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ÂNGELA MARGARIDA MARTINS DE CASTRO

DIRECT ORAL ANTICOAGULANTS: A THERAPEUTIC ADHERENCE STUDY AND THE THROMBIN GENERATION ASSAY

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PROFESSOR DOUTOR RUI MIGUEL TERENAS LANÇA BAPTISTA
DOUTOR JOÃO MARIANO PEGO

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DIRECT ORAL ANTICOAGULANTS: A THERAPEUTIC ADHERENCE STUDY AND THE THROMBIN GENERATION ASSAY

Ângela Castro[3]; Cátia Ferreira MD[1]; João Pego MD[2]; Rui Baptista[1,3], MD, PhD

1. Cardiology A Department, Centro Hospitalar e Universitário de Coimbra, Portugal
2. Clinical Pathology Department, Centro Hospitalar e Universitário de Coimbra, Portugal
3. Faculty of Medicine, University of Coimbra, Portugal

Rui Miguel Terenas Lança Baptista, MD, PhD
Azinhaga de Santa Comba, 3000-548, Coimbra
rui.baptista@fmed.uc.pt

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LIST OF ACRONYMS

AF – Atrial Fibrillation
APTT – Activated Partial Thromboplastin Time
BMI – Body Mass Index
CAT – Calibrated Automated Thrombogram
DOAC – Direct Oral Anticoagulants
ECA-II – Ecarin Chromogenic Assay II
ETP – Endogenous Thrombin Potential
GFR – Glomerular Filtration Rate
HP – Height Peak
INR – International Normalized Ratio
LT – Lag Time
MI – Myocardial Infarction
OAC – Oral Anticoagulants
PE – Pulmonary Embolism
PT – Prothrombin Time
SD – Standard Deviation
SMAQ – Simplified Medication Adherence Questionnaire
ST – Start Tail
TF – Tissue Factor
TGA – Thrombin Generation Assay
TTP – Time To Peak
VI – Velocity Index
VKA – Vitamin K Antagonists
VTE – Venous Thromboembolism
WHO – World Health Organization

ABSTRACT

Introduction: The use of direct oral anticoagulants (DOAC) is increasing, mainly due to their fixed therapeutic doses and limited monitoring requirements as well as their pharmacokinetic profile. The thrombin generation assay (TGA) is a promising tool to monitor patients on DOAC treatment, besides the ecarin chromogenic assay (ECA-II) for dabigatran and the chromogenic antifactor-Xa assay for rivaroxaban, apixaban and edoxaban which efficacy is already well known.

Aims: We aimed to assess the rate of medication adherence of patients on undergoing treatment with DOAC and evaluate whether there is a correlation between medication adherence and TGA parameters and DOAC concentrations.

Methods: This cross-sectional study included 20 senior patients on DOAC treatment for atrial fibrillation (AF) and pulmonary embolism (PE) prophylaxis. Patients completed the Simplified Medication Adherence Questionnaire (SMAQ) for assessing adherence to medication. DOAC concentrations were measured using STA®-ECA-II and STA®-Liquid Anti-Xa. TGA parameters were also measured using ST Genesia® analyzer. It was studied the correlation between: TGA parameters and DOAC concentrations, DOAC concentrations and SMAQ results, SMAQ results and TGA parameters. It was also tested if there were any predictors of greater adherence to medication.

Results: The mean age was 75.5±7.4 years; 60% were female and three quarters (n=15) of patients were polymedicated. The majority of the patients were taking apixaban (n=12, 60%), while 4 patients were on edoxaban, 2 on rivaroxaban and 2 on dabigatran. All patients were on appropriate DOAC doses according to clinical criteria. Regarding the adherence to medication, it was identified a rate of adherence of 40% (n=8). There were not recognized predictors of low adherence. Although all the TGA parameters were significantly correlated with DOAC concentrations, thrombin height peak (HP) showed the best correlation (r=-0.742, p<0.001). Endogenous thrombin potential (ETP) was also strongly correlated with DOAC levels (r=-0.649, p=0.002) which may represent the variable haemorrhagic risk in each patient, taking into account that previous studies have shown that ETP is strongly associated with bleeding risk. Regarding the SMAQ, higher scores (low adherence) were moderately correlated with higher ETP (r^2=0.460, p=0.04). No statistically difference was found in the mean of plasmatic concentrations of DOAC between the non-adherent group ($\bar{x}$=97.25 ng/ml) and the adherent one ($\bar{x}$=130.87 ng/ml) (p=0.985).

Conclusion: Our findings support the concept that TGA parameters are potentially useful for monitoring treatment with DOAC and adherence to anticoagulation treatment. Howsoever, future studies combining clinical data with TGA are needed to implement the use of TGA as routine in the clinical laboratory. Adherence to DOAC is a concerning problem that requires incisive adherence-improving strategies and personalized treatment to patient's needs and preferences.

Keywords: DOAC; Anticoagulant; Thrombin Generation Assay; Treatment Adherence; Blood coagulation.

RESUMO

Introdução: O uso dos Anticoagulantes Orais Diretos (DOAC) tem vindo a aumentar, principalmente devido às suas doses terapêuticas fixas e necessidade limitada de monitorização, assim como ao seu perfil farmacocinético. O Teste de Geração de Trombina (TGA) é um instrumento promissor para a monitorização de doentes sob terapêutica com DOAC, para além do ensaio cromogénico da ecarina (ECA-II) usado para o dabigatrano e o ensaio cromogénico anti fator Xa para o rivaroxabano, apixabano e edoxabano, cujas eficácias já estão bem documentadas.

Objetivos: O nosso objetivo é aferir a taxa de adesão à terapêutica dos doentes sob tratamento com DOAC e avaliar se existe uma correlação entre a adesão à medicação e os parâmetros do TGA e concentrações de DOAC.

Métodos: Este estudo transversal incluiu 20 doentes seniores sob tratamento com DOAC para a Fibrilhação Auricular (FA) e profilaxia de Embolia Pulmonar (PE). Os doentes completaram o Questionário Simplificado de Adesão à Terapêutica (SMAQ) para aferir a sua adesão à medicação. A concentração de DOAC foi medida através do STA®-ECA-II e o STA®-Liquid Anti-Xa. Os parâmetros do TGA foram também medidos através do ST Genesia® analyzer. Foi estudada a correlação entre: parâmetros do TGA e concentrações de DOAC; concentrações de DOAC e resultados do SMAQ; resultados do SMAQ e parâmetros do TGA. Foi também estudado se existiam preditores de adesão à medicação.

Resultados: A média de idades foi de 75,5±7,4 anos; 60% eram do sexo feminino. Três quartos (n=15) dos doentes estavam polimedicados. A maioria dos doentes estava medicado com apixabano (n=12, 60%), enquanto que 4 estavam sob edoxabano, 2 sob rivaroxabano e 2 sob dabigatrano. Todos os doentes estavam medicados com doses apropriadas de DOAC de acordo com critérios clínicos. No que refere à adesão à terapêutica, foi identificada uma taxa de adesão de 40% (n=8). Não foram reconhecidos preditores de baixa adesão. Apesar de todos os parâmetros do TGA estarem significativamente correlacionados com as concentrações de DOAC, o pico de altura da trombina (HP) mostrou ter a melhor correlação ($r=-0,742$, $p<0,001$). O potencial endógeno da trombina (ETP) também estava fortemente correlacionado com os níveis plasmáticos de DOAC ($r=-0,649$, $p=0,002$) o que pode representar a variável de risco hemorrágico para cada doente, tendo em conta que estudos prévios mostraram que o ETP está fortemente associado a risco de hemorragias. Relativamente ao SMAQ, pontuações elevadas (baixa

adesão) correlacionaram-se com um ETP elevado (r^2=0,460, p=0,04). Não foi encontrada diferença estatisticamente significativa nas médias das concentrações plasmáticas de DOAC entre os grupos não-aderente ($\bar{x}$=97,2500 ng/ml) e aderente ($\bar{x}$=130,8750 ng/ml) à medicação (p=0,985).

Conclusão: Os nossos resultados apoiam o conceito de que os parâmetros do TGA são potencialmente úteis para monitorizar o tratamento com DOAC e a adesão à terapêutica anticoagulante. Contudo, são necessários estudos futuros que combinem dados clínicos com o TGA para implementar o uso do mesmo como rotina na prática clínica laboratorial. A adesão aos DOAC é um problema sisudo que requer estratégias incisivas de melhoria da adesão assim como um tratamento personalizado às necessidades e preferências dos doentes.

Palavras-chave: DOAC; Anticoagulante; Teste de Geração de Trombina; Adesão ao tratamento; Coagulação sanguínea.

INTRODUCTION

Oral anticoagulants (OAC) are the basis for prevention and treatment of thromboembolic disorders. The main indications for oral anticoagulation are (1) atrial fibrillation (AF), (2) venous thromboembolism (VTE) and (3) prevention of surgical and medical thromboembolism.[1]

Limitations of previous OAC have led to the development of newer therapies. In the last years, the development of direct oral anticoagulants (DOAC) that dose-dependently inhibit thrombin or inhibit the activated factor Xa has led them to be preferred over warfarin and another vitamin K antagonists (VKA). Despite being highly effective, its use is limited by a fine therapeutic index that requires frequent monitoring and dose adjustments.

The use of DOAC comprehends the main advantages of having (1) rapid onset/offset of action, (2) fixed therapeutic doses according to the indication and some individual clinical criteria, (3) no need for controlling the level of anticoagulation through the International Normalized Ratio (INR), (4) expectable pharmacokinetic and pharmacodynamic profiles and (5) less drug-drug interactions.[2,3] Multiple studies also confirmed similar or less bleeding risk although maintaining equivalent or better efficacy than VKAs.[4] However, the lack of frequent monitoring related to DOAC may induce a reduction in adherence, which can lead to potentially severe consequences.[1]

Due to the fact that monitoring is not necessary, the availability of tests for assessment of the anticoagulant effect is low. However, they might be required in specific situations such as overdose, bleeding, urgent surgery, need for reversal of anticoagulation or suspected non-compliance with therapy. Therefore, new assays to estimate the bleeding risk and efficacy of these drugs are undoubtedly in demand.[5]

The most commonly available tests to monitor these patients are Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT). However, these tests, which only evaluate the initial phase of coagulation, are poorly sensitive and specific for DOAC and not suited to represent the balance of coagulation that occurs in vivo[6,7], although some DOAC do affect their values.

The test of choice for measure the DOAC anticoagulant effect is the ecarin chromogenic assay (ECA-II) for dabigatran and the chromogenic antifactor-Xa assay for rivaroxaban, apixaban and edoxaban. Although these tests show an excellent linear correlation with the dosage and are useful for measuring plasma concentrations of DOAC, they are not an overall test, which means that they do not reflect the state of the coagulation system as a whole.[8]

Henceforth, promising new data have arisen on the thrombin generation assay (TGA), which measures the ability of plasma to generate thrombin, the final result of the coagulation

cascade and a proven target for anticoagulant therapies.[8] To date, TGA has been used mainly to compare the efficacy of different anticoagulant drugs in healthy volunteers but also to predict ischemic and bleeding adverse events during anticoagulant therapy while monitoring the efficacy of anticoagulant drugs[5].

It is well known that the safety and effectiveness of these drugs is highly reliant on the patients' adherence to medication. The World Health Organization (WHO) defines adherence as "the extent to which a person's behaviour – taking drugs, following a diet, and/or executing lifestyle changes – corresponds with consensus to the recommendations from a health care provider"[24]. It is also known that in chronic conditions such as AF, where patients take these drugs as a preventive measure and symptoms are recurrently intermittent or not present, rates of nonadherence can be as high as 50%[9]. Some studies also demonstrate the association between full adherence to therapies and lower rate of major cardiovascular events and cost savings.[12]

Therefore, non-adherence to DOAC remains a matter of concern, once they have a short half-life which overvalues the impact of treatment breaches. Additionally, the absence of routine monitoring may result in increased risk for stroke, bleeding and death.[10] Even though nonadherence is a subject of concern, the studies related with adherence to DOAC are inconsistent and scarce.

In this study, we globally aimed to study the adherence of patients to DOAC therapy. We specifically aimed to assess if TGA is a useful instrument to monitor DOAC treatment, as we hypothesized that TGA parameters are correlated with DOAC plasmatic concentrations. Additionally, we specifically aimed to study whether there was a correlation between adherence, assessed by a validated questionnaire, and the efficacy of anticoagulation, measured by TGA, ECA-II and chromogenic antifactor Xa assay. We hypothesized that low adherence was correlated with lower levels of anticoagulation, assessed by several parameters. Finally, we specifically aimed to assess which parameters of the patient's personal and medical history were most closely related with adherence to therapy. We hypothesized that a distinct set of clinical and demographical parameters can influence adherence.

MATERIAL AND METHODS

Study Design

We conducted this cross-sectional, single-center study at the Centro Hospitalar e Universitário de Coimbra.

Study Population

We consecutively enrolled 20 patients treated with DOACs for AF and pulmonary embolism (PE) prophylaxis. Patients included in the study were taking one of four DOAC: apixaban, edoxaban, rivaroxaban or dabigatran. All patients were on appropriate DOAC dosing, according to the recommended clinical reduction criteria.

Data Collection

The demographic and clinical variables were collected in an anonymized database: gender, age, marital status, education level, household, height (cm), weight (kg), sedentary lifestyle, dyslipidaemia, diabetes, hypertension, history of stroke or myocardial infarction (MI), coronary disease, congestive heart failure, smoking and alcoholic habits, creatinine levels (mg/dL), estimated glomerular filtration rate (GFR) ($mg/mL/m^2$) and current medication. Data were collected after authorization and written informed consent signed by patients.

Study Protocol

After signing the informed consent and collecting the clinical and adherence questionnaire (SMAQ, see below), a blood sample was collected by phlebotomy into a tube containing 0.109M trisodium citrate as an anticoagulant. Plasma samples were then centrifuged twice at 2000-2500 g for 10 minutes at room temperature within 60 minutes of blood collection and subsequently stored at -70°C or lower. Before analyzing the sample, it was defrosted in a 37°C water bath for the time needed to obtain complete thawing. Plasma was then homogenized and samples were processed immediately after homogenization.

DOAC concentrations were then measured in valley phase, using STA®-ECA-II for dabigatran and STA®-Liquid Anti-Xa with specific calibration for apixaban, edoxaban and rivaroxaban on the STA® line equipment. These are chromogenic assays which specifically measures the anticoagulant effect of direct thrombin inhibitors.

TGA parameters were also measured in the same phase (lag time (LT), endogenous thrombin potential (ETP), thrombin height peak (HP), time to peak (TTP), velocity index (VI) and Start Tail (ST). The method used in this study was performed with the STG® - DrugScreen kit, that provides quantitative determination of thrombin generation in plasma using a fluorogenic method on the ST Genesia® analyzer, an entirely automatized system that allows acquisition and interpretation of the thrombin generation curves in a calibrated Thrombogram®. Unlike traditional clotting assays, the Thrombogram represents the generation and inhibition of thrombin in a dynamic manner.

The STG® - DrugScreen contains 4 reagents: (1) STG® - DrugScreen - trigger reagent with a high concentration of human recombinant tissue factor and phospholipids vesicles, lyophilized; (2) STG® - RefPlasma DS - reference plasma; (3) STG® - QualiTest Low DS - control plasma containing citrated hypocoagulable human plasma; (4) STG® - QualiTest Norm DS - control plasma comprised of citrated normal human plasma.

TGA

The TGA has the ability to assess the stability between the action of the procoagulant driver and the anticoagulant driver to evaluate both thrombin generation and decay.[7] In our study, it was used the ST Genesia® analyzer, which is an automated analyzer, based on the fluorescence principle, as well as the Calibrated Automated Thrombogram (CAT) system (the most studied method for thrombin generation)[13], with the main difference of being designed to support clinical applications in vitro while CAT's main purpose is for research and development. ST Genesia® offers a fully automated system to measure thrombin generation in platelet-poor plasma and aims at standardizing and automating the TGA in order to make it widely accessible and available for use in-vitro diagnostics.

ST Genesia® assay depends on a fluorogenic substrate to monitor thrombin activity in plasma. With this assay, there is required STG-Drugscreen®, STG-Thrombiclean® which is a decontamination solution and STG-Cal&Fluo® which include (1) STG-Thrombical – a calibrator with a fixed amount of thrombin; (2) STG-Fluostart which contains calcium and fluorogenic substrate important for initiation of thrombin generation; (3) STG-Fluoset, a fixed amount of fluorochrome which allows eliminating optical interferences.

Then, fluorescence readings are converted into thrombin generation curves by software. The curve obtained by calibration is necessary to convert raw fluorescence units into thrombin concentration units, expressed in nM.[13,15,16]

The final curve describes the variation of thrombin amount during the activation of coagulation cascade, and several parameters are obtained: (1) TTP reflects the time to the

high peak of thrombin generation, (2) ETP is the area under the curve and mirrors the total amount of thrombin generation, (3) HP is the maximum amount of generated thrombin, (4) LT translates to the time expended to begin clot formation, (5) VI is the maximum speed of thrombin generation (VI = HP/(TTP-LT)) and ST represents at which time all formed thrombin is inactivated. ETP is considered the best parameter to assess risk of bleeding or thrombosis.[2,13]

In an abbreviated way, a prolonged lag time, a reduced ETP and a reduced peak height can describe an hypocoagulable state.[17]

An infra-therapeutic concentration was considered when plasmatic concentrations of DOAC were: (1) inferior to 28-155 ng/ml for dabigatran (110 mg 2id) or inferior to 40-215 ng/ml for dabigatran (150 mg 2id); (2) less than 10-40 ng/ml for edoxaban (60 mg 1id); (3) below to 41-230 ng/ml for apixaban (5 mg 2id); (4) inferior to 12-137 ng/ml for rivaroxaban (20 mg 1id).

SMAQ

The SMAQ is a sociodemographic questionnaire that was validated as a method of measuring adherence to therapy, initially to evaluate adherence to antiretroviral treatment by Knobel et al.[18] It was based on the Morisky scale that contained four questions: (1) “Do you ever forget to take your medicine?”; (2) “Are you careless at times about taking your medicine?”; (3) “When you feel better, do you sometimes stop taking your medicine?”; (4) “If sometimes you feel worse when taking the medicine, do you stop taking it?”.

The SMAQ includes six questions:

1. Do you ever forget to take your medicine? Yes/No;
2. Are you careless at times about taking your medicine? Yes/No;
3. If at times you feel worse, do you stop taking your medicine? Yes/No;
4. Thinking about the last week, how often have you not taken your medicine? Never, 1-2 times, 3-5 times, 6-10 times, >10 times;
5. Did you not take any of your medicine over the last weekend? Yes/No;
6. Over the past 3 months, how many days have you not taken any medicine at all? ≤2, >2.

This instrument has been properly translated and validated to Portuguese language.[18,19] Through SMAQ, a patient was considered **nonadherent** when there was a positive response to any of the qualitative interrogations, more than two doses missed over the past week, or more than two days of total non-medication during the past three months.[18]

The SMAQ is a valid marker of patient’s non-adherence to medication, which led us to choose this questionnaire for this study. The main advantages of being inexpensive, reliable (adequate internal consistency and reproducibility), easily applicable in daily medical practice

and fast (under 5 minutes). Its disadvantages include both the dependence on memory and social desirability bias, with a propensity to overestimate adherence, which are common problems in all self-reported reviews.[18] Even so, it showed acceptable levels of sensitivity and specificity when compared with other more objective measures which makes it an instrument that may be applied in most clinical settings.[18]

Results related to SMAQ answers were dichotomized to adherent or non-adherent, using the criteria explained previously as well as organized based on score, being 0 equivalent to "non low adherence answer", 1 equivalent to "1 low adherence answer" and so on. According to the dichotomized scale, if a patient answered in the direction of a low adherence in only one question was automatically considered non-adherent. In this way, the organization by scores and posterior analysis using the cut-offs of the mean/median allows us a better approximation to reality.

We also tested if there were predictors of adherence to medication (clinical factors such as number of daily drugs, dose frequency of DOAC, previous cardiovascular events such as MI and stroke as well as demographic factors such as marital status – married versus unmarried –, level of education – with or without a degree –, gender, age and household). We used the conventional cut off of SMAQ considering as non-adherent if score higher than 0 and another cut-off taking into account the median of the population to improve the sensibility which resulted in non-adherent as if score greater or equal to 2.

Ethical considerations

All ethical procedures for this type of study were fulfilled, especially regarding for confidentiality and anonymity. All information collected that can identify patients will be kept confidential, safeguarding the principles of respect, beneficence, non-maleficence and justice. The written consent was written in an easy-to-understand language with all the necessary information about the research and the possibility of leaving the study at any time and it followed the Helsinki Declaration of the World Medical Association and its updates.

Statistical approach and power calculations

Continuous variables were expressed as mean ± SD (standard deviation). Median and interquartile range were used if the distribution was not normal, assessed by the use of the Kolmogorov-Smirnov test. Categorical variables were presented as frequency and percentages. DOAC concentrations were squared before all statistical analyses to improve the normality of distributions. The student's unpaired T-test was used for comparisons among groups of different DOAC. Categorical variables were compared using Chi-squared

test or Fisher's exact test and continuous variables by student's unpaired T-test. The existence of correlation between variables was assessed by computing the corresponding Spearman and Pearson correlation tests. For predictors' analysis, logistic regression was used. All data were analysed using IBM SPSS Statistics software 24.0[20] with the level of significance set at $p<0.05$.

RESULTS

Global characterization of the sample

The baseline profile of the sample is shown in Table 1. There was a female preponderance, with 12 (60%) females and 8 males (40%), with a mean age of 75.5±7.4 years. The mean body mass index (BMI) was 24.9±3.5. Relatively to marital status, 85% (n=17) of the patients were married, 5% (n=1) divorced and 10% (n=2) widowers. Regarding the education level, 40% (n=8) had a primary education, 45% (n=9) a secondary education and 15% (n=3) a superior degree. In relation to household, 10% (n=2) of patients lived alone, 80% (n=16) with one person, 5% (n=1) with 2 people and 5% (n=1) with four.

None of the patients had history of smoking, 30% of patients (n=6) had alcoholic habits, 15% (n=3) had diabetes *mellitus* type 2, 75% (n=15) hypertension, 10% (n=2) a prior stroke and 15% (n=3) a history of MI. The mean creatinine level was 1.05±0.39 mg/dl and the mean estimated GFR was 61.06±26.16 mg/mL/1.73m^2.

Table 1. Baseline characteristics of the sample

	Frequency
Age	75.5±7.4
Sex n(%)	
Female	12 (60)
Male	8 (40)
BMI	24.9±3.5
Marital status n(%)	
Married	17 (85%)
Divorced	1 (5%)
Widowers	2 (10%)
Education level n(%)	
Primary	8 (40%)
Secondary	9 (45%)
Degree	3 (15%)
Household n(%)	
Alone	2 (10%)
1 person	16 (80%)
2 people	1 (5%)
4 people	1 (5%)
Alcohol use n(%)	6 (30)
Tobacco use n(%)	0 (0)
Medical conditions n(%)	
Diabetes	3 (15)
Hypertension	15 (75)
Stroke	2 (10)
MI	3 (15)
Creatinine mg/dl	1.05±0.39
GFR mg/ml/m^2	61.06±26.16

BMI - Body Mass Index; MI - Myocardial Infarction; GFR - Glomerular Filtration Rate.

The majority of the patients were taking apixaban (n=12, 60%), while 4 patients were on edoxaban, 2 on rivaroxaban and 2 on dabigatran. All patients were on appropriate DOAC doses considering clinical dose reduction criteria. Three quarters (n=15) of patients were polymedicated. Table 2 shows the characterization of the sample according to medication.

Table 2. Characterization of the sample according to medication

	Frequency
OAC n(%)	
Apixaban	12 (60)
Edoxaban	4 (20)
Rivaroxaban	2 (10)
Dabigatran	2 (10)
Medication n(%)	
ACEI/ARB	12 (60)
CCB	6 (30)
BB	11 (55)
MRA	1 (5)
Diuretics	9 (45)
Statins	17 (85)
Alopurinol	3 (15)
Amiodarone/Flecainide	5 (25)
Antiplatelet agents	1 (5)
Oral antidiabetics	2 (10)
Insulin	1 (5)
Levothyroxine	2 (10)
Benzodiazepines	6 (30)
Antidepressants	4 (20)
Antiepiletics	0 (0)
PPI	7 (35)

OAC - Oral Anticoagulants; ACEI/ARB - angiotensin-converting-enzyme inhibitor/ angiotensin II receptor blockers; CCB - Calcium Channel Blockers; BB - Beta Blockers; MRA - Mineralocorticoid Receptor Antagonists; PPI - proton-pump inhibitor.

Characterization of the sample according to adherence to medication

The answers to SMAQ are represented in Table 3. There was a rate of adherence to treatment of 40% (n=8) and non-adherence of 60% (n=12), according to the adherence/non-adherence criteria established previously based on the validation of SMAQ. Organizing the answers by scores, 40% (n=8) were on score 0, 20% (n=4) on score 1, 35% (n=7) on score 2 and 5% (n=1) on score 3.

Table 3. Answers to SMAQ

	n	%
Patients undergoing DOAC	**20**	**100**
	n	**%**
SMAQ 1 – Do you ever forget to take your medicine?		
Yes	7	35
No	13	65
SMAQ 2 – Are you careless at times about taking your medicine?		
Yes	10	50
No	10	50
SMAQ 3 – If at times you feel worse, do you stop taking your medicine?		
Yes	0	0
No	20	100
SMAQ 4 – Thinking about the last week, how often have you not taken your medicine?		
Never	19	95
1-2 times	1	5
3-5 times	0	0
6-10 times	0	0
>10 times	0	0
SMAQ 5 – Did you not take any of your medicine over the last weekend?		
Yes	3	15
No	17	85
SMAQ 6 – Over the past 3 months, how many days have you not taken any medicine at all?		
≤2	20	100
>2.	0	0

DOAC - Direct Oral Anticoagulants; SMAQ - Simplified Medication Adherence Questionnaire.

Description of DOAC concentrations and TGA parameters

There was a wide range of DOAC concentrations measured through STA®-ECA-II and STA®-Chromogenic antifactor-Xa assay as well as TGA results. Three (15%) patients had a therapeutic concentration of DOAC under the recommended, all undergoing apixaban. These patients were treated with 5 mg b.i.d. Table 4 shows the characterization of TGA parameters and figure 1 displays the variation between DOAC concentrations.

Table 4. Description of TGA parameters

	LT	HP	TTP	ETP	V_{max}
Mean	2.89	238.80	5.59	1382.90	166.85
Median	2.54	214.00	5.48	1363.00	113.10
Standard Deviation	1.01	94.39	1.84	170.03	127.01
Percentiles					
25	2.05	158.80	3.74	1244.25	66.43
50	2.54	214.00	5.48	1363.00	113.10
75	3.88	325.60	7.32	1512.50	249.53

LT - lag time; HP - heightpeak; TTP - time to peak; ETP - endogenous thrombin potential; V_{max} - velocity index

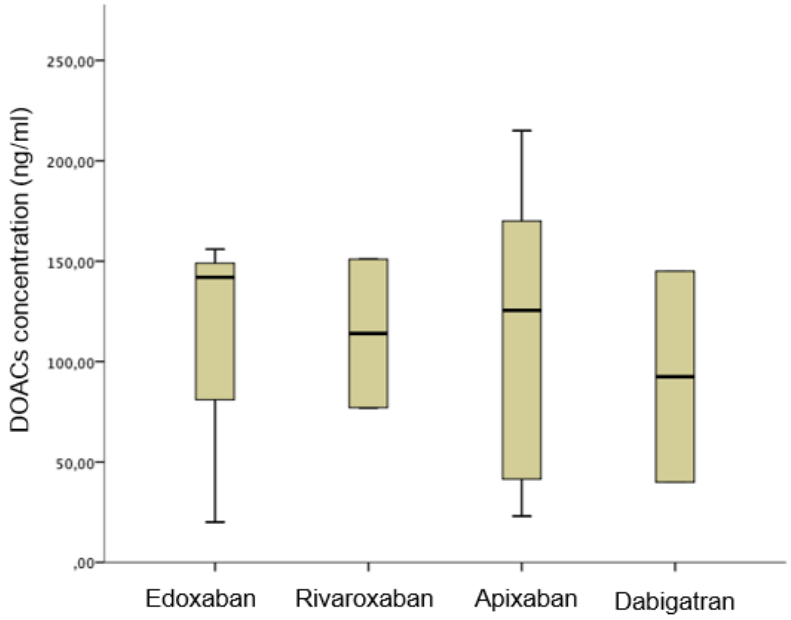

Figure 1. Description of DOAC concentrations

The comparison between groups showed that there were no statistically significant differences between groups of different DOAC in what concerns to TGA parameters and concentrations of DOAC. There was also no difference between the maximum therapeutic dose of DOAC and the adjusted one considering clinical criteria.

Correlations study and mean comparisons

Correlation between TGA parameters and DOAC concentrations

A moderate correlation was found between DOAC concentration and therapeutic concentration ($r=0.547$, $p=0.013$). A strong correlation was found between DOAC concentrations and LT ($r=0.557$, $p=0.011$), HP ($r=-0.742$, $p<0.001$), TTP ($r=0.639$, $p=0.002$), ETP ($r=-0.649$, $p=0.002$), VI ($r=-0.603$, $p=0.005$) and ST ($r=0.713$, $p<0.001$). Figures 2 and 3 show the correlation between DOAC concentrations and HP as well as ETP, respectively.

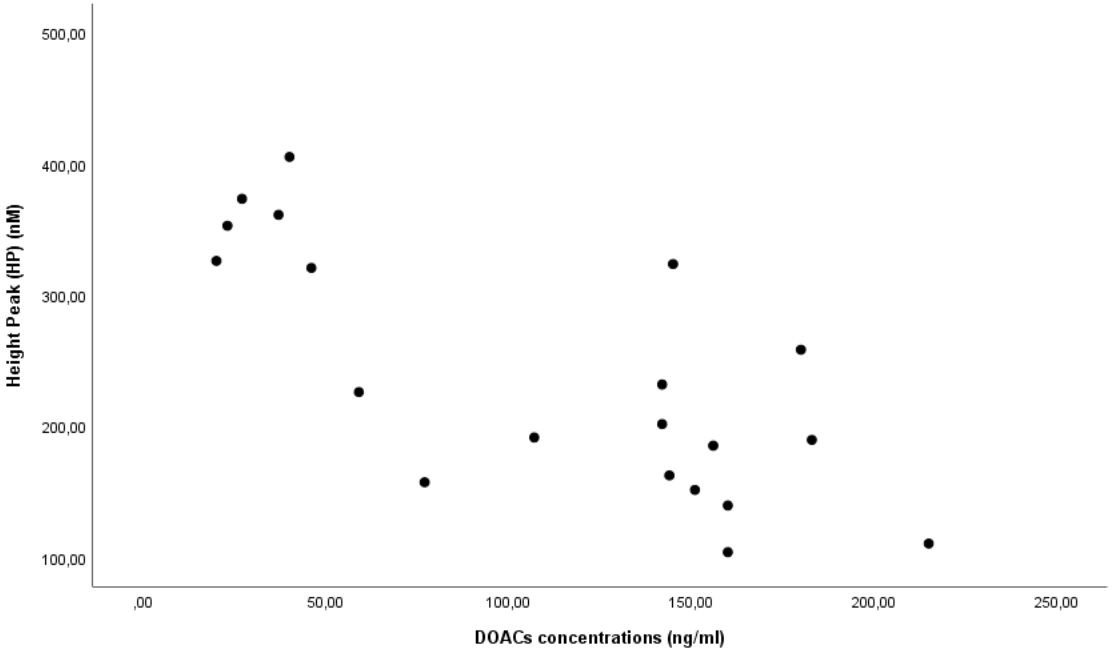

Figure 2. Correlation between DOAC concentrations and HP (r=-0.742, p<0.001).

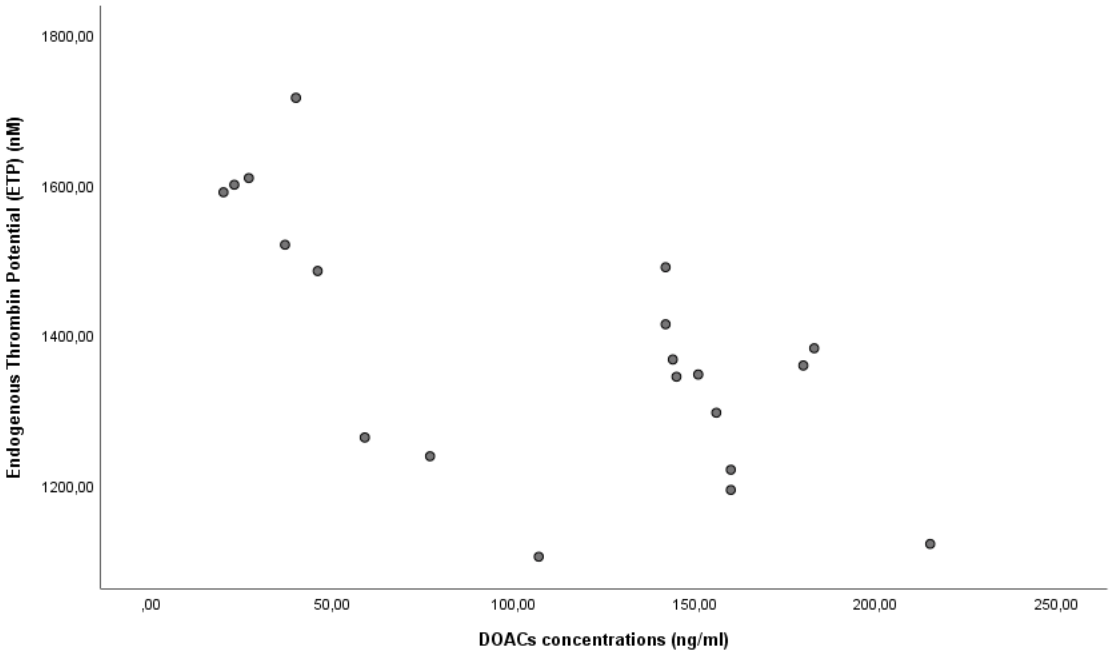

Figure 3. Correlation between DOAC concentrations and ETP (r =-0.649, p=0.002)

Correlation between TGA/DOAC concentrations and adherence

No statistically significant correlation was found between DOAC plasmatic concentrations and being adherent or not by SMAQ criteria (r=-0.248, p=0.292). Also, no correlation was found with the score obtained through SMAQ (r=-0.375, p=0.103). Finally, no significant correlation was found between the SMAQ score and therapeutic concentrations (r=-0.129, p=0.588) or between being adherent/non-adherent by SMAQ and therapeutic concentrations (r=-0.057, p=0.811).

Comparing the means of DOAC plasma concentrations between the non-adherent group ($\bar{x}$=97.3 ng/ml) and the adherent one ($\bar{x}$=130.9 ng/ml), according to the criteria validated for SMAQ, no statistically difference was found (p=0.985). However, it was found a moderate non-linear correlation between SMAQ score and ETP (r^2=0.460, p=0.041). Figure 4 shows this correlation.

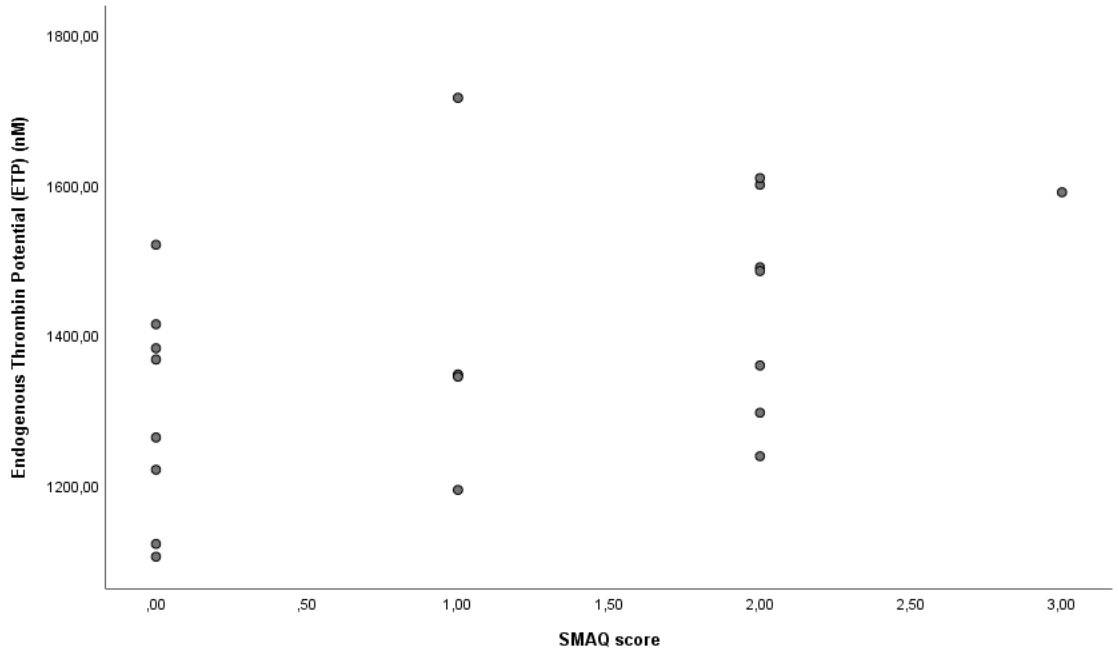

Figure 4. Correlation between adherence score and ETP

Study of predictors of low adherence to medication

Using logistic regression, no clinical or demographic factors were predictors of adherence to DOAC, as presented in Table 5. We also tested if there were predictors for therapeutic concentrations – besides SMAQ score – using all parameters tested for SMAQ as well as creatinine, GFR, weight and height. However, no predictors were found.

Table 5. Predictors of Adherence to DOACs

	OR	Significance	95% CI Lower	95% CI Upper
Using the conventional cut-point of SMAQ (adherent/non-adherent)				
Age	1.037	0.573	0.914	1.176
Gender	0.500	0.459	0.080	3.127
Education level	2.549	0.206	0.599	10.856
Marital status (married vs not married)	3.667	0.327	0.273	49.288
Household	0.378	0.285	0.063	2.254
Previous MI/stroke	0.600	0.650	0.066	5.447
Number of daily DOACs pills	0.200	0.187	0.018	2.181
Number of daily drugs	0.837	0.314	0.591	1.184
Using the median cut-point				
Age	0.931	0.300	0.813	1.066
Gender	0.840	0.852	0.134	5.261
Education level	1.000	1.000	0.278	3.601
Marital state (married vs not married)	1.400	0.799	0.105	18.615
Household	0.368	0.345	0.046	2.933
Previous MI/stroke	1.667	0.650	0.184	15.130
Number of daily DOACs pills	0.200	0.125	0.026	1.562
Number of daily drugs	1.000	1.000	0.716	1.397

DOAC - Direct Oral Anticoagulants; CI - Confidence Interval; OR - Odds Ratio; SMAQ - Simplified Medication Adherence Questionnaire; MI - Myocardial Infarction;

When comparing adherent to non-adherent patients on anticoagulant therapy (Table 6), the mean age was found to be higher in nonadherent patients, although this difference was not statistically significant (p = 0.593).

Table 6. Comparison of patient characteristics considering adherence

	Adherence n (%)	Non-adherence n (%)	p-value
Age (mean±SD)	74.38±4,470	76.25±8.976	0.593
Gender n(%)			0.648
Feminine	4 (20%)	8 (40%)	
Masculine	4 (20%)	4 (20%)	
Education level n(%)			0.242
Non-degree	8 (40%)	9 (45%)	
Degree	0	3 (15%)	
Marital status (married vs not married) n(%)			0.537
Married	6 (30%)	11 (55%)	
Not-married	2 (10%)	1 (5%)	
Household n(%)			1.000
Living with one person	1 (5%)	1 (5%)	
Living with more than one person	7 (35%)	11 (55%)	
Previous MI/stroke n(%)	2 (10%)	2 (10%)	1.000
DOAC dose posology n(%)			0.325
1id	1 (5%)	5 (25%)	
2id	7 (35%)	7 (35%)	
Number of daily drugs n(%)			0.603
<5	1 (5%)	4 (20%)	
≥5	7 (35%)	8 (40%)	

SD - Standard Deviation; MI - Myocardial Infarction; DOAC - Direct Oral Anticoagulants.

All patients with a college degree had a numerically worse adherence to therapy, although this difference was also not statistically significant (p=0.242) and a prior history of MI or stroke did not appear to be statistically related to better or worse adherence to therapy.

Regarding DOAC posology, the proportion of non-adherent patients to therapy was higher in the group with a simplified scheme (one daily), although this difference was not statistically significant (p=0.325).

Finally, the proportion of non-adherence to therapy was higher in the group with less than 5 daily drugs, although this difference was not statistically significant (p=0.603).

DISCUSSION

The main findings of this study were: (1) all parameters of TGA, specially thrombin HP and ETP, were strongly correlated with DOAC concentrations, which supports their usage as parameters to monitor DOAC treatment; (2) self-reported adherence to DOAC therapy was low (40%), and a lower adherence was correlated with a higher ETP, reinforcing the fact that adherence to treatment is a concerning problem and that TGA may be useful in evaluating these situations; and (3) we did not identified demographical or clinical predictors of adherence to medication.

One of the main advantages of DOAC usage is their predictable pharmacokinetics, making regular control of their anticoagulant activity redundant. However, in some situations, it may be interesting to know if the patient is anticoagulated, and "how strong" is that anticoagulation. However, the tests that are used for this assessment are heterogenous and not widely available. Here, we assessed the usefulness and performance of a new coagulant test, the TGA, on an unselected cohort of patients under DOAC.

Although all the TGA parameters were significantly correlated with DOAC concentrations, the thrombin high-peak (HP) showed the best correlation, which makes it a potentially valuable parameter to monitor DOAC activity on thrombin generation. Taking into consideration that previous studies have shown that ETP is strongly associated with bleeding risk,[8,13] ETP may be valuable as a marker of haemorrhagic risk in each patient. Even though ETP and HP are usually well correlated, HP seems to be a more sensitive indicator of the plasma thrombin generating capacity.[13] In summary, and according to previous studies, a short LT, a high HP, a short TTP, a high VI and a high ETP describes a hypercoagulable state.[7]

One additional consideration is that anticoagulants are very effective in preventing thrombosis, but at the expense of an increase of the bleeding risk. Being so, the dose needed to prevent thrombosis, still preserving the bleeding risk, varies between individual patients. This happens because their coagulation asset is different, which is verified by the relatively large variation of thrombin generation parameters and DOAC concentrations we found. Hence, tailoring drug dosages according to the necessity of individual patients may be important in specific subgroups of patients (such as patients with diabetes, non-alcoholic fatty liver diseases and coagulopathy induced by chronic liver disease, myeloproliferative neoplasms, inflammatory bowel diseases and in emergency settings – trauma, massive bleeding, haemophilia, liver transplantation).[7] TGA seems to be an important instrument in this matter. TGA has been extensively explored and its efficacy has been proved to further comprehend the coagulation mechanisms in various clinical conditions. However, future studies combining clinical data with TGA results are needed to implement the use of TGA as

routine in the clinical laboratory.[7] If confirmed in larger future studies, we may predict that TGA could be used in the daily clinical practice to monitor anticoagulant drug efficacy.[5]

The real application of what we found in clinical trials and what we might expect in real-world setting may differ due to a variety of reasons, one of them adherence. In our study, we found a rate of adherence of 40%, that although low, is in line with what has been reported in previous studies. In fact, despite evidence of improved outcomes from having a good adherence to medication, the average medication compliance rates in developed countries are estimated to be just 50%.[11,21] Recent data concerning anticoagulant therapy in routine clinical practice indicates that long-term adherence is generally a problem, not negligible in the case of DOAC.[22] This challenge is well supported by the World Health Organization, stating that in developed countries the rate of adherence to treatment in patients with chronic diseases is on average 50%, which has an impressive impact on the population.[23]

Silent chronic conditions, such as AF, where patients can't sense the nature and severity of their illness, represent a noteworthy challenge. For adherence to occur, symptoms must be sufficiently severe to arouse the need for treatment, be perceived as being resolvable and acute, and remedial action must effect a rapid and noticeable reduction in symptoms.[21] According to the American Heart Association, more than 65% of people with AF don't recognize the seriousness of their illness and may be less adherent to their medication.[24] Finally, it has also been shown that self-reported medication adherence to cardiovascular disease medications is less than 40%, or even lower in elderly population.[25]

In our study, we verified a wide range of DOAC concentrations, as well as TGA results. We may speculate that this fact was, in part, responsible for the lack of correlation between DOAC plasmatic concentrations and SMAQ results. However, the SMAQ score was correlated with ETP, which may represent the fact that a patient that is not adherent to medication is not well hypocoagulated, once higher ETP reflects a hypercoagulable status. With this, we can predict that TGA may be also useful in monitoring adherence to anticoagulant therapy.

Regarding this variability in DOAC concentrations that we faced, because DOAC have a short half-life, it is critical to know the precise time at which patient took the medication. However, in "real-world", this is difficult and challenging, unlike in clinical trials, once doctors have to estimate the drug intake time based on patient information. We can speculate that this fact also affected our results. In real world conditions, monitoring of DOAC concentrations using this type of tests should be interpreted considering the wide variations in data, which reflects the variability in patient-reported loading time and interpatient inconsistency. As other studies have reported, and also supported by the results of our study, monitoring DOAC treatment with these techniques in controlled conditions has some underlying discrepancies compared to ideal conditions.[27] Even so, anti-factor Xa

chromogenic assay and ECA-II have been proved to have an excellent linearity with plasma concentrations of DOAC.[28] Since measurement of plasma concentrations of DOAC by these methods may not be as linear in clinical practice as in clinical trials, and since we have found a strong correlation between plasma concentrations and TGA, this last assay may be an important tool to help increasing applicability in monitoring DOAC effect in real world.

Regarding the analysis of adherence predictors, we couldn't identify predictors of low adherence, contrary to what has been published in different studies. This may be related to the fact that our sample consisted of patients with very homogeneous characteristics among them, namely, all elderly, retired, middle class, non-illiterate and Caucasians.

The WHO recognizes two distinct categories of reasons for non-adherence: preventable reasons, like poor health literacy as one of the most common and cost prohibitive; and nonpreventable ones, as serious mental illness, serious adverse effects and polypharmacy.[23] Although, age, gender, education, occupation, income, race and religion have not been absolutely associated with treatment adherence in patients undergoing DOAC for AF,[22] the WHO report that socioeconomic factors such as occupation, social support, habitational conditions, costs of treatment, age, marital status and literacy may interfere in adherence to medication,[23] contrary to what was found in our study. Some studies claim that satisfactory social support, living with others and being married have been shown to be associated with better adherence.[21,23] The most frequently identified barriers to adherence to OAC treatment were poor literacy, poor patient-physician relationships, forgetfulness due to employment or social environment, prior bleeding events and fear of recurrent ones and changes in daily routine.[22] Medication costs represent a key reason of non-adherence in all fields of medicine and seem to be a particularly important factor in adherence to DOAC therapy once these drugs are expensive, especially considering their life-time course associated. Also, it has been reported that polypharmacy, most common in elderly patients, has a negative impact on adherence, diverging from the results in this study.[21]

Relatively to DOAC posology, the conclusions are contradictory. Some studies suggest that a single-dose regimen improves adherence to medication, however, in these cases, failure of a single dose may imply greater risk in terms of cardioembolic predisposition, while, in a double-dose regimen, the failure of a dose is associated with lower risk. This is due to the fact that DOAC have similar half-lives of about 12 hours, leading some authors to propose that the regimen more appropriate to the pharmacokinetic profile of DOAC is the double-dose one, which is associated with lower adherence to medication, resulting in a complex decision.[26,27]

The **limitations** of this study included the reduced number of patients, the homogeneous characteristics among them and the fact that the study is unicentric, as well as the memory bias inherent to the application of an adherence questionnaire. We were also unable to

measure time since diagnosis to SMAQ completion and it was shown that adherence rates to AF-specific medications tend to decline with time.[28] We also have to include the limitations related to TGA as the fact that this still isn't a part of the routine clinical practice, due to the lack of an international standardized procedure and high inter-laboratory variability. However, when applying a protocol like we did, the coefficient of variation was inferior to 10% for most of the parameters, which translate into adequate validation criteria.[8] The absence of validated specific questionnaires for adherence to treatment with DOAC is also relevant to mention because it could have improved the specificity of this study. Since this is a growing concern these days, we may think that a more specific questionnaire related to this topic would be a useful tool in monitoring adherence to therapy with these new anticoagulants.

The solution for addressing non-adherence requires a multifaceted strategy. Possible measures include: (1) patient education to the importance of adherence to anticoagulant treatment and to modalities of administration, emphasizing the importance of full adherence to the prescribed treatment, explaining the increased thromboembolic risk to which the patient is exposed in case of failure of DOAC intake because of the rapid decrease of the anticoagulant effect that will occur; (2) involvement of the closest family of the patient; (3) instruments like timetables for follow-up, electronic systems, technological applications that allow patients to remember their medication; (4) good quality health services, good patient-doctor relationship based on confidence and improved communication and active involvement of other health professionals; (5) use of combination drug formulations (polypill) that facilitates therapeutic regimens and reduce costs.[21] Patients should also be motivated to share their preferences with their clinician since OAC adherence can be promoted if therapies are personalized to patients' needs and preferences.[22]

Adherence to medication is a key factor in the effective prevention and treatment of chronic conditions[20] and the need for incisive adherence-improving strategies are increasingly recognized.

CONCLUSION

In this study, we globally aimed to study the adherence of patients to DOAC therapy. We specifically aimed to assess if TGA is a useful instrument to monitor DOAC treatment, as we hypothesized that TGA parameters are correlated with DOAC plasmatic concentrations. Additionally, we specifically aimed to study whether there was a correlation between adherence, assessed by a validated questionnaire, and the efficacy of anticoagulation, measured by TGA, ECA-II and chromogenic antifactor Xa assay. We hypothesized that low adherence was correlated with lower levels of anticoagulation, assessed by several parameters. Finally, we specifically aimed to assess which parameters of the patient's personal and medical history were most closely related with adherence to therapy. We hypothesized that a distinct set of clinical and demographical parameters can influence adherence.

Our findings globally support that adherence is low, as assessed by a validated questionnaire. Additionally, TGA parameters are potentially useful for monitoring treatment with DOAC and the correlation between ETP and the SMAQ score supports SMAQ as a valid tool to assess adherence in these patients. Finally, in our cohort we did not identify clinical predictors of adherence. Future studies combining clinical data with TGA results are needed to implement the use of TGA as routine in the clinical laboratory.

More importantly, adherence to DOAC is a concerning problem that requires incisive adherence-improving strategies. A more specific questionnaire related to this topic maybe could be a useful tool in monitoring adherence to therapy with these new anticoagulants as well as personalized treatment to patient's needs and preferences.

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SCIENTIFIC PRESENTATION

Part of this study was submitted to the European Society of Cardiology 2019 Annual Congress which will take place in Paris, between August 31 and September 4.

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SUPPLEMENTAL MATERIAL

I. ABSTRACT SUBMITTED TO EUROPEAN SOCIETY OF CARDIOLOGY 2019 ANNUAL CONGRESS

Assessment of thrombin generation of patients under direct oral anticoagulants

C. Ferreira[1], F. Marques[1], M. Castro[1], A. Correia[2], I. Rasteiro[2], J. Pego[2], R. Baptista[1], H. Pinho[1], I. Rocha[1], M. Alves[1], C. Domingues[1], A. Freitas[1]

(1) University Hospitals of Coimbra, Cardiology A, Coimbra, Portugal

(2) University Hospitals of Coimbra, Clinical patology, Coimbra, Portugal

BACKGROUND: The use of direct oral anticoagulants (DOAC) is increasing, mainly to limited monitoring requirements and predictable pharmacokinetics. However, assessment of the anticoagulant effect may be required in specific situations. The test of choice for measurement of their anticoagulant effect is the dilute thrombin time for dabigatran and the antifactor 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. The Thrombin Generation Test (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. We aimed to assess the anticoagulant effect of DOAC on each individual TGT parameter to assess suitability of these parameters for monitoring the global anticoagulant effect.

METHODS: We prospectively included 19 senior patients on DOAC for atrial fibrillation. TGT parameters were measured at through (lag time, endogenous thrombin potential (ETP), thrombin peak height, time to peak, and velocity index). TGT parameters and DOAC concentrations were correlated. The patients also completed the simplified medication adherence questionnaire (SMAQ) for assessing adherence to medication.

RESULTS: The mean age was 76±7 years; 42% were male. The majority of the patients were taking apixaban (n=11, 58%), while 4 patients were on edoxaban, 2 on rivaroxaban and 2 on dabigatran. All patients were on appropriate DOAC doses. Although all the TGT parameters were significantly correlated with DOAC concentration (Figure 1), thrombin peak height showed the best correlation ($r=-0.82$, $p<0.001$). Conversely, ETP was weakly correlated with DOAC levels ($r=-0.69$, $p=0.001$). Taking into consideration that previous

studies have shown that ETP is strongly associated with bleeding risk, this may represent the variable hemorrhagic risk in each patient.
Patients treated with appropriate dose reduction (n=7, 35%) had a similar DOAC plasmatic concentration compared to patients treated with a regular DOAC dose. In addition, no differences were found between the groups in the different TBT parameters, signaling that dose reduction is effective on avoiding supratherapeutic DOAC levels.
Regarding the SMAQ adherence questionnaire, low adherence was correlated with lower DOAC concentrations (r=-0.46, p=0.04), but not with the remaining TGT parameters.

CONCLUSION: Thrombin peak height was strongly correlated with DOAC concentration and is potentially a useful parameter to monitor DOAC dosing. Conversely, ETP may be valuable as a marker of bleeding risk. The dose needed to prevent thrombosis, but still prevent bleeding, may vary in each patient. Therefore, tailoring drug dosages to fit the needs of individual patients may be crucial in specific subgroups.

II. CONSENTIMENTO INFORMADO, LIVRE E ESCLARECIDO (de acordo com a Declaração de Helsínquia e a Convenção de Oviedo)

Título do estudo: Avaliação da Geração de Trombina em doentes sujeitos a terapia com Anticoagulantes Orais Diretos (dabigatrano, rivaroxabano, apixabano e edoxabano).
Enquadramento: Realizado no serviço de Cardiologia e Patologia Clínica do Centro Hospitalar e Universitário de Coimbra.

Informação aos doentes participantes no estudo

Gostaríamos de contar com a sua colaboração neste estudo de investigação que tem como Responsável o especialista em Cardiologia – Prof. Dr. Rui Baptista e o especialista em Patologia Clínica – Dr. João Mariano Pego do Serviço de Cardiologia e Patologia Clínica do Centro Hospitalar e Universitário de Coimbra.

Com este estudo pretendemos avaliar o contributo de um novo teste para o diagnóstico, prognóstico e tratamento de doentes com alterações da coagulação. Para tal, queríamos pedir consentimento para utilizar a amostra de sangue para efetuar o novo teste e comparar o resultado com os resultados dos testes comuns. Não haverá benefícios imediatos, embora a longo prazo possam existir benefícios indiretos resultantes de um melhor conhecimento dos testes e do seu valor clínico. Não haverá benefícios económicos, nem o participante será ressarcido das despesas de deslocação ao hospital, nem pelas faltas ao Serviço, nem por danos resultantes da sua participação no estudo, uma vez que as determinações dos parâmetros serão efetuadas numa amostra colhida no decorrer da avaliação clinica e de pedidos de análises pelo médico assistente, não sendo o doente submetido a qualquer tipo de procedimento médico.

Termo de consentimento informado

Eu, abaixo-assinado, fui informado sobre o estudo de investigação acima mencionado. Foi-me garantido que todos os dados relativos à identificação dos participantes neste estudo são confidenciais. Sei que posso recusar-me a participar no estudo sem nenhum tipo de penalização por este facto. Compreendi a informação que me foi dada, tive oportunidade de fazer perguntas e as minhas dúvidas foram esclarecidas. Aceito participar de livre vontade no estudo acima mencionado e autorizo a utilização do meu sangue para a realização das análises que fazem parte deste estudo, bem como a recolha de alguns dados clínicos que são importantes para interpretar os resultados. Também autorizo a divulgação dos resultados obtidos no meio científico, com anonimato garantido.

III. Simplified Medication Adherence Questionnaire

(Knobel *et al.* 2002; Translated and adapted by Goés e Santos (2015))

1) Você, alguma vez se esqueceu de tomar seus remédios? Sim/Não

2) Você se descuida, às vezes, sobre tomar os seus remédios? Sim/Não

3) Às vezes, se você se sente mal, você para de tomar seus remédios? Sim/Não

4) Pensando na semana passada. Quantas vezes você deixou de tomar os remédios? Nunca/ 1-2 vezes/ 3-5 vezes/ 6-10 vezes/ >10 vezes

5) Você não tomou nenhum dos seus remédios durante o fim de semana passado? Sim/ Não

6) Nos últimos três meses, quantos dias você deixou de tomar todos os remédios? 0-2 dias/ 3 dias ou mais