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# Clinical Significance of Positive Platelet Immunofluorescence Assay in Adult Immune Thrombocytopenia: a Cross Sectional Study

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## Abstract

**Background:** Immune thrombocytopenia is a hematologic disorder characterized by low platelet count and variable bleeding manifestations due to immune-mediated platelet destruction and/or suppression of platelet production. This study was performed to evaluate characteristics and clinical presentations of adult patients with immune thrombocytopenia and to explore the clinical value of platelet antibodies assay among Iranian patients.

**Patients and Methods:** In this cross sectional case series study 46 adult patients with immune thrombocytopenia and platelet count of < 100 x 109/L, referred to the Taleghani Medical Center, Tehran, Iran, between 2007 and 2009 were evaluated. The platelet autoantibodies were measured by means of indirect platelet suspension immunofluorescence test.

**Results:** According to our results, 7 patients (15.2%) displayed positive platelet antibody assay. There was a significant negative correlation between platelet count and antibody level ($r = -0.59$; $p < 0.001$). Additionally, a positive correlation between platelet count and patients' age ($r = 0.302$; $p = 0.042$) was detected. Twenty patients (56.5%) were symptomatic at presentation and the most common bleeding signs were petechia, purpura and epistaxis. Results indicated no significant correlation between increased platelet antibody level and bleeding manifestations except for hematuria ($r = 0.435$; $p = 0.02$) and epistaxis ($r = 0.382$; $p = 0.015$).

**Conclusion:** Patients with positive antibody assay have been reported to have more bleeding symptoms and lower platelet counts. In our series, the increased antibody level was correlated with thrombocytopenia. However, among our patients with positive antibody assay no significant difference in bleeding manifestations was observed except for hematuria and epistaxis.

**Keywords:** Immune thrombocytopenia; platelet, autoantibodies, platelet immunofluorescence test.

## Introduction

Immune thrombocytopenia (ITP) is a relatively common hematologic disorder manifested by low platelet count due to immune-mediated platelet destruction and/or suppression of platelet production. The abbreviation ITP wich originally stood for idiopathic thrombocytopenic purpura is now used for immune thrombocytopenia. A large proportion of ITP cases are categorized as primary since they occur in the absence of any clinically apparent alternative etiologies of low platelet count [1,2]. According to recent studies, the estimated annual incidence is 100 new cases per 1 million population, with adults accounting for about half of that number [3].

The clinical features of immune thrombocytopenia in adults are quite distinct from those seen in childhood. Adult ITP predominantly affects women between the ages of 30 and 60 years while there is a gender balance with increasing age [4]. Although ITP in adult patients often has an insidious onset with no prodromal illness; it typically tends to follow a chronic course. Moreover, the clinical status of ITP is heterogeneous. Signs and symptoms range from common asymptomatic

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patterns or mild mucocutaneous bleeding through serious hemorrhage [5].

According to the American Society of Hematology (ASH) guidelines, the diagnosis of ITP remains one of exclusion and principally is based on medical history, physical examination and ancillary laboratory data such as complete blood count and peripheral blood smear. This guideline does not recommend bone marrow examination in patients with the typical features of ITP, irrespective of the age of the patient [6]. Further diagnostic studies are generally not recommended as the routine work-up in patients suspected of ITP 6. Considering the fact that a diagnosis of exclusion bears inherent difficulties [5, 7], detection of antibodies directed against platelet epitopes may have a higher degree of diagnostic value. However, the importance of platelet antibodies assay in the clinical course of ITP has not been clearly established [8].

Up to now various methods have been applied for detection of anti platelet antibody among ITP patients. ELISA, flow cytometry, monoclonal antibody immobilization of platelet antigen (MAIPA), enzyme-linked immunospot (ELISPOT) assay, lymphocytotoxicity test (LCT) and SPRCA assay (solid phase red cell adherence assay) are some of these applied methods [9-15]. It has been shown that flow cytometry assay using donor platelets as target cells can detect autoantibodies in 70% of patients with ITP and it is more sensitive than SPRCA assay (50%-65% sensitivity) for antibody detection among these patients 9. However, SPRCA method with 100% specificity is more specific [9]. MAIPA is another specific method for detection of platelet antibody that can differentiate immune from non-immune thrombocytopenia 10. Flow cytometry assay is more rapid and sensitive than modified MAIPA [14].

We therefore conducted a case series to elucidate the clinical significance of platelet auto antibodies in adult patients with ITP. This study aimed to determine demographic characteristics and presenting manifestations of Iranian patients with ITP as well as to explore the laboratory assays which might be relevant to diagnosis and management of ITP.

## Patients and Methods

This cross-sectional case series was performed on newly presenting adults (aged 14 years or above) with ITP attending the Taleghani University Hospital in Tehran, Iran, between 2007 and 2009. The diagnostic criteria of ITP were the presence of isolated thrombocytopenia with platelet counts of 100 × $10^9$/L or lower, normal or increased percentage of bone marrow megakaryocytes, absence of splenomegaly and exclusion of other potential causes of thrombocytopenia [6].

A total of 46 adult patients who fulfilled the proposed criteria, were evaluated. The patients' charts were evaluated including a thorough medical history and physical examination in addition to the other parameters such as patients' age, gender and the bleeding signs and symptoms at presentation. In order to exclude the secondary etiologies contributing to thrombocytopenia and/or increased risk of bleeding, we analyzed the laboratory data involving complete blood counts

**Table 1:** Characteristics of antibody-positive and antibody-negative ITP patients.

| Variable | Ab-positive group (n=7) | Ab-negative group (n=39) | *p*-value |
|---|---|---|---|
| **Mean age, ( years)( Mean ± SD)** | 40.3 ± 25* | 38.62 ± 19 | 0.839, T-test |
| **Gender, (Males / Females)** | 3/4 | 17/22 | 0.65, $Chi^2$ test |
| **Platelet count (×$10^9$/L)( Mean ± SD)** | 10.42 ± 11.35 | 48.74 ± 28.6 | 0.001, T-test |
| **Platelet antibody titer (Mean ± SD)** | 1 / 4.17 ± 8.042 | - | <0.001, T-test |

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(both differential and reticulocyte count), liver and renal function tests, thyroid profile, anti-nuclear antibody (ANA), HIV test and bone marrow findings if available. Also, patients were excluded if they were under pharmacotherapy. The study protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences and patients signed informed consent before enrollment.

We measured the level of platelet autoantibodies in sera of patients (Phase II assays) as well as the platelet count. Specimens were collected at onset of diagnosis and prior to initiation of therapy. Circulating platelet antibody detection was performed by means of indirect platelet suspension immunofluorescence test (IPSIFT) based on Rose et al. method [13]. This method (second generation) was the only available test in Iran at the time of our study. Fluorescein - labeled anti- immunoglobulins (DAKO, Denmark) including anti-IgG, anti-IgM and anti- IgA, were used as secondary antibodies. Subsequently, immunofluorescence data were interpreted by a Leica fluorescence microscope. In this study we analyzed the results based on the semi quantitative estimation and sera with the platelet antibody levels above the cut-off point of 1:16 were assumed as antibody- positive.

**Statistical analysis**

Statistical analysis of data was performed using SPSS software. For all continuous variables, mean values and standard deviations (SD) were calculated. Independent sample T-test and Chi square test were applied to compare continuous (with normal distribution) and categorical variables between the two groups. The Pearson's correlation test was used to explore the relationship between continuous variables such as platelet counts and platelet antibody levels. Also, Spearman's correlation test was applied to find the correlation between continuous and categorical variables such as platelet counts and bleeding signs.

## Results

A total of 46 patients with ITP were studied, among which 26 (56.5%) were female resulting in a female to male ratio of 1.3/1. The median age of patients was 38.9 ±19.7 years ranging from 14 to 86 years (p = 0.188).

The clinical signs included petechiae, purpura (41%), epistaxis (41%), hematuria (11%), GI bleeding (9%) and bleeding from gums (26%) and conjunctiva (7%). There was no record of intra cranial hemorrhage (ICH) or hemoptysis.

According to the results, peripheral platelet counts ranged from less than $1\times10^9$ /L to up to

**Table 2:** Distribution of clinical bleeding signs in antibody-positive and antibody-negative ITP patients.

| Bleeding signs | Ab-positive patients (n=7) | Ab-negative patients (n=39) | *p*-value |
|---|---|---|---|
| **Overall bleeding diathesis, n (%)** | 6 (85.7) | 20 (51.3) | 0.091, $Chi^2$ test |
| **GI bleeding, n (%)** | 2 (28.6) | 2 (5.1) | 0.104, $Chi^2$ test |
| **Epistaxis, n (%)** | 6 (85.7) | 13 (33.3) | 0.015, $Chi^2$ test |
| **Gingival bleeding, n (%)** | 4 (57.1) | 8 (20.5) | 0.65, $Chi^2$ test |
| **Hematuria, n (%)** | 3(42.9) | 2(5.1) | 0.02, $Chi^2$ test |
| **Petechiae, purpura, n (%)** | 5 (71.4) | 14 (35.9) | 0.091, $Chi^2$ test |
| **Conjuctival bleeding, n (%)** | 2(28.6) | 1(2.6) | 0.056, $Chi^2$ test |

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100×10⁹/L with the mean of 42.91 ×10⁹/L ± 30.03 (p = 0.598).

The platelet antibody was not demonstrable in 33 patients (71.7%), while the antibody titer of 1:8 was detected in 6 patients (13%) and the titer of 1:16 and 1:32 were reported in 5 (10.9%) and 2 (4.3%) of patients, respectively. Considering the antibody level of 1:16 as the cut-off point, 7 (15.2%) of the study subjects disclosed a positive platelet antibody result while, 39 patients (84.8%) had a negative assay.

The main characteristics of antibody-positive and antibody-negative ITP patients are illustrated in table 1. According to our findings, there was a statistically significant negative correlation between platelet count and antibody titer among ITP patients (r = - 0.59; p < 0.001) (Figure1).

Evaluation of clinical signs revealed that 26 patients (56.5%) were symptomatic at presentation. There was no significant correlation between antibody levels and the overall bleeding tendency. However, considering each sign separately, epistaxis (r = 0.382; p =0.015) and hematuria (r = 0.435; p =0.02) correlated with the platelet antibody titers.

The clinical bleeding signs in ITP patients according to the results of platelet antibody analysis are outlined in table 2. In the studied group (n = 46), results displayed a statistically positive correlation between platelet counts and patients' age (r = 0.302; p =0.042) (Figure 2). However, among the antibody-positive group (n = 7) there was no significant correlation between increased antibody level and patients' age (p =0.121). Additionally, no significant correlation was detected between the platelet antibodies and patients' gender (p =0.65).

**Clinical Outcome and Follow Up**

For each patient, the observation period started right after the initial diagnosis and they were followed for about 12 months. Among 39 patients with negative serum platelet antibody, 13 patients missed the follow up and 17 patients never required therapy. Of the 9 patients who were treated with corticosteroids, 5 cases responded to

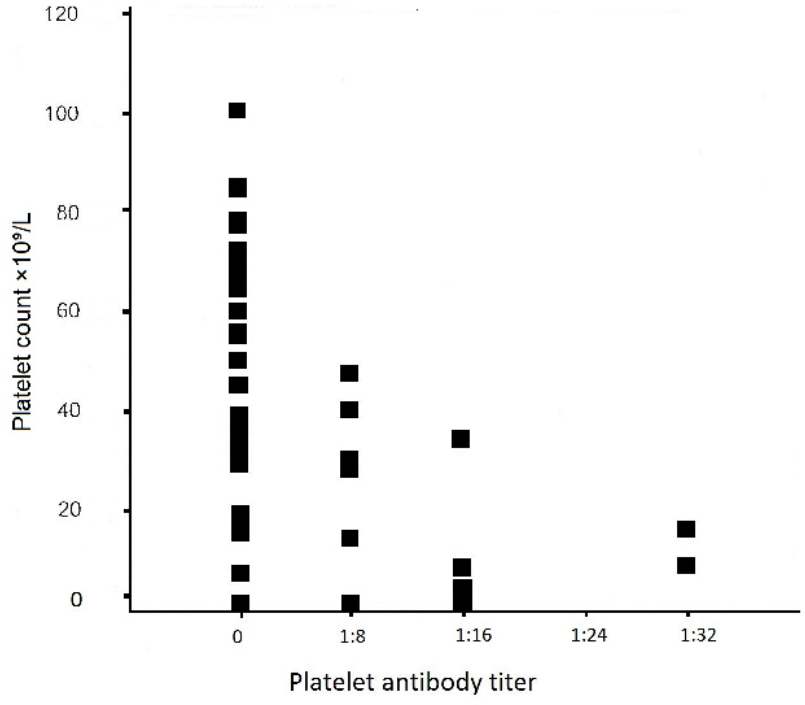



**Figure1:** Correlation between antibody titer and platelet count among ITP patients. (Spearman r = - 0.59; p < 0.001)

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therapy, while the others failed the treatment and 3 of them underwent splenectomy.

Out of the 7 patients with positive platelet antibody assay, 3 patients missed the follow up. Among the others, 3 patients were treated with corticosteroids; while 2 cases had complete response, splenectomy was performed for one nonresponder patient. Also, none of the patients underwent bone marrow examination for ITP diagnosis.

## Discussion

The pathogenic effect of platelet autoantibodies in ITP has been clearly established. Furthermore, a positive antibody assay provides strong evidence for the presence of immune thrombocytopenia. This study determined demographic characteristics and presenting manifestations of the Iranian patients with ITP.

The platelet antibodies involved in ITP most often are directed toward certain platelet membrane glycoproteins, either the GP IIb/IIIa or GP Ib/IX complexes. Nevertheless; some patients disclose autoantibodies against the multiple platelet antigenic targets [16].

Numerous methods have been devised for detection of platelet antibodies since Harrington et al. [17] and Shulman et al. 18 demonstrated the autoimmune pathophysiology of ITP [9-15]. The current techniques are based on detection of immunoglobulins on platelets, either by direct assays on patients' platelets or through an indirect test on normal platelets after exposure to patients' sera.

The sensitivity and specificity of these techniques in detection of anti platelet antibody is different. Folw cytometry assay with 70% sensitivity and SPRCA method with 100% specificity have been determined as the most sensitive and specific techniques, respectively for antibody detection in ITP patients [9,10,14]. The LCT sensitivity and specificity have been reported as 94.73% and 100%, respectively in a study among Thai patients [15]. That study showed lower sensitivity and specificity of LCT and SPRCA in detecting platelet antibodies than that of flow cytometry [15]. In addition to the routine

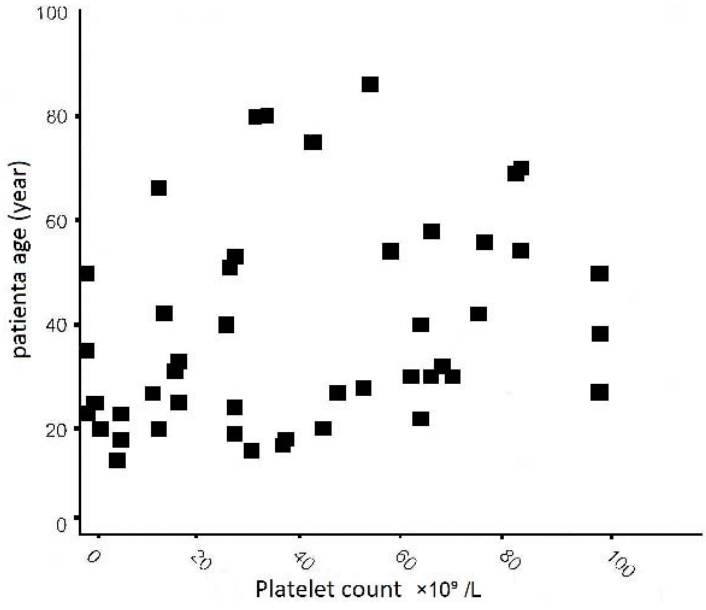



**Figure2:** Correlation between platelet count and age among ITP patients.
(Pearson r = 0.302; p =0.042)

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and currently in use methods, recently a novel assay based on peptide aptamer technology has been proposed for the detection of anti- platelet antibodies. This assay sensitivity has been reported to be about 90% and gives better results than the MAIPA method for detecting antibody in neonatal alloimmune thrombocytopenia [19].

This study set out with the aim of evaluating characteristics, clinical features and also laboratory findings in adult ITP patients. In addition, the clinical value of platelet antibodies assay in proposed patients was determined. In the present study, we measured the autoantibody levels by means of indirect platelet suspension immunofluorescence test (IPSIFT) in 46 adult patients with ITP.

Some literature in the 1970s have reported a sensitivity of about 90% for direct platelet-associated IgG assays in ITP patients . Whereas, recent studies using antigen-specific assays, demonstrated platelet antibodies in around 50–60% of the ITP cases [2]. In our study, [7] patients (15.2%) displayed a positive platelet antibody result which is statistically close to the findings of similar studies carried out by Phase II platelet antibody assays[20]. Possible factors influencing the detection rate include: limited sensitivity of the indirect antibody assay, along with technical problems associated with the test; presence of alternative platelet destruction mechanisms which are not attributed to autoantibody such as direct T-cell cytotoxicity [21] and complement mediated lyses [22]. Furthermore, some patients suspected of having ITP satisfying ASH diagnostic guidelines may not have primary immune thrombocytopenia. Hence, there is a need for additional evaluations and modifications to develop diagnostic criteria that can be used as a routine [23,24].

The ITP phenotype is heterogeneous varing from mild bleeding through frank hemorrhage. We observed that the majority of studied patients (56.5%) were symptomatic at presentation and the most common bleeding signs were petechia, purpura and epistaxis. There was no significant correlation between antibody titer and the overall bleeding risk (p =0.091). However, a positive correlation was demonstrated between increased platelet antibody level and the clinical signs including epistaxis (r = 0.382; p = 0.015) and hematuria (r = 0.435; p =0.02), compared to the patients with negative antibody assay. These findings suggest that platelet autoantibodies initiate antigen shedding and inhibit specific glycoprotein functions, leading to bleeding tendency in some ITP patients [25].

According to our findings, there was a statistically significant negative correlation between platelet count and antibody titer among ITP patients (r = - 0.59; p < 0.001). This is in agreement with previously reported data [26].

A possible explanation might be the fact that antibody-coated platelets are destroyed prematurely by reticuloendothelial system. The resulting shortened life span of platelets in the circulation along with the immune mediated ineffective thrombopoiesis, contributes to decreased platelet count in ITP patients [2]. Likewise, some studies have addressed elevated platelet counts following immunosuppressive therapy in most ITP patients [27].

Considering the demographic patterns of the studied patients, we noted a significant positive correlation between platelet count and patients' age (r = 0.302; p =0.042). However, no significant correlation was detected between the platelet antibody level and patients' age as well as the gender.

Comparing with previous published data there was a considerably less ITP patients with positive assay in our study which may be explained, at least in part, by the relatively low sensitivity of the implicated assay [9].

The severity of thrombocytopenia is associated to some extent but not completely with the propensity to bleed which necessitates more factors to be evaluated [28,29]. On the other hand, increased platelet antibodies level is only one of several factors involved in development of the clinical status of ITP. Further evaluations will be needed to modify the current clinical guidelines for diagnosis of ITP as well as to improve the diagnostic modalities in order to specifically target the pathogenic mechanisms of immune thrombocytopenia.

## Conclusion

It has been suggested that detection of platelet antibody might help to predict the course of ITP. In fact, patients with positive antibody assay have been reported with more bleeding symptoms and lower platelet counts. In our series, the increased antibody level was correlated with thrombocytopenia. However, among our patients

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with positive antibody assay no significant difference in bleeding manifestations was observed except for hematuria and epistaxis.

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