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Research Article

# The Effect of Anti-Nuclear Antibody Positivity on Immune Thrombocytopenic Purpura and its Clinical Course

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## Abstract

### Objective

Immune Thrombocytopenic Purpura (ITP) is an autoimmune-mediated disease. Patients with primary ITP are occasionally found to have an Antinuclear Antibody (ANA) titer ≥1:160. Our objective was to study the effect of ANA on ITP and its clinical course.

### Methods

This was a retrospective chart review of 765 adult patients with ITP, followed regularly at St. Paul's Hospital, Vancouver, Canada; 120 patients who had an ITP diagnosis and had ANA testing during their initial work-up were included. An ANA titer ≥1:160 was correlated with clinical presentation including steroid responsiveness, platelet count at presentation, and autoimmune disease development. This study took place in St. Paul's Hospital, Vancouver, British Columbia, Canada between the months of June 2014, till January 2015.

### Results

Of 24 patients with ANA titers ≥1:160, 10(58.8%) were steroid-resistant, compared to 9(29.0%) of 31 patients with ANA titers <1:160(P=0.044). Five (20.8%) patients with ANA titers ≥1:160 developed other autoimmune diseases after diagnosis with ITP, compared to only one (1%) patient with an ANA titer <1:160(P=0.001). Patients with ANA titers ≥1:160 had a lower platelet count than patients with ANA titers <1:160, (P<0.001).

### Conclusion

We analyzed the relationship between high ANA and ITP clinical course. Compared to patients with ANA titers <1:160, those with ANA titers ≥1:160 were steroid-resistant, had a higher risk of developing other autoimmune diseases, and presented with a lower platelet count.

**Keywords:** ANA; ITP; SLE; Steroids

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## Introduction

Immune Thrombocytopenic Purpura (ITP) is defined as an autoimmune disease characterized by isolated thrombocytopenia with a platelet count ≤100×109/L resulting from accelerated destruction of antibody-coated platelets by tissue macrophages, predominantly in the spleen. ITP can be primary with no identifiable or specific precipitating factor, while secondary ITP is associated with an underlying disease [1]. ITP is classified into three phases based on disease duration: newly diagnosed, less than 3 months; persistent, between 3 and 12 months; and chronic, more than 12 months [1]. While the majority of affected children have secondary short-lived ITP, adults usually have primary chronic ITP [2].

In primary ITP, it is believed that the patient's B-cells produce IgG antibodies against platelet membrane glycoproteins, resulting in antibody-mediated destruction [3]. A subset of patients with ITP are positive for Antinuclear Antibodies (ANA) and antiphospholipid antibodies, in addition to antiplatelet antibodies. However, this does not qualify them as having secondary ITP if they do not meet the criteria for Systemic Lupus Erythematosus (SLE) and antiphospholipid syndrome [4,5]. The efficacy of regular testing for ANA in patients presenting with a low platelet count remain controversial in children and young adults [6].

Additionally, ANA-positive patients have been reported to have an unusual response to treatment [7]. A pilot study by Abbasi et al., [7] showed that adult patients with ITP who are ANA-positive have a poor response to steroid therapy; close monitoring was suggested for these patients [7]. Furthermore, Khellaf et al., [8] suggested the use of hydroxychloroquine as a second-line drug in patients with SLE-associated ITP as well as ANA-positive ITP. This previous study showed that 50% of patients with ANA-positive ITP without definite SLE showed a good response and tolerated hydroxychloroquine well [8]. Moreover, Hazzan et al., [9] studied the risk of developing SLE in ITP patients and found that all patients who developed SLE were women and ANA-positive; however, 64% of ANA-positive ITP patients did not develop SLE [9].

In this single-center chart review, we reviewed and analyzed the data of adult patients with ITP, focusing on ANA positivity and its effect on treatment and future prognosis. To determine the relationship of ANA positivity on treatment outcome and future prognosis, we retrospectively reviewed the data of adult patients with ITP. We hypothesized that patients with a higher degree of ANA positivity would have worse outcomes and prognosis.

## Materials and Methods

This is a retrospective chart review of patients at St.Paul's Hospital, Vancouver, British Columbia, Canada between the months of June 2014, till January 2015. We conducted a retrospective chart review of 765 adult patients with ITP, who were regularly followed up at St. Paul's Hospital, Vancouver, British Columbia, Canada. Patients included were all diagnosed with primary ITP, aged ≥15 years, and who had their ANA titers tested at the time of diagnosis. Exclusion criteria

included diagnosis of hepatitis C, hepatitis B, or human immunodeficiency virus, as well as diagnosis of autoimmune diseases prior to the diagnosis of ITP.

Data extracted included date of birth, age at time of diagnosis, gender, ANA titer, follow-up period, treatment used (if any), response to treatment, platelet level before and after treatment, diagnosis of other autoimmune disease, signs and symptoms of autoimmune disorders at the time of diagnosis of ITP, direct family history of autoimmune diseases, complete blood count with differential, C-reactive protein, and erythrocyte sedimentation rate.

Diagnosis of ITP was made by a hematologist, and patients who subsequently developed other autoimmune diseases were diagnosed strictly by rheumatologists. ANA titer was considered positive if the ANA titer was ≥1:160, as many centers consider ANA titer 1:80 as equivocal. Treatment was based on the treating hematologist's preference; however, steroids were the first line of therapy. Patients were considered steroid-resistant only if a steroid trial of at least 2 weeks was attempted and the patient did not improve. Other patients were considered steroid-dependent when it was not possible to taper the steroid dose without a drop in platelet count. Other treatment methods included intravenous immunoglobulin, azathioprine, rituximab, and romiplostim; moreover, splenectomy was performed in some patients.

Data were analyzed using a chi-square test, Fisher's exact test, or Wilcoxon rank-sum test as appropriate. P value <0.05 was considered statistically significant.

This analysis was performed in accordance with requirements of the Institutional Research Ethics Boards at St. Paul's Hospital, and ethical approval obtained.

## Results

In total, 765 charts were reviewed. One hundred and twenty patients were included based on criteria and availability of ANA testing. Out of 120 patients, 24 patients were ANA positive. ANA titers for these patients are summarized in table 1.

| ANA Titer | All Patients (n=120) | ANA<1:160(n=96) | ANA ≥1:160(n=24) |
|---|---|---|---|
| 0 | 76(63.3) | 76(79.2) | 0(0.0) |
| 1:40 | 10(8.3) | 10(10.4) | 0(0.0) |
| 1:80 | 10(8.3) | 10(10.4) | 0(0.0) |
| 1:160 | 11(9.2) | 0(0.0) | 11(45.8) |
| 1:320 | 4(3.3) | 0(0.0) | 4(16.7) |
| 1:640 | 6(5.0) | 0(0.0) | 6(25.0) |
| 1:1280 | 3(2.5) | 0(0.0) | 3(12.5) |

**Table 1:** Antinuclear Antibody (ANA) titers in patients with immune thrombocytopenic purpura.

*ANA, antinuclear antibody

The majority (n=80; 66.7%) of patients diagnosed with ITP were women. While 20(25%) female patients with ITP were found to have ANA titers ≥1:160, only 4(10%) male patients were found to have ANA titers ≥1:160. Moreover, a higher percentage (83.3%) of patients with ANA titers ≥1:160 were women compared with that among patients with ANA titers <1:160(62.5%). The mean age at the time of diagnosis of ITP was 41.0 years (standard deviation [SD] 17.6). Moreover, the mean age at the time of diagnosis was non-significantly lower in patients with ANA titers ≥1:160(37.8 years, SD 19.2) than that in those with ANA titers <1:160(41.8 years, SD 17.2).

Patients included in this study were treated according to their symptoms, including C, prolonged epistaxis, overt hematuria, and gingival bleeding associated with a platelet count of ≤30,000/mL.

While the majority of patients (n=69, 57.5%) did not need any intervention, the remainder of patients required intervention to control their symptoms. Steroids were adequate to control the symptoms of most patients needing intervention (n=32, 26.7%). However, in a number of patients (n=19, 15.8% of all ITP patients), steroids failed to control these symptoms. When comparing all ANA-positive patients with ANA-negative patients in our study, we found that significantly more patients (n=10, 58.8%) with ANA titers ≥1:160 were steroid-resistant than those with ANA titers <1:160(n=9, 29.0%; P=0.044). Patients who've failed steroid thearapy have been treated with various modalities. The different treatment modalities used are summarized in table 2. The response to steroid therapy is summarized in table 3.

| Treatment Used | All Patients (n=120) | ANA <1:160(n=96) | ANA ≥1:160(n=24) |
|---|---|---|---|
| None | 69(57.5) | 64(66.7) | 5(20.8) |
| IVIG | 3(2.5) | 1(1.0) | 2(8.3) |
| Rituximab | 2(1.7) | 1(1.0) | 1(4.2) |
| Steroids | 32(26.7) | 22(22.9) | 10(41.7) |
| Azathioprine | 2(1.7) | 0(0.0) | 2(8.3) |
| Romiplostim | 1(0.8) | 0(0.0) | 1(4.2) |
| Splenectomy | 11(9.2) | 8(8.3) | 3(12.5) |

**Table 2:** Treatments for patients with immune thrombocytopenic purpura.

*ANA, antinuclear antibody

| Variable | All | ANA <1:160(n=96) | ANA ≥1:160(n=24) | P Value |
|---|---|---|---|---|
| Steroid resistance | | | | |
| Responsive | 29(60.4) | 22(71.0) | 7(41.2) | 0.044 |
| Resistant/dependent | 19(39.6) | 9(29.0) | 10(58.8) | |
| Recent diagnosis of other autoimmune disorders | | | | |
| No | 114(95.0) | 95(99.0) | 19(79.2) | 0.001 |
| Yes | 6(5.0) | 1(1.0) | 5(20.8) | |
| Platelet level before treatment | | | | |
| Missing, n(%) | 2(1.7) | 1(1.0) | 1(4.2) | |
| Median (IQR) | 57.000/mL (17.000/mL, 94.000/mL) | 65.000/mL (30.000/mL, 103.000/mL) | 16.000/mL (13.000/mL, 49.000/mL) | <0.001 |
| Mean (SD) | 59.900/mL (41.900/mL) | 66.300/mL (41.500/mL) | 33.200/mL (32.700/mL) | |
| Range | (0.000/mL, 146.000/mL) | (0.000/mL, 146.000/mL) | (2.000/mL, 126.000/mL) | |

**Table 3:** Clinical characteristics based on high or low Antinuclear Antibody (ANA) titers.

All values are n(%), except as otherwise indicated. IQR, Interquartile Range; SD, Standard Deviation

Only one patient had a direct family history of SLE; this patient had an ANA titer of 1:80. However, 5(20.8%) patients with ANA titers ≥1:160 had developed further autoimmune diseases after being diagnosed with ITP, compared to only one (1%) patient with an ANA titer <1:160(P=0.001); these diseases included systemic lupus erythematosus and anti-phospholipid syndrome. Moreover, patients with ANA

titers ≥1:160 had a significantly lower platelet count (mean, 33.000/mL) than patients with ANA titers <1:160 (mean, 59.9; P<0.001). Table 3 summarizes these clinical characteristics.

## Discussion

This study explored the relationship between a positive ANA titer (≥1:160) and ITP clinical course and outcome. Three main aspects of patients with a positive ANA titer were statistically compared to that of patients with an equivocal or negative ANA titer: steroid resistance, progression to other autoimmune disease, and platelet level at presentation.

It is well accepted that steroids are the first-line treatment modality in ITP. Several studies that evaluated a total of 442 patients with ITP found that 81% of hematologists used steroids as a first-line treatment [10-14].

Patients with an ANA titer ≥1:160 were more likely to have steroid resistance than patients with an ANA titer <1:160. The 2008 study of Abbasi et al., [7] also supports this finding, as the platelet count remained significantly lower after treatment in ANA-positive patients. Additionally, Khellaf et al [8] suggested hydroxychloroquine as a second-line therapy in patients with an ANA titer ≥1:160, as the patients included in their study were all steroid-resistant. In contrast to our findings, a retrospective chart review of 1791 ITP case showed no difference in response to steroids between ANA-negative and ANA-positive ITP patients; nevertheless, ANA-positive patients had a shorter remission period post-splenectomy [15].

Moreover, a higher percentage of ITP patients with ANA titers ≥1:160 showed progression to other autoimmune diseases compared to the percentage in those with ANA titers <1:160. This agrees with the findings of Hazzan et al., [9] who studied the risk of progression to SLE in ITP patients with positive ANA titers. He found that all patients who showed progression to SLE had ANA titers ≥1:160, despite the fact that the majority of ITP patients with ANA titers ≥1:160 did not develop SLE. In contrast, Altintas et al. studied 36 adults with ITP and positive ANA titers, and only one of them developed Sjogren syndrome, leading them to the belief that ANA positivity alone is not a good predictor for the risk of developing further autoimmune diseases [16].

Finally, ITP patients with ANA titers ≥1:160 presented with a much lower platelet count than did patients with ANA titers <1:160. We were unable to find any previous reports comparing platelet count at presentation according to the ANA titer.

Thus, our study findings indicate that ANA titers ≥1:160 is a poor prognostic factor for ITP outcomes, as it is associated with steroid resistance, progression to other autoimmune disease, and a lower platelet count at presentation.

We believe that our study could have been more valuable if it would have been a prospective study, as it may shed the light on aspects we could not have anticipated. Moreover, we believe our biggest disadvantage was that the majority of ITP patients did not have their ANA levels measured which led to a relatively small sample size.

## Conclusion

ITP is a disease with a variable presentation; however, ANA titer levels could be a useful predictor for prognosis. Patients with ITP and ANA titers ≥1:160 were prone to exhibit steroid resistance, have a higher risk of developing other autoimmune diseases, and to present with a lower platelet count than patients with ANA titers <1:160.

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