# Protein C, Protein S, and Anti-Thrombine III as Natural Anticoagulant

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## INTRODUCTION

Hemostatic disorders, which could cause (venous or arterial) thrombosis or bleeding, are often found during hospital care. Common manifestations of thrombosis are inner vein thrombosis, cardiac infarct, stroke, or even recurrent miscarriage. In addition to hemorrhological defect, certain clinical conditions such as diabetes mellitus or hypercholesterolemia are risk factors that also play a role in the hemostasis system.

The hemostasis system depends on vascular endothelial conditions, platelet function (in this case platelet aggregation), coagulation function, anti-coagulation, fibrinolysis, and anti-fibrinolysis. If one of these conditions or functions is disturbed, the hemostatic system may also be disturbed.[1]

Coagulation function requires coagulant proteins. In general, coagulant proteins are classified into three groups with different functions, as follows: 1. procoagulants; 2 anticoagulants, and 3. protein fibrinolysins. There are several serine enzymes with 2 main cofactors, factors V and VIII. Active anticoagulant proteins are neutralized by the natural anti coagulant system.[1,3]

In this summary, we will discuss various proteins that are the natural anticoagulants, protein C, protein S, and antithrombine III by the synthesis, function, and the role in the hemostasis system, and if there is a reduction or dysfunction of various proteins with common manifestations and the treatment recommended by the most up-to-date reference.

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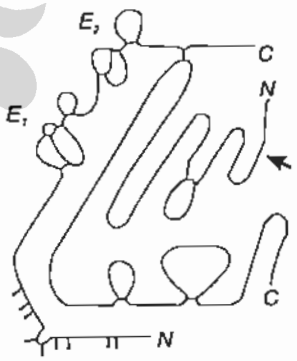
## ANTICOAGULANT PROTEINS

There are various known anticoagulant proteins, including those belonging to the *Protein C Pathway* as well as proteinase inhibitors. Proteins that are included in the C protein pathway are protein C, protein S, and thrombomodulin. Most proteins in this group depend on vitamin K (Vitamin K-dependent/VKD). Proteinase inhibitor include $a_2$-Macroglobulin; the serine proteinase inhibitor family, such as: $a_1$-Proteinase Inhibitor, antithrombin III, C1 esterase inhibitor, protein C inhibitor, Heparin II co-factor as well as Tissue Factor Inhibitor.[3]

Protein C, protein S, and antithrombin III are known as natural anti-coagulants.[2-4]

## PROTEIN C

Protein C is an anticoagulant synthesized in the liver as a single chain precursor that then undergoes matura-




Protein C
(Mr 62.000)

Figure 1. The Structure of Protein C and Its Binding Site

tion in the plasma as a double chain. Protein C contains two glycoprotein chains consisting of a single heavy chain (41kDa) that has a catalytic linkage site associated with a light chain (21kDa) that has a lipid binding site (Figure 1).[3] Just like other proteins that contain vitamin K, protein C function depends on the post-translation modifi-

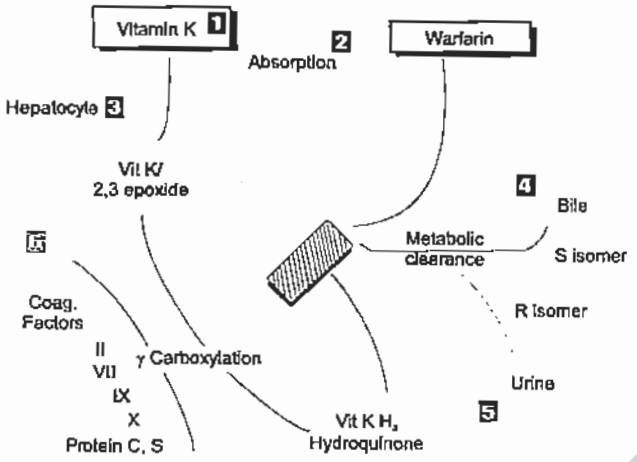



Figure 2. Vitamin K Metabolism and the Role of Warfarin

cation, including gamma-carboxylation and several glutamic acid residue. This step is highly needed for the protein to bind the phospholipids surface.[2,3]

The plasma protein C level is 4 mg/mL, and its circulatory half-life ranges from 8 to 10 hours, which is a lot shorter than other K-dependent procoagulant proteins. This difference plays a role in the hypercoagulable state that occurs during the use of anticoagulants from the coumarine group (Figure 2).[2-5] In neonates, the plasma C protein level drops to 35% (ranging around 17-53%) from the normal adult level. This level will increase to over 50% of the adult level at 6 months, to then return lower than the normal adult value until ten years of age.[6]

Protein C is a zymogene that is stimulated into the active form by thrombine by stimulating the $Arg^{169}$-$Leu^{170}$ peptide link in the heavy chain to release dodecapeptide. This thrombine-mediated activation process usually takes a long time. In vivo, the process is facilitated by the co-factor thrombomoduline. Thrombomoduline is an endothelial cell-derived protein that lies on the endothelial surface, and strongly binds thrombine. After creating a bind, the procoagulant function of thrombine is inhibited. The thrombin-linked thrombomoduline would then activate C proteins.[2,3,5]

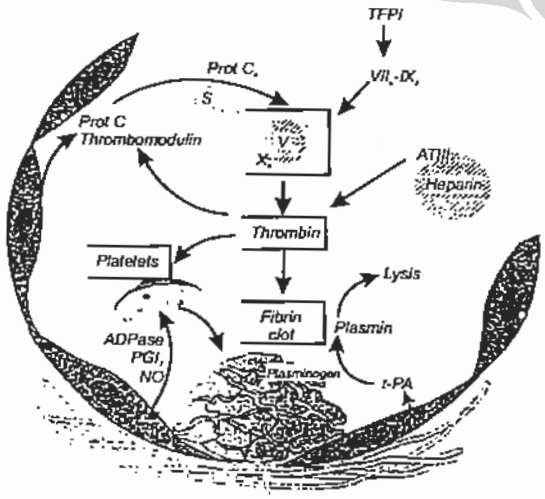



Figure 3. Normal Control of Thrombosis

Activated Protein C (APC) plays a role as an anticoagulant by inhibiting the proteolysis factors Va and VIIIa, which then inhibits the formation of two key coagulation enzymes, factors Xa and thrombine.[2,3] The activity of APC is strengthened by the aid of another vitamin-K dependent protein, protein S (Figure 3).[5] APC also indirectly increases fibrinolysis.

## ABNORMAL PLASMA PROTEIN C LEVELS

Protein C deficiency is found in 0.14-0.5% of the general population. Up to 3% of patients suffer from primary venous thrombosis without malignancy, and up to 9% of patients with venous thrombosis are under 70 years. Patients usually suffer from primary venous thrombosis at an age of 10-50 years.[6]

Protein C deficiency may be hereditary or acquired. Hereditary protein C deficiency is found as heterozygotic and homozygotic conditions.[3,6] If heterozygote, the risk for venous thrombosis reaches 7 times normal condition. The protein C level in heterozygotes ranges from 35 to 65% of the normal adult level. Patients with homozygotic protein C deficiency are commonly found as neonates with fulminate purpura and disseminated intravascular coagulation (DIC).[3,6]

There are two types of protein C deficiency. The first type is a deficiency in the number and function of protein C. The second type is where the plasma protein C level remains normal, but is dysfunctional.[3-6] Type II may be caused by mutations at the thrombomoduline binding site, at the serine protease site, or at glutamate-rich sites.[4]

Acquired protein C deficiency is often caused by reduced synthesis due to liver disease, disseminated intravascular coagulation, widespread venous thrombosis, infection, major operations, adult respiratory distress syndrome, and uremic hemolytic syndrome. In addition, it is also found in patients under treatment with L-asparginase due to reduced liver synthesis.[3,4,6,7] Protein C levels have also been reported to be reduced due to auto-antibodies.[6] The most common cause is due to the use of oral anticoagulants and vitamin K deficiency. Oral anticoagulants also disturb laboratory measurements of protein C levels, since it reduces the plasma protein C level to 50% of normal levels,[2] thus it has been recommended that administration oral anticoagulants be terminated ten days prior to the evaluation.[6] Warfarin also increases the risk of skin necrosis if administered without prior

administration of heparin.[2]

Protein C levels have been reported to increase during pregnancy and the use of oral contraception. In patients with nephritic syndrome, plasma protein C levels may be increased, reduced, or remain normal.[6] There also have been reports that protein C levels are lower in women than in man, and in pre-menopause women, the level is a little lower than in post-menopausal women.[6] In patients with renal failure undergoing hemodialysis, there is an increase in protein C level and function, associated with reduced risks of thrombosis in patients undergoing hemodialysis. The activity of protein C is also reduced in antiphospholipid syndrome.[8]

## EVALUATION OF PLASMA PROTEIN C

Protein C is usually evaluated using assay techniques. Aside from screening, protein C function must also be evaluated. If blood coagulation is evaluated based on PTT, factor VIII is also evaluated. Increased factor VIII can reduce protein C during blood clot evaluation based on PTT. Factor VIII may be disregarded if chromogenicity is being evaluated. Increased factor VIII is usually associated with acute reaction phase. If during an evaluation there is reduced protein C function, protein C antigen levels must be evaluated to differentiate type I and type II.

If there is reduced protein C levels, the possible primary disease should be determined. Evaluations must be repeated after the primary disease is resolved. Administration of oral anticoagulants such as coumarine must be terminated 10 days prior to evaluation and laboratory tests required to determine the primary disease should be performed. In cases of liver disease, liver function tests, such as enzyme evaluation and serum albumin levels are evaluated. Screening for disseminated intravascular coagulation, such as D-dimer or fibrin degradation products (FDPs), PT, PTT, fibrinogen, and platelet levels should be evaluated. In cases of hereditary protein C deficiency, DNA evaluation does not provide satisfactory results. Thus, another approach may be required, such as evaluation of protein C levels in families with history of thrombosis.[6]

## MANAGEMENT OF PROTEIN C DEFICIENCY

In cases of protein C deficiency, fresh frozen plasma may be used as a replacement. Protein C concentrates are now also available at a high cost.[2,6] Protein C concentrate is best used in cases of homozygotic protein C deficiency, but is not used in heterozygotes.[2] This form of management is only taken as a life-saving measure, and one of the main treatments is liver transplantation.[2] On the other hand, it is recommended that protein C deficiency during pregnancy be treated with anticoagulant treatment, such as heparin.[9,10] In non-pregnant patients, oral anti-coagulants may be administered to reduce coagulation factors, even though this would reduce protein C levels.

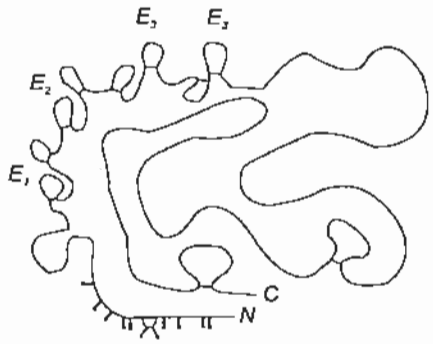
## ACTIVATED PROTEIN C

Activated protein C (APC) plays a role in inhibiting protein FVa proteolysis, with the mediation of protein S. FVa is a cofactor of FXa in thrombine formation from prothrombine. Factor V is a polypeptide with a molecular weight of 330 kDa, which would be rapidly activated by thrombine into FVa. APC destroys FVa by forming a link that would reduce FVa activity on various sites on the molecule. The half-life from APC is 20 minutes. The first binding site is arginine. Replacing arginine with another product causes resistance towards APC linkage. If one of the arginine sites is replaced by glutamine, APC resistance ensues, which is often known as Leiden factor V. Adding APCs in the plasma of patients with Leiden factor V fails to induce the anticoagulant response.[2,3,11]

Hereditary APC resistance (APCR) appears in homozygotic and heterozygotic forms. Clinical studies found that APC resistance is found in 40% of patients with idiopathic venous thrombosis.[2,12] APCR is also associated with thrombosis during pregnancy,[2,13] and factors that play a role in the development of pre-eclampsia.[13-15] Coagulation activation in ischemic heart disease undergoing thrombolytic treatment is also associated with an increased rate of APCR.16 However, there have been reports that APCR is not correlated in young patients with cardiac infarct.[17,18]

## PROTEIN S

Protein S was discovered in 1977. It is a VCD glycoprotein with a molecular weight reaching 70 kDa an is not a zymogene.[2,3,4,5,19] The protein is synthesized within hepatocytes, endothelial cells, and megakaryocytes,[2,6] and has also been reported to be synthesized by neural tumor cells.[3] There are three genes coding protein S, both of which are located on chromosome 3.[3,20] Approximately 50-60% of protein S is bind with binding protein C4b, and the rest are in free form.[2-6,19,20] Only free protein S has anticoagulant function, while those bound to C4b inhibits free protein S as an anticoagulant. C4b may increase up to 4 times in inflammation due to acute phase




*Protein S*

*(Mr 70.700)*

Figure 4. Protein S

reactants.[2-6,19,20]

Normal plasma S levels range around 20-25 ìg/mL, and its half-life is approximately 42 hours.[3] At birth, babies have a lower protein S level up to 36% (ranging from 12-60%) of the normal adult value. Protein S levels increases to normal levels at 6 months of age. Infant protein S is found in free form, due to very low C4b levels. This assists the effort to deal with low protein S levels in neonates.[2,6]

The function of protein S is to act as cofactor for APC in inhibiting the activation of factors VIIIa and Va. In addition to acting as an anticoagulant, protein S also has a mitogenic activity and increases the proliferation of smooth muscle cells in the aorta. This creates the speculation that protein S contributes in the proliferation of smooth muscle cells that causes aterogenesis.[3]

## ABNORMAL PROTEIN S LEVELS

Hereditary protein S deficiency is found in 0.7% of the general population. There are as many as 2% in unselected patients with venous thrombosis, and increases to 7.6% in patients with thrombosis under the age of 70 years.[6] However, there has been a report that in patients under 45 years of age with idiopathic venous thrombosis, the number reaches 5-10%.[2] Heterozygotic patients has a strong tendency for venous thrombosis, while homozygotic patients usually present severe clinical conditions or fulminate purpura in neonates, up to the point of death.[6,12] Protein S deficiency is associated with deep vein thrombosis and surface thrombophlebitis in 33% of cases, and pulmonary embolism in 38% of cases.[2,21-23] Low S protein levels are also found in young patients with recurrent stroke, where 10 out of 13 patients were female.[20]

Acquired protein S deficiency may be due to the use of estrogen in oral contraceptives, pregnancy, estrogen replacement treatment, and l-asparginase therapy. In addition, it is also found in liver disease, active thrombosis, disseminated intravascular coagulation, and major surgery. In liver disease, the level is sometimes normal, which is possible due to extra-hepatic synthesis. Protein S is also reduced during HIV infection, nephrotic syndrome, and Crohn's disease, or ulcerative colitis. There has also been a report of protein S deficiency due to auto-antibody disease, even though this is rare. Varicella virus infection has also been reported to be a cause.[2,3,5,6,9] The level of Protein S in patients with diabetes mellitus type 1 is low, while in diabetes mellitus type 2, the level increases according to cholesterol level.[2] Administration of coumarine in patients with protein S deficiency causes necrosis of the skin and lipid tissue.[2] Women have a slightly lower level of protein S compard to men. Premenopausal women also have a slightly lower level compared to post-menopausal women.[6]

There are three types of protein S deficiency. The first type is due to a reduction in the number and function of protein S. In type II, the total protein S count and free protein S remain normal, but the function is reduced. In type III, the function and number of free protein S is reduced, while the total count remains normal.

## EVALUATION OF PROTEIN S DEFICIENCY

Evaluation of function is used for screening. Usually, immunoassay is used to measure the level of protein S. If there is reduced function, the next step is to measure the level of free protein S. If the level is also reduced, total protein S level is then assessed. Factor VIII can influence the evaluation of protein S using blood clot evaluation based on PTT. Patients that are to undergo protein S evaluation should terminate their use of oral contraceptives and should not be pregnant for 1-2 months prior to the evaluation. Patients are also not allowed to use oral anticoagulants such as coumarin for 10 days prior to the assessment.[6]

Just as for protein C evaluation, all patients with conditions that may reduce the level of protein S should undergo treatment for their condition and undergo a repeat evaluation. To determine whether the protein S deficiency is hereditary or acquired, the same procedure may be used as for protein C.[6]

## TREATMENT FOR PROTEIN S DEFICIENCY

Administration of fresh frozen plasma may assist increasing plasma protein S.[2] To prevent thrombosis during pregnancy, administration heparin or low molecular-weight heparin is recommended for patients with protein S deficiency during pregnancy.[9,10] Warfarin may also be administered during the pre-conception period.[10] Another report states that warfarin is unable to prevent recurrent stroke.[20] Administration of warfarin, like that of protein C, must be preceeded with administration of heparin.[2]

### Antithrombine III

This molecule was first isolated by Abilgaard in 1968 as a member of the large family of serine-protease inhibitors. It is also known as a natural inhibitor. Antithrombine III (AT III) is most widely studied compared of the other natural anticoagulants, and plays a role in several coagulation pathways.[2] There are two types of AT III, ATIIIα and ATIIIβ, which accounts for 5-10% of the plasma AT III. ATIIIβ has the strongest ability to bind with heparin. AT III is mostly synthesized within hepatocytes, including DCV.[3,24] A mature protein weighs 58.[2] kDa and consists of 432 amino acid glycoproteins and three internal disulfide linkages.[2]

The normal plasma AT III level is kdkdkdk with a half life of 61-72 hours.[3] AT levels in neonates drop to 63% (ranging from 39 to 87%) compared to the normal adult level. In the age of 6 months, the level returns to the normal adult level. The reduction in antithrombine level is balanced by a high level of alpha2-macroglobulin, a natural thrombine inhibitor.[6] The AT III level does not change in patients undergoing hemodialysis, but falls in cases of nephrotic syndrome.8 AT III levels in premenopausal women is lower than in men, while that of post-menopausal women is higher than that of men.[6]

AT III inhibits the coagulation of all serine enzymes such as factors XIIa, XIa, IXa, Xa, thrombine, as well as other factors by forming a serine-protease complex.[24-26] Thus, the name AT III is actually incorrect (a misnomer), since it does not only inhibit thrombine. Its main target is to inhibit the formation of factor Xa and thrombine.[2,24] AT III plays a role in the three coagulation pathways: the extrinsic, intrinsic and combined pathways (Figure 5).[6,27]

Heparin facilitates AT III interaction with protease as a target up to 2000 times.[2,24] After the serine-protease complex is formed, heparin is then released to rebind with other AT IIIs. In addition, the heparan sulphate within endothelial cells can strengthen the inhibitory action of AT III on blood vessels. The interaction between heparan sulphate and AT III also releases

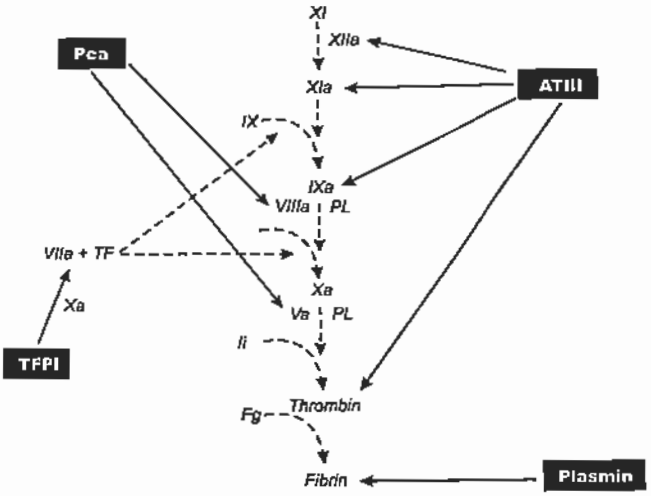



Figure 5. The Role of AT III in the Coagulation Process

prostacycline as a platelet-aggregationinhibitor.[6,24] Okajima reported that AT III is also able to inhibit leukocyte activation in laboratory animals.[28]

### Abnormal Antithrombine III Levels

Antithrombine deficiency is found in 0.17% of the general population, and reaches 1.1% of all unselected patients with venous thrombo-emboli, and 5% of patients with thrombosis under the age of 70 years.[2,6] Homozygote AT III deficiency is life-threatening, since it causes severe venous thrombosis,[2,6] while 20% of patients with heterozygote AT III deficiency may avoid the risk of thrombosis.[2]

There are two types of AT III deficiency. Type I is associated with reduced AT III levels and function, while in type II, the level may be normal, but the function is reduced. Type II is further classified into the following three groups: 1. abnormal thrombine reactive binding site, 2. abnormal heparin binding site, 3. abnormality of both sites. In type IIb, the risk of thrombosis is relatively lower than in the other groups.[6]

Acquired AT III deficiency may occur during pregnancy, the use of contraceptive pills, and during surgery. These three conditions aggravate the condition of patients with heterozygote AT III deficiency.[2] Reduced AT III is also associated with reduced synthesis, such as in liver disease, and L-asparginase treatment as well as malignancy,[29,30] increased used such as in disseminated intravascular coagulation and sepsis/infection, or increased output such as in nephrotic syndrome and colitis.[2,6,20] Oral contraceptives, particularly estrogen, can bind AT III, thus reducing plasma levels.[2,30] Administration of full-dose heparin can temporarily reduce AT III levels in few days, which would then increase to normal after termination of heparin administration.[6]

### Evaluation of Antithrombine III Levels

Function is first assessed as a screening test. If there is reduced function, antigen evaluation to calculate the number of AT III is then performed in order to determine the type of AT III deficiency. Just like in the evaluation for protein C and S, the possibility of all diseases that can cause AT III reduction should be eliminated in order to be able to establish the diagnosis of hereditary deficiency.[6,30]

### Management of Antithrombine III Deficiency

Heparin is used to manage AT III deficiency, even though high doses are sometimes required. At this moment, we could also use AT III concentrate (R/Kybernin) to increase plasma levels. AT III concentrates can also be used to manage disseminated intravascular coagulation and sepsis, which provides insignificant yet satisfactory results.[30,31] In cases of symptomatic hereditary disorder, warfarin is administered throughout life.[2]

## CONCLUSION

Based on the discussion above, we know that as natural anticoagulants, the mechanism of action and target of protein C, protein S, and antithrombine III are very unique. However, we also know that the three are linked to heparin in the treatment of deficiency, aside from fresh frozen plasma.

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