# EDITORIAL

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# Protein C and Protein S Deficiency – Practical Diagnostic Issues

## Niedobór białka C i białka S – praktyczne problemy diagnostyczne

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## Abstract

Protein C (PC) and protein S (PS) are vitamin K-dependent glycoproteins, that act as natural anticoagulants. The proteolytic activation of PC by thrombin occurs on the surface of endothelial cells and involves thrombomodulin and endothelial PC receptor. In the presence of PS, phospholipids and calcium, activated PC (APC) inactivates membrane bound factor V (FVa) and FVIIIa by their cleavage at the specific arginine residues. PC and PS deficiencies are inherited as autosomal dominant disorders associated with recurrent venous thromboembolism (VTE) and, in most cases, derived from heterozygous missense mutations (78% and 63%, respectively). Heterozygous PC deficiency is found in 6% of families with inherited thrombophilia, in 3% of patients with a first-time deep vein thrombosis (DVT) and in 0.2–0.3% of healthy individuals. The PS deficiency is detected more commonly than PC deficiency and its prevalence has been estimated with a less than 0.5% in the general European population and 2% to 12% of selected groups of thrombophilic patients. Approximately 75% of PC-deficient patients have type I deficiency and 95% of PS-deficient patients develop type I and type III of PS deficiency. The diagnosis of PC and PS deficiencies is challenging due to many preanalytical and analytical factors affecting the PC/PS levels. Molecular analysis of the PC and PS genes (*PROC* and *PROS1*, respectively) involves direct gene sequencing and if negative, multiplex ligation-dependent probe amplification (MLPA) method is performed. Patients with low PC and PS levels and the known mutation within *PROC* or *PROS1* genes combined with other genetic or environmental thrombosis factors are at increased risk of recurrent thromboembolic events and require lifelong oral anticoagulation (**Adv Clin Exp Med 2013, 22, 4, 459–467**).

**Key words:** protein C, protein S, deficiency, venous thromboembolism, mutation.

## Streszczenie

Białko C (*protein C, PC*) i białko S (*protein S, PS*) to zależne od witaminy K glikoproteiny będące naturalnymi inhibitorami krzepnięcia krwi. Proteolityczna aktywacja PC przez trombinę zachodzi na powierzchni komórek śródbłonka w obecności trombomoduliny i śródbłonkowego receptora PC. Aktywne PC (APC) z udziałem kofaktora, którym jest PS, w obecności fosfolipidów i wapnia, degraduje czynnik Va i FVIIIa przez ich cięcie w specyficznych resztach argininowych. Niedobory PC i PS są podłożem występowania i nawrotów żylnej choroby zakrzepowo-zatorowej (ż.ch.z.z.). Niedobory PC/PS są dziedziczone w sposób autosomalny dominujący i w większości przypadków pochodzą z heterozygotycznych mutacji typu zmiany sensu (odpowiednio: 78 i 63%). Heterozygotyczny niedobór PC występuje u 6% rodzin z trombofilią, u 3% pacjentów, z pierwszym epizodem zakrzepicy żył głębokich oraz u 0,2–0,3% osób zdrowych. Niedobór PS pojawia się częściej niż niedobór PC, a jego występowanie szacuje się na mniej niż 0,5% w zdrowej populacji europejskiej i 2–12% u pacjentów z zakrzepicą. U około 75% pacjentów z niedoborem PC występuje niedobór typu I, a u 95% pacjentów z niedoborem PS rozwija się typ I i typ III niedoboru. Diagnostyka niedoborów PC i PS jest trudna ze względu na występowanie wielu czynników fazy przed- i analitycznej wpływających na stężenia PC i PS. Analiza molekularna genów PC i PS (odpowiednio: *PROC* i *PROS1*) obejmuje ich bezpośrednie sekwencjonowanie genów, a w przypadku wyniku negatywnego stosuje się metodę multipleksowej amplifikacji sondy zależnej od ligacji (*multiplex ligation-dependent probe amplification, MLPA*). Pacjenci z małymi stężeniami PC i PS oraz mutacją w genach *PROC* lub *PROS1* w połączeniu z innymi genetycz-

nymi i środowiskowymi czynnikami sprzyjającymi zakrzepicy są narażeni na zwiększone ryzyko nawracających epizodów zakrzepowo-zatorowych, co jest wskazaniem do stosowania przewlekłej antykoagulacji (**Adv Clin Exp Med 2013, 22, 4, 459–467**).

**Słowa kluczowe:** białko C, białko S, niedobór, zakrzepica żylna, mutacja.

Protein C (PC) and protein S (PS) are vitamin K-dependent glycoproteins that act as natural anticoagulants. Basic characteristics of PC ad PS are summarized in Table 1.

## Role of Protein C and Protein S in Human Physiology

PC is a precursor of the serine protease, activated protein C (APC). Its proteolytic activation by thrombin occurs on the surface of endothelial cells and involves thrombomodulin and endothelial PC receptor (EPCR). PC activation is enhanced approximately 20-fold *in vivo* when PC is bound to the EPCR [1]. In the presence of PS, phospholipids and calcium, APC inactivates membrane bound activated factor (F)V and FVIII by their cleavage at the specific arginine residues [2]. Only free PS that constitutes 40% of the total protein amount possesses APC cofactor activity [3].

Protein S also exerts a direct APC-independent inhibitory effect on the prothrombinase complex by binding to FXa and to FVa, and thus results in impaired prothrombin activation [4]. In addition, PS stimulates tissue factor pathway inhibitor (TFPI) in the inactivation of FXa [5].

Growing evidence indicates that PC, APC and PS, besides their known anticoagulant properties, have multiple actions such as anti-apoptotic and anti-inflammatory activities, regulation of gene expression and stabilization of endothelial barrier protection [6, 7]. This activity appears to be mediated by two key receptors, protease activated receptor 1 (PAR1) and EPCR [6, 8]. Cytoprotective signaling induced by APC can be observed not only in the endothelium but also in many other cell types therein neurons, which may be a very promising tool for providing neuroprotective acute and chronic therapies [9].

## PC and PS Deficiencies

Low levels of PC were first described by Griffin et al. in 1981 [10]. PS deficiency was first reported in 1984 by Comp and Esmon [11]. Both deficiencies were associated with recurrent venous thromboembolism (VTE). PC and PS deficiencies are inherited as autosomal dominant disorders and, in most cases, derived from heterozygous mutations.

Heterozygous PC deficiency is found in 6% of families with inherited thrombophilia, in 3% of patients with a first-time deep vein thrombosis (DVT) and in 0.2–0.3% of healthy individuals [12–14]. PC/PS homozygosity or compound heterozygosity is extremely rare (1/4 million of living infants) and results in neonatal purpura fulminans (PF) and disseminated intravascular coagulation (DIC) presented within hours of birth [15, 16].

PC deficiency is classified as type I (quantitative defects of PC: low antigen levels, reduced activity) and type II (qualitative defects: IIa, normal antigen concentrations, reduced activity in both amidolytic and clotting functional assays, and IIb, normal antigen concentrations, reduced clotting activity) [12, 17]. Approximately 75% of PC-deficient patients have type I deficiency and 95% of the remainding have type IIa deficiency, but type IIb is very rare [18].

The PS deficiency prevalence has been estimated with a less than 0.5% in the general European population and 2% to 12% of selected groups of thrombophilic patients [19]. Currently, PS deficiency is detected more commonly than PC deficiency in the general population and among patients with VTE.

PS deficiency is classified as type I (low total and free antigen, reduced activity), type II (normal total and free antigen, reduced activity) and type III (normal total antigen, reduced free antigen and activity) [12, 20]. Type I and type III deficiencies account for 95% of cases of PS deficiency.

Acquired PC and PS deficiency can develop with vitamin K deficiency, liver disease, treatment with vitamin K antagonists, severe and chronic inflammation, autoimmune syndromes, nephritic syndrome, or DIC [14, 21]. Of note, PC and PS deficiency cannot be reliably measured in patients receiving warfarin or acenocoumarol. Importantly, PS deficiency, but not PC deficiency, is commonly observed during pregnancy starting from the first weeks. Moreover, low PS levels can be found in patients with AIDS [22] and acute varicella infection [23].

**Table 1.** Main characteristics of protein C (PC) and protein S (PS) [3, 14, 18, 26]

**Tabela 1.** Charakterystyka białka C (PC) i białka S (PS) [3, 14, 18, 26]

| | PC | PS |
|---|---|---|
| History (Historia odkrycia) | isolated from bovine plasma by Johan Stenflo in 1976 and named protein C because it was the third protein to elute from DEAE-Sepharose | described by Di Scipio in 1977, named protein S in reference to its isolation and characterization in Seattle |
| Gene characterization (Charakterystyka genu) | gene *PROC* located on chromosome 2, position 2q13-q14, spans 11kb long including 9 exons and 8 introns and encodes a 461 amino acids protein | active gene *PROS1* (or PSα) and transcriptionally inactive pseudogene *PROS2* (or PSα) both located on chromosome 3, position 3p11.1-3q11.2 and share approximately 97% similarity; *PROS1* gene spans 80 kb long including 15 exons and 14 introns and encodes a 672 amino acid protein |
| Synthesis (Synteza) | hepatocytes, endothelial cells, mouse renal tubular cells | hepatocytes, endothelial cells, human testis Leydig cells, vascular smooth muscle cells and megakaryocytes |
| Molecular weight (Masa cząsteczkowa) | 62 kDa | 71 kDa |
| Protein (Białko) | the zymogenic form of plasma PC is activated by thrombin in the presence of thrombomodulin and endothelial protein C receptor leding to activated PC (APC) generation | 60% bound to C4bBP-β chain (inactive), 40% free PS (physiologically active), together total PS |
| Concentration in plasma (Stężenie w osoczu) | 3–5 μg/ml | 20–25 μg/ml |
| Half-life (Okres półtrwania) | 6–8 hours | 42 hours |
| Reference range (Zakres referencyjny) | PC 70–140% | free PS: female 55–124%, male 74–146%; total PS: female 60–140%, male 75–140% |
| Assays (Testy) | amidolytic PC assays for routine screening for PC deficiency, more specific than coagulation assays; immunoassays (turbidimetric, nefelometric, ELISA) | immunoassays for free and total PS, and clotting assays for PS activity |
| Physiological variability (Zmienność fizjologiczna) | early childchood PC = 40%; increase by more than 20% in pregnancy and in the old age; remain elevated in the postpartum period | early childhood PS=60%, total PS is increasing with age; free PS levels are independent of age; pregnancy free PS 30–50%, lower level in women than men |
| Pathophysiological variability (Zmienność patofizjologiczna) | VKA-based anticoagulant therapy, vitamin K deficiency, DIC, severe infections, liver disease, fresh thrombosis, oral contraceptive use, autoantibody presence | VKA-based anticoagulant therapy, vitamin K deficiency, DIC, liver disease, oral contraceptive use, autoantibody presence |

APC – activated PC.
DIC – disseminated intravascular coagulation.
VKA – vitamin K antagonist.

APC – aktywowane PC.
DIC – zespół rozsianego wewnątrznaczyniowego krzepnięcia.
VKA – antagonista witaminy K.

## Genetic Background of PC and PS Deficiencies

Recently, Caspers et al. have shown that PC and PS deficiencies exhibit similar mutation profiles and are most frequently caused by missense mutations (78% and 63%, respectively) followed by nonsense mutations (11% and 9%), splice-site mutations (7% and 13%), small duplications/insertions/deletions (2% and 9%) and large deletions (3% and 6%) [13]. These data are in concordance with data published in the HGMD mutation database (http://www.hgmd.org). The detection rate of

mutations is 70–80% for PC deficiency and around 50% in families with PS deficiency [13, 24, 25].

The lower mutation rate detection within the *PROS1* gene may be explained by the fact that PS levels are also influenced by non-genetic factors like age, sex, pregnancy, oral contraceptive use, or vitamin K intake [13, 26]. Moreover, some polymorphisms within the *PROC* and *PROS1* genes, or within vitamin K-dependent gamma-carboxylation-related genes, may affect PC and PS activity or antigen concentrations [27, 28].

## Clinical Manifestations and Treatment of PC and PS Deficiencies

The risk of developing thrombosis among individuals with genetic defect in *PROC* or *PROS1* genes varies significantly and depends on multiple gene-gene or gene-environment interactions. People with hereditary PC/PS deficiency have about a 2- to 11-fold increased risk for VTE developing, with its main clinical presentations of DVT and pulmonary embolism (PE) in comparison with those without a deficiency [29]. This means that DVT or PE will occur in approximately 1 of every 100 to 500 people with one of these deficiencies annually [29]. Less common manifestations of PC/PS deficiency are superficial, cerebral, visceral, or axillary vein thrombosis [26].

Depending on the phenotype, approximately half of the PC/PS deficient subjects become symptomatic at 55 years of age, whereas others will never experience any complications [26, 30]. The development of VTE in PC/PS deficient patients may be provoked by concomitant thrombophilic defects (FV Leiden, prothrombin G20210A, increased levels of FVIII, IX, and XI, hyperhomocysteinemia) and exposure to transient risk factors (obesity, surgery, trauma, immobilization, chronic illnesses, pregnancy, or female hormone intake) [29, 31].

The large European Prospective Cohort on Thrombophilia (EPCOT) study has shown that 4.5% of asymptomatic relatives of patients with confirmed thrombophilia, developed first VTE during the 5.7 years of follow-up [32]. The annual incidence of VTE in PC- (0.7%) and PS- (0.8%) deficient patients was higher than in cases of FV Leiden [32].

Patients with provoked episodes are labeled as a low risk category and receive time-limited anticoagulation, usually 3 to 6 months of warfarin [33]. Warfarin should be administered initially with an additional injectable anticoagulant (usually low-molecular-weight heparin, LMWH) until an international normalized ratio (INR) of 2.0–3.0 is reached on two consecutive days. Heparin should be given for at least 5 days to prevent skin necrosis, which is a rare adverse effect that occurs during early warfarin treatment in patients with PS/PC deficiency [26]. Patients with idiopathic events are considered at high risk for VTE recurrence and benefit from indefinite duration anticoagulation [33, 34].

Patients with hereditary PC/PS deficiency have a high risk of VTE recurrence. A retrospective study in a large cohort of families has shown that annual incidences of first recurrence after a first episode of VTE were 6.0% (95% CI, 3.9–8.7) in PC-deficient patients and 8.4% (95% CI, 5.8–11.7) in PS-deficient patients [30]. This risk has been increased 1.4-fold by concomitance of other thrombophilic defects e.g. FV Leiden, prothrombin G20210A compared to patients without concomitant thrombophilic defects [30].

Severe PC/PS deficiency when plasma PC/PS concentrations are almost undetectable is associated with PC/PS homozygosity or compound heterozygosity. This extremely rare condition causes PF and DIC and is characterized by general clotting in the microvasculature [15, 16]. PF originates with red or purpuric lesions which rapidly progress to form palpable black eschars on the back of the head and buttocks. Coagulation studies have shown markedly elevated D-dimer, an undetectable plasma PC activity, thrombocytopenia, hypofibrinogenaemia, and prolongation of the prothrombin time [15].

Neonatal PF can be monitored only with PC replacement in the form of fresh frozen plasma (FFP) or a human plasma-derived, viral inactivated PC concentrate (Ceprotin) [15]. Ceprotin is manufactured by Baxter BioScience (Glendale, CA, USA) and has been licensed in the United States and Europe.

Replacement therapy with PC concentrate can be used at a dose of 100 U/kg followed by 50 U/kg every 6 hours to maintain a PC level of about 50%. If PC concentrate is unavailable, FFP is recommended at a dose 10–15 ml/kg every 8–12 h. The most affected infants have been managed for long-term secondary prophylaxis with PC concentrate or therapeutic anticoagulation using either LMWH or high-intensity warfarin [15]. Monitoring with D-dimer levels for evidence of coagulation activation is useful to confirm adequate replacement or anticoagulation therapy [35].

Recombinant APC (Xigris; Eli Lilly, Indianapolis, IN, USA) has also been used to treat PF in a child with severe PC deficiency and 24 μg/kg/h dose has been administrated [36]. However,

potential hemorrhagic risk may be involved during this treatment [37].

Although PC/PS deficiency is associated primarily with an increased risk for VTE, some studies have shown that arterial thrombosis manifested by myocardial infarction (MI) or ischemic stroke may be caused by PC/PS deficiency with other predisposing factors involvement [38–40]. Sayin et al. has found that left main coronary artery (LMCA) thrombus accompanied by embolization of the left anterior descending artery (LAD) in a 37-year-old man with normal coronary arteries was caused by PC and PS deficiency [38]. In this case cigarette smoking was considered to be a contributory factor leading to endothelial dysfunction. A large family cohort study demonstrated that hereditary PC (PC antigen < 63% and/or activity < 64%) or PS (PS antigen < 68%) deficiency is associated with increased risk of arterial thrombosis, defined as MI, ischemic stroke or transient ischemic attack, before age 55 years, but not in older subjects [40].

## Indications for PC/PS Deficiencies Diagnostic Testing

1. VTE without obvious cause < 45–50 years.
2. VTE in patients with a family history of thrombosis.
3. Recurrent VTE.
4. Thrombosis at an unusual location.
5. Developing VTE during pregnancy, use of oral contraceptives or hormone replacement therapy.

In asymptomatic patients with PC/PS deficiency thromboprophylaxis is not recommended.

## Blood Sample Collection and Processing for PC/PS Deficiency Testing

For routine screening of PC deficiency the amidolytic PC assay is recommended as more functional and specific than coagulation assays [41]. PC antigen is generally assayed by ELISA [18]. Immunoassays for free and total PS are preferred for screening, and clotting assays for PS activity (Table 1). If the results of functional and antigenic assays do not confirm the diagnosis unequivocally, genetic testing is indicated [14].

Testing should be done at least several weeks (3–6 months) after an acute clotting event to allow acute-phase reactant proteins to return to baseline [29, 42].

PC/PS deficiency should not be diagnosed or excluded on the basis of assays performed when the patient is taking vitamin K antagonist (VKA) [18]. To avoid false-positive test results, plasma samples should be taken after temporary interruption of oral anticoagulant therapy for at least 10 days [42] and, in some cases, bridging anticoagulation with alternative agents such as LMWH [26]. Positive test results should be confirmed by a second blood sample.

In pregnant women, positive test results should be established after the postpartum period [42]. To prove inherited deficiency, testing of family members is recommended.

Both patient and family members should receive genetic counseling prior to genetic testing, and such testing should only be performed after obtaining consent.

Additional factors influencing plasma PC/PS levels are summarized in Table 1.

## Molecular Diagnosis

Genetic analysis by DNA sequencing is the important tool in the diagnosis of PC and PS deficiencies. The presence of the PS pseudogene requires careful primer design to avoid amplification of pseudogene fragments [14]. When no point mutations within *PROC* or *PROS1* genes have been found by routine methods, the multiplex ligation-dependent probe amplification (MLPA) is a useful technique for the detection copy number variation involving duplication or deletions from one or more exons to the whole gene [24, 25]. It should be noted, however, that genetic analysis of patients with PC levels above 70% and PS levels above 55% is not recommended because of the very low likelihood of detection a mutation associated with inherited PC or PS deficiencies [13].

### Thromboprophylaxis with LMWH (Dalteparin, Enoxaparin and Nadroparin) or Unfractionated Heparin (UFH) According to Polish Guidelines for the Prevention and Treatment of Venous Thromboembolism, 2012

Both suggested thromboprophylaxis in asymptomatic individuals with a PC/PS deficiency and thromboprophylaxis indicated after VTE event includes following groups of patients:

- in pregnancy and puerperium,
- in the perioperative period,
- at the time of internal medicine hospitalization (thromboprophylaxis should not be used in patients at high-risk of bleeding, e.g., liver damage

INR > 1.5, thrombocytopenia < 50 × $10^3$/ul, serious bleeding in last 3 months).

After VTE event prophylactic or intermediate doses of LMWH should be used in patients who have previously interrupted anticoagulation. Therapeutic doses (after two incidents), adjusted or intermediate, should be used in patients who have previously applied anticoagulation.

Prophylactic PC concentrate should be administrated prior the invasive procedures with high risk of thrombosis, bleeding or prior the childbirth, at the initial dose of 100 U/kg followed by 30–50 U/kg every 12–24 h until PC > 100% (minimal PC activity 20–50%).

Indications for extended anticoagulation after one episode of VTE:

– anticoagulant therapy for up to 2 years without other thrombophilia,

– life-long if other thrombophilia is present (coexistence of PC/PS heterozygous deficiency with FV Leiden or prothrombin 20210A).

## PS and PC Deficiencies with Known Genetic Background in Polish Patients

So far the authors identified two mutations responsible for PC deficiency and two mutations responsible for PS deficiency in Polish patients who have been referred to the Centre for Coagulation Disorders, John Paul II Hospital, Krakow, Poland.

A missense mutation in a *PROC* gene, a heterozygous nucleotide substitution G > C in codon 109 resulting in the replacement of glycine for arginine (G109R) associated with PC deficiency was described for the first time in a 28-year-old male Polish patient, who was admitted due to acute MI with anterior wall ST-segment elevation (STEMI) 8 h after the pain onset. Coronary angiography revealed a thrombus in the LMCA leading to an 80% diameter stenosis and distal embolization of LAD; whereas the remaining vessels were normal. The patient received clopidogrel with a glycoprotein IIb/IIIa inhibitor (abciximab) and unfractionated heparin with the subsequent intravenous infusion. Twenty-four hours after angiography enoxaparin at therapeutic doses was initiated with clopidogrel and aspirin. After 7 days, a repeat coronary angiography showed no evidence of residual thrombus in LMCA or LAD. The patient was discharged on dual antiplatelet therapy with enoxaparin (maintained for 6 months) and after a year clopidogrel was discontinued. The patient remains on aspirin indefinitely. PC antigen values were reduced to 46% and 59% on the two respective occasions (reference range, 65–140%). In this case the patient's risk factors including heavy smoking, hypercholesterolemia, and obesity in combination with genetic thrombophilic factor caused the LMCA thrombus [43].

The second *PROC* mutation discovered in a Polish patient was a heterozygous nucleotide T > C substitution in codon 106 resulting in the replacement of cysteine by arginine (C106R) which has not been reported so far in the literature. A 29-year-old, obese male patient experienced first-ever proximal DVT of the left leg following trauma and the subsequent high-risk PE with hypotonia that was successfully treated with tenecteplase, a fibrinolytic agent. The patient started therapy with acenocoumarol with a target INR value of 2 to 3. His family history of was negative. On two separate occasions PC activity was 42% (reference range, 70–140%), while PC antigen were 44% and 41% (reference range, 65–140%), respectively. The diagnosis of the type I PC deficiency was established (Wypasek 2013, unpublished data).

The first Polish case of PS deficiency with genetic characterization has been reported in a young male patient, who experienced idiopathic DVT at the age of 24 and was treated with enoxaparin for 5 months. A few months later he developed edema of the right calf and right popliteal vein thrombosis was diagnosed. He started acenocoumarol at a daily dose of 2–3 mg with a target INR of 2 to 3. Family history revealed idiopathic DVT complicated with post-thrombotic syndrome in the patient's mother at the age of 45 and two episodes of DVT in her sister. The free PS level was 18.9% in the patient (male reference range 74–146%) and 17.6% in the patient's mother (female reference range 55–124%). Similar results were obtained on two separate occasions. Total PS in both patients were reduced to 40 and 44%, respectively (reference range 70–120%). Type I PS deficiency was diagnosed. MLPA analysis profile suggested a large deletion involving exons 1 to 12 of the *PROS1* gene, present at the heterozygous state in both the proband and his mother [44].

Another report associated with PS deficiency was the Heerlen polymorphism, a missense serine 501 to proline exchange (S501P) resulting in thymine to cytosine transition in exon 13 of the *PROS1* gene. The association between the PS Heerlen and venous thromboembolism (VTE) is not clearly established. The prevalence of the S501P mutation is high in the healthy European population (0.5%) and data on the association between PS Heerlen and thrombosis are inconsistent [45]. It was shown that the PS Heerlen may be involved in the occurrence of VTE when other genetic risk factors like FV Leiden, prothrombin G20210A or antiphospholipid antibodies are present [46].

PS Heerlen has been reported in a 50-year-old man with several thrombotic episodes of deep and superficial veins and a highly positive thrombotic family history. He was successfully treated with enoxaparin for 3 months but refused anticoagulation with VKA taking only low-dose aspirin. The free PS levels were reduced to 52.8% and 55.8% on the two separate occasions (reference range 74–146%). Total PS was 110.5% (reference range 75–140%). Type III PS deficiency combined with FV Leiden and primary antiphospholipid syndrome was diagnosed [47].

The current evidence indicates that screening for inherited thrombophilia, including deficiencies in natural anticoagulants like PC and PS, should be conducted in young patients with VTE and in selected cases of stroke or MI in particular if a family history for thrombosis is positive. Patients with low PC and PS levels and the known mutation within *PROC* or *PROS1* genes combined with other genetic or environmental thrombosis factors are at increased risk of recurrent thromboembolic events and require lifelong oral anticoagulation even after the first episode. Given positive family history including deaths from VTE among first degree relatives, genetic counseling in such families should be implemented and appropriate thromboprophylaxis in high-risk states should be considered in asymptomatic carriers.

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