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

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A B S T R A C T

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 characteristics 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,
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 dosing 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 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.

2.3. 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 Cockcroft-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/r² 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-assy coefficient of variation (CV%). |
|---|---|---|---|---|
| Reagents | Bologna (A) | Cremona (B) | Padua (C) | Florence (D) |
| Thrombin Siemens | Thrombin Stago | Hyphen Hemoctot | Hyphen Hemoctot |
| Liquid antiXa Stago | Liquid antiXa Stago | Hyphen DxAl | – |
| Liquid antiXa Stago | Liquid antiXa Stago | – | Hyphen DxAl |
| Coagulometer | STA compact (Stago) | STA-R (Stago) | CA7000 (Sysmex) | ACL TOP 700 (Werfen) |
| Diluted thrombin time (dTT) 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 | 2.2–6.2 | – |
| Anti-FXa rivaroxaban inter-assay CV% | 0.6–4.4 | 1.0–4.3 | – | – |
| Anti-FXa apixaban intra-assay CV% | 1.3–2.4 | 1.1–3.6 | 1.5–6.6 | 2.2–6.9 |
| Anti-FXa apixaban inter-assay CV% | 1.7–3.6 | 2.0–4.5 | – | – |
33% at peak and from 29% to 49% at trough. For apixaban 2.5 mg, CV values ranged from 21% to 42% at peak and from 44% to 68% at trough (Table 4). For rivaroxaban 20 mg, CV values ranged from 32% to 49% at peak and from 40% to 103% at trough. For rivaroxaban 15 mg, CV values ranged from 46% to 52% at peak and from 30% to 79% at trough (Table 5). There were no major differences between the clinics.

Total inter-individual variability expressed as overall mean concentration (min–max), irrespective of the clinics, is in Fig. 1. The overall mean concentrations and ranges for all drugs were lower at trough than at peak.

Total inter-individual variability expressed as overall CV (irrespective of clinics) is in Fig. 2. For dabigatran 110 mg, overall CV value was 60% at trough and 43% at peak. For dabigatran 150 mg, overall CV value was 61% at trough and 43% at peak. For rivaroxaban 110 mg, overall CV value was 60% at trough and 43% at peak. For apixaban 2.5 mg, overall CV value was 59% at trough and 43% at peak. For apixaban 5 mg, overall CV value was 49% at trough and 35% at peak. For apixaban 2.5 mg, overall CV value was 59% at trough and 35% at peak. Overall CV values for all drugs were lower at peak than at trough. Dabigatran showed the highest overall CV value. We also calculated the percentage of patients outside the 10° and 90° percentile to assess for the potential bradiprotaxa. The overall CV value was 60% at trough and 43% at peak. For rivaroxaban 110 mg, overall CV value was 60% at trough and 43% at peak. For apixaban 2.5 mg, overall CV value was 59% at trough and 35% at peak. For apixaban 5 mg, overall CV value was 49% at trough and 35% at peak. For apixaban 2.5 mg, overall CV value was 59% at trough and 35% at peak. Overall CV values for all drugs were lower at peak than at trough. Dabigatran showed the highest overall CV value. We also calculated the percentage of patients outside the 10° and 90° percentile to assess for the potential of under- or over-anticoagulation. The cumulative percentage of patients outside the 10–90 percentile was 26% and 22% at trough or peak, respectively, with negligible between-drug differences.

### 3.3. Correlation of DOAC plasma concentrations vs. creatinine clearance

The r² values are in Table 6. In general, there was an inverse correlation between plasma DOAC concentrations and creatinine clearance. The r² values at trough were small for rivaroxaban or apixaban and slightly higher for dabigatran.

### 4. Discussion

DOAC inter-individual variability has been inconsistently evaluated in published reports and guidelines, and differently commented as being either low or high [5–14]. Furthermore, few studies investigated all the DOAC currently licensed for treatment of patients with atrial fibrillation. Our multicenter study, investigating a relatively high number of patients, shows a relatively high inter-individual variability for the three DOAC in patients treated with different dosages. On average, the drug concentration levels varied more than 20-times among the patients for dabigatran, nearly 15-times for rivaroxaban and 7-times for apixaban. This variability, observed in real life patients, compares favorably with that already reported for dabigatran [11,12,18] or rivaroxaban [11] for results stemming from the clinical trials. Variability was similarly high if assessed within each clinic or evaluated as a whole, suggesting that it cannot be accounted for by the variability of the different laboratory assays. Furthermore, variability was considerably higher in patients treated with the lowest dose of DOAC. This observation may have important practical implications since on the one hand it supports the strategy of assigning lower doses to patients with specific clinical characteristics, while on the other hand it shows that the same patient may reveal a greater variability with respect to anticoagulation, regardless of the clinical criteria adopted to assign drug dosage. It is intriguing that patients taking the same oral dose of single DOAC may present with highly variable plasma concentrations. Different DOAC metabolism patterns in individual patients may be the likely explanation for this variability. Our data show a greater variability at trough than at peak levels. Although the reason for this is not known, drug absorption is...
more likely predictable than elimination, since the latter is probably affected by a greater number of variables than the former [19].

A crucial role in metabolism is assigned to the kidney’s ability to clear DOAC from circulation even though this is not the only mechanism for elimination. Although dabigatran, rivaroxaban and apixaban show different rates of renal excretion (nearly 80%, 35% and 27%, respectively), it can be expected that their plasma concentrations at steady state do correlate with calculated creatinine clearance. No formal assessment of this correlation has however yet been reported. Our results show that while rivaroxaban or apixaban plasma concentrations are not correlated with creatinine clearance at trough, the relatively high inter-individual dabigatran variability can only in part be accounted for by a degree of inverse correlation between its plasma concentrations and creatinine clearance. In line with current recommendations, patients with creatinine clearance lower than 30 mL/min were not prescribed dabigatran in this study. Despite this, a certain degree of inverse correlation between dabigatran plasma concentrations and creatinine clearance was observed, supporting the idea that circulating dabigatran is regulated by renal function even at near-normal creatinine clearance levels and suggesting that prescribing dabigatran to patients at the lower end of normal range for creatinine clearance may prompt excessive residual plasma concentrations. Indeed, post-hoc analysis of RE-LY trial [17] showed that trough plasma concentrations of dabigatran were associated far more with the relative risk of hemorrhage than with the relative risk of ischemic stroke, suggesting that the real concern for dabigatran might be safety rather than efficacy.

The results of our study may have practical implications for patient management. First, there is an urgent need to establish drug-specific cut off levels to flag concern about the occurrence of clinical (hemorrhage or thrombosis) events in treated patients. It is reasonable to assume that given the relatively high inter-individual variability, it will be difficult to set

Fig. 1. Distribution of DOAC plasma concentrations in treated patients. Horizontal bars represent mean (middle bar) and min–max values recorded for all patients. Numbers of patients are within brackets.
precise cut off values applicable to the general population of treated patients. Information on anticoagulant levels from real world patients are needed to increase the scanty data presently available [18].

Secondly, there is a need to establish cut off drug levels to be applied to patients receiving treatment after stopping anticoagulation in the event of surgery or invasive procedures. It is currently thought that owing to the relatively short DOAC half-life, residual circulating drug levels are reasonably low when anticoagulation is discontinued two days before surgery, provided renal function is normal. To this end, it is recommended renal function be assessed by measuring serum creatinine and calculating clearance by means of the Cockroft Gault formula. The safety of such an assessment is based on the assumption that DOAC plasma concentrations are correlated with creatinine clearance. Our study shows that this assumption does not hold true, particularly for rivaroxaban and apixaban (i.e., the anti FXa drugs that show a minor dependency on renal function), and may therefore lead to misleading interpretation of anticoagulant status in individual patients who may thus be at risk of bleeding during surgery. It is our view that, instead of proposing measurement of indirect parameters such as renal and liver function, direct measurement of the effect of drug anticoagulation is much safer and should be performed just before surgery or invasive procedure. Consequently, specific tests should be available in real time in each hospital to guide the management of patients in the different clinical conditions. The lower limits of drug detection, in our experience through the methods used, is associated to the nearly complete absence of residual anticoagulant activity. While the cost incurred by measuring the anticoagulant effect of DOAC is probably slightly higher than that of measuring serum creatinine, the strategy would make patient management much safer.

Intra-individual variability is an interesting parameter since it provides information as to whether single DOAC time-point measurements may or may not characterize individual patients. If DOAC are metabolized at the same rate by individual patients, their concentrations at steady-state should be fairly constant over time and this might be taken as a characteristic feature for each patient. As shown by our study and elsewhere in the literature [12] this does not hold true for dabigatran where intra-individual variability is considerably higher (average CV, 55%). Rivaroxaban and apixaban showed, respectively, intermediate (average CV, 33%) and relatively small (average CV, 19%) variability. Accordingly, single DOAC measurement can hardly provide an estimation of the average anticoagulation achieved in individual patients. It should be realized that estimating the average anticoagulation achieved with DOAC is difficult as it depends on the adsorption, metabolism and clearance of the drug that may vary over time. However, it can be assumed that when DOAC measurement is performed after drug discontinuation and soon before surgery or invasive procedures even a single DOAC measurement may be useful to see whether there are still excess circulating levels that may increase the risk of bleeding.

In conclusion, this collaborative study involving four large Italian anticoagulation clinics shows that the inter-individual variability of plasma concentrations estimated in a relatively large number of patients with atrial fibrillation is relatively high for the three DOAC: dabigatran (average CV = 66%), rivaroxaban (CV = 52%) and apixaban (CV = 46%). The relatively high inter-individual variability cannot be accounted for by the rate of renal clearance the three DOAC are (although to a different extent) subjected to since the correlation of their plasma concentrations and creatinine clearance was relatively poor. The possible influence on this poor correlation brought about by the hepatic function and other confounding variables such as gender and body mass index has not been formally assessed. However, owing to the real nature of this study, investigating relatively large numbers of patients, it is unlikely that the above effects (if any) can affect the conclusion that the poor observed correlation argues against using creatinine clearance as the sole laboratory parameter to assess whether or not residual circulating DOAC is sufficiently low to ensure low risk of bleeding during surgery and/or invasive procedures. Intra-individual variability is also relatively high for dabigatran, suggesting that single DOAC measurement cannot be used to estimate the level of anticoagulation reached at the steady-state in treated patients. All in all, the above observations may have important practical implications for the management of patients treated with DOAC.

Table 6
Correlation (r value), coefficient of determination (r²) and statistical significance (p) of DOAC plasma concentrations (at peak or trough) vs. creatinine clearance.

<table>
<thead>
<tr>
<th>Drug and dose (mg)</th>
<th>C trough (r²)</th>
<th>p</th>
<th>C peak (r²)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabigatran 110</td>
<td>-0.25/0.0625</td>
<td>0.04</td>
<td>-0.12/0.014</td>
<td>ns</td>
</tr>
<tr>
<td>Dabigatran 150</td>
<td>-0.32/0.1024</td>
<td>0.03</td>
<td>-0.18/0.0324</td>
<td>ns</td>
</tr>
<tr>
<td>Rivaroxaban 20</td>
<td>-0.18/0.0324</td>
<td>0.03</td>
<td>-0.15/0.0225</td>
<td>ns</td>
</tr>
<tr>
<td>Rivaroxaban 15</td>
<td>-0.05/0.0081</td>
<td>ns</td>
<td>0.07/0.0049</td>
<td>ns</td>
</tr>
<tr>
<td>Apixaban 5</td>
<td>-0.03/0.0009</td>
<td>ns</td>
<td>-0.17/0.0289</td>
<td>ns</td>
</tr>
<tr>
<td>Apixaban 2.5</td>
<td>-0.02/0.0004</td>
<td>ns</td>
<td>-0.01/0.0001</td>
<td>ns</td>
</tr>
</tbody>
</table>

Addendum

ST and AT conceived the study, reviewed the data and wrote the manuscript. CL, VP, RA, CD, PC, LS, RP, OP, DP enrolled patients and supervised laboratory measurement. ST, CD and OP analyzed the data. GP and all the authors revised and accepted the final version of the manuscript.
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