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ORIGINAL ARTICLE

Low drug levels and thrombotic complications in high-risk atrial fibrillation patients treated with direct oral anticoagulants

S. TESTA,* O. PAOLETTI,* C. LEGNANI,† C. DELLANOCE,* E. ANTONUCCI,‡ B. COSMI,† V. PENGO,§ D. POLI,¶ R. MORANDINI,* R. TESTA,** A. TRIPODI†† and G. PALARETI‡

**Hemostasis and Thrombosis Center, Ospedale di Cremona, Cremona; †Angiology and Blood Coagulation, University Hospital of Bologna; ‡Arianna Anticoagulazione Foundation, Bologna; §Department of Cardiac, Thoracic and Vascular Sciences, Cardiology Clinic, Thrombosis Center, University of Padua, Padua; ¶Thrombosis Center, AOU Careggi, Firenze; **Clinical Laboratory and Molecular Diagnosis, INRCA-IRCCS National Institute, Ancona; and ††Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS CàGranda Maggiore Hospital Foundation, Milano, Italy*

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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.

Summary. *Background:* Direct oral anticoagulants (DOACs) are administered at fixed doses without the need for dose adjustment according to laboratory testing. High interindividual variability in drug blood levels has been shown with all DOACs. To evaluate a possible relationship between DOAC C-trough anticoagulant levels and thromboembolic events, 565 consecutive naive patients with atrial fibrillation (AF) were enrolled in this study performed within the START Laboratory Registry. *Methods:* DOAC-specific measurements (diluted thrombin time or anti-activated factor II calibrated for dabigatran; anti-activated FX calibrated for rivaroxaban or apixaban) at C-trough were performed locally at steady state within 15–25 days after the start of treatment. For each DOAC, the interval of C-trough levels, from the limit of quantification to the highest value, was subdivided into four equal classes, and results were attributed to these classes; the median values of results were also calculated. Thromboembolic complications occurring during 1 year of follow-up were recorded. *Results:* Thromboembolic events (1.8%) occurred in 10 patients who had baseline C-trough levels in the lowest class of drug levels. The incidence of thromboembolic events among patients with DOAC C-trough levels in the lowest level class was 2.4%, and that in the remaining groups was 0%. The patients with thrombotic complications also had a higher mean CHA_2DS_2-VASc score than that of the total patient population: 5.3 (95% confidence interval [CI] 4.3–6.3 versus 3.0 (95% CI 2.9–3.1). *Conclusion:* In this study cohort, thrombotic complications occurred only in DOAC-treated AF patients who had very low C-trough levels, with a relatively high CHA_2DS_2-VASc score. Larger studies are warranted to confirm these preliminary observations.

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

Introduction

Direct oral anticoagulants (DOACs) have been introduced into 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]. At present, the available drugs include dabigatran, a selective anti-activated factor II (FIIa) molecule, and three direct anti-activated FX (FXa) inhibitors: apixaban, edoxaban, and

Correspondence: Sophie Testa, Hemostasis and Thrombosis Center, Ospedale di Cremona, Viale Concordia 1, 26100 Cremona, Italy
Tel.: +39 37 240 5453
E-mail: s.testa@asst-cremona.it

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rivaroxaban. All of these agents have shown non-inferiority, and some of them even superiority, as 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 a low level of food–drug interactions. Thanks to their pharmacological profiles, this class of drugs is administered at a fixed dose in relation to clinical indications, individual characteristics, and renal function, without current indications for dose adjustment based on laboratory testing [7]. Nevertheless, high interindividual variability in drug blood levels has been shown with all DOACs, and *post hoc* analyses of phase III trial results showed an association between DOAC plasma levels and thrombotic and bleeding complications during follow-up [8–16].

More recently, some studies have underlined the usefulness of DOAC measurements in addressing specific treatment approaches. Special clinical settings, such as bleeding or thromboembolic complications, surgery, invasive maneuvers, thrombolytic therapy in patients with acute stroke, and drug–drug interactions, may require DOAC measurement in plasma [17–19]. In addition, because a specific antidote for dabigatran is available and anti-FXa antidotes are expected to be introduced into clinical practice soon, DOAC plasma measurements are considered to be useful to ensure the appropriate administration of DOACs in order to prevent overuse of these new, expensive medications [20,21]. Taking into account both the high interindividual variability and the association between DOAC C-trough plasma levels and bleeding and thromboembolic complications, DOAC measurements at steady state could provide additional useful information to improve the efficacy and safety of these drugs. Moreover, phase IV clinical trials have shown higher interindividual variability as 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 has an attempt been made to define a therapeutic range [22,23].

The aim of the present study, performed within the framework of the START Laboratory Register, a branch of the START Register (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 during 1 year of follow-up.

Methods

Patients

This was an observational multicenter study in patients with NVAF treated with dabigatran, rivaroxaban, or apixaban. It was conducted in four anticoagulation clinics (Ancona, Bologna, Cremona, and 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 times since June 2013, and, during the period of patient enrollment, the drugs available and reimbursed by the national health system were dabigatran, rivaroxaban, and apixaban. After giving their informed consent, 565 consecutive naive patients with NVAF, aged > 18 years, seen at the anticoagulation clinics from 1 January 2015 to 31 December 2015, were enrolled in the study.

One hundred and eighty-five patients were taking dabigatran (82 and 103 taking 150 mg or 110 mg twice daily, respectively), 172 were taking rivaroxaban (100 and 72 taking 20 mg or 15 mg once daily, respectively), and 208 were taking apixaban (154 and 54 taking 5 mg or 2.5 mg twice daily, respectively). Patients were evaluated at enrollment, and received type and dosage of DOACs on the basis of clinical characteristics at the discretion of the attending physician, following recommendations issued by the Italian regulatory agency. All patients with renal function, estimated according to creatinine clearance (CrCl), calculated with the Cockroft–Gault formula, of less than 30 mL min^{-1} 1.73 m^{-2} were excluded because they were not eligible for DOAC treatment.

Baseline characteristics (demographic characteristics, clinical characteristics, risk factors, CHA_2DS_2-VASc score, HAS-BLED score, weight, body mass index, kidney and liver function, and concomitant medications) were recorded in a structured database. Follow-up, as defined by FCSA guidelines, included clinical evaluation within the first month and then every 3 months for 1 year. Patient compliance and adherence to anticoagulant treatment was evaluated by manual counting of pills at each visit.

All bleeding and thromboembolic complications were registered for 1 year of follow-up. In this study, we report data only on the relationship between DOAC levels and thromboembolic complications; the analysis of DOAC C-trough levels and bleeding will be reported in a subsequent article.

Thromboembolic complications, such as stroke, transient ischemic attack (TIA), peripheral embolism, acute myocardial infarction (AMI), deep vein thrombosis (DVT), and pulmonary embolism, were recorded. Thrombotic complications were adjudicated by the local investigators on the basis of clinical signs and symptoms combined with objectively confirmed diagnostic radiology and laboratory test results (colour Doppler ultrasound, magnetic resonance imaging, computed tomography, electrocardiography, and laboratory markers).

Laboratory assay

Plasma samples were collected within the first 15–25 days of treatment at C-trough level, obtained at 12 h from the last dose intake for dabigatran and apixaban, and at 24 h from the last dose intake for rivaroxaban. Plasma samples were collected in vacuum plastic tubes (Vacutainer; Becton

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Dickinson, Plymouth, UK) containing 3.2% trisodium citrate (9 : 1 v/v, 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 was performed. DOAC levels, expressed as drug concentration-equivalent (ng mL^{-1}), were measured with commercial specific coagulation tests that, when compared with liquid chromatography tandem mass spectrometry, have previously demonstrated good performance. Diluted thrombin time or anti-FIIa assays, calibrated for dabigatran, and specific anti-FXa assays, calibrated for apixaban and rivaroxaban [18,25,26], were used to measure DOAC plasma levels. All tests were performed locally, within 3 months from plasma collection, with Stago (Asnieres-sur-Seine, France), Hyphen (Neuilly-sur Oise, France) and Siemens (Marburg, Germany) reagents on an STA R (Stago) and a CA 7000 (Siemens), according to the manufacturer's instructions, as described in previously [27].

The limits of quantification (LOQs) were evaluated by retesting a pooled normal plasma 10 times with each assay. The limits of quantification (LOQ) were evaluated retesting a pooled normal plasma 10 times with each assay. Raw data, expressed as seconds or OD/min (Y1, Y2, ...) were used to calculate the SD (standard deviation). Then, raw data were transformed as follows: $Y1' = Y1 + 10SD$ ($Y2' = Y2 + 10SD, \ldots$) or $Y1' = Y1 - 10SD$ ($Y2' = Y2 - 10SD, \ldots$) for clotting and chromogenic assays, respectively. Each transformed raw data ($Y1'$, $Y2'$, ...) was used to calculate the drug concentration (ng/ml) on the calibration curves (X1, X2,....). The mean value of X1, X2, ... was used as LOQ for each drug. Measured DOAC concentrations below LOQ were substituted with the LOQ values.

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

Statistical analysis

Descriptive analysis was performed. Continuous variables are expressed as mean and SD, or median and range. Categorical variables are expressed as frequencies and percentages. The incidence of adverse events was calculated and is given with a 95% confidence interval (CI). A *P*-value of < 0.05 was considered to be statistically significant. SPSS for Windows version 22 (SPSS, Chicago, IL, USA) was used for data processing.

Ethics

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

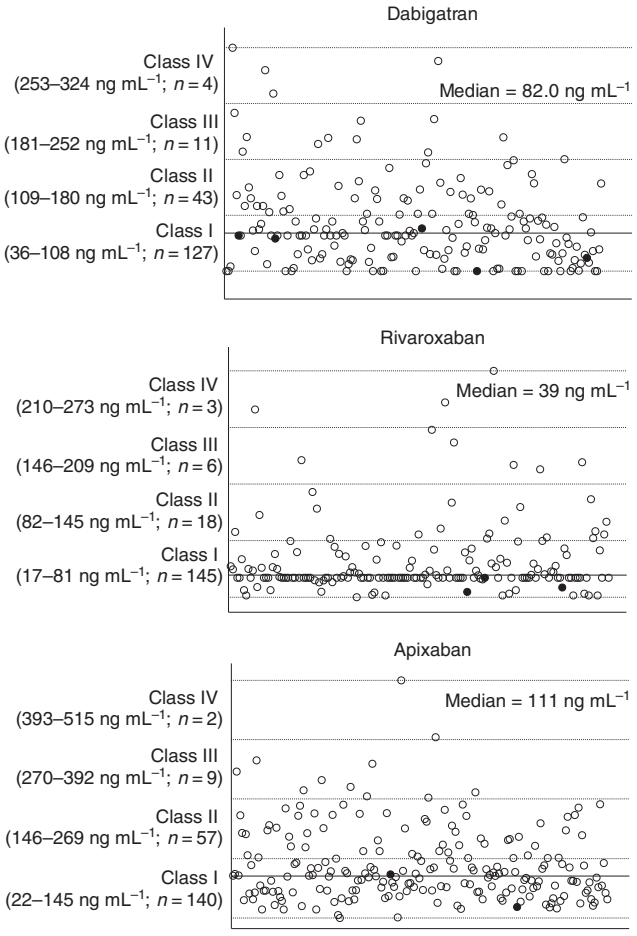

Fig. 1. Dabigatran, rivaroxaban and apixaban plasma levels distributed into the four classes of drug levels, calculated for each direct oral anticoagulant drug by dividing into four equal classes the results from the limit of quantification to the highest concentration. Patients with thromboembolic events are identified as filled circles.

Results

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

The median age was 80 years (range, 44–97 years) and was not different among patients treated with the three drugs. There were 315 males and 250 females. The median CrCl was 69.0 (range, 33–149 mL min^{-1} 1.73 m^{-2}). All patients showed normal liver function, according to aspartate transaminase and alanine transaminase measurements. The median CHA_2DS_2-VASc score was 3 (range, 0–9), without significant differences among patients treated with the three drugs. The median DOAC C-through plasma levels were 82 ng mL^{-1} (range, 36–324 ng mL^{-1}) in dabigatran-treated patients, 39 ng mL^{-1}

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Table 1 Main clinical characteristics, thrombotic complications and direct oral anticoagulant (DOAC) plasma levels at C-trough for: all patients, patients with thrombotic complications, and patients without thrombosis

	Dabigatran	Rivaroxaban	Apixaban	Total
Patients (*n*)	185	172	208	565
Age (years), median (range)	78 (44–94)	82 (57–97)	80 (49–94)	80 (44–97)
Gender (M/F), *n*	105/80	95/77	115/93	315/250
BMI, median (range)	26.9 (17.4–43.3)	25.5 (16.6–34.7)	26.2 (16.4–40.1)	26.2 (16.4–43.3)
Daily dose of drug (no. of patients)	2 × 150 mg (82) 2 × 110 mg (103)	20 mg (100) 15 mg (72)	2 × 5 mg (154) 2 × 2.5 mg (54)	–
Creatinine clearance (mL min^{-1} 1.73 m^{-2}), median (range)	70.5 (39–149)	66.5 (36–117)	69.0 (33–117)	69.0 (33–149)
CHA_2DS_2VASc score, median (range)	3 (0–7)	3 (0–7)	3 (0–9)	3 (0–9)
DOAC plasma levels (ng mL^{-1}), median (range)				
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)

BMI, body mass index; F, female; M, male.

(range, 17–273 ng mL^{-1}) in rivaroxaban-treated patients, and 111 ng mL^{-1} (range, 22–515 ng mL^{-1}) in apixaban-treated patients.

The total interindividual variability, expressed as overall percentage coefficient of variation (CV%), was 64.4 for dabigatran, 86.4 for rivaroxaban, and 58 for apixaban.

Adherence, evaluated by the manual counting of pills, was high, with agreement of > 90% between consumed and expected doses for the three drugs. During 1 year of 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 three were females; their mean age was 81.2 years (SD 9.9 years), and two of them were also treated with aspirin 100 mg daily and amiodarone (Table 2). Five thrombotic complications (four strokes and one AMI) occurred in patients treated with dabigatran; three events (two AMIs and one TIA) occurred in patients treated with rivaroxaban; and two events (one DVT and one systemic embolism) occurred in patients treated with apixaban. The mean CrCl was 63.7 mL min^{-1} (SD 12.9 mL min^{-1}) in patients with complications. Types and site of thrombosis and DOAC levels are shown in Tables 1 and 2.

All 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 (no. 412; Fig. 1), with an incidence of 2.4% (95% CI 1.2–4.4) among the patients with results in that class; in eight of these patients, the drug level was below the median value (Fig. 1). The 10 patients with thromboembolic complications had a mean CHA_2DS_2-VASc score that was significantly higher than that of the entire study population: 5.3 (95% CI 4.3–6.3) versus 3.0 (95% CI 2.9–3.1); $P < 0.0001$. The CHA_2DS_2-VASc score was > 3 in 291 of the 565 investigated patients (51.5%), and 127 of them had drug levels within the lowest level class. The incidence of thromboembolic events in the latter patients was 7.9%, as compared with 0% in patients with a similar clinical risk profile but with drug levels in the higher classes.

Table 2 Thromboembolic complications, CHA_2DS_2-VASc score, and direct oral anticoagulant (DOAC) C-trough levels

Patient	Drug	Daily dose	CHA_2DS_2-VASc score	ASA	Amiodarone	CrCl (mL min^{-1} 1.73 m^{-2})	DOAC C-trough (ng mL^{-1})	Thromboembolic complication
1	Dabigatran	150 mg × 2	5	Yes	Yes	79	36	Stroke
2	Dabigatran	110 mg × 2	7	No	No	67	67	Stroke
3	Dabigatran	110 mg × 2	3	No	Yes	53	53	Stroke
4	Dabigatran	110 mg × 2	4	No	No	67	78	Stroke
5	Dabigatran	150 mg × 2	7	No	No	76	91	AMI
6	Rivaroxaban	20 mg	7	No	No	69	39	TIA
7	Rivaroxaban	15 mg	5	No	No	56	23	AMI
8	Rivaroxaban	15 mg	5	No	No	47	28	AMI
9	Apixaban	2.5 mg × 2	6	Yes	No	44	113	Systemic embolism
10	Apixaban	5 × 2 mg	4	No	No	79	45	DVT

AMI, acute myocardial infarction; ASA; acetylsalicylic acid; CrCl, creatinine clearance; DVT, deep vein thrombosis; TIA, transient ischemic attack.

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Discussion

DOACs have been shown to be more safe and effective for the treatment of VTE and stroke prevention in NVAF than VKAs. They offer advantages over VKAs, because dose adjustment based on laboratory testing is not needed [1–5]. Nevertheless, thromboembolic and bleeding complications, thromboembolic and bleeding complications, accounting for nearly 3 and 4 × 100 patient-years respectively [6,28], may occur during DOAC treatment.

The possibility that an insufficient anticoagulant effect can be associated with thromboembolic complications during DOAC treatment is suggested by some evidence, as follows: first, the relationship between C-trough levels and complications as shown by Food and Drug Administration reports on DOAC phase III clinical studies [8–10,11]; second, the high interindividual variability reported in both phase III and phase IV clinical trials [12–16]; and third, case series showing these types of complication and the relevance of drug measurements [29–31].

Pharmacological studies have demonstrated a sufficiently predictable DOAC anticoagulant effect in standard clinical conditions and in selected patient populations. However, in general clinical practice, patients differ from those enrolled in clinical trials, because they are older, are mainly affected by comorbidities, and are often being treated with several additional drugs. These factors may also explain the high observed interindividual variability [31]. As a consequence, the fixed dose calculated only on the basis of clinical characteristics and renal function may not always be the optimal choice for all patients [32].

In this observational study, conducted on 565 patients treated with dabigatran, rivaroxaban, or apixaban, we evaluated the occurrence of thromboembolic events during 1 year of follow-up.

All patients enrolled had a CrCl of > 30 mL min^{-1}, nearly half of them had a CHA_2DS_2-VASc score of > 3.0, and 40.5% were treated with low doses, as recommended by drug regulatory agencies on the basis of their clinical characteristics. For all drugs, we confirmed the high interindividual variability, which was even higher than 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 comorbidities, cotherapies, and age. During 1 year of follow-up, 10 thromboembolic events were recorded, at a rate of 1.8%; all of the events occurred in patients whose C-trough measurement was in the lowest class of anticoagulation levels and who had a 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 a CHA_2DS_2-VASc score of > 3 and a 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 were performed at steady state during the 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. However, adherence, as assessed by counting of pills during the periodic 3-month follow-up visits, was considered to be good, and we therefore consider it unlikely that the thrombotic complications were associated with persistently low drug plasma levels resulting from lack of adherence.

Current clinical indications exclude specific anticoagulant measurements for routine DOAC dose adjustment, because: (i) the anticoagulant effect is considered to be predictable on the basis of pharmacokinetic characteristics; and (ii) phase III clinical studies have shown that fixed dosing is effective and safe. However, whereas interindividual variability is small among healthy individuals or uncomplicated patients, as shown in phase II trials (overall CV% of 16–40%) [33–35], this variability increases considerably in phase IV (postmarketing) studies [16], up to a CV% of > 80%, as shown in this study population.

Only recently has the need for specific measurements in special situations been suggested [17–19,21,36]. Although randomized clinical trials have shown efficacy and safety of DOACs without laboratory dose adjustments, some patients 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, which may be different from the biological interindividual variability. Presently, only for dabigatran has a therapeutic range already been proposed [22,23].

Very recently, it has been suggested that only a randomized prospective trial could answer the clinical question regarding the usefulness or otherwise of routine DOAC measurements [36]. Unfortunately, we believe that it is very unlikely that such a trial will be performed in the short term. In the meantime, and for pragmatic reasons, we propose that the results of the present study should be taken into due consideration to improve patient management, particularly for those at higher cardiovascular risk. In fact, according to previous observations [23], the measurement of DOACs could improve the risk/benefit profile by identifying poor responders [37]. This information could be crucial for reducing the risk of subsequent cardiovascular events.

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-FXa drugs. In our prospective

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investigation, only patients with low plasma levels (in the lowest level class) developed thrombotic events.

A limitation of the present study, besides the relatively small number of patients, is the observation of, among thrombotic complications, three AMIs that may also be associated with other concomitant risk factors and not necessarily with the drug levels.

In conclusion, our data show a relationship between low C-trough DOAC levels and the 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 efforts to achieve more effective and safer anticoagulation with DOACs, a target that has recently been advocated [38]. 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 and C. Dellanoce were 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 the acquisition of data. R. Testa was responsible for the 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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