Original Article

Association of Protein C, S and Antithrombin III Deficiencies with Thrombosis and Stroke

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DOI
10.21276/apalm.3796

Article History
Received: 26-12-2025
Revised: 01-02-2026
Accepted: 12-03-2026
Published: 06-04-2026

How to cite this article
Chauhan NB, Patel P, Prajapati J, et al. Association of Protein C, S and Antithrombin III Deficiencies with Thrombosis and Stroke. Ann Pathol Lab Med. 2026;13(4):A205-A211.

Abstract

Background: Isolated deficiencies of Protein C, Protein S, Antithrombin III and Plasminogen have been implicated as a cause of thrombosis. Patients with recurrent, familial, or early onset thrombosis are more likely to have these deficiencies.
Method: The present study is conducted retrospectively over a period of one year to know the relationship between deficiency of natural anticoagulants (Protein C, Protein S, and Antithrombin III) with thrombosis and stroke. We have taken 130 cases, and levels of natural anticoagulants were measured for each case with an automated coagulation analyzer.
Aims and objectives: 1) To study the frequency of protein C, protein S and antithrombin III in patient of thrombosis and/or stroke 2) To observe the pattern and distribution of natural anticoagulant deficiencies among these patients 3) To correlate findings with clinical presentation.
Result: A total of 130 patients with thrombotic events or stroke were evaluated for natural anticoagulant deficiencies, of whom 48.46% showed one or more deficiencies. Protein S deficiency was the most common abnormality, occurring alone or in combination, particularly in the 21–40 years age group. Deep venous thrombosis was the most frequent clinical presentation and showed a strong association with anticoagulant deficiencies. Deficiencies were slightly more prevalent in females. Combined deficiencies were more common in younger adults, while older patients more often had no detectable deficiency.
Conclusion: Protein S, protein C, and antithrombin III deficiencies are associated with a high risk of thrombosis. Screening for these deficiencies, followed by thromboprophylaxis, may help in reducing episodes of provoked venous thromboembolism.

Keywords: protein C; protein S; antithrombin III; thrombosis; stroke

Introduction

Each step of the coagulation cascade is regulated by a specific natural protein inhibitor, which helps prevent excessive clot formation and uncontrolled thrombosis. These proteins are tissue factor pathway inhibitor (TFPI), antithrombin III, protein C, and protein S.[1] Thrombophilia refers to conditions, whether genetic or acquired, that elevate the risk of developing blood clots.[2] It is a condition characterized by a predilection for clots to occur spontaneously in circulation as a result of enhanced coagulation.[3] It is characterized by onset at an early age, frequent recurrence and family history.[4]

Inherited (hereditary) thrombophilia describes a genetic predisposition that increases an individual's risk of developing thrombosis.[5] The most clearly defined heritable thrombophilias are the factor V Leiden variant (F5 G1691A), the prothrombin gene variant (F2 G20210A), protein C deficiency, protein S deficiency, and antithrombin III deficiency.[2]

Protein C (PC)

Protein C is a vitamin K–dependent anticoagulant protein. It is converted to its active form, activated protein C (APC), by thrombin when thrombin is bound to thrombomodulin on the endothelial cell surface. In the presence of protein S and calcium ions, APC inactivates coagulation factors Va and VIIIa, thereby limiting clot formation.[5] The normal plasma range is 0.70–1.40 iu/ml.[6]

Protein S (PS)

Protein S is also a vitamin K-dependent protein that acts as a cofactor for activated PC.[6] PS circulates in two forms: an inactive form bound to C4b-binding protein (60% of total PS) and an unbound or free form (40% of total PS). Only free PS has PC cofactor activity.[5] Free protein S is considered the most accurate parameter and is most closely linked to the risk of thrombosis. The normal range is 0.60 to 1.35 iu/ml.[6]

Antithrombin III

Antithrombin III is the principal natural inhibitor of thrombin as well as coagulation factors IXa, Xa, and XIa. Antithrombin deficiency, which may be either inherited or acquired, is identified in about 2% of patients with thrombosis. Normal antithrombin levels range from 0.75 to 1.25 iu/ml.[6]

Materials and Methods

The present study was a retrospective study conducted in the Hematology section of the Department of Pathology, B. J. Medical College, Ahmedabad, over a period of one year from January 2024 to December 2024. A total of 130 samples from patients with thrombosis and/or stroke were included.

Detailed clinical data of age, sex, clinical histories were retrieved from the Laboratory Information system (LIS) and laboratory request form. Blood samples were collected in 3.2% sodium citrate vacutainers by clinicians and sent to the hematology laboratory. The samples were then centrifuged to obtain platelet-poor plasma and transferred into cuvettes labelled with patient's name, age, sex and MRD number.

Plasma samples were stored in the blood bank freezer at temperatures of −80 °C and were analyzed weekly (every Wednesday) using a fully automated coagulation analyzer (ACL TOP 500, Instrumentation Laboratory, USA). Thus, the maximum storage duration prior to analysis did not exceed seven days.

Protein C, Protein S, and Antithrombin III levels were measured using reagent kits manufactured by Instrumentation Laboratory Company, Bedford, USA. Protein C was assessed using the HemosIL Protein C reagent kit (lot numbers- N0733458, N0835085 and N0249476). Protein S was measured using the HemosIL Free Protein S reagent kit (lot numbers- B35361, B36422 and B37137), while Antithrombin III was analyzed using the HemosIL Liquid Antithrombin reagent kit (lot numbers- N1029722, N0431293 and N0633341). All reagents were color sensitive and were stored at 2–8 °C as per manufacturer's instructions.

Reference ranges for Protein C, Protein S, and Antithrombin III were based on laboratory-established ranges validated in our institution using routine internal quality control procedures. The cut-off values for defining deficiency of Protein C, Protein S and Antithrombin III were 70%, 60%, and 75%, respectively, with values below these thresholds considered deficient.

Inclusion Criteria: All the patients with thrombosis and/or stroke who provided sample for analysis.

Exclusion Criteria: Clotted samples, Sample quantity insufficient, Hemolyzed samples, Lipemic samples

Results

Total of 130 patients with documented thrombotic events and/or stroke were evaluated for deficiencies of natural anticoagulants, namely Protein C, Protein S and Antithrombin III.

Patients were commonly presented with complaints of upper or lower limb weakness with or without pain, difficulty in walking, pedal edema, slurring of speech, convulsion, altered sensorium, headache, and giddiness, some also reported abdominal pain, fever, vomiting and breathlessness.

A history of recurrent pregnancy loss was observed in five patients: three with cerebral venous sinus thrombosis, one with deep venous thrombosis, and one with ischemic stroke. Among these patients, two demonstrated one or more thrombophilia

marker deficiencies.

A history of prolonged immobilization was documented in two patients (1.54%). One patient was a 63-year-old male who demonstrated combined Protein C and Antithrombin III deficiency, while the other was a 16-year-old male with Protein S deficiency.

Two patients in the present study were known carriers of the Factor V Leiden mutation. Both patients were 28 years old, one male and one female, and presented with ischemic stroke. Both were additionally found to have Protein S deficiency, indicating the presence of combined inherited thrombophilia. Factor V Leiden mutation testing was not performed uniformly across the study cohort, these two patients had documented positive results from prior evaluations available in their medical records.

Of the 130 patients, 68 (52.31%) were males and 62 (47.69%) were females. Deficiency of one or more natural anticoagulants was observed in 30 males (44.12%) and 33 females (53.23%). Overall, 63 patients (48.46%) demonstrated isolated or combined anticoagulant deficiencies, while 67 patients (51.54%) showed no detectable deficiency. The distribution of individual and combined deficiencies is shown in Figure 1.

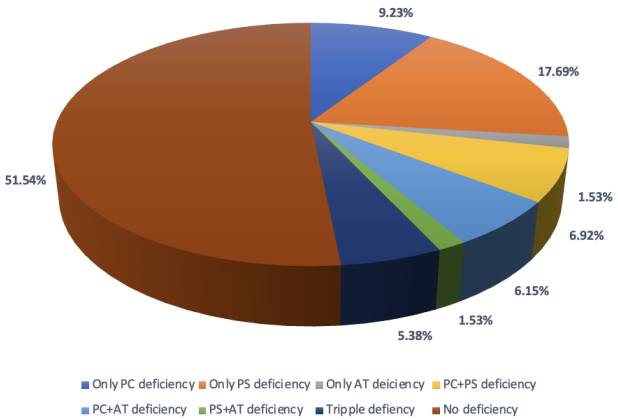

Figure 1: Distribution of individual and combined deficiencies of natural anticoagulant deficiencies in the study population. Abbreviations: PC- Protein C, PS- Protein S, AT- Antithrombin.

Age-wise distribution of Protein C, Protein S, and Antithrombin III deficiencies is presented in Table 1. Deficiencies were noted across all age groups, with a higher frequency among young and middle-aged adults (21–30 and 31–40 years). Protein S deficiency was the most common abnormality, occurring both as an isolated defect and in combination with other deficiencies. Combined deficiencies were more frequent in the third and fourth decades, whereas isolated Antithrombin III deficiency was uncommon. Older age groups showed a higher proportion of patients without detectable deficiency.

The distribution of anticoagulant deficiencies according to thrombotic events is summarized in Table 2. Deep venous thrombosis was the most frequent presentation and showed a high proportion of associated deficiencies. Cerebral venous sinus thrombosis and ischemic stroke were also commonly associated with anticoagulant deficiencies.

Abbreviations: PC- Protein C, PS- Protein S, AT- Antithrombin, def- deficiency, IJV- Internal jugular vein, IVC- Inferior Vena Cava

Discussion

Ischemic heart disease and stroke are the leading global causes of death, accounting for approximately 7 million and 6.2 million deaths annually, respectively. Thrombosis may result from congenital or acquired factors; although inherited thrombophilia often presents early, first thrombotic events can also occur later in life. Accurate prevention and management therefore require detailed clinical assessment and comprehensive diagnostic evaluation.[5] This study aimed to evaluate the frequency, distribution, and clinical correlation of Protein C, Protein S, and Antithrombin III deficiencies in patients with thrombosis and/or stroke.

In the present study, 130 patients with thrombosis or stroke were evaluated. Out of which 68 (52.31%) were males and 62 (47.69%) were females, which was comparable with study by Gupta M et al[7] (52.07% males and 47.93% females), Vashist et al[8] (57.5% males and 42.5% females) and K. Ghosh et al[9] (58.33% males and 41.66% females).

Overall, 48.46% of patients demonstrated one or more natural anticoagulant deficiencies (alone or combined), which was comparable with Mishra MN & Bedi VS[10] (56%), Ichiyama et al[11] (40%), K Ghosh et al[9] (34%), Gupta M et al[7] (38%) and Mishra et al[12] (38.8%) studies.

Table 1: Age-wise distribution of Protein S, Protein C and Antithrombin III deficiencies.

Age group	Total samples	Only PC Deficient	Only PS Deficient	Only AT Deficient	PC+PS	PC+AT	PS+AT	Triple deficiency	No deficiency
0–10	6	1	0	0	1	0	0	2	2
11–20	15	3	1	0	2	1	0	1	7
21–30	37	2	9	0	3	2	1	2	18
31–40	31	4	5	1	1	0	0	1	19
41–50	25	2	5	1	2	3	0	1	11
51–60	8	0	1	0	0	1	0	0	6
61–70	5	0	1	0	0	1	1	0	2
71 onwards	3	0	1	0	0	0	0	0	2
Total	130	12	23	2	9	8	2	7	67

Table 2: Distribution of natural anticoagulant deficiencies in various thrombotic events.

Presentation	Total cases	Only PC def.	Only PS def.	Only AT def.	PC+PS def.	PC+AT def.	PS+AT def.	Triple def.	No def.	Total deficient
Brain infarct (stroke)	43	3	13	0	1	1	0	0	25	18 (41.86%)
Cerebral venous sinus thrombosis (CVST)	37	4	5	1	3	0	0	3	21	16 (43.24%)
Deep venous thrombosis (DVT)	18	4	2	0	2	1	1	1	7	11 (61.11%)
Portal vein thrombosis	11	1	1	0	1	2	1	2	3	8 (72.72%)
CVST + Brain infarct	6	0	1	0	1	0	0	0	4	2 (33.33%)
Thrombosis with Budd–Chiari syndrome	4	0	0	0	1	2	0	1	0	4 (100%)
Cerebral arterial thrombosis	3	0	0	0	0	0	0	0	3	0 (0%)
Arterial thrombosis (axillary & brachial)	2	0	0	0	0	1	0	0	1	1 (50%)
IVC thrombus	1	0	0	0	0	0	0	0	1	0 (0%)
IJV thrombosis with axillary & subclavian artery thrombosis	1	0	0	0	0	0	0	0	1	0 (0%)
Pulmonary thromboembolism	2	0	0	1	0	1	0	0	0	2 (100%)
Atherosclerotic changes in leg veins	1	0	1	0	0	0	0	0	0	1 (100%)
Pulmonary vein thrombosis	1	0	0	0	0	0	0	0	1	0 (0%)
Total	130	12	23	2	9	8	2	7	67 (51.54%)	63 (48.46%)

The frequency of individual deficiencies - Protein S (30%), Protein C (27.69%), and AT III (14.61%). When compared with other studies, present study shows higher levels of natural anticoagulant abnormalities. Present study shows that Protein

S deficiency was the most common followed by Protein C, which was also comparable with study by Mishra et al[12], Mishra MN & Bedi VS[10] and Agrawal et al[13] studies. Isolated AT III deficiency was less common than Protein C and Protein S deficiency which was compared with study by Mishra MN & Bedi VS[10] and Agrawal et al[13], Bhattacharyya et al[14], Bhakuni et al[15] and Shein et al[16] studies. Shipra Singhal et al.[17] reported findings consistent with the present study regarding protein C and protein S deficiencies, although antithrombin III deficiency was more frequent in their study. Combined deficiencies were identified in 20% of patients, higher than the 5–10% range reported in most other studies[7, 13, 16, 18]. Table 3 and 4 showing the comparison across various studies.

These discrepancies are likely because of differences in cohort selection across studies, including variation in the proportion of arterial versus venous thrombotic events, inclusion of pediatric populations, and differences in defining inherited thrombophilia (transient reductions in plasma levels versus genetically confirmed deficiencies). The present study includes a broad spectrum of venous and arterial thrombotic events, such as cerebral venous sinus thrombosis, Budd–Chiari syndrome, portal vein thrombosis, and pulmonary thromboembolism. The findings of the present study support this heterogeneity, with natural anticoagulant deficiencies being most frequently observed in patients with Budd–Chiari syndrome (100%), portal vein thrombosis (72.72%), and deep vein thrombosis (61.11%). These thrombotic conditions have been reported to be associated with inherited anticoagulant deficiencies in several studies, including those by Bhattacharyya et al[14] and Agrawal et al[13]. The age distribution of our cases may contribute to the elevated prevalence. Nearly half of the deficient patients were between 21 and 40 years of age–a group in which inherited thrombophilia is more likely to manifest clinically. Comparable findings have been documented by Pai et al[19] and Mishra et al[12] who emphasized the predominance of hereditary thrombophilia markers in young patients presenting with thrombosis.

Prolonged immobilization, although documented in a small proportion of patients, represents an important acquired risk factor and may have acted synergistically with underlying anticoagulant deficiencies in precipitating thrombotic events.

The coexistence of Protein S deficiency and Factor V Leiden mutation observed in the present study is clinically significant, as combined thrombophilic defects have been reported to increase thrombotic risk compared with isolated abnormalities.[20] Ischemic stroke at a young age in these patients suggests a synergistic effect of multiple prothrombotic factors. However, as Factor V Leiden testing was not routinely performed, the prevalence of combined thrombophilia in this cohort may be underestimated.

The present study addresses several important gaps in the existing Indian literature on thrombophilia. Region-specific data from western India regarding the prevalence and pattern of natural anticoagulant deficiencies remain limited, with most published Indian studies originating from northern or southern regions.[7, 9, 14] Previous Indian studies have largely focused on isolated venous thromboembolism or selected younger patient populations, often excluding arterial thrombosis and thrombosis at unusual sites.[7, 10, 13, 14] Consequently, data integrating both venous and arterial thrombotic events within a single cohort are limited.[7, 10] The present cohort is distinct in that it includes a wide spectrum of thrombotic presentations, including cerebral venous sinus thrombosis, portal vein thrombosis, Budd–Chiari syndrome, and ischemic stroke, which have been reported to show stronger associations with inherited thrombophilia.[13, 14, 19] As a tertiary care referral center, our institution also manages a higher number of patients with severe, recurrent, or thrombosis occurring at unusual anatomical sites, which may also explain the higher prevalence and distinct distribution of Protein C, Protein S, and Antithrombin III deficiencies observed in this cohort compared to previously published Indian studies.[7, 9, 10, 13, 14]

A key strength of this study is the inclusion of diverse thrombotic presentations. However, its retrospective design limits control over confounding factors such as acute-phase reactions, liver disease, anticoagulant therapy, and pregnancy, which may affect protein levels. In addition, genetic confirmation of deficiencies (e.g., *SERPINC1*, *PROC*, and *PROS1* mutations) was not performed, limiting differentiation between inherited and acquired causes.

Table 3: Comparison of protein C, S and Antithrombin III deficiencies with other studies.

Study	Protein C deficiency	Protein S deficiency	AT III deficiency
Present study (n=130)	27.69%	30%	14.61%
Mishra et al[12] (n=85)	4.7%	9.4%	7%
Mishra MN & Bedi VS 2010[10] (n=78)	7.7%	16.6%	6.4%
Bhakuni et al.[15] (n=1950)	10.76%	8.7%	2.66%
Bhattacharyya et al.[14] (n=78)	21.1%	19.0%	6.4%
Vashist et al.[8] (n=40)	42.5%	12.5%	–
Shipra Singhal et al.[17] (n=306)	21.67%	30%	30%

Conclusion

Natural anticoagulant deficiencies are common among patients presenting with thrombosis or stroke in our tertiary care setting, with nearly half demonstrating reduced levels of Protein C, Protein S, or Antithrombin III. Protein S deficiency

Table 4: Comparison of protein C, S and Antithrombin III and combined deficiencies with other studies.

Study	Protein C deficiency	Protein S deficiency	AT III deficiency	Combined deficiency
Present study (n=130)	9.23%	17.69%	1.53%	20%
Agrawal et al.[13] (n=166)	7.22%	12.04%	4.8%	9.63%
Shen et al.[16] (n=85)	32.9%	18.8%	3.2%	3.5%
Gupta M et al.[7] (n=121)	18.2%	10.7%	0%	5%
P.K. Gupta et al.[18]	22.5%	30%	–	12.5%

was most prevalent, followed by Protein C and AT III deficiencies. The high frequency of abnormalities in patients with CVST, DVT, portal vein thrombosis, and Budd–Chiari syndrome highlights the importance of evaluating these pathways in young patients and those presenting with thrombosis at unusual anatomical sites. These results underline the need for repeat testing after recovery, genetic confirmation when feasible, and standardized laboratory protocols to reliably identify inherited thrombophilia. While our data support the contribution of natural anticoagulant deficiencies to thrombotic risk in younger patients, larger studies are required to establish the true prevalence and clinical significance of these abnormalities in the Indian population. Early identification allows appropriate counselling, selection of long-term anticoagulation strategies, and prophylaxis in high-risk situations, potentially reducing recurrence of thrombotic events.

Acknowledgements: I extend my gratitude to my mentors, peers and laboratory team for their support and assistance during this study.

Funding: None

Competing Interests: No conflict of interest

Statement of Ethical Approval for the study: Ethical approval was obtained from the college's ethical committee.

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