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REVIEW ARTICLE

Thrombophilia Testing - a Systematic Review

Lars Asmis [1], Peter Hellstern [2]

[1] *Center of Perioperative Thrombosis and Hemostasis, Zurich, Switzerland*
[2] *Center of Hemostasis and Thrombosis Zurich, Zurich, Switzerland*

SUMMARY

Background: **Thrombophilia testing is controversial, not least because of its high cost. Because comprehensive valid testing requires standardized blood collection close by the specialized laboratory, and interpretation of findings together with clinical data, often only part of the necessary laboratory analyses can be performed in remote central laboratories. Restrictive indications for testing, as have been recommended by previous reviews on the topic, have been based on incomplete analytics, studies with small case numbers, or short observation periods, and on an inappropriate, simple risk stratification for venous thromboembolism (VTE), further subdivided into provoked and unprovoked events.**

Methods: **The authors reviewed four electronic databases for all peer-reviewed and in-press articles about thrombophilia, VTE, obstetric complications, and arterial thrombosis. After confirmation for relevance to the topic, 201 articles were accepted for inclusion in this article. This review summarizes the studies relevant to the evaluation of thrombophilic conditions, and their combination with each other and with clinical risk factors, to stratify individual risk for thromboembolism and obstetric complications.**

Results: **Thrombophilia testing requires highly skilled personnel for laboratory analysis and interpretation. Clinical conditions that influence the results as well as special preanalytical, analytical, and postanalytical aspects must be considered if valid results are to be obtained. Tests involved include the natural anticoagulants antithrombin, protein C, and protein S; the procoagulants fibrinogen (dysfibrinogen), prothrombin (mutation G20210A), factor V (Leiden mutation), factor VIII/von Willebrand factor/blood group ABO, factor IX, and factor XI; the antiphospholipid antibodies to detect an antiphospholipid syndrome and potentially additional uncertain thrombophilic conditions. The risks of thrombophilic conditions and clinical risk factors for VTE are cumulative or even supra-additive. Scores from thrombophilic conditions and other genetic and nongenetic risk factors permit estimation of risk for first and recurrent VTE. Therapeutic strategies can be derived from this risk stratification.**

Conclusions: **Thrombophilia testing is indicated when the results have potential to influence the type and duration of treatment. Indications include certain patients after VTE; or patients without previous VTE but with positive family history regarding VTE or thrombophilia before major surgery, pregnancy, combined oral contraceptives, or hormone replacement therapy. Whether or not thrombophilia is present should help determine anticoagulation, hormonal contraception, or hormone replacement.**

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Correspondence:
Peter Hellstern, MD and Professor
Center of Hemostasis and Thrombosis Zurich
Weinbergstrasse 157
8006 Zurich
Switzerland
Phone: + 41 44 360 8070
Fax: + 41 44 360 8071
Email: peter.hellstern@haemoclot.ch

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Supplementary Data

Table S1. Thrombophilic conditions - prevalence, relative risk of first and recurrent venous thromboembolism, and laboratory diagnosis.

Thrombophilic condition	Prevalence in general population	Significant HR, OR, or RR for 1st VTE compared with controls	Significant HR, OR, or RR for recurrent VTE	Assays *
Inherited AT deficiency	**1:500 - 1:5,000 [38,39]**	**HR = 10.2 [12] OR = 16 [40]**	**HR = 3.6 [12,40]**	**Anti Xa-based chromogenic assay, anti-IIa-based chromogenic assay,** antigen assay, turbidimetrically, ELISA. Possibly SERPINC1 gene analysis
Inherited AT deficiency types IIHBS	**30% of all inherited AT deficiencies [36,37]**	**HR = 0.23 vs. other types of AT deficiency [36] RR = 0.28 vs. type I AT deficiencies [37]**	**unknown**	**Anti Xa-based chromogenic assay, anti-IIa-based chromogenic assay,** antigen assay, turbidimetrically, ELISA. Possibly SERPINC1 gene analysis
Inherited AT deficiency Cambridge II Ala384Ser, G1246T (rs121909548)	**1:500 [41]**	**OR = 9.7 [41] RR = 0.42 vs. type I AT deficiency [37]**	**OR ns [41]**	**Anti Xa-based chromogenic assay, anti-IIa-based chromogenic assay,** antigen assay, turbidimetrically, ELISA. Possibly SERPINC1 gene analysis
Mild AT deficiency (70 - 80 U/dL)	**1.5% < 80 U/dL [43]**	**unknown**	**HR = 3.4 < 70 vs. > 80 U/dL [42] HR = 3.7 < 70 vs. ≥ 80 U/dL [43]**	**Anti Xa-based chromogenic assay, anti-IIa-based chromogenic assay,** antigen assay, turbidimetrically, ELISA
PC deficiency	**1:400 [39]**	**OR = 7.5 [40]**	**OR = 2.9 [40]**	**Chromogenic assay, APTT or PT-based clotting assay, antigen assay, ELISA**
PS deficiency	**1:300 [39]**	**HR = 9.6 [12] OR = 5.3 [40]**	**OR = 2.5 [40]**	**PT-based clotting assay, free PS, turbidimetrically, ELISA**
PS Heerlen Ser460Pro (rs121918472)	**1:250 [55]**	**OR = 6.5 [55]**	**unknown**	**PT-based clotting assay, free PS, turbidimetrically, ELISA. PS Heerlen DNA-based method**
Homozygous fibrinogen C10034T (rs2066865)	**1:17 [58] 1:18 [59]**	**OR = 2.4 [58] HR = 1.6 heterozygous + homozygous [23]**	**HR = 1.1 [16] HR = 1.7 [21]**	**PCR-based methods,** non-PCR based FRET assay
Inherited dysfibrinogenemia	**1:6,000 [63]**	**unknown**	**unknown**	**Clottable fibrinogen, Clauss' method; antigen, turbidimetrically.** DNA-based method, sequencing
Heterozygous PTM	**3% in Europe and in the USA [68]**	**OR = 3.1 [70] OR = 2.8 [69]**	**OR = 1.7 [71] RR = 1.3 [72] OR ns [73]**	**PCR-based methods, non-PCR based FRET assay**
Homozygous PTM	**1:1670 [69], 1:1430 in Europe [68]**	**OR = 11.2 [69]**	**unknown**	**PCR-based methods,** non-PCR based FRET assay
Heterozygous FVL G1691A (rs6025)	**4.4% [75], 3 - 15% in european whites [76]**	**OR = 5.0 [70] OR = 4.3 [69]**	**OR = 1.4 [71] RR = 1.4 [72] OR = 1.5 [73] HR = 1.6 [16] HR = 3.4 [22] HR = 1.6 [21]**	**FVL specific APC resistance by APTT or prothrombin-based clotting assay; PCR-based methods,** non-PCR based FRET assay
Homozygous FVL G1691A (rs6025)	**1:760 [75], 1:5,000 in whites [76], 1:485 [69]**	**OR = 79 [77] OR = 9.4 [70] OR = 11.4 [69] OR = 13.9 [78]**	**OR = 2.6 [73]**	**FVL specific APC resistance by APTT or prothrombin-based clotting assay; PCR-based methods,** non-PCR based FRET assay

Table S1. Thrombophilic conditions - prevalence, relative risk of first and recurrent venous thromboembolism, and laboratory diagnosis (continued).

Thrombophilic condition	Prevalence in general population	Significant HR, OR, or RR for 1st VTE compared with controls	Significant HR, OR, or RR for recurrent VTE	Assays *
Heterozygous FVL + heterozygous FV deficiency ("pseudo-homozygous" FVL)	**1:20,000 [96]**	**Like homozygous FV Leiden**	**Like homozygous FV Leiden**	**FVL specific APC resistance by APTT or prothrombin-based clotting assay PCR-based methods; FV one-stage clotting assay.** Non-PCR based FRET assay
Heterozygous FVL + heterozygous PTM	**1:1,000 [98, 1:617 [99] 1:1,666 [100]**	**OR = 20.0 [75] OR = 3.4 [69]**	**RR = 2.6 [98]**	**APC resistance (screening), clotting assay; PCR-based methods,** non-PCR based FRET assay
High FVIII (and high VWF)	**1:9 > 150 IU/dL [103] 1:10 > 150 IU/dL [104]**	**OR = 6.2 [103] OR = 6.7 < 100 vs. > 150 IU/dL [104] OR = 8.0 < 86 vs. > 174 - 196 IU/dL [66]**	**RR = 2.7 > 234 IU/dL [105] RR = 2.9 > 234 IU/dL [106] HR = 3.4 ≤ 100 vs. > 200 IU/dL [107] HR = 2.2 ≥ 203 IU/dL [108]**	**FVIII, one-stage clotting assay,** chromogenic assay, antigen ELISA. VWF, antigen immunoassay, turbidimetrically; activity, turbidimetrically
AB0 blood group non-O	**55% in Europe and the USA [111,114]; 54% in Europe and the USA [112]; 60% in Denmark [113]**	**OR = 1.8 [111] OR = 2.0 [112] HR = 1.4 [113] OR = 1.9 [20] RR = 1.8 [115] OR = 1.8 [116]**	**HR = 1.9 [117]**	**Agglutination assay,** DNA-based methods
High FIX	**1:10 > 129 IU/dL [118]**	**OR = 2.3 > 129 IU/dL [118] OR = 1.7 < 92 vs. > 154 IU/dL [66]**	**RR = 1.6 > 134 IU/dL [105] OR ns [119]**	**One-stage clotting assay,** antigen ELISA
High FXI	**1:10 > 120 IU/dL [120] 1:5 ≥ 157 IU/dL [119]**	**OR = 2.2 > 120 IU/dL [120] OR = 1.9 > 120 IU/dL [121] OR = 1.8 < 104 vs. ≥ 157 IU/dL [119] OR = 1.9 < 87 vs. > 134 IU/dL [66]**	**RR = 2.4 > 133 IU/dL concomitantly high TAFI [106]**	**One-stage clotting assay,** antigen ELISA
FXI gene polymorphisms rs2036914 and rs2289252	**27% rs2036914 risk allele CC, 49% rs2036914 risk allele CT, 17% rs2289252 risk allele TT, 47% rs2289252 risk allele TC [123]**	**OR = 1.3 rs2036914 OR = 1.5 rs2289252 [123] OR = 1.6 rs2289252, OR = 1.24 rs2036914 [124] HR = 1.55 rs2036914 [23]**	**HR = 1.8 rs2289252 [125]**	**DNA-based methods**
Increased aPL, APS	**4.1% positive for aCL IgG or IgM [127]; 0.9% positive for LA, 3.4% positive for aß2GPI [128]; 3.2% for aCL or aß2GPI IgG or IgM positive [129]; APS 1:2000 [130]**	**OR = 6.1 for positive LA OR = 1.4 for positive aCL [132]**	**RR = 1.5 for aCL positive, RR = 2.8 for LA positive [133] HR = 2.7 for any type of aPL HR = 4.5 with 2 or 3 types of aPL [134] OR = 7.3 for isolated positive LA [135]**	**LA, DRVVT, APTT-based, SCT; aCL, aß2GPI, CLIA, ELISA**

Table S1. Thrombophilic conditions - prevalence, relative risk of first and recurrent venous thromboembolism, and laboratory diagnosis (continued).

Thrombophilic condition	Prevalence in general population	Significant HR, OR, or RR for 1st VTE compared with controls	Significant HR, OR, or RR for recurrent VTE	Assays *
Hyperhomocysteinemia	**5%, 95th percentile of controls [141]**	**OR = 2.5 > 95th percentile [141] OR = 3.3 > 14.8 µmol/L [142] OR = 1.27 per 5 µmol/L increase in prospective and 1.6 in retrospective studies [143] OR = 1.1 retinal vein occlusion, for 1 µmol/L increase [145]**	**RR = 2.7 > 8.8 µmol/L in F and > 11.6 µmol/L in M [147] HR = 1.8 > 90th percentile [148] HR ns [149]**	**Electrochemical assay,** nephelometric immunoassay, colorimetric enzyme assay, HPLC
Hypofibrinolysis by increased PAI-1 or increased TAFI	**PAI-1 20% of controls > 57 ng/mL, upper quintile [151] TAFI 9% of controls > 122 U/dL, 90th percentile [153]**	**OR PAI-1 ns [151] OR = 1.5 PAI-1, top vs. bottom quartile [154] OR = 1.5 PAI-1, upper vs. lower tertile [152] OR = 1.7 TAFI, > 122 U/dL [153] OR = 1.4 TAFI, top vs. bottom quartile [154]**	**RR = 1.7 TAFI, ≥ 110 U/dL [106]**	**Antigen ELISAs**
High lipoprotein(a)	**7% > 30 mg/dL [155]**	**OR = 3.2 > 30 vs. < 30 mg/dL [155] OR = 1.5 [156] HR ns [157]**	**RR ns [158]**	**Immunoassay, turbidimetrically,** nephelometrically, ELISA, electroimmunoassay
PZ deficiency	**1:40 [159]**	**OR = 2.1 [160]**	**unknown**	**Antigen assay, ELISA**
Sticky platelet syndrome	**14% with 0.4 µM of epinephrine, 7% with 0.5 µM of ADP [162]; 15% [163]**	**OR ns [165]**	**unknown**	**Light transmission platelet aggregation with low epinephrine and ADP concentrations**

* **First line assays in bold.**

aCL, anti-cardiolipin antibodies; aß2GPI, anti-ß2-Glycoprotein I antibodies; ADP, adenosine diphosphate; APC, activated protein C; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; APTT, activated partial thromboplastin time; AT, antithrombin; CLIA, chemiluminescent immunoassay; DRVVT, diluted Russel's viper venom time; ELISA, enzyme-linked immunosorbent assay; F, factor; F, females; FRET, fluorescence resonance energy transfer; FVL, factor V Leiden; HBS, heparin binding site; HPLC, high performance liquid chromatography; IU, international units; HR, hazard ratio; LA, lupus anticoagulant; M, males; ns, not significant; OR, odds ratio; PC, protein C; PCR, polymerase chain reaction; PS, protein S; PT, prothrombin time; PTM prothrombin mutation G20210A (rs1799963), PZ, protein Z; RR, relative risk, risk ratio; SCT, silica clotting time; TAFI, thrombin activatable fibrinolysis inhibitor; VTE, venous thromboembolism.

Table S2. Studies demonstrating additive or supra-additive effects of combined thrombophilic and clinical risks in determining an individual's overall risk for venous thromboembolism.

	Family history	Can-cer	Surgery, trauma, immobile, travel	COC, HRT, preg-nancy	Age	Smoking	BMI	FFG rs2066865	FXI, F11 rs2036914, rs2289252	FIX	FVIII/ABO blood group	PTM
FVL	[13,15, 17,167]	[13]	[13,14,17, 22,24,169]	[13,17, 169]	[167, 170]	[23,167]	[13,17, 23,152, 167,170]	[13,16,17, 21,23,152, 168]	[13,16,17, 21,23,152, 167,168]		[13,15-17, 21,23,110, 112,113, 152,167-173]	[13,15-18, 21,23,152, 167,168, 170,173]
PTM	[13,15, 17,167]	[13]	[13,17]	[13,17]	[167, 170]	[18,23, 167]	[13,17, 18,23, 167,170]	[13,16-18, 21,23,152, 168]	[13,16-18, 21,23,152, 167,168]		[13,15-18, 21,23,113, 152,167, 168,170]	
FVIII/ ABO blood group	[13,15, 17,167]	[13]	[13,14,17, 24,169]	[13,17]	[167, 170]	[18,23, 167]	[13,17, 18,23, 152,167, 170]	[13,16-18, 21,23,152, 168]	[13,16-18, 21,152, 167,168]	[21, 105]		
FIX			[14]									
FXI, F11 rs2036914, rs2289252	[13,17, 167]	[13]	[13,17]	[13,17, 169]	[167]	[18,23, 167]	[13,17, 18,23, 152,167]	[13,16-18, 21,23,152, 168]				
FGG rs2066865	[13,17]	[13]	[13,17]	[13,17]		[18,23]	[13,17, 18,23, 152]					
BMI	[13,17, 167]	[13]	[13,17]	[13,17]	[167, 170]	[18,23, 167]						
Smoking												
Age												
COC, HRT, pregnancy	[13,17, 190]	[13]	[13,17, 169]									
Surgery, trauma, immobile, travel	[13,17, 190]	[13]										
Cancer	[13]											

BMI, body mass index; COC, combined oral contraceptives; F, factor; F11, factor XI gene; FGG, γ-fibrinogen gene; FVL, factor V Leiden; HRT, hormone replacement therapy; PTM, prothrombin mutation G20210A.