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Phenotyping of CYP 4501A2 Activity by Total Overnight Salivary Caffeine Assessment (TOSCA) in Patients on Warfarin Treatment: A Cross-Sectional Study

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Abstract

Warfarin is an oral anticoagulant, commonly used for primary and secondary prevention of venous and arterial thromboembolic events. The drug is characterized by narrow therapeutic index, widespread individual variability in clinical response, and high rates of adverse events, particularly bleeding complications. For these reasons, a close monitoring of the dosage, using the frequent assessment of coagulation status by means of International Normalized Ratio value, is mandatory. Warfarin is metabolized by hepatic cytochrome P-450. High CYP 450 activity may lead to low drug concentration and requires high warfarin doses to reach efficacy; conversely, low CYP 450 activity is responsible for high drug concentration and needs for low doses to avoid potential toxicity risks. The major isoforms of CYP involved in the metabolism of warfarin sodium are CYP1A2 (for the R-warfarin) and CYP2C9 (for the S-warfarin). The probes for testing CYP1A2 are phenacetin and caffeine while for CYP2C9 tolbutamide. Although S-warfarin has major activity, it was decided to exclude its phenotyping for ethical issues, being mandatory to use a drug (tolbutamide). Instead, it was chosen to test the 1A2 isoform, as the activity of the latter isoform could be investigated by using caffeine contained in the caffeinated beverages. Specifically, a single-point concentration of salivary caffeine (total overnight salivary caffeine assessment [TOSCA]) after an overnight period of the caffeinated beverages abstinence was utilized. In the present study, 75 nonsmoker patients regularly receiving warfarin sodium were enrolled. The results have showed a significant association of the warfarin dose with TOSCA values (coefficient = –0.15, standard error = 0.04, 95% confidence interval = –0.24 to –0.06, $t = -3.23$, $P = .002$). In conclusion, the phenotyping of CYP1A2 by TOSCA could be useful, if further proven, to help manage patients on warfarin in order to lessen severe adverse events.

Keywords

phenotyping, CYP 4501A2, TOSCA, warfarin

Introduction

Vitamin K antagonists (VKAs) are commonly used for primary and secondary prevention of venous and arterial thromboembolic events. Warfarin sodium is the most widely prescribed VKA. The chemical name of warfarin sodium is 3-(α-acetonylbenzyl)-4-hydroxycoumarin sodium salt, which is a racemic mixture of the R- and S-enantiomers.[1] In humans, the R-enantiomer exhibits an estimated eudismic potency ratio approximately 3.4 times less than of S-enantiomer, but its contribution for action may be important.[2]

Warfarin acts by inhibiting the synthesis of vitamin K-dependent clotting factors, which include Factors II, VII, IX, and X, and the natural anticoagulant proteins C and S.[3-5] Approximately 99% of the drug is bound to plasma proteins.[6] Warfarin is metabolized by hepatic cytochrome P-450.[7] The half-life of racemic warfarin ranges from 36 hours to 42 hours,[8]

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with the mean half-life of 51.1 hours for R-warfarin and 33.1 hours for S-warfarin. Thus, the clearance of R-warfarin is lower than that of S-warfarin.[9]

Adverse events of warfarin therapy include bleeding episodes involving in particular, the gastrointestinal (GI) and urinary tract and the risk of cerebral hemorrhage is the most fearsome complication in particular in elderly patients.[10,11]

As known, the relationship between the dose of warfarin and the response is modified by genetic and environmental factors (diet and drugs) that can influence the absorption, pharmacokinetics, and pharmacodynamics. It is difficult to reach a stable therapeutic effect of warfarin sodium and for this reason, it is recommended a close monitoring of the dosage, by using the frequent assessment of coagulation status.[12] In a large population, the recommended maintenance warfarin dose is from 2.5 mg/day to 10 mg/day with a maximum to 15 mg/day; however, the effective daily dose varies from 0.5 mg to 60 mg from patient to patient.[13,14]

Consequently, it could be important evaluating the phenotype of CYP 450 activity to obtain an optimal dose regimen. In fact, high CYP 450 activity leads to low drug concentration and requires high warfarin doses to reach drug efficacy; conversely, low CYP 450 activity is responsible for high drug concentration and needs for low doses to avoid potential toxicity risks.[15] Therefore, it could be advisable to foresee the metabolic pattern of the treated patients for avoiding both the events.

It is well known that the warfarin metabolism can be influenced by several factors comprising race, gender, age, diet, and concomitant drugs. Thus, the evaluation of metabolic pattern is recommended and useful to better foresee the potential bleeding risk in these patients. The major isoforms of CYP involved in the metabolism of warfarin sodium are CYP1A2 (for the R-warfarin) and CYP2C9 (for the S-warfarin).[7,16] The probes for testing CYP1A2 are phenacetin[17] and caffeine,[18] while for CYP2C9 the probe for testing is tolbutamide.[19]

Although S-warfarin has major activity, it was decided to exclude its phenotyping for ethical issues, being mandatory to use a drug (tolbutamide). The choice of phenotyping CYP1A2 activity fell upon testing R-warfarin. Concerning the latter enantiomer, 2 options were available: phenacetin and caffeine. As phenacetin showed the same ethical limitations of tolbutamide, the caffeine option was obliged because coffee is a usual beverage of our population. That said, owing to the minor power of probe of caffeine compared to that of phenacetin,[20] it was necessary that the patients assumed a satisfactory intake of coffee for a reliable assessment of metabolic profile. On the basis of recent evidence, no difference should be attributed to the type of probe chosen as it has been clearly demonstrated that the metabolic profiles are similar (within the 20%), except for CYP2A6, not involved in the warfarin metabolism and 3A4.[15] Finally, the isoform CYP3A4 is responsible for high incidence of drug–drug interactions with consequent source of pharmacokinetic bias.[7]

In this research, total overnight salivary caffeine assessment (TOSCA) was used for phenotyping the CYP1A2 activity. Total overnight salivary caffeine assessment was determined by means a single-point concentration of salivary caffeine after an overnight period of the caffeinated beverages abstinence. The pharmacokinetic and analytical strengths of the test were extensively validated in a previous study.[21]

The aim of this research was to investigate any relationship between the dose of warfarin sodium and the CYP1A2 activity, evaluated by TOSCA, in patients on warfarin treatment in order to likely reduce inefficacy or bleeding risk.

Patients and Methods

Seventy-five nonsmoker patients on current treatment with warfarin sodium were included in this observational study. Patients were enrolled from April 2015 to February 2016 at the outpatient clinic of the Department of Clinical Medicine and Surgery, "Federico II" University, Naples, Italy.

In Table 1, age of the enrolled patients and information concerning diseases for which they were on anticoagulant therapy were reported. In Table 2, warfarin dose and cotherapies were described. The warfarin dose considered for each patient was that allowing to maintain the international normalized ratio (INR) value in the stable recommended range of 2 to 3 confirmed by repeated measurements.[22] Total overnight salivary caffeine assessment was performed at this steady-state condition (INR within therapeutic range for at least 4 weeks). Patients were advised not to assume drugs and/or food able to interfere with the investigated metabolic pathway at least 2 weeks before performing TOSCA test in order to avoid any potential pharmacokinetic interactions. In particular, among the inhibitors, fluvoxamine, quinolones, and grapefruit juice were excluded; and among the inducers, omeprazole, phenobarbital, rifampicin, *Hypericum perforatum*, and Brussels sprouts were excluded. In addition, patients with impaired laboratory liver tests were kept out from the study.

As a previous study has fixed mean caffeine intake from caffeinated beverages at 3.02 mg/kg[23] and taking into account that the content of caffeine in typical Italian caffeinated coffee is almost 50 mg/cup,[24] patients were allowed to assume 2 to 4 cups of coffees per day, corresponding to 100 mg to 200 mg of caffeine, according to their usual habit.

During the day before the test, patients were allowed to consume their caffeinated beverage/food in the late afternoon (prior to 2000 hours). At 0800 hours of the following day, a sample of 2 cc of saliva was collected after a unique and short mouth rinsing and having chewed parafilm for several minutes. Saliva specimens were rough centrifuged, and the supernatant was stored frozen until analysis.

Caffeine was measured in the saliva by the enzyme-multiplied immunoassay technique (EMIT) assay from Siemens Healthcare Diagnostics Inc (Tarrytown, New York) on a CD×90 automated analyzer (Tema Ricerca, Castenaso, Italy). The analytical performance parameters of the EMIT caffeine assay were the following: the lower limit of quantitation was 0.15 mcg/mL; the intraday and interday precision were 4.0 and 3.9%, respectively; and the range of the standard curve was 0 to 30 mcg/mL.[21,25]

Table 1. Demographic Characteristics of the Patients With Related Disease.

Patient	Gender	Age (Years)	Diseases
1	F	37	Recurrent DVT
2	M	59	DVT and SVT
3	F	35	DVT, PE, CVT
4	M	44	Prosthetic aortic valve
5	F	62	DVT and PE
6	F	48	DVT and PE
7	M	41	Recurrent SVT
8	F	88	DVT
9	F	76	DVT, SVT, and AF
10	M	59	DVT and PE
11	F	68	DVT and PE
12	M	76	AF
13	M	61	DVT
14	F	25	CVT
15	M	48	DVT
16	M	68	DVT
17	F	36	DVT
18	F	31	Portal vein thrombosis
19	F	81	Recurrent SVT and Budd-Chiari syndrome
20	F	79	AF and AT
21	M	43	Recurrent DVT
22	M	57	DVT and PE
23	M	62	Acute myocardial infarction and stroke, CVT
24	M	69	Thrombosis of portal and mesenteric veins
25	M	52	AF
26	M	58	DVT and PE
27	M	51	Aortic valve and SVT
28	M	49	DVT
29	M	44	DVT and CVT
30	F	40	CVT
31	M	55	AT
32	M	67	DVT
33	M	81	AF
34	M	62	DVT and PE
35	F	56	DVT and AF
36	M	80	DVT and PE
37	M	72	Recurrent DVT
38	F	68	SVT
39	M	67	DVT and SVT
40	M	63	DVT and PE
41	F	48	DVT and PE
42	M	60	PE
43	M	74	DVT and PE
44	F	61	PE
45	F	82	PE and DVT
46	F	50	DVT
47	F	32	DVT
48	F	70	SVT and DVT
49	M	70	Recurrent TIA and recurrent SVT
50	M	65	DVT
51	M	24	DVT
52	F	44	CVT
53	M	61	Recurrent SVT
54	M	73	DVT
55	F	74	DVT and SVT
56	M	66	DVT and AF
57	M	61	DVT and SVT
58	F	54	DVT
59	M	77	DVT
60	F	57	AT
61	F	40	PE
62	M	76	DVT of upper limbs
63	M	41	DVT and PE
64	F	66	PE
65	F	55	DVT and PE
66	F	40	DVT
67	M	59	DVT
68	F	29	CVT
69	F	64	DVT
70	M	64	DVT
71	M	56	Thrombosis of portal and mesenteric veins
72	M	80	DVT and AF
73	F	51	SVT
74	M	42	DVT and SVT
75	M	62	DVT and PE

Abbreviations: AF, atrial fibrillation; AT, arterial thrombosis; CVT, cerebral vein thrombosis; DVT, deep venous thrombosis; PE, pulmonary embolism; SVT, superficial venous thrombosis; TIA, transient ischemic attacks.

The study was carried out according to the Declaration of Helsinki and its amendments, which include written informed consent by all participants before their enrolment, and was approved by our Medical School Ethics Committee.

Statistics

First, data of every studied variable were analyzed in order to assess the distribution, by the Shapiro-Wilk (S-W) test. Accordingly, both TOSCA values and doses were expressed as median plus 25 to 75 interquartile range (IQR), having the S-W test rejected normality ($P < .0001$ and $P = .0125$, respectively). Vice versa, age was normally distributed ($P = .29$) and was expressed as mean $\pm$ SD. The prediction of the dose of warfarin sodium by TOSCA values was estimated by linear regression (least squares method), calculating coefficient, standard error, 95% confidence interval (CI), t value, and P. To evaluate the effect size in the regression, R^2 was calculated, and values of 0.01, 0.09, or 0.25 were considered small, medium, or large, respectively. In order to study the phenotype distribution of CYP1A2, its values were broken into percentiles (<25 rapid metabolizers, 25-75 intermediate metabolizers, >75 slow metabolizers). Dealing with differences of TOSCA values between more than 2 groups, the analysis of variance (ANOVA) Kruskal-Wallis with Conover test for the post hoc analysis was used.

The relationship between TOSCA values and warfarin doses in the 3 subgroups was assessed by the Spearman coefficient of rank correlation (ρ). P value <.05 was considered significant.

Table 2. Warfarin Dose, TOSCA Value, CYP1A2 Phenotype, and Co-Therapy of the Patients.

Patient	Warfarin Dose (mg/day)	TOSCA (mcg/mL)	CYP1A2 Phenotype	Co-Therapy
1	5.89	0.00	R	None
2	3.74	1.57	S	Rosuvastatin, lisinopril, hydrochlorothiazide, lansoprazole
3	7.73	0.00	R	Atorvastatin, rabeprazole
4	7.05	1.23	S	Nifedipine, olmesartan, hydrochlorothiazide, carvedilol
5	6.25	0.28	I	Ramipril, methylprenisolone, amitryptiline, colecalciferol, ranitidine
6	4.76	0.20	I	Metformin, repaglinide, ezetimibe, levothyroxine, ranitidine, lisinopril
7	4.99	1.98	S	None
8	0.53	5.70	S	Pantoprazole, simvastatin, citalopram, telmisartan, hydrochlorothiazide
9	4.1	0.40	I	Rabeprazole, levothyroxine, irbesartan, metformin, amiodarone, tizanidine, pravastatin, levosulpiride, tramadol
10	5.05	0.20	I	Rabeprazolo, irbesartan, verapamil, atorvastatin, acetylsalicylic acid, clopidogrel
11	6.69	0.40	I	Atorvastatin, ramipril, levetiracetam, metformin, colecalciferol
12	1.25	1.80	S	Digoxin, rabeprazole, hydroxychloroquine, furosemide, carvedilol, dutasteride, canreonate
13	9.2	1.00	S	Quinazide, carvedilol, metformin, pravastatin
14	7.3	0.40	I	None
15	2.32	0.20	I	Rabeprazole, ramipril, prednisone, clopidogrel, amlodipine, irbesartan, atorvastatin, 5-aminosalicylic acid
16	7.23	0.20	I	Lansoprazole, pravastatin, allopurinol, cilazapril, hydrochlorothiazide
17	5.71	0.60	I	Topiramate, hydroxychloroquine, colecalciferol
18	2.0	0.10	R	Lansoprazole, leflunomide
19	5.8	0.70	I	Losartan, pravastatin, pantoprazole, hydrochlorothiazide
20	3.3	0.80	I	Levothyroxine, carvedilol, simvastatin, furosemide, digoxin, irbesartan, hydrochlorothiazide
21	1.42	0.30	I	None
22	5.71	0.60	I	Doxazosin, levothyroxine, enalapril, hydrochlorothiazide, silodosin, dutasteride
23	5.35	0.70	I	Carvedilol, pravastatin, isosorbide mononitrate
24	6.43	0.80	I	Metformin, telmisartan, hydrochlorothiazide, atorvastatin, linagliptin, febuxostat, carbidopa, pramipexole
25	1.96	0.60	I	Metformin, pantoprazole, ezetimibe, simvastatin, amlodipine, irbesartan, hydrochlorothiazide, allopurinolo, bisopropolol
26	1.78	1.30	S	Colecalciferol, ramipril, citalopram, rabeprazole
27	3.39	0.50	I	Carvedilol, candesartan, digoxin
28	2.46	0.50	I	Pantoprazole, allopurinol, magaldrate, ursodeoxycholic acid
29	8.21	0.20	I	Rabeprazole, carvedilol, hydroxyurea, carbamazepine
30	3.75	0.50	I	Topiramate, propanolol
31	4.91	0.10	R	Clopidogrel, pravastatin, ranitidine, silodosine, hydroxyurea, L-propionil carnitine, allopurinol
32	7.85	0.50	I	Levothyroxine, irbesartan, bisoprolol, pantoprazole
33	3.83	0.30	I	Atenolol, atorvastatin, rabeprazole
34	5.12	0.50	I	Felodipine, ramipril, simvastatin
35	5.71	080	I	Levothyroxine, losartan, hydrochlorothiazide, flecainide, ranitidine, metoprolol, ezetimibe, colecalciferol
36	3.57	0.80	I	Tapazole, ramipril, hydrochlorothiazide, atenolol, allopurinol, pravastatin
37	2.14	1.60	S	Rabeprazole, nebivolol, nimodipine, L-acetylcarnitine, dutasteride, ramipril, alfuzosin, metformin, rosuvastatin
38	4.1	0.70	I	Lansoprazole, repaglinide, allopurinol, colecalciferol, valsartan, hydrochlorothiazide
39	4.91	3.60	S	Irbesartan, hydroxychloroquine, dutasteride, silodosine
40	2.5	0.90	S	Allopurinol, amlodipine, fluvastatin
41	7.5	0.60	I	None
42	3.9	0.30	I	Levothyroxine, pantoprazole, sildenafil, amiloride/hydrochlorotiazide, irbesartan, dambrisentan, flecainide
43	5.0	0.60	I	Levothyroxine, rabeprazole, telmisartan, simvastatin, alfuzosine
44	3.75	0.80	I	Pantoprazole, pravastatin, denosumab, abatacept, N-acetylcysteine
45	1.6	3.80	S	Pantoprazole, carvedilol, furosemide, allopurinol, atorvastatin, digoxin, amlodipine, calcitriol, memantine
46	5.89	0.40	I	Simvastatin, ezetimibe, pantoprazole, levothyroxine, nebivolol
47	9.28	0.00	R	Hydroxychloroquine, methylprednisolone, colecalciferol, lansoprazole
48	5.0	1.50	S	Ezetimibe
49	4.2	0.20	I	Rabeprazole, colchicine, prednisone, colecalciferolo, telmisartan, amlodipine, simvastatin, tamsulosin, dutasteride

(continued)

Table 2. (continued)

Patient	Warfarin Dose (mg/day)	TOSCA (mcg/mL)	CYP1A2 Phenotype	Co-Therapy
50	6.25	0.00	R	Ramipril, ursodeoxycholic acid
51	3.03	0.20	I	None
52	7.5	0.60	I	Ezetimibe, rosuvastatin, levothyroxine, lansoprazole
53	10.0	0.20	I	Simvastatin, allopurinol
54	1.96	0.40	I	Ramipril, finasteride, silodosine
55	2.14	0.00	R	Levothyroxine, losartan, hydrochlorothiazide, metoprolol
56	6.44	1.30	S	Pantoprazole, atorvastatin, bisopropolol, perindopril, febuxostat
57	3.75	1.50	S	Lansoprazole, ramipril, amlodipine, tamsulosin
58	6.96	0.00	R	Clopidogrel, rabeprazole, atorvastatin, topiramate
59	5.17	0.40	I	Carvedilol, atorvastatin
60	7.14	0.10	R	Pantoprazole, ramipril, amlodipine, clopidogrel, atorvastatin cilostazol
61	10.0	0.20	I	Hydroxychloroquine, calcifediol, pantoprazole
62	4.64	1.10	S	Flecainide, bisopropolol, valsartan, pantoprazole
63	2.5	4.60	S	Simvastatin, ramipril
64	6.6	0.70	I	Irbesartan, hydrochlorothiazide, ranitidine
65	4.73	0.30	I	None
66	6.07	0.30	I	None
67	5.0	0.60	I	Atorvastatin
68	15.0	1.30	S	Acetazolamide, topiramate, flunarizine, rabeprazole
69	5.7	0.70	I	Lovastatin, atenolol, levothyroxine, olmesartan, amlodipine, ranitidine
70	4.28	0.50	I	Pravastatin, candesartan, bisopropolol
71	6.78	0.80	I	None
72	0.86	3.40	S	None
73	9.64	0.20	I	None
74	5.89	0.30	I	Irbesartan
75	3.75	0.60	I	Levothyroxine, simvastatin, silodosine

Abbreviations: I, intermediate metabolizer; R, rapid metabolizer; S, slow metabolizer; TOSCA, total overnight salivary caffeine assessment.

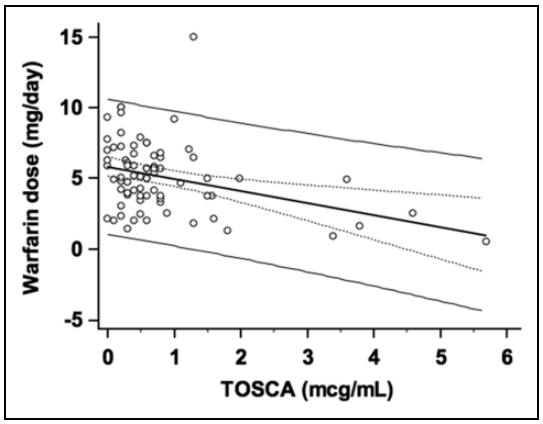

Figure 1. Correlation between warfarin dose and TOSCA value (thick, continuous, and dotted lines represent regression, 95% prediction, and 95% confidence intervals, respectively), linear regression (least squares method). TOSCA indicates total overnight salivary caffeine assessment.

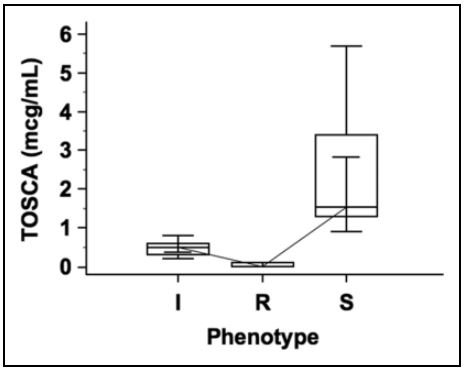

Figure 2. Distribution of the 3 phenotypes (metabolizers) showing no overlap using ANOVA Kruskal-Wallis. ANOVA indicates analysis of variance; I, intermediate; R, rapid; S, slow.

Results

The age of our patients, 43 males and 32 females, was 57.9 $\pm$ 15.0 years.

The median values of TOSCA and dose were 0.50 mcg/mL (IQR = 0.20-0.80) and 5.0 mg/day (IQR = 3.6-6.6), respectively. Figure 1 shows a significant association between the warfarin dose and TOSCA value (coefficient = –015, standard error = 0.04, 95% CI = –0.24 to –0.06, $t = -3.23$, $P = .002$ and $R^2 = 0.12$).

The cutoff of TOSCA values classifying rapid, intermediate, and slow metabolizers were <0.22, 0.22 to 0.80, and >0.80, respectively. The phenotype distribution of CYP1A2 in our population was 19 rapid metabolizers (25.3%), 38 intermediate metabolizers (50.7%), 18 slow metabolizers (24.0%; Table 2 and Figure 2). The medians (IQRs) of the 3 groups, that is, rapid, intermediate, and slow metabolizers, were different one

from the other: 0.20 (0-0.20), 0.55 (0.40-0.70), and 1.53 (1.23-3.58), respectively, $P \leq .001$.

Among subgroups, corresponding to phenotypes, slow metabolizers showed a strong correlation between TOSCA values and warfarin doses, $\rho = -0.57$ ($P = .008$).

Discussion

Although widely used for the prevention and treatment of thromboembolic diseases, warfarin is well noted for its narrow therapeutic index, large individual variability in clinical response, high rates of adverse events, and sometimes serious and frequent adjustment of therapeutic dose for each patient. Furthermore, polymorphisms of enzymatic isoforms involved in warfarin metabolism have been shown to be important in determining individual variability in patients on warfarin therapy. From this perspective, warfarin treatment is considered well suited as a model for studying individualized medicine.[26] As previously emphasized, frequent monitoring of the patient during warfarin therapy is commonly performed by the INR value. Currently, the INR represents a simple and relatively economical tool for clinicians to individualize warfarin dose, which is related to different metabolic profiles. The time required to reach the therapeutic INR range may differ significantly among patients, from days to months.

For all isoforms of CYP 450 have been generally identified 3 phenotypes of metabolism extent: slow, intermediate, and rapid metabolizers although ethnic differences have been observed.[15] Specifically, the distribution of CYP1A2 was confirmed in patients from Italy, China, and a State of the United States (Arkansas) with percentages of slow, intermediate, and rapid ranging 12 to 13, 51 to 67, and 20 to 37, respectively.[27] This trimodal distribution of the CYP1A2 activity was confirmed in our population.

Several approaches have been performed for CYP1A2 phenotyping. A previous research has measured caffeine metabolites in a spot urine sample collected after 4 hours to 5 hours of coffee consumption by using the high-performance liquid chromatography (HPLC) assay considered more complex than EMIT.[28] Data of the literature confirmed a strong correlation coefficient (0.97) for HPLC versus EMIT caffeine assay.[29] Similarly, in another study, the saliva was collected 5 hours to 7 hours after a "drug" administration (caffeine), by using the ^{13}C-caffeine breath test requiring 5 determinations of breath samples (before and at 15, 30, 45, and 60 mintes after caffeine ingestion).[30] Total overnight salivary caffeine assessment is fast, economic, and requires one sampling point for CYP1A2 phenotyping. It utilizes coffee and "no drug;" and the matrix is saliva, which is simple to collect. On the basis of these characteristics, the test is easy to perform.

While all data showed an evident inverse correlation between TOSCA values and warfarin doses confirming a potential role of CYP1A2 phenotyping for a more accurate evaluation of warfarin response, the separate evaluation in the 3 subgroups of phenotypes confirmed the negative relationship for slow metabolizers. This aspect is not of secondary importance as in the warfarin treatment, the issue of the drug-related complications (ie, GI bleeding) is crucial. Vice versa, regarding the lack of statistical significance between TOSCA values and warfarin doses in the other 2 groups, the narrow range of dispersion of TOSCA values could be a likely explanation. Despite the introduction of direct oral anticoagulant drugs (DOACs), warfarin treatment remains useful for several indications (mechanical valve replacement, severe thrombophilic conditions), and it has been reported that interruption/discontinuation of warfarin therapy increases the risk of thromboembolism.[31]

Moreover, in the metabolism of DOACs, both CYP 450 system (by several isoforms) and P-glycoprotein are involved, except for dabigatran using only P-glycoprotein, and therefore potential drug–drug interactions can be expected.[32] In addition, in some clinical occasion, it could be necessary to switch patients from DOACs to warfarin therapy and the management of these anticoagulants when transitioning from or back to warfarin, or to low-molecular-weight heparin around surgery or in case of major hemorrhage, requires knowledge of their pharmacokinetics and mechanism of action.[33]

Multiple clinical factors for determining optimal warfarin dosage, namely age, weight, and use of interacting co-medications, although reported in Tables 1 and 2, were not statistically evaluated due to the suggested poor clinical burden. In fact, realistically, it would not be possible to evaluate each of the clinical factors in trials to optimize warfarin dosing.[34]

This study is characterized by the following potential drawbacks: (i) the reduced number of enrolled patients taking into account the well-known wide pharmacokinetic variability of warfarin, but this research is a pilot study; (ii) the lack of the CYP1A2 genotyping, burdened by invasiveness, difficulty of the analytical tool, and high costs; moreover, a previous study highlighted the importance of pharmacogenetic research of CYP1A2 concerns smokers and thus it does not seem to be appropriate to our population.[35]

As previously emphasized, the clinical utility of the CYP1A2 phenotyping by a simple, reliable, noninvasive test such as TOSCA, if confirmed in large-scale studies enrolling patients from different ethnicity, alimentary habit, and life style, could be used to help the clinical management of patients on warfarin treatment.

Authors' Note

GT conceived the study, participated in research design, in performing statistics, and in writing the paper. DC participated in research design and the analytical tools, in performing statistics, and in writing the paper. PC and AG participated in collecting clinical and laboratory data. TM participated in the analytical tools. AT participated in collecting data.

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Declaration of Conflicting Interests

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