

# Utility of current thrombophilia screening in young patients with stroke and TIA

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## ABSTRACT

**Introduction** Approximately 40% of strokes in young adults are cryptogenic. The diagnostic yield of thrombophilia screening remains controversial. We aimed to determine utility of current thrombophilia testing for young patients with stroke and transient ischaemic attack (TIA).

**Methods** We present a retrospective review of all patients with stroke and TIA ≤60 years presenting to University College London Hospital stroke unit and daily TIA clinic from 1 January 2015 to 1 August 2016. Consecutive clinical records and thrombophilia tests, including factor V Leiden (FVL), prothrombin G20210A mutation (PGM), antiphospholipid antibody (APA), and protein S, C and antithrombin (AT) levels, were reviewed.

**Results** The mean age of 628 patients with stroke and TIA was 49.1 years (SD 9.2). Thrombophilia testing was performed in 360 (57%) patients, including 171 with stroke and 189 with TIA. Positive tests were found in 50 (14%) patients, of whom 24 patients were <50 years. Positive results were found in 36 (10%) with acute ischaemic stroke, 4 (1%) with haemorrhagic stroke and 10 (3%) with TIA. Thirteen patients (4%) had homozygous/heterozygous FVL or PGM, and 27 (7.5%) had positive APA (anticardiolipin antibody, anti-β2 glycoprotein antibody or lupus anticoagulant). Of 27 (7.5%) patients with protein C, S or AT deficiency, 10 (2.8%) had primary deficiency, presumed hereditary with other secondary causes excluded. 9% of patients with protein C, S or AT and 27% with APA were followed by confirmatory testing.

**Conclusion** Thrombophilia testing was positive in only 14% of cases overall. Thrombophilia mutations and protein C, S or AT abnormalities were found rarely and were very uncommon in patients with TIA. Follow-up of abnormal results was generally poor for all groups, which further limited the impact of the thrombophilia testing policy.

## BACKGROUND

In comparison with older individuals, young stroke pathogenesis is more pleomorphic, with a higher representation of uncommon causes such as dissections, cardiac arrhythmia and thrombophilia.<sup>1 2</sup> These aetiological differences often necessitate additional investigations, including complex thrombophilia screening, but the utility of extensive thrombophilia work-up in young patients with stroke and

transient ischaemic attack (TIA) is not established.

Thrombophilias are broadly defined as inherited or acquired coagulation disorders predisposing to thrombosis.<sup>3 4</sup> Antithrombin (AT), protein C and S deficiency, factor V Leiden (FVL) and factor II mutations are rare inherited thrombophilias associated with an increased risk of venous thrombosis, but their relation to arterial ischaemic stroke and TIA is less well established.<sup>5–16</sup> Accurate diagnosis and clinical relevance of AT, protein C and S deficiency to acute stroke are quite complex, since assays are usually influenced by active illness, concurrent medical conditions, and medications such as oral contraception and anticoagulation.<sup>17–24</sup> Hence, repeat testing is recommended in order to distinguish false-positive diagnoses from genuine AT, protein C and protein S deficiencies.<sup>25</sup>

Antiphospholipid syndrome is an autoimmune thrombophilia characterised by recurrent arterial or venous thrombosis and/or pregnancy morbidity, and presence of persistent antiphospholipid antibodies (APA).<sup>26</sup> A large prospective study showed that antiphospholipid syndrome manifests as stroke and TIA in 2.4% and 2.3% of cases, retrospectively.<sup>27</sup> There are three types of APA with different phospholipid binding sites: lupus anticoagulant (LA), anticardiolipin antibody (ACL) of IgG or IgM subtype, and anti-β2 glycoprotein 1 (anti-β2GPI) of IgG or IgM subtype. The interpretation of positive APA results in the context of arterial thrombosis requires careful consideration of antibody type, isotype and titre, in the individual clinical context.<sup>26</sup> Among APAs, the specificity for arterial thrombosis is higher for LA and anti-β2GPI.<sup>28–31</sup> In addition, among patients with thrombosis, the highest risk of recurrence is the relatively small cohort positive for triple APA.<sup>32</sup> Since APA can be transiently positive after acute illness, it is recommended to repeat it at



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least 3 months after stroke to distinguish definite antiphospholipid syndrome.<sup>26</sup> In our study, we evaluated acute stroke admissions potentially eligible for more intensive cholesterol treatment.

Thrombophilia testing in young patients with stroke often includes protein C, S and AT levels, genotyping for FVL and prothrombin G20210A mutation (PGM), and APA testing. This complex and costly testing is often routinely sent despite limited evidence linking hereditary thrombophilia to arterial thrombosis; moreover, in practice, only a small proportion of initial abnormal results are further followed up by appropriately timed repeat testing.

The present study aimed to determine our current practice of assessing positive thrombophilia testing in the context of young stroke and TIA.

## METHODS

We performed a retrospective evaluation of consecutive cases within a single regional hyperacute stroke unit and daily TIA clinic at University College London Hospital, the main referral centre for North Central London. We reviewed consecutive medical and laboratory records of all patients with acute ischaemic stroke (AIS), haemorrhagic stroke and TIA 60 years and under presenting to the service from 1 January 2015 to 1 August 2016. The standard thrombophilia panel included genotyping for FVL and PGM, AT activity, protein C and S antigen and activity, and APA testing.

APA testing was conducted for LA (using dilute Russell viper venom time and/or Taipan venom time methodology according to whether the patient was anticoagulated), ACL IgG/IgM and anti- $\beta$ 2GPI IgG/IgM (ELISA). Laboratory criteria for positive APA were considered in accordance with the Miyakis criteria, which include the following: (1) LA present; (2) ACL (either IgG or IgM) in medium or high titre, that is, >40 GPL units or MPL units (defined as the cardiolipin-binding activity of 1 g/ml of affinity-purified IgG or IgM ACL), or >99th centile; and (3) anti- $\beta$ 2GPI (IgG or IgM) in titre >99th centile. ACLs with low titres were excluded.

In all those with initial positive thrombophilia testing (defined as homozygous or heterozygous FVL or PGM, presence of APA, low protein S, C and AT), clinical and laboratory records were reviewed by the stroke and haematology physicians for acquired causes, stroke characteristics and whether appropriate follow-up had been arranged. The Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification was used to subtype ischaemic stroke and the ABCD2 score was used for TIA risk assessment.

The estimated final costs for thrombophilia testing were based on the University College London Hospital NHS Trust's pathology pricing model at the time of the study, 1 January 2015–1 August 2016, and are outlined in online supplementary table 1.

## RESULTS

Six hundred and twenty-eight patients were  $\leq 60$  years: 383 (61%) AIS, 202 (32%) TIA and 43 (6.8%) haemorrhagic stroke. The mean age was 49.1 years (SD 9.2).

Three hundred and sixty (57%) patients had thrombophilia testing: 171 (40%) stroke and 189 (94%) TIA. Abnormal results were returned in 70 (19%) patients. Twenty were later excluded from this cohort on secondary screening, 17 with acquired protein C, S or AT deficiency, and 3 with negative confirmatory APA testing of initial positive APA (figure 1).

A total of 50 (14%) patients, including 27 (8%) patients with positive APA, 13 (4%) homozygous or heterozygous for FVL or PGM, and 10 (3%) presumed genuine protein C, S or AT deficiency, were analysed: 36 (10%) AIS, 4 (1%) haemorrhagic stroke and 10 (3%) TIA. The median vascular risk factor of these 50 patients was 1 (IQR 0–5) (table 1). Of 50 patients with positive thrombophilia testing, 36 had AIS and 10 had TIA, 2 (4%) had carotid artery stenosis >70%, 6 (13%) had lacunar syndrome, 14 (30%) were cardioembolic, 4 (9%) had vertebral or internal carotid artery dissection and 20 (43%) had other determined aetiology.

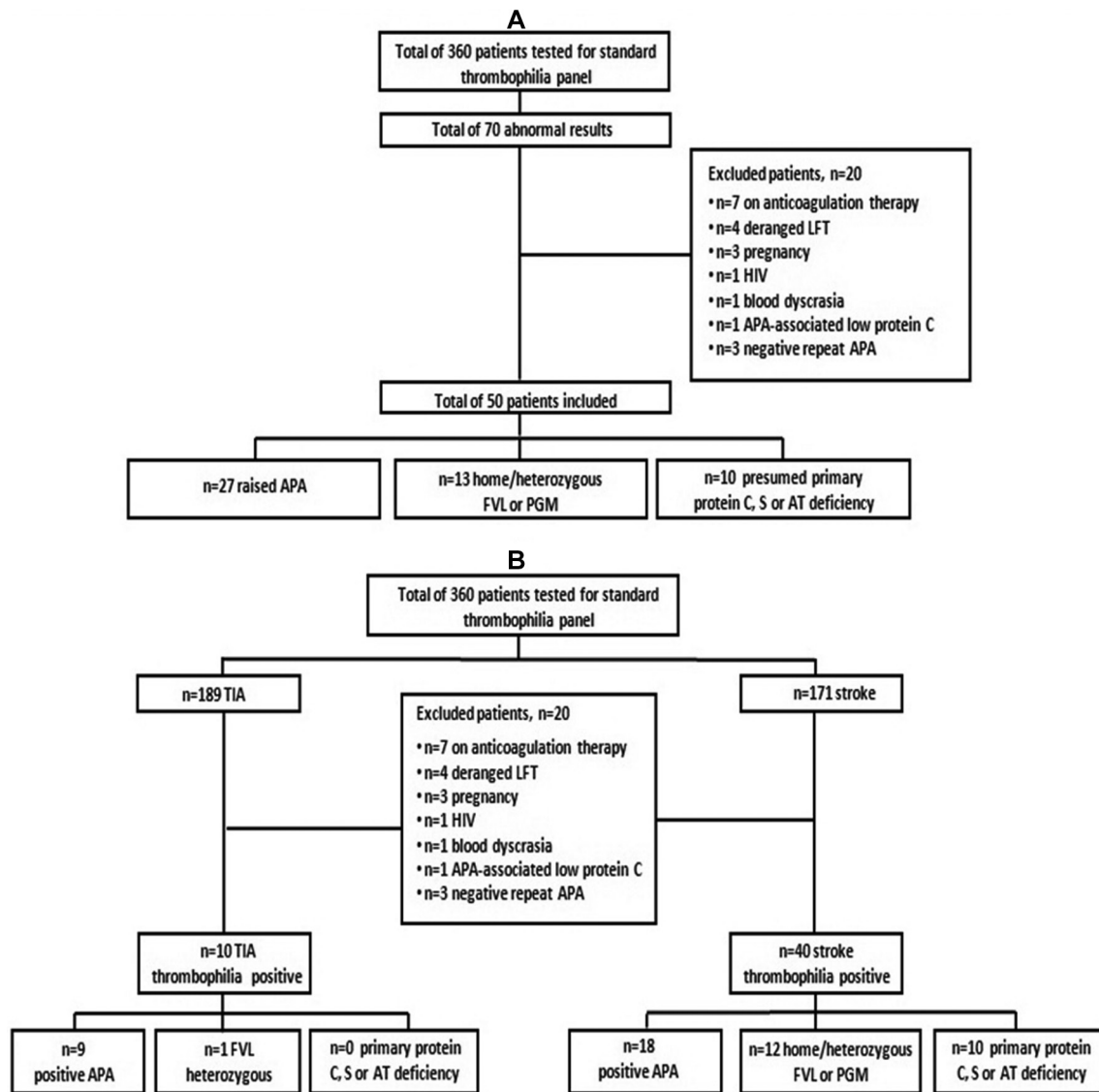
Twenty-four of 243 patients (9.9%) <50 years were thrombophilia testing-positive. There was no significant difference in those <50 years vs 51–60 years ( $p=0.15$ ).

Of 27 (8%) positive for an APA, 20 (6%) were single antibody-positive, 5 (1%) double antibody-positive and 2 (0.6%) triple antibody-positive (figure 2). Four patients who were on anticoagulation therapy were included, although APA was not repeated after stopping anticoagulation and false positivity could not be confirmed. Three cases with medical history of systemic lupus erythematosus (SLE) and antiphospholipid syndrome were also on anticoagulation therapy.

Of 360 patients tested for standard thrombophilia, 94% were screened for both LA and ACL antibodies and 3% for 'triple APA' including LA, ACL and anti- $\beta$ 2GPI antibodies. Of 30 patients with initial positive APA, only 8 (27%) were followed by repeat APA testing.

Of 189 thrombophilia-tested TIA cases, 10 (5%) were positive and 9 (5%) with APA. Four presented with amaurosis fugax, all of whom were APA-positive. Thrombophilia mutations and protein C, S or AT deficiency were rarely positive in this group (0.5%). The positive cases included two cardioembolic, four small vessel disease, one case with SLE and three cases with a cryptogenic aetiology at the time of initial work-up (table 2). The median ABCD2 score of positive cases was 4.5 (IQR 1–5).

The thrombophilia panel cost per patient ranged from £245.67 to £373.19 (online supplementary table 1). More than 94% of patients had thrombophilia test that included FVL, PGM, ACL, LA, protein C and AT functional assays, and free protein S quantification tests. Additional confirmatory tests included protein C and AT quantification, protein S functional assay and anti- $\beta$ 2GPI antibody.



**Figure 1** Outcome of thrombophilia testing for the whole cohort (A) and separated for TIA and stroke (B). APA, antiphospholipid antibody; AT, antithrombin; FVL, factor V Leiden; LFT, liver function test; PGM, prothrombin G20210A mutation; TIA, transient ischaemic attack.

## DISCUSSION

There is no clear consensus on the age range of ‘young adult stroke’; most studies of this age group include young adults from 15 to 50 years. We included patients with stroke and TIA of 60 years and under into the category of ‘young adult stroke and TIA’, and analysed 51–60 years and <50 years age groups separately. This subgroup analysis showed an even distribution of positive thrombophilia testing among 51–60 and <50 age groups. Young-onset stroke and TIA are more heterogeneous than for older patients, and typical aetiologies include both traditional cardiac and large vessel atheroma, as well as a higher prevalence of ‘rarer’ aetiologies including arteriopathies, inflammatory or otherwise, cardiomyopathy and prothrombotic states. Based on the TOAST classification of ischaemic stroke, ‘stroke of other determined origin’ is traditionally deemed correspondingly highest in

the younger age group. This was also represented in our thrombophilia-screen positive AIS and TIA cohorts, with 61% of the diagnosis falling into ‘stroke with other, determined or undetermined origin’ category. Nine per cent of cases with cervical artery dissection were included into this category that likely resulted from trauma and were coincidentally positive to thrombophilia screen.

In practice, patients with young stroke and TIA undergo additional investigations, including complex thrombophilia screening, despite weak association with arterial thrombosis and hereditary thrombophilia. In our institution, prior to this study, complex thrombophilia screening was indicated for all patients with AIS and TIA younger than 50 independent of vascular risk factors profile. There was no selection bias. Similarly, in our cohort screened for thrombophilia, only 3.6% of the individuals were carriers of FVL or PMG. This

**Table 1** Baseline characteristics of thrombophilia screen-positive patients (n=50)

Characteristics	All (n=50)	Positive APA (n=28)	FVL/PGM genotype (n=13)	Protein C, S or AT deficiency (n=10)
Age, mean (SD), years	49 (9.2)	50 (7)	50 (5)	49 (9)
Male sex, n (%)	28 (57)	18 (37)	7 (14)	4 (8)
Hypertension, n (%)	25 (51)	12 (24)	6 (12)	6 (12)
Diabetes mellitus, n (%)	6 (12)	2 (4)	4 (8)	0
Hyperlipidaemia, n (%)	11 (22)	5 (10)	3 (6)	3 (6)
Smoking, n (%)	18 (36)	12 (22)	3 (6)	3 (6)
Peripheral vascular disease, n (%)	1 (2)	1 (2)	0	0
Previous stroke/TIA, n (%)	7 (14)	2 (4)	4 (8)	1 (2)
Family history of stroke/IHD, n (%)	8 (16)	4 (8)	2 (4)	2 (4)
History of VTE, n (%)	1 (2)	1 (2)	1 (2)	0
Ischaemic heart disease, n (%)	4 (8)	2 (4)	2 (4)	0
Atrial fibrillation*, n (%)	1 (2)	1 (2)	1 (2)	0
Patent foramen ovale, n (%)	4† (8)	2 (4)	2 (4)	1 (2)
Other cardiac disease, n (%)	3‡ (6)	3 (6)	0	0
History of cancer, n (%)	3 (6)	2 (4)	2 (4)	0

\*Atrial fibrillation detected by either 12-lead ECG or Holter.

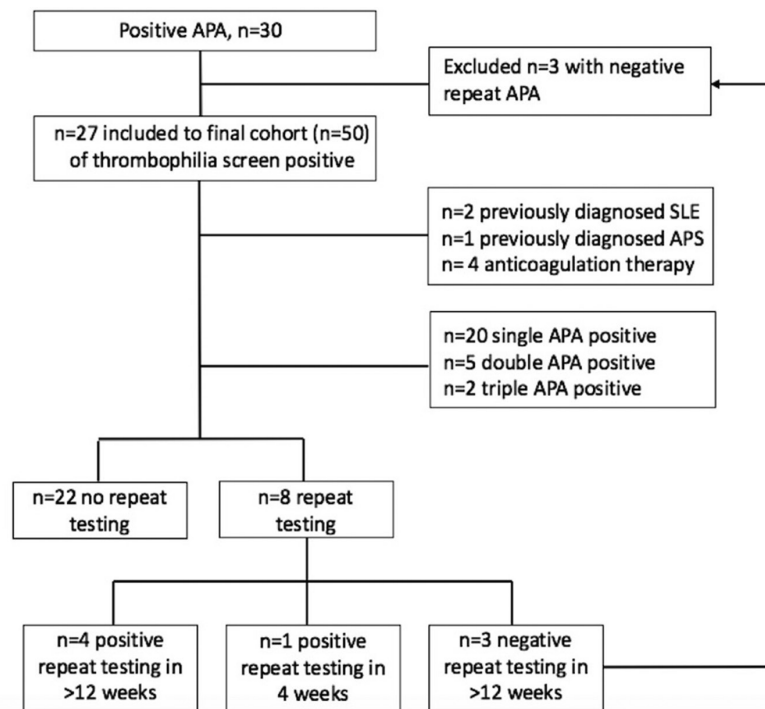
†One case both with FVL mutation and raised APA.

‡Cases with metallic valve replacement and cardiomyopathy.

APA, antiphospholipid antibodies; AT, antithrombin; FVL, factor V Leiden; IHD, ischaemic heart disease; PGM, prothrombin G20210A mutation; TIA, transient ischaemic attack; VTE, venous thromboembolism.

association was even less for patients presenting with TIA (0.5%). Moreover, more than 60% of patients with stroke and TIA with low levels of AT, protein C and S also had concurrent acquired causes, such as anticoagulation, liver

function derangement and infection. In addition, only a small proportion of those with low levels of AT, protein C and S were repeated, an important step in distinguishing genuine deficiency.



**Figure 2** Outcome of antiphospholipid antibody testing-positive cohort. APA, antiphospholipid antibody; APS, antiphospholipid syndrome; SLE, systemic lupus erythematosus.



**Table 2** Thrombophilia test-positive stroke and TIA

Characteristics	All (n=50)	Positive APA (n=28)	FVL/PGM genotype protein C, S or AT deficiency (n=23)
Acute ischaemic stroke, n (%)	36 (72)	16 (32)	20 (40)
Intracranial haemorrhage, n (%)	4 (8)	2 (4)	2 (4)
Transient ischaemic attack, n (%)	10 (20)	9 (18)	1 (2)
Amaurosis fugax, n (%)	4 (8)	4 (8)	0
Non-ocular TIA, n (%)	6 (12)	5 (10)	1 (2)

APA, antiphospholipid antibody; AT, antithrombin; FVL, factor V Leiden; PGM, prothrombin G20210A mutation; TIA, transient ischaemic attack.

Compared with hereditary thrombophilia, antiphospholipid syndrome is a more established risk for arterial thrombosis.<sup>8 11–16 27–31</sup> Patients with triple positivity for LA, ACL and anti-β2GPI have been shown to be at the highest risk for arterial and venous thrombosis, and for obstetric complications.<sup>32</sup> However, only 3% of our thrombophilia-screened cohort had triple APA testing. There is evidence that persistent APA is associated with the highest incidence of thrombosis,<sup>33</sup> although only 27% of our positive initial APA cohort was followed up by confirmatory APA testing.

AT, protein C and protein S deficiencies are rare in the general population, with an estimated frequency of about 1:5000.<sup>7 9 10 34–36</sup> Similarly, FVL and PMG present in 5% and 1%–5.5% of the Caucasian population but are rare in other ethnic groups.<sup>34 37 38</sup> Although those hereditary thrombophilias increase the risk of venous thrombosis, their association with arterial ischaemic stroke and TIA is less well established.<sup>5–16</sup> More than 94% of patients with stroke and TIA in the thrombophilia-tested cohort had genotyping and protein C, S and AT tests, and similar to previous findings 13% of patients with ischaemic stroke and only 0.5% of patients with TIA were either carriers of FVL/PGM genotype or protein C-deficient, S-deficient or AT-deficient after exclusion of secondary causes of deficiency (table 2), indicating low-value hereditary thrombophilia screening especially in young TIA work-up. Consequently, based on the costing scheme outlined in online supplementary table 1, limited testing focusing on triple APS testing rather than full inherited thrombophilia panel only would potentially save £267.13 per patient. Those tested positive on initial testing for protein C, S or AT deficiency should be followed up by confirmatory testing. Low numbers of confirmatory testing may reflect the ‘hub and spoke’ model of hyperacute stroke care at regional centres in London, whereby initial positive testing at the regional centre may not have been effectively communicated to the stroke unit performing follow-up.

The standard thrombophilia panel could also be stratified by including multiple triple APA testing and excluding thrombophilia mutations screening. Based on our costs at the time of the study, this more focused testing would save £267.13 per patient.

Limitations of this study include its retrospective single-centre design. Attributable risks of thrombophilia screening and interaction between the other risk factors such as contraceptive pill use, migraine and smoking were not estimated. Statistical comparison between risk factors of thrombophilia-screened cohort and not-screened cohort was not assessed. However, our review is of real-life practice, highlighting that current thrombophilia testing should be particularised to patients with young stroke and TIA without conventional cardiovascular risk factors. Initial positive tests should be repeated to distinguish genuine thrombophilia from common false-positive conditions.

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**Data sharing statement** All available data can be obtained by contacting the corresponding author.

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## REFERENCES

1. Ji R, Schwamm LH, Pervez MA, *et al.* Ischemic stroke and transient ischemic attack in young adults: risk factors, diagnostic yield, neuroimaging, and thrombolysis. *JAMA Neurol* 2013;70:51–7.
2. Putaala J, Metso AJ, Metso TM, *et al.* Analysis of 1008 consecutive patients aged 15 to 49 with first-ever ischemic stroke. *Stroke* 2009;40:1195–203.
3. Weston-Smith S, Revell P, Savidge GF. Thrombophilia. *Br J Hosp Med* 1989;41:368–71.
4. Boekholdt S, Kramer M. Arterial thrombosis and the role of thrombophilia. *Semin Thromb Hemost* 2007;33:588–96.
5. Juul K, Tybjaerg-Hansen A, Steffensen R, *et al.* Factor V Leiden: the copenhagen city heart study and 2 meta-analyses. *Blood* 2002;100:3–10.
6. Sode BF, Allin KH, Dahl M, *et al.* Risk of venous thromboembolism and myocardial infarction associated with factor V Leiden and prothrombin mutations and blood type. *CMAJ* 2013;185:E229–E237.

7. Maclean PS, Tait RC. Hereditary and acquired antithrombin deficiency. *Drugs* 2007;67:1429–40.
8. Nagaraja D, Christopher R, Tripathi M. Plasma antithrombin III deficiency in ischaemic stroke in the young. *Neurol India* 1999;47:155.
9. Corral J, Hernandez-Espinosa D, Soria JM, et al. Antithrombin Cambridge II (A384S): an underestimated genetic risk factor for venous thrombosis. *Blood* 2007;109:4258–63.
10. Blajchman MA. An overview of the mechanism of action of antithrombin and its inherited deficiency states. *Blood Coagul Fibrinolysis* 1994;5(Suppl 1):S5–12.
11. Gómez-Aranda F, López Dominguez JM, Rivera Fernández V, et al. Stroke and familial protein S deficiency. *Stroke* 1992;23:299.
12. Vasse M, Guegan-Massardier E, Borg J-Y, et al. Frequency of protein Z deficiency in patients with ischaemic stroke. *The Lancet* 2001;357:933–4.
13. Mayer SA, Sacco RL, Hurllet-Jensen A, et al. Free protein S deficiency in acute ischemic stroke. A case-control study. *Stroke* 1993;24:224–7.
14. Limperger V, Klostermeier UC, Kenet G, et al. Clinical and laboratory characteristics of children with venous thromboembolism and protein C-deficiency: an observational Israeli-German cohort study. *Br J Haematol* 2014;167:385–93.
15. Heller C, Becker S, Scharrer I, et al. Prothrombotic risk factors in childhood stroke and venous thrombosis. *Eur J Pediatr* 1999;158 Suppl 3:S117–S121.
16. Martinez HR, Rangel-Guerra RA, Marfil LJ. Ischemic stroke due to deficiency of coagulation inhibitors. Report of 10 young adults. *Stroke* 1993;24:19–25.
17. Anzola GP, Magoni M, Ascari E, et al. Early prognostic factors in ischemic stroke. The role of protein C and protein S. *Stroke* 1993;24:1496–500.
18. D'Angelo A, Landi G, Viganò D'Angelo S, et al. Protein C in acute stroke. *Stroke* 1988;19:579–83.
19. Viganò S, Mannucci PM, D'Angelo A, et al. Protein C antigen is not an acute phase reactant and is often high in ischemic heart disease and diabetes. *Thromb Haemost* 1984;52:263–6.
20. Rodeghiero F, Tosetto A. The VITA Project: population-based distributions of protein C, antithrombin III, heparin-cofactor II and plasminogen—relationship with physiological variables and establishment of reference ranges. *Thromb Haemost* 1996;76:226–33.
21. Faioni EM, Boyer-Neumann C, Franchi F, et al. Another protein S functional assay is sensitive to resistance to activated protein C. *Thromb Haemost* 1994;72:648–51.
22. Takano K, Yamaguchi T, Uchida K. Markers of a hypercoagulable state following acute ischemic stroke. *Stroke* 1992;23:194–8.
23. Tohgi H, Kawashima M, Tamura K, et al. Coagulation-fibrinolysis abnormalities in acute and chronic phases of cerebral thrombosis and embolism. *Stroke* 1990;21:1663–7.
24. Bushnell CD, Goldstein LB. Diagnostic testing for coagulopathies in patients with ischemic stroke. *Stroke* 2000;31:3067–78.
25. Baglin T, Gray E, Greaves M, et al. Clinical guidelines for testing for heritable thrombophilia. *Br J Haematol* 2010;149:209–20.
26. Keeling D, Mackie I, Moore GW, et al. Guidelines on the investigation and management of antiphospholipid syndrome. *Br J Haematol* 2012;157:47–58.
27. Gómez-Puerta JA, Cervera R. Diagnosis and classification of the antiphospholipid syndrome. *J Autoimmun* 2014;48–49:20–5.
28. de Laat HB, Derksen RH, Urbanus RT, et al. beta2-glycoprotein I-dependent lupus anticoagulant highly correlates with thrombosis in the antiphospholipid syndrome. *Blood* 2004;104:3598–602.
29. Urbanus RT, Siegerink B, Roest M, et al. Antiphospholipid antibodies and risk of myocardial infarction and ischaemic stroke in young women in the RATIO study: a case-control study. *Lancet Neurol* 2009;8:998–1005.
30. Galli M, Luciani D, Bertolini G, et al. Anti-beta 2-glycoprotein I, antiprothrombin antibodies, and the risk of thrombosis in the antiphospholipid syndrome. *Blood* 2003;102:2717–23.
31. Galli M, Luciani D, Bertolini G, et al. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. *Blood* 2003;101:1827–32.
32. Pengo V, Ruffatti A, Legnani C, et al. Clinical course of high-risk patients diagnosed with antiphospholipid syndrome. *J Thromb Haemost* 2010;8:237–42.
33. Forastiero R, Martinuzzo M, Pombo G, et al. A prospective study of antibodies to beta2-glycoprotein I and prothrombin, and risk of thrombosis. *J Thromb Haemost* 2005;3:1231–8.
34. Crowley MP, Hunt BJ. Venous thromboembolism and thrombophilia testing. *Medicine* 2017;45:233–8.
35. Dahlbäck B, Villoutreix BO. The anticoagulant protein C pathway. *FEBS Lett* 2005;579:3310–6.
36. Macias WL, Yan SB, Williams MD, et al. New insights into the protein C pathway: potential implications for the biological activities of drotrecogin alfa (activated). *Crit Care* 2005;9(Suppl 4):S38.
37. De Stefano V, Chiusolo P, Paciaroni K, et al. Epidemiology of factor V Leiden: clinical implications. *Semin Thromb Hemost* 1998;24:367–79.
38. Hessner MJ, Luhm RA, Pearson SL, et al. Prevalence of prothrombin G20210A, factor V G1691A (Leiden), and methylenetetrahydrofolate reductase (MTHFR) C677T in seven different populations determined by multiplex allele-specific PCR. *Thromb Haemost* 1999;81:733–8.