



Prothrombin G20210A mutation, antithrombin, heparin cofactor II, protein C, and protein S defects

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Prothrombin G20210A mutation and thrombosis

The prothrombin G20210A mutation was first described by Poort et al in 1996 [1]. In this initial study, 28 subjects from families with a documented history of venous thrombosis were assessed. Specifically, the 3' untranslated region of the prothrombin gene was assessed by polymerase chain reaction by direct sequencing. It was found that 18% of this patient population had a prothrombin gene G20210A mutation in the 3' untranslated region. A healthy control group also was studied, and the mutation was present in only 1%. Poort et al concluded that harboring this prothrombin gene mutation imparted a three-fold increased risk of thrombosis. Functional prothrombin assays also were performed, and it was noted that 87% of individuals with this prothrombin gene G20210A mutation had prothrombin levels greater than 115% of normal. It initially was thought that functional prothrombin levels could be used as a screening test for this gene mutation; however, subsequent studies have suggested that there is no correlation between functional (plasma) prothrombin levels and those harboring this gene mutation. At present, there is no satisfactory screening test. This defect is autosomal dominant. The gene mutation now is known to be associated with both arterial and venous thrombosis. Subsequently, Brown et al [2] reported a study of 504 patients with venous thromboembolism, and 2.6% of this population was positive for the prothrombin G20210A gene mutation; those harboring the

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prothrombin gene mutation were concluded to have a two-fold enhanced risk for thrombosis, whereas, in this same population, individuals with factor V Leiden mutation had a 5.8-fold increased risk of thrombosis. Three of the 504 patients with thrombosis were heterozygous for both prothrombin gene mutation and factor V Leiden mutation. Rosendaal et al [3] reported a multicenter trial (11 centers) in nine different countries, assessing 5527 patients. They found 111 heterozygous carriers of the prothrombin gene mutation and noted that the incidence was approximately 3% in Southern Europeans and 1.7% in Northern Europeans. They also noted that prothrombin gene mutation, similar to the factor V Leiden mutation, seems to be rare in individuals of African and Asian descent.

The first study addressing arterial thrombotic risk was that by Ferraresi et al [4], wherein 132 patients with venous thrombosis and 195 patients with coronary artery or cerebrovascular thrombosis were assessed for the prothrombin gene mutation. A 4% incidence of the mutation was found concomitantly in a healthy Italian control population. It was noted that the prothrombin gene mutation was increased and found in 16% of patients with venous thrombosis but was not increased in patients with the aforementioned arterial thrombotic events. This finding is different from that reported by De Stefano et al [5], however, wherein 72 patients with thrombotic stroke, aged less than 50 years, in the absence of other risk factors were assessed for the gene mutation. The findings from these stroke-affected patients were compared to findings in 198 thrombosis-free individuals. In the stroke population, there were seven heterozygotes and two homozygotes for the prothrombin gene mutation (12.5%) and five heterozygotes in the control population (2.5%). It was concluded that harboring the prothrombin gene mutation rendered a greater-than-five-fold increased risk for cerebrovascular thrombotic or occlusive disease. More recent studies have found the prothrombin gene mutation also to play a role in patients with cerebral venous thrombosis. Martinelli et al [6] found the gene mutation in 20% of unselected patients with cerebral venous thrombosis and concluded that harboring this gene increases the risk of cerebrovascular venous thrombosis by 10.2-fold. In women ingesting oral contraceptives and harboring this mutation, the risk was increased to 150-fold. In a similar study, Reuner et al [7] found the prothrombin gene mutation in 8.9% of patients with cerebral venous thrombosis and concluded that heterozygosity for this gene mutation increased the risk of cerebral venous thrombosis by at least five-fold. From the studies thus far reported, it seems the prothrombin G20210A gene mutation is a common hereditary cause of venous and, slightly less commonly, arterial thrombosis. This defect always must now be considered when assessing patients for unexplained venous or arterial thrombosis.

Antithrombin defects

Antithrombins (ATs) were first described in 1939 by Brinkhous et al [8]. The first large survey of ATs was reported by Seegers et al in 1952 [9]. Egeberg, in 1965, first noted the association between AT deficiency and thromboembolic

complications in a Norwegian family [10]. Antithrombin is an alpha-2-globulin composed of 432 amino acids with a molecular weight of approximately 58,000 daltons [11,12]. It is synthesized in liver and endothelial cells [13,14]. Its specific characteristics have been described in detail [15,16]. The gene for AT has been localized to the long arm of chromosome 1 (1q23–1q25) and is composed of seven exons and six introns spanning nearly 16 kb [17]. Antithrombin inactivates thrombin and other serine proteases in a progressive, irreversible manner after second-order kinetics [18]. Also, AT inactivates other serine proteases, including factor Xa, IXa, XIa, XIIa, and kallikrein, although with less efficiency than for inhibition of thrombin [19–24]. It has been suggested that most of the inhibition of the serine protease procoagulant system is ascribed to AT and less to other inhibitors, but this concept is controversial [11,25]. Neutralization of thrombin and factor Xa by AT occurs by the interaction of heparin with AT, or alternatively, the interaction of heparin with the particular serine protease involved [26–29]. Another proposed mechanism is that a molecule of heparin may bind to AT and thrombin [30]. In the presence of heparin, the preferential target of AT is thrombin, followed by factor Xa. Proposed mechanisms of action of AT are summarized in Figs. 1 and 2. The physiologic range of AT in normal human blood is narrow; the usual plasma

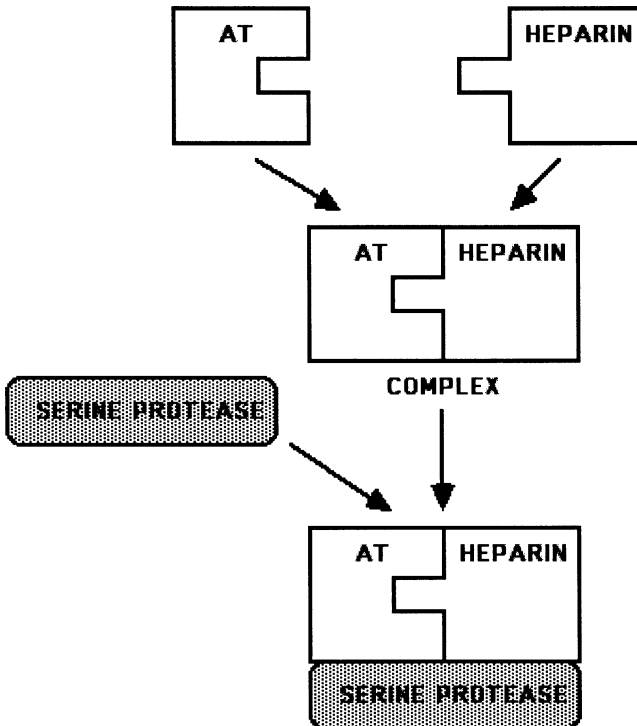


Fig. 1. Antithrombin activity (model I).

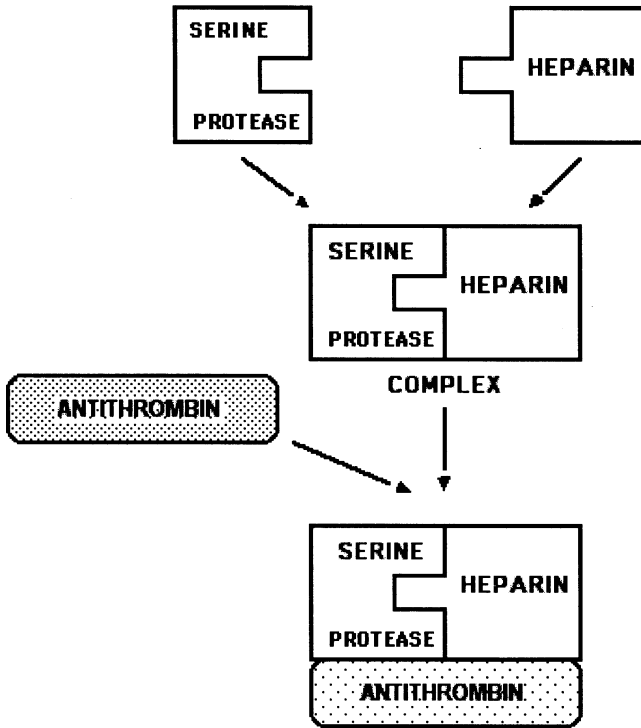


Fig. 2. Antithrombin activity (model II).

concentration is approximately 150 $\mu\text{g}/\text{mL}$ [31–33]. Only moderate decreases of AT often are associated with thrombosis or thromboembolus [31,34]. Infants have approximately 50% of normal adult AT levels; however, adult levels are attained by the age of 6 months [35]. The mechanisms by which a potential deficiency of AT may occur are (1) a defect in synthesis that may occur in the congenital form and in several acquired forms, such as liver disease; (2) increased consumption of AT, resulting from the generation of pathologic levels of serine proteases as happens in disseminated intravascular coagulation, extensive deep venous thrombosis, massive pulmonary embolization, and diffuse small and large venous and arterial thrombo-occlusive events; (3) loss of AT from the intravascular compartment, as may happen in some renal diseases; and (4) increased protein catabolism [31,34]. These mechanisms are listed in Box 1.

Pathologic decreases in antithrombin

Hereditary defects of antithrombin

“Hereditary thrombophilia” initially was the term used to describe congenital AT deficiency; however, this term now is used generically for all the hyper-

Box 1. Mechanisms of AT deficiency

- Decreased synthesis
 - Congenital form
 - Acquired form
- Dysfunctional synthesis
 - Congenital form
 - Acquired form
- Increased consumption
 - Disseminated intravascular coagulation
 - Deep venous thrombosis
 - Pulmonary embolus
 - Diffuse vaso-occlusive diseases
- Proteinuria and nonselective loss of AT-III
- Increased nonselective protein catabolism

coagulable or prethrombotic disorders caused by a hereditary defect. Hereditary thrombophilia also includes congenital heparin cofactor II, protein C, protein S, activated protein C resistance (factor V Leiden and other factor V mutations), plasminogen, and other similar defects. Hereditary deficiency of AT usually is inherited as an autosomal-dominant disorder; however, variant inheritance also has been described [25]. Most patients with hereditary AT deficiency are heterozygous, although kindreds with homozygous deficiency have been reported [36]. Two main types of AT deficiency have been described. In most patients with classic hereditary type I deficiency of AT, there is reduced synthesis of AT [25,31,37]; however, hereditary deficiency of AT also may be from a dysfunctional AT molecule [25,31,37,38]. The absence (quantitative) form (type I) and dysfunctional (qualitative) form (type II) exist. Type I may be subdivided into heterozygous (decreased AT) or homozygous (absence) of AT. Type I AT deficiency may result from gene deletion or from frameshift mutations resulting in a truncated protein. The truncated protein is unstable and therefore undetectable in AT assays [39]. Type II AT deficiency is characterized by normal antigen levels but decreased functional activity. Type II deficiency typically results from point mutations. Point mutations can be categorized as those that affect the heparin-binding domain or those that affect the thrombin-binding domain of the AT molecule [40]. Finazzi et al [41] and Girolami [42] have noted that heterozygous patients with defects in the heparin-binding domain do not have a severe thrombotic tendency. The prevalence of thrombosis among these individuals is less than 6%. By contrast, individuals who are homozygous for abnormalities in the heparin-binding domain are affected severely. Several subclassification schemes for AT deficiency have been proposed, although no one classification is adopted widely. Sas [43] has proposed a classification that incorporates clinical, biochemical, and molecular features of AT deficiency.

Given the wide variety of defects that clinically manifest as AT deficiency, one can appreciate why functional AT assays are the preferred method to be used in screening for AT deficiency. The best single test for screening purposes is the AT-heparin cofactor assay by synthetic substrate [44].

The prevalence of hereditary deficiency of AT seems to be between 1 in 2000 to 1 in 5000 [45,46]. The prevalence of hereditary AT deficiency in a general patient population with thrombotic or thromboembolic events is approximately 3% to 8% [31,47,48].

Patients with hereditary deficiency of AT have a marked increased risk of venous thrombotic events and pulmonary embolism [6,31,34,49–52]. These events typically appear in the mid- to late teenage years. Approximately two thirds of published cases of AT deficiency have a thrombotic event between ages 10 and 35 [34]. The most common sites of thrombosis are the deep veins of the lower extremities, followed by the iliofemoral veins; however, other characteristic sites of thrombosis include the mesenteric veins, vena cava, renal veins, and retinal veins [30]. Cerebral venous thrombosis and Budd-Chiari syndrome also have been noted [53,54]. The usual presentation is that of recurrent deep venous thrombosis with or without pulmonary embolization [6,31,34]. Arterial thrombotic events are not seen commonly in AT deficiency, although rare cases have been reported [55]. A defect of AT need not be severe for thrombotic events to occur; some individuals have deep venous thrombosis and pulmonary emboli at between 50% and 70% biologic (functional) activity, whereas others with lower AT levels may not have thrombosis [22,56–60]. Thrombotic events in patients with AT deficiency are sometimes first precipitated by other factors such as surgery, trauma, pregnancy, oral contraceptive use, hormone replacement therapy, or infection [61]. Box 2 summarizes the characteristics of hereditary AT deficiency.

Acquired antithrombin deficiency

Causes of acquired AT deficiency include acute thrombosis, disseminated intravascular coagulation, liver disease, nephrotic syndrome, oral contraceptive use, L-asparaginase treatment, and rarely, after heparin administration [31,37]. Approximately 70% of individuals with deep venous thrombosis or pulmonary embolus have decreased levels of AT before the initiation of anticoagulant therapy; however, most of these individuals have decreased AT levels owing to consumption when the thrombus developed, and only a few individuals, 3% to 8%, have had the thrombotic event because of a congenital deficiency of AT [47]. There is a general correlation between the severity of the intravascular thrombotic event (such as deep venous thrombosis and pulmonary emboli) and the degree of decrease in AT levels in most acquired cases. Generally, the intensity of decrease correlates with disseminated intravascular coagulation, iliofemoral thrombosis, pulmonary embolization, bilateral calf thrombosis, and single-calf thrombosis in descending order of decrease of AT levels [62,63].

Many reports have appeared about AT levels in patients taking oral contraceptives [64–68]. Many conflicting data exist and are summarized in an excellent

Box 2. Characteristics of hereditary AT deficiency

Clinical features

- Autosomal-dominant trait
- Absence form and dysfunctional forms exist
- Venous thrombosis begins in mid- to late teenage years
- Pulmonary emboli are common
- Mesenteric vessels are particularly susceptible
- Thrombosis occurs commonly in heterozygotes
- Thrombosis may occur at levels below 75%

Laboratory features

- Low AT levels by biologic activity
- Low immunologic AT levels in absence form
- Normal immunologic levels in dysfunctional form
- Global tests of coagulation are normal
- Tests of fibrinolysis are normal
- Template bleeding time is normal
- Platelet aggregation is normal
- Activated PTT may not prolong adequately or at all during intravenous heparin therapy

Therapy

- Oral anticoagulants
- Porcine heparin/low-molecular-weight heparin: long-term antiplatelet agents (aspirin plus dipyridamole)
- Antithrombin concentrates
- Heparin may be ineffective

review giving sensible perspective to the issue of oral contraceptives, thromboembolism, and the hemostasis system [69]. Estrogens may reduce AT levels approximately 15%, and greater reductions are correlated with higher doses of estrogens [56,57]. Also, there is specific binding between estrogens or other steroids and AT [70]. One might deduce a potential relationship between decreases in AT levels and thrombotic tendencies in women taking oral contraceptives; however, the data are too conflicting for any firm conclusions regarding the clinical relevance of decreased AT levels in women taking oral contraceptive agents.

Patients with proteinuria lose AT in the urine along with other plasma proteins [71]. There is a well-recognized risk of deep vein thrombosis and pulmonary embolism in patients with nephrotic syndrome [72]; however, these patients have many other conditions also constituting high-risk factors for thrombosis, including high concentrations of various clotting factors, urinary loss of other coagulation inhibitors (such as protein C), prolonged bed rest, and stasis [73,74]. It is unclear whether there is a causal relationship between decreases of AT in patients with nephrotic syndrome and the increased risk of thrombosis and thromboembolic disease.

Antithrombin levels often are decreased in patients with chronic liver disease [31,32] and in patients with acute hepatitis [75–77]; however, the importance of this finding is unclear because patients with chronic liver disease and hepatic failure have no apparent increased risk of thrombosis. The decrease in AT may be counterbalanced by the severe defects in hemostasis and subsequent “hypocoagulability” often associated with acute and chronic liver disease [78–80].

Pathologic consumption of AT is expected in conditions associated with abnormal acceleration of procoagulant activity and the pathologic generation of thrombin and other serine proteases, such as occurs in disseminated intravascular coagulation [75–77,81–84]. Several studies have shown significant decreases in AT levels in patients with disseminated intravascular coagulation. Mechanisms of acquired AT defects are:

- Disseminated intravascular coagulation
- Extensive deep vein thrombosis
- Pulmonary embolus
- Chronic liver disease
- Acute hepatitis
- Diffuse arterial thrombosis
- Proteinuria
- Oral contraceptives
- Hormone replacement therapy

Treatment of antithrombin defects

The mainstay of treatment of patients with AT deficiency and acute thrombotic events is low- molecular-weight heparin (LMWH) or unfractionated (UF) porcine heparin. The indications for thrombolytic therapy for acute thrombosis are the same as for patients without AT deficiency. Antithrombin concentrates have become available for treatment of both congenital and acquired AT-deficiency states [85]. Although controlled trials are lacking, available data suggest that AT concentrates used with or without concomitant heparin administration are safe and effective in the prevention of thrombosis in high-risk settings and for treatment of acute thrombotic episodes in AT-deficient patients [86]. Antithrombin concentrates are especially useful in patients with recurrent thromboses despite therapeutic anticoagulation with other modalities. A dose of 50 U/kg given intravenously has been recommended to correct levels in individuals with AT activities in the range of 50% at baseline. Repeat doses of 60% of the loading dose may be given every 24 hours to maintain activity levels greater than 80% [87]. Life-long anticoagulant therapy is indicated in individuals with congenital deficiency of AT who manifest recurrent thrombotic episodes. Prophylactic treatment of asymptomatic individuals is controversial. Usually, treatment of these individuals is reserved for situations in which the risk of thrombosis is increased, such as after surgery or during pregnancy. Particular attention must be

paid to AT-deficient individuals during pregnancy. All AT-deficient patients should be maintained on subcutaneous porcine heparin or LMWH throughout pregnancy. Warfarin is to be avoided because of its teratogenic effects. Antithrombin concentrates are best reserved for use during labor, delivery, or obstetric complications [88].

Antithrombin concentrates are probably the specific antiprocoagulant therapy of choice for patients with fulminant disseminated intravascular coagulation [31,77,89,90], but prospective randomized clinical trials using AT concentrates in patients with acute disseminated intravascular coagulation and patients with “consumption” resulting from acute hepatic failure are needed to determine if efficacy is present and equal to or better than current modalities of therapy. The clinical use of AT concentrates has been reviewed recently by Vinazzer [91].

Heparin cofactor II defects

Heparin cofactor II (HC-II) was first discovered by Briginshaw and Shanberge [92] when they noticed that besides the rapid inhibition of thrombin by heparin, which could be reversed by addition of polybrene or protamine, there was a slow, time-dependent inhibition representing an irreversible decrease in thrombin; this second inhibitory effect was called heparin cofactor A. Briginshaw and Shanberge also noted that unlike the activity of AT, their heparin cofactor A had no inhibitory effect on factor Xa. Later, Tollifsen et al [93] further isolated and characterized this thrombin-inhibiting glycoprotein and named it HC-II. These investigators demonstrated that not only heparin but also dermatan sulfate accelerated the thrombin-inhibiting activity of HC-II and noted that HC-II inhibited the amidolytic and proteolytic activities of thrombin by forming a covalent 1:1 molar complex with thrombin [94,95]. Heparin cofactor II is a glycoprotein with a molecular weight of approximately 64,000 daltons. The inhibitory activity of HC-II is accelerated by heparin, including heparins with low AT affinity, dermatin sulfate, the semisynthetic heparinoid pentosan polysulfate, dextran sulfate, and other sulfated polysaccharides. Unlike AT, HC-II is not capable of significant inhibition of factors Xa, XIa, IXa, or plasmin, and in addition to thrombin inhibition, HC-II inhibits chymotrypsin. The inhibition of thrombin by HC-II is not limited to the activity of thrombin on fibrinogen, but thrombin-induced platelet aggregation and release also are inhibited [95].

Hereditary deficiency of heparin cofactor II

Many assays are available for assessing HC-II activity, and concomitant with assay availability have been the finding of congenitally deficient patients and the definition of acquired HC-II deficiency [95]. Shortly after the availability of specific assays, the first case of hereditary HC-II was reported by Tran et al in

1985 [96]; the patient was a 42-year-old woman with left middle cerebral artery thrombosis and an HC-II level that was 50% of normal. Two of four additional family members had experienced thrombotic events and also were found to have low HC-II levels. Since this original report, other patients and families have been found. In the families in whom molecular defects in HC-II (dysfunctional HC-II) have been searched for, none has yet been found. All hereditary deficiencies are currently quantitative (type I) and not qualitative. Heparin cofactor II deficiency seems to be inherited as an autosomal-dominant defect, with heterozygous individuals having approximately 50% of normal HC-II levels. Thrombotic tendencies are associated with levels less than 60%. Some studies have shown low HC-II activity in asymptomatic individuals and families, and because of this finding, it seems the clinical manifestations of hereditary HC-II deficiency can span from arterial or venous thrombosis to asymptomatic states. Heparin cofactor II deficiency seems to be a rare cause of unexplained thrombosis. In Bertina et al's [97] series of 277 patients with unexplained venous thrombosis, only three were associated with decreased HC-II levels. Mateo et al [98] reported an increased risk of venous thrombosis in carriers of natural anticoagulant deficiencies in the family studies of the Spanish Multicenter Study on Thrombophilia (EMET study). The prevalence of hereditary thrombophilia in Spain was assessed in a multicenter study in which 583 individuals from 114 families with natural anticoagulant deficiencies were analyzed. In this particular study, there was no increased thrombotic risk in those deficient in HC-II. Hudecek et al [99] also concluded that HC-II is probably not a serious risk factor for thromboembolic disease, but they described a child with thrombotic complications and a congenital deficiency of HC-II.

In another study from Spain, however, Mateo et al [98] assessed clinical and laboratory characteristics of 2132 consecutive unselected patients with venous thromboembolism. Although previous studies from this group regarding prevalence of biologic abnormalities causing venous thrombosis and the clinical characteristics of the thrombotic patient were conflicting, the study of 2132 consecutive individuals revealed that the overall prevalence of protein deficiencies was 12.85%. Antiphospholipid antibodies were found in 4.08%. Ten patients had AT deficiency, 68 had protein C deficiency, 155 had protein S deficiency, 16 had plasminogen deficiency, 8 had HC-II deficiency, and 1 had dysfibrinogenemia. Bernardi et al [100] reported on 305 patients with juvenile thromboembolic episodes who were screened for the presence of HC-II deficiency. The heterozygous deletion of two bases was found in the exon 5 of the HC-II gene in two unrelated patients. This molecular lesion, causing a frameshift and elongated translation, was concluded to affect the core of the molecule and caused the complete unfolding of the protein, which is in accordance with the observed type I deficiency. The corresponding region of the AT gene is affected by a cluster of frameshift mutations, suggesting that HC-II and AT might share similar mutational patterns. In one patient, the HC-II gene alteration was associated with the factor V Leiden mutation, and in the other, type I protein C deficiency. The tracing of the single defects in several family members indicated that the mu-

tations became clinically manifest only when present in the doubly heterozygous condition. This study provides two examples, based on molecular findings, of the interplay of risk factors that is potentially useful to define a role for HC-II deficiency in inherited thrombophilia.

Lopaciuk et al [101] assessed the prevalence of HC-II deficiency in patients with a history of venous thrombosis. This study noted that although several pedigrees have been reported in which 50% decreases in plasma HC-II levels were associated with venous or arterial thrombosis, the role of HC-II deficiency in inherited thrombophilia remains unresolved. This study was performed to determine the prevalence of HC-II deficiency among patients with a history of venous thrombosis. Heparin cofactor II antigen was measured by electroimmunoassay in 122 unrelated patients with first-episode deep vein thrombosis occurring before the age of 45 and in 114 healthy volunteers. Of the control subjects, one had a low HC-II concentration (37%), whereas in the remaining 113, levels ranged from 65% to 180%, with the mean value of $98.6\% \pm 20.6\%$. In patients with thrombosis, the mean HC concentration was $99.9\% \pm 28.0\%$; individual values ranged from 52% to 180%. Seven patients (5.7%) exhibited values beneath the lower limit of the normal range (65%). The authors of this study concluded that HC-II deficiency is more prevalent among patients with venous thromboembolism than in healthy subjects. Jaeken et al [102] reported an association of hyperprolinemia type I and HC-II deficiency with CATCH 22 syndrome, and they suggest that this association is evidence for a contiguous gene syndrome involving the proline oxidase gene. In this study, increased proline levels were found in the plasma of a girl with slight psychomotor retardation, epilepsy, obesity, scoliosis, hypocalcemia, variable lymphocytopenia, and facial dysmorphism suggestive of CATCH 22 syndrome. Fluorescence in situ hybridization indicated the presence of a submicroscopic 22q11 deletion, confirming the diagnosis. Further investigation revealed evidence that the patient was heterozygous for HC-II deficiency and for hyperprolinemia type I, a proline catabolic disorder caused by proline oxidase deficiency. This association extends the CATCH 22 syndrome and suggested that expression of the proline oxidase gene depends on the chromosome 22q11 region. Kondo et al [103] reported a Japanese patient with type I HC-II deficiency who had angina pectoris and coronary artery disease. Polymerase chain reaction–based sequence analysis showed that the propositus' gene for HC-II (HC-II Awaji gene) had a thymine insertion after codon (GAT) for Asp88 in exon II, resulting in a frameshift mutation. Consequently, the abnormal HC-II Awaji protein was suggested to have an altered amino acid sequence from position 89 and to terminate at 107, thus being composed of the NH₂-terminal one fifth of normal HC-II and dysfunctional for thrombin inhibition. The molecular weight and pI value of HC-II Awaji were calculated to be 12,040 and 3.6, respectively, without post-translational modification. Polymerase chain reaction followed by the Tsp509I digestion showed that half of the polymerase chain reaction products derived from the propositus and his sister was cleaved, suggesting that his sister also had the same mutant allele. Crossed-immunoelectrophoresis and Western blot analyses of plasma and urine from the propositus and of plasma from his sister did

not provide evidence for the existence of the abnormal HC-II, suggesting that little truncated HC-II was circulating in the patient's blood. Stable-expression assay using human kidney 293 cells transfected with the expression vector containing cDNA encoding wild-type or Awaji-type HC-II, however, showed that mutant and wild-type HC-II was secreted into culture medium normally. These results suggest that the abnormal HC-II Awaji protein is secreted normally but rapidly degraded in the circulating blood.

Acquired heparin cofactor II deficiency

Because HC-II is a normal physiologic inhibitor of thrombin, it is expected that with significant activation of the procoagulant system and subsequent thrombin generation, HC-II would be consumed, much like AT. Heparin cofactor II is decreased markedly in disseminated intravascular coagulation. Heparin cofactor II activity has been studied in patients with nephrotic syndrome and found to remain normal, unlike AT activity, which commonly is decreased [94,95,104]. Heparin cofactor II is decreased when systemic activation of coagulation occurs, but it seems to not decrease with local activation, as in deep venous thrombosis.

In a study reported by Reverdiau-Moalic et al [105], blood coagulation proteins were determined in 285 healthy fetuses from 19 to 38 weeks' gestation and compared with those of 60 normal full-term newborns and 40 adult control subjects. Prolongation of the coagulation screening tests, prothrombin time, activated partial prothrombin time, and thrombin clotting time throughout intrauterine life in fetuses was explained by low levels of vitamin K-dependent factors (II, VII, IX, and X), contact factors (XI, XII, prekallikrein, and high-molecular-weight kininogen), factor V, factor VIII, and fibrinogen. Low levels of AT, HC-II, protein C, protein S, and tissue factor pathway inhibitor also were found, and these combined changes probably contributed to a satisfactory hemostatic balance. Some of these parameters were evaluated by immunologic and functional assays to detect possible "fetal" proteins. An increase in factor levels was observed after the thirty-fourth week of intrauterine life for most of the coagulation activators and inhibitors, but only factors V and VIII reached adult values at birth. This study, therefore, showed that fetal hemostasis is a dynamic system that evolves gradually toward the neonatal state and then toward the adult state and demonstrates that, like other endogenous inhibitors, the HC-II level is low in the fetus and is slow to reach adult values. Bellart et al [106] assessed the plasma levels of HC-II during the third trimester and 72 hours after delivery in pregnant women who were either normotensive or had essential hypertension, gestational hypertension, or pre-eclampsia. It was found that HC-II levels in the pre-eclampsia group were depressed. The clinical relevance of this finding is the potential utility of HC-II plasma levels in the differential diagnosis between nonproteinuric hypertension and pre-eclampsia. This study suggests and the authors conclude that HC-II may be a good marker for pre-eclampsia. Bellart et al

[107] also have assessed coagulation and fibrinolytic parameters in normal pregnancy and in pregnancy complicated by intrauterine growth retardation. The plasma levels of coagulation and fibrinolysis parameters in the third trimester of gestation and 72 hours after delivery were assessed. Antithrombin, thrombin-AT complexes, HC-II, protein C, protein S, tissue plasminogen activator, D-dimer, and plasminogen activator inhibitor levels in uncomplicated pregnancies and in pregnancies complicated by intrauterine growth retardation have been determined. Normal pregnant women ($n = 63$) and women whose pregnancy was complicated by intrauterine growth retardation ($n = 10$) formed the study population. Coagulation and fibrinolysis parameters were estimated using commercial tests. There were no differences in AT, HC-II, and protein S levels between normal and intrauterine growth retardation pregnancies.

Andersen et al [108] assessed plasma levels of protein C antigen (PC:Ag) and activity (PC:Act), tissue factor pathway inhibitor, protein S, AT, HC-II, and resistance to activated protein C before, during, and after elective gastric surgery to compare patients with and without gastric malignancy. Blood was collected from a forearm vein of two age-matched patient groups undergoing elective gastric surgery: nine patients with and nine patients without gastric malignancy. The plasma levels of the above-mentioned parameters were determined preoperatively, intraoperatively, and postoperatively on days 1 and 7. On the first and seventh postoperative day, plasma levels of HC-II were significantly lower in patients undergoing an operation for gastric malignancy than in those undergoing an operation for benign disorders, but levels of tissue factor pathway inhibitor, PC:Act, PC:Ag, AT, protein S, and activated protein C did not differ in the postoperative period. The day-to-day variation was also similar in the two patient groups.

Chan et al [109] studied 22 consecutive pediatric patients who underwent cardiopulmonary bypass. Fibrinogen; factors II, V, VII, VIII, IX, and XII; prekallikrein; protein C; protein S; AT; HC-II; alpha 2-macroglobulin; plasminogen; alpha 2-antiplasmin; tissue plasminogen activator; plasminogen activator inhibitor; thrombin-AT complexes; D-dimer; heparin (by both anti-Xa assay and protamine titration); and activated clotting time were assayed perioperatively. The timing of the sampling was as follows: before heparin, after heparin, after initiation of cardiopulmonary bypass, during hypothermia, after hypothermia, after protamine reversal, and 24 hours after cardiopulmonary bypass. Plasma concentrations of all hemostatic proteins, including HC-II, decreased by an average of 56% immediately after the initiation of cardiopulmonary bypass due to hemodilution. During cardiopulmonary bypass, most of the procoagulants, inhibitors, and some components of the fibrinolytic system (plasminogen, alpha 2-antiplasmin) remained stable; however, plasma concentrations of thrombin-AT-complexes and D-dimers increased during cardiopulmonary bypass, showing that significant activation of the coagulation and fibrinolytic systems occurred. Mechanisms responsible for the activation of hemostasis are complex.

Cardigan et al [110] investigated changes in the hemostatic system in the pulmonary vein during cardiopulmonary bypass compared with blood that

circulated through the bypass circuit. Paired samples were taken from the pulmonary vein and central venous pressure line during the perioperative period from 10 patients. Plasma levels of factor VII ($P < 0.001$), prekallikrein ($P < 0.05$), AT ($P < 0.001$), and HC-II ($P < 0.005$) were decreased in the pulmonary vein after 20 minutes of bypass compared with preoperative levels. Andersson et al [111] studied and reported on thrombin-AT complexes and thrombin-heparin cofactor complexes (T-HC-II) during normal delivery; the median thrombin-AT level in 10 women increased from 4.1 to 7.8 times the median normal reference level. There was great individual variation, and levels 42 and 56 times the normal median were found in two women shortly after normal delivery. The median T-HC-II levels increased only moderately from 2.3 to 3.1 times the median normal reference. The median thrombin-AT level was 2.5 times the median normal reference, and the median T-HC-II level was 5.6 times the median normal reference value. The values were stable during the first 4 days postpartum, and there was little difference between those who delivered vaginally or by cesarean section. D-dimer values were above the normal reference value in all women and higher in women who delivered by cesarean section. Increasing thrombin-AT levels during labor and delivery indicated generation of thrombin, which was inactivated mainly by AT. The T-HC-II levels increased less during delivery. In the early postpartum period, the T-HC-II levels were relatively more increased than the thrombin-AT levels. These results suggest that intravascularly generated thrombin is inactivated preferably by AT, even in parturient women. In the postpartum period, formation of T-HC-II complexes was more evident, possibly reflecting extravascular inactivation of thrombin.

A collaborative group study [112] evaluated the effects of two regimens of transdermal estradiol (E2) combined with progestin on the balance between procoagulant factors and inhibitors; 255 women in physiologic menopause for 1 to 5 years were allocated randomly to 1 year of treatment with cyclic transdermal E2 (50 $\mu\text{g}/\text{d}$ for 21 days) plus medroxyprogesterone acetate (MPA) (10 mg/day from days 10 to 21), continuous transdermal E2 (50 $\mu\text{g}/\text{day}$ for 28 days) plus MPA (10 mg/day from days 14 to 25), or placebo. Fibrinogen, factor VII, factor VIII:C, AT, protein C, protein S, HC-II, and plasminogen activator inhibitor levels were measured at baseline and after 6 and 12 cycles. One hundred sixty-seven women taking the treatment for at least six cycles were evaluable. The continuous-treatment group had significantly lower final values of HC-II and other factors (fibrinogen, factor VII, AT, protein S) than the placebo group. Levy et al [113] assessed 51 consecutive patients with premature lower-extremity atherosclerosis for atherogenic risk factors and primary or acquired hypercoagulability, which might contribute to early ischemia and revascularization failure. Laboratory tests included plasma assays of (1) natural anticoagulants, lipoprotein (a), and anti-cardiolipin antibodies and (2) fibrinolytic activators and inhibitors at baseline and stimulated after 20 minutes of upper-extremity venous occlusion. Forty-six (90%) of these 51 patients had laboratory abnormalities. One or more deficiencies were found in 15 patients (30%) and included AT ($n = 5$), protein C ($n = 8$), protein S ($n = 4$), and HC-II ($n = 2$). These data strongly support the hypothesis that the

convergence of atherogenic risk factors and hypercoagulability, including that due to HC-II deficiency, plays an important role in early ischemia and poor results reported for lower-extremity vascular procedures in young adults.

O'Driscoll et al [114] noted that plasma HC-II is decreased in a variety of hemolytic conditions. Because the risk of thrombosis is recognized to be increased in both thalassemia major (TM) and intermedia (TI), HC-II levels were compared in 20 untransfused patients with TI and 20 regularly transfused patients with TM to determine the influence of transfusion on HC-II. Additionally, untransfused patients with TI have been commenced on regular red cell transfusion, and the effects on correction of low HC-II levels have been investigated. Heparin cofactor II levels were significantly lower in the untransfused patients with TI (mean, 0.56 ± 0.06 U/mL) compared with patients with TM (mean, 0.85 ± 0.1 U/mL; $P < 0.001$). Levels in TI were significantly less than in healthy age-matched controls ($P < 0.001$) and correlated with hemoglobin (Hb) values ($r = 0.8$), whereas levels in TM were at the lower end of the normal range. Heparin cofactor II antigen showed a parallel reduction to HC-II activity, indicating that reduction in HC-II is not a consequence of increased thrombin consumption. Three patients with TI were started prospectively on hypertransfusion programs that resulted in a slow normalization of their levels, taking 2 to 3 months, suggesting that low HC-II levels are related to increased red cell turnover and can be normalized once this turnover has been suppressed by hypertransfusion. The thrombotic risk for patients with low HC-II levels in the presence of hemolysis, in principle, might be decreased by such transfusion regimens. Rodeghiero and Tassetto [115] studied population-based distributions of protein C, AT, HC-II, and plasminogen. They assessed relationships with physiologic variables and established reference ranges. Protein C, AT, HC-II, and plasminogen levels from the first 4000 subjects enrolled in the VITA Project were analyzed. Serum triglycerides and total cholesterol, together with plasma fibrinogen, were found to influence the functional plasma level of protein C, AT, HC-II, and plasminogen. Menopause increased AT concentration, whereas oral contraceptive use increased HC-II and plasminogen. PT and PTT ratio, gender, age, smoking, body mass index, high-density lipoprotein cholesterol, and blood group had minor effects. The effect of these variables should be taken into account for both clinical and epidemiologic purposes, using appropriate reference ranges or covariance analysis for adjustment.

Heparin cofactor II defects clearly may cause venous or arterial thrombosis; however, this defect, whether inherited or acquired, seems to be rare and should be considered after more common prothrombotic (thrombophilic) defects have been ruled out.

Protein C defects

Protein C is a vitamin K–dependent protein that inhibits the coagulation system primarily through inactivation of factors V and VIII:C, the cofactors required for activation of thrombin and factor Xa [116]. This serine protease is

inhibited by AT [117] and enhanced by protein S [118]. The presence of protein C is essential to maintain hemostatic balance. Deficiencies of protein C may be either hereditary or acquired [119].

The hereditary deficiency is autosomal dominant and characterized by recurrent venous thrombosis and thromboembolism beginning in adolescence [120–122]. Most homozygous patients die of thromboembolic disease in infancy [123,124]. Both absence and dysfunctional forms of the disorder are observed. Type I disease is characterized by reduction in both antigenic and functional levels and in type II, functional levels are decreased much more than antigenic levels [37,125–129].

Homozygous patients have been managed successfully with infusions of fresh-frozen plasma or certain factor IX concentrates (containing large amounts of protein C and S), together with heparin [121,124,130]. Maintenance of an International Normalized Ratio of 3.5 or higher may be necessary to prevent recurrence of severe skin necrosis [131]. Considering the need for life-long anticoagulation, the need for high-dose warfarin, the difficulties attendant to maintenance of a consistent level of anticoagulation, and the risks of major hemorrhage, LMWH seems to be preferred for long-term management [132].

Anticoagulation is the treatment of choice in patients with heterozygous protein C deficiency. Heparin or LMWH is used according to accepted guidelines for acute thrombotic events [133,134]. Long-term anticoagulation with warfarin is indicated after an acute event and as prophylaxis. Warfarin-induced skin necrosis is a major therapeutic problem [135–137]. With the institution of warfarin, the reduction of protein C (half-life, 6 hours) occurs at a faster rate than the reduction in the other vitamin K–dependent factors II (half-life, 72 hours), VII, and X. This reduction results in a transient hypercoagulable state predisposing to thrombosis, including skin necrosis [134,138]. Recent studies have demonstrated that this problem can be controlled by maintaining full anticoagulation with heparin until the PT is well into the therapeutic range (target International Normalized Ratio, 3.0–3.5). Maintaining a therapeutic PT is, subsequently, essential to avoiding recurrent thrombosis. Despite therapeutic anticoagulation with warfarin, there are treatment failures. Long-term therapy with heparin or LMWH may be required. Protein C concentrates are now available for treatment failures.

Therapy is monitored with the appropriate laboratory test, depending on the pharmacologic agent selected. The target International Normalized Ratio for warfarin therapy is 3.0 to 3.5. Therapy with LMWH is weight adjusted and does not require monitoring, except for a platelet count every 2 to 3 days for the first 2 weeks of treatment. Although the measurement of the biologic and immunologic activity of protein C is required for diagnosis [139], follow-up analysis is not required. When evaluating a patient with unexplained thrombosis, however, it is essential to obtain the laboratory tests for protein C deficiency before therapy is initiated because warfarin immediately reduces the vitamin K–dependent hepatic production of protein C. The levels of both protein C and S decrease to 40% to 60% of normal immediately and return to approximately 70% of normal after

several weeks of therapy. Measurement of the protein C and S levels should not take place until several weeks after initiation of warfarin therapy. On repeat evaluation of protein C and S, if levels greater than 60% are not detected, congenital deficiency should be considered. Characteristics of hereditary protein C deficiency are outlined in Box 3.

Acquired protein C defects

Acquired deficiency is detected in disseminated intravascular coagulation, in the presence of acute thromboembolic disease, in severe liver disease (decreased hepatic synthesis), hemolytic uremic syndrome, and thrombotic thrombocytopenia purpura [121]. Treatment of acquired protein C deficiency requires a precise diagnosis and treatment of the underlying cause.

Anticoagulation is the treatment of choice in patients with acquired protein C deficiency and thrombosis. Heparin or LMWH is used according to accepted guidelines for acute thrombotic events [140,141]. Long-term anticoagulation with warfarin is indicated after an acute event and as subsequent secondary prophylaxis in those with acquired deficiency. Warfarin-induced skin necrosis is a major therapeutic problem [135–137]. With the institution of warfarin, the reduction of protein C (half-life, 6 hours) occurs at a faster rate than the reduction in the other vitamin K–dependent factors (half-life, 72 hours) II, VII, and X. This reduction

Box 3. Features of hereditary protein C deficiency

Clinical features

- Autosomal-dominant trait
- Absence form and dysfunctional forms exist
- Homozygous patients often die of thrombosis in early infancy
- Deep venous thrombosis and pulmonary embolus begin in mid- to late teenage years
- Warfarin-induced skin necrosis is seen often
- Clotting is common in heterozygotes
- Congenital deficiency may account for 5% to 10% of all patients with early clotting problems

Laboratory features

- Low biologic protein C levels
- Low immunologic protein C levels in absence form
- Normal immunologic protein C levels in dysfunctional form

Therapy

- Heparin for acute thrombotic events
- Warfarin: long term (with initial heparin)
- Heparin/LMWH: long term
- Prothrombin complex concentrates?

results in a transient hypercoagulable state predisposing to thrombosis, including skin necrosis [138,142]. Recent studies have demonstrated that this problem can be controlled by maintaining full anticoagulation with heparin until the PT is well into the therapeutic range (target International Normalized Ratio, 3.0–3.5). Maintaining a therapeutic PT is, subsequently, essential to avoiding recurrent thrombosis. Despite therapeutic anticoagulation with warfarin, there are treatment failures. Long-term therapy with heparin or LMWH may be required. Protein C concentrates are now available for treatment failures.

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Protein S defects

Protein S is a cofactor for the protein C–induced inactivation of factor V [14] and the protein C–induced inactivation of factor VIII:C [112]. Protein S also is a cofactor in the protein C acceleration of fibrinolysis [143] and seems to have anticoagulant functions independent of protein C by direct inhibition of procoagulant enzyme complexes [144,145]. Congenital protein S deficiency is autosomal dominant and is fairly common, identified in as many as 10% of patients younger than 45 presenting with deep vein thrombosis [146]. Incidence in other selected groups has varied from 1.5% to 7% [147,148]. Simmonds et al [149] recently reported analysis of a 122-member family in which 44 members were identified with the protein S gene mutation substitution, Gly-295 to Val. The probability of remaining thrombosis-free was 0.97 for unaffected family members and 0.5 for those with the mutation. Homozygotes have a severe propensity to thrombosis and may present with purpura fulminans soon after birth [150]. Heterozygous patients are at high risk for thrombosis throughout life. An asymptomatic variant also may exist [151,152]. Management of patients with protein S deficiency is similar to that of patients with protein C deficiency. Acute thrombosis is managed with heparin anticoagulation according to accepted guidelines dependent on the site and severity of disease. Long-term anticoagulation with warfarin is indicated after heparin therapy for an acute event and for prophylaxis [133,134]. Follow-up of patients involves repeated clinical evaluation in the acute setting, and monitoring is dependent on the form of anti-

coagulation selected. Low–molecular-weight heparin requires only periodic evaluation of the platelet count. Warfarin is monitored with the PT to maintain a target International Normalized Ratio of 2.0 to 3.0. Repeat analysis of the protein S level usually is not required if evaluation is performed before initiation of warfarin therapy. Subsequent measurement should not take place until several weeks have passed, as discussed with protein C. In patients who fail to respond to warfarin, long-term therapy with UFH or LMWH may be required. As with other thrombophilic disorders, the patient and family members should be counseled regarding the need for diagnostic screening and the need to intervene in high-risk circumstances. These circumstances include but are not limited to the avoidance of oral contraceptives and the control of obesity and prophylactic anticoagulation at the time of surgery, prolonged immobility, pregnancy, and the puerperium. Characteristics of hereditary protein S deficiency are summarized in Box 4.

Acquired protein S deficiency

Acquired deficiency has been documented in a variety of conditions, including disseminated intravascular coagulation [153], type I [154] and type II [155] diabetes mellitus, pregnancy [156], oral contraceptive use [157], nephrotic syndrome [158], liver disease [159], and essential thrombocythemia [160].

Box 4. Protein S defects

Clinical characteristics

- Autosomal, dominant and recessive forms
- Type I and type II exist (opposite nomenclature)
- Homozygotes often die in utero or “neonatal purpura fulminans”
- Venous thrombosis in mid-late teens
- DVT/PE most common
- Mesenteric and other unusual sites also
- Thrombosis common in heterozygotes
- Warfarin-induced skin necrosis

Laboratory characteristics

- Low protein S levels by functional assay
- Type I: low free PS; normal C4b-bound PS
- Type II: low free PS and low C4b-bound PS
- Screening/global test of hemostasis normal

Treatment

- Heparin/LMW heparin for acute events
- Protein C concentrates ?
- Oral anticoagulants
- Clopidogrel ?

Management of patients with protein S deficiency is similar to management of those with protein C deficiency. Acute thrombosis is managed with heparin anticoagulation according to accepted guidelines dependent on the site and severity of disease. Long-term anticoagulation with warfarin is indicated after heparin therapy for an acute event and for prophylaxis [140,141]. Follow-up of patients involves repeated clinical evaluation in the acute setting, and monitoring is dependent on the form of anticoagulation selected. Low-molecular-weight heparin requires only periodic evaluation of the platelet count. Warfarin is monitored with the PT to maintain a target International Normalized Ratio of 2.0 to 3.0. Repeat analysis of the protein S level usually is not required if evaluation is performed before initiation of warfarin therapy. Subsequent measurement should wait for several weeks, as discussed with protein C. In patients who fail to respond to warfarin, long-term therapy with subcutaneous UFH or LMWH may be required. As with other thrombophilic disorders, the patient and family members should be counseled regarding the need to intervene in high-risk circumstances. These circumstances include but are not limited to the avoidance of oral contraceptives and the control of obesity and prophylactic anticoagulation at the time of surgery, prolonged immobility, pregnancy, and the puerperium. The protein C and protein S systems are summarized in Fig. 3.

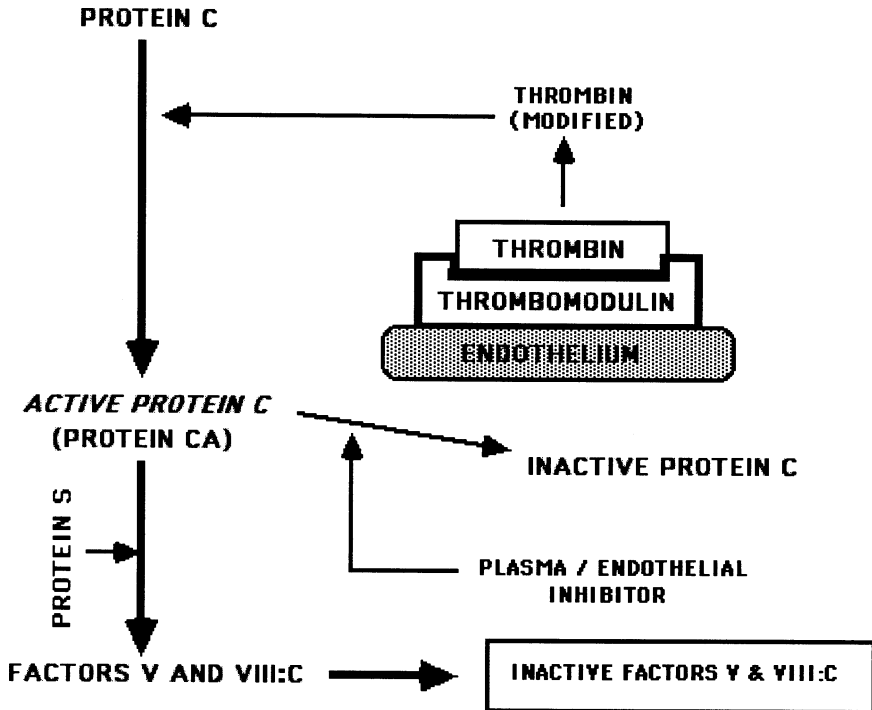


Fig. 3. Protein C and protein S activity.

Summary

These defects are not as common as factor V Leiden, but they are more common than many other hereditary procoagulant defects. The incidence of the prothrombin gene (G20210A) mutation is not yet known with certainty, but it may approach or even exceed that of factor V Leiden. These defects also seem less common than hereditary sticky platelet syndrome; however, they are all common enough that they always should be considered in any individual with unexplained thrombosis and should be part of the work-up for patients with thrombotic disorders. Of the defects discussed herein, prothrombin G20210A mutation seems, thus far, to be more common than AT, protein C, protein S, or HC-II defects. Assessment of prothrombin gene mutation should be part of the primary evaluation of patients with unexplained thrombosis.

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