Section: Miscellaneous

Original Research Article

AUTOIMMUNE BIOMARKERS IN IMMUNE THROMBOCYTOPENIA AND ITS CORRELATION WITH DISEASE MANIFESTATION

Abdul Mateen Athar[1], Seetharam Anandram[2], Michael Pusharani[3], Tiji Alphonse[4], Gaurav Raj[5], Muhammad Mohsin[6]

[1]Associate Professor, Department of Medicine, St. Johns Medical College & Hospital, Sarjapur Road Koramangala Bengaluru, India.
[2]Associate Professor, Department of Clinical Hematology, St. Johns Medical College & Hospital, Sarjapur Road Koramangala Bengaluru, India.
[3]Senior Resident, Department of Medicine, St. Johns Medical College & Hospital, Sarjapur Road Koramangala Bengaluru, India.
[4]Specialty doctor in Haematology, Gloucestershire Hospital NHS Foundation Trust, Great Western Road, Gloucester.
[5]Intern, Kempegowda Institute of Medical Sciences, Banashankari 2nd Stage, Bengaluru, India.
[6]Consultant Haematologist, Barnsley Hospital NHS Foundation Trust, Gawber Road, Barnsley, United Kingdom.

Received : 16/06/2025
Received in revised form : 05/08/2025
Accepted : 23/08/2025

Keywords:
Immune Thrombocytopenia, autoimmunity, biomarkers.

Corresponding Author:
Dr. Abdul Mateen Athar,
Email: mateenathar@yahoo.co.in

DOI: 10.47009/jamp.2025.7.5.11

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2025; 7 (5); 51-55

ABSTRACT

Background: Immune thrombocytopenia (ITP) is an autoimmune disorder mediated by platelet antibodies, which accelerate platelet destruction and result in low platelet counts. Several studies have suggested that patients with ITP have a higher prevalence of positive autoimmune markers, including antinuclear antibody (ANA), red cell direct antiglobulin test (DAT), antithyroid peroxidase antibodies, and reduced levels of complement C3 and C4. Certain biomarkers may also be prognostically useful in this disorder. In this context, the present study was conducted to correlate biomarkers with the clinical presentation and severity of ITP. **Methodology:** This prospective observational study was conducted at St. John's Medical College Hospital (SJMCH). We analysed and correlated clinical features with biomarkers, as well as with the WHO and IBLS grades. **Results :**A significant p-value was found for ANA (51%), DCT (33%), APLA (41%), and ANTI-TPO (48%). As disease grade increased, severity worsened, and autoimmune biomarkers were more frequently positive, with a significant p-value of 0.001*. The percentages and levels of Immunoline, complement C3, and C4 were not statistically significant. Hence, Immunoline and complement C3 and C4 levels were not found to be important indicative biomarkers for assessing severity in ITP. These results demonstrate that most patients with ITP have additional autoantibodies present, suggesting wider immune dysregulation. **Conclusion:** In this study, patients with bleeding manifestations tended to have a greater prevalence of autoimmune biomarkers. Both the WHO Bleeding and IBLS scores correlated well with clinically severe disease, and these results were statistically significant. Both scores are helpful clinical tools for monitoring bleeding and may aid the development of laboratory parameters that correlate with underlying bleeding propensity in ITP.

INTRODUCTION

Immune thrombocytopenia is an autoimmune disorder mediated through platelet antibodies, which are thought to accelerate platelet destruction and inhibit their production, resulting in decreased platelet counts. This can cause spontaneous bruising, petechial rash, mucosal bleeding, or even life-threatening haemorrhage.[1]

Immune thrombocytopenia is one of the most common hematological problems in India.[2] Bleeding symptoms in ITP patients present either in mild forms, such as bleeding in the skin and mucosal regions, or more severe, life-threatening forms, such as gastrointestinal or intracranial bleeding. The degree of thrombocytopenia does not always correlate with disease severity.[2] The pathological mechanisms underlying ITP are unclear, and diagnosis remains a process of exclusion.[3] Biomarker results suggest that many patients with ITP have a state of immune dysregulation extending beyond platelet autoantibodies and that specific biomarkers may be prognostically valuable in this disorder.[4] Several studies have found that patients

ISSN (O): 2687-5365; ISSN (P): 2753-6556

with ITP have a higher prevalence than the general population for positive autoimmune markers, including ANA, DAT, antithyroid peroxidase antibodies, and reduced levels of complement C3 and C4,[5,6,7,8,9,10] In this context, the present study describes the clinical features of adult ITP, its severity, and its correlation with biomarkers.

MATERIALS AND METHODS

This prospective, observational study was carried out at St. John's Medical College Hospital, Bangalore, from November 2021 to March 2023. All patients attending the inpatient or outpatient services of St John's Medical College Hospital with ITP were included. Inclusion criteria were age above 18 years, primary ITP diagnosis, and satisfaction of the 2019 ASH Clinical Practice Guidelines for ITP diagnosis.[11] Patients were excluded if they had a diagnosis of systemic lupus erythematosus, rheumatoid arthritis, or antiphospholipid antibody syndrome at the time of ITP diagnosis. After obtaining clearance from the Institutional Ethics Committee, the study was initiated. After informed consent, patients with ITP were tested for autoimmune markers, based on the hypothesis that there may be an autoimmune phenotype associated with ITP, not for suspicion of other underlying diseases.

In addition to laboratory information, demographics, and clinical information, including age, sex, and date of initial clinic evaluation, were collected.

The severity of bleeding manifestation was assessed using IBLS- Bleeding Score and the WHO bleeding scale.[12]

Statistical Analysis: Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp, Armonk, NY). Continuous variables are presented as mean ± SD, and categorical variables as frequency and percentage. The Kolmogorov-Smirnov test was used to assess normality. Inferential statistics, such as the Chi-square/Fisher Exact test, were applied to test associations. The Kruskal-Wallis test was used to evaluate differences in variables between different grades. A p-value <0.05 was considered statistically significant.

RESULTS

The study involved 100 patients aged 18–79 years, of whom 77 (77%) were female and 23 (23%) were male, with a female: male ratio of 4:1. Most patients were between 31 and 40 years (30% of cases). The mean age was 38.19 ± 12.53 years. There was a tendency for severe disease in the 21–40 years age group. The maximum number of ITP cases were new cases/acute (41%), followed by chronic (37%) and persistent (22%). Most patients received corticosteroids (n=92), with 31 patients receiving other immunosuppressants (Romiplostim, Rituximab, Dapsone, IVIg). The most common presenting symptoms were fatigue (67%), followed by dyspnea (31%), with only 4% presenting with altered sensorium. On examination, most patients had pallor (61%), 11% had icterus, and 28% had no findings.[13]

According to the WHO Bleeding score, among 100 ITP patients, most were in Grade 3 (32%), followed by Grade 2 (30%), Grade 1 (16%), Grade 0 (12%), and Grade 4 (10%). There was a maximum number in Grade 3, followed by Grade 2, and the fewest in Grade 4, where disease severity is considered highest. According to the IBLS Bleeding grade, most patients were in Grade 2 (66%), followed by Grade 1 (22%) and Grade 0 (12%).

Haematological and Biomarker Results

Table 1: Comparison of hematologic parameters according to WHO Bleeding Grades

(Mean±SD)	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Overall	P value
Hemoglobin	13.32±0.99	10.25±1.52	7.24±1.81	7.0±1.65	4.96±1.31	8.15±2.84	0.001*
Platelets	75250.0±9789.83	46406.25±17269.79	16020.0±15851.41	10287.50±14017.25	8300.0±6700.75	25383.0±26412.08	0.001*
Total Counts	9007.42±4901.7867	8764.18±4226.06	9071.73±4986.54	8397.37±3577.41	7903.80±3747.53	8682.22±4253	0.998
Neutrophils	71.08±13.26	78.0±10.60	71.40±12.29	74.31±11.84	68.90±10.34	73.10±11.92	0.234
Lymphocytes	21.42±16.18	16.18±8.68	21.56±9.79	22.18±16.84	25.60±8.73	21.29±12.40	0.198
PlateletDistribution width	12.84±3.02	12.37±2.95	12.28±3.27	12.46±2.87	13.09±2.32	12.50±2.93	0.945
Mean Platelet volume	10.84±2.21	10.35±2.60	10.75±3.36	10.60±1.96	10.47±1.16	10.62±2.51	0.902
TSH	5.91±9.10	2.05±2.17	1.93±2.12	2.40±2.96	2.21±2.16	2.61±3.99	0.359
USG Liver	14.33±2.11	14.12±1.44	14.19±1.65	14.79±1.95	14.48±2.05	14.42±1.81	0.740

ISSN (O): 2687-5365; ISSN (P): 2753-6556

USG Spleen	9.71±1.33	10.77±1.39	10.32±2.04	10.31±1.78	10.70±1.48	10.35±1.73	0.293

Statistically significant (p<0.05, Kruskal-Wallis test)

Severe decrease in hemoglobin concentration was found in Grade 4 (4.96 g/dL), followed by Grades 3 (7.0 g/dL), 2 (7.24 g/dL), with mild decline in Grade 1 (10.25 g/dL), and normal levels in Grade 0 (p=0.001*). A parallel severe decrease in platelet count was demonstrated across increasing WHO grades (p=0.001*) as shown in Table 1.

Other parameters like total counts, neutrophils, lymphocytes, platelet distribution width, mean platelet volume, TSH, and ultrasound findings did not reach statistical significance.

Table 2: Distribution of autoimmune biomarkers according to Bleeding Grade (WHO)

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Overall	P value
DCT (Positive)	02	02	03	17	09	33	0.001*
ANA							
0(Negative)	10	11	15	13	0	49	0.007*
1.0(1+)	0	02	09	09	04	24	
2.0(2+)	0	02	03	06	04	15	
3.0(3+)	02	01	03	04	02	12	
Immunoline (Positive)	04	02	04	02	03	15	0.116
APLA(N=72) (Positive)	0	03	01	27	10	41	0.001*
Anti TPO							
Normal	10	15	23	04	0	52	0.001*
Elevated	02	01	07	28	10	48	
C3							
Normal	10	12	14	19	06	61	0.170
High	02	02	15	11	03	33	
Low	0	02	01	02	01	06	
C4							
Normal	08	10	13	19	06	56	0.282
High	03	04	15	13	03	38	
Low	0	02	01	0	01	04	

Table 2 shows that autoimmune markers DCT, ANA, ANTI TPO, and APLA all showed statistically significant associations with disease grade (each p=0.001*) where as Immunoline, C3, and C4 levels were not statistically significant.

Table 3: Comparison of hematologic parameters by IBLS grade

(Mean±SD)	Grade 0	Grade 1	Grade 2	Overall	P value
Hemoglobin	13.32±0.99	9.92±1.62	6.62±1.71	8.15±2.84	0.001*
Platelets	75250.0±9789.84	47795.45±15443.30	8845.45±7900.93	25383.0±26412.08	0.001*
Total Counts	9007.42±4901.78	8963.45±3929.16	8529.35±4293.02	8682.22±4253.36	0.798
Neutrophils	71.08±13.26	76.95±11.97	72.18±11.55	73.10±11.92	0.202
Lymphocytes	21.42±10.45	17.64±10.31	22.48±13.24	21.29±12.40	0.189
Platelet distribution width	12.84±3.02	12.94±3.23	12.30±2.84	12.50±2.93	0.537
Mean platelet volume	10.84±2.21	11.0±2.77	10.45±2.50	10.62±2.51	0.552
TSH	5.91±9.10	2.12±2.78	2.17±2.34	2.61±3.99	0.072
USG Liver	14.33±2.10	14.23±1.62	14.49±1.83	14.42±1.81	0.792
USG Spleen	9.72±1.33	10.61±1.51	10.38±1.85	10.35±1.73	0.206

Statistically significant (p<0.05, Kruskal-Wallis test)

Trends were similar to the WHO score, with a significant decrease in hemoglobin and platelet count with higher grade (each p=0.001*) as shown in Table 3.

Table 4: Comparison of autoimmune markers based on IBLS grade

	Grade 0N(%)	Grade 1N(%)	Grade 2N(%)	Overall N(%)	P value
DCT (Positive)	02	03	28	33	0.024*
ANA					
0	10	14	25	49	0.011*

ISSN (O): 2687-5365; ISSN (P): 2753-6556

1.0	0	02	22	24	
2.0	0	04	11	15	
3.0	02	02	08	12	
Immunoline (Positive)	04	04	07	15	0.102
APLA (Positive)	0	05	36	41	0.001*
Anti TPO					
Normal	10	16	26	52	0.002*
Elevated	02	06	40	48	
C3					
Normal	10	16	35	61	0.137
High	02	04	27	33	
Low	0	02	04	06	
C4					
Normal	08	12	36	56	0.111
High	03	06	29	38	
Low	0	03	01	04	

Statistically significant (p<0.05, Fisher's Exact test)

Similar findings were observed: significant associations for DCT (p=0.024*), ANA (p=0.011*), APLA (p=0.001*), and ANTI TPO (p=0.002*); complement C3 and C4 were not significant as shown in Table 4.

Table 5: Prevalence of autoimmune markers

Autoimmune markers	No. of cases	Percentage
All positive	02	02
>3 Positive	34	34
<3 positive	49	49
Negative for all	15	15
Total	100	100

Table 5 shows that out of 100 patients tested for all seven autoimmune markers, 2% had all markers positive, 34% had more than 3 positive markers, and 15% were negative for all markers. The most prevalent positive markers were ANA (51%) and antithyroid peroxidase antibody (48%).

APLA, DCT, Immunoline, C3 & C4 were present in 41%, 33%, 15%, 6%, and 4% of the samples, respectively.

There was significant overlap between—29 of 51 ANA with APLA & Anti-TPO (56%).

A severe decline in platelet count was seen in higher grades of both WHO and IBLS bleeding scores, which was statistically significant (p=0.001*).

DISCUSSION

This study investigated the prevalence of autoimmune biomarkers and their association with clinical severity in adults with immune thrombocytopenia (ITP). Our findings reinforce the concept that ITP is not a purely platelet-specific disorder but rather represents a broader state of autoimmune dysregulation.

A high prevalence of autoimmune markers—including ANA, DCT, APLA, and anti-TPO—was observed in our cohort. The detection of these markers correlated strongly with increasing disease severity, as measured by both the WHO Bleeding scale and IBLS grade. This is consistent with previous studies reporting that most ITP patients possess additional autoantibodies beyond antiplatelet antibodies, supporting a model of generalized immune dysregulation.[5,6,7,9] ANA positivity, in particular, has been reported at highly variable frequencies in ITP cohorts, depending on population, methodology, and inclusion criteria. Our rates fall within the higher range noted in the literature.[16] The significance of such findings is underscored by reports associating ANA and APLA positivity with higher thrombotic risk as well as the potential to predict reduced remission rates with antithyroid peroxidase antibody.

In our study, certain biomarkers such as immunoline, complement C3, and C4 did not show a significant association with disease severity or bleeding grade. This observation agrees with evidence that while the presence of platelet-directed autoantibodies is central to the pathogenesis of ITP, tests for broader markers of immune activation or complement consumption are not consistently predictive or prognostically relevant.

The grading of bleeding using validated systems, including the WHO Bleeding scale and IBLS, showed strong correlation with decreased hemoglobin and platelet counts as well as higher rates of autoimmune biomarker positivity at greater severity. Recent comparative studies confirm the clinical utility, responsiveness, and consistency of these bleeding grading systems in assessing disease severity, risk stratification, and outcomes in ITP. Notably, while low platelet counts generally correspond to increased bleeding risk, clinical manifestations can be modulated by factors such as platelet function and the presence of other

ISSN (O): 2687-5365; ISSN (P): 2753-6556

autoimmune pathologies, again highlighting the complexity of ITP pathogenesis.

These findings have direct implications for clinical management. The detection of autoimmune biomarkers may help identify patients with more severe disease or those at higher risk for thrombotic events—a complication increasingly recognized in the ITP population, particularly among patients with antiphospholipid antibodies or ANA positivity. At present, routine testing for all possible autoimmune markers at diagnosis is not universally recommended. Still, targeted assessment may inform prognosis and therapeutic choices in selected individuals, such as those being considered for second-line therapies or with other autoimmune features.

Limitations of the present study include the single-center design and potential selection bias, which may limit generalizability. Not all patients were tested for the full autoantibody panel, possibly underestimating true prevalence. Further research is warranted to clarify the prognostic value of individual biomarkers and their role in therapeutic decision-making.

In conclusion, our data contribute to a growing body of evidence highlighting the frequent co-occurrence of autoimmune markers in ITP and their correlation with clinical severity. The use of standardized clinical scores supports comprehensive bleeding assessment and may facilitate risk-adapted and biomarker-driven management of this heterogeneous disorder.

CONCLUSION

Patients with bleeding manifestations of ITP tended to have a greater prevalence of autoimmune biomarkers. Both WHO Bleeding and IBLS scores correlated well with clinically severe disease, and this association was statistically significant. Both scores are helpful clinical tools for monitoring bleeding and may aid in developing laboratory parameters that correlate with underlying bleeding propensity in ITP.

REFERENCES

1. McMillan R, Wang L, Tomer A, Nichol J, Pistillo J. Suppression of in vitro megakaryocyte production by antiplatelet autoantibodies from adult patients with chronic ITP. Blood. 2004;103(4):1364-1369.
2. Kohli R, Chaturvedi S. Epidemiology and Clinical Manifestations of Immune Thrombocytopenia. Hamostaseologie. 2019;39(3):238-249.
3. Matzdorff A, Meyer O, Ostermann H, et al. Immune Thrombocytopenia - Current Diagnostics and Therapy.
4. Zufferey A, Kapur R, Semple JW. Pathogenesis and Therapeutic Mechanisms in Immune Thrombocytopenia (ITP).
5. Pratt EL, Tarantino MD, Wagner D, Hirsch Pescovitz O, Bowyer S, Shapiro AD. Prevalence of elevated antithyroid antibodies and antinuclear antibodies in children with immune thrombocytopenic purpura. Am J Hematol. 2005;79(3):175-179.
6. Aledort LM, Hayward CPM, Chen M-G, Nichol JL, Bussel J; ITP Study Group. Prospective screening of 205 patients with ITP, including diagnosis, serological markers, and the relationship between platelet counts, endogenous thrombopoietin, and circulating antithrombopoietin antibodies. Am J Hematol. 2004;76(3):205-213.
7. Demir C, Esen R, Atmaca M, Efe S. Prevalence of autoantibodies related to some autoimmune disorders in patients with chronic idiopathic thrombocytopenic purpura. Clin Appl Thromb Hemost. 2011;17(6): E114-E118.
8. Årfors L, Winiarski J, Lefvert AK. Prevalence of antibodies to cardiolipin in chronic ITP and reactivity with platelet membranes. Eur J Haematol. 1996;56(4):230-234.
9. Kurata Y, Miyagawa S, Kosugi S, et al. High-titer antinuclear antibodies, anti-SSA/Ro antibodies, and antinuclear RNP antibodies in patients with idiopathic thrombocytopenic purpura. Thromb Haemost. 1994;71(2):184-187.
10. Frison L, Lombardi A, Caputo I, Semenzato G, Fabris F, Vianello F. Relevance of antiphospholipid antibody profile in the clinical outcome of ITP: a single-centre study. Hematology. 2019;24(1):134-138.
11. Rodeghiero F, et al. Standardization of terminology, definitions, and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood. 2009;113(11):2386-2393.
12. LK, Psaila B, Provan D, et al. The immune thrombocytopenic purpura (ITP) bleeding score: assessment of bleeding in patients with ITP. Br J Haematol. 2007;138(2):245-248.
13. Sajwani FH, Al Tunaiji HO. Demographic and clinical analysis of hospitalized patients with thrombocytopenia. J Appl Hematol. 2014; 5:58-64.
14. Frederiksen H, Schmidt K. The incidence of idiopathic thrombocytopenic purpura in adults increases with age. Blood. 1999;94(3):909-913.
15. Chakrabarti P, et al. How do patients and physicians perceive immune Thrombocytopenia (ITP) as a disease? Results from Indian analysis of ITP World Impact Survey (I-WISh). Journal of Patient-Reported Outcomes. 2022; 6:24.
16. Hollenhorst MA, Al-Samkari H, Kuter DJ. Markers of autoimmunity in immune thrombocytopenia: prevalence and prognostic significance. Blood Adv. 2019;3(22):3515-3521.

ISSN (O): 2687-5365; ISSN (P): 2753-6556