroxaban	Liquid antiXa Stago	Liquid antiXa Stago	Hyphen DiXal	-
Apixaban	Liquid aXa Stago	Liquid aXa Stago	–	Hyphen DiXal
Calibrators				
Dabigatran	Hyphen Biomed	Hyphen Biomed	Hyphen Biomed	Hyphen Biomed
Rivaroxaban	Calibrator Stago	Calibrator Stago	Biophen Stago	–
Apixaban	Calibrator Stago	Calibrator Stago	–	Biophen apixaban
d-TT intra-assay CV%	2.4–5.1	2.7–5.8	2.8–3.6	1.4–7.6
dTT inter-assay CV%	1.9–7.3	3.1–7.9	4.2–8.1	3.1–6.0
Anti-FXa rivaroxaban intra-assay CV%	0.5–2.2	0.8–3.3	2.2–2.6	–
Anti-FXa rivaroxaban inter-assay CV%	0.6–4.4	1.0–4.3	2.2–6.2	–
Anti-FXa apixaban intra-assay CV%	1.3–2.4	1.1–3.6	–	1.5–6.6
Anti-FXa apixaban inter-assay CV%	1.7–3.6	2.0–4.5	–	2.2–6.9