Inherited and acquired thrombophilia as a modifier of clinical course of chronic immune thrombocytopenia

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Abstract:
Patients with immune thrombocytopenia (ITP) exhibit striking heterogeneity in bleeding manifestations even at similar platelet counts. We report the prevalence and impact of thrombophilia marker expression in chronic ITP patients. For the present study, patients with chronic ITP were clinically assessed at regular intervals using the bleeding assessment tool for ITP, and bleeding was compared among patients with and without thrombophilia marker expression (thrombophilia markers analyzed included clot-based assays for protein C, protein S, Pro C Global®, FVIII levels, and lupus anticoagulant assay). Thirty-six patients (25.5%) tested positive for at least one thrombophilia marker, and the remaining 105 patients (74.5%) were negative for all markers. Patients expressing at least one thrombophilia marker had significantly less bleeding than those without. We conclude that a part of heterogeneity in the clinical presentation of chronic ITP can be explained by the presence of thrombophilia.

Keywords: Acquired thrombophilia, chronic immune thrombocytopenia, inherited thrombophilia

Introduction
It is a common observation that patients with immune thrombocytopenia (ITP) exhibit striking heterogeneity in bleeding manifestations even at similar platelet counts. For instance, many patients have bleeding manifestations at platelet counts below 30 × 10⁹/L (or lower) while others do not exhibit any bleeding even at lower platelet counts. Furthermore, registry data showing four to five times higher thromboembolic events in patients of ITP (despite low platelet counts) compared to the healthy population.¹² Some of the mechanisms that explain this heterogeneity include increased generation of platelet and red cell microparticles,³⁴ complement activation, nitric oxide depletion, and impact of various treatment modalities such as steroids, intravenous immunoglobulins, and splenectomy.⁶⁻¹⁰ The presence of various thrombophilia markers (such as protein C, protein S, antiphospholipid antibodies, and FVIII) has not been evaluated, particularly in patients with chronic ITP as a modifier of the clinical bleeding. In the present study, we report the prevalence and impact of thrombophilia markers in chronic ITP patients.

Methods
Patients diagnosed chronic ITP (age 15–45 years of age) were eligible. Patients with acute or persistent ITP, pregnancy, and patients with secondary ITP were excluded from the study. Eligible patients at the Hematology Department of Nil Ratan Sircar Medical College, Kolkata, India, were recruited from July 2012 to July 2014, with study patients followed up at...
regular intervals till July 2015. All patients clinically assessed at regular intervals using the bleeding assessment tool (ITP-Bleeding Assessment Tool, version 1.0). Baseline thrombophilia profile obtained for all patients at study entry (clot-based assays for protein C, protein S, Pro C Global®, FVIII levels, and lupus anticoagulant assay – done on CA-500 fully automated coagulation analyzer, kits from Siemens Inc.). Patients expressing at least one thrombophilia marker were compared to those without for the number of times patients in each group presented with a bleeding episode. As the total number of visits could be different in both groups, we derived the parameter bleeding episodes/visit to estimate the severity of bleeding in each group, calculated as follows:

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\text{Total number of bleeding episodes during the period of follow-up (mild or severe)} = \frac{\text{Total number of times patient attended the outdoor during the period of follow-up}}{\text{BE/visit}}
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For analyses, the severity of bleeding was defined as no bleeding (bleeding score zero at all domains, i.e., the skin, mucosa, and organ), mild bleeding (skin and mucosal bleeding 0–2 and organ bleeding 0–1), and severe bleeding (skin and mucosa >2 and organ bleeding >1).

The treatment decisions were based on the treating physician’s discretion.

Statistical analysis of experimental data was performed by the Biostatistics Department, NRS Hospital using MedCalc Statistical Software version 17.2 (File name medcalcssetup32.msi). The Chi-square test was used for the comparison of groups with or without thrombophilia marker expression. All tests were two-tailed, and the level of statistical significance was set at \( P < 0.05 \).

**Results and Discussion**

ITP is a heterogeneous disease with patients exhibiting varying bleeding severities despite the similar extent of thrombocytopenia. The reason for this heterogeneity is, however, not known. In the present study, we evaluated whether the presence of thrombophilia in patients with chronic ITP can modify the bleeding severity in such patients.

A total of 154 patients were found eligible and followed-up for a median of 21.2 months. Thirteen patients were excluded (two patients developed ANA positivity that was initially negative and 11 patients lost to follow-up). A final analysis of 141 patients was done. Thirty-nine patients were male (25.3%) and the remaining 115 patients (74.7%) were female. Therapies given during the study period included steroids, dapsone, rituximab, and eltrombopag.

**Thrombophilia marker evaluation**

Of the 141 patients, 36 patients (25.5%) tested positive for at least one thrombophilia marker and the remaining 105 patients (74.5%) were negative for all markers. Raised factor VIII was found in 21 patients (14.8%), lupus anticoagulant positive in 16 patients (11.3%), low protein S was found in eight patients (5.7%), low protein C in five patients (3.5%), and abnormal Pro C global® in 15 patients (10.6%) [Table 1]. In a similar study by Wong et al., 81% of the 167 chronic ITP patients had positivity for at least one thrombophilia marker, with high FVIII levels in 48%, low PC and PS in 5% and 22%, respectively, and positive lupus anticoagulant in 40% of patients tested. These rates for thrombophilia marker positivity are higher than the normal population and comparable to those found in patients with deep-vein thrombosis.

**Impact of thrombophilia marker expression on bleeding tendency**

Few studies have evaluated the impact of thrombophilia marker in patients with hemophilia A. Escuriola Ettingshausen et al. evaluated pediatric hemophilia A patients and found the prevalence of activated protein C resistance to be 6.5% and protein C deficiency in 1.1% of the total 124 patients studied, and the patients expressing thrombophilia markers had milder bleeding phenotypes and required significantly less factor concentrates. Studies by other investigators have also corroborated similar findings. However, no studies till date have reported the impact of thrombophilia marker expression on bleeding phenotypes in patients with chronic ITP. We, in the present study, evaluated the impact of thrombophilia marker expression on bleeding phenotype of chronic ITP patients. This was done using bleeding score assessment and calculating bleeding episodes/visits (as described in the material and method section).

Mild bleeding episodes/visit (MBE/visit): patients with at least one thrombophilia marker expression had 0.22 MBE/visit, as compared to those with none (0.49 MBE/visit), that was statistically significant (\( P < 0.001 \)). Subgroup analyses showed this difference in bleeding tendency was significant for patients expressing low protein C and S, abnormal Pro C Global® and lupus positivity, but not for FVIII abundance [Table 2].

Severe bleeding episodes/visit (SBE/visit): patients with any thrombophilia marker expression had 0.15 SBE/visit, as compared to those with none (0.39 SBE/visit), and
that was statistically significant ($P < 0.001$). Subgroup analyses showed this difference in bleeding tendency was significant for patients expressing low protein C and S, abnormal Pro C Global®, and lupus positivity, but not for FVIII abundance (Table 1).

We found a reduction of bleeding tendency in patients with chronic ITP who expressed thrombophilia marker compared to those without, similar to disease-modifying role of thrombophilia marker expression in patients with hemophilia A. Patients with low protein C and S, abnormal Pro C Global®, and lupus positivity (but not high FVIII levels) had lower bleeding tendencies than those with normal levels.

The increased incidence of thrombotic episodes has been shown in ITP patients in large registries.¹² In the present study, one patient developed documented deep-vein thrombosis involving the right femoral vein extending up to the popliteal vein. This patient was positive for lupus anticoagulant and had high FVIII levels.

The present study has certain limitations. First, we have performed the coagulation tests once at baseline and that acquired causes of deranged procoagulant factor levels were not evaluated. Furthermore, the coagulation tests were done with proper controls; however, these tests have not been repeated. It needs further evaluation whether deranged thrombophilia markers in chronic ITP patients have an etiologic association. Second, the number of patients recruited for the study is small owing to limited research grant availability.

**Conclusion**

We conclude that at least part of the heterogeneity in the clinical presentation of chronic ITP can be explained by the presence or absence of thrombophilia and that workup of patients with ITP should include thrombophilia evaluation, the results of which may play a role in therapeutic decision-making.

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**Conflicts of interest**

There are no conflicts of interest.

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