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Cost-Effective Use of the Protein S Algorithm in Thrombophilia Testing

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REVIEW

Cost-Effective Use of the Protein S Algorithm in Thrombophilia Testing

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Background: One of the most complex risk factors for the laboratory assessment of thrombophilia is Protein S (PS). The testing algorithm for PS employs the plasma-based assays of free PS antigen, total PS antigen, and PS activity creating a complex diagnostic scheme that can lead to misdiagnosis if incorrectly used, and a potential waste of resources and money.

Content: This paper compares the recently published evidence-based algorithm from the International Society for Hemostasis and Thrombosis (ISTH) with several commonly performed nonevidence-based testing schemes, to demonstrate the efficiency of the evidence-based algorithm for diagnostic efficiency with improved patient care and increased cost savings for the laboratory.

Summary: Significant savings (31%–60%) can be realized when the evidence-based algorithm is used in place of other testing modalities of initial PS activity testing (31%) or testing with all 3 assays simultaneously (60%). This study utilizing the PS testing evidence-based algorithm as part of a thrombophilia evaluation demonstrates that the appropriate testing methods can be used to limit wasteful practices while achieving the maximum level of information in this time of limited resources and need for increase monetary savings.

IMPACT STATEMENT

The laboratory assessment of thrombophilia can be a challenging due to several factors ranging from preanalytical variables to complex diagnostic schemes. Testing for Protein S (PS) deficiency employs the plasma-based assays of free PS antigen, total PS antigen, and PS activity creating a complex diagnostic scheme that can lead to misdiagnosis if incorrectly used and a potential waste of resources and money. This review shows that using an evidence-based algorithm as part of a thrombophilia evaluation demonstrates that the appropriate testing methods can be used to limit wasteful practices in this time of limited resources while ensuring patient care.

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BACKGROUND

Thrombophilia is a complex disease usually resulting in venous thromboembolism (VTE) (1). As a complex disease, the cause has been associated with a combination of both genetic and acquired and/or environmental risk factors (1, 2). The current standard of practice for the laboratory assessment of the primary genetic risk factors associated with thrombophilia is the 5 major genetic abnormalities that cause significant disease expression (1, 2). Two common but lower risk factors are specific point mutations (Factor V Leiden and Prothrombin UT-20210), while the remaining 3 factors are genetic abnormalities associated with plasma regulatory components: Antithrombin (AT), Protein C (PC), and Protein S (PS) (2–5). Criteria, methods, and testing algorithms for the evaluation of these plasma thrombophilic factors have been developed and presented for optimal risk assessment (3–5). One of the most complex risk factors evaluated in the assessment of thrombophilia is PS (5, 6). PS plays a prominent role in the regulation of clot formation, and as such, decreases in levels of plasma PS are associated with an increased risk of VTE (5, 6). To assess the role of PS in increased thrombotic risk, plasma PS levels are determined during the laboratory evaluation of thrombophilia (5).

Recently, evidence-based recommendations for laboratory testing algorithms for these specific plasma genetic risk factors have been published for the assessment of thrombophilic abnormalities, most recently from the International Society of Hemostasis and Thrombosis (ISTH) (3–5). These algorithms were developed to increase the likelihood of obtaining accurate plasma levels while minimizing delays in patient care to correct diagnosis and increasing cost efficiency. Because of the complex nature of PS in plasma, the PS testing algorithm is multifaceted, resulting in misdiagnosis and monetary and resource loss if incorrectly used (5). This paper evaluates cost efficiency of using the published evidence-based algorithm compared to other commonly used nonevidence-based testing paradigms.

Mechanism of Action of Protein S

PS is a vitamin K-dependent glycoprotein, but in contrast to most of the other vitamin K-dependent plasma proteins, it is not a serine protease enzyme, rather functioning as a cofactor in the protein C regulatory system and the Tissue Factor Pathway Inhibitor (TFPI) mechanism [for a brief review see ref. (6)]. Hepatocytes and endothelial cells are the major synthetic sources of plasma PS but it is also stored in the endothelial cell and the alpha granules of platelets (5, 6). Plasma PS circulates as an unbound fraction (in normal individuals at approximately 40% and termed free PS) and a bound fraction (approximately 60% is complexed to complement protein, C4b binding protein) (5–7). PS has 2 main antithrombotic mechanisms: its apparent main mechanism is a cofactor for Activated Protein C (APC)-dependent anticoagulant activity (inactivation of factors Va and VIIIa) (5–7). The other important function is to serve as a cofactor for enhancing factor VIIa and factor Xa inhibition by TFPI (6). Both of these mechanisms require free PS.

Laboratory Tests for Protein S

To assess plasma levels of PS, 3 assay types have been developed (2, 5, 7). Each assay has individual drawbacks that can potentially cause erroneous results. Therefore, PS levels must be interpreted carefully to allow for an accurate diagnosis of a true PS deficiency (5, 7). Analytical and mainly preanalytical variables can have detrimental effects on each type of PS assay (5, 7–10).

Free PS antigen assay (free PS Ag) is an immunologic measure of the unbound PS fraction using a monoclonal antibody based-test method [the most common ones are automated Latex

Immunologic Assays (LIA)] (5, 7, 11, 12). This test is used as a surrogate marker for PS function (activity). PS activity assay is a clotting-based assay that relies on the ability of PS to function as a cofactor for APC (5, 7, 11–14). This assay has been reported to be difficult to perform properly and fraught with the numerous technical problems (11–13). Total PS antigen assay is an immunological assay (usually by LIA or ELISA methodologies) to determine both the free and bound fractions (total) of PS in plasma (5, 7, 11, 14, 15).

Clinical Aspects of Protein S Deficiency

PS plays a role in many physiological conditions where altered states of plasma PS levels are found (5, 7). In addition, improper preparation of plasma specimens for testing can also generate lower, erroneous results (5, 7, 13). Therefore, assessment of the patient's status and the sample itself requires awareness during the testing process, as well as interpretation of results (5, 7, 13). Acquired clinical conditions can lead to abnormal or erroneous PS results, making diagnosis of a genetic deficiency more difficult [see reference (5) for a list]. In addition, some PS assays can have interference from plasma-based substances, drugs, or certain physiological or pathological conditions (5, 7, 13). All of these clinical conditions and issues must be resolved before completing testing and/or interpretation.

The classification of the types of PS deficiencies is based only on PS ability to regulate the APC-dependent anticoagulant properties (5–7, 13). Type I deficiency is the classical quantitative deficiency due to a significant gene defect resulting in decreased synthesis and low PS levels in plasma. The levels of PS activity, as well as free and total PS antigen, are all decreased by approximately 50% in these heterozygous individuals (5, 7). The Type I deficiency of PS accounts for about 85% all PS deficiencies. Type II deficiency is the classical qualitative deficiency associated with a loss of function mutation (5, 7). In individuals with a Type II PS deficiency, the gene mutation is usually a point mutation resulting in a circulating dysfunctional protein, bringing about a decrease in PS activity with normal free and total PS antigen levels. These individuals account for about 1%–2% of all PS deficiencies (1, 7, 16). Type III deficiency is also a quantitative deficiency uniquely caused by an altered distribution in binding between free PS and C4BP-bound PS (5, 7). The cause of this type of deficiency and differentiation between Types I and III remains uncertain (5). However, it has been shown that in some families and patients, they can change from Type III to Type I (5). The percentage of patients with Type III PS deficiency constitute about 15% of all patients with a PS deficiency.

The final diagnosis of PS deficiency is a plasma PS antigen and/or activity level below the appropriate reference interval (RI) on 2 or more occasions at least 4 weeks apart (5, 7). The type of PS deficiency is determined after the relevant PS assays have been performed and repeated. (5). There does not appear to be a difference in the clinical phenotype (clinical presentation) between the different PS types (5, 6), nor a difference in the treatment method and duration for the 3 different types of PS deficiency (5, 6).

ISTH Proposed Algorithm for PS Evaluation (5)

The current ISTH recommended algorithm for PS evaluation is based on evidence from studies published over the last 10 years using epidemiological studies and clinical laboratory testing research to develop the algorithm presented by the subcommittee on Plasma Coagulation Inhibitors of the ISTH (Fig. 1). The algorithm is a multistep process and summarized as follows:

Prior to all testing, the ordering provider and laboratory technical personnel must assess the patient and the sample for causes of acquired deficiencies, interfering substances, and preanalytical integrity. In this algorithm, the first PS assay to

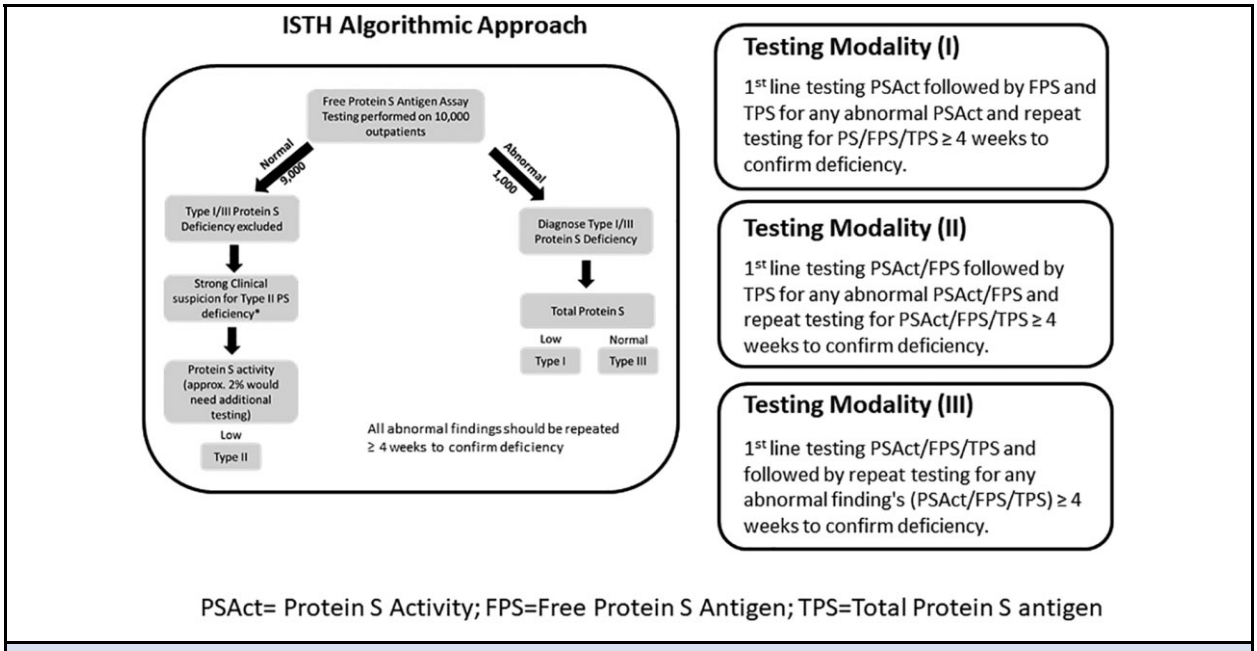

Fig. 1. Approaches to testing Protein S (PS).

be performed is free PS antigen assay (FPS Ag), the surrogate marker of PS activity. If the result is within the RI, then both Types I and III have been excluded; if there is a strong suspicion of a qualitative defect (Type II), then a PS activity (PS Act) assay is performed. If the PS Act is normal, then all 3 types of PS deficiency are excluded. If the PS Act is abnormal, then a Type II is present. If the FPS Ag assay is abnormal, a total PS antigen assay (TPS Ag) could be performed to distinguish between Type I (decreased total PS antigen) and Type III (normal total PS antigen). All tentative deficiencies must be confirmed by repeating with a new sample after a minimum of 4 weeks to confirm a PS deficiency.

CONTENT

Cost–Benefit Analysis of the ISTH Algorithmic Protocol

This new testing algorithm should reduce delays in correct diagnosis and/or misclassification of PS deficiency leading to potential adverse patient outcomes. In addition, this algorithm has the potential to improve laboratory efficiency and decrease costs associated with PS testing, including a significant cost savings by employing less reflex and repeat testing (13).

For these model comparisons, we chose 10 000 patients as a starting number. While FPS Ag will not detect the rare Type II defect, the incidence of these types of defects has been reported to be from 5% to <1% of all genetic mutations of PS deficiencies (16, 17). For the purpose of our model comparison, we assumed 2% of all cases would be Type II PS deficiencies and require further testing for PS activity based on the clinical suspicion. As PS deficiency has been reported in 1%–15% of patients with VTE (18), we assumed that 10% of patients tested would be abnormal, leading to additional testing. Since PS Act assay have a false positive rate of 10%–15%, which, when added to the true positives (assumed 12%), would result in at least a 20% abnormal rate for PS Act.

Table 1. Cost breakdown of the ISTH evidence-based algorithmic approach to Protein S (PS) testing[a].

		Outpatients (*n*)	Cost/test	Cost ($)
Type I/III PS deficiency	Initial workup			
	FPS	10 000	$20.00	200 000
	TPS	1000	$15.00	15 000
	Repeated testing			
	FPS	1000	$20.00	20 000
	TPS	1000	$15.00	15 000
Type II PS deficiency	Initial workup			
	PSAct	200	$20.00	4000
	Repeated testing			
	FPS	200	$20.00	4000
	PSAct	200	$20.00	4000
Total cost ($)				262 000 ($26.20/patient)

Abbreviations: Free PS antigen (FPS), Total PS antigen (TPS), and PS activity (PSAct).
[a]Assume that 10% of the FPS would be abnormal and 2% of the normal FPS would require additional testing based on clinical suspicion of Type II PS deficiency.

The assumptions for test cost are based on reagent cost, cost of full time equivalent technical staff, and other costs associated with laboratory testing (e.g., quality control, proficiency testing, administrative cost, overhead costs, etc.), we queried several laboratories for the "research" cost for these assays. Although costs varied among laboratories, we estimated the cost to perform either PSAct or FPS Ag assays to be $20/test each and TPS Ag to be $15/test.

We evaluated several different nonevidence-based testing algorithms, either "advertised" or performed by different laboratories, as the method for PS comparison with the proposed ISTH algorithmic method (Fig. 1). Table 1 shows the cost breakdown of the ISTH evidence-based algorithmic approach. Using the ISTH algorithm approach, each patient would receive the initial FPS Ag assay (total cost of $200 000) with 1000 of those patients having an abnormal result, which would be followed up with a TPS Ag assay (cost $15 000) to differentiate between Types I and III. Each of those 1000 patients would be repeated after 4 weeks with both tests to confirm PS deficiency and type (total cost for initial and repeat testing is $250 000). For those patients with suspected of Type II PS deficiency (2% or 200 patients), a PS Act assay would be performed at a cost of $4000 and both the FPS Ag and PS Act assays would be repeated after 4 weeks for a total cost of $12 000 for the evaluation of Type II PS deficiency. The total cost for testing for PS using the ISTH evidence-based algorithm is $262 000 (or $26.20/patient).

Tables 2–4 show the cost breakdown of 3 other testing algorithms currently being performed by clinical laboratories (testing modalities I, II, and III). In testing modality I (Table 2), the PS Act assay is the initial test performed on the 10 000 patients. With the high rate of falsely abnormal test results with the PS Act assay, the initial number of "confirmation" tests (FPS Ag and TPS Ag) would be 2000 patients for an additional $70 000. The required 4 weeks confirmation of PS deficiency

Table 2. Cost breakdown of testing modality I for Protein S.

	Outpatients (*n*)	Cost/test	Cost ($)	Cost savings[b]/10 000 patients (%)
Testing modality (I)[a]				
Initial workup				
PSAct	10 000	$20.00	200 000	
FPS	2000	$20.00	40 000	
TPS	2000	$15.00	30 000	
Repeated testing				
PSAct	2000	$20.00	40 000	
FPS	2000	$20.00	40 000	
TPS	2000	$15.00	30 000	
Total cost			380 000 ($38.00/patient)	$118 000 (−31%)

Abbreviations: Free PS antigen (FPS), Total PS antigen (TPS), and PS activity (PSAct).
[a] PSAct testing initially and assume that 20% of the PS would be abnormal and require additional workup.
[b] Cost savings compared to the cost breakdown of the ISTH evidence-based algorithmic approach.

Table 3. Cost breakdown of testing modality II for Protein S (PS).

	Outpatients (*n*)	Cost/test	Cost ($)	Cost savings[b]/10 000 patients (%)
Testing modality (II)[a]				
Initial workup				
PSAct	10 000	$20.00	200 000	
FPS	10 000	$20.00	200 000	
TPS	2000	$15.00	30 000	
Repeated testing				
PSAct	2000	$20.00	40 000	
FPS	2000	$20.00	40 000	
TPS	2000	$15.00	30 000	
Total cost			540 000 ($54.00/patient)	$278 000 (−51%)

Abbreviations: Free PS antigen (FPS), Total PS antigen (TPS), and PS activity (PSAct).
[a] Testing both PSAct & FPS assume that 20% would be abnormal and require additional workup.
[b] Cost savings as comparted to the cost breakdown of the ISTH evidence-based algorithmic approach.

would be an additional $110 000 for all 3 PS assays (PS Act, FPS Ag, and TPS Ag). The total testing cost with this testing modality would be $380 000 for 10 000 patients (or $38/patient).

In testing modality II (Table 3), both PS Act and FPS Ag are initially performed to determine all 3 of the deficiency types (Types I, II, and III). Those 2000 patients with abnormal results would then be tested for TPS Ag to differentiate between Types I and III. The total cost for this initial screening algorithm would come to $430 000. Confirmation of PS deficiency at 4 weeks would require all 3 tests (PS Act, FPS Ag, and TPS Ag) to be repeated for an additional cost of $110 000. The total testing cost with testing modality II would be $540 000 for the 10 000 patients (or $54/patient).

Table 4. Cost breakdown of testing modality III for Protein S (PS).

	Outpatients (*n*)	Cost/test	Cost ($)	Cost savings[b]/10 000 patients (%)
Testing modality (III)[a]				
Initial workup				
PSAct	10 000	$20.00	200 000	
FPS	10 000	$20.00	200 000	
TPS	10 000	$15.00	200 000	
Repeat testing				
PSAct	2000	$20.00	40 000	
FPS	2000	$20.00	40 000	
TPS	2000	$15.00	30 000	
Total cost			660 000($66.00/patient)	$398 000 (−60%)

Abbreviations: Free PS antigen (FPS), Total PS antigen (TPS), and PS activity (PSAct).
[a] PSAct/FPS/TPS testing performed assume that 20% of the would be abnormal and require additional workup.
[b] Cost savings comparted to the cost breakdown of the ISTH evidence-based algorithmic approach.

In testing modality III (Table 4), all 3 PS assays (PS Act, FPS Ag, and TPS Ag) are performed at the initial testing for an initial cost of $600 000, followed by repeat testing of the 2000 initially abnormal test results, increasing this test modality cost to a total of $660 000 for the 10 000 patients (or $66/patient).

Cost Comparison

The recommended evidence-based algorithmic approach for PS testing to determine PS deficiency would cost the laboratory an estimated 31% less than testing with modality I (the PS activity assay first protocol). The ISTH algorithm would cost 51% less than testing with modality II (the free PS antigen and PS activity assays protocol). Finally, a 60% savings in testing with the ISTH algorithm compared to modality III (testing all 3 tests initially). Overall, using the evidence-based algorithm does lead to significant cost savings for this initial, reflex, and repeat testing scheme.

SUMMARY

Accurate diagnosis in PS deficiency has long been a challenging field. While any of the algorithms discussed here may ultimately lead to the correct diagnosis, the new ISTH testing algorithm afford diagnostic efficiency with both improved patient care (shorter time to diagnosis) and decreased overall cost. The former is particularly relevant given the substantial number of false positive results observed with the PS activity assays (10%–15% of normal donors), which may persist even on confirmation, potentially leading to misdiagnosis and/or a delay in appropriate patient care. However, the ISTH algorithm does acknowledge that some Type II patients (<1%–5%) may be missed, if clinical suspicion is low (7, 8). In addition to improved patient care for PS deficiency, this model of the evidence-based ISTH algorithm supports significant cost savings to laboratories performing this testing scheme. The exact amount saved will vary depending on volume, ordering practices, cost per test, and demographics of the population. As many laboratories do not perform all 3 assays, additional savings

could be seen with reduction in the number of referral tests. However, the challenging logistics of specimen management and aliquoting of samples should also be considered. Multiple tests may need to be performed at different times on the same sample, which is even more onerous for laboratories performing some, but not all, of the assays and sending out the remaining sample for referral testing. Finally, another cost saving step, while not specifically recommended by the ISTH, their algorithm does allow for laboratories and/or clinicians to choose not to order a total PS antigen assay to distinguish between Types III and I as this information will not significantly change therapy for the patient.

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REFERENCES

1. Lijfering WM, Brouwer JL, Veeger NJ, Bank I, Coppens M, Middeldorp S, et al. Selective testing for thrombophilia in patients with first venous thrombosis: results from a retrospective family cohort study on absolute thrombotic risk for currently known thrombophilic defects in 2479 relatives. Blood 2009;113:5314–22.
2. Marlar RA, Gausman JN. Laboratory testing issues for protein C, protein S, and antithrombin. Int J Lab Hematol 2014;36:289–95.
3. Van Cott EM, Orlando C, Moore GW, Cooper PC, Meijer P, Marlar RA; Subcommittee on Plasma Coagulation Inhibitors. Recommendations for clinical laboratory testing for antithrombin deficiency; communication from the SSC of the ISTH. J Thromb Haemost 2020;18: 17–22.
4. Cooper PC, Pavlova A, Moore GW, Hickey KP, Marlar RA. Recommendations for clinical laboratory testing for protein C deficiency, for the subcommittee on plasma coagulation inhibitors of the ISTH. J Thromb Haemost 2020;18:271–7.
5. Marlar RA, Gausman JN, Tsuda H, Rollins-Raval MA, Brinkman HJM. Recommendations for clinical laboratory testing for protein S deficiency: communication from the SSC committee plasma coagulation inhibitors of the ISTH. J Thromb Haemost 2021;19:68–74.
6. Brinkman HJM, Ahnstrom J, Castoldi E, Dahlback B, Marlar RA. Pleiotropic anticoagulant functions of protein S, consequences for the clinical laboratory. Communication from the SSC of the ISTH. J Thromb Haemost 2021;19: 281–6.
7. Marlar RA, Gausman JN. Protein S abnormalities: a diagnostic nightmare. Am J Hematol 2011;86:418–21.
8. Gosselin RC, Marlar RA. Preanalytical variables in coagulation testing: setting the stage for accurate results. Semin Thromb Hemost 2019;45: 433–48.
9. Marlar RA, Rollins-Raval MA. Sources and solutions for spurious test results in coagulation. Int J Lab Hematol 2019;41(Suppl 1):162–9.
10. Adcock DM, Hefner DM, Kottke-Marchant K, Marlar RA, Szamosi DI, Warunek DJ. Clinical and Laboratory Standards Institute (CLSI) Collection, transport and processing of specimens for testing plasma-based coagulation assays and molecular hemostasis assays. Approved Guideline, 5th Ed. CLSI; 2008: H21A5.
11. Duebgen S, Kauke T, Marschall C, Giebl A, Lison S, Hart C, et al. Genotype and laboratory and clinical phenotypes of protein S deficiency. Am J Clin Pathol 2012;137:178–84.
12. Johnston AM, Aboud M, Morel-Kopp MC, Coyle L, Ward CM. Use of a functional assay to diagnose protein S deficiency; inappropriate testing yields equivocal results. Intern Med J 2007;37:409–11.
13. Marlar RA, Potts RM, Welsh C. Accuracy of diagnosis of protein S deficiency by protein S activity and antigen assays. J Clin Ligand Assay 2005;28:130–5.

14. Ten Kate MK, Platteel M, Mulder R, Terpstra P, Nicolaes GA, Reitsma PH, et al. PROS1 analysis in 87 pedigrees with hereditary protein S deficiency demonstrates striking genotype-phenotype associations. Hum Mutat 2008;29:939–47.
15. Zoller B, Garcia de Frutos P, Dahlback B. Evaluation of the relationship between protein S and C4b-binding protein isoforms in hereditary protein S deficiency demonstrating type I and type III deficiencies to be phenotypic variants of the same genetic disease. Blood 1995;85:3524–31.
16. Inherited thrombophilia: memorandum from a joint WHO/International Society on Thrombosis and Haemostasis meeting. Bull World Health Organ 1997;75: 177–89.
17. Castoldi E, Hackeng TM. Regulation of coagulation by protein S. Curr Opin Hematol 2008;15:529–36.
18. Melissari E, Monte G, Lindo VS, Pemberton KD, Wilson NV, Edmondson R, et al. Congenital thrombophilia among patients with venous thromboembolism. Blood Coagul Fibrinolysis 1992;3:749–58.