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Full Length Article

High detection rates of antithrombin deficiency and antiphospholipid syndrome in outpatients aged over 50 years using the standardized protocol for thrombophilia screening

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ABSTRACT

Introduction: Thrombophilia screening has limited detection efficiency. We assessed the detection rate when a standardized approach to thrombophilia-screened outpatients was used.

Methods: We analyzed 1185 patients (36.5% males, median age: 43 years [IQR 33-54]) referred to a single center from January 2014 to October 2017 with 11 different clinical indications for thrombophilia screening, which was performed in the adherence to published guidelines. Factor V Leiden, prothrombin G20210A mutation, antithrombin (AT), protein C, protein S deficiencies and antiphospholipid syndrome (APS) were determined.

Results: The overall positivity rate was 37.1% (95% CI 34.3%–39.7%). The highest positivity rate was found in women following VTE during pregnancy/childbirth (64.1%) and provoked VTE patients with positive family history (52.9%). In patients aged > 50 years (32.5%), APS was found at a similar rate as in younger subjects (11.4% vs 10.1%), while AT deficiency was detected more frequently in the older group (5.7% vs 2.4%, $p = 0.003$).

Conclusions: Standard indications for thrombophilia screening lead to detection rates of 37% or more. Frequent detection of APS and AT deficiency among older patients, which often implies a need for long-term anticoagulation and could impact clinical practice patterns, suggests a benefit of thrombophilia screening in this population in selected clinical circumstances.

1. Introduction

Thrombophilia, both genetically determined and acquired, increases the risk of venous thromboembolism (VTE) [1]. Thrombophilia can be found in approximately 50% of patients with VTE [2]. Thrombophilia testing include factor V Leiden (FVL) and prothrombin (FII) G20210A mutations, with a prevalence in VTE patients up to 20% and 6%, respectively [1,3], along with plasma levels of natural anticoagulants, i.e. antithrombin (AT), protein C (PC) and protein S (PS), with the prevalence estimated at 0.5–4.9%, 3%, 2–12% of patients, respectively, after the first incident of VTE [1,3–5]. Antiphospholipid syndrome (APS) is also part of thrombophilia screening. The overall frequency of APS antibodies is 6% for pregnancy morbidity, 13.5% for ischemic stroke, 11% for myocardial infarction (MI), and 9.5% for deep vein thrombosis (DVT) patients [1,3,4].

Although no strong recommendations exist regarding a target population which benefits from thrombophilia detection, there are some clinical characteristics which are highly suggestive for thrombophilia, namely thrombosis at a young age (< 50 years), especially in association with weak provoking factors (minor surgery, combination oral contraceptives, or immobility) or unprovoked VTE, strong family history of VTE (first-degree family members affected at a young age), recurrent VTE events, in particular at a young age, and VTE in unusual sites such as splanchnic or cerebral veins [1]. Few studies evaluated all the above mentioned indications in terms of the rates of positive results among consecutive outpatients in a tertiary center.

The so far published rates of positive results differ with regard to clinical adherence to thrombophilia screening guidelines [6–9]. Kwon

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et al. showed that the rate of positive results was as low as 13.8% among all patients who were tested for thrombophilia during a 3-year period [10], whereas Meyer et al. reported 20% rate of positive results among patients with acute VTE [11]. Roldan et al. showed that in a selected group of 3613 symptomatic VTE patients with confirmed indication for thrombophilia screening, the positivity rate reached 34%, with the highest prevalence of FVL mutation [12].

To our knowledge, there have been no studies that compared detection rates at thrombophilia screening in consecutive outpatients with various indications beyond unprovoked or recurrent VTE. The goal of the study was to assess the detection rates of thrombophilia using a standardized approach to thrombophilia screening with 11 different clinical indications with the focus on subjects aged 50 years or more.

2. Methods

2.1. Patients

We assessed thrombophilic factors in 1185 consecutive adult Caucasian outpatients referred to the Center for Coagulation Disorders at the John Paul II Hospital in Krakow, Poland, due to suspected thrombophilia from January 2014 to October 2017. All analyzed patients fulfilled at least one of the following indications for thrombophilia testing developed in our center based on available expert opinions and guidelines [6–8]. This unified approach to thrombophilia testing was started in January 2014.

All patients were categorized with regard to the indication for thrombophilia screening, as follows:

1) first unprovoked pulmonary embolism (PE);
2) first unprovoked DVT;
3) ischemic stroke at age < 50 years;
4) MI at age < 50 years;
5) recurrent VTE (≥2 episodes);
6) positive family history of VTE and provoked VTE;
7) ≥2 miscarriages;
8) thrombosis at unusual site (e.g. splanchnic veins or cerebral sinus veins thrombosis);
9) VTE episode during pregnancy/childbirth (within 4 weeks);
10) VTE while on oral contraceptives or on hormone replacement therapy;
11) other, e.g. asymptomatic patients with positive family history without a prior thrombotic event.

The diagnosis of DVT was established by a positive finding of color duplex sonography (visualization of an intraluminal thrombus in calf, popliteal, femoral or iliac veins), whereas PE was diagnosed on the basis of a positive finding on computed tomography angiography. Unprovoked VTE was diagnosed in the absence of the following transient risk factors: trauma, surgery, prolonged hospitalization (3 days or more), pregnancy, childbirth or the oral contraceptive use. The acute ischemic stroke diagnosis was made based on clinical symptoms according to the World Health Organization definition [13] and brain imaging. MI was defined as typical chest pain and elevated cardiac troponin levels. Patients who experienced VTE following trauma, surgery, hospitalization and pregnancy or childbirth within 12 weeks prior to the onset of VTE-related symptoms were eligible, only if their family history of VTE was positive, that is, a documented VTE episode in a first-degree relative below 50 years of age. Recurrent miscarriages were defined as two or more consecutive miscarriages. The diagnosis of cerebral sinus veins thrombosis was based on computed tomographic angiography, magnetic resonance imaging, or magnetic resonance angiography. The diagnosis of splanchnic vein thrombosis was based on color duplex sonography, computed tomography, or magnetic resonance imaging.

All patients provided their written informed consent before thrombophilia screening.

2.2. Laboratory investigations

Thrombophilia screening was performed 4 weeks or more after a thrombotic event. Six basic thrombophilic factors determined involved FVL, FII G20210A mutation, deficiencies of AT, PC, and/or PS, together with APS. FVIII activity and plasma homocysteine (tHcy) were measured optionally, at the discretion of the attending physician. FVL and FII G20210A mutations were determined by the Real-Time PCR with the use of TaqMan Genotyping Assays (QuantStudio Dx Real-Time PCT Instrument, ThermoFisher Scientific, Waltham, Massachusetts, USA). Plasma PC activity was quantified using a chromogenic assay (Siemens Healthcare Diagnostics); deficiency was defined as activity of < 70%. PS levels were measured using an immunoturbidimetric assay (INNOVANCE® Free PS Ag, Siemens Healthcare Diagnostic); deficiency was defined as levels of < 60% for women and < 67% for men. AT activity was measured using factor Xa inhibition or thrombin-inhibition based assays (Siemens Healthcare Diagnostics Marburg, Germany); deficiency was defined as activity < 83%.

To detect APS, the 5 variables were determined. Estimation of lupus anticoagulant was performed using clot-based assays [7]. Anticardiolipin and β_2GP-I antibodies were determined by immunoenzymatic assays (INOVA Diagnostics, San Diego, California, USA). Reference ranges for IgG antibodies were up to 15.0 GPL and 8.0 SGU, respectively, and for IgM antibodies up to 17.0 MPL and 10.0 SMU, respectively. APS was diagnosed as recommended in 2006 [14].

Plasma FVIII activity was determined by the coagulometric assay using a deficient plasma (Siemens Healthcare Diagnostics) and levels of 150% or more were considered elevated. tHcy was determined in plasma by the enzymatic assay (Roche Diagnostics, Mannheim, Germany). Hyperhomocysteinemia was defined as fasting tHcy ≥ 15 μmol/l [15,16].

In case of decreased levels of PS, PC or AT, repeated testing to confirm the initial abnormality was performed after 1–6 months while off anticoagulation.

2.3. Statistical analysis

Categorical variables are presented as numbers and percentages. Continuous variables are expressed as mean ± standard deviation or median and interquartile range (IQR), as appropriate. Normality was assessed by the Shapiro–Wilk test. Equality of variances was assessed using the Levene's test. Differences between groups were compared using the Welch's *t*-test or the Mann-Whitney *U* test, depending on the equality of variances for normally distributed variables. Categorical variables were analyzed using the chi^2 test or Fisher's exact test as appropriate. To investigate intergroup differences between multiple groups, Kruskal-Wallis ANOVA was used. Associations between the variables were expressed as odds ratios with 95% confidence intervals. Two-sided *P*-values < 0.05 were considered statistically significant. All calculations were done with STATISTICA 12.0 software (StatSoft Inc., Tulsa, USA).

3. Results

We analyzed 1185 consecutive outpatients (752 women, 63.5%), aged 18 to 84 (median 43) years, with indications for thrombophilia screening. The most common indications were recurrent VTE (15.6%), ischemic stroke at the age < 50 years (12.8%) and first unprovoked PE episode (11.6%; Table I). As many as 214 asymptomatic individuals (18.1%) were screened due to positive family history without a prior thrombotic event or adverse pregnancy outcome (Table I).

The overall rate of any positive test of the 6 key parameters reached 37.1% (95% confidence interval [CI] 34.3%–39.7%), with the highest incidence of heterozygous FVL (13.3%), APS (10.5%), FII G20210A

Table I
The rate of positive thrombophilia screening with regard to the main indication.

Variable	Screening indications, n(%)												p-Value
	First unprovoked PE	First unprovoked DVT	AIS < 50 years old	MI < 50 years old	≥2 recurrent VTE	Positive family history and provoked VTE	≥2 miscarriages	Thrombosis in unusual site	VTE during pregnancy/ childbirth	VTE on oral contraceptives or hormone replacement therapy	Other	Total	
All	137 (11.6%)	125 (10.6%)	152 (12.8%)	47 (4.0%)	185 (15.6%)	34 (2.8%)	88 (7.4%)	98 (8.3%)	39 (3.3%)	66 (5.6%)	214 (18.1%)	**1185 (100%)**	
Males	71 (16.4%)	64 (14.8%)	56 (12.9%)	25 (5.8%)	78 (18.0%)	23 (5.3%)	–	33 (7.6%)	–	–	73 (16.9%)	**433 (36.5%)**	
Females	66 (8.8%)	61 (8.2%)	96 (12.7%)	22 (2.9%)	107 (14.2%)	11 (1.5%)	88 (11.7%)	65 (8.6%)	39 (5.2%)	66 (8.8%)	141 (18.8%)	**752 (63.5%)**	
Age, years (IQR)													
All	41 (31–47)	38 (32–46)	46.5 (38–53)	43 (33–51)	59 (52–65)	45.5(36–50)	31 (28–35)	43 (35–54)	33 (28–37)	40.5 (28–47)	43 (32–58)	**43 (33–54)**	< 0.0001
Males	42.0 (34–47)	41(32–49.5)	47 (40–52.5)	37 (31–48)	56 (47–61)	44 (34–49)	–	44.(35–53)	–	–	43 (35–60)	**45 (36–55)**	< 0.0001
Females	37.5 (31–47)	36 (32–43)	46.5 (37–54)	43 (37–51)	61 (56–66)	51 (45–55)	31 (28–35)	41 (35–54)	33 (28–37)	40 (31–47)	43 (30–58)	**41 (32–54)**	< 0.0001
FVL mutation													
All	21 (15.3%)	23 (18.4%)	7 (4.6%)	5 (10.6%)	27 (14.6%)	7 (20.6%)	8 (9.1%)	20 (20.4%)	12 (30.8%)	13 (19.7%)	27 (12.6%)	**170 (13.3%)**	0.0009
Males	12 (16.9%)	14 (21.9%)	4 (7.1%)	3 (12.0%)	19 (22.9%)	6 (26.1%)	–	11 (33.3%)	–	–	5 (6.9%)	**74 (17.1%)**[Δ]	0.02
Females	9 (13.6%)	9 (14.8%)	3 (3.1%)	2 (9.1%)	8 (7.8%)	1 (9.1%)	8 (9.1%)	9 (13.9%)	12 (30.8%)	12 (19.7%)	22 (15.6%)	**95 (12.6%)**[Δ]	0.002
FII G20210A													
All	8 (5.8%)	4 (3.2%)	5 (3.3%)	1 (2.1%)	10 (5.4%)	2 (5.9%)	3 (3.4%)	1 (1.0%)	1 (2.6%)	3 (4.6%)	11 (5.1%)	**49 (4.1%)**	0.76
Males	4 (5.6%)	3 (4.7%)	2 (3.6%)	0	6 (7.2%)	2 (8.7%)	–	0	–	–	2 (2.7%)	**19 (4.4%)**	0.52
Females	4 (6.1%)	1 (1.6%)	3 (3.1%)	1 (4.6%)	4 (3.9%)	0	3 (3.4%)	1 (1.5%)	1 (2.6%)	2 (3.3%)	9 (6.4%)	**29 (3.9%)**	0.82
PC deficiency													
All	4 (2.9%)	2 (1.6%)	2 (1.3%)	1 (2.1%)	1 (0.5%)	2 (5.9%)	0	0	0	0	10 (4.7%)	**22 (1.9%)**	0.02
Males	2 (2.8%)	1 (1.6%)	1 (1.8%)	1 (4.0%)	0	2 (8.7%)	–	0	–	–	3 (4.1%)	**10 (2.3%)**	0.56
Females	2 (3.1%)	1 (1.6%)	1 (1.0%)	0	1 (0.98%)	0	0	0	0	0	7 (5.0%)	**12 (1.6%)**	0.096
PS deficiency													
All	2 (1.5%)	6 (4.8%)	2 (1.3%)	0	1 (0.5%)	1 (2.9%)	3 (3.4%)	3 (3.1%)	4 (10.3%)	1 (1.5%)	10 (4.7%)	**33 (2.8%)**	0.04
Males	1 (1.4%)	3 (4.7%)	1 (1.8%)	0	0	1 (4.4%)	–	1 (3.0%)	–	–	0	**7 (1.6%)**	0.6
Females	1 (1.5%)	3 (4.9%)	1 (1.0%)	0	1 (0.98%)	0	3 (3.4%)	2 (3.1%)	4 (10.3%)	1 (1.6%)	10 (7.1%)	**26 (3.5%)**	0.06
AT deficiency													
All	6 (4.4%)	3 (2.4%)	4 (2.6%)	0	13 (7.0%)	1 (2.9%)	1 (1.1%)	3 (3.1%)	1 (2.6%)	2 (3.0%)	7 (3.3%)	**41 (3.5%)**	0.28
Males	3 (4.2%)	2 (3.1%)	1 (1.8%)	0	8 (9.6%)	1 (4.4%)	–	1 (3.0%)	–	–	4 (5.5%)	**20 (4.6%)**	0.53
Females	3 (4.6%)	1 (1.6%)	3 (3.1%)	0	5 (4.9%)	0	1 (1.1%)	2 (3.1%)	1 (2.7%)	2 (3.3%)	3 (2.1%)	**21 (2.8%)**	0.9
APS													
All	7 (5.1%)	13 (10.5%)	16 (10.5%)	6 (12.8%)	25 (13.5%)	5 (14.7%)	10 (11.4%)	6 (6.2%)	7 (17.9%)	7 (10.6%)	23 (10.9%)	**125 (10.5%)**	0.35
Males	4 (5.6%)	3 (4.8%)	3 (5.4%)	3 (12.0%)	7 (8.4%)	2 (8.7%)	–	1 (3.0%)	–	–	7 (9.7%)	**30 (6.9%)**[Δ]	0.9
Females	3 (4.6%)	10 (16.4%)	13 (13.5%)	3 (13.6%)	18 (17.7%)	3 (27.3%)	10 (11.4%)	5 (7.8%)	7 (17.9%)	7 (11.5%)	1 (0.7%)	**80 (10.6%)**[Δ]	0.3
Total													
All	**48 (35.0%)**	**51 (40.8%)**	**36 (23.7%)**	**13 (27.7%)**	**77 (41.6%)**	**18 (52.9%)**	**25 (28.4%)**	**33 (33.7%)**	**25(64.1%)**	**26 (39.4%)**	**88 (41.1%)**	**440 (37.1%)**	**0.006**
Males	**26 (36.6%)**	**26 (63.4%)**	**12 (21.4%)**	**7 (28.0%)**	**40 (48.2%)**	**14 (60.9%)**	**–**	**14 (42.4%)**	**–**	**–**	**21 (28.8%)**	**160 (37.0%)**	**0.1**
Females	**22 (33.3%)**	**25 (41.0%)**	**24 (25.0%)**	**6 (27.3%)**	**37 (36.3%)**	**4 (36.4%)**	**25 (28.4%)**	**19 (29.2%)**	**25(64.1%)**	**24 (39.3%)**	**52 (36.9%)**	**263 (35.0%)**	**0.02**
Elevated FVIII[a]													
All	51 (38.4%)	47 (39.8%)	36 (24.2%)	15 (32.6%)	78 (42.2_ %)	12 (37.5%)	20 (22.7%)	33 (35.5%)	16 (41.0%)	20 (30.8%)	58 (28.0%)	386 (33.6%)	0.002 0.84
Males	25 (37.3%)	20 (33.3%)	15 (27.8%)	7 (28.0%)	26 (31.3%)	7 (31.8%)	–	8 (25.8%)	–	–	17 (23.9%)	125 (30.1%)	
Females	26 (39.4%)	27 (46.6%)	21 (22.1%)	8 (38.1%)	52 (53.1%)	5 (50.0%)	20 (22.7%)	25 (40.3%)	16 (41.0%)	20 (30.8%)	41 (30.2%)	261 (35.6%)	0.0002

(*continued on next page*)

Table I (*continued*)

Variable	Screening indications, n(%)											p-Value
	First unprovoked PE	First unprovoked DVT	AIS < 50 years old	MI < 50 years old	≥ 2 recurrent VTE	Positive family history and provoked VTE	≥ 2 miscarriages	Thrombosis in unusual site	VTE during pregnancy/ childbirth	VTE on oral contraceptives or hormone replacement therapy	Other	Total	
Elevated tHcy[b]													
All	32 (24.6%)	22 (20.2%)	21 (14.8%)	7 (16.3%)	37 (20.0%)	13 (40.6%)	0	18 (20.5%)	1 (2.6%)	7 (11.9%)	34 (17.9%)	192 (17.9%)	< 0.0001
Males	22 (32.4%)	13 (24.5%)	11 (20.8%)	3 (13.0%)	20 (24.1%)	8 (36.4%)	–	10 (33.3%)	–	–	18 (27.3%)	105 (26.9%)	0.69
Females	3 (4.6%)	10 (16.4%)	13 (13.5%)	3 (13.6%)	18 (17.6%)	3 (27.3%)	0	5 (7.8%)	1 (2.6%)	7 (11.5%)	16 (11.4%)	79 (11.5%)	0.0002

AIS, acute ischemic stroke; AT, antithrombin activity < 83%; APS, anti-phospholipid syndrome; DVT, deep vein thrombosis; FII, prothrombin; FVL, Factor V Leiden; FVIII, Factor FVIII > 150%; MI, myocardial infarction; PC, protein C activity < 70%; PE, pulmonary embolism; PS, protein S level < 67% for men and 60% for women; tHcy, plasma homocysteine ≥ 15 μmol/l; VTE, venous thromboembolism.
Bolded text indicates the rate of positive thrombophilia screening with regard to the main indication in total number of patients.

[a] FVIII measured for 1148 patients.

[b] tHcy measured for 1073 patients.

[Δ] Denotes a statistically significant difference between males and females patients in the given variable (see the Results section for exact *p*-values).

mutation (4.1%) and AT deficiency (3.5%; Table I).

The highest rates of positive results of basic 6 thrombophilia tests were found in women following VTE during pregnancy/childbirth (n = 25, 64.1%) and in patients with positive family history and provoked VTE episode (n = 18, 52.9%). The positivity rate was higher in the former group (p = 0.003).

FVL and APS were most often detected in patients following VTE during pregnancy/childbirth (n = 12, 30.8%; and n = 7, 17.9%, respectively). The highest prevalence of deficiencies of AT, PC or PS were observed in patients with ≥2 recurrent VTE (7.0%), positive family history and provoked VTE (5.9%), and VTE during pregnancy/childbirth (10.3%), respectively (Table I).

Forty-three (3.6%) patients positive for thrombophilia had 2 or more abnormalities, including 4 (0.3%) with three abnormalities. The highest co-incidence included FVL and APS (n = 14, 1.2%).

Gender-specific analysis showed that the screened men were older than women (45 [IQR 36-55] vs 41 [IQR 32-54] years, respectively, p = 0.003). In women the most frequent indications for testing were recurrent VTE (n = 107, 14.2%) and ischemic stroke at the age < 50 years (n = 96, 12.7%; Table I). The highest rate of positive test results was observed in women with VTE during pregnancy/childbirth (n = 25, 64.1%) and after first unprovoked DVT (n = 25, 41.0%). The most prevalent indication in male patients was recurrent VTE (n = 78, 18.0%), followed by first unprovoked PE (n = 71, 16.4%; Table I). Men after first unprovoked DVT (n = 26, 63.4%) and those with VTE and positive family history (n = 14, 60.9%) had the highest rate of positive test results (Table I). In men FVL was more often detected than in females (n = 74 [17.1%] vs n = 95 [12.6%;] p = 0.03). As expected, APS was more frequently detected in females (n = 80 [10.6%] vs n = 30 [6.9%]; p = 0.003).

When we compared patients aged ≤50 years and older (Table II), the former group was most frequently referred to thrombophilia screening after first unprovoked PE (n = 112, 14.0%), followed by first unprovoked DVT (n = 101, 12.6%). The highest rate of positive results in this age group was found in women following VTE during pregnancy/childbirth (n = 25, 64.1%) and in patients with positive family history of VTE and provoked VTE (n = 16, 61.5%; Table II).

Among individuals > 50 years old, the most common indication for thrombophilia testing were recurrent VTE (n = 141, 36.6%) and history of ischemic stroke at the age < 50 (n = 56, 14.6%). In the older age group, the highest rate positive test results were observed when estrogen-related or recurrent VTE occurred (n = 4, 44.4% and n = 62, 44.0%, respectively).

For both age categories (i.e. ≤50 and > 50 years), the most frequently observed thrombophilias were FVL mutation (14.6% and 13.8%) and APS (10.1% and 11.4%, respectively). In patients > 50 years old, compared with younger subjects, AT deficiency was higher (and n = 22 [5.7%] vs n = 19 [2.4%]; p = 0.003), whereas the rate of PS deficiency was lower (n = 30 [3.8%] vs n = 3 [0.8%]; p = 0.004). No differences in thrombophilic factors, including PC deficiency, APS diagnosis, related to sex were observed between two age groups (Table II).

Assessment of plasma tHcy and FVIII activity was performed in 1073 (90.6%) and 1148 (96.9%) patients, respectively. We identified 192 (17.9%) subjects with elevated tHcy, 386 (33.6%) with FVIII activity > 150% and 98 (8.5%) cases in whom FVIII activity was > 200%. When the 6 basic thrombophilias were combined with determination of FVIII and tHcy, 85.9% (95% CI 83.92%–87.88%) of screened patients were found positive in any of the 8 tests.

4. Discussion

In the current study on 1185 consecutive outpatients, we investigated detection rates of thrombophilia using our predefined institutional indications for thrombophilia screening, based on so far published guidelines, and we found that the overall prevalence of

Table II
The rate of positive results in thrombophilia screening testing in regard to the initial diagnosis and age.

Variable	Screening indications, n/%												*p*-Value
	First unprovoked PE	First unprovoked DVT	AIS < 50 years old	MI < 50 years old	≥2 recurrent VTE	Positive family history and provoked VTE	≥2 miscarriages	Thrombosis in unusual site	VTE during pregnancy/ childbirth	VTE on oral contraceptives or hormone replacement therapy	Other	Total	
≤50 years old	112 (14.0%)	101 (12.6%)	96 (12.0%)	35 (4.4%)	44 (5.5%)	26 (3.6%)	88 (11.0%)	66 (8.3%)	39 (4.9%)	57 (7.1%)	136 (17.0%)	**800 (67.5%)**	
> 50 years old	25 (6.5%)	24 (6.2%)	56 (14.6%)	12 (3.1%)	141 (36.6%)	8 (2.1%)	0	32 (8.3%)	0	9 (2.3%)	78 (20.3%)	**385 (32.5%)**	
Females													
≤50 years old	54 (48.2%)	52 (51.5%)	60 (62.5%)	16 (45.7%)	22 (50.0%)	5 (19.2%)	88 (100%)	45 (68.2%)	39 (100%)	54 (94.7%)	91 (66.9%)	**521 (65.1%)**	< 0.0001
> 50 years old	12 (48.0%)	9 (37.5%)	36 (64.3%)	6 (50.0%)	85 (60.3%)	6 (75.0)	0	20 (62.5%)	0	7 (77.8%)	50 (64.0%)	**231 (60.0%)**	0.26
FVL mutation													
≤50 years old	18 (16.1%)	21 (20.8%)	3 (3.1%)	4 (11.4%)	7 (15.9%)	7 (26.9%)	8 (9.1%)	11 (16.7%)	12 (30.8%)	11 (19.3%)	15 (11.0%)	**117 (14.6%)**	0.001
> 50 years old	3 (12.0%)	2 (8.3%)	4 (7.1%)	1 (8.3%)	20 (14.2%)	0	0	9 (28.1%)	0	2 (8.33%)	12 (15.4%)	**53 (13.8%)**	0.23
FII G20210A													
≤50 years old	5 (4.5%)	3 (2.97%)	4 (4.2%)	0	3 (6.8%)	2 (7.7%)	3 (3.4%)	0	1 (2.6%)	3 (5.3%)	10 (7.4%)	**34 (4.3%)**	0.38
> 50 years old	3 (12.0%)	1 (4.2%)	1 (1.8%)	1 (8.3%)	7 (5.0%)	0	0	1 (3.1%)	0	0	1 (1.3%)	**15 (3.9%)**	0.41
PC deficiency													
≤50 years old	3 (2.7%)	1 (0.99%)	0	1 (2.9%)	0	2 (7.7%)	0 (0.0%)	0	0	0	6 (4.4%)	**13 (1.6%)**	0.03
> 50 years old	1 (4.0%)	1 (4.2%)	2 (3.6%)	0	1 (0.7%)	0	0	0	0	0	4 (5.1%)	**9 (2.3%)**	0.56
PS deficiency													
≤50 years old	2 (1.8%)	5 (4.95%)	1 (1.04%)	0	1 (2.3%)	1 (3.9%)	3 (3.4%)	3 (4.6%)	4 (10.3%)	1 (1.8%)	9 (6.6%)	**30 (3.8%)**[Δ]	0.22
> 50 years old	0	1 (4.2%)	1 (1.8%)	0	0	0	0	0	0	0	1 (1.3%)	**3 (0.8%)**[Δ]	0.62
AT deficiency													
≤50 years old	5 (4.5%)	2 (1.98%)	1 (1.04%)	0	1 (2.3%)	0	1 (1.1%)	2 (3.1%)	1 (2.6%)	2 (3.5%)	4 (2.9%)	**19 (2.4%)**[Δ]	0.84
> 50 years old	1 (4.0%)	1 (4.2%)	1 (1.8%)	0	12 (8.5%)	1 (12.5%)	0	1 (3.1%)	0	0	3 (3.9%)	**22 (5.7%)**[Δ]	0.75
APS													
≤50 years old	5 (4.5%)	13 (13.0%)	11 (11.5%)	3 (8.6%)	3 (6.8%)	4 (15.4%)	10 (11.4%)	4 (6.2%)	7 (18.0%)	5 (8.8%)	16 (11.9%)	**81 (10.1%)**	0.43
> 50 years old	2 (8.0%)	0	5 (8.9%)	3 (25.0%)	22 (15.6%)	1 (12.5%)	0	2 (6.3%)	0	2 (22.2%)	7 (9.0%)	**44 (11.4%)**	0.22
Total													
≤50 years old	**38 (33.9%)**	**45 (44.6%)**	**20 (20.8%)**	**8 (22.9%)**	**15 (34.1%)**	**16 (61.5%)**	**25 (28.4%)**	**20 (30.3%)**	**25 (64.1%)**	**22 (38.6%)**	**60 (44.1%)**	**294 (36.8%)**	**0.001**
> 50 years old	**10 (40.0%)**	**6 (25.0%)**	**14 (25.0%)**	**5 (41.7%)**	**62 (44.0%)**	**2 (25.0%)**	**0**	**13 (40.6%)**	**0**	**4 (44.4%)**	**28 (35.9%)**	**146 (37.9%)**	**0.78**
Elevated FVIII#													
≤50 years old	36 (32.7%)	38 (37.6%)	15 (16.0%)	10 (28.6%)	15 (34.1%)	8 (33.3%)	20 (22.7%)	19 (31.2%)	16 (41.0%)	15 (26.8%)	25 (18.8%)	217 (27.9%)	0.002
> 50 years old	15 (65.2%)	9 (39.1%)	21 (38.2%)	5 (45.5%)	63 (46.7%)	4 (50.0%)	0	14 (43.8%)	0	5 (55.6%)	33 (44.6%)	169 (45.7%)	0.68
Elevated tHcy*													
≤50 years old	20 (18.9%)	18 (20.9%)	9 (10.1%)	4 (12.1%)	8 (18.2%)	9 (36.0%)	0	11 (18.6%)	1 (2.6%)	5 (10.0%)	17 (14.2%)	102 (14.1%)	0.00013
> 50 years old	12 (50.0%)	4 (17.4%)	12 (22.6%)	3 (30.0%)	29 (23.8%)	4 (50.0%)	0	7 (24.1%)	0	2 (22.2%)	17 (24.3%)	90 (25.9%)	0.13

Abbreviations: see Table I.
[Δ] Denotes a statistically significant difference between patients ≤ 50 and > 50 years old in the given variable (see the Results section for exact *p*-values).

thrombophilia reached 37% in all age (> 50 and ≤50 years old) and sex groups. We showed substantial detection rates, including APS, not only in unprovoked or recurrent VTE, but on other patients including those with arterial thromboembolism, miscarriages, provoked VTE combined with positive family history. The highest positivity rate was observed when patients were screened due to VTE during pregnancy/childbirth and those with positive family history and provoked VTE, but not in those with recurrent or unprovoked VTE, which is an unexpected finding. This observation indicates that the two indications should be included in the routine thrombophilia screening strategies. We detected thrombophilia in a substantial proportion of patients > 50 years old, with AT deficiency being more common in this group and with APS at similar rates both in patients ≤ 50 and > 50 years old. Hence, we demonstrated that patients aged 50 years or more should be screened for thrombophilia if there are appropriate indications.

Most of previous studies on thrombophilia screening were focused on VTE patients. In a registry-based study on 21,367 VTE patients, Roldan et al. demonstrated that the positivity rate in a retrospectively selected group of 3618 VTE Spanish patients with confirmed screening indications was 34% [10,12]. However, this study only included patients with symptomatic VTE [12]. In turn, Kwon et al. [10] evaluated outpatient thrombophilia screening practices at a tertiary academic medical center. They performed a retrospective review of the outpatient electronic medical records and coagulation laboratory data collected from 2081 patients treated in New York City. The low positivity rate (13.8%) of patients due to limited clinician adherence to screening guidelines has been reported [10]. Meyer et al. identified 1314 US patients with confirmed acute VTE and reported 20% rate of positive results [11]. In this study more than half of patients were tested in situations known to increase the potential for inaccurate results e.g. on anticoagulation therapy or during the acute VTE period [11]. In the current study such situations were excluded. None of the above studies included tHcy and FVIII evaluation for laboratory screening of thrombophilia, which renders the present study closer to everyday practice in most centers. The current study based on institutional guidelines presented a comprehensive analysis of the detection rates in a wide range of indications for thrombophilia screening, showing a substantial rate of positive results in most indications other than the most commonly studied VTE related indications, in particular ischemic stroke at a young age.

As expected, the most frequent abnormality in our cohort (13.3% of all patients) regardless of the indication for testing was FVL, which agrees with previous reports in white patients [11,12]. The second most common detected thrombophilia in our study was APS diagnosed in 10.5% of screened patients, similarly to the study by Roldan et al. [12]. The incidence of APS in every screening indication was substantial, ranging from 5.1% in the group of first unprovoked PE to 17.9% in women with VTE during pregnancy/childbirth. Among deficiencies of the natural anticoagulants, AT deficiency was the most frequently detected, present in 3.5% of the studied patients. Diagnosis of AT deficiency and APS is of particular importance given indications for long-term anticoagulation in most of those individuals after a first thrombotic event [17,18]. Meyer et al. demonstrated FII G20210A mutation to be the second most common thrombophilia in VTE patients diagnosed in clinics in Colorado, USA [11]. In the current cohort this mutation was less prevalent (4.1%) and similar in all indications for thrombophilia screening. That confirms that the prevalence of the FII G20210A mutation largely depends on the geographical location [3].

Contrary to our expectations, the highest positivity rate was observed in patients screened due to VTE during pregnancy/childbirth or with positive family history and provoked VTE, which highlights the role of screening patients with provoked VTE in the presence of weak triggers [1]. Moreover, in our study each of the 6 key thrombophilias was more frequently present in women following VTE during pregnancy/childbirth. Roldan et al. showed 51% of pregnant women tested positive for thrombophilia screening, however the highest prevalence (53%) of thrombophilia was observed among patients < 50 years old with recurrent VTE [12]. Our study strongly indicates that women who experienced pregnancy related VTE should be screened for thrombophilia even if this type of VTE is perceived as provoked episodes with limited duration of anticoagulation in most cases. The same holds true for patients with positive family history, which appears to be of value even if trauma or surgery provoked VTE in young or middle-aged individuals.

All basic thrombophilias, including the 2 major prothrombotic mutations, were frequently detected in young stroke patients from our cohort even if the relevance of these factors other than APS is highly controversial [19]. Recently, the FVL mutation has been shown to increase the risk of transient ischemic attack or amaurosis fugax [20]. In our study the most common thrombophilia detected in young acute ischemic stroke patients was APS in 44.4% of all positive results in this group, which indicates that this disease should be looked for in such subjects to ensure appropriate anticoagulant treatment. As expected, a higher rate of APS was demonstrated in women than in men. Of importance, the prevalence of APS did not differ between younger and older patients. The high prevalence of APS among patients aged 50 years or more is of major importance given the subsequent need for oral anticoagulation regardless of history of cardiovascular risk factors or the absence or presence of VTE events [21].

In our study, men and women had similar positivity rates in thrombophilia testing. It is well known that men have two-fold higher risk of developing first and recurrent VTE [22]. Several hypotheses have been proposed to explain the association, like more advanced age or the presence of other risk factors in male population, however in thrombophilias it did not prove relevant [23].

Recent studies have shown that prevalence of any thrombophilia decreased with age which concerns mostly the hereditary thrombophilias, like FII G20210A mutation or deficiencies of natural anticoagulants [12,24]. Surprisingly, in the current study the rate of AT deficiency was higher in patients > 50 years old compared with younger subjects. It proves that the older patients should not be ruled out from genetic testing if guidelines-based indications are found. From our experience young and middle-aged survivors of VTE are referred to further laboratory work-up a few years after the event, often at the aged of > 50 years. Therefore a personal history of VTE as well as positive family history of VTE should be taken into account even in older patients. Thrombophilia testing among patients > 50 years of age may identify those who require chronic anticoagulation for high-risk thrombophilia, including > 10% of patients with APS and may facilitate family counseling directed at first-degree family members.

The issue of tHcy and FVIII determination deserves a special comment. Hyperhomocysteinemia is associated with higher risk of DVT in the general population [25], and predicts VTE recurrences [26]. High FVIII levels constitute a prevalent, dose-dependent risk factor for VTE, and patients with FVIII > 200 IU/d were at higher risk of recurrences [27,28]. Most of our cohort was tested for FVIII and hyperhomocysteinemia; one-third was positive for elevated FVIII, almost 18% for elevated tHcy. Although the practice of hyperhomocysteinemia treatment with the combination of folic acid, B6-, and B12-vitamins supplementation may vary in different centers [29], the evidence that elevated tHcy and FVIII are associated with thrombosis and its recurrence is strong. Nonetheless, it remains to be established whether assessment of tHcy, and particularly FVIII, would yield any clinically relevant information (even if their optional determination was frequently chosen during thrombophilia screening in the present study).

Our study has several limitations. There was no control group in which another approach to thrombophilia testing was used. We did not confirm deficiencies of natural anticoagulant proteins by genetic testing, though two positive results were required following exclusion of other acquired cause of such abnormalities e.g. liver injury. Some cut-off values e.g. AT could be suboptimal as suggested recently [30].

Our study demonstrates new aspects of everyday thrombophilia

screening in outpatients, suggesting the need for testing older patients if indicated and those with provoked VTE with positive family history of VTE. We found high detection rates of APS and AT deficiency among older screened patients that implicate long-term anticoagulation. Further studies are needed to elucidate a clinical relevance of thrombophilia testing in the current clinical practice.

Conflict of interest statement

None of the authors declare conflict of interest.

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AU designed the study, edited and revised the manuscript, SGM was main author of the main body and result analyses. AS performed statistics analyses, EW and MK edited and revised the manuscript. All authors have finally approved manuscript to be published.

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References

[1] J.M. Connors, Thrombophilia testing and venous thrombosis, N. Engl. J. Med. 377 (2017) 2298.
[2] S. Middeldorp, Evidence-based approach to thrombophilia testing, J. Thromb. Thrombolysis 31 (2011) 275–281.
[3] P.M. Mannucci, M. Franchini, Classic thrombophilic gene variants, Thromb. Haemost. 114 (2015) 885–889.
[4] L. Andreoli, C.B. Chighizola, A. Banzato, G.J. Pons-Estel, G. Ramire de Jesus, D. Erkan, Estimated frequency of antiphospholipid antibodies in patients with pregnancy morbidity, stroke, myocardial infarction, and deep vein thrombosis: a critical review of the literature, Arthritis Care Res. 65 (2013) 1869–1873.
[5] E. Wypasek, J. Corral, M. Alhenc-Gelas, W. Sydor, T. Iwaniec, M. Celinska-Lowenhoff, et al., Genetic characterization of antithrombin, protein C, and protein S deficiencies 8 patients, Pol Arch Intern Med. 127 (2017) 512–523.
[6] T. Baglin, E. Gray, M. Greaves, B.J. Hunt, D. Keeling, S. Machin, et al., Clinical guidelines for testing for heritable thrombophilia, Br. J. Haematol. 149 (2010) 209–220.
[7] V. Pengo, A. Tripodi, G. Reber, J.H. Rand, T.L. Ortel, M. Galli, et al., Update of the guidelines for lupus anticoagulant detection. Subcommittee on lupus anticoagulant/antiphospholipid antibody of the scientific and standardisation committee of the International Society on Thrombosis and Haemostasis, J Thromb Haemost. 7 (2009) 1737–1740.
[8] A.N. Nicolaides, H.K. Breddin, P. Carpenter, S. Coccheri, J. Conard, V. De Stefano, et al., Thrombophilia and venous thromboembolism. International consensus statement. Guidelines according to scientific evidence, Int. Angiol. 24 (2005) 1–26.
[9] E.M. Van Cott, M. Laposata, M.H. Prins, Laboratory evaluation of hypercoagulability with venous or arterial thrombosis, Arch. Pathol. Lab. Med. 126 (2002) 1281–1295.
[10] A.J. Kwon, M. Roshal, M.T. DeSancho, Clinical adherence to thrombophilia screening guidelines at a major tertiary care hospital, J. Thromb. Haemost. 14 (2016) 982–986.
[11] M.R. Meyer, D.M. Witt, T. Delate, S.G. Johnson, M. Fang, A. Go, et al., Thrombophilia testing patterns amongst patients with acute venous thromboembolism, Thromb. Res. 136 (2015) 1160–1164.
[12] V. Roldan, R. Lecumberri, J.F. Muñoz-Torrero, V. Vicente, E. Rocha, B. Brenner, et al., Thrombophilia testing in patients with venous thromboembolism. Findings from the RIETE registry, Thromb. Res. 124 (2009) 174–177.
[13] S. Hatano, Experience from a multicentre stroke register: a preliminary report, Bull. World Health Organ. 54 (1976) 541–553.
[14] S. Miyakis, M.D. Lockshin, T. Atsumi, D.W. Branch, R.L. Brey, R. Cervera, et al., International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS), J. Thromb. Haemost. 4 (2006) 295–306.
[15] M. Cattaneo, Hyperhomocysteinemia and venous thromboembolism, Semin. Thromb. Hemost. 32 (2006) 716–723.
[16] A. Undas, E. Stepień, D. Plicner, L. Zielinski, W. Tracz, Elevated total homocysteine is associated with increased platelet activation at the site of microvascular injury: effects of folic acid administration, J. Thromb. Haemost. 5 (2007) 1070–1072.
[17] C.B. Chighizola, T. Ubiali, P.L. Meroni, Treatment of thrombotic antiphospholipid syndrome: the rationale of current management-an insight into future approaches, J Immunol Res 2015 (2015) 951424.
[18] F.N. Croles, J. Borjas-Howard, K. Nasserinejad, F.W.G. Leebeek, K. Meijer, Risk of venous thrombosis in antithrombin deficiency: a systematic review and Bayesian meta-analysis, Semin. Thromb. Hemost. 44 (2018) 315–326.
[19] J.G. Morris, S. Singh, M. Fisher, Testing for inherited thrombophilias in arterial stroke: can it cause more harm than good? Stroke 41 (2010) 2985–2990.
[20] S.H. Pahus, A.T. Hansen, A.M. Hvas, Thrombophilia testing in young patients with ischemic stroke, Thromb. Res. 137 (2016) 108–112.
[21] K. Schreiber, S. Sciascia, P.G. de Groot, K. Devreese, S. Jacobsen, G. Ruiz-Irastorza, et al., Antiphospholipid syndrome, Nat Rev Dis Primers. 4 (2018) 18005.
[22] S.M. Bleker, M. Coppens, S. Middeldorp, Sex, thrombosis and inherited thrombophilia, Blood Rev. 28 (2014) 123–133.
[23] J. Douketis, A. Tosetto, M. Marcucci, T. Baglin, B. Cosmi, M. Cushman, et al., Risk of recurrence after venous thromboembolism in men and women: patient level meta-analysis, BMJ 342 (2011) d813.
[24] L. Weingarz, J. Schwonberg, M. Schindewolf, C. Hecking, Z. Wolf, M. Erbe, et al., Prevalence of thrombophilia according to age at the first manifestation of venous thromboembolism: results from the MAISTHRO registry, Br. J. Haematol. 163 (2013) 655–665.
[25] M. den Heijer, T. Koster, H.J. Blom, G.M. Bos, E. Briet, P.H. Reitsma, et al., Hyperhomocysteinemia as a risk factor for deep-vein thrombosis, N. Engl. J. Med. 334 (1996) 759–762.
[26] S. Eichinger, A. Stumpflen, M. Hirschl, C. Bialonczyk, K. Herkner, M. Stain, et al., Hyperhomocysteinemia is a risk factor of recurrent venous thromboembolism, Thromb. Haemost. 80 (1998) 566–569.
[27] P.V. Jenkins, O. Rawley, O.P. Smith, J.S. O'Donnell, Elevated factor VIII levels and risk of venous thrombosis, Br. J. Haematol. 157 (2012) 653–663.
[28] J. O'Donnell, E.G. Tuddenham, R. Manning, G. Kemball-Cook, D. Johnson, M. Laffan, High prevalence of elevated factor VIII levels in patients referred for thrombophilia screening: role of increased synthesis and relationship to the acute phase reaction, Thromb. Haemost. 77 (1997) 825–828.
[29] E. Lonn, S. Yusuf, M.J. Arnold, P. Sheridan, J. Pogue, M. Micks, et al., Homocysteine lowering with folic acid and B vitamins in vascular disease, N. Engl. J. Med. 354 (2006) 1567–1577.
[30] J. Sokol, J.F. Timp, S. le Cessie, A. van Hylckama-Vlieg, F.R. Rosendaal, P. Kubisz, et al., Mild antithrombin deficiency and risk of recurrent venous thromboembolism: results from the MEGA follow-up study, J. Thromb. Haemost. 16 (2018) 680–688.