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MORE EARLY BLEEDS ASSOCIATED WITH HIGH BASELINE DIRECT ORAL ANTICOAGULANT LEVELS IN ATRIAL FIBRILLATION: THE MAS STUDY

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Abstract:
Treatment with direct oral anticoagulants (DOAC) in atrial fibrillation (AF) patients is effective and safe. However, bleeding complications still occur. Whether the measurement of DOAC levels may further improve treatment efficacy and safety is still an open issue. In the "Measure and See" (MAS) Study (#NCT03803579) venous blood was collected 15-30 days after DOAC initiation in AF patients who were then followed for one year to record the occurrence of major and clinically relevant non-major bleeding. DOAC plasma levels were measured in one laboratory, and results were kept blind to patients and treating doctors. Trough DOAC levels were assessed in 1657 patients [957 (57.7%) and 700 treated with standard and low-dose, respectively]. Fifty bleeding events were recorded during 1606 years of follow-up (3.11% pt/yrs). Fifteen bleeding events (4.97% pt/yrs) occurred in patients with C-trough standardized values in the highest activity class (> 0.50); whereas 35 events (2.69% pt/yrs) occurred in those with values in the two lower classes (□ 0.50, p= 0.0401). Increasing DOAC levels and low-dose DOAC use were associated with increased bleeding risk in the first three months of treatment. 19% of patients receiving low doses had standardized activity values in the highest class. More bleeding occurred in patients treated with low (4.3% pt/yrs) than standard (2.2% pt/yrs; p= 0.0160) dose DOAC. Early measurement of DOAC levels in AF patients identified many subjects with high activity levels despite the low doses use and had more bleeding risk during the first 3 months of treatment.

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Data Sharing Statement

Raw data and scripts used for analysis are available upon request to the Authors at osf.io (https://osf.io, Center for Open Science, Charlottesville, VA). For original data, please contact c.legnani@fondazionearianna.org

Short Title

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ABSTRACT

Treatment with direct oral anticoagulants (DOAC) in atrial fibrillation (AF) patients is effective and safe. However, bleeding complications still occur. Whether the measurement of DOAC levels may further improve treatment efficacy and safety is still an open issue. In the "Measure and See" (MAS) Study (#NCT03803579) venous blood was collected 15-30 days after DOAC initiation in AF patients who were then followed for one year to record the occurrence of major and clinically relevant non-major bleeding. DOAC plasma levels were measured in one laboratory, and results were kept blind to patients and treating doctors. Trough DOAC levels were assessed in 1657 patients [957 (57.7%) and 700 treated with standard and low-dose, respectively]. Fifty bleeding events were recorded during 1606 years of follow-up (3.11% pt/yrs). Fifteen bleeding events (4.97% pt/yrs) occurred in patients with C-trough standardized values in the highest activity class (> 0.50); whereas 35 events (2.69% pt/yrs) occurred in those with values in the two lower classes (≤ 0.50, p= 0.0401). Increasing DOAC levels and low-dose DOAC use were associated with increased bleeding risk in the first three months of treatment. 19% of patients receiving low doses had standardized activity values in the highest class. More bleeding occurred in patients treated with low (4.3% pt/yrs) than standard (2.2% pt/yrs; p= 0.0160) dose DOAC. Early measurement of DOAC levels in AF patients identified many subjects with high activity levels despite the low doses use and had more bleeding risk during the first 3 months of treatment.

KEY POINTS

- A relationship between high baseline DOAC levels and early bleeding events in one-year follow-up was found.
- Early measurement allows to identify subjects with high DOAC levels and, hopefully, to adjust treatment to reduce bleeding events.

INTRODUCTION

Clinical trials and clinical practice data confirmed the efficacy and safety of direct oral anticoagulants (DOACs) for stroke prevention in patients with non-valvular atrial fibrillation (AF) [1-8]. Metaanalysis studies showed that DOAC, compared to warfarin, had lower rates of stroke or systemic embolism, and comparable rates of major bleeding complications (MB)[9], with the advantage of a lower incidence of intracranial hemorrhages (ICH) but a higher risk of gastrointestinal bleeding. As such, the risk of bleeding in DOAC-treated AF patients is still a relevant factor potentially limiting a wider use of anticoagulation in these patients, particularly important for elderly patients, who are the most prevalent setting of AF patients and who have the higher baseline risk of bleeding. Therefore, the action to improve the clinical management of DOAC-treated patients to further reduce the risk of bleeding complications during treatment is to be pursued.

The MAS study (Measure And See) was designed to investigate the possible relationship between plasma DOAC levels, measured at the beginning (at steady state) of treatment in AF patients, and the subsequent occurrence of thrombotic and bleeding complications. The results related to thrombotic events have recently been published [10]. In that report, it was shown that most thrombotic complications recorded in one-year follow-up occurred in patients whose standardized activity levels were in the lowest class. The present article aims to analyze the MAS study results regarding the relationship between the measured baseline DOAC plasma levels and the occurrence of bleeding events in one-year follow-up recorded in the investigated AF patients.

MATERIAL AND METHODS

As detailed elsewhere [10], the MAS (NCT03803579) is an observational, prospective cohort, multicenter study of patients with AF who started treatment with a DOAC (apixaban, dabigatran, edoxaban, or rivaroxaban). The study was promoted and funded by the “Arianna Anticoagulazione” Foundation (Bologna, Italy) and conducted in Anticoagulation Clinics affiliated with the Italian Federation of Anticoagulation Centers (FCSA).

Patient population

Patients with non-valvular AF, aged over 18 years, who had initiated a DOAC treatment within one month, were enrolled in the study between 27 August 2018 and 10 November 2022. Patients without indication for electrical cardioversion, who did not have other indications for

anticoagulant therapy, who agreed to have blood sampling and accepted a follow-up for at least one year, were included in the study after signing a written informed consent. The choice of the DOAC drug and dose was left to the discretion of treating physicians.

A code was given to each participant center, and an anonymous identifying code was given to each patient, used to collect clinical information, to identify plasma samples and to record the results of DOAC level measurements. The following baseline characteristics were recorded in a specific electronic database: patient identification number, date of birth, gender, type and dose of DOAC used, weight, body mass index, kidney function [estimated by creatinine clearance (CrCl) according to the Cockroft-Gault formula], liver function (assessed by aspartate aminotransferase and alanine aminotransferase), diabetes, CHA_2DS_2-VASc score, previous stroke/TIA, other comorbidities, concomitant medications (with special attention to antiplatelet drugs). Data were stored in the database located at a section of Aruba cloud rented by the Arianna Anticoagulazione Foundation, which guaranteed storage, backup, and maintenance of the database.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Independent review board approval was obtained prior to all study-related activity from the Ethics Committee (EC) of the coordinating center (Cremona) (approval number 14725; 02/05/2018) and from the ECs of all other centers. The promoter of the study provided the measures to safeguard the subject's privacy and the protection of personal data according to the EU GDPR 2016/679 and Italian law.

Blood sampling and DOAC measurement

All patients had venous blood sampled at a steady state (within the first 2-4 weeks of initiation of treatment) immediately before the subsequent intake of the drug (C-trough). The participating centers could decide whether to collect an additional blood sample on the same day, 2 hours after the last drug intake (C-peak). Blood samples were also used to perform ancillary laboratory tests (including blood cell count, CrCl, and liver enzymes). Blood samples for DOAC measurement were collected in vacuum tubes (Vacutainer; Franklin Lakes, NJ, USA) containing 3.2% trisodium citrate (9:1 v/v, blood/anticoagulant). Blood was centrifuged within 1 h of collection at 2000-x g for 20 min [11], and plasma samples were aliquoted (0.5 mL) in cryovials, identified locally to maintain the patient's anonymity. The vials were then frozen and stored in the freezer (-80°C) [11] at the participating centers and later centralized at the biobank of the Arianna Anticoagulazione Foundation (Bologna). Finally, the aliquots were transferred to the Hemostasis

and Thrombosis Center of Cremona Hospital, where the DOAC measurements were performed. Shipment of plasma samples was carried out by express courier in dry ice.

DOAC levels, expressed as drug concentration-equivalent (ng/mL), were measured by chromogenic assays using STA-ECA II (DiagnosticaStago, Asnieres-sur-Seine, France) for dabigatran, and STA-Liquid anti-Xa (DiagnosticaStago) for apixaban, edoxaban, and rivaroxaban [12-14]; hemolyzed samples were discarded and not tested. Tests were calibrated using commercial plasmas with certified DOAC concentration as supplied by the same manufacturer and performed on STA-R Max instrument (Diagnostica Stago). The results of DOAC levels, identified with the patient identity code, were transmitted to the central database repository, and were not communicated to patients, participating centers, or attending physicians. The few patients who changed the drug or dose during follow-up were censored when the treatment was changed.

Follow-up and outcomes

The clinical follow-up was organized by the participating centers following the guidelines defined by FCSA, including a clinical evaluation within the first month of treatment and a clinical check-up every 3 to 4 months for one year. All thromboembolic and bleeding complications, death, and other events were recorded during the 12-month follow-up.

The present report analyzes the data regarding the relationship between DOAC levels and the occurrence of bleeding complications during follow-up. Predefined study outcomes were the major bleeding (MB), defined as to the International Society for Thrombosis and Haemostasis [15], and clinically relevant non-major bleeding (CRNMB) [16]. An independent Adjudication Committee evaluated the adverse events occurring during follow–up.

Statistical analysis

Sample size. Was calculated for thrombotic complications (detailed elsewhere) [10].

Analysis plan. Since the absolute DOAC plasma concentrations at trough and peak are drug and dose-dependent, the measured absolute values were standardized by subtracting from the original values the mean value of all results of each DOAC divided by the standard deviation. Standardized values represent the distance of each value from the drug mean distribution and may be, therefore, pooled to evaluate the effect of drug levels, irrespective of the DOAC type and administration (i.e., once- or twice-in-day).

The outcome incidence rates were computed for all patients with at least one measured

plasma DOAC concentration (C-trough or C-peak). Patients were censored at the end of the study, after the occurrence of a qualifying clinical event, when the initial anticoagulant treatment was stopped or modified when they moved to another clinical center or were lost to follow-up.

For the primary analysis, we assumed that the bleeding risk after the inception of anticoagulant therapy is not constant over time, but it is higher in the first months and declines in the following period [17]. We, therefore, stratified all observations in two different time strata, 1-3 months and >3 months, to allow for possibly time-varying hazards or incidence rates. We used a time-stratified Cox regression model to model the occurrence of the study outcomes as a function of C-trough or C-peak standardized DOAC levels. The regression model included as possible confounders the HAS-BLED score, body mass index, creatinine clearance estimated by the Cockcroft and Gault method, concomitant antiplatelet use, low-dose DOAC, and enrolling Center.

As an exploratory analysis, we subsequently evaluated the incidence of bleeding events stratified by standardized values divided into three categories ($\leq$ -0.5, representing low DOAC levels; -0.49 - 0.5, representing intermediate DOAC levels; and > 0.5, representing high DOAC levels). Data were analyzed with the use of Prism software (Version 9.3.1, GraphPad Software Incorporated, San Diego, CA) and SPSS software (version 11.0 SPSS Inc., IBM, Armonk, NY), and R (version 4.3.1, R Foundation for Statistical Computing, Vienna).

Independent review board approval was obtained prior to all study-related activity from the Ethics Committee (EC) of the coordinating center (Cremona) (approval number 14725; 02/05/2018) and from the ECs of all other centers.

RESULTS

Characteristics of patient population

The flow chart of the study population is shown in Figure 1. A total of 1718 patients, who started a DOAC treatment for non-valvular AF, were included in the study. After the exclusions (detailed in Figure 1), 1657 patients had blood sampling for DOAC levels measurement performed 15 to 30 days from the start of treatment (steady state). The clinical history for one year follow-up was collected in 1345 patients and for a shorter period for the remaining patients: 139 because the study was stopped before one-year follow-up for these patients; 173 patients were censored for the occurrence of thrombotic (21) or bleeding complications (50), 37 for death for other causes, 64 for other reasons, detailed in Figure 1. The main demographic and clinical characteristics of the 1657 investigated patients are shown in Table 1. The DOAC drugs used to treat the patients, with the proportions of those using standard [n. 957 (57.8%) or low n. 700 (42.2%)] dose, are shown in the Table. The appropriateness of low and standard-dose prescriptions by the treating physicians was analyzed according to the criteria reported by Steffel et al. [18]. In total, the dose was appropriate in 1441 subjects (87%); an inappropriate standard dose was calculated in 76 (4.6%) patients (6 apixaban, 57 edoxaban, 13 rivaroxaban), whereas a low dose was inappropriate in 140 (8.4%) patients (82 apixaban, 31 edoxaban, 27 rivaroxaban) (Table 1).

Plasma samples for DOAC measurement were available in all patients at C-trough, and in 1303 patients at C-peak. Results [mean±standard deviation and min-max] of DOAC levels, at C-trough and at C-peak, are shown in Supplemental Table S1.

DOAC activity levels and bleeding events during follow-up

During a total follow-up of 1606 years, bleeding outcomes occurred in 50 patients (30 MB and 20 CRNMB), with an incidence of 3.1% pt/yrs. Table 2 shows some characteristics, types of treatment, bleeding events, and DOAC measurement results of the patients who bled. The MB events were 12 gastrointestinal bleeding, 5 intracranial (1 fatal), 9 hemoglobin falls > 2 g/dL, and 4 in various sites (2 fatal). The CRNMB were: 7 epistaxis, 4 gastrointestinal, 4 intramuscular, and 5 in different sites. Eighteen of the 30 MB and 11 of the 20 CRNMBs (29/50; 58%) occurred in patients treated with low-dose DOACs. Among the patients who had bleeding the dosing was evaluated as

inappropriate in 2 (2.6%) patients among those receiving inappropriate standard dose, and in 10 (7.1%) among those treated with inappropriate low dose.

The baseline characteristics of patients included in the study (with or without bleeding events during follow-up) are shown in Supplemental Table S2

. Multivariate analysis (Table 3) showed that increasing DOAC C-trough levels and use of low-dose DOACs were both independently associated with increased bleeding risk in the first three months, but not in the subsequent study period. None of the others considered patients' characteristics were associated with the study's bleeding outcomes.

Using standardized C-trough and C-peak values it was possible to distribute the patients in three activity classes: at low (≤0.50), intermediate (-0.49 to 50), or high (> 0.50) activity. As shown in Table 4, at C-trough (Panel A) the incidence of bleeding was 4.97% pt/yrs (95%CI 2.8-8.2) in the highest DOAC level class and 2.69% pt/yrs (95%CI 1.9-3.7) in the lower classes (p= 0.0401). The incidence of bleeding was not statistically different between the three standardized activity classes at C-peak (Panel B). Figure 2 shows the incidence of bleeding, stratified by study period, for the three above classes. From the figure, it appears evident that the significant association between C-trough levels and bleeding events is mainly driven by the sharp increase in bleeding incidence in patients showing standardized C-trough values above 0.5. Altogether, 46 deaths were recorded (2.8%), three of whom were related to bleeding complications (Supplemental Table S3).

As shown in Table 5, 21 bleeding events occurred in patients treated with standard dose DOAC (2.2% pt/yrs) and 29 in subjects receiving low dose (4.3% pt/yrs; p= 0.0160), without significant differences whether the dose was appropriate or inappropriate. Although treated with low dose DOAC, 133 patients had standardized C-trough values in the highest-value class and had the highest rate of bleeding events (8.3% pt/yrs).

DISCUSSION

In the MAS study, which involved 1657 AF patients treated with one of the four available DOACs, blood was sampled within 2-4 weeks of initiation of DOAC treatment. Patients were then prospectively followed to record all thrombotic, bleeding, and other complications occurring in the subsequent one-year follow-up. During follow-up, DOAC activity levels in the collected plasma samples were measured in one laboratory (coordinating study center); the test results were kept blind to patients and attending physicians and merged with the correspondent patients only at the end of the study. The original measured values were also converted into drug/dosage standardized values to allow a pooled analysis. The present article analyzes the relationship between the measured DOAC levels and occurrence of bleeding complications.

An important result of our study was that the incidence of bleeding complications during the first three months of treatment was significantly higher (p= 0.0401) in patients with standardized C-trough values in the highest activity class compared to that in the lower value classes; after three months the difference was no longer statistically significant. In particular, about 30% of all recorded bleeding events occurred in patients with standardized levels in the highest DOAC plasma level class. Another relevant result is that more than half of all bleeding complications occurred in subjects who were treated with low-dose DOAC. This finding was in part expected since the patients with conditions exposing to higher risk of drug accumulation and bleeding preferably receive low-dose treatment. However, the use of low dose could not substantially prevent the bleeding risk but, what is more, it did not always avoid high levels of plasma DOAC concentration as found in 19% of subjects treated in this way.

In line with the registration trials, DOACs are administered to AF patients at fixed doses, based on patients' characteristics, such as age, comorbidity, body weight, renal function, and associated interfering drugs, without dose-adjustment based on measured DOAC concentrations. However, a high inter-individual variability of DOAC levels has been shown for all DOACs and all doses used [19-25]. Our results show that adoption of low-dose treatment cannot guarantee to avoid too high DOAC activity levels, thus predisposing to higher bleeding risk during the first three months of therapy, a period at higher risk of bleeding with oral anticoagulants [17]. Interestingly, after the first trimester, the risk of bleeding events was not associated with either baseline DOAC concentration or use of low-dose treatment, suggesting that other factors than the levels of anticoagulant activity may explain long-term bleeding in arguably frail patients.

The measurement of DOAC activity has so far been recommended only in particular situations, such as bleeding or thrombotic complications, before urgent need of surgery or invasive procedures, use of antidotes, and also suggested in special patient populations, such as those with frailty, under or overweight, or using highly interfering concomitant drugs [26-28]. The MAS study found [10] that an early detection of low activity DOAC levels was associated with higher risk of subsequent thrombotic complications and in the present report it is found that high activity levels at baseline are associated with occurrence of bleeding events during the first three months of treatment. We are aware that studies have indicated that empirical dose changes may be associated with worse outcomes [29-33]. Based on our results, however, it seems reasonable to consider further investigation determine whether the use of DOAC activity levels could guide safer DOAC treatment.

Limitations

The present report of the MAS study results on bleeding events has the same limitations declared in our previous article on thrombotic complications. In addition, we acknowledge that the DOAC levels, measured only once in our study (at steady state after the initiation of treatment), may have changed for various conditions and new risk factors may have occurred during follow-up thus increasing the risk of bleeding. Furthermore, we recognize that the finding of high baseline DOAC levels is associated with an increasing risk of bleeding limited only to the first few months of treatment; as such, confirming that the risk of bleeding complications during an anticoagulant treatment is multicausal.

The prospective, observational, and multicentre design, the centralization of DOAC measurement tests, and the blindness of all results to patients and treating physicians for one year follow-up are strengths of the study.

Conclusion

Our results show a relationship between high DOAC levels measured at steady state in AF patients and early occurrence of bleeding events during follow-up; furthermore, they show that treatment with low-dose DOAC does not always allows avoiding high levels of drug activity, thus exposing the patients to a higher bleeding risk. Together with the results of our previous study [10], which focused on thrombotic complications, we supposethat measuring anticoagulant levels at the beginning of DOAC treatment, in special settings of AF patients, especially in those prescribed with low dose treatment, might contribute to avoid a persistent too high or too low DOAC activity

levels and, possibly, to reduce the rate of bleeding or thrombotic events. However, before influencing clinical practice, our results need to be confirmed and expanded with studies in which DOAC treatment in AF patients is assessed at steady state and dose adjusted, if needed, according to the measured levels.

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Authorship contributions

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Conflict of Interest Disclosures

The authors declare no conflicts of interest related to the present study.

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Table 1 Baseline characteristics of included patients.

Patients, n	1657
Participating centres, n	27
Age, median (min-max), years	80 (47-100)
Males, n (%)	896 (54.1)
BMI, median (min-max)	26.2 (14.9-68.1)
Haemoglobin, median (min-max), (g/dL)	13.3 (8.0-18.8)
Platelets, median (min-max), ($X10^3/\mu L$)	218 (52-700)
Creatinine clearance, median (min-max), mL/min	58.0 (13-246)
History of cerebrovascular ischemic disease/ peripheral arterial emboli, n (%)	186 (11.2)
History of cardiovascular disease, n (%)	284 (17.1)
History of gastrointestinal bleeding, n (%)	22 (1.3)
History of cancer, n (%)	227 (13.7)
Hypertension, n (%)	1472 (88.8)
Diabetes, n (%)	375 (22.6)
Liver cirrhosis, n (%)	14 (0.8)
Chronic kidney disease, n (%)	197 (11.9)
Hypothyroidism/Hyperthyroidism, n (%)	165 (10.0)/62 (3.7)
Smokers, n (%)	190 (11.5)
Alcohol intake, n (%)	58 (3.5)
Mental disorders, n (%)	52 (3.1)
Family/social support, n (%)	1410 (85.1)
Drug daily dose, n (%)	
- Apixaban [Standard dose] [Low dose]	521 (31.5) [336 (65.5)] [185 (35.5)]
- Dabigatran [Standard dose] [Low dose]	221 (13.3) [100 (45.3)] [121 (54.7)]
- Edoxaban [Standard dose] [Low dose]	583 (35.2) [283 (48.6)] [300 (51.4]
- Rivaroxaban [Standard dose] [Low dose]	332 (20.0) [238 (71.7)] [94 (28.3)]
Prescribing accuracy of DOACs[19]:	
- Appropriate, n (%)	1441 (87.0)
- Inappropriate low dose	140 (8.4)
- Inappropriate standard dose	76 (4.6)
Prior AVK treatment, n (%)	512 (30.9)
Use of antiplatelet drugs, n (%)	382 (23.0)
Number of associated drugs, median (min-max)	3 (0-9)
- Antihypertensives, n (%)	933 (56.3)
- Antiarrhythmics, n (%)	695 (41.9)
- Gastroprotectors, n (%)	655 (39.5)
- Antidyslipidemics, n (%)	585 (35.3)
- Thyroid disease drugs, n (%)	210 (12.7)
- Anxiolytics, n (%)	175 (10.6)
- Psychotropics, n (%)	137 (8.3)
- Painkillers, n (%)	67 (4.0)
- Steroids, n (%)	47 (2.8)
- Antiepileptic drugs, n (%)	28 (1.7)
- Nitrates, n (%)	13 (0.8)
- Immunosuppressants, n (%)	11 (0.7)
- Antivirals, n (%)	7 (0.4)

Polytherapy ≥3, n (%)	1196 (72.2)
CHA_2DS_2VASc score, median (min-max)	4 (0-8)
CHA_2DS_2VASc score ≥4, n (%)	1072 (64.7)
HAS-BLED in all patients, median (min-max)	3 (0-6)
HAS-BLED score ≥3, n (%)	996 (60.1)

BMI: Body-Mass Index; DOAC: direct oral anticoagulants; VKA: vitamin K antagonist

Table 2 Details of all patients with bleeding outcomes.

Sex/Age	Type of bleeding outcome	DOAC dose	Inappropriate DOAC prescription	C-trough level (ng/mL)	C-peak level (ng/mL)	History of bleeding	HAS-BLED score
Major bleeding							
M/86	Gastrointestinal	Apixaban 2.5 mg/BID	No	337	NA	No	3
M/93	Retroperitoneal (Fatal)	Apixaban 2.5 mg/BID	No	218	369	No	4
F/86	Hemoglobin fall	Apixaban 2.5 mg/BID	Yes	206	415	No	4
F/77	Hemoglobin fall	Apixaban 2.5 mg/BID	Yes	175	349	No	3
F/70	Intracranial	Apixaban 2.5 mg/BID	Yes	164	155	Yes	5
M/88	Gastrointestinal	Apixaban 2.5 mg/BID	Yes	106	115	Yes	4
M/88	Gastrointestinal	Apixaban 2.5 mg/BID	Yes	87	221	No	2
F/76	Hemoglobin fall	Apixaban 2.5 mg/BID	Yes	47	213	No	2
M/90	Hemoglobin fall	Apixaban 2.5 mg/BID	Yes	44	79	No	4
M/73	Hemoglobin fall	Apixaban 5mg/BID	No	235	308	No	3
M/81	Intracranial	Apixaban 5mg/BID	Yes	120	187	No	3
M/83	Gastrointestinal	Apixaban 5mg/BID	No	109	102	No	3
M/76	Gastrointestinal	Apixaban 5mg/BID	No	55	NA	No	2
M/84	Gastrointestinal	Dabigatran 110 mg/BID	No	143	299	No	2
F/84	Gastrointestinal	Edoxaban 30 mg	No	169	312	No	4
F/83	Intracranial (Fatal)	Edoxaban 30 mg	No	147	NA	No	3
F/83	Hemoglobin fall	Edoxaban 30 mg	No	87	196	No	3
F/83	Intracranial	Edoxaban 30 mg	No	23	207	No	5
M/69	Gastrointestinal	Edoxaban 30 mg	Yes	10	85	No	2
F/83	Hematuria in K (Fatal)	Edoxaban 30 mg	No	8	159	No	4
M/85	Gastrointestinal	Edoxaban 60 mg	No	65	NA	No	1
M/74	Intraocular	Edoxaban 60 mg	No	30	381	No	1
M/65	Intracranial	Edoxaban 60 mg	No	25	254	No	1
M/80	Hemoglobin fall	Edoxaban 60 mg	No	25	23	No	2
M/80	Intraarticular	Edoxaban 60 mg	No	24	422	Yes	3

M/78	Gastrointestinal	Rivaroxaban 15 mg	No	57	NA	No	3
M/87	Hemoglobin fall	Rivaroxaban 15 mg	No	21	231	No	4
M/71	Gastrointestinal	Rivaroxaban 20 mg	No	58	NA	No	2
M/60	Hemoglobin fall	Rivaroxaban 20 mg	No	30	205	No	1
F/74	Gastrointestinal	Rivaroxaban 20 mg	No	26	NA	No	2
Not major clinical relevant bleeding							
F/82	Vaginal	Apixaban 2.5 mg/BID	No	134	276	No	4
F/88	Intramuscular	Apixaban 2.5 mg/BID	Yes	127	NA	No	3
F/85	Gastrointestinal	Apixaban 2.5 mg/BID	No	84	186	No	3
M/74	Gastrointestinal	Apixaban 5mg/BID	No	72	154	No	3
M/81	Hematuria	Apixaban 5mg/BID	No	43	92	No	2
M/71	Intramuscular	Dabigatran 150 mg/BID	No	136	200	No	2
M/79	Epistaxis	Dabigatran 150 mg/BID	No	46	95	No	3
F/79	Epistaxis	Edoxaban 30 mg	No	52	250	No	3
M/75	Epistaxis	Edoxaban 30 mg	No	42	259	No	3
F/89	Intramuscular	Edoxaban 30 mg	No	39	154	No	3
M/77	Epistaxis	Edoxaban 30 mg	No	28	109	No	3
M/86	Epistaxis	Edoxaban 30 mg	No	26	98	No	4
F/80	Gastrointestinal	Edoxaban 30 mg	No	19	217	No	4
F/84	Epistaxis	Edoxaban 30 mg	Yes	18	188	No	4
F/75	Gengivorrhagia	Edoxaban 30 mg	No	17	277	No	3
F/83	Gastrointestinal	Edoxaban 60 mg	No	131	NA	No	2
M/84	Epistaxis	Edoxaban 60 mg	No	39	192	No	2
M/69	Intramuscular	Edoxaban 60 mg	No	21	245	No	2
M/81	Hematuria	Rivaroxaban 20 mg	No	28	NA	No	2
F/90	Vaginal	Rivaroxaban 20 mg	Yes	25	NA	No	3

Table 3 Effect of standardized plasma DOAC levels on bleeding outcomes, adjusted for potential confounders.

	First model (C-trough), n=1657		Second model (C-peak), n=1298	
Characteristic	HR	95% CI	HR	95% CI
- Standardized C-trough DOAC levels, first 3 months	1.36	1.02 – 1.78	-	-
- Standardized C-trough DOAC levels, >3 months	0.97	0.66 – 1.45	-	-
- Standardized C-peak DOAC levels, first 3 months	-	-	1.10	0.70 – 1.74
- Standardized C-peak DOAC levels, >3 months	-	-	0.71	0.45 – 1-13
- HAS-BLED score	1.04	0.66 – 1.62	1.14	0.67 – 1.96
- BMI, Kg/m2	1.00	0.93 – 1.06	1.00	0.93 – 1.07
- Glomerular filtration rate, ml/min	1.00	0.99 – 1.02	1.01	0.99 – 1.02
- Low vs standard dose DOAC, first 3-months	3.61	1.28 – 10.2	3.30	1.06– 10.3
- Low vs standard dose DOAC, >3 months	1.40	0.61 – 3.23	2.17	0.82 – 5.74
- Antiplatelet treatment (yes vs. no)	1.06	0.66 – 1.62	1.23	0.51 – 3.00

Both models were estimated using time-varying Cox regression.

BMI: Body-Mass Index; CI: confidence interval; DOAC: Direct oral anticoagulant; HR: hazard ratio. The inclusion of enrollment Center as a potential confounder was not significant (p>0.9 for both models) and it is not reported as it did not materially change estimates

Table 4 Patient distribution in three classes of standardized values around the mean value for all anticoagulant drugs (0) assessed at C-trough (panel A) or at C-peak (Panel B), with the number of patients and of bleeding complications recorded in each class. The equivalent DOAC plasma levels for each class are also reported.

Panel A

Classes of standardized C-trough values	Equivalent DOAC C-trough plasma levels (ng/mL)							N. patients	Follow-up years	N. bleeding complication	Incidence (% pt/yrs) [95%CI]
	Apixaban		Edoxaban		Rivaroxaban	Dabigatran					
	2.5 mg/BID	5 mg/BID	30 mg	60 mg	20 mg	110 mg/BID	150 mg/BID				
≤ -0.50 (low levels)	≤ 77	≤ 94	≤ 23	≤ 24	≤ 22	≤ 78	≤ 68	531	513	15	2.92 (1.7-4.8)
-0.49 to 0.50 (intermediate levels)	78-146	95-160	24-54	25-58	23-52	81-138	69-128	804	790	20	2.53 (1.6-3.9)
> 0.50 (high levels)	> 149	> 163	> 55	> 60	> 53	> 143	> 132	322	302	15	4.97 (2.8-8.2)
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CI: confidence interval; DOAC: direct oral anticoagulant; pt/y: patient/years

Panel B

Classes of standardized C-peak values	Equivalent DOAC C-peak plasma levels (ng/mL)							N. patients	Follow-up years	N. bleeding complication	Incidence (% pt/yrs) [95%CI]
	Apixaban		Edoxaban		Rivaroxaban	Dabigatran					
	2.5 mg/BID	5 mg/BID	30 mg	60 mg	20 mg	110 mg/BID	150 mg/BID				
≤ -0.50 (low levels)	≤ 189	≤ 202	≤ 149	≤ 197	≤ 165	≤ 172	≤ 159	454	443	14	3.16 (1.7-5.3)
-0.49 to 0.50 (intermediate levels)	191-294	203-291	151-232	205-309	166-260	173-277	165-248	476	457	14	3.06 (1.7-5.1)
> 0.50 (high levels)	> 304	> 297	> 233	> 310	> 268	> 279	> 267	368	360	11	3.06 (1.5-5.5)
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CI: confidence interval; DOAC: direct oral anticoagulant; pt/y: patient/years

Table 5 Distribution of measurement results [n. (%)] in the three classes of standardized C-trough values in AF patients who received appropriate or inappropriate standard or low dose DOAC and incidence of bleeding events in the classes.

	Standardized C-trough value classes			
	Low levels	Intermediate levels	High levels	
	≤ -0.50	From -0.49 to 0.50	> 0.50	All
DOAC doses and incidence of bleeds in the classes, n. (% pt/yrs)				
Standard dose	309 (32.3)	459 (48.0)	189 (19.7)	957
- Appropriate, n. (%)	294 (17.7)	413 (24.9)	174 (10.5)	881 (53.2)
Incidence of bleeding events, n. (% pt/yrs)	6/287 (2.1)	8/415 (1.9)	5/167 (3.0)	19/869 (2.2)
- Inappropriate, n. (%)	15 (0.9)	46 (2.8)	15 (0.9)	76 (4.6)
Incidence of bleeding events, n. (% pt/yrs)	0/16 (0)	2/44 (4.5)	0/14 (0)	2/75 (2.8)
Low dose	222 (31.7)	345 (49.3)	133 (19.0)	700
- Appropriate, n. (%)	180 (10.5)	282 (17.0)	98 (5.9)	560 (33.8)
Incidence of bleeding events, n. (% pt/yrs)	6/177 (3.4)	7/272 (2.6)	7/88 (7.9)	20/537 (3.7)
- Inappropriate, n. (%)	42 (2.5)	63 (3.8)	35 (2.1)	140 (8.4)
Incidence of bleeding events, n. (% pt/yrs)	3/40 (7.5)	3/60 (5.0)	3/33 (9.1)	9/133 (6.8)

Figure 1 Patient flowchart

Figure 2 Incidence of bleeding events, stratified by the first 3 months and > 3 months of the study period, for the three standardized DOAC C-trough level classes.

Figure 1

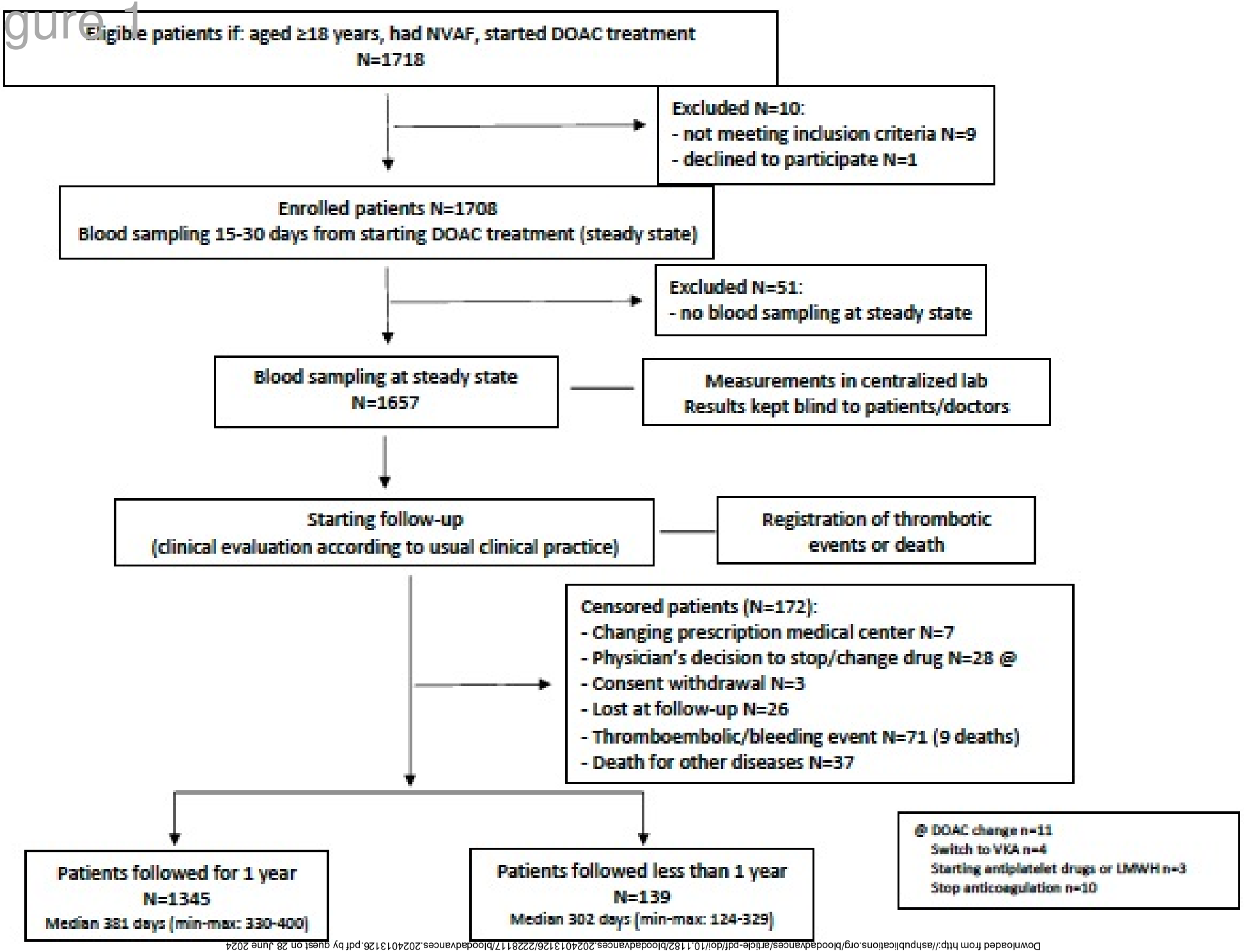

Figure 2