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LOW DRUG LEVELS AND THROMBOTIC COMPLICATIONS IN HIGH RISK ATRIAL FIBRILLATION PATIENTS TREATED WITH DIRECT ORAL ANTICOAGULANTS

Short title: DOAC plasma levels and thromboembolic events

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Essentials

- Direct oral anticoagulants (DOACs) do not require laboratory monitoring currently.
- DOAC specific measurements were performed at trough in patients with atrial fibrillation.
- Patients who developed thromboembolic events showed lower DOAC plasma levels.
- This study supports the concept of measuring DOAC levels at steady state.

Abstract

Background. Direct oral anticoagulants(DOACs) are administered at fixed dose without need for dose adjustment by lab testing. A high inter-individual variability in the drug blood levels was shown with all DOACs. To evaluate a possible relationship between DOAC C-trough anticoagulant levels and thromboembolic events,565 consecutivenaïve patients withatrial fibrillation (AF), were enrolled in this study performed within the START-Laboratory Registry.

Methods. DOAC specific measurements [diluted thrombin time (dTT) or anti-FIIa calibrated for dabigatran; anti-FXa calibrated for rivaroxaban or apixaban] at C-trough were performed locally at steady state within 15-25 days from starting treatment. For each DOAC, the interval of C-trough levels,from the limit of quantification to the highest value, was subdividedinto4 equal classes and results were attributed to these classes; the median values of results were also calculated. Thromboembolic complications occurring for 1 year follow up were recorded.

ResultsThromboembolic events (1.8%) occurred in 10 patients who had baseline C-trough levels in the lowest class of drug levels. The incidence of thromboembolic eventsamong patients with DOAC C-trough results in the lowest level class was 2.4%, while it was 0% in the remaining groups. The patients with thrombotic complications also had a mean CHA_2DS_2-VASc score higher than the total patient population:5.3(4.3-6.3 95%-confidence interval-CI) vs 3.0 (2.9-3.1 95% CI).

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ConclusionInthis study cohort, thrombotic complications occurred only in DOAC-treated AF patients who hadvery low C-troughlevels, with relatively high CHA_2DS_2-VASc score. Larger studies are warranted to confirm these preliminary observations.

Keywords: atrial fibrillation, direct oral anticoagulants, coagulation test, cardiovascular risk, thromboembolism

INTRODUCTION

Direct oral anticoagulants (DOACs) have been introduced in clinical practice for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation (NVAF) and for the prevention and treatment of venous thromboembolism (VTE) [1]. Presently, available drugs include dabigatran, a selective anti-factor IIa molecule and three direct anti-factor Xa inhibitors: apixaban, edoxaban and rivaroxaban. All these agents have shown non-inferiority, and some of them even superiority, compared with vitamin K antagonists (VKAs) in terms of efficacy and safety in phase III clinical studies [2-6]. DOAC specific characteristics are the rapid onset of action, a short half-life, a predictable anticoagulant effect in standard conditions and low food/drug interactions. Thanks to their pharmacological profiles, this class of drugs is administered at fixed dose in relation to clinical indications, individual characteristics and renal function, without current indications for dose adjustment based on laboratory testing [7]. Nevertheless, a high inter-individual variability in the drug blood levels was shown with all DOACs and post-hoc analyses of phase III trials showed an association between DOAC plasma levels and thrombotic and bleeding complications during follow up [8-16].

More recently, some studies underlined the usefulness of DOAC measurements to address specific treatment approaches. Special clinical settings, such as on the occasion of bleeding or thromboembolic complications, surgery or invasive maneuvers, thrombolytic therapy in patients with acute stroke, drug to

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drug interactions may require DOAC measurement in plasma[17-19]. In addition, because a specific antidote for dabigatran is available and anti-Xa antidotes are expected to be soon introduced in clinical practice, DOAC plasma measurements are considered useful to ensure their appropriate administration to prevent over-use of these new expensive medications [20, 21]. Taking into account both the high inter-individual variability and the association between DOAC C-trough plasma levels and bleeding and thromboembolic complications, DOAC measurements at steady-state could represent an additional information useful to improve efficacy and safety of these drugs. Moreover, phase IV clinical trials have shown a higher inter-individual variability if compared with phase III studies, confirming that real world patients differ from the selected populations enrolled in randomized trials [12-16]. Moreover, only for dabigatran an attempt to define therapeutic range has been proposed [22, 23].

Aim of the present study, performed within the frame of activity of the START Laboratory Register, a branch of the START-Registry (Survey on anTicoagulatedpAtientsRegisTer) (NCT 02219984). [24], was to evaluate a possible relationship between DOAC C-trough anticoagulant levels, measured at steady state within the first month of treatment, and thromboembolic events observed for 1 year follow up.

Methods

Patients

This is an observational multicenter study in patients with NVAF treated with dabigatran, rivaroxaban or apixaban. It was conducted in four Anticoagulation Clinics (Ancona, Bologna, Cremona, Padova) affiliated with the Italian Federation of Anticoagulation Clinics (FCSA) and participating in the START-Registry [24]. DOACs have been introduced in Italy at different time from June 2013, and during the period of patient's enrolment the drugs available and reimbursed by the national health system were dabigatran, rivaroxaban and apixaban. After giving their informed consent, a total of 565 consecutive naive patients with NVAF,

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aged more than 18 years, seen at the anticoagulation clinics from January 1st 2015 to 31th December 2015, were enrolled in the study.

A total of 185 patients were on dabigatran (82 and 103 taking 150mg or 110mg twice-daily, respectively), 172 on rivaroxaban (100 and 72 taking 20mg or 15mg once-daily, respectively), and 208 on apixaban (154 and 54 taking 5mg or 2.5mg twice-daily, respectively). Patients were evaluated at enrolment and received type and dosage of DOACs based on clinical characteristics at the discretion of the attending physician, following recommendations issued by the Italian regulatory agency. All patients with a renal function, estimated by creatinine clearance (CrCl), calculated with Cockroft Gault formula, less than 30 ml/min/1.73 m^2, were excluded because not eligible for DOAC treatment.

Baseline characteristics (demographic, clinical, risk factors, CHA_2DS_2-VASc Score, HAS-BLED, weight, body mass index, kidney and liver function, concomitant medications) were recorded in a structured data base. Follow-up, as defined by FCSA guidelines, included clinical evaluation within the first month and every 3 months for one year. Patient's compliance and adherence to anticoagulant treatment was evaluated by manual counting pills at each visit.

All bleeding and thromboembolic complications were registered for one year follow up. In this study, we report data only on the relationship between DOAC levels and thromboembolic complications, while the analysis on DOAC C-trough levels and bleedings will be reported in a subsequent study.

Thromboembolic complications, such as stroke, transient ischemic attack (TIA), peripheral embolism, acute myocardial infarction (AMI), deep vein thrombosis and pulmonary embolism (DVT/PE), were recorded. Thrombotic complications were adjudicated by the local investigators on based on clinical signs and

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symptoms combined with objectively confirmed diagnostic radiology and laboratory tests (colour doppler ultrasounds, magnetic resonance imaging, computed tomography, electrocardiography, laboratory markers).

Laboratory assay

Plasma samples were collected within the first 15-25 days of treatment at C-trough level, obtained at 12 hours from the last dose intake for dabigatran and apixaban, and at 24 hours for rivaroxaban. 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 hour from collection at 2000g for 20 minutes and plasma was quickly frozen and stored at -80°C until testing. DOAC levels, expressed as drug concentration-equivalent (ng/ml), were measured using commercial specific coagulation tests that, when compared with liquid chromatography tandem mass spectrometry (LC-MS/MS) have previously demonstrated good performance. Diluted thrombin time (dTT) or anti-FIIa, calibrated for dabigatran, and specific anti-FXa assays calibrated for apixaban and rivaroxaban [25-27] were used to measure DOAC plasma levels. All tests were performed locally, within three months from plasma collection, using Stago, Hyphen and Siemens reagents on STA R (Stago, France) and CA 7000 (Siemens, Germany), according to manufacturer's indications as described in a previous study [28].

The limits of quantification (LOQ) were evaluated repeating a pooled normal plasma 10 times with each assay. Raw data (expressed as seconds or OD/min) were reported on the X-axis of the calibration curves to obtain Y0 values and LOQ was computed as follows: LOQ=Y0+10SD and LOQ=Y0-10SD for clotting and chromogenic assays, respectively.Measured DOAC concentrations below LOQ were substituted with the LOQ values.

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For each drug, the range of obtained measurements from LOQ to the highest value recorded was divided into 4 equal classes and the patient results were distributed among these classes, ranging from that with the lowest (class I) to that with the highest levels (class IV) (Figure 1).

Statistical analysis

Descriptive analysis was performed. Continuous variables are expressed as mean and standard deviation (SD), or median and range. Categorical variables are expressed as frequencies and percentages. The incidence of adverse events was calculated and given with 95% confidence interval (CI). The $p < 0.05$ was considered statistically significant. The SPSS software for Windows, version 22 (SPSS Inc, Chicago, IL) is used for data processing.

Ethics

The study protocol of START-Registry was approved by the local ethics committees and was conducted in accordance with the Declaration of Helsinki.

Results

Main clinical characteristics and DOAC levels, detailed for each drug, are shown in Table 1; 565 naïve NVAF patients, starting oral anticoagulant treatment with DOACs, were enrolled in the study from January 1st2015 to 31th December 2015 and followed-up for one year. No patient was lost at one year follow up.

Median age was 80 (from 44 to 97) years and was not different among patients treated with the 3 drugs. Males were 315 while female 250. Median CrCl was 69.0 (33-149). All patients showed normal liver function, as estimated by aspartate transaminase (AST) and alanine transaminase (ALT). Median CHA_2DS_2-VASc score was 3 (0-9), without significantly differences among the three drugs. Median (range) DOAC C-

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through plasma level was 82 ng/mL (36-324) in dabigatran-treated patients, 39 ng/mL (17-273) in rivaroxaban and 111 ng/mL (22-515) in apixaban.

Total inter-individual variability, expressed as overall CV%, was 64.4 for dabigatran, 86.4 for rivaroxaban and 58 for apixaban.

Adherence, evaluated through the manual counting of pills, resulted high, with an agreement greater than 90%, between consumed and expected doses for the three drugs. For one year follow up, 10 thromboembolic events were observed, with an incidence of 1.8% (95% CI 0.8-3.2) of the total population. Seven patients were males and 3 females, mean age(SD) was 81.2 (9.9) years, two of them were also treated with aspirin 100mg/day and amiodarone (Table 2). Five thrombotic complications (4 stroke and 1 acute myocardial infarction, AMI) occurred in patients treated with dabigatran; three events (2 AMI and 1 TIA) in patients on rivaroxaban and two events (1 DVT and 1 systemic embolism) in patients on apixaban. Mean (SD) CrCl was 63.7 (12.9) ml/min in patients with complications.Type and site of thrombosis and DOAC levels are detailed in Tables 1 and 2.

All the 10 thromboembolic complications (Table 2) occurred after the first 6 months of treatment in patients whose C-trough drug levels were in the lowest level class calculated for each drug (n. 412; Figure 1), with an incidence of 2.4% (95% CI 1.2-4.4) among the patients with results in that class; in 8 of these patients the drug level was below the median value (Figure 1). The 10 patients with thromboembolic complications had mean(SD)CHA_2DS_2-VASc score significantly higher if compared with the entire study population: 5.3(4.3-6.3 95% CI) vs 3.0 (2.9-3.1 95% CI);$p<0.0001$. The CHA_2DS_2-VASc score was > 3 in 291 out of the 565 investigated patients (51.5%) and, among them, 127 had drug levels within the lowest level

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class. The incidence of thromboembolic events in the latter patients was 7.9%, compared to 0% in patients with similar clinical risk profile but with drug measurement in the higher classes.

Discussion

DOACs have been shown safe and effective for the treatment of VTE and stroke prevention in NVAF when compared to VKAs. They offer advantages over VKAs because dose-adjustment by lab testing is not needed [1-5]. Nevertheless, thromboembolic and bleeding complications, accounting for nearly 3-4% patient-years respectively [6, 29], may occur during DOAC treatment.

The possibility that an insufficient anticoagulant effect can be associated to thromboembolic complications during DOAC treatment is suggested by some evidence. First, the relationship between C-trough levels and complications as shown by FDA reports on DOAC phase III clinical studies [8-10][[11]. Second, the high inter-individual variability reported both in phase III and IV clinical trials [12-16]. Third, case series showing this type of complications and relevance of drug measurements [30-32].

Pharmacological studies have demonstrated a sufficiently predictable DOAC anticoagulant effect in standard clinical conditions and in selected patient populations. However, in the general clinical practice, patients differ from those enrolled in clinical trials because they are older, mainly affected by comorbidities and often treated with several additional drugs. The high observed inter-individual variability may also be due to the above reasons [32]. As a consequence, the fixed dose calculated only on clinical characteristics and renal function may not always be the optimal choice for all patients [33].

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In this observational study, conducted on 565 patients treated with dabigatran, rivaroxaban or apixaban, we evaluated the occurrence of thromboembolic events for one year follow up.

All patients enrolled had CrCl>30 ml/min, nearly half of them had CHA_2DS_2-VASc score > 3.0 and 40.5% were treated with low dosages, as recommended by drug regulatory agencies based on their clinical characteristics. For all drugs, we confirmed the high inter-individual variability, even wider when compared to that recorded in randomized clinical trials, thus confirming that unselected patients, the so called "real world patients", are more complex and less homogeneous because of co-morbidities, co-therapies and age. During one year follow-up, 10 thromboembolic events were recorded, at a rate of1.8%; all the events occurred in patients whose C-trough measurement was in the lowest class of anticoagulation level and with high cardiovascular risk score. Our data seem to indicate that the combination of high cardiovascular risk with low anticoagulant levels may expose patients to a greater risk of thrombotic complications. In fact, the incidence of thrombotic complications in patients with CHA_2DS_2-VASc score > 3 and C-trough level in the lowest class was as high as 7.9%, whereas no complications were recorded in patients with high cardiovascular risk but higher anticoagulant levels.

Measurements have been performed at steady state during the first initial phase of treatment and all thromboembolic complications occurred after the first 6 months of treatment. Unfortunately, specific measurements at the time of acute event occurrence were not available.Problems with adherence to therapies, especially in the patients with low drug levels, cannot be excluded. Adherence, however, assessed by count of pills during the 3-month periodical follow up visits, was considered good, and we therefore consider it unlikely that the thrombotic complications were associated with persistently low drug plasma levels due to adherence.

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Current clinical indications exclude specific anticoagulant measurements for routine DOAC dose adjustment because: a) anticoagulant effect is considered predictable based on pharmacokinetic characteristics, and b) phase III clinical studies showed that fixed dosing proved effective and safe. However, while inter-individual variability was small in healthy individual or uncomplicated patients, as shown in phase II trials (overall CV was 16-40%) [34-36], this variability increases considerably in phase IV (post-marketing) studies [16], up to a CV > 80%, as shown like in this study population.

Only recently, needs for specific measurements have been suggested in special situations [17, 19, 21, 25, 37]. Though randomized clinical trials have shown efficacy and safety of DOACs without laboratory dose adjustments, some patients however have very low or high anticoagulant levels at steady state. Currently, the clinical significance of these extreme drug levels is still unknown. However, for each drug and clinical indication, it would be advisable to properly define specific therapeutic ranges that may be different from the biological inter-individual variability. Presently, only for dabigatran a therapeutic range has already been proposed [22, 23].

Very recently, it has been suggested that only a randomized prospective trial may answer the clinical issue about usefulness or not of routine DOAC measurements [37]. Unfortunately, we believe that such a trial will hardly be performed in the short term. In the meantime, and for pragmatic reason, we propose that the results of the present study should be kept into due consideration to improve patients' management, particularly for those at higher cardiovascular risk. In fact, according to previous observations [23], the measurement of DOACs could improve risk/benefit profile identifying poor responders [38]. This information could be crucial to reduce subsequent cardiovascular events.

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Different dabigatran therapeutic schemes have been proposed according to patient C-trough plasma levels [23] and prospective studies, with a similar target, are needed for all anti-Xadrugs. In our prospective investigation, only patients with low plasma levels (in the lowest level class) developed thrombotic events.

Limit of the present study, besides the relative small number of the patient population, is the observation, among thrombotic complications, of 3 AMI that may also be associated to other concomitant risk factors and not necessarily to the drug levels.

In conclusion, our data show a relationship between low C-trough DOAC levels and occurrence of thrombotic events in NVAF patients, and support the concept of assessing the anticoagulant levels at steady state as a tool to contribute to the efforts for a more effective and safer anticoagulation with DOACs, a target recently advocated [39].The relatively small number of patients enrolled represents a limit of the present study and these preliminary results need to be confirmed by larger and specifically designed clinical studies.

Addendum

A. S. Testa was responsible for the study design and manuscript preparation; O. Paoletti was responsible for patient identification and manuscript approval; C. Legnani was responsible for patient identification and data analysis; C. Dellanoce was responsible for patient identification and data analysis; E. Antonucci was responsible for data analysis; B. Cosmi, V, Pengo, D. Poli, A. Tripodi and G. Palareti were responsible for manuscript revision and approval; R. Morandini was responsible for acquisition of data; R. Testa was responsible for acquisition of data and manuscript approval.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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Table 1 Main clinical characteristics, thrombotic complications and DOAC plasma levels at C-trough for: all patients, patients with thrombotic complications and patients without thrombosis. Results are reported as median (min-max).

	Dabigatran	**Rivaroxaban**	**Apixaban**	**Total**
Patients (n)	185	172	208	565
Age (years)	78 (44-94)	82 (57-97)	80 (49-94)	80 (44-97)
Gender (M/F)	105/80	95/77	115/93	315/250
BMI	26.9 (17.4-43.3)	25.5 (16.6-34.7)	26.2 (16.4-40.1)	26.2 (16.4-43.3)
Drug daily dose **(n. of patients)**	2x150 mg (82) 2x110 mg (103)	20 mg (100) 15 mg (72)	2x5 mg (154) 2x2.5 mg (54)	---
Creatinine clearance ($mL/min/1.73m^2$)	70.5 (39-149)	66.5 (36-117)	69.0 (33-117)	69.0 (33-149)
CHA_2DS_2VASc	3 (0-7)	3 (0-7)	3 (0-9)	3 (0-9)
DOAC plasma levels (ng/ml)				
All patients	82 (36-324)	39 (17-273)	111 (22-515)	---
Patients with thrombosis	67 (36-91)	28 (23-39)	79 (45-113)	---
Patients without thrombosis	82 (36-324)	39 (17-273)	111 (22-515)	---
Thrombosis n (%)	5 (2.7) (4 Strokes, 1 AMI)	3 (1.7) (2 AMI, 1 TIA)	2 (1.0) (1 DVT, 1 Systemic Embolism)	10 (4 Strokes, 3 AMI, 1 TIA, 1 DVT, 1 Systemic Embolism)

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Table 2 Thromboembolic complications, CHA_2DS_2VASc score and DOAC C-trough levels

Pt	Drug	Posology (mg/die)	CHA_2DS_2VASc	ASA	Amiodaron	CrCl ($mL/min/1.73m^2$)	DOACs C-trough ng/ml	Thromboembolic Complication
1	Dabigatran	150mgx2	5	yes	yes	79	36	Stroke
2	Dabigatran	110mgx2	7	no	no	67	67	Stroke
3	Dabigatran	110mgx2	3	no	yes	53	53	Stroke
4	Dabigatran	110mgx2	4	no	no	67	78	Stroke
5	Dabigatran	150mgx2	7	no	no	76	91	AMI
6	Rivaroxaban	20mg	7	no	no	69	39	TIA
7	Rivaroxaban	15mg	5	no	no	56	23	AMI
8	Rivaroxaban	15mg	5	no	no	47	28	AMI
9	Apixaban	2.5mgx2	6	yes	no	44	113	Systemic Embolism
10	Apixaban	5x2mg	4	no	no	79	45	DVT

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Legend to Figure 1

Dabigatran, Rivaroxaban and Apixaban plasma levels distributed into the four classes of drug levels, calculated for each DOAC drug by dividing into 4 equal classes the results from the limit of quantification to the highest concentration. Patients with thromboembolic events are identified as filled circles.

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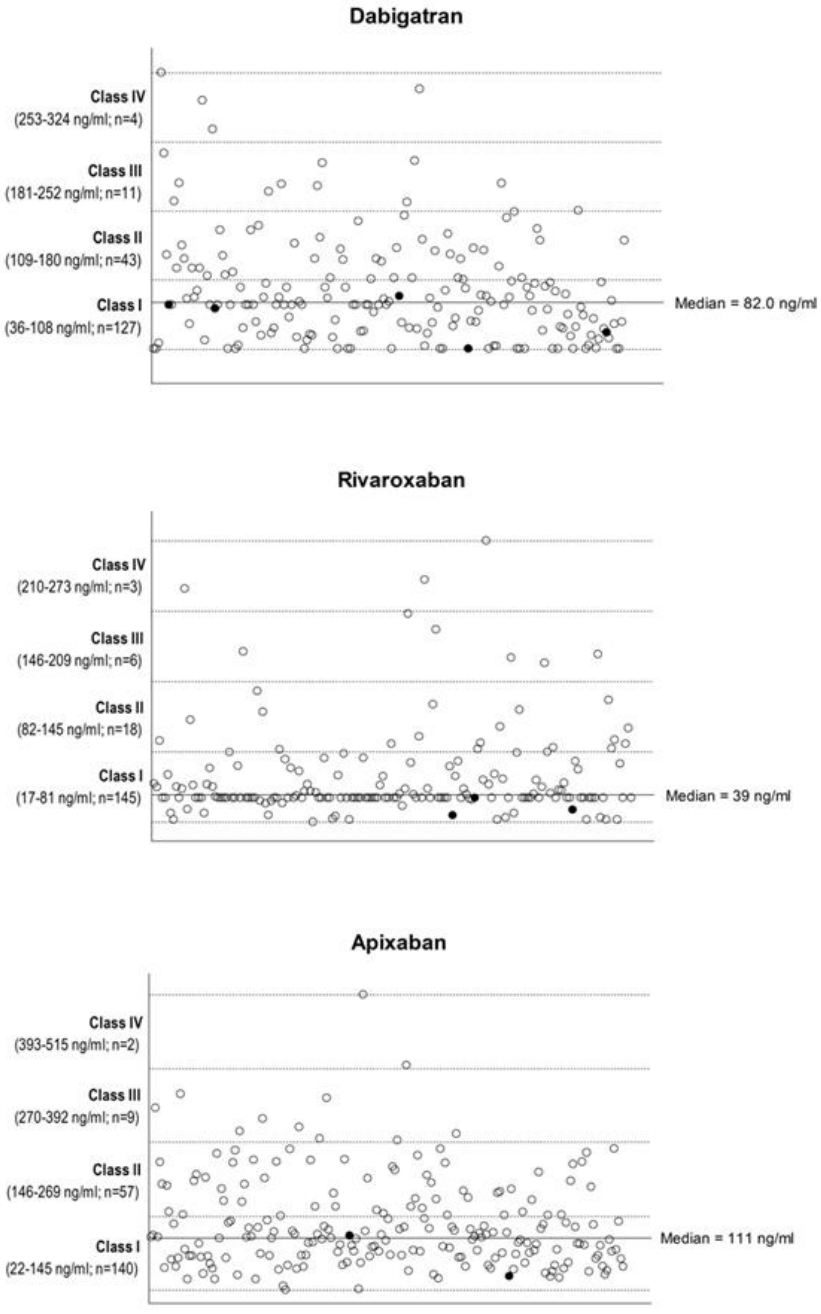
Figure 1



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