COLLEGE of AMERICAN PATHOLOGISTS

Coagulation-Based Thrombophilia Testing for Protein C and Protein S Deficiency

SYNOPSIS AND RELEVANCE

Protein C (PC) and Protein S (PS) testing is particularly susceptible to clinical conditions that may lead to temporary reductions of levels and mistaken diagnoses of inherited deficiencies. These situations include increased consumption during acute illnesses and thrombotic states, liver disease causing coagulation factor hyposynthesis, vitamin K deficiency or antagonist (eg, warfarin), and, specific for PS, pregnancy. In addition, clot-based PC and PS activity tests are susceptible to interference from heparin, oral direct factor Xa inhibitors, and oral and parenteral direct thrombin inhibitors.

INSIGHTS

Who to test:

- Thrombophilia testing, including PC and PS, should not be performed for patients with very low risks of VTE recurrence (post-surgery and trauma) or at high risk of recurrence (spontaneous, unprovoked VTE).
- Clinical judgement should guide whether to perform thrombophilia testing in situations with less intense provoking factors, or venous thromboses in unusual locations (visceral or central nervous system).

When to test:

- PC and PS testing should not be performed during acute illnesses and thrombotic events, during treatment with warfarin and other anticoagulants, pregnancy, or when the PT/INR is elevated.
- PC and PS testing on outpatients while not anticoagulated reduces the likelihood of detecting misleading acquired deficiencies.

What tests to perform:

- Chromogenic PC activity assays maximize sensitivity to detect type I and type II deficiencies and avoid interference from LA and anticoagulants, except warfarin.
- Free PS antigen assays avoid interference from LA, Factor VIII, Factor V Leiden, and anticoagulants, except warfarin, but will not detect rare type II deficiencies.
- PS clot-based activity assays are vulnerable to numerous interferences that can cause false low or high results but could be used in settings of high clinical suspicion for type II deficiencies.

Test with limited clinical utility:

- PC antigen, total PS antigen, and PS/PC genotyping

Repeat testing:

- Any abnormally low PC and PS results should be confirmed by repeat testing under conditions that minimize risk of a temporary deficiency before diagnosing a patient with inherited PC and PS deficiency.

BACKGROUND

The consensus among hemostasis experts has evolved to limit the clinical circumstances warranting thrombophilia testing after a VTE.[1] Patients with surgery provoked VTEs are at very low risk of a recurrence and should not be tested. There is limited high quality evidence to guide
decisions regarding duration of anticoagulation therapy based on thrombophilia test results in patients with provoked VTEs from other transient risk factors such as pregnancy, combined hormonal contraception, and medical illnesses requiring hospitalization. Patients with unprovoked VTEs are at increased risk of recurrence regardless of thrombophilia test results, and they are candidates for indefinite anticoagulation.

Standard laboratory evaluation of selected patients for thrombophilia risk factors associated with VTE consists of a lupus anticoagulant (LA) panel, and anticardiolipin and B2GP1 IgG and IgM serologies for acquired risks, and a panel of five tests for inherited risks: antithrombin, activated PC resistance/Factor V Leiden, prothrombin gene mutation G20210A, PC, and PS.[1]

Hepatic synthesis of PC and PS is dependent upon the reduced form of vitamin K for post-translational gamma carboxylation to produce fully functional regulators of coagulation. Activated Protein C (aPC) degrades factors V_a and $VIII_a$ resulting in decreased generation of factor X_a and thrombin. The prevalence of inherited heterozygous deficiency of PC is approximately 0.2% in blood donors and about 3% in patients with VTEs.[2] Type I deficiencies (low PC activity and antigen) are much more common than Type II forms (decreased PC activity, normal antigen). The two types of deficiencies are indistinguishable in terms of clinical presentations and management.

Functional PC assays will identify both quantitative (type I) and qualitative (type II) deficiencies. There is rarely clinical value to following up a repeatedly low PC activity result with a PC antigen measurement to distinguish Type I from Type II deficiency. Clot-based PC activity assays, but not chromogenic PC activity assays, are vulnerable to

interference from anticoagulants and lupus anticoagulants. In a chromogenic assay, PC in a patient's plasma is activated with copperhead snake venom, and aPC cleaves a synthetic peptide releasing a chromogen and increasing light absorption (OD), which is calibrated with PC standards.[3]

About 60% of PS is bound to a complement regulatory protein C4b binding protein (C4bBP). The other 40% is free PS, which functions as a cofactor for aPC to accelerate breakdown of factors V_a and $VIII_a$. Estimates of heterozygous PS deficiency prevalence in the general population (0.013-0.3%) and in VTE patients (approximately 2%) vary widely due to clinical and analytical variability.[2]

Type I PS deficiencies (low activity, low free antigen, and low total antigen levels) dominate, and type II deficiencies (low activity, normal free antigen, and normal total antigen levels) are rare. Type III deficiency is unique to PS where total PS antigen is normal, while free PS antigen and PS activity are both low. At least 95% of PS deficiencies are Types I and III.[4] PS deficiency levels
associated with increased VTE risk are considerably lower (eg, less than 30%), than the lower limit of laboratory reference ranges, and are independent of whether a patient's deficiency is type I or type III.[6] Many laboratories do not offer total PS antigen testing due to the limited clinical utility.[2,7]

Clot-based PS activity methods can detect Type I and II inherited deficiencies. However, they are vulnerable to inaccurate results due to numerous interferences including from anticoagulants (positive bias), Factor V Leiden and elevated factor VIII (negative bias), and poor precision.[4,5]

Free PS (fPS) antigen methods are resistant to these interferences, but they do not detect rare type II deficiencies. Laboratory practices vary. The International Society on Thrombosis and Haemostasis recommends screening for PS deficiency with fPS antigen and reserving PS activity testing for when there is a high suspicion for a deficiency despite a normal fPS level.[7] Laboratory directors should consult with their clinical colleagues to determine a screening strategy for PS deficiency for their patients. Due to the limited clinical utility, routine testing for PS activity and total PS antigen should be discouraged.

There are additional clinical conditions that will cause temporary reductions in PC and PS that are not test method dependent. Detection of acquired deficiencies can be avoided by not testing during acute illnesses, compromised liver function, anticoagulation with warfarin, or vitamin K deficiency.[3] An elevated prothrombin time and international normalized ratio (PT/INR) indicates inhibition of coagulation factor synthesis or rapid consumption, and it is a clear warning that acquired deficiencies of PC and PS are likely. Ideally, testing should be deferred until the PT/INR normalizes. C4bBP is an acute phase reactant that increases in response to inflammation and during pregnancy, causing a shift to increased bound and inactive PS and decreased free and active PS. It is advisable not to perform PS testing for thrombophilia during pregnancy.[4]

Interpreting PC and PS results is challenging, and repeat testing is necessary to have confidence in initially abnormally low results.[3]

REFERENCES

1. Connors JM. Thrombophilia testing and venous thrombosis. *N Engl J Med*. 2017;377(12):1177-1187. doi:10.1056/NEJMra1700365
2. Middeldorp S. Inherited thrombophilia: a double-edged sword. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):1-9. doi:10.1182/asheducation-2016.1.1
3. Eby C. Laboratory diagnosis of inherited thrombophilia. In: Kottke-Marchant K. *Algorithmic Approach to Hemostasis Testing*. 2nd ed. CAP Press.2016:299.
4. Marlar RA, Gausman JN. Laboratory testing issues for protein C, protein S, and antithrombin. *Int J Lab Hematol*. 2014;36(3):289-295. doi:10.1111/ijlh.12219
5. Smock KJ, Plumhoff EA, Meijer P, et al. Protein S testing in patients with protein S deficiency, factor V Leiden, and rivaroxaban by North American specialized coagulation laboratories. *Thromb Haemost*. 2016;116(1):50-57. doi:10.1160/TH15-12-0918
6. Brouwer JL, Veeger NJ, van der Schaaf W, Kluin-Nelemans HC, van der Meer J. Difference in absolute risk of venous and arterial thrombosis between familial protein S deficiency type I and type III. Results from a family cohort study to assess the clinical impact of a laboratory test-based classification. *Br J Haematol*. 2005;128(5):703-710. doi:10.1111/j.1365-2141.2005.05371.x
7. Marlar RA, Gausman JN, Tsuda H, Rollins-Raval MA, Brinkman HJM. Recommendations for clinical and laboratory testing for protein S deficiency: Communication from the SSC committee plasma coagulation inhibitors of the ISTH. *J Thromb Haemost.* 2021;19(1):68-74. doi:10.1111/jth.15109