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CLINICAL AND PHARMACOLOGICAL CRITERIA FOR DOSE REDUCTION IN THOSE EXPOSED TO DIRECT ORAL ANTICOAGULANTS: EVALUATION OF THE QUALITY OF ANTICOAGULATION WITH THROMBIN GENERATION TEST

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ABSTRACT

BACKGROUND The use of direct oral anticoagulants (DOACs) is increasing because its prescription in fixed doses does not require routine monitoring. However, assessment of the anticoagulant effect may be needed in specific situations to prevent ischemic or bleeding events. The Thrombin Generation Test (TGT) is a promising tool to monitor patients on DOACs. We aimed to assess the anticoagulant effect of DOACs on TGT and to compare the population under a full dose to patients with dose reduction in order to weigh TGT as a valuable clinical tool.

METHODS A cross-sectional study of 20 senior patients on DOAC therapy for atrial fibrillation (AF) and pulmonary embolism (PE) prophylaxis was conducted. TGT parameters were measured at through [lag time, endogenous thrombin potential (ETP), thrombin peak height, time to peak, and velocity index]. A structured questionnaire was used to collect individual patient data and potential drug-drug interactions (DDIs). Spearman's rank correlation coefficients were used to explore the relation between TGT parameters and DOAC concentration. P-values < 0.05 were considered statistically significant.

RESULTS We split the patients into two groups according to their DOAC dose: 13 patients with a median age of 72.08 years and a GFR of 68.70 mg/mL/1.73m^2 on a full dose (FD group); and 7 patients with a median age of 81.86 years and a mean GFR of 50.14 mg/mL/1.73m^2 with a 50% dose reduction (DR group). All patients were polymedicated. All the TGT parameters significantly correlated with DOAC concentration: peak height showed the best correlation ($r=-0.74$, $p<0.001$) while ETP was the least correlated ($r=-0.65$, $p=0.002$). Patients from the DR group had similar DOAC plasmatic concentration and TGT parameters when compared to those in the FD group. The drugs that correlated with alterations in the TGT were proton-pump inhibitors (PPIs), digoxin and allopurinol, while amiodarone led to a statistically significant increase in anti-factor Xa activity.

CONCLUSION The correlation between peak height and DOAC concentration means it is potentially a useful parameter to monitor DOAC dosing. Conversely, ETP may be valuable as a marker of bleeding risk. Patients with adequately reduced DOAC dose have similar TGT parameters as those with a full dose. Therefore, tailoring drug dosages to fit the needs of individual patients is crucial to diminish ischemic events while not increasing bleeding risk. Our results corroborate previous findings showing that DOACs have fewer DDIs than vitamin K antagonists. However, close monitoring may be required in specific patient profiles.

KEYWORDS DOACs, anticoagulation, thrombin generation test, clinical characteristics, dosing, drug-drug interactions.

INTRODUCTION

In the past years we have witnessed a paradigm shift in the prescription of anticoagulants, with a progressively higher number of patients switching from vitamin-K antagonist (VKA) anticoagulants to direct oral anticoagulants (DOACs). These drugs have been preferred over VKA mainly because of (1) their predictable and consistent pharmacokinetic and pharmacodynamic profiles, (2) the fact that they can be administered in fixed doses, (3) less drug-drug interactions (DDIs) and (4) the lack of need to monitor the levels of anticoagulation on a regular basis through international normalized ratio (INR) testing.[1,2] When compared to VKA, several studies have found that DOACs offer similar or even better efficacy whilst maintaining or diminishing the risk of bleeding.[3] Nevertheless, clinical characteristics such as old age, low weight and renal insufficiency need to be accounted for, as they are risk factors to bleeding side effects.[4]

Most of these patients are polymedicated, reinforcing the need to study and document DDIs of DOACs, as the one reported by Liesenfeld *et al.*[5] between dabigatran and proton pump inhibitors (PPIs). As a result, several parameters need to be taken into account when determining the appropriate dose of DOACs for a patient, making prescription challenging and increasing the probability of an adverse event.[6] Without measurements of the plasma concentration of the DOAC, DDIs are extremely difficult to detect and will only be so if a complication occurs.[7] Hence, some authors believe that these patients would benefit from close monitoring in order to prevent a major bleeding and offer individualized treatment.[8] Traditional coagulation tests to monitor anticoagulant activity include prothrombin time (PT) and activated partial thromboplastin time (aPTT), which are very useful for following those under VKA and non-fractioned heparin therapy, respectively.[1] The same principle does not apply for DOACs, since PT and aPTT use clot formation as their endpoint, making them incapable of assessing the whole coagulation system, as 95% of thrombin is formed after clotting has occurred.[9] The test of choice for measurement of DOAC effect is the dilute thrombin time for dabigatran and the anti-factor Xa assay for rivaroxaban, apixaban and edoxaban. However, both tests do not truly represent the achieved anticoagulation because they measure the inhibitory activity exerted against an individual factor and not as a whole. A global coagulation test such as thrombin generation test (TGT) may be the answer since it measures total thrombin formation, the final result of the coagulation cascade, thus offering a more sensitive measurement of anticoagulation intensity for those medicated with DOACs.[3] Despite its use as a research tool for many years now, TGT is not yet part of the routine clinical armamentarium.

In this study, we aimed to test if patients medicated with clinically-indicated dose reduction strategies had comparable anticoagulation levels with patients medicated with full-dose DOAC, thus exploring if candidates with dose reduction benefit from it by maintaining a balance between the risk of thrombotic and hemorrhagic events. We hypothesized that patients with clinically-driven dose reduction strategies had comparable levels of anticoagulation to patients without indication for dose reduction, due to the pharmacodynamic profile of DOACs. Additionally, we also wanted to specifically weight the DDIs of DOACs by measuring the concentration and function of anticoagulation through TGT. We

theorized that some drugs, namely CYP3A4 and P-glycoprotein inhibitors, enhance or diminish DOAC concentration and function, making patients who are taking these drugs candidates for tailored dosing.

MATERIALS AND METHODS

Study design and participants

We conducted a cross-sectional study of 20 senior patients on DOAC therapy for atrial fibrillation (AF) and pulmonary embolism (PE) prophylaxis. At the time of their outpatient clinic consultation, the patients were informed about the study that was being conducted and asked if wanted to participate. After signing the informed consent, a blood sample and collection of personal and clinical data were taken through a structured questionnaire. The patients were distributed in two groups according to their DOAC dosage: one with the patients on clinically-indicated full dose (FD group) and the other with the patients that were treated with a recommended dose reduction (DR group). Each patient was taking one of the four DOACs (apixaban, edoxaban, rivaroxaban or dabigatran) in either full dose, 5 mg bid, 60 mg od, 20 mg od or 150 mg bid, respectively) or clinically-recommended reduced dose (2.5 mg bid, 30 mg od, 15 mg od, 110 mg bid, respectively). All patients were on appropriate doses, as clinically mandated: 13 (65%) on full-dose and 7 (35%) with a reduced dose.

Clinical questionnaire

Personal and clinical data were gathered during the clinical interview and complemented with data collected from the patient file. Patients were asked: (1) whether they had a sedentary lifestyle; (2) if they had diseases that enhanced cardiovascular risk such as dyslipidemia, diabetes mellitus, arterial hypertension, coronary artery disease, a previous stoke or acute myocardial infarction; (3) if they were current or past smokers; (4) and if they had alcoholic habits in the present or the past. Through the clinical patient file, we gathered data regarding creatinine plasma levels, LDL cholesterol levels and previous echocardiograms. Additionally, a Simplified Medication Adherence Questionnaire (SMAQ) to assess their therapeutic adherence was used.

Blood collection

Each patient had blood drawn by phlebotomy and collected into a tube containing 0.109M trisodium citrate as an anticoagulant. Plasma samples were then centrifuged two times at 2000-2500 g for 10 minutes at room temperature within 60 minutes of blood collection and subsequently stored at -70°C or lower.

DOAC concentration

We assessed dabigatran plasma concentration with STA®-ECA II since it measures the anticoagulant effect of direct thrombin inhibitors; and STA®-Liquid Anti-Xa, a test that measures the

residual factor Xa activity (which will be inversely proportional to the DOAC concentration) to assess edoxaban, rivaroxaban and apixaban values in the plasma.

Thrombin Generation Test (TGT)

TGT has the ability to assess both thrombin generation and decay by evaluating the balance between the action of the procoagulant driver and the anticoagulant driver, making this test a candidate to investigate either hypo- or hypercoagulability.[10] Since the first thrombin generation test was created in 1953, several methods have been produced in order to improve its efficacy but one has stood out by being the most frequently studied, i.e. the Calibrated Automated Thrombography (CAT).[11] This assay[11-13] requires two wells with the same plasma, that can be either platelet-poor or platelet-rich, with $CaCl_2$ and a fluorogenic substrate. Tissue factor and phospholipids are added in the measurement well to induce thrombin formation by triggering the coagulation cascade, while in the calibration well only a known constant amount of thrombin activity is added to the non-clotting solutions. Both samples are then monitored through the conversion of thrombin into the fluorescent signal, aminomethylcoumarin (AMC), by a software that calculates the amount of thrombin formed during the experiment, thus creating the thrombin generation curve. The curve (Fig. 1) is characterized by various parameters that are useful for the interpretation of the results, such as lag time, peak height, time to peak, velocity index and endogenous thrombin potential (ETP). Lag time is the time expended to start clot formation; peak height is the maximum concentration of active thrombin that was reached, whereas time to peak is the time needed to achieve it; velocity index matches the slope of thrombin generation curve; and ETP is reflected by the area under the curve and embodies the total amount of thrombin generation formed, therefore being projected as the best parameter to assess the risk of bleeding or thrombosis.[1,3,11] To sum up, a hypocoagulable state can be determined by a prolonged lag time, as well as a reduced ETP and peak height; whereas the opposite results would indicate an hypercoagulable state.[14]

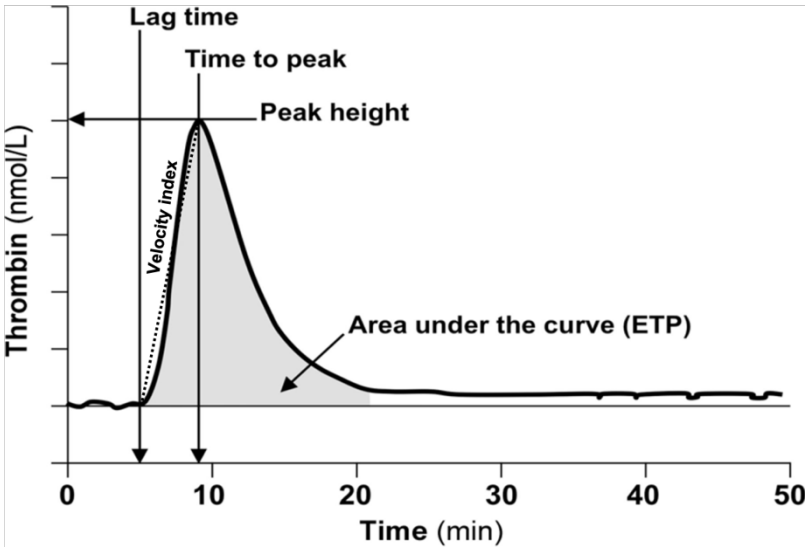

Figure 1 Thrombin generation curve. Adapted from Tripodi (2016)[9]

Blood samples were processed immediately after thawing and homogenization. Thawing was obtained by placing the sample in a 37°C water bath for exclusively the time needed to obtain complete thawing. Plasma was then homogenized and subsequently processed.

The measurement used in this study was performed with the STG®-DrugScreen kit. Quantitative determination of thrombin generation in plasma was provided by using a fluorogenic method on the ST Genesia®, a totally automatized system that allows acquisition and interpretation of the thrombin generation curves in a calibrated Thrombogram®. ST Genesia® was based on the same principles as the CAT system. The key difference between the two systems is that ST Genesia® focus is to support clinical applications in the lab, while CAT's main purpose is research. The STG®-DrugScreen contains 4 reagents: (1) trigger reagent with a high concentration of human recombinant tissue factor and phospholipids; (2) reference plasma; (3) control plasma with citrated hypocoagulable human plasma; and (4) control plasma comprised of citrated normal human plasma.

Data analysis

The normality of the distribution was assessed using the Kolmogorov-Smirnov test and histogram analysis. The clinical characteristics were compared using Pearson's chi-square test for categorical variables and independent Student's T-test for continuous variables. Spearman's rank correlation coefficients were used to explore relationships between TGT parameters and DOAC concentration. A p-value < 0.05 was considered statistically significant in all the performed statistical tests. SPSS v.21 for Windows (IBM) was used for statistical analysis.

RESULTS

Demographic characteristics

The baseline characteristics, comorbidities, type of DOAC and concomitant medication of both groups are depicted in Table 1 and Table 2. The mean age of the FD group was 72.08 years and 69.2% were female, while the population of the DR group had a mean age of 81.86 years and 57.1% were male. The mean glomerular filtration rate (GFR) was 68.7 mg/mL/1.73m^2 in the FD group and 50.14 mg/mL/1.73m^2 in the DR group. The most common comorbidity in both groups was arterial hypertension (69.2% of the FD group and 85.7% of the DR group). The majority (76.9%) of the FD group were anticoagulated with apixaban, while in the DR group there was not a preferential DOAC. All patients were polymedicated: statins (92.3%) and beta blockers (61.5%) were the most common drugs in the FD group; whereas in the DR group angiotensin-converting-enzyme inhibitor or angiotensin II receptor blockers (85.7%), diuretics (71.4%) and statins (71.4%) were the most co-prescribed medications.

Table 1 Baseline characteristics and comorbidities of both groups

	Full-dose group (n = 13)	**Dose-reduction group (n = 7)**	**p value**
Age (years) mean±SD	72.08±6.2	81.86±5.0	0.002
Female n (%)	9 (69.2)	3 (42.9)	0.274
BMI (kg/m2) mean±SD	24.95±7.72	24.89±2.34	0.971
Creatinine plasma levels (mg/dL) mean±SD	0.97±0.31	1.16±0.50	0.353
GFR (mg/mL/1.73m^2) mean±SD	68.7±26.78	50.14±22.65	0.156
Comorbidities			
Diabetes mellitus n (%)	2 (15.4)	1 (14.3)	0.999
Arterial hypertension n (%)	9 (69.2)	6 (85.7)	0.613
Stroke n (%)	2 (15.4)	0	0.521
Acute myocardial infarct n (%)	2 (15.4)	1 (14.3)	0.999

BMI – body mass index; GFR – glomerular filtration rate.

Table 2 Type of DOAC and concomitant medication of both groups

	Full-dose group (n = 13)	Dose-reduction group (n = 7)	p value
Type of DOAC			
Apixaban n (%)	10 (76.9)	2 (28.6)	0.108
Edoxaban n (%)	2 (15.4)	2 (28.6)	0.108
Rivaroxaban n (%)	1 (7.7)	1 (14.3)	0.108
Dabigatran n (%)	0	2 (28.6)	0.108
Concomitant medication			
ACEI/ARB n (%)	6 (46.2)	6 (85.7)	0.158
CCB n (%)	3 (23.1)	3 (42.9)	0.613
BB n (%)	8 (61.5)	3 (42.9)	0.642
Diuretics n (%)	4 (30.8)	5 (71.4)	0.160
Statins n (%)	12 (92.3)	5 (71.4)	0.270
Allopurinol n (%)	0	3 (42.9)	0.031
Amiodarone n (%)	2 (15.4)	2 (28.6)	0.587
Antiplatelet agents n (%)	1 (7.7)	0	0.999
Digoxin n (%)	0	2 (28.6)	0.111
Metformin n (%)	1 (7.7)	1 (14.3)	0.999
Insulin n (%)	1 (7.7)	0	0.999
Benzodiazepines n (%)	4 (30.8)	2 (28.6)	0.999
SSRI n (%)	3 (23.1)	1 (14.3)	0.999
PPI n (%)	5 (38.5)	2 (28.6)	0.999
Levotiroxin n (%)	0	2 (28.6)	0.111

DOAC – direct oral anticoagulant; ACEI – angiotensin-converting enzyme inhibitor; ARB – angiotensin II receptor blocker; CCB – calcium channel blocker; BB – beta blocker; MRA – mineralocorticoid receptor antagonist; SSRI – selective serotonin reuptake inhibitor; PPI – proton pump inhibitor.

Coagulation tests

Although all the TGT parameters significantly correlated with DOAC concentration, thrombin peak height showed the best correlation ($r=-0.74$, $p<0.001$). Conversely, ETP showed the weakest, although significant, correlation with DOAC levels ($r=-0.65$, $p=0.002$).

No statistically significant differences were found in the TGT parameters and DOAC concentration between the FD group and the DR group (Table 3), indicating that tailoring doses in clinically-indicated patients is effective in avoiding supra- or infratherapeutic DOAC levels.

Table 3 TGT parameters and DOAC concentration in both groups

	FD group	DR group	p value
lag time (min) mean±SD	2.58±1.0	3.45±0.8	0.064
peak height (nM) mean±SD	240.76±97.1	235.16±96.5	0.903
time to peak (min) mean±SD	5.31±2.0	6.11±1.6	0.365
ETP (nM.min) mean±SD	1388.46±160.4	1372.57±199.7	0.848
velocity index (nM/min) mean±SD	157.40±102.3	184.41±172.0	0.662
DOAC concentration (IU/mL) mean±SD	102.08±71.8	126.71±41.6	0.345

FD – full dose; DR – dose reduction; ETP – endogenous thrombin potential.

Clinical factors and anticoagulation levels

Age (dichotomized at 80 years) was not related to alterations in the TGT parameters, neither was bodyweight (cutoff of 60 kg). On the other hand, as expected, impaired renal function (cutoff GFR of 50 ml/min/1.73m^2) was associated with an increase in lag time (t=-2.453; p=0.027) and in time to peak (t=-2.242; p=0.041).

Concerning the DDIs of DOACs, we found a positive correlation with (1) amiodarone, which led to an increase in anti-factor Xa activity (t=-3.092, p=0.006) (Fig. 2); (2) PPIs, resulting in a decrease in the velocity index (t=2.358; p=0.03); (3) digoxin, causing an increase in both peak height (t=-2.173; p=0.043) and velocity index (t=-4.245; p<0.001); (4) allopurinol, which was correlated with a decrease in peak height (t=2.363; p=0.034); and (5) SSRIs, leading to a decrease in anti-factor Xa assay (t=2.252; p=0.037) (see Fig. 3). A comparison was not possible for mineralocorticoid receptor antagonist (MRA), antiplatelet agents and insulin, since we only had one patient taking each of these drugs. We found no correlation between the remaining pharmacological classes of our patients and the TGT.

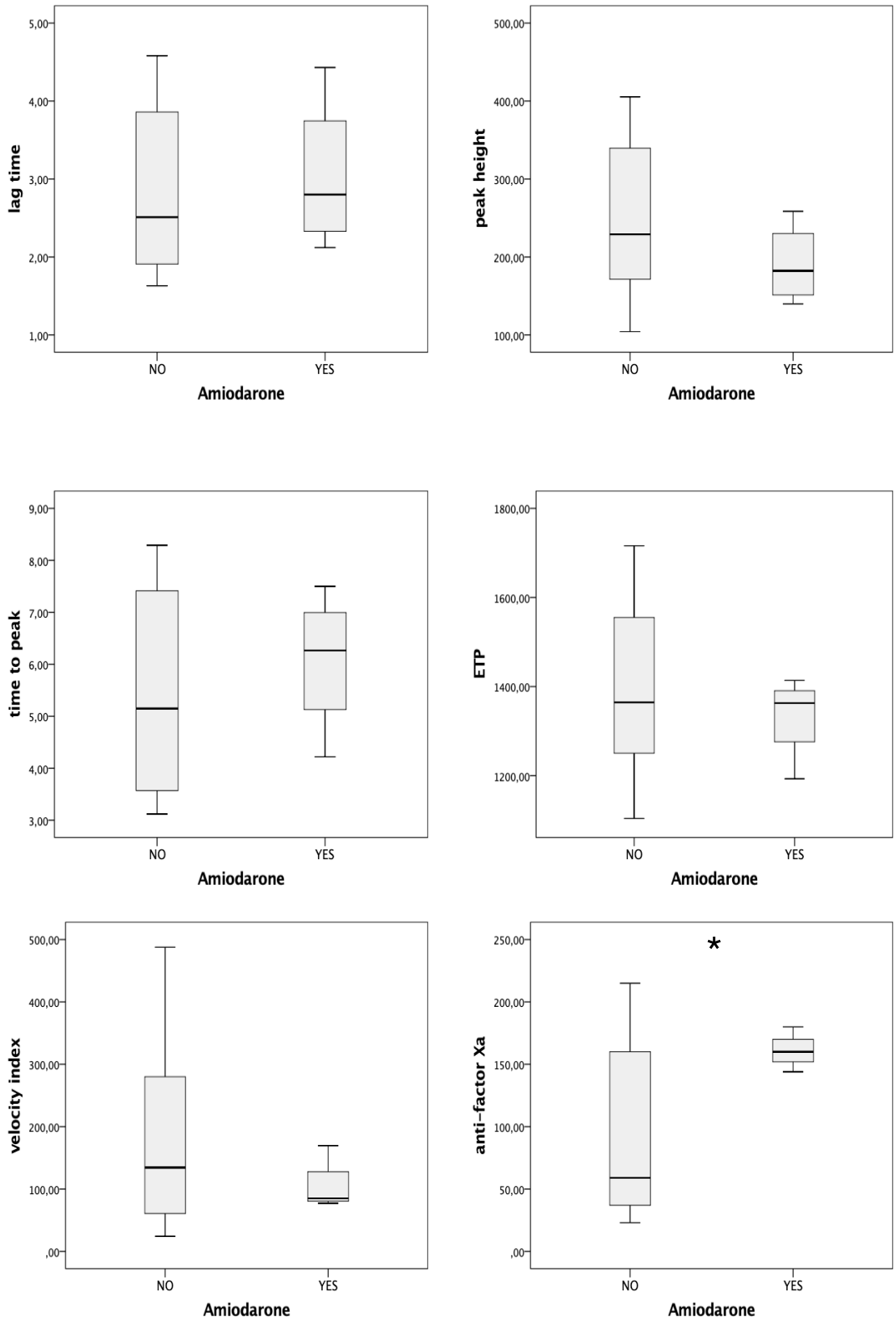

Figure 2 Boxplot figures showing effects of amiodarone therapy in TGT parameters and anti-factor Xa activity.
TGT – thrombin generation test; ETP – endogenous thrombin potential; * – statistically significant differences.

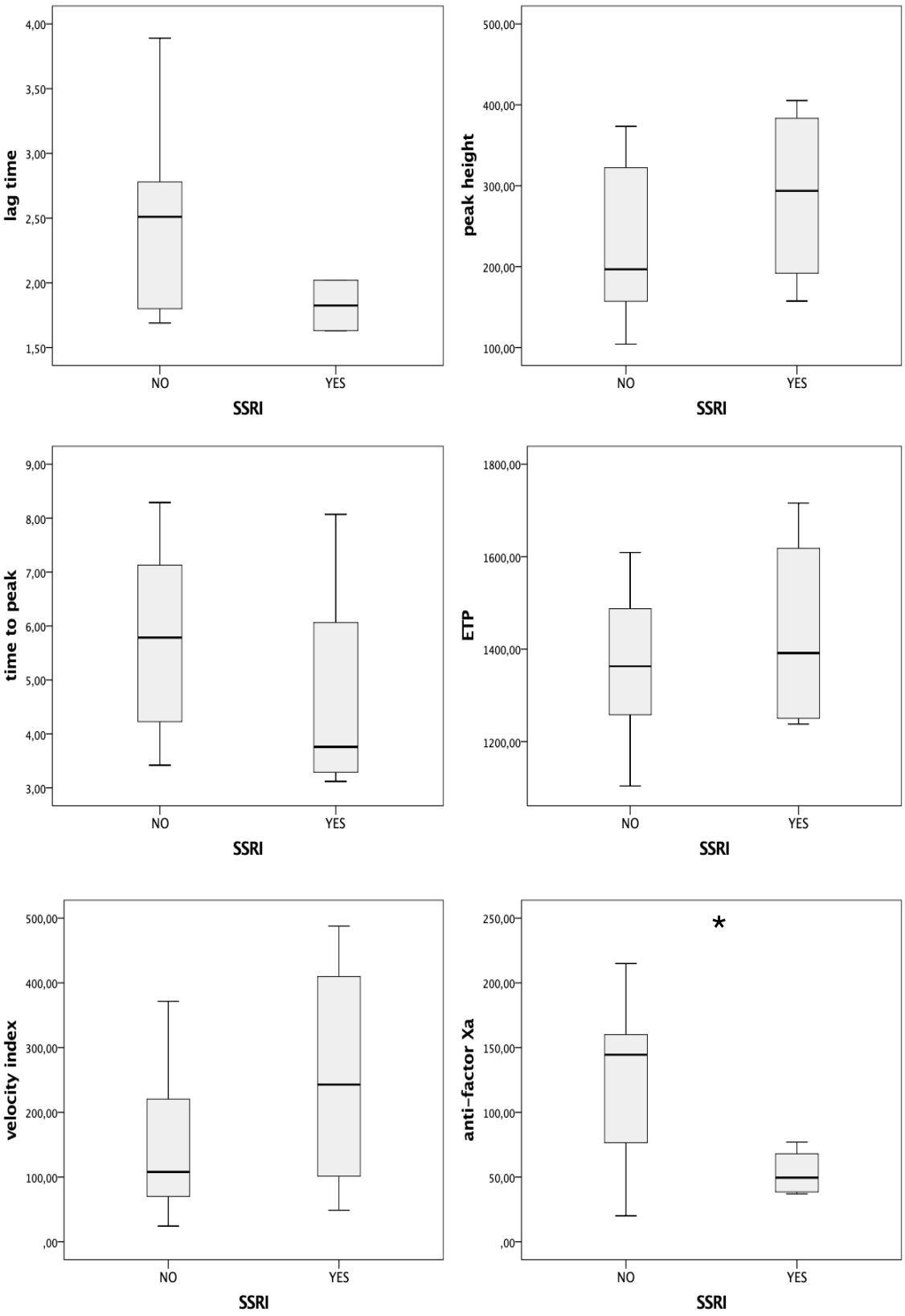

Figure 3 Boxplot figures showing effects of SSRI therapy in TGT parameters and anti-factor Xa activity. SSRI – selective serotonin reuptake inhibitor; TGT – thrombin generation test; ETP – endogenous thrombin potential; * – statistically significant differences.

DISCUSSION

In this study, we globally aimed to use TGT parameters to compare anticoagulation levels among patients treated with a DOAC at a full dose to patients medicated with a clinically-indicated dose reduction strategy. We found no statistically significant differences in TGT parameters between groups, signaling that dose reduction is effective on avoiding supra- or infratherapeutic drug levels. Thus, tailoring the DOAC dose based on clinical criteria to fit the needs of individual patients helps balancing the thrombotic and bleeding risk. The same strategy has been postulated by other authors, although not on the basis of TGT parameters.[15]

TGT is a promising tool to monitor patients on DOAC, as it evaluates thrombin generation (procoagulant driver) and inhibition (anticoagulant driver), thus evaluating the balance between the two. As suggested by other authors,[16] we confirmed that the DOAC anticoagulant effect can be measured using TGT. In fact, a significant correlation between TGT parameters and DOAC concentration was found. From all TGT parameters, peak height showed the best correlation with DOAC concentration, thus making it a potentially useful parameter to monitor DOAC dosing. On the other hand, ETP was the parameter that correlated the least with DOAC levels. We believe that this is because the hemorrhagic risk is variable in each patient and cannot be interpreted solely based on anticoagulation levels.

Our study also evaluated the effect of other comorbidities in TGT parameters. Since DOACs are excreted in variable amounts through the kidneys, GFR is an important laboratory parameter when treating these patients. Patient with renal insufficiency tend to accumulate the drug in the circulation, thereby increasing the risk of bleeding.[17] Not surprisingly, we found that impaired renal function (GFR < 50 ml/min/1.73m^2) was associated with a shift in the TGT curve, namely an increase in lag time ($t=-2.453$; $p=0.027$) and in time to peak ($t=-2.242$; $p=0.041$). Therefore, these data confirm the importance of adjusting the DOACs dose to renal function and might signal the interest of measuring TGT variables in patients with rapid shifts in GFR.

Despite previous studies have identified bodyweight under 60 kg as a predictor for bleeding events,[18] we could not find any correlation between bodyweight and alterations in the TGT parameters.

All DOACs are substrates of P-glycoprotein, while edoxaban, apixaban and rivaroxaban also undergo CYP3A4 metabolism. Furthermore, drugs that impair hemostasis may increase the probability of a hemorrhagic event if given in concomitance with a DOAC.[19] Therefore, we aimed to study the possible DDIs of DOACs by evaluating the effects of co-medication of specific drugs through the use of TGT and DOAC concentration.

In spite of the fact that DDIs between amiodarone and DOACs have been reported, results were not always concordant. A sub analysis of the ARISTOTLE trial could not find significant differences in major bleeding events among those with the association and those who were not on amiodarone.[20]

Furthermore, a review by Stöllberger,[7] considering DDIs of DOACs in elderly patients, had inconsistent results regarding the effects of a comedication with amiodarone and DOACs: it affected the bioavailability of dabigatran but showed only small to moderate effects on exposure at steady state; a non-significant interaction with apixaban; a modest effect on edoxaban. On the other hand, a retrospective cohort study that took place in Taiwan found a significantly superior number of hemorrhagic events in patients under amiodarone plus a DOAC.[21] In our study, we did not find a significant difference in the thrombin generation curves of patients under amiodarone, compared with those without amiodarone. Interestingly, we demonstrated that amiodarone increases anti-factor Xa activity, thus favoring a hypocoagulable state. Moreover, it can be seen in Fig. 2 that patients under amiodarone tend to have higher lag time and time to peak, as well as reduced peak height and velocity index, in comparison to patients not taking amiodarone. It is well known that TGT is influenced by many factors beyond pharmacologic interactions, so a definitive conclusion cannot be made based solely on this result. However, the association between amiodarone and a DOAC is relatively common, particularly in patients with AF, making these individuals candidates for close monitoring and eventually dose-reduction.

The interaction between PPIs and DOACs, specifically dabigatran, has been widely studied. By increasing gastric pH, PPIs might decrease bioavailability of dabigatran, although this finding has not been associated with relevant clinical events.[5,7] When PPI users were analyzed with TGT, we found a decrease in the velocity index but no significant alterations in the other parameters, which is in accordance with the literature.

Digoxin, a P-glycoprotein competitor, is a relatively common co-prescribed medication with DOACs for AF, representing 28.6% of our DR group. In our study, we found that digoxin was associated with a significant increase in both peak height and velocity index, signaling that these patients produce more thrombin. Through the literature we learned that Stangier *et al.* [22] concluded that the plasma levels of dabigatran were not particularly affected with the co-administration of digoxin; and that Chang *et al.*[21] reported a marginal lower bleeding rate associated with the drug, thus making it a likely safe combination.

We examined 3 patients with concomitant use of allopurinol and a DOAC, all of whom had a GFR of under 45 mL/min/1.73m^2 and 2 of them were in the DR group. In the TGT analysis, we found a statistically significant decrease in peak height, which means these patients had a lower concentration of thrombin when compared to their peers. To the best of our knowledge, no association has been made between the DDIs of allopurinol and DOACs. Since all 3 individuals had impaired renal function, this hypocoagulable state might be more associated to their GFR than with a DDI between allopurinol and DOACs.

It has been hypothesized that the concomitant use of SSRI and a DOAC increases bleeding risk because these antidepressants induce a qualitative platelet defect due to their effect on serotonin. Our analysis found divergent results: a statistically relevant decrease of anti-factor Xa activity in the patients

that were under SSRI, indicating that these 4 patients (3 in the FD group and 1 in the DR group) were actually less prone to a bleeding event. Regarding the TGT parameters, no significant modification was found. However, in Fig. 3 we can see that ETP and peak height are higher in patients taking SSRI and lag time and time to peak are reduced in comparison with the population that did not take SSRI, which is in accordance to the conclusions of an analysis from the ROCKET AF trial[23] that suggest the addition of SSRIs to a DOAC does not predispose for an hemorrhagic event.

Atorvastatin, by being both an inhibitor of P-glycoprotein and CYP3A4, has been portrayed as a drug that could potentially be associated with a higher incident of major bleeding events when used concomitantly with a DOAC. However, that was not the case in our population. Our study found no statistically significant variations in the TGT of patients under the two drugs, which represented the majority of individuals in both groups (92.3% of the FD group and 71.4% of the DR group). We found similar results in the literature, signaling that atorvastatin does not increase bleeding risk and can be used in concomitance with a DOAC.[7,21]

Concerning CCB, we found no correlation between those under a drug of this class and the TGT. That might be explained by the fact that verapamil and diltiazem have different inhibitory potencies of P-glycoprotein, thus having distinctive effects on the bioavailability of DOACs.

In summary, the results obtained regarding DDIs reinforce the idea that DOACs have fewer DDIs than VKA but may still need dose adjustment in patients taking specific drugs, more so in individuals with renal impairment or other clinical criteria for dose reduction.

This study has several limitations. First, the small sample size limits our conclusions regarding the clinical characteristics and DDIs more frequently associated with the need for dose reduction. Additionally, not all patients had recent blood analysis so information such as creatinine plasma levels was missing in some individuals. Finally, we did not measure the plasma levels of other drugs, therefore we are only based on patient information regarding its administration (and do not really know if they were taking the drug). Despite its use as a research tool for many years, TGT is still not part of routine clinical practice. This is probably due to the lack of an international standardized procedure, which correlates into a high inter-laboratory variability.[24] However, when preanalytical variables are diminished by applying a protocol such as the one developed by R. Loeffen *et al.*[25], the coefficient of variation was <10% for most of the parameters of thrombin generation, translating into adequate validation criteria.

There is a need for further studies regarding the DDIs of DOACs, particularly regarding amiodarone as it is a frequently prescribed drug in AF patients. Although we could not find statistically significant changes in the TGT curve, we believe there could be a higher risk of bleeding among patients with this combination. On the other hand, our study corroborated the findings from Quinn *et al.*[23] regarding DDIs between SSRI and DOACs and we believe it might be considered a safe combination.

In conclusion, patients meeting dose reduction criteria benefit from dose adjustment. This fact is supported by the similar results in the TGT obtained in the FD group and the DR group. We believe

that this test will be of grand importance in the near future, by being able to measure the formation of thrombin in the plasma, helping clinicians to adjust the dose of DOAC according to the patients' comorbidities and possible DDIs in order to find the perfect balance between ischemic and bleeding risk.

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