A cross-sectional study on thrombopoietin levels in immune thrombocytopenia and its correlation with platelet count, megakaryocytes, and treatment response

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Abstract:

INTRODUCTION: Thrombopoietin (TPO) being the major regulator of megakaryopoiesis is expected to show a compensatory increase in immune thrombocytopenia (ITP), however, it is not so observed. This study was undertaken to measure TPO levels in ITP and assesses its association with platelet count, megakaryocytes, and response to steroid therapy.

MATERIALS AND METHODS: A total of 41 cases of ITP and twenty controls with normal platelet count were enrolled in this prospective study. Complete blood count, bone marrow examination, and ELISA for serum TPO were measured. Response to steroid therapy was evaluated for thirty cases.

RESULTS: The TPO levels were increased in 80.5% of patients in comparison to the controls. The degree of rise, however, was variable. On analyzing low, normal, and high TPO levels with reference to platelet and megakaryocyte count no statistically significant difference was observed. Raised TPO levels were seen with significant lowering of functional megakaryocytes. The mean TPO levels in nonresponders were higher than responders but highly variable and statistically nonsignificant.

CONCLUSION: Quantitative alterations in TPO are in a way regulated by qualitative efficacy of megakaryocytes rather than platelet or megakaryocyte count. Nonresponders with markedly increased TPO levels (due to qualitative megakaryocyte injury) are less likely to respond to TPO receptor agonist.

Keywords: Immune thrombocytopenia, megakaryocyte, thrombocytopenia, thrombopoietin

Introduction

Primary immune thrombocytopenia (ITP) is an autoimmune disease characterized by isolated thrombocytopenia in the absence of other causes or disorders that may be associated with thrombocytopenia. The diagnosis of ITP is made on clinical suspicion and after exclusion of various causes associated with thrombocytopenia. The main mechanism of pathogenesis is immune-mediated peripheral destruction of platelets (antibody as well as cell-mediated). In some patients, megakaryocytic suppression and injury have been reported.

Thrombopoietin (TPO) is the major regulator of megakaryopoiesis. TPO has been reported to play role at various stages of platelet production including stem cell induction for differentiation into megakaryocytic progenitors, differentiation of these to megakaryocytes and the final proliferation.
and maturation of megakaryocytes including the release of platelets.\[5,4\]

This study was undertaken to measure TPO levels in ITP patients and assesses its association with platelet count and megakaryocyte count and their functional activity.

**Materials and Methods**

Assuming a standard deviation of 90 pg/ml and margin of error within 30 pg/ml along with confidence interval of 95%, the sample size comes to be 34 (formula used \( n = (\text{standard deviation} \times 1.96 / \text{margin of error})^2 \)). A total of 41 cases of ITP and 20 controls with normal platelet count were enrolled in this prospective study. The institutional ethics committee approved the study, and all patients had given written informed consent before enrolment. All the case underwent detailed clinical evaluation and laboratory workup. The investigations included complete blood count on ADVIA 2120 (Siemens diagnostics) along with thorough peripheral blood smear examination. Investigations for HIV, hepatitis B virus surface antigen, hepatitis C virus, *Helicobacter pylori* and autoimmune disorders were done to exclude these possible etiologies. Bone marrow examination\[6\] was done to exclude other hematological conditions contributing to thrombocytopenia. In all the cases, megakaryocyte count per high power fields was assessed. Forty megakaryocytes were counted in all patients, and the percentage of functional megakaryocytes was assessed. Morphologically evident budding of platelets from megakaryocyte cytoplasm was considered as a marker of functional megakaryocyte response.\[6\] To avoid observational bias two independent observers evaluated all the cases, and their findings averaged. In case of difference of opinion third observer independently reviewed the slides. All the observers were blinded for serum TPO values. The TPO assay was done using the human enzyme-linked immunosorbent kit for TPO, 96 wells (SEA135HU, cloud-clone corp. Houston, USA) with a detection range of 15.6–1000 pg/ml, detection limit of <5.8 pg/ml, intraassay coefficient of variance (CV) <10%, and interassay CV <12%. Thrombocytopenia was defined as platelet count <100 × 10⁹/L.\[11\]

Thirty cases were available for follow-up to treatment response after 3 weeks of steroid therapy. Steroid (prednisolone) was given at a standard dose of 2 mg/kg daily. The response was defined as rise in platelet count by >50 × 10⁹/L in 3 weeks. The cases that achieve response were known as responders.\[7\]

**Statistical analysis**

Continuous groups were compared by Student’s t-test and Mann–Whitney U-test as applicable. Groups were also compared by Kruskal–Wallis (H) ANOVA followed by Z-test for multiple comparisons. Categorical groups were compared by Chi-square test. A two-tailed \( P < 0.05 \) was considered statistically significant. Analyses were performed on SPSS software (Windows version 17.0, IBM, United States).

**Results**

The mean age of patients was 15.18 ± 12.33 years. The male:female ratio was 1.15:1. The most common symptoms were petechiae and purpura (75.6%; 31 cases) followed by epistaxis (39%; 16 cases) and melena (31.7%; 13 cases).

The mean platelet count in patients was found to be 24.93 ± 22.82 × 10⁹/L. In the bone marrow smears mean megakaryocyte count was found to be significantly higher in patients (\( P < 0.001 \)) in comparison to controls. Twenty-seven out of 41 patients (65.9%) had increased megakaryocyte count, while, 14 cases (34.1%) had normal megakaryocyte count. The percentage of functional megakaryocytes was markedly reduced. Mean TPO level in controls was 108.15 ± 14.36 pg/ml. The median TPO levels in cases was 250.72 with interquartile range of 148.50–401.79 pg/ml. Ten cases (24.4%) had values greater than third quartile, ranging from 451.7 to 1662.08 pg/ml. The patients were subdivided according to TPO levels and platelet count, megakaryocyte count, and percentage of functional megakaryocytes were compared between the subgroups [Table 1].

Of the 41 patients, follow-up was available for 30 cases. The nonresponders accounted for 16.6% (\( n = 5 \)) cases. The laboratory parameters in these are shown in Table 2. The mean TPO levels were higher in the nonresponder group (527.05 pg/ml) as compared to the responders (mean TPO level 319.14 pg/ml), however, the difference was not statistically significant (\( P = 0.824 \)).

**Discussion**

ITP is characterized by immune-mediated peripheral destruction of platelets. An expected compensatory response by the marrow shall require an increase in TPO levels. In majority 80.5% of our patients, TPO levels were increased as compared to the controls. Out of 33 patients with increased TPO levels, majority (70%) (\( n = 23 \)) had TPO levels within the interquartile range, i.e., the degree of increase in comparison to control was modest as previously reported.\[8–13\] In ITP patient’s megakaryocytes are increased in number, and their dense expression of c-Mpl is postulated to absorb large amounts of TPO, thereby reducing its level in comparison to cases with hypo proliferative thrombocytopenia.\[14\] However, 30% (\( n = 10 \)) of these patients had TPO levels
Higher than the third quartile. Such high TPO levels have usually been reported in hypoproliferative thrombocytopenia \(^{10-15}\) as well as in occasional cases of ITP. \(^{10}\) One of these patients subsequently developed marrow aplasia. However, it may be an overestimation to quote this single case for an explanation to higher TPO levels in these patients. Another plausible cause could be quantitative and qualitative immune-mediated injury to megakaryocytes. On analysis of megakaryocyte count in these patients, the mean megakaryocyte count was 3.2/hpf, with a variable range of 0.6–7.8/hpf. The quantitative variation in megakaryocyte number is thus less likely to be responsible for higher TPO levels in these patients. Four patients (9.8%) each, however, had low or normal TPO level in coherence with previous reports. \(^{15-17}\)

To identify possible causes of these variations the TPO level were analyzed in relation to platelet and megakaryocyte count [Table 1]. However, no statistical significant difference was observed emphasizing the fact that the platelet or megakaryocyte count alone or in combination does not determine the feedback for TPO. \(^{6,11}\) On analyzing the TPO levels in relation to functional megakaryocytes, patients with increased TPO level had significantly lower functional megakaryocytes \((P = 0.002)\) in comparison to those with lower TPO level. We have for the first time shown this association of TPO level with functional activity in ITP patients. The significantly lower number of functional megakaryocytes in combination with raised TPO levels supports the hypothesis of functional impairment of proplatelet formation in ITP cases. \(^{18}\) The morphological finding of smooth nonbudding membrane of megakaryocyte has been shown to indicate functional defect of megakaryocyte. \(^{6,19}\) The TPO levels hence appear to be governed by the functional megakaryocyte mass rather than count of platelets or megakaryocytes. Furthermore, this highlights the interplay of functional efficacy of TPO and megakaryocytes in ITP cases.

We have also studied the association between TPO levels with response to treatment with corticosteroids. The mean TPO levels in nonresponders are higher than responders but are highly variable and statistically nonsignificant. Since in our study, only five were nonresponders, further studies with a larger sample size are warranted. Since a significant difference in functional megakaryocytes in different TPO level groups was seen further work may be done to assess differentiation stages of megakaryocytes in ITP.

In our previous work, we have demonstrated possible role of eltrombopag in steroid nonresponsive ITP cases. However, response to this thrombopoietin receptor agonist therapy was seen in 80% of cases only. \(^{20}\) The evaluation of functional megakaryocytes in ITP patients may provide help in predicting TPO response in steroid nonresponsive patients. The nonresponders with markedly raised TPO levels (due to qualitative megakaryocyte injury) are less likely to respond to TPO receptor agonists. \(^{13}\)

### Table 2: Clinical and laboratory characteristics of nonresponders

| Age/sex | Duration of symptoms (months) | TPO level (pg/ml) | Platelet count (10\(^9\)/L) |
|---------|-----------------------------|------------------|-----------------------------|
| 19/female | 0.5 | 890.89 | 10 |
| 4/female | 3 | 141.98 | 2 |
| 32/male | 5 | 229.95 | 2 |
| 20/female | 8 | 115 | 20 |
| 12/female | 72 | 1257.42 | 41 |

TPO=Thrombopoietin

**Conclusion**

Quantitative variations in TPO are in a way regulated by qualitative efficacy of megakaryocytes rather than the numerical platelet or megakaryocyte count alone. Hence, when economically feasible, measurement of TPO levels be included in workup algorithm of steroid nonresponsive ITP patients before considering TPO receptor agonist therapy. Evaluation of proportion of functional megakaryocytes may also be act as an alternative. This might help in better prognostication of treatment response.

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Conflicts of interest
There are no conflicts of interest.

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