Impact of Thrombophilia Testing on Clinical Management: A Retrospective Cohort Study

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Abstract

Thrombophilia management is based on the personal and family history of thrombosis. Current guidelines recommend performing thrombophilia testing only when the results will change clinical management. To investigate to what extent treatment recommendations changed following thrombophilia testing, clinical and laboratory data of 255 patients with and without venous thromboembolism who underwent thrombophilia screening were assessed retrospectively. A local score based on clinical indicators for thrombophilia was used to assess the pretest probability of thrombophilia. A total of 144 patients (57.6%) were found to have a clear thrombophilic phenotype, of which 78 were predicted to have definite thrombophilia and considered for indefinite anticoagulation; 66 were likely to have thrombophilia and were considered for indefinite or prolonged anticoagulation. Eighty-three (32.5%) could not be clearly classified and 28 (11%) were asymptomatic. A thrombophilic risk factor was diagnosed in 98 (38.4%) patients; this included 64 of 144 (44.5%) patients with a clear thrombophilic phenotype and 26 of 83 (31.3%) patients who could not be easily classified. Treatment recommendations changed in 57 of 255 (22%) patients following thrombophilia testing. Eight patients were switched from direct oral anticoagulants to vitamin K antagonists due to confirmed triple-positive antiphospholipid syndrome. In 49 patients, the anticoagulant dose was either increased ($n = 3$) or treatment was prolonged ($n = 46$) following diagnosis of high-risk thrombophilia. Clinically, assessing thrombophilia probability score before thrombophilia testing improves thrombophilia management recommendations.

Keywords

- risk assessment
- thrombophilia
- anticoagulation therapy
- thrombophilia testing

Introduction

Thrombophilia is characterized by a predisposition to venous thromboembolism (VTE) and is caused by either inherited or acquired disorders of the hemostatic system that result in a transient or permanent hypercoagulable state.[1,2] Clinical manifestations, or symptoms, associated with thrombophilia often include VTE at a young age (<50 years), unprovoked VTE or VTE associated with weak provoking factors, recurrent thrombotic events, VTE at an atypical site, and there is often a family history of VTE.[1–4] In addition, some types of thrombophilia are associated with pregnancy loss and complications.[5]

Well-defined hereditary thrombophilic conditions include the factor V Leiden (FVL) and prothrombin (F2G20210A) gene mutations as well as deficiencies of the natural inhibitors antithrombin (AT), protein C (PC), and protein S (PS).[6] Acquired thrombophilias include persistent antiphospholipid antibodies (APA)

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(i.e., antiphospholipid syndrome [APS]), including anticardiolipin (aCL), anti-beta-2 glycoprotein 1 (β2GP1), and lupus anticoagulant (LA), as well as paroxysmal nocturnal hemoglobinuria, and the somatic JAK2 mutation.[2,3,7]

Laboratory thrombophilia testing is recommended in patients with clinical symptoms of thrombophilia, but it is not recommended to assess the basal risk of thrombosis.[1,2,5,8–10] This is because (1) not all factors causing thrombophilia have been identified and (2) the presence of a positive thrombophilia test result does not accurately predict an individual's thrombotic risk due to the high interpatient variability in clinical phenotype. Even for monogenic thrombophilias such as FVL and F2G20210A, the risk of thrombosis and VTE recurrence shows a high degree of variability.[11,12] Furthermore, thrombophilia testing is not recommended in patients who develop thrombosis provoked by typical risk settings. This is based on clinical data showing low rates of VTE recurrence in these patient populations.[13] In general, current guidelines recommend thrombophilia testing only if it will change the clinical management in terms of the type, intensity, and/or duration of anticoagulation.[2,5,9,13–16]

Once a diagnosis of VTE is made, treatment with anticoagulation is initiated to interrupt hypercoagulability and prevent further thrombus growth.[14,17] This primary treatment course includes a short period of high-dose followed by standard-dose anticoagulation for 3 to 12 months, depending on the location and size of the thrombus. Depending on the patient's risk of recurrence, primary treatment is followed by secondary prophylaxis to prevent a recurrence. High-risk and intermediate-risk patients, characterized by cumulative recurrence rates over 5 years of up to 30% and 14 to 30%, respectively, benefit from prolonging secondary VTE treatment to 2 to 5 years or even indefinitely.[10–12] Of note, some forms of anticoagulation therapy such as direct oral anticoagulants (DOACs) are known to interfere with clotting-based thrombophilia tests, which may result in false positives or false negatives and complicate result interpretation. To mitigate this, anticoagulation may be stopped where clinically appropriate prior to blood collection, and/or anticoagulation levels may be measured at the time of testing to ensure that they fall below the interference thresholds according to the test reagent manufacturer.[18]

The assessment of thrombotic risk which is used to delineate these high-risk patients is based on the patient's personal and family history of thrombosis and is supported by the results of thrombophilia testing. However, it is not clear to what extent the results of thrombophilia testing alter treatment recommendations that were initially planned based only on clinical criteria. To investigate this, pre-test and post-test treatment recommendations were retrospectively assessed in a cohort of 255 patients referred for thrombophilia workup by their primary physicians.

Methods

Patients and Pre-Test Assessment of Thrombophilia

This retrospective study was performed at the Institute of Experimental Hematology and Transfusion Medicine, University Hospital Bonn, Germany. Written informed consent was waived due to the retrospective nature of the study. The collection of data from the medical records and consent waiver were sanctioned by the Ethics Committee of the Faculty of Medicine, University of Bonn, as well as according to the Declaration of Helsinki. All authors had access to primary data.

The medical records of consecutive patients referred to our thrombophilia center between November 2019 and February 2023 were retrospectively reviewed. Patients were included if they had been referred for thrombophilia screening based on the decision of their primary care physicians. Exclusion criteria were any previous laboratory-confirmed thrombophilia, VTE within 6 weeks prior to blood sampling, arterial thrombosis, as well as cardiovascular and malignant diseases (►**Fig. 1**).

The clinical probability of thrombophilia was evaluated using a novel, local scoring system based on the patient's personal and family history of VTE. Briefly, two points were awarded if the patient had a history of unprovoked (spontaneous) VTE, and one point each was given for the following conditions: VTE triggered by a mild, transient risk factor; recurrent VTE; VTE with atypical localization; first incidence of VTE at the age of <50 years; and symptomatic thrombophilia in a first-degree relative (►**Table 1**). The clinical probability of thrombophilia was classified as unlikely in patients with a score of 0, indeterminate in patients with a score of 1, likely in patients with a score of 2, or definite in patients with a score of 3 or more.

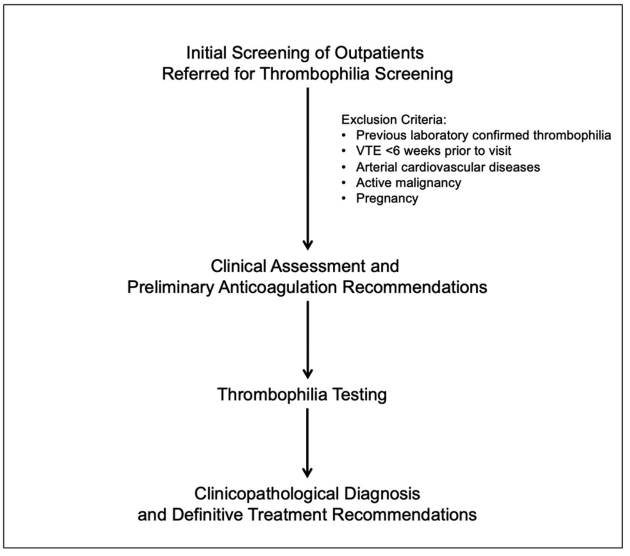

Fig. 1 Study design. Patients referred to our ambulatory thrombophilia center between November 2019 and February 2023 were screened for study participation. Preliminary recommendations for anticoagulant management were made based on the clinical probability of thrombophilia. Finally, treatment recommendations were modified in some cases based on the results of thrombophilia testing.

Table 1 Clinical thrombophilia probability score

Condition	Points
Unprovoked VTE	2
VTE triggered by a minor transient thrombosis risk factor	1
Recurrent VTE events	1
VTE at atypical sites	1
First thrombosis at age < 50 y	1
Symptomatic thrombophilia in a first-degree relative	1

Abbreviation: VTE, venous thromboembolism.
Note: A score of 0 corresponds to unlikely probability of thrombophilia; 1 corresponds to indeterminate probability; 2 corresponds to likely probability, and 3 or more corresponds to definite probability of thrombophilia.

Thrombophilia Testing

Blood samples were obtained by venipuncture of an appropriate arm vein using a 21-gauge winged infusion set (Sarstedt, Numbrecht, Germany). The first 2 mL of blood was collected into EDTA tubes and processed for molecular genetic analysis. For coagulation analysis, blood was collected into sodium citrate tubes (10.5 mmol/L final concentration, Sarstedt). Plasma was prepared by centrifugation (2,600 × g, 10 minutes) within 1 hour of blood collection and assayed within 4 hours. Plasma samples used for the functional detection of LA were centrifuged twice.

Plasma levels of AT (Berichrom Antithrombin III (A)), PC (Berichrom Protein C), free PS (Innovance Free PS Ag Assay), and coagulation factor VIII (FVIII) were determined using an Atellica Coag 360 coagulation analyzer and appropriate reagents (all Siemens Healthineers, Marburg, Germany). Of note, both the AT and PC assays used are chromogenic tests and thus less sensitive to interference by anticoagulation than clotting-based tests, while the free PS antigen assay includes a liquid latex reagent also associated with low sensitivity to interfering substances. APA testing followed international guidelines[17] and included functional coagulation assays using LA-sensitive (screen) as well as LA-insensitive (confirm) reagents (activated partial thromboplastin time: actin FSL and actin FS; dilute Russell viper venom time: LA1 and LA1; all Siemens) as well as two immunoassays including an aCL IgG/IgM enzyme-linked immunosorbent assay (ELISA; AESKU diagnostics, Wendelsheim, Germany) and an anti-β2GP1 IgG/IgM ELISA (DiaPharma Group, West Chester, Ohio, United States). In accordance with the revised Sapporo classification criteria, an APA test was considered positive if the initial positive APA test was confirmed after at least 12 weeks.[19] The FVL and F2-G20210A mutations were analyzed using in-house methods as previously described.[20,21] Preanalytics and reference values were covered by accreditation with the national accreditation body and were performed according to ISO standards.

For patients taking DOACs, blood samples were collected 12 hours (apixaban, dabigatran) or 24 hours (edoxaban, rivaroxaban) after the last dose. Patients on vitamin K antagonists (VKA) were tested when they were switched to low-molecular-weight heparin (LMWH) for medical reasons such as tooth extraction or other planned procedures. DOAC and LMWH levels were measured at the time of testing to ensure that there was no clinically significant effect on thrombophilia test results.

Anticoagulant Management Strategies

Prior to thrombophilia testing, patients who were scored as having a definite probability of thrombophilia were considered for indefinite anticoagulant treatment with low-dose DOACs. Indefinite anticoagulation is administered without a defined endpoint, with the reassessment of the risk:benefit ratio every 2 to 5 years. After testing for thrombophilia, patients meeting the criteria for APS were considered for indefinite anticoagulation with VKA at a therapeutic INR of 2 to 3.[5,22] Patients with a high-risk thrombophilia diagnosis, including inherited deficiencies of AT, PC, PS, homozygosity for FVL or F2G20210A, or compound heterozygosity for FVL and F2G20210A, were considered for DOAC at the standard prophylactic dose. For all other patients with a definite probability of thrombophilia, the pre-test recommendation of indefinite anticoagulation with a DOAC at a low prophylactic dose remained unchanged.

Patients clinically assessed as likely to have thrombophilia and who had a history of at least one VTE were initially considered for prolonged or indefinite anticoagulation. Indefinite anticoagulation was considered in patients with spontaneous thrombosis or thrombosis at an atypical site. Prolonged anticoagulation was defined as continued anticoagulation for 1 to 5 years in patients who have completed maintenance therapy for VTE. The confirmation of a high-risk thrombophilia diagnosis changed the recommendation from indefinite anticoagulation with a low-dose DOAC to a standard-dose DOAC. Patients with confirmed triple-positive APS were switched from DOAC to VKA. The diagnosis of a low-risk thrombophilia or a negative thrombophilia screen did not change the initial recommendation (►**Fig. 2A**).

Patients who were classified as having an indeterminate probability of thrombophilia based on a positive family history in a first-degree relative, but no personal history of thrombosis, had a pre-test recommendation of routine prophylaxis, which is typically prescribed to all adult patients in high-risk situations such as major surgery and prolonged hospitalization. In patients with an established thrombophilic risk factor in a first-degree relative but a negative personal thrombophilia screen, the recommendation was routine prophylaxis in high-

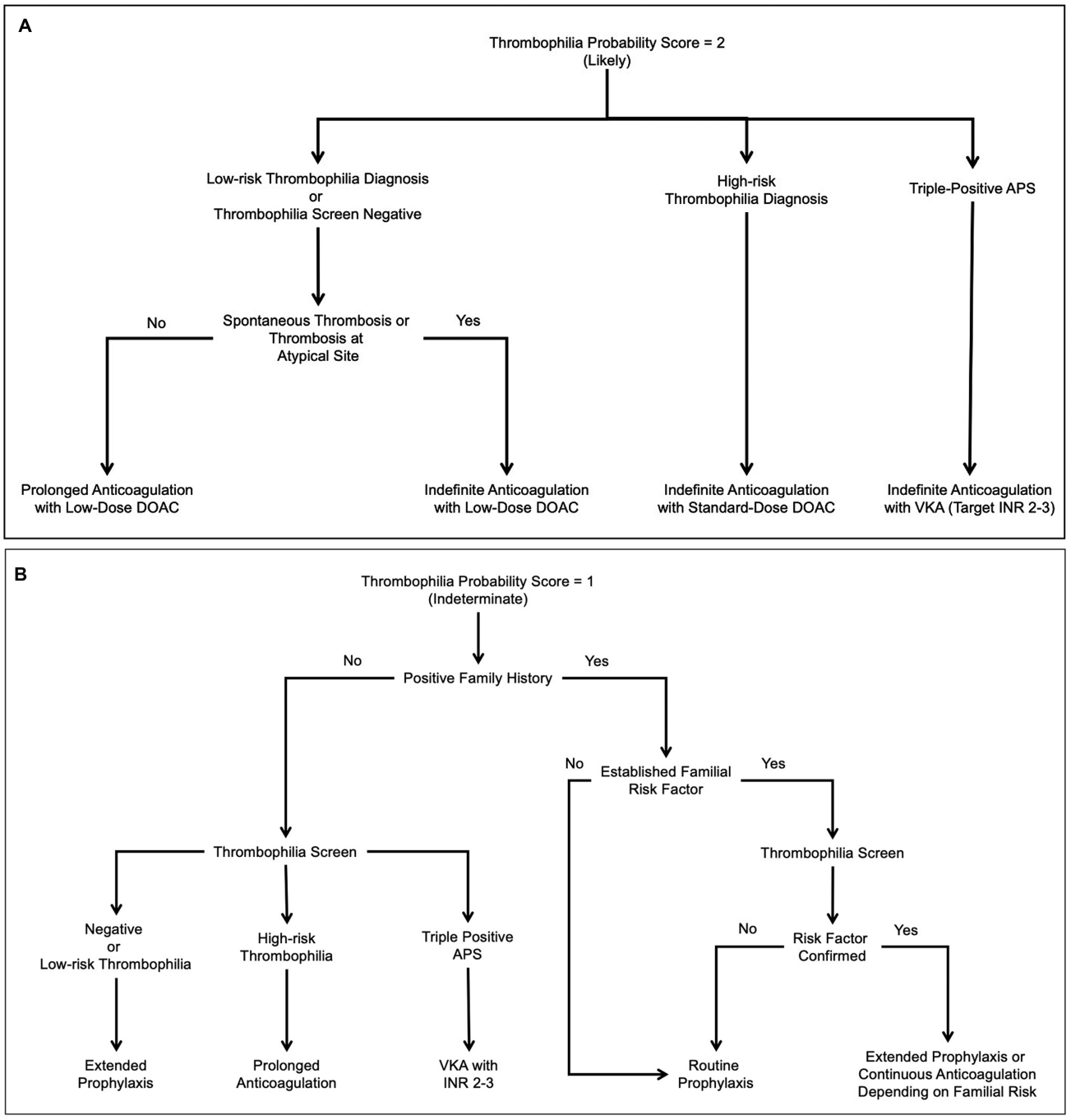

Fig. 2 Treatment algorithms. Based on thrombophilia test results, treatment recommendations were modified in patients initially classified as having a (**A**) likely or (**B**) indeterminate probability of thrombophilia.

risk situations, whereas a confirmed diagnosis of the familial thrombophilia changed the treatment recommendation to extended prophylaxis or even to indefinite anticoagulation as primary prophylaxis in patients with a strong family history of (especially high-risk) thrombophilia (►**Fig. 2B**). Extended prophylaxis was defined as routine prophylaxis extended to low-risk situations such as long-distance travel or bed rest >3 days. Extended prophylaxis was the pre-test recommendation for all other patients who fell in the indeterminate category. The diagnosis of a high-risk thrombophilia changed this recommendation to indefinite anticoagulation or, in APS patients, to VKA therapy.

Patients for whom the probability of thrombophilia was unlikely were recommended for routine prophylaxis. The diagnosis of a high-risk thrombophilia changed this recommendation to extended prophylaxis.

Results

Patient Characteristics and Clinical Probability of Thrombophilia

A total of 255 patients, 140 (54.9%) female, with a mean age of 47 years (range: 8 months to 83 years, standard deviation: 17.2 years) were included in the study. Of these, 72

(28%) had a history of pulmonary embolism (PE), 84 (33%) had a history of deep vein thrombosis (DVT), and 39 (15%) patients developed thrombosis at an atypical site (►Table 2). Fifty-seven (22%) patients reported a family history of thrombophilia in a first-degree relative including 26 with an established diagnosis (FVL, $n=15$; F2G20210A, $n=5$; AT deficiency, $n=2$; PC deficiency, $n=4$) in the index patient (►Table 2).

In the total study cohort, 78 (30.6%) had a thrombophilia probability score of 3 or higher, 66 (25.9%) had a score of 2, 83 (32.5%) had a score of 1, and 28 (11%) received a score of 0. In the patients with a definite probability of thrombophilia, spontaneous (unprovoked) thrombosis (75.6%) and age <50 years at the time of first thrombosis (64.1%) were the most common indicators (►Fig. 3). Of those with a likely probability of thrombophilia, unprovoked thrombosis and thrombosis provoked in low-risk settings were the most common indicators, with frequencies of 30 and 36%, respectively, followed by thrombosis at the age of <50 years. The majority (68%) of patients with an indeterminate probability of thrombophilia reported a family history of thrombosis and/or thrombophilia, but no personal signs or symptoms of thrombophilia.

Results of Thrombophilia Testing

Thrombophilia testing was positive in 98 (38.4%) patients (►Table 3). The most common thrombophilia diagnoses were FVL mutation ($n=39$, 15.3%), F2G20210A mutation ($n=12$, 4.7%), followed by triple positive APS ($n=8$, 3.1%). Rare thrombophilia disorders such as AT ($n=3$, 1.2%), PC ($n=4$, 1.6%), and PS ($n=2$, 0.78%) deficiencies were also detected.

The highest frequencies of positive thrombophilia test results were observed in the cohorts of patients clinically scored as having definite and likely probabilities of thrombophilia, reaching 42.3 and 47.0%, respectively (►Table 3). In particular, the number and proportion of thrombophilia diagnoses associated with a high risk of thrombosis and VTE recurrence, such as homozygous FVL or F2G20210A, compound heterozygous FVL and F2G20210A mutations, and triple-positive APS, was higher in these groups. In the group of patients who could not be clearly classified according to clinical phenotype, 31.3% of patients were diagnosed with thrombophilia, including four patients with high-risk conditions such as AT, PC, and PS deficiency. Of the 21 patients with an established familial thrombophilia diagnosis, the thrombophilia test results either confirmed ($n=8$) or excluded ($n=13$) the diagnosis of thrombophilia in our patients. The frequency of positive thrombophilia test results was lowest (21.4%) in the cohort of patients considered unlikely to have thrombophilia based on their clinical phenotype. In this group, heterozygous mutations for FVL and F2G20210A were detected in one patient each, respectively. In addition, one patient each was found to have PC deficiency and triple-positive APA, respectively.

Impact of Thrombophilia Diagnosis on Anticoagulant Management

Across all patient subgroups, anticoagulation recommendations following thrombophilia screening differed in 57 (22%) patients as compared to pre-screening treatment plans (►Fig. 4). Of the 105 patients who were initially prescribed indefinite anticoagulation with low-dose DOACs, change to anticoagulation with VKA was recommended in

Table 2 Thrombotic history of study patients

Parameter	Total, $n=255$	Definite, $n=78$	Likely, $n=66$	Indeterminate, $n=83$	Unlikely, $n=28$
VTE instances, *n*	214	108	78	24	4
VTE/patient, mean (range)	0.8 (0–3)	1.5 (1–3)	1.2 (0–3)	0.3 (0–2)	0
Thereof unprovoked, *n* (mean/patient)	75 (0.35)	48 (0.65)	27 (0.4)	0	0
Provoked in mild risk situation, *n* (mean/patient)	54 (0.21)	21 (0.28)	25 (0.37)	8 (0.1)	0
Provoked in high-risk situation, *n* (mean/patient)	26 (0.1)	5 (0.07)	8 (0.12)	9 (0.1)	4 (0.14)
Age (years) at first VTE, mean (range)	51.7 (1–82)	43.7 (14–77)	50.5 (1–82)	51.2 (22–76)	61.5 (52–73)
PE, *n*	72	30	28	10	4
DVT, *n*	84	42	40	7	1
Cerebral venous sinus thrombosis, *n*	6	4	2	0	0
Retinal vein thrombosis, *n*	4	4	0	0	0
Splanchnic vein thrombosis, *n*	4	3	1	0	0
Portal vein thrombosis, *n*	9	8	1	0	0
Others, *n*	14	8	5	0	1

Abbreviations: DVT, deep vein thrombosis; PE, pulmonary embolism; VTE, venous thromboembolism.

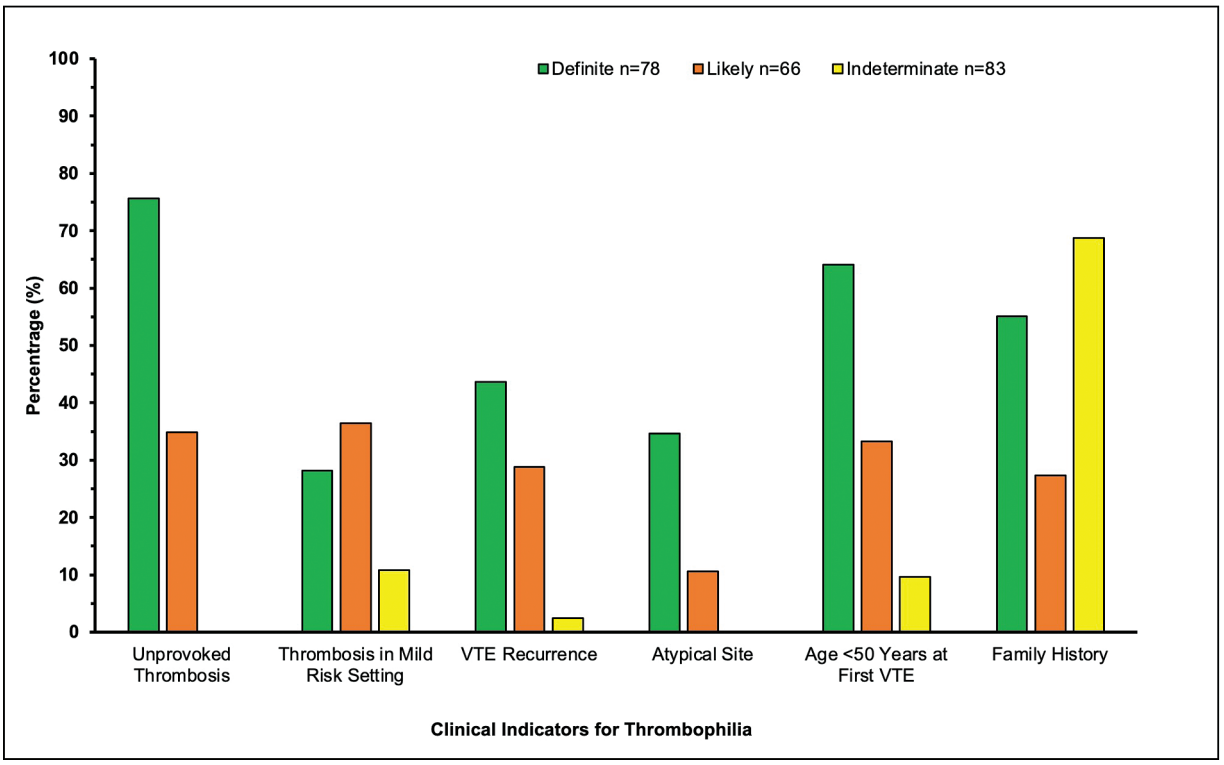

Fig. 3 Prevalence of thrombophilia symptoms. The relative frequencies of indicators for thrombophilia are shown for patients in whom the probability of thrombophilia was clinically classified as definite ($n = 78$, green), likely ($n = 66$, orange), or indeterminate ($n = 83$, yellow).

Table 3 Thrombophilia diagnoses

Diagnosis	Definite, $n = 78$	Likely, $n = 66$	Indeterminate, $n = 83$	Unlikely, $n = 28$	Total, $n = 255$
Heterozygous FVL, *n* (%)	12 (15.4)	12 (18.2)	11 (13.2)	2 (7.1)	37 (14.5)
Homozygous FVL, *n* (%)	1 (1.3)	–	–	–	1 (0.4)
Heterozygous F2G20210A, *n* (%)	3 (3.8)	–	5 (6)	2 (7.1)	10 (3.9)
Homozygous F2G20210A, *n* (%)	–	1 (1.5)	–	–	1 (0.4)
Compound heterozygous FVL/F2G20210A, *n* (%)	1 (1.3)	–	–	–	1 (0.4%)
AT deficiency, *n* (%)	1 (1.3)	2 (3)	1 (1.2)	–	4 (1.6)
PC deficiency, *n* (%)	0	1 (1.5)	2 (2.4)	1 (3.6)	4 (1.6)
PS deficiency, *n* (%)	1 (1.3)	–	1 (1.2)	–	2 (0.8)
Triple-positive APS, *n* (%)	6 (7.7)	4 (6)	1 (1.2)	1 (3.6)	12 (4.7)
LA positive, *n* (%)	1 (1.3)	1 (1.5)	1 (1.2)	–	3 (1.2)
APA positive, *n* (%)	3 (3.8)	2 (3.0)	3 (3.6)	–	8 (3.1)
Heterozygous FVL and triple-positive APS, *n* (%)	1 (1.3)	–	–	–	1 (0.4)
FVIII > 250%, *n* (%)	3 (3.8)	7 (10.6)	1 (1.2)	–	11 (4.3)
None, *n* (%)	44 (56.4%)	35 (53.0)	57 (68.7)	22 (78.6)	159 (62.4)

Abbreviations: APA, antiphospholipid antibodies; APS, antiphospholipid syndrome; AT, antithrombin; FVL, factor V Leiden; LA, lupus anticoagulant; PC, protein C; PS, protein S.

5 patients due to the diagnosis of APS, and standard-dose anticoagulation with DOACs in 3 patients due to severe AT or PC deficiency. Of the 41 patients initially prescribed prolonged anticoagulation, 3 patients were diagnosed with triple-positive APS and were subsequently recommended to switch to VKA. In eight patients, indefinite

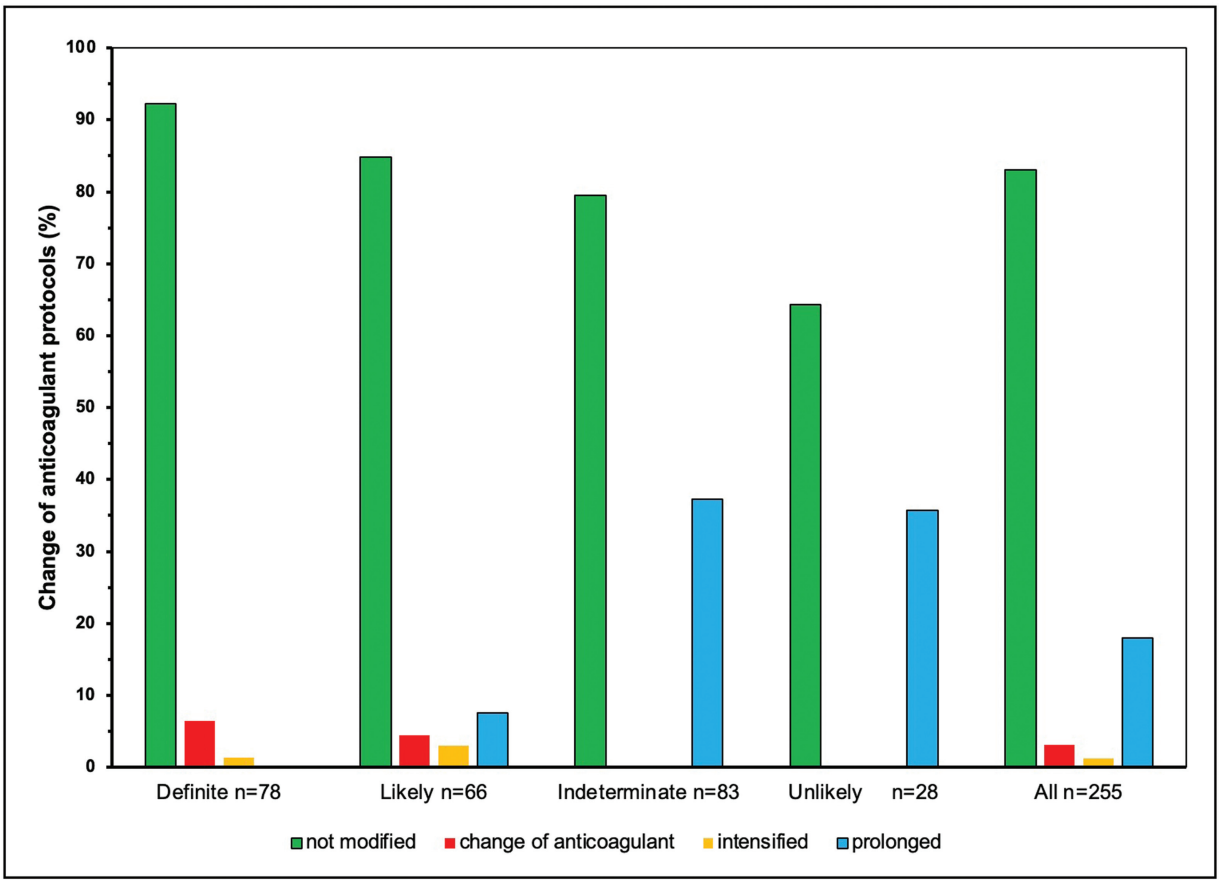

Fig. 4 Impact of thrombophilia testing on treatment recommendations. Green bars indicate patients for whom the initial therapeutic recommendations made based on the clinical probability of thrombophilia were not changed following thrombophilia testing. Red bars indicate patients for whom thrombophilia testing led to a change in treatment recommendations. Changes include intensification (orange) or prolongation (blue) of anticoagulant treatment.

anticoagulation with DOACs (one standard dose and seven low dose) was recommended following the confirmation of a high-risk thrombophilia diagnosis, including homozygous FVL or PC or PS deficiency. In the group of seven patients who could not be clearly assigned either to prolonged anticoagulation or extended prophylaxis during the pre-test clinical evaluation, thrombophilia testing revealed a diagnosis of a high- or intermediate-risk thrombophilia in five patients, leading to a post-test recommendation for prolonged anticoagulation.

Of the 90 patients who were recommended only routine prophylaxis, the diagnosis of an intermediate risk factor led to a post-test recommendation for extended prophylaxis in 21 patients and indefinite low-dose anticoagulation in 5 patients. Four of these patients were members of a family with a strong history of recurrent thrombosis, and all patients were carriers of high-risk inhibitor deficiencies (including PC and AT deficiencies).

Discussion

The impact of thrombophilia testing on clinical decision making remains controversial. Current guidelines recommend performing testing only when the test results will alter patient management.[1–3,5,16] To investigate how and to what extent thrombophilia test results alter management decisions in daily clinical practice, pre-test and post-test recommendations were compared in a cohort of 255 patients referred to our ambulatory hemostasis and thrombosis clinic for thrombophilia screening.

The clinical diagnosis of thrombophilia is based on the presence of several key indicators (i.e., clinical manifestations).[1–4] In theory, the greater the number of indicators present, the higher the probability of thrombophilia. According to this hypothesis, the likelihood of thrombophilia prior to thrombophilia testing was estimated using a scoring system based on clinical criteria. A past medical history of unprovoked (i.e., spontaneous) VTE was assigned 2 points, while all other clinical indicators, such as thrombosis triggered by minor VTE risk factors, were assigned 1 point each (►**Table 1**). Of note, a substantial proportion of patients who experienced thrombosis in the setting of only minor, transient VTE risk factors cannot be clearly dichotomized into provoked and unprovoked VTE, which can complicate treatment decisions. On the other hand, unprovoked (i.e., spontaneous) VTE is a strong indicator for thrombophilia, justifying a score of 2. This is supported by the fact that of the 82 patients with unprovoked VTE, 59 had at least one additional clinical indicator for thrombophilia.

Table 4 Treatment recommendations before/after thrombophilia screening

Cohorts	Anticoagulation with VKA	Indefinite anticoagulation with standard-dose DOAC	Indefinite anticoagulation with low-dose DOAC	Prolonged anticoagulation	Extended prophylaxis	Routine prophylaxis
Definite score > 2 $n = 78$	0/5	0/1	78/72	0/0	0/0	0/0
Likely score = 2 $n = 66$	0/3	0/2	26/25	40/36	0/0	0/0
Indeterminate score = 1 $n = 83$	0/0	0/0	1/2	1/4	19/31	62/46
Unlikely score = 0 $n = 28$	0/0	0/0	0/0	0/0	0/10	28/18
All	0/8	0/3	105/100	41/40	19/41	90/64

Abbreviations: DOAC, direct oral anticoagulant; VKA, vitamin K antagonist.

The distinct thrombophilic phenotype of patients who received a thrombophilia probability score of 3 or higher clearly classifies these patients as being at high risk for (recurrent) thrombosis, with an estimated VTE risk greater than 5% per year or greater than 30% over 5 years, justifying administration of prolonged or indefinite anticoagulation according to current guidelines.[1,9,13–15] Currently, low-dose DOACs are preferred for prolonged or indefinite anticoagulation, as they have been shown to be as effective as standard-dose DOACs or VKAs, but are associated with a comparatively lower bleeding risk.[13,14] Therefore, indefinite low-dose DOAC treatment was generally recommended for patients with a thrombophilia probability score of 3 or higher (exceptions to treatment recommendations were of course considered on a case-by-case basis, such as in the setting of medication intolerance or patient preference), while prolonged anticoagulation was recommended for patients with a score of 2. A positive thrombophilia test result was obtained in 45% of these patients, a rate which is comparable with data from other recent studies.[3,14] Triple-positive APS was identified in 7.6% of these patients, leading to the initiation of VKA treatment in accordance with current guidelines.[3] Based on the identification of high-risk thrombophilia diagnoses, including AT and PC deficiencies and homozygous FVL or F2G20210A mutations, the therapeutic recommendations were changed either from prolonged to indefinite anticoagulation or from low-dose to standard-dose DOACs in 7.8 and 1.6% of patients, respectively (►**Table 4**). In 45% of patients, the diagnosis of thrombophilia was confirmed based on a positive thrombophilia test result by identifying the underlying mechanisms. In the remaining 55% of these patients for whom the laboratory thrombophilia screen was negative, a general diagnosis of "thrombophilia of unknown origin" was made due to the distinct thrombophilic phenotype identified based on the clinical history and physical examination, and the pre-test treatment recommendation was not changed. As previously mentioned, DOAC and LMWH levels were measured at the time of testing to ensure that thrombophilia test results were free from anticoagulation interference and thus could be reliably interpreted.

The probability of thrombophilia was found to be indeterminate in 83 patients. The most common clinical findings in this cohort included VTE triggered by minor risk factors, recurrent VTE in the setting of typical thrombosis risk factors, or a family history of thrombophilia. None of these patients had received a pre-test recommendation for prolonged or indefinite anticoagulant treatment. The majority of these patients (74%) were recommended routine prophylaxis. The remaining 26% of patients were considered for extended prophylaxis, that is, routine prophylaxis extended to low-risk situations such as long-distance travel or bed rest mainly due to a history of prior VTE triggered by a low-risk situation. A positive thrombophilia test result was obtained in 31% of these patients, including a diagnosis of high-risk thrombophilia in 6%. In one patient who developed VTE after a long-haul flight, the diagnosis of triple-positive APS led to the initiation of VKA therapy. Indefinite low-dose DOAC treatment was initiated in four patients with AT, PC, or PS deficiency. Overall, thrombophilia test results led to a different treatment recommendation in 21.5% of these patients, including changing from indefinite

to prolonged anticoagulation in 7.2% and extended prophylaxis to routine prophylaxis in 14.5%.

In the absence of any clinical indicators for thrombophilia, the diagnosis of thrombophilia was clinically excluded and no anticoagulant treatment was considered, except for routine prophylaxis. Interestingly, thrombophilia testing in this small group of 28 patients revealed high-risk diagnoses including PC or AT deficiency in one patient each, and intermediate-risk thrombophilia in 4 patients. These results highlight the dilemma of whether to perform thrombophilia testing and how to interpret test results in apparently healthy individuals. There is no evidence that carriers of hereditary thrombophilias—including disorders associated with a high risk of VTE—with no personal or family history of thrombosis benefit from the initiation of continuous anticoagulation as primary prophylaxis. With this in mind, a post-test recommendation was included in these cases to extend routine prophylaxis to low-risk situations, although evidence from clinical trials to support such an approach is not yet available.

Of note, recent guidelines explicitly recommend against testing patients for thrombophilia in the absence of any clinical indicators, as the results would not result in a change in clinical management.[1,5,16] However, our retrospective study has three main advantages in this regard: (1) the inclusion of patients with a thrombophilia probability score of 0 provides a "healthy" control group; (2) this allows for representative distribution across the full spectrum of the scoring system (0–6 points), thus highlighting the heterogeneity of patients referred for thrombophilia workup; and (3) these data additionally served as an internal quality improvement initiative, allowing us to assess our thrombophilia test usage and appropriateness in light of the new guidelines. Ultimately, these data offer valuable clinical context in certain areas where the guidelines relied on limited evidence.

This study was notably limited by its retrospective nature. All patients included in this study were treated by the same attending physician at an institute within an academic medical center. This has the potential to introduce bias, as there is natural variability in treatment philosophies and clinical decision making from physician to physician or institute to institute. To mitigate this (and avoid potential errors during the data collection process), all data were reviewed by a second attending physician from the same institute prior to the final analysis. In addition, there is unfortunately no laboratory assay available that is 100% sensitive for all functional deficiencies in the inhibitors AT, PC, and free PS in the case of certain mutations, which may result in missed diagnoses in rare cases.[23–25]

The data presented in this study demonstrate that the pre-test clinical assessment of the probability of thrombophilia improves personalized treatment recommendations, supporting and extending recent guideline recommendations. In patients with a pre-test clinical phenotype of thrombophilia, a positive test result confirms the diagnosis of thrombophilia and potentially leads to altered treatment decisions. However, a negative thrombophilia test result does not necessarily exclude thrombophilia (nor does it necessarily denote a lower risk of VTE recurrence) and thus does not ultimately change treatment recommendations. Likewise, in patients for whom the probability of thrombophilia cannot be determined on a clinical basis, a positive thrombophilia test result confirms the diagnosis and may alter treatment decisions, while a negative result does not definitively rule out thrombophilia. In patients with a low (or zero) pre-test probability of thrombophilia, thrombophilia testing carries a risk of overdiagnosis and should be avoided.

Future large-scale, prospective studies at multiple centers would be required to achieve maximum objectivity, although the application of a thrombophilia probability score in our study ensured that all patients in the study cohort were evaluated and treated with the same approach. A potential prospective study design should include treatment recommendations based on the existing guidelines to evaluate the impact of thrombophilia testing on clinical decision making, in addition to an evaluation of the management of patients who do not clearly meet the criteria for thrombophilia. This would both provide important clinical context to the current guidelines and improve practice standardization in the diagnosis and management of thrombophilia.

Data Sharing Statement

For original data, please contact bernd.poetzsch@ukbonn.de.

Authors' Contributions

H.L.M. and B.P. designed the research and co-wrote the manuscript; H.L.M., H.R., and B.P. collected and analyzed the data; H.R. performed statistical analysis; H.R. and J.M. reviewed and edited the manuscript; B.P. supervised study activities. All authors have read and agreed to the final version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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