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Thrombotic events associated with low baseline direct oral anticoagulant levels in atrial fibrillations: the MAS study

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Abstract:
Though effective and safe, treatment with direct oral anticoagulants (DOAC) in atrial fibrillation (AF) is still associated with thrombotic complications. Whether the measurement of DOAC levels may improve treatment efficacy is an open issue. We carried out the observational, prospective, multicenter study [MAS Study (NCT03803579)]. Blood was collected 15-30 days after starting DOAC treatment in AF patients who were followed for one year. Plasma samples were centralized for DOAC level measurement. Patients' DOAC levels were converted into drug/dosage standardized values to allow a pooled analysis in a time-dependent, competitive-risk model. The measured values were transformed into standardized values (representing the distance of each value from the overall mean) by subtracting the DOAC-specific mean value from the original values and dividing by the standard deviation. Trough and peak DOAC levels were assessed in 1657 and 1303 patients, respectively. Twenty-one thrombotic complications were recorded during 1606 years of follow-up (incidence of 1.31% patient/years). 17/21 thrombotic events occurred in patients whose standardized activity levels were below the mean of each DOAC (zero); the incidence was the highest (4.82% patient/years) in patients whose standardized values were in the lowest class (below zero, □ -1.00). Early measurement of DOAC levels in AF patients allowed us to identify most of the subjects who, having low baseline DOAC levels, subsequently developed thrombotic complications. Further studies are warranted to assess whether thrombotic complications may be reduced by measuring baseline DOAC levels and modifying treatment when indicated.

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Thrombotic events associated with low baseline direct oral anticoagulant levels in atrial fibrillations: the MAS study

Short Title

Low initial DOAC level and thrombotic events in AF

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KEY POINTS

- A relationship between low baseline DOAC levels and thrombotic events in one-year follow-up was found.
- Early measurement allows to identify subjects with low DOAC levels and, hopefully, to adjust treatment to avoid future thrombotic events.

ABSTRACT

Though effective and safe, treatment with direct oral anticoagulants (DOAC) in atrial fibrillation (AF) is still associated with thrombotic complications. Whether the measurement of DOAC levels may improve treatment efficacy is an open issue. We carried out the observational, prospective, multicenter study [MAS Study (NCT03803579)]. Blood was collected 15-30 days after starting DOAC treatment in AF patients who were followed for one year. Plasma samples were centralized for DOAC level measurement. Patients' DOAC levels were converted into drug/dosage standardized values to allow a pooled analysis in a time-dependent, competitive-risk model. The measured values were transformed into standardized values (representing the distance of each value from the overall mean) by subtracting the DOAC-specific mean value from the original values and dividing by the standard deviation. Trough and peak DOAC levels were assessed in 1657 and 1303 patients, respectively. Twenty-one thrombotic complications were recorded during 1606 years of follow-up (incidence of 1.31% patient/years). 17/21 thrombotic events occurred in patients whose standardized activity levels were below the mean of each DOAC (zero); the incidence was the highest (4.82% patient/years) in patients whose standardized values were in the lowest class (below zero, $\leq$ -1.00). Early measurement of DOAC levels in AF patients allowed us to identify most of the subjects who, having low baseline DOAC levels, subsequently developed thrombotic complications. Further studies are warranted to assess whether thrombotic complications may be reduced by measuring baseline DOAC levels and modifying treatment when indicated.

INTRODUCTION

Over the last years, clinical trials, metanalyses and clinical practice confirmed the efficacy and safety of direct oral anticoagulants (DOAC) for stroke prevention in patients with non-valvular atrial fibrillation (AF).[1-8] Recent observational studies in AF patients showed that DOAC, compared to warfarin, had lower rates of stroke, systemic embolism, comparable rates of major bleedings (MB),[9] and advantages in terms of risk reduction of intracranial bleeding and systemic embolism even in the elderly and frail populations.[10,11] However, a non-negligible incidence of thrombotic and bleeding events has been recorded in clinical trials and in observational studies in patients receiving DOAC.[5,7,8] Hence, the issue of improving the clinical management of DOAC-treated patients and further reducing the risk of complications during treatment is relevant.

DOACs are administered to AF patients based on patient characteristics such as age, comorbidity, body weight, and renal function without dose adjustment based on DOAC concentration measurements. This is mainly based on results of pharmacokinetic studies, which indicated a predictable anticoagulant response and an effective prevention of excessive drug concentration.[12-16] Furthermore, the registration trials were conducted at dose regimens adjusted for some patient characteristics or for concomitant use of associated interacting drugs, and not for measured DOAC levels.

Consequently, The measurement of DOAC levels has 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 treated for epilepsy.[17-19] However, studies focusing on the measurement of plasma DOAC levels showed high inter-patient variability for all the DOAC and for all the doses used to treat patients.[20-24]

Moreover, post-marketing studies showed that the variability of DOAC levels was even higher than that reported in phase II and III studies.[25-29] Indeed, observational studies reported that some of the patients treated at fixed doses, may have relatively high or low DOAC plasma levels, thus supporting the issue of assessing whether an early or periodical measurement might contribute to improving the quality of treatment and reducing risks of complications.[30,31]

In addition, very recent studies showed a relationship between low DOAC levels (generally measured after the events), the risk of ischemic stroke and its severity,[32] and the risk of stroke recurrences.[33] Finally, a pilot prospective multicenter study evidenced low plasma levels of DOAC in patients with AF, measured at the time of embolic stroke.[34] Whether DOAC concentration

measurement may be useful to better tailoring the dose and optimizing the risk-benefit of treatment remains an unsolved clinical problem.

The present study aimed to investigate whether low DOAC plasma levels, assessed at steady-state within the first month of treatment, are associated with thrombotic events during a one-year follow-up.

MATERIAL AND METHODS

The Measure and See Study (MAS) (NCT03803579) is an observational, prospective cohort, multicenter study of patients with AF, who started treatment with one of the available DOAC (dabigatran, apixaban, edoxaban, and rivaroxaban) for therapy and prevention of thrombotic complications. 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

Consecutive patients with AF, without rheumatic mitral valve disease or mechanical heart valves, aged over 18 years, seen at the anticoagulation clinics from 27 August 2018 to 10 November 2022, who had started within one month anticoagulation with a DOAC, were enrolled in the study. Patients suitable for electrical cardioversion, or who have refused blood sampling, or did not accept follow-up for at least one year or who had other clinical indications for anticoagulant therapy were excluded from the study.

The choice of DOAC and dose used was left to the discretion of treating physicians. The study protocol included a recommendation for the participant centers to include patients, who were treated following the rules defined for each drug by the Italian regulatory agency (AIFA) and current clinical practice.

Each patient was given a unique anonymous identifying code to ensure anonymity, which was used to collect clinical information and identify biological samples. The following baseline characteristics were recorded in a specific electronic database: patient identification number, date of birth, gender, type of drug used and dose, weight, body mass index, kidney function [estimated by creatinine clearance (CrCl) according to the Cockroft-Gault formula], liver enzymes ~~function~~ (~~assessed by~~ aspartate aminotransferase and alanine aminotransferase), diabetes, CHA_2DS_2-VASc score, previous stroke/transient ischemic attack (TIA), other comorbidities, concomitant

medications, (with particular attention to antiplatelet drugs). Data were stored in the database located at a section of the Aruba cloud rented by the Arianna Anticoagulazione Foundation, which guaranteed the database's storage, backup, and maintenance.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Independent review board approval was obtained before all study-related activity from the Ethics Committee (EC) of the coordination center (Cremona) (approval number 14725; 02/05/2018) and from the ECs of all other centers. Written informed consent was obtained from each patient before enrolment. The study promoter provided 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

The study required a mandatory plasma collection to measure the DOAC level for each enrolled patient. Venous blood sampling had to be performed at a steady state (within the first 2-4 weeks of initiation of treatment) and obtained at the time of trough (C-trough), immediately before the subsequent drug intake. It was up to the discretion of participating centers, depending on their availability and organization, to perform additional blood collection on the same day, 2 hours after the last intake (C-peak). Further blood samples were used to perform ancillary laboratory tests (including blood cell count, CrCl, and liver enzymes). Plasma samples for DOAC measurement were collected in vacuum tubes (Vacutainer; Franklin Lakes, NJ, USA) containing 1/10 volumes of 3.2% trisodium citrate. Blood was centrifuged within one hour of collection at 2000x g for 20 min (controlled room temperature), and plasma samples were aliquoted in cryovials, identified locally to maintain patient's anonymity, in volumes that allow for optimal DOAC testing ; sample vials were stored frozen (-80°C)[35] at the participating centers and later centralized to the biobank of the Arianna Anticoagulazione Foundation (Bologna). Finally, aliquots were transferred (dry-ice and express courier) to the Haemostasis and Thrombosis Center of Cremona Hospital for DOAC measurement.

DOAC levels, expressed as drug concentration-equivalent (ng/mL), were measured by chromogenic assays using STA-ECA II (Diagnostica Stago, Asnieres-sur-Seine, France) for dabigatran, and STA-Liquid anti-Xa (Diagnostica Stago) for apixaban, edoxaban, and rivaroxaban (hemolyzed samples were discarded).[36-38] Tests were calibrated using commercial plasmas with certified DOAC concentration supplied by the same manufacturer and performed on STA-R Max instrument (Diagnostica Stago). The results of DOAC levels for each patient were transmitted to

the central database repository, and were not communicated to patients, participating centers, or attending physicians.

Follow-up and outcomes

Participating centers were instructed to organize blood sampling and clinical follow-up as requested by the study. Follow-up, as defined by FCSA guidelines, included 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 predefined study outcomes were all thromboembolic complications, including objectively documented ischemic cerebral vascular events, systemic emboli, the occurrence of acute venous thromboembolism (VTE), acute myocardial infarction (AMI), and thrombotic and cardiovascular deaths. An independent adjudication committee, that was unaware of patient name, the results of DOAC levels in the collected samples, and the enrolling center, assessed all the adverse events occurring during follow–up. The present report analyses the data regarding the relationship between DOAC levels and the occurrence of thromboembolic complications during follow-up. The relationship between DOAC levels and bleeding events will be analyzed and reported separately.

Statistical analysis

Sample size. Based on previous studies,[12,25] we hypothesized that the annualized risk of the primary study outcome may be fourfold as high in the lowest quintile of DOAC distribution as compared to the highest. We assumed an annualized risk of primary study outcome of 1.25% patient/years (pt/y) and 5% pt/y in patients in the highest and lowest quintile of DOAC plasma concentration, respectively. Under this assumption, a total sample size of 1315 patients would be sufficient to refute a null hypothesis of equivalence with alpha and beta errors of 0.05 and 0.2, respectively.

Analysis plan. Since the absolute C-trough and C-peak DOAC plasma concentrations are drug and dose-dependent, we standardized each measured absolute value using their drug- and dose-specific mean and 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 (OID or BID). Supplemental Figure 1

shows the correlation between unstandardized (absolute) and standardized plasma drug concentrations.

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 or after the occurrence of a qualifying event. For the primary analysis, we used a Cox regression model allowing for competing risk according to Fine & Gray[39] to model the occurrence of the primary study outcomes as a function of standardized drug concentration as a continuous value. The regression model included as possible confounders CHA_2DS_2VASc score, body mass index, creatinine clearance, concomitant antiplatelet use, use of low-dose DOAC, and enrolling Center. Deaths occurring for causes other than thromboembolic events were considered as competing events. Akaike information criteria (AIC) was used to evaluate the goodness of fit of the Fine & Gray model. As a sensitivity analysis, we also explored the risk of the primary study outcomes in patients below the first quintile of the absolute drug concentration, after adjustment for the above-stated possible confounders.

As an exploratory analysis, we evaluated the incidence of the primary thrombotic outcome stratified by standardized values. For this analysis, Kaplan-Meier survival curves were plotted to estimate the cumulative incidence of thrombotic outcomes in patients with standardized values in the lowest class compared to those in the higher classes, and HR and their 95% CI were calculated. The variable CHA_2DS_2VASc score ($\geq$4 vs <4) was arbitrarily categorized according to the median values as cut-off. 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). 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).

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. Ten patients were excluded for not meeting inclusion criteria (9) or declined to participate (1), and 51 because no blood sampling at steady state was available. 1657 patients had blood sampling for DOAC levels measurement 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 in 139 because the study was stopped (median follow-up 302 days [min-max: 124-329]). A total of 173 patients were censored before one year of follow-up for the following reasons: they had changed the reference medical center (7), the consent to the study was withdrawn (3), the physician decided to change/stop the treatment (28), they were lost at follow-up (26), for thromboembolic or bleeding events (72) (including 9 deaths), death due to other diseases (37). The main demographic and clinical characteristics of the 1657 investigated patients are shown in Table 1.

Plasma samples for DOAC measurement were available for all patients at C-trough, and in 1303 patients at C-peak. Results [mean±standard deviation (SD) and min-max] of DOAC levels, at C-trough and at C-peak, are shown in Supplemental Table 1, in addition with the number of plasma samples analyzed for the different DOAC.

DOAC concentration levels and thrombotic events during follow-up

During a total follow up of 1606 years, thromboembolic outcomes occurred in 21 patients (incidence of 1.31% pt/y): 6 strokes (1 fatal), 1 TIA, 1 VTE, 12 AMI (5 fatal) and 1 superficial vein thrombosis were recorded. Details of patients, who had thrombotic outcomes during follow-up are shown in Table 2. Altogether, 46 deaths were recorded (2.8%), 6 of whom were related to thrombotic complications (Supplemental Table 2). DOAC plasma level was the most important independent predictor of the occurrence of the primary study outcome according to the best-fitting model (Table 3), even after adjustment for other possible confounders and enrollment centers. Patients with C-peak crude concentrations below the first quintile were particularly at risk of the primary study event (Supplemental Table 3).

As shown in Figure 2, patients with thrombotic outcomes had C-trough DOAC values below the mean value for each drug in 17 (1.7% pt/y) cases, whereas 4 (0.69% pt/y) cases had values above the mean value. At C-peak, their values were below the mean in 13 (1.8% pt/y) cases and above in 4 (0.70% pt/y) cases. Using standardized C-trough values, patients were distributed into classes of increasing levels (Table 4). The highest incidence of thrombotic events (4.82% pt/y) occurred among the 89 patients with standardized DOAC values in the lowest class (≤ -1.00) compared to the mean value; the incidence of events decreased sharply in the other classes. The incidence of events in the lowest class of standardized levels was significantly different than in the sum of all the other classes (4.82% pt/y vs 1.12% pt/y; p= 0.0039). The Kaplan-Meier curves of

cumulative thrombotic outcomes occurring in patients in the lowest class of standardized DOAC levels, assessed at C-trough, compared with those in all the higher classes are shown in Figure 3.

DISCUSSION

The MAS Study is the first, observational, multicenter study, which measured DOAC plasma levels in AF patients at the beginning (steady state) of DOAC treatment and prospectively followed-up to record thrombotic complications occurring within one year. DOAC levels were kept blind to patients and to attending physicians and merged with the individual patients only at the end of the study. The main findings of the study are that 17 out of 21 patients who experienced thrombotic outcomes had low plasma activity of the drug at the beginning of treatment; furthermore, the highest incidence of thrombotic complications (4.82% pt/y) occurred in patients whose standardized levels were in the lowest and most distant class from the overall mean.

Efficacy of DOAC treatment for stroke prevention in AF has been largely documented[5-9] however, a non-negligible incidence of thrombotic events has been recorded in registration trials and clinical practice in patients receiving DOAC.[9-11] These results pose the question of how to improve clinical management of DOAC-treated AF patients to further reduce the risk of complications. DOAC are administered to AF patients at fixed dose, either standard or low dose in relation to patient characteristics (mainly age and renal function), without the need for laboratory monitoring. However, high inter-individual variability has been confirmed in trials and observational studies,[25,27-29,40-42] showing that some patients may have low anticoagulant levels and therefore are more exposed to an increased risk of thrombotic complications, especially if at high cardiovascular risk. In a previous study[30] we observed a relationship between low DOAC levels and thrombotic events, particularly in patients with high CHA_2DS_2VASc score. Very recently, a monocentric observational study[33] reported early stroke recurrence in 3% of AF patients with a previous stroke, for whom low plasma levels of apixaban and dabigatran had been detected at steady state.

In the present study, which involved 1657 AF patients treated with the four DOAC currently on the market, we collected data on thrombotic cardiovascular events occurring for a one-year follow-up after blood sampling taken at a steady state to measure the DOAC levels. We found that more than 80% of thrombotic complications occurred in patients with standardized values present in the classes below zero (which is the overall mean of DOAC levels). Interestingly, both C-peak and C-trough standardized DOAC plasma concentrations had similar predictive capability in our study; however, C-trough measurements are generally easier to obtain.

Though these results seem to indicate that measurement of DOAC levels at a steady state in AF patients may help avoiding most thrombotic complications that may occur during treatment, our results clearly show that it would be unsuitable to measure the drug levels in all patients with AF who start a DOAC treatment to avoid (or reduce) the relatively few thrombotic complications that were recorded during one-year follow-up (21 events, 1.31% pt/y). Nevertheless, it appears clinically relevant to try to improve the efficacy of DOAC treatment by further lowering the incidence of severe thrombotic complications, which would seem achievable by adjusting the daily dose or changing the drug in patients at high thrombotic risk who show low DOAC levels when measured at the beginning of treatment. To this aim, we suppose that different combinations of patient characteristics might be helpful in identifying a criterion which would allow the minimum number of patients to be tested together with the maximum number of thrombotic events that potentially would be avoided.

Limitations

Our study has limitations. The enrolment was strongly hit by the COVID-19 pandemic, that dramatically blocked many activities at the participating centers. We admit that the study may present problems of generalizability since about 75% of patients were included in only one clinical center (ST, Cremona). The four available DOAC are not similarly used in our country and, consequently, the number of investigated patients was not equal for each drug. Although rivaroxaban tablets should be taken with food to increase absorption (Product Monograph, Bayer, revision April 2023) in this study blood sampling was performed before the administration of the subsequent DOAC dose, and we are not aware whether patient blood sampling was performed before or after food intake for individual patients, DOAC concentration was measured only once (i.e., 15-30 days after the initiation of treatment). We therefore cannot exclude possible intra-individual changes in DOAC levels and problems in adherence to treatment during follow-up. However, a previous study reported that the intra-individual variability of DOAC levels was substantially lower than the inter-individual variability.[28] On the other hand, testing DOAC at different time points during follow up would have been unpractical. However, our study showed an association between very low DOAC levels assessed at the beginning of treatment and occurrence of thrombotic complications in the one-year follow-up, and therefore our results were not influenced by possible DOAC level changes after the time-point of measurement. It is well known that association with potent inducer agents of DOAC catabolism (such as antiepileptic

drugs) may significantly reduce DOAC concentration.[43] The concomitant use of antiepileptic drugs in our study was reported in 28 of the investigated patients, 5 of them had standardized C-trough values in the lowest class around the overall mean (≤ - 1.00), and none of these patients had thrombotic complications during follow-up. All in all, we believe that an accurate analysis of the many factors that may interfere with DOAC concentration would be needed to determine which patients should be tested in future studies. For example, DOAC dosing is still uncertain in some groups of patients, such as those with an extreme body weight. Weight and Body-Mass Index (BMI) are important variables in drug distribution and plasma concentration levels and in individuals with very low or high BMI DOAC measurements may be considered.[19] In our study population, the number of subjects with BMI <18.5 or >50 (criteria suggested by Steffel et al to define patients with extreme body weight[19]) were small (39 and 1, respectively). The relatively low number of subjects with extreme BMI may have contributed to the non-significant result of BMI as predictor of thrombotic outcomes in the competitive risk score analysis (Table 3).

The strengths of the study are its prospective and multicentric design, the centralization of laboratory tests, which avoided inter-laboratory variations, and the blindness of all test results to patients and treating physicians that avoided possible treatment adjustment during follow-up.

Future directions

While we are aware that the results of this study are not sufficient to modify current clinical practice, we believe that our findings may pave the way to future studies aimed to definitively assess whether measuring DOAC plasma levels at steady state in selected patients may reduce the incidence of thrombotic complications during follow-up in the setting of AF patients.

Conclusion

In conclusion, our data show a relationship between low DOAC levels measured at steady state and occurrence of stroke, TIA and VTE, and other thrombotic cardiovascular events in AF patients. The results support the clinical utility of measuring DOAC concentration at the beginning of treatment in special settings of AF patients. Laboratory measurement at steady state may allow to avoid a persistent treatment at insufficient DOAC concentration in patients who are at a very high thrombotic risk. Our results need to be confirmed and expanded with further clinical studies.

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Conflict of Interest Disclosures

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

Data Sharing Statement

For original data, please contact c.legnani@fondazionearianna.org

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Table 1 Baseline characteristics of included patients

Patients, n	1657
Participating centers, 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)
Hemoglobin, median (min-max), (g/dL)	13.3 (8.0-18.8)
Platelets, median (min-max), (<109/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]	521 (31.4) [336 (65.5)]
- Dabigatran [Standard dose]	221 (13.3) [100 (45.3)]
- Edoxaban [Standard dose]	583 (35.2) [283 (48.6)]
- Rivaroxaban [Standard dose]	332 (20.0) [238 (71.7)]
Prescribing accuracy of DOACs[19]:	
- Appropriate, n (%)	1459 (88.0)
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 (%)	29 (1.8)
- 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)

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

Table 2 Details of all thrombotic outcomes

Sex/ Age	Thrombotic Outcome	DOAC dose	Weight (Kg)	BMI	Creatinine (mg/dL)	Creatinine clearance mL/min	C-trough level (ng/mL)	C-peak level (ng/mL)	CHA_2DS_2VASc score	Previous Stroke/TIA	Inappropriate DOAC prescription	Use of antiplatelet drugs	Use of interfering drugs
M/78	Stroke	Apixaban 5 mg/BID	90	24.9	1.31	69	94	182	4	Yes	No	No	No
F/78	Stroke	Apixaban 2.5 mg/BID	41	18.2	1.31	23	73	84	7	Yes	Yes	No	No
M/71	SVT	Apixaban 5 mg/BID	84	25.6	1.20	67	110	150	4	No	No	Yes	No
F/83	Fatal AMI	Apixaban 2.5 mg/BID	46	18.7	0.69	44	33	219	4	No	No	No	Yes
M/86	AMI	Apixaban 5 mg/BID	86	29.7	1.08	58	106	238	4	No	No	No	No
M/82	AMI	Apixaban 5 mg/BID	78	26.4	1.00	63	169	368	4	No	No	Yes	No
M/83	AMI	Apixaban 2.5 mg/BID	112	34.6	1.74	50	97	NA	5	No	No	No	No
M/78	Fatal AMI	Apixaban 5 mg/BID	66	23.4	1.24	45	32	80	6	No	No	Yes	No
M/62	Stroke	Dabigatran 150 mg/BID	85	28.7	0.85	108	68	248	2	No	No	No	No
M/81	DVT	Dabigatran 110 mg/BID	73	28.5	1.27	47	90	165	5	Yes	No	No	No
M/67	AMI	Dabigatran 110 mg/BID	83	28.1	1.30	65	37	60	3	No	No	Yes	No
M/66	AMI	Dabigatran 150 mg/BID	102	32.9	1.37	75	34	NA	4	No	No	Yes	No
M/89	AMI	Dabigatran 110 mg/BID	67	21.9	0.91	51	128	NA	3	No	No	No	No
F/83	Stroke	Edoxaban 30 mg	54	23.1	0.75	48	23	101	5	Yes	No	No	No
F/79	Stroke	Edoxaban 30 mg	67	26.2	0.87	55	35	102	5	No	Yes	No	No
M/79	AMI	Edoxaban 30 mg	60	18.5	1.44	35	20	247	5	No	No	Yes	No
M/70	Fatal AMI	Edoxaban 60 mg	64	26.6	1.10	57	40	296	4	No	No	Yes	No
F/72	Fatal AMI	Edoxaban 60 mg	72	28.8	1.00	57	52	NA	3	No	No	No	No
F/79	Fatal Stroke	Rivaroxaban 15 mg	55	22.9	0.60	63	17	121	5	No	Yes	No	No
F/89	TIA	Rivaroxaban 15 mg	75	29.3	1.15	39	13	9	5	No	No	Yes	No
M/81	Fatal AMI	Rivaroxaban 20 mg	104	34.0	0.57	150	43	189	6	No	No	Yes	No

AMI: acute myocardial infarction; BID: bis in die; BMI: Body-Mass Index; DOAC: direct oral anticoagulants; DVT: deep vein thrombosis; NA: not available; SVT: superficial vein thrombosis; TIA: transient ischemic attack

Table 3 Effect of standardized plasma DOAC levels on the primary thrombotic outcome endpoint, adjusted for potential confounders.

	First Model (C-trough), n=1657		Second model (C-peak), n=1298	
Characteristic	HR	95% CI	HR	95% CI
Standardized trough DOAC	0.56	0.37 - 0.86	-	-
Standardized peak DOAC	-	-	0.19	0.06-0.66
CHA_2DS_2VASc score	2.01	1.02-3.97	2.07	1.26-3.39
BMI, Kg/m^2	0.93	0.80-1.08	0.95	0.82-1.09
Glomerular filtration rate, ml/min	1.02	1.00-1.05	1.02	0.99-1.05
Low-dose vs Standard dose DOAC	3.49	0.76-16.0	2.72	0.55-13.5
Antiplatelet treatment (yes vs. no)	0.28	0.03-2.53	0.25	0.03-1.81

Both models were estimated using the Fine & Gray competitive risk regression model. The AIC was 118.4 and 106.1 for the models using C-trough and C-peak, respectively.

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 classes of standardized C-trough values around the mean value (0) for all anticoagulant drugs, with the number of patients and of thrombotic complications recorded in each class. The equivalent DOAC plasma levels at trough for each class are also reported.

Classes of standardized C-trough values	Equivalent DOAC C-trough plasma levels (ng/mL)							N. patients	Follow up years	N. thrombotic complication	Incidence (% pt/y) [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				
≤ -1.00	≤ 41	≤ 61	≤ 8	≤ 7	≤ 6	≤ 46	≤ 37	89	83	4	4.82 (1.3 – 12.3)
From -0.99 to -0.50	44-77	63-94	9-23	8-24	8-22	51-78	38-68	442	430	7	1.63 (0.6 – 3.3)
From -0.49 to 0	78-113	95-128	24-39	25-42	23-37	81-109	69-99	525	513	6	1.17 (0.4 – 2.5)
From 0.01 to 0.50	114-146	129-160	40-54	43-58	38-52	115-138	101-128	279	277	3	1.08 (0.2 – 3.2)
From 0.51 to 1.00	149-179	163-195	55-70	60-73	53-67	143-163	132-163	129	119	1	0.84 (0.02 – 4.7)
> 1.00	187-468	199-445	72-202	79-284	70-331	175-311	165-367	193	183	0	0
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CI: confidence interval; DOAC: direct oral anticoagulant; pt/y: patient/years

FIGURE LEGENDS

Figure 1 Flowchart of the study

Figure 2 Black dots represent the C-trough DOAC values, assessed at steady state, in atrial fibrillation patients who experienced thrombotic outcomes within the subsequent year of follow-up. Dotted lines represent the mean values of each drug.

Figure 3 The Kaplan-Meier cumulative event rates for the thrombotic outcomes in patients with DOAC levels in the standardized class $\leq$ - 1.00 (continuous line) and in patients with DOAC levels in the standardized classes > - 1.00 (dotted line) at C-trough.

Figure 1

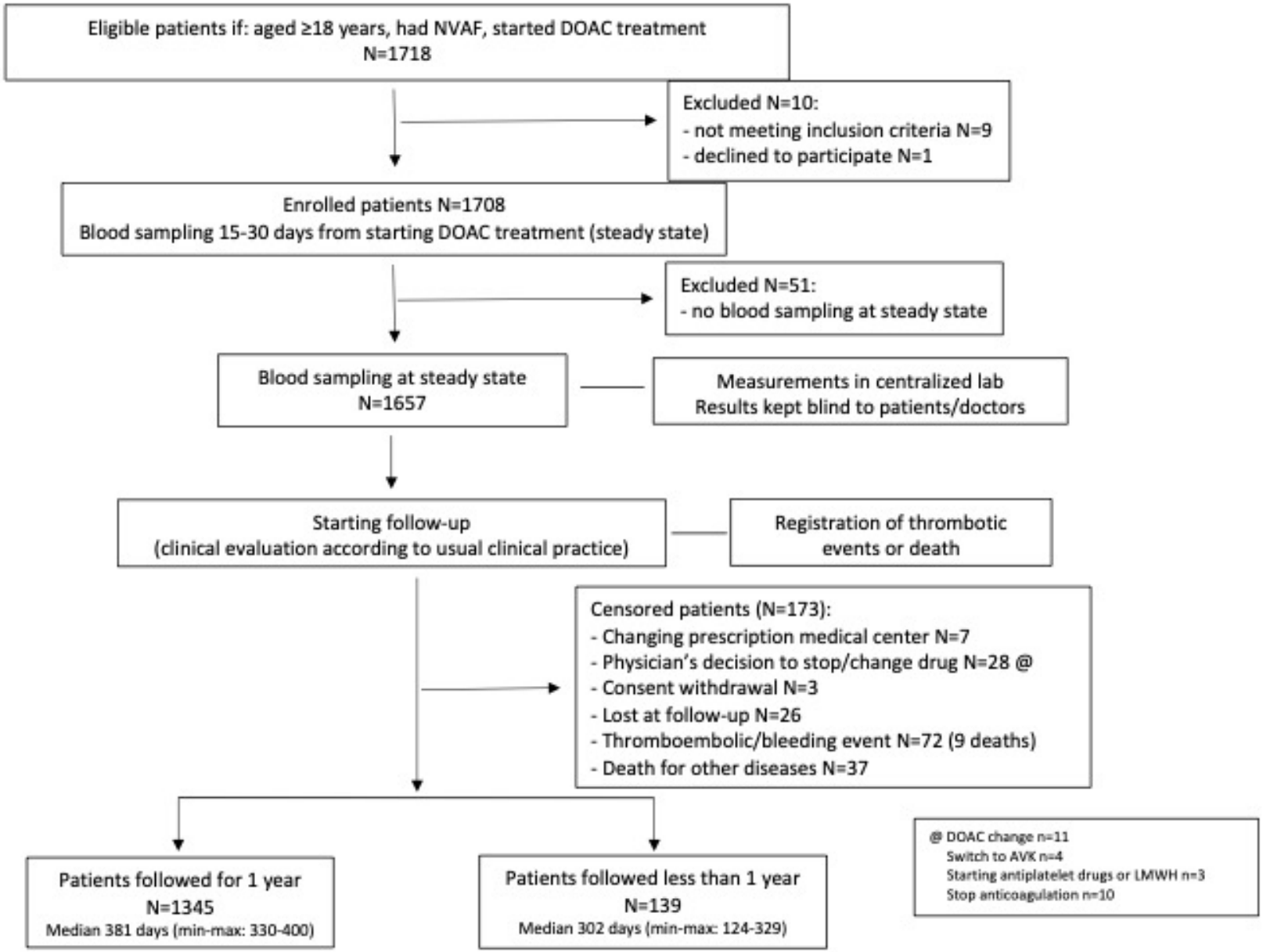


Figure 2

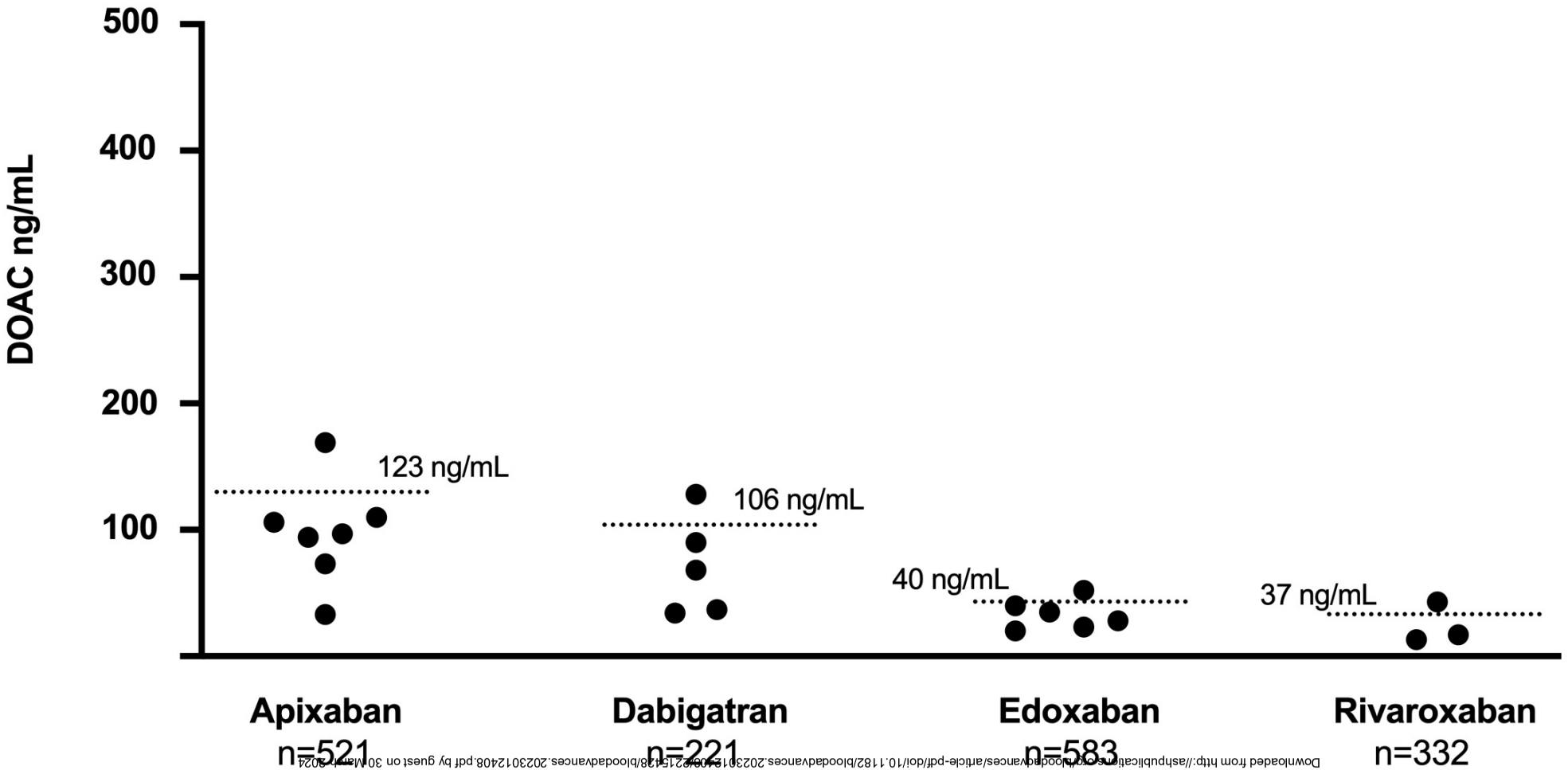
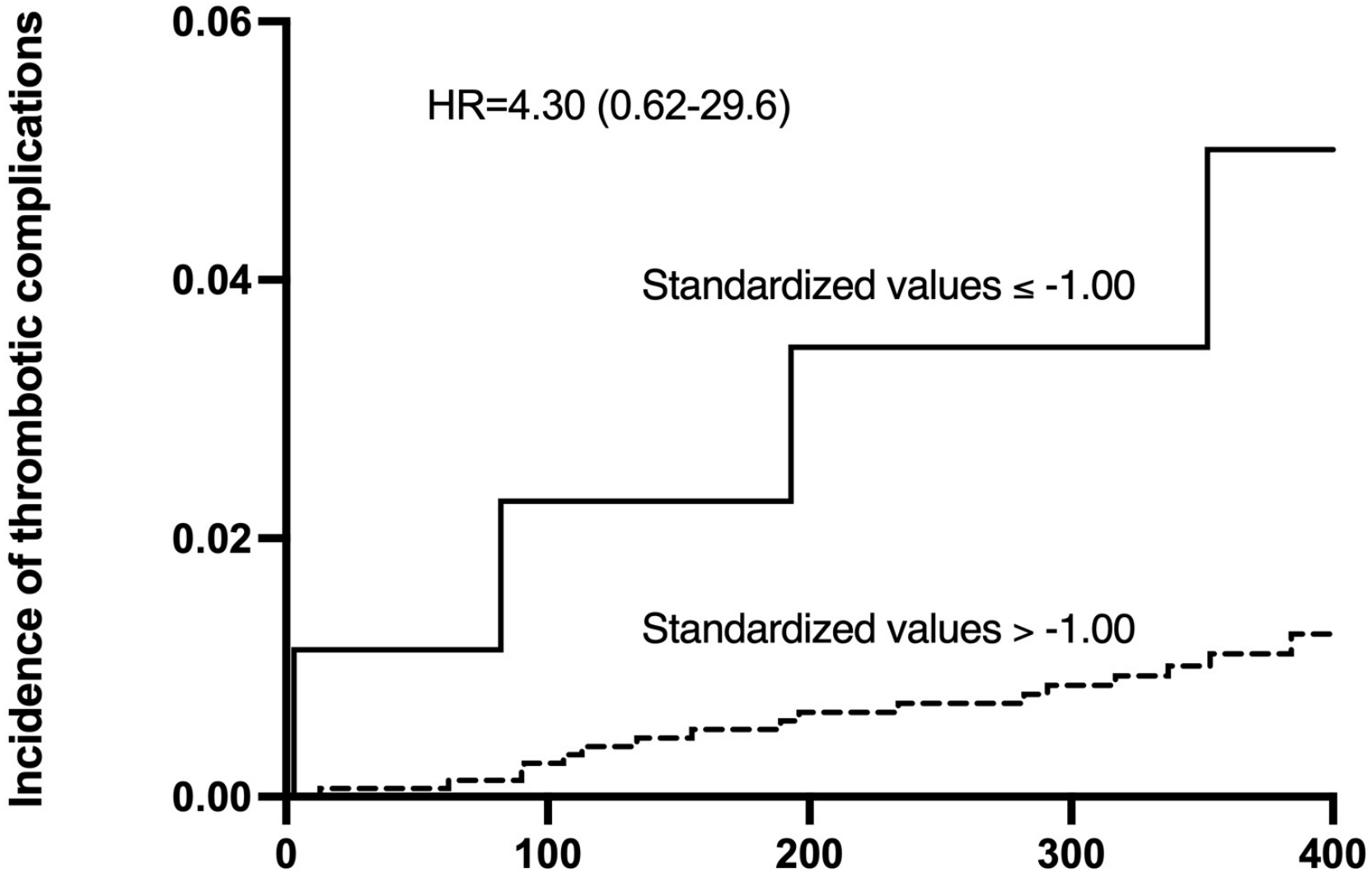


Figure 3



Number at risk by time

- Standardized values ≤ -1.00	89	85	81	76	24
- Standardized values > -1.00	1568	1528	1489	1408	513