Anticoagulation in Italian patients with venous thromboembolism and thrombophilic alterations: findings from START2 register study

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Background - Randomised control trials have assessed the efficacy and safety of direct oral anticoagulants in the prophylaxis and treatment of venous thromboembolism (VTE). Positive but limited results have been reported in patients with inherited thrombophilia. Using an Italian, multicentre, prospective registry of consecutive patients presenting with symptomatic, acute VTE, we aimed to assess which factors are involved in making the choice of the drug that best fits the patient's risk profile in a large real-world setting of VTE patients.

Materials and methods – We investigated 4,866 VTE patients who took oral anticoagulants in the period between 2012 and April 2018 to prevent a new thromboembolic episode.

Results – The large majority of patients who underwent thrombophilic screening, regardless of the results obtained, were prescribed direct oral anticoagulants rather than conventional anticoagulant therapy (p<0.001). During anticoagulation, bleeding events occurred more frequently in patients on conventional anticoagulant therapy (4.2%) than in those receiving direct oral anticoagulants (1.8%) and an increase in bleeding events was observed in patients who tested positive at the thrombophilic screening. Overall, a higher number of recurrent VTE was observed in patients not screened for thrombophilia (n=36; 1.7%) than in those screened (n=20; 0.7%; adjusted odds ratio: 2.2; 95% confidence interval: 1.2-4.1).

Discussion – The present data confirm previous findings from other post-marketing registries and suggest that the choice of oral anticoagulation is strongly driven by patients' characteristics and VTE manifestations. Factors leading to the prescription of thrombophilic screening may identify a patient with a lower risk of VTE recurrence during anticoagulation.

Keywords: *anticoagulation, thrombophilia, thrombosis, bleeding.*

INTRODUCTION

Venous thromboembolism (VTE) most commonly manifests as lower extremity deep vein thrombosis and/or pulmonary embolism and has an annual incidence of 1-2 cases per 1,000 population[1]. Anticoagulation is the cornerstone of VTE therapy. For several decades, low molecular weight heparins and vitamin K-antagonists (VKA) have been the mainstays

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of anticoagulant prophylaxis and treatment for VTE. In the past few years, direct oral anticoagulants (DOAC), which directly inhibit either thrombin (dabigatran) or factor Xa (rivaroxaban, apixaban, and edoxaban), have been introduced into clinical practice for the prevention and treatment of VTE[2]. The introduction of DOAC has revolutionised the management of VTE[3-6]. Their oral administration, rapid onset and offset of action, fewer food and drug interactions and predictable anticoagulant effects have resolved many of the drawbacks associated with conventional anticoagulant therapy (CAT). Following the convincing results of randomised clinical trials, DOAC have begun to be widely used in the treatment of VTE[7]. Some guideline groups have given consideration to preference of DOAC versus warfarin in the setting of atrial fibrillation and VTE treatment[8,9]. This class of drugs has been broadly adopted in practice, becoming more frequently prescribed than VKA in Europe and North America[10]. The choice of anticoagulant should consider medical issues such as efficacy, safety, renal and hepatic function, and concurrent medications. In addition, practical issues such as availability, familiarity of use, patients' preference, and cost should be considered.

Thrombophilia is an inherited or acquired state causing a susceptibility to thrombosis. Testing for inherited thrombophilia is controversial and a clear indication on the clinical usefulness and benefits of such testing is lacking[9,11,12]. Nevertheless, thrombophilia testing is widely performed almost routinely[13]. Despite this widespread testing, there is no specific guidance on DOAC use in patients with inherited thrombophilia. A limited number of thrombophilic patients have been enrolled in randomised clinical trials, but evidence for a non-inferiority of DOAC was shown only in one of them[4,14]. The important role of observational studies in depicting "the real world", outside of trial settings, is being increasingly recognised[15-17]. Indeed, observational studies may generate relevant evidence, complementing that from randomised clinical trials, and provide context to trial results.

The START2-Register (Survey on anticoagulated patients register) is an ongoing, Italian, multicentre, prospective registry of consecutive patients presenting with symptomatic acute VTE confirmed by objective tests (ClinicalTrials.gov identifier: NCT02219984)[18].

In a real-world setting of a large cohort of VTE patients, we aimed to assess which factors are considered when making decisions regarding the drug that best fits the patient's risk profile according to thrombophilia testing.

MATERIALS AND METHODS

START2-Register design

The START-Register is an observational, multicentre, dynamic cohort study that includes adults (≥18 years) who start anticoagulation therapy, whatever the drug and dosage used. The aim of the START-Register is to collect data on the effectiveness and safety of anticoagulant treatments, on determinants of adverse events in anticoagulated patients, as well as on their quality of life and compliance with treatment. The registry was approved on October 2011 (n=142/2010/O/Oss) by the ethical committee of the coordinating institution ("S. Orsola-Malpighi" University Hospital, Bologna, Italy). All patients gave their written informed consent before enrolment. The study, which is ongoing and actively recruiting, is registered with ClinicalTrials.gov identifier: NCT02219984. Here we present the results of the cohort of patients with VTE. Data collected from January 2012 to April 2018 were used for the purpose of the present analysis.

Patients' eligibility

Patients aged ≥18 years who at the time of consideration for inclusion in the registry have been receiving anticoagulation therapy for no more than 30 days are eligible. Because the START-Register was originally proposed as a prospective, observational study, dedicated to the follow-up of anticoagulated patients, mainly focusing on what happens in long-term (not initial anticoagulation) treatment, patients who have a very short life-expectancy at the screening visit are not included in the registry. Therefore, patients with a life-expectancy <6 months, or not residents in the participant region, or planning to leave the region within the next 6 months are not eligible. Patients already enrolled in phase II or III clinical studies are excluded. Participants are required to enrol their patients consecutively, without any *a priori* exclusion criteria other than life-expectancy or geographical inaccessibility. The definition of the time-frame for enrolment (e.g. 1 week every month, or the first month of the year) is left at each participant's

discretion, as long as it provides a random enrolment of patients.

Baseline data

The patient's clinical features are recorded by participants on web-based case report forms. Baseline data include demographic and clinical characteristics of the patients, clinical indication for treatment, associated risk factors for thromboembolic complications or bleeding, routine laboratory data, type of anticoagulant drug used and dose or expected therapeutic range (in cases treated with VKA), and use of concomitant drugs. For patients treated with VKA, all International Normalised Ratio (INR) controls, the subsequent dosing prescriptions and information about possible clinical events and changes in the medical history occurring during treatment are automatically captured via the informatics platform.

Serum creatinine levels are measured by local hospital laboratories, and creatinine clearance is calculated using the Cockroft-Gault formula[19]. Renal failure is defined according to the National Kidney Foundation stratification[20]. In patients with VTE, assessment of the site and presence of provoking factors is mandatory; determination of the presence of biochemical or molecular risk factors is optional.

Follow-up data

Participants are required to follow up enrolled patients regularly, at least quarterly, by phone call or outpatient visit. An outpatient follow-up visit is mandatory at least annually. Participants are required to provide detailed clinical reports of any relevant clinical outcomes occurring in enrolled patients. For patients on VKA, all the INR results obtained during treatment are recorded in the electronic database of the registry, and time spent within (TTR), below or above the therapeutic range (computed according to Rosendaal's method)[21] can be calculated whenever indicated.

Events

Although all changes in medical history are recorded, specific outcomes for patients treated for VTE are new or recurrent deep vein thrombosis, pulmonary embolism, superficial vein thrombosis, major bleeding and non-major, but clinically relevant bleeding episodes. Participants are required to provide detailed clinical reports of clinically relevant outcomes (deep vein thrombosis, pulmonary embolism, major bleeding) occurring in enrolled patients to allow adjudication of the reported events by a central Adjudication Committee. Major bleeding events are defined as recommended by the International Society on Thrombosis and Haemostasis[22]. Clinically relevant non-major bleeding is defined as overt bleeding that does not meet the criteria for major bleeding, but is associated with the need for medical intervention, contact with a physician, changes in dosing of VKA, or with discomfort or impairment of activities of daily life[23]. A diagnosis of stroke requires the abrupt onset of focal neurological symptoms lasting at least 24 h, and is supported by congruent ischemic lesions on computed tomography or magnetic resonance imaging. Systemic embolism is defined by symptoms consistent with an acute loss of blood flow to a peripheral artery, and is supported by objective evidence of embolism. Acute myocardial infarction is defined by thoracic symptoms and levels of troponin elevated ≥2 times the upper limit of normal or significant Q waves. Pulmonary embolism is diagnosed by the detection of new intraluminal filling on spiral computed tomography scanning, pulmonary angiogram or ventilation/perfusion lung scan. Deep vein thrombosis is diagnosed by the detection of a thrombus in a deep vein by abnormal compression ultrasound.

Thrombophilia screening

Thrombophilic screening was not routinely performed in the study, but left to the judgement of clinicians. In patients tested the following inherited risk factors were investigated: factor V (FV) Leiden and factor II (FII) A20210, which may have been tested during the acute VTE event; because the screening test for natural anticoagulants may be confounded by the presence of oral anticoagulants, antithrombin, protein C and protein S deficiencies should be tested 2 weeks after discontinuation of VKA therapy or 4 days after termination of DOAC treatment[24-26]. All the analyses were performed according to local protocols.

Statistical analysis

For each patient, time (in days) on anticoagulation was obtained. Student t-tests and non-parametric tests were used to compare means and medians of continuous variables. Median and interquartile ranges were used to present continuous variables not normally distributed. Categorical variables were compared using the chi-square

test (two-sided) and Fisher's exact test (two-sided). Logistic regression was used to calculate odds ratios (OR) with 95% confidence intervals (95% CI) adjusted for variables that were significant at univariate analyses. SPSS software (version 22, SPSS Inc. Chicago, IL, USA) was used for the statistical management of the data, and a 5% two-tailed significance level was used for all tests.

RESULTS

Patients' characteristics

At the time of the present analysis 90 participants, equally distributed in northern, central and southern Italy, had enrolled 4,866 acute VTE patients who were anticoagulated for treatment of the event and prevention of a new episode. As shown in **Table I**, males were slightly less represented (49.3%) than females. The median age was 66 years (interquartile range: 50-77) and about one-fifth of all patients were >80 year old (905; 18.6%). Among patients >80 years old, 376 were prescribed a DOAC (16.5% of the all patients on DOAC).

The demographic, clinical, and laboratory characteristics of the patients and the incidence of risk factors for VTE recurrence and bleeding are shown in **Table I**. The most represented sites of VTE were deep veins of the lower limbs with/without pulmonary embolism (67.8%), isolated

Table I - *Clinical characteristics of START2-Register patients with venous thromboembolism at enrolment, who had or had not undergone thrombophilia screening and who had positive or negative results*

	All patients	Screening no	Screening yes	p	Screening negative	Screening postitive	p
Patients, n (%)	4,866	2,140 (44.0)	2,726 (56.0)		2,280 (83.6%)	446 (16.4%)	
Males, n (%)	2,399 (49.3)	1,023 (47.8)	1,376 (50.5)	0.064	1,128 (49.5)	248 (55.6)	0.02
Median age (IQR), years	66 (50-77)	68 (52-78)	65 (49-76)	<0.001	67 (51-77)	54.5 (43-67)	<0.001
Presenting VTE, n (%)							
DVT upper limb	238 (4.9)	105 (4.9)	133 (4.9)	<0.001	117 (5.1)	16 (3.6)	<0.001
DVT lower limb ± PE	3,303 (67.9)	1,540 (72.0)	1,763 (64.7)		1,450 (63.6)	313 (70.2)	
Isolated PE	822 (16.9)	295 (13.8)	527 (19.3)		474 (20.8)	53 (11.9)	
Splanchnic VT	109 (2.2)	40 (1.9)	69 (2.5)		54 (2.4)	15 (3.4)	
Cerebral VT	78 (1.6)	27 (1.3)	51 (1.9)		41 (1.8)	10 (2.2)	
SVT (%)	316 (6.5)	133 (6.2)	183 (6.7)		144 (6.3)	39 (8.7)	
Unprovoked (%)	3,334 (69.0)	1,366 (64.4)	1,968 (72.6)	<0.001	1,614 (71.1)	354 (80.3)	<0.001
Provoked (%)	1,498 (31.0)	756 (35.6)	742 (27.4)		655 (28.9)	87 (19.7)	
Recurrent VTE, n (%)	1,214 (24.9)	473 (22.1)	741 (27.2)	<0.001	533 (23.4)	208 (46.6)	<0.001
Oral contraceptive therapy*, n (%)	302 (47.4)*	118 (46.8)	175 (47.5)	n.s.	133 (48.5)	41 (44.6)	n.s.
Active cancer, n (%)	380 (7.8)	155 (7.2)	225 (8.3)	n.s.	213 (9.4)	12 (2.9)	<0.001
History of major bleeding, n (%)	134 (2.8)	75 (3.5)	59 (2.2)	0.005	50 (2.3)	9 (2.3)	n.s.
ICVD, n (%)	283 (6.7)	114 (7.3)	169 (6.3)	n.s.	159 (7.1)	10 (2.5)	0.001
Previous stroke/TIA, n (%)	257 (5.3)	119 (5.6)	138 (5.1)	n.s.	129 (5.7)	9 (2.2)	0.002
Diabetes, n (%)	432 (8.9)	200 (9.3)	232 (8.5)	n.s.	204 (9.1)	28 (6.8)	n.s.
Renal failure, n (%)							
GFR 30-60	1,081 (22.3)	521 (24.5)	560 (20.6)	<0.001	517 /22.7)	43 (9.6)	<0.001
GFR <30	139 (2.9)	73 (3.4)	66 (2.4)		64 (2.7)	2 (0.4)	
History of falls, n (%)	74 (1.5)	36 (1.7)	38 (1.4)	n.s.	34 (1.5)	4 (0.9)	n.s.
Antiplatelet drugs, n (%)	489 (10.0)	211 (9.9)	278 (10.2)	n.s.	252 (11.1)	26 (9.4)	0.001

*Only women aged <50 years. IQR: interquartile ranges; VTE: venous thromboembolism; DVT: deep venous thrombosis; SVT: superficial venous thrombosis; PE: pulmonary embolism; VT: venous thrombosis; GFR: glomerular filtration rate; ICVD: ischaemic cardiovascular disease; n.s.: not significant.

pulmonary embolism (16.9%), superficial veins (6.5%), and veins of upper limbs (4.9%). As a whole, 1,214 patients presented with a recurrent episode (24.9%), 380 with active cancer (29.3%), and 322 (47.6%) of women aged less than 50 years were taking oral contraceptive therapy. Women were more frequently elderly (median age 69 *vs* 64; p<0.001), with a personal history of provoked VTE (36.8% *vs* 25.0%; p<0.001) and presented with a lower estimated glomerular filtration rate (<60 mL/min: 32.3% *vs* 17.6%; p=0.01). Men more frequently had a personal history of recurrences (26.6% *vs* 23.3%; p<0.001) and of cardiovascular ischemic disease (8.1% *vs* 5.3%; p<0.001).

Results of screening for genetic thrombophilia

Screening for genetic thrombophilia was performed in 2,756 (56%) patients. Patients investigated were younger (median age: 65 *vs* 68; p<0.001) and had a lower prevalence of estimated glomerular filtration rate <60 mL/min (n[%]: 626 [23.0%] *vs* 594 [27.8%]; p<0.001). In addition, investigated patients more frequently suffered from unprovoked (72.6 *vs* 64.4%; p<0.001) and recurrent (27.2 *vs* 22.1%; p<0.001) events (**Table I**).

A positive result was obtained in 446 (16.4%) patients. Patients carrying a genetic thrombophilia were younger (median age: 54.5 *vs* 67; p<0.001) and more frequently suffered from an unprovoked (80.3 *vs* 71.1%; p<0.001) or recurrent (46.6 *vs* 23.4%; p<0.001) VTE. In addition, positive patients were less likely to have a circumstantial risk factor, such as active cancer, previous cerebrovascular ischaemic disease, and renal failure, or were assuming an antiplatelet drug.

The types of genetic thrombophilia identified are presented in **Table II**. As expected, FV Leiden and FII A20210 mutation were the most frequently found risk factors (overall 11.2%), whereas deficiency of a natural anticoagulant was identified in 91 patients (3.3%). Forty-nine patients (1.8%) were found to carry multiple defects. Overall, more thrombophilic patients were prescribed a DOAC (p<0.001).

Choice of oral anticoagulant treatment

At the time of the present analysis, the majority of VTE patients in the START2-Register (n=2,591; 53.2%) were still treated with conventional anticoagulant therapy (CAT) consisting of parenteral low molecular weight heparins with transition to a VKA, whereas DOAC were prescribed to 2,275 patients (46.8%) for long-term therapy. Antithrombotic treatment choices during the frame of time of enrolment are summarised in **Figure 1**. Starting from the 2013 patients begun to be treated with DOAC, especially those who underwent thrombophilic screening, regardless of the results obtained.

Overall, the anticoagulant therapy was interrupted in 1,578 (31.7%) of patients and more frequently in those assuming CAT (**Table III**), regardless of whether they underwent thrombophilic screening or the findings of such screening. Among CAT-treated patients, the quality of anticoagulation control (TTR <60%) was inadequate more frequently in those who underwent thrombophilic screening with negative results. (**Table III**) On multivariate analysis, a DOAC was prescribed more frequently in male patients (OR: 1.25; 95% CI: 1.05-1.48), in those with an

Table II - *Laboratory findings in patients investigated for genetic thrombophilia*

	All patients (n=2,726)	CAT (n=1,339; 49.1%)	DOAC (n=1,387; 50.9%)
None	2,280 (83.6)	1,167 (87.2)	1,113 (80.3)*
FII A20120	101 (3.7)	47 (3.5)	54 (3.9)
FV Leiden	205 (7.5)	69 (5.2)	136 (9.8)
Antithrombin	22 (0.8)	8 (0.6)	14 (1.0)
Protein C	28 (1.0)	15 (1.1)	13 (0.9)
Protein S	41 (1.5)	17 (1.3)	24 (1.7)
Multiple defects	49 (1.8)	16 (1.2)	33 (2.4)

CAT: conventional anticoagulant therapy; DOAC: direct oral anticoagulant; FII: factor II; FC: factor V. *p<0.001

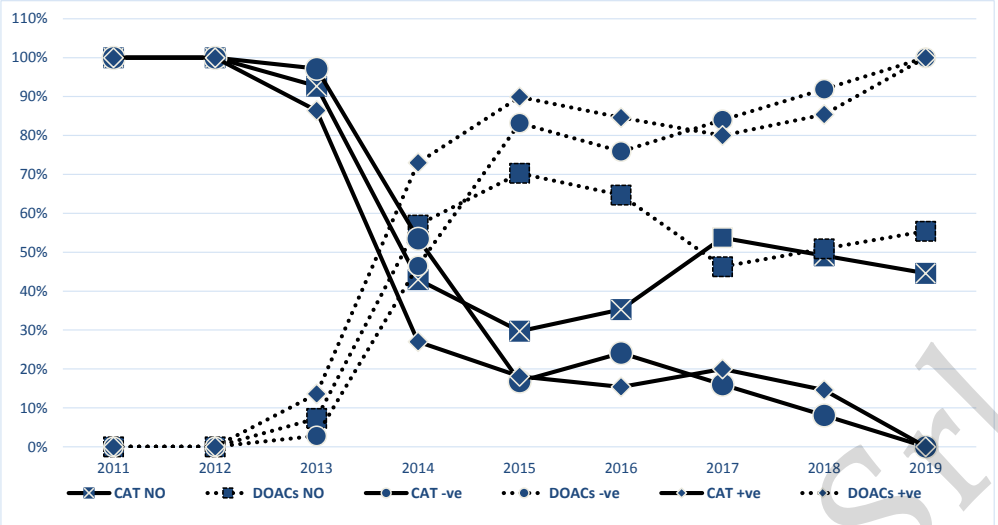

Figure 1 - Prescription pattern of the anticoagulant regimen prescribed according to year of enrolment in the register
no: not screened; -ve: negative at the screening; +ve: positive at the screening.

Table III - *Type of anticoagulant regimen prescribed and follow-up of the therapy in START2 patients*

	All patients	Screening no	Screening yes	p	Screening negative	Screening postitive	p
Patients	4,866	2,140	2,726		2,280	446	
Type of therapy, n (%) **CAT** **DOAC**	 2,591 (53.2) 2,275 (46.8)	 1,252 (58.5) 888 (41.5)	 1,339 (49.1) 1,387 (50.9)	 <0.001	 1,167 (51.2) 1,113 (48.8)	 172 (38.6) 274 (61.4)	 <0.001
Stop of therapy, n (%) **CAT** **DOAC**	1,578 (32.4) 1,037 (40.0) 541 (23.8)§	701 (32.8) 468 (37.4) 233 (26.2)§	877 (32.2) 569 (42.5) 308 (22.2)§	n.s. 0.008 0.03	787 (34.5) 514 (44.0) 273 (24.5)§	90 (20.2) 55 (32.0) 35 (12.8)§	<0.001 0.003 <0.001
TTR <60%*, n (%)	412 (15.9)	135 (10.8)	277 (20.7)	0.05	257 (22.0)	20 (11.6)	0.003
N. of dose modifications >30%* Median (IQR)	6 (3-11)	5 (3-9)	7 (3-14)	<0.001	7 (3-14)	6 (3-12)	n.s.

§p<0.001 CAT *vs* DOAC; *only patients on CAT.
CAT: conventional anticoagulant therapy; DOAC: direct oral anticoagulant; TTR: time in therapeutic range; IQR: interquartile range; n.s.: not significant.

idiopathic (OR: 1.51; 95% CI: 1.25-1.83) or recurrent (OR: 2.11; 95% CI: 1.74-2.57) VTE, and in those investigated for genetic thrombophilia (OR: 2.11; 95% CI: 1.85-2.54), independently of the results. Patients with an active cancer (OR: 0.50; 95% CI: 0.36-0.71), a history of ischaemic cardiovascular disease (OR: 0.54; 95% CI: 0.38-0.78) or assuming an antiplatelet drug (OR: 0.74; 95% CI: 0.56-0.97) had a lower rate of a DOAC prescription.

Outcomes

Thrombotic and haemorrhagic complications during the anticoagulant therapy are reported in **Table IV**. As far as concerns thrombotic episodes, a significantly higher rate of recurrent VTE was recorded in patients who were prescribed a DOAC, regardless of whether they underwent the screening or not. Overall, an increased number of recurrent VTE was observed in patients not screened for thrombophilia (n=36; 1.7%) than in those who underwent such screening (n=20; 0.7%; OR: 2.3; 95% CI: 1.3-4.0). The multivariate risk of a recurrent VTE was significantly higher in patients who were prescribed a DOAC, an antiplatelet drug, or not screened for thrombophilia (**Table V**). As for arterial thrombosis, the risk was higher in hypertensive patients, if bleeding had occurred before enrolment, and in those on CAT (**Table V**).

Table IV - *Clinical outcomes during anticoagulant therapy*

Patients	All patients		Screening no		Screening negative		Screening positive	
Total	4,866		2,140		2,280		446	
Age median (IQR)	66 (50-77)		68 (52-78)		67 (51-77)		54 (43-67)	
Type of OAC	CAT	DOAC	CAT	DOAC	CAT	DOAC	CAT	DOAC
Number	2,591	2,275	1,252	888	1,167	1,113	172	274
Age median (IQR)	66 (50-77)	66 (50-77)	68 (51-78)	67 (52-78)	67 (52-77)	67 (51-77)	50 (41-64)	56 (45-69)*
Bleeding, n (%)	109 (4.2)	42 (1.8)**	54 (4.3)	17 (1.9)§	43 (3.7)	19 (1.7)^	12 (7.0)	6 (2.2)°
Major, n (%)	33 (1.3)	19 (0.8)	18 (1.4)	9 (1.0)	12 (1.1)	7 (0.6)	3 (1.8)	3 (1.1)
Rate (100 pt. years)	1.1	1.2	1.7	1.4	0.7	1.0	1.2	1.4
CRMN, n (%)	76 (2.9)	23 (1.0)**	36 (2.9)	8 (0.9)§	31 (2.8)	12 (1.1)^	9 (5.2)	3 (1.1)°°
Rate (100 pt. years)	2.4	1.5	3.3	1.4	1.8	1.7	3.6	1.4
Thrombosis, n (%)	30 (1.2)	44 (1.9)#	18 (1.4)	28 (3.2)+	11 (0.9)	13 (1.2)	1 (0.6)	3 (1.1)
Arterial, n (%)	16 (0.7)	2 (0.1)^	9 (0.7)	1 (0.1)	6 (0.5)	1 (0.1)	1 (0.6)	0
Rate (100 pt. years)	0.5	0.1	0.9	0.2	0.3	0.2	0.4	--
Venous, n (%)	14 (0.5)	42 (1.8)**	9 (0.7)	27 (3.1)**	5 (0.4)	12 (1.1)	0	3 (1.1)
Rate (100 pt. years)	0.5	2.7	0.9	4.3	0.3	1.7	--	1.4

*p=0.004; **p<0.001; §p=0.001; ^p=0.003; °p=0.013; #p=0-034; +p=0.009; °°p=0.01. OAC: oral anticoagulation; CAT: conventional anticoagulant therapy; CRMN: clinically relevant non-major bleeding.

A bleeding episode occurred more frequently in patients on CAT (4.2%) than on DOAC (1.8%) and an increase was observed in CAT-treated patients who tested positive at the thrombophilic screening. In addition, a higher bleeding rate was associated with the use of antiplatelet drugs (OR: 1.9; 95% CI: 1.1-3.5). The rate of cerebral bleeding was non-significantly higher in the CAT group (n=10; 0.4%) than in the DOAC-treated group (n=4; 0.2%). Likewise, a trend for a higher number of gastrointestinal bleeds was observed in the CAT group (n=24; 0.9%) as compared to the DOAC-treated group (n=10; 0.4%; OR: 2.1; 95% CI: 1.0-4.3). On multivariate analysis, female sex, a recurrent VTE at enrolment, the occurrence of a recurrent VTE, the use of antiplatelet drugs, and the prescription of CAT were significantly associated with the occurrence of bleeding (**Table V**).

DISCUSSION

The study presents data obtained from a large series of consecutively enrolled patients with a new VTE in real life and suggests a widespread practice of ordering inherited thrombophilia testing notwithstanding the negative advice of several guidelines[27-29], which restrict testing to specific clinical situations and patients. Overall, patients submitted to the screening were younger, more frequently suffered from unprovoked and recurrent events, had better kidney function, and a lower risk of bleeding. These findings suggest that among anticoagulated patients with VTE, thrombophilic screening was preferentially recommended to patients presenting with some clinical

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Table V - *Multivariate analysis for variables associated with adverse events during anticoagulation*

Variables	p	OR	95% CI
Venous thromboses			
Use of antiplatelet therapy	0.006	2.63	1.33-5.22
Anticoagulant therapy			
CAT		ref.	
DOAC	<0.001	5.21	2.77-9.78
Screening for thrombophilia			
Not done		ref.	
Tested	<0.001	3.04	1.72-5.35
Arterial thromboses			
Hypertension	0.020	3.77	1.24-11.52
Previous bleeding	0.046	3.61	1.02-12.71
Anticoagulant therapy			
DOAC		ref.	
CAT	0.039	4.74	1.08-20.72
Haemorrhages			
Anticoagulant therapy			
DOAC		ref.	
CAT	<0.001	2.69	1.79-4.04
Presenting VTE			
1st episode		ref.	
Recurrence	0.002	1.82	1.25-2.66
Female sex	0.005	1.69	1.18-2.44
Thrombosis in follow-up	0.014	3.33	1.28-8.67
Use of antiplatelet therapy	0.038	1.67	1.03-2.70

OR: odds ratio; 95% CI: 95% confidence interval; CAT: conventional anticoagulant therapy; DOAC: direct oral anticoagulant; VTE: venous thromboembolism; ref.: reference group.

characteristics. Although ordered excessively, the practice of screening was directed more to patients presenting with clinical characteristics suggestive of inherited thrombophilia[24].

An increase in overall DOAC prescription rates over the study period was recorded. In agreement with previous findings[10], DOAC prescription were equipoised and slightly overcame standard therapy with low molecular weight heparins and VKA. To the best of our knowledge, this is the first study in Italy that assessed the prescription patterns of DOAC, well after the introduction of all DOAC. The results of the present study suggest that practice patterns of anticoagulant prescription for VTE prevention in patients who have undergone screening for thrombophilia changed dramatically during the years under study, with a rapid increase in the use of DOAC. In contrast, the rate of prescription of the two anticoagulant regimens was equipoiseed in patients who did not undergo screening, Indeed, multiple logistic regression analysis showed that patients on DOAC submitted to screening more frequently presented with an idiopathic VTE (OR: 1.4; 95% CI: 1.1-1.8), an estimated glomerular filtration rate >60 mL/min (OR: 1.5; 95% CI: 1.1-1.8), and a negative history of major bleeding (OR: 2.4; 95% CI: 1.5-3.8). The present data confirm previous findings[29-31] and further show that in real life an important subgroup of patients preferentially received treatment with DOAC.

The rates of complications, both haemorrhagic and thrombotic, recorded in patients on DOAC were similar to those in other registries[30,31], whereas in the CAT group the rate of VTE recurrences was lower but consistent with other Italian data[32].

Randomised clinical trials, meta-analyses and real-world analyses that investigated outcomes of patients showed that DOAC decrease the risk of major bleeding events when compared to CAT[33-35]. The present study confirms a higher bleeding rate among patients on CAT. Overall, patients presenting with previous major bleeding were more frequently prescribed DOAC (76/2,275) than CAT (58/2,591; OR: 1.5; 95% CI: 1.1-2.1). Among patients on CAT, those likely to have poor anticoagulation control - based on TTR and the number of changes above 30% of the prescribed dose of the VKA administered - had a significantly higher bleeding risk. A concomitant higher prescription of antiplatelet therapy in patients on CAT further strengthened this finding. Patients who tested positive to thrombophilic screening showed better anticoagulant control. This investigation does, in fact, confirm previous studies[36,37] and further shows that the quality of anticoagulation obtained and the intensity of anticoagulation predicted the risk of bleeding in patients on CAT.

During the treatment-period, arterial thrombosis occurred more frequently in patients on CAT, whereas patients on DOAC had a significantly increased rate of VTE recurrences.

In our setting, VTE patients with concurrent atherosclerotic co-morbidities at risk of arterial thrombosis were more frequently prescribed CAT than DOAC. The trend towards warfarin prescription was consistent during all phases of enrolment and suggested a physician-driven preference in

vulnerable patients at highest risk of arterial thrombotic complications.

The coexistence of a traditional cardiovascular risk factor for atherosclerosis or atherosclerotic vascular disease has been suggested to be associated with a risk of VTE[38,39]. The significant association with the use of antiplatelet drugs further confirms these data. Patients investigated for inherited thrombophilia were younger and had a relatively lower prevalence of concomitant atherosclerotic co-morbidities or isolated pulmonary embolism. These findings are consistent with widespread indications from the literature[24,40] and suggest that the prescription of thrombophilic screening may identify a patient with a lower risk of VTE recurrence during anticoagulation. In addition, patients with an episode of proximal VTE have a higher risk, substantiating that the initial presentation has an impact on the chance of VTE recurrence[43]. Finally, patients who were prescribed a DOAC were more likely to suffer from a recurrent and unprovoked episode of VTE. As a result, a patient's personal history of VTE was confirmed to be a strong predictor of VTE recurrence.

The strengths of our study include analysis of a large, contemporary group of patients from one country. The subjects investigated are representative of a real-world population of patients with VTE.

However, the study suffers from a number of limitations. The START-Register has an observational design and does not have the stringent inclusion/exclusion criteria restrictions of randomised control trials. Extreme caution is required in interpreting results of direct comparisons between drugs because of the intrinsic limitations and the risk of bias of the study. This registry enrolled Italian patients and may not be representative and account for national differences. The restriction of study centres to Italy may limit extrapolation of the findings to other geographical areas. The choice of which anticoagulant to prescribe was entirely at the physician's discretion. As a consequence, independent variables, such as drug availability and reimbursement, might have had a role, affecting drug choice.

CONCLUSIONS

The present data confirm previous findings from other post-marketing registries suggesting that the choice of oral anticoagulation is strongly driven by patients' characteristics and VTE manifestations, although physicians' discretion may play a role. Thrombophilia screening is widely performed, and implementation of guidance is needed to improve current thrombophilia screening practice.

AUTHORSHIP CONTRIBUTIONS

MM, EA, and GP conceived and designed the study; MM, EA, GDA, and GP analysed and interpreted data; LM, WA, EB, BC, AF, GM, DM, CP, DP, and ST collected data; GDA and MM performed statistics; LM, WA, EB, BC, AF, GM, DM, CP, DP, and ST recruited study materials or patients; EG drafted the article or parts of it; MM, EA, and GP critically revised the article for important intellectual content; EA and GP planned administrative, technical, or logistic support; GP was the guarantor of the study; MM and EA supervised the study.

The Authors declare no conflicts of interest.

REFERENCES

1. White RH. The epidemiology of venous thromboembolism. Circulation 2003; **107** (Suppl. 1): 4-8.
2. Ageno W, Gallus AS, Wittkowsky A, et al. Oral anticoagulant therapy: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012; **141**: e44S-e88S
3. Schulman S, Kearon C, Kakkar AK, et al. Dabigatran versus warfarin in the treatment of acute venous thromboembolism. N Engl J Med 2009; **361**: 2342-52.
4. Bauersachs R, Berkowitz S D, Brenner B et al. Oral rivaroxaban for symptomatic venous thromboembolism. N Engl J Med 2010; **363**: 2499-510.
5. Agnelli G, Buller HR, Cohen A, et al. Oral apixaban for the treatment of acute venous thromboembolism. N Engl J Med 2013; **369**: 799-808.
6. Büller HR, Décousus H, Grosso MA, et al. Edoxaban versus warfarin for the treatment of symptomatic venous thromboembolism. N Engl J Med 2013; **369**: 1406-15.
7. Barnes GD, Lucas E, Alexander GC, Goldberger ZD. National trends in ambulatory oral anticoagulant use. Am J Med 2015; **128**: 1300-5.
8. Lip GYH, Banerjee A, Boriani G, et al. Antithrombotic therapy for atrial fibrillation: CHEST Guideline and Expert Panel Report. Chest 2018; **154**: 1121-201.
9. Kearon C, Akl EA, Ornelas J, et al. Antithrombotic therapy for VTE disease: CHEST Guideline and Expert Panel Report. Chest 2016; **149**: 315-52.
10. Huisman MV, Rothman KJ, Paquette M, et al; GLORIA-AF Investigators. The changing landscape for stroke prevention in AF: findings from the GLORIA-AF Registry phase 2. J Am Coll Cardiol 2017; **69**: 777-85.
11. Hicks LK, Bering H, Carson KR, et al. The ASH Choosing Wisely campaign: five hematologic tests and treatments to question. Blood 2013; **122**: 3879-83.
12. Baglin T, Gray E, Greaves M, et al. Clinical guidelines for testing for heritable thrombophilia. Br J Haematol 2010; **149**: 209-20.
13. Favaloro EJ, McDonald D, Lippi G. Laboratory investigation of thrombophilia: the good, the bad, and the ugly. Semin Thromb Hemost 2009; **35**: 695-710.

14. Goldhaber SZ, Eriksson H, Kakkar A, et al. Efficacy of dabigatran versus warfarin in patients with acute venous thromboembolism in the presence of thrombophilia: findings from RE-COVER®, RE-COVER™ II, and RE-MEDY™. Vasc Med 2016; **21**: 506-14.
15. Ligthelm RJ, Borzi V, Gumprecht J, et al. Importance of observational studies in clinical practice. Clin Ther 2007; **29**: 1284-92.
16. Rawlins M. De testimonio: on the evidence for decisions about the use of therapeutic interventions. Lancet 2008; **372**: 2152-61.
17. Silverman SL. From randomized controlled trials to observational studies. Am J Med 2009; **122**: 114-20.
18. Antonucci E, Poli D, Tosetto A, et al. The Italian START-register on anticoagulation with focus on atrial fibrillation. PLoS One 2015; **10**: e0124719.
19. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; **16**: 31-41.
20. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002; **39**: S1-266
21. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briet E. A method to determine the optimal intensity of oral anticoagulant therapy. Thromb Haemost 1993; **69**: 236-9.
22. Schulman S, Kearon C. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost 2005; **3**: 692-4
23. Buller HR, Cohen AT, Davidson B, et al. Idraparinux vs standard therapy for venous thromboembolic disease. N Engl J Med 2007; **357**: 1094-104.
24. Connors JM. Thrombophilia testing and venous thrombosis. N Engl J Med 2017; **377**: 1177-87.
25. Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. Thromb Haemost 2018; **16**: 209-19.
26. Favresse J, Lardinois B, Sabor L, et al. Evaluation of the DOAC-Stop® procedure to overcome the effect of DOAC on several thrombophilia screening tests. TH Open 2018; **2**: e202-9.
27. Cohen AT, Gitt AK, Bauersachs R, et al. The management of acute venous thromboembolism in clinical practice. Results from the European PREFER in VTE Registry. Thromb Haemost 2017; **117**: 1326-37.
28. Jun M, Lix LM, Durand M, et al. Comparative safety of direct oral anticoagulants and warfarin in venous thromboembolism: multicentre, population based, observational study. BMJ 2017; **359**: j4323.
29. Sindet-Pedersen C, Langtved Pallisgaard J, Staerk L, et al. Comparative safety and effectiveness of rivaroxaban versus VKAs in patients with venous thromboembolism. A Danish nationwide registry-based study, Thromb. Haemost 2017; **117**: 1182-91.
30. Kreutz R, Mantovani LG, Haas S, et al. XALIA-LEA: An observational study of venous thromboembolism treatment with rivaroxaban and standard anticoagulation in the Asia-Pacific, Eastern Europe, the Middle East, Africa and Latin America. Thromb Res 2019; **176**: 125-32.
31. Keller L, Marten S, Hecker J, et al. Venous thromboembolism therapy with rivaroxaban in daily-care patients: results from the Dresden NOAC registry. Int J Cardiol 2018; **257**: 276-82.
32. Palareti G, Antonucci E, Migliaccio L, et al. Vitamin K antagonist therapy: changes in the treated populations and in management results in Italian anticoagulation clinics compared with those recorded 20 years ago. Intern Emerg Med 2017; **12**: 1109-19.
33. Ruff CT, Giugliano RP, Braunwald E, et al. Comparison of the efficacy and safety of new oral anticoagulants with warfarin in patients with atrial fibrillation: a meta-analysis of randomised trials. Lancet 2014; **383**: 955-62.
34. Lip GYH, Keshishian A, Kamble S, et al. Real-world comparison of major bleeding risk among non-valvular atrial fibrillation patients initiated on apixaban, dabigatran, rivaroxaban, or warfarin. A propensity score matched analysis. Thromb Haemost 2016; **116**: 975-86.
35. Yao X, Abraham NS, Sangaralingham LR, et al. Effectiveness and safety of dabigatran, rivaroxaban, and apixaban versus warfarin in nonvalvular atrial fibrillation. J Am Heart Assoc 2016; **5**: e003725.
36. Palareti G, Leali N, Coccheri S, et al. Bleeding complications of oral anticoagulant treatment: an inception-cohort, prospective collaborative study (ISCOAT). Italian study on complications of oral anticoagulant therapy. Lancet 1996; **348**: 423-8.
37. Connolly S, Pogue J, Eikelboom J, et al. Benefit of oral anticoagulant over antiplatelet therapy in atrial fibrillation depends on the quality of international normalized ratio control achieved by centers and countries as measured by time in therapeutic range. Circulation 2008; **118**: 2029-37.
38. Cavallari I, Morrow DA, Creager MA, et al. Frequency, predictors, and impact of combined antiplatelet therapy on venous thromboembolism in patients with symptomatic atherosclerosis. Circulation 2018; **137**: 684-92.
39. Gaertner S, Cordeanu EM, Mirea C, et al. Increased risk and severity of unprovoked venous thromboembolism with clustering cardiovascular risk factors for atherosclerosis: results of the REMOTEV registry. Int J Cardiol 2018; **252**: 169-74.
40. Colaizzo D, Amitrano L, Iannaccone L, et al. Gain-of-function gene mutations and venous thromboembolism: distinct roles in different clinical settings. J Med Genet 2007; **44**: 412-6.
41. Boutitie F, Pinede L, Schulman S, et al. Influence of preceding length of anticoagulant treatment and initial presentation of venous thromboembolism on risk of recurrence after stopping treatment: analysis of individual participants' data from seven trials. BMJ 2011; **342**: d3036.

APPENDIX

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