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

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Authors
Charles Eby, MD, FCAP*, Paul F. Lindholm, MD, FCAP
*Lead author, Washington University School of Medicine, St. Louis, MO

Editors
Richard W. Brown, MD, FCAP*, Ron B. Schifman, MD, FCAP, Barbara Blond, MBA, Thomas Long, MPH
*Lead editor, Memorial Hermann Health System, Houston, TX

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 hypothesis, 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.

Following an acute venous thromboembolism event (VTE), thrombophilia testing, especially for PC and PS, should be delayed to an outpatient setting when anticoagulation therapy can be safely interrupted. To minimize risks of false deficient results, abnormally low PC and PS levels should be confirmed by repeated testing. There is minimal clinical value in measuring PC antigen, total PS antigen, or genetic testing.

OBJECTIVES
As a result of participating in this activity, participants will be able to:
1. Appreciate the limited utility of thrombophilia testing for patients with post-surgical and unprovoked VTEs.
2. Recognize the pitfalls in measuring PC and PS in patients on warfarin and other anticoagulation therapies.
3. Identify acquired patient clinical factors that could explain temporary low PC and PS results.
4. Employ strategies to prevent unnecessary PC and PS testing.
5. Understand the correct application of functional and antigenic testing for PC and PS.
6. Recommend repeat testing to confirm initial abnormally low PC and PS results.

BACKGROUND
The consensus among hemostasis experts has evolved to limit the clinical circumstances warranting thrombophilia testing after a VTE. 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.

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 Vα and VIIIα resulting in decreased generation of factor Xα and thrombin. The prevalence of inherited heterozygous deficiency of PC is approximately 0.2% in blood donors and about 3% in patients with VTEs. 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.  

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 and VIII. 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.

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 II. 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.

Many laboratories do not offer total PS antigen testing due to the limited clinical utility.

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.

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. 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. 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.

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

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.

**INTERVENTIONS**
1. Consider revising the test menu for PC testing to one test: chromogenic PC activity.
2. Consult with local hematology subject experts to revise the test menu for PS testing: free PS antigen is the preferred first line test.
3. Consider a reflex panel in which a normal PT/INR is required before proceeding with ordered PC and PS testing.
4. Consider flagging all abnormal PC and PS results for clinical review with feedback to the ordering provider about possible erroneous (abnormally low) results if concurrent warfarin therapy, other anticoagulants, acute medical conditions, or a prolonged PT/INR, and recommend retesting later, as an outpatient ideally off of anticoagulation therapy.
5. Consider adding a comment to all abnormal PC and PS results to warn about the effect of warfarin or other anticoagulants, and the effect of an active clotting event or an elevated PT/INR.
6. Consider developing a clinical guideline that discourages ordering of PC and PS on inpatients due to the potential for misleading results.
7. Consider providing retrospective feedback to providers who order PC and PS tests in patients who are on oral anticoagulation, have elevated PT/INRs, or tested during an acute clotting event.
8. Clot-based PC and PS assays should be avoided for patients who are receiving direct thrombin inhibitors and direct Xa inhibitors, as these treatments can falsely elevate PC and PS activities.

**QUESTIONS AND ANSWERS**

**QUESTION 1 OBJECTIVE**
Recognize the pitfalls in measuring PC and PS in patients on warfarin.

**QUESTION 1**
Testing PC and PS levels in a patient taking warfarin should be avoided because:

A. The level may be decreased but not represent an underlying deficiency.
B. The warfarin may interfere with the assay through competitive binding.
C. Warfarin levels fluctuate based on timing, dosage, and metabolism.
D. Patients on warfarin bruise easily following venipuncture.

**The correct answer is A.** The PC and PS levels may be reduced due to warfarin effect and not because of any underlying deficiency, thus making the patient potentially falsely appear to be PC and PS deficient.

**B is incorrect.** Warfarin does not impact the assay through competitive binding.

**C is incorrect.** Warfarin levels do fluctuate based on timing dosage and metabolism, but this variability is not the major reason to avoid PC and PS testing in this setting.

**D is incorrect.** Patients on warfarin may bruise easily but bruising is not a contraindication for PC and PS testing.

**REFERENCES**
https://labtestsonline.org/tests/protein-c-and-protein-s

**QUESTION 2 OBJECTIVE**
Understand the application of functional or antigenic testing for PC and PS.

**QUESTION 2**
Performing a functional test for PC rather than an antigenic test can be a better strategy because the PC functional assay:
A. Is less expensive.
B. Is not affected by warfarin.
C. Can screen for both type I and type II PC deficiency.
D. Can differentiate thrombophilia due to intrinsic pathway defects from cases due to extrinsic.

The correct answer is C. This is an advantage of the functional assay. It should be abnormal in either type of deficiency.

A is incorrect. This may be true in some settings but is not the major reason to choose a functional test.
B is incorrect. Warfarin may impact either test.
D is incorrect. The two tests relate to aspects of PC and would not differentiate between intrinsic and extrinsic pathway defects.

REFERENCE

QUESTION 3 OBJECTIVE
Employ strategies for limiting unnecessary PC and PS testing in patients on warfarin.

QUESTION 3
Strategies for improving evaluation of thrombophilia would include:
A. Removing PC and PS assays from your laboratory’s test menu.
B. Reducing the price of testing by 20%.
C. Evaluating the PT/INR prior to performing testing.
D. Perform testing in triplicate to reduce error.

The correct answer is C. An elevated PT/INR could be the result of warfarin and would signal a potential problem with subsequent testing.
A is incorrect. PC and PS can be very helpful in the evaluation of thrombophilia.
B is incorrect. While this may seem attractive to whoever pays for the tests, this would not improve evaluation of thrombophilia.
D is incorrect. While testing in triplicate could result in more accurate results that would not necessarily improve the evaluation of thrombophilia.

REFERENCE
https://labtestsonline.org/tests/protein-c-and-protein-s

MODULE REFERENCES AND SUGGESTED READINGS