

Short Research Article

Title page

Can histochemical c-mpl positivity in bone marrow be a predictor for splenectomy in immune thrombocytopenia?

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Table Number: 1

Figure number:1

Word count: 771

Abstract

Purpose: Splenectomy is used as the second line therapy in patients with immune thrombocytopenia (ITP). However, there is no parameter predicting splenectomy decision. Thrombopoietin is the main regulator of platelet count through its receptor c-mpl . The aim of the present study was to evaluate immune histochemical Cloned Myeloid Leukemia Virus (c-mpl) positivity in bone marrow specimens of ITP patients with or without splenectomy indications.

Methods: Pre-splenectomy bone marrow was stained for c-mpl, that was taken from 24 patients with ITP and who had splenectomy as well as bone marrow samples from 30 patients with ITP who did not have splenectomy.

Results: c-mpl negativity was higher in splenectomized patients (n: 23) compared to patients without splenectomy (n:18). A significant difference was found for platelet counts before and after splenectomy. Our study show that, c-mpl positivity was statistically significant in patient group who did not have splenectomy . In the patient group who had the splenectomy, c-mpl was not associated with refractory status.

Conclusion: the significant level of c-mpl negativity might be the useful parameter for splenectomy indication in patients with immune thrombocytopenia.

Keywords: immune, thrombocytopenia, splenectomy, c-mpl positivity

Introduction: Splenectomy is used as the second line therapy in patients with immune thrombocytopenia (ITP). More than 80% of patients with ITP have an excellent response to surgery, and approximately 60-70% of them have a long-term stable response. Identification of predictive factors to splenectomy might be useful to avoid unnecessary surgical intervention, which has a significant morbidity rate. For many patients, the spleen is the primary site of platelet sequestration; but sometimes liver destruction may also exist and even be predominant. It was previously suggested that, after splenectomy, patients with mainly splenic sequestration had a better outcome than those with mixed or hepatic predominant sequestration (1,2). However, there is no parameter predicting splenectomy decision. Thrombopoietin (TPO) is the main regulator of platelet count through its receptor c-mpl. TPO acting via its receptor, the cellular homologue of the myeloproliferative leukemia virus oncogene (Mpl), is the major cytokine regulator of platelet number (3,4). The aim of the present study was to evaluate immune histochemical Cloned-mpl positivity in bone marrow specimens of ITP patients with or without splenectomy indications.

Material-Method: Bone marrow specimens were taken from 24 patients who were diagnosed with ITP and who had splenectomy (15 female, 9 male, mean age 50±16) before splenectomy and 30 patients who were diagnosed with ITP but did not have splenectomy (15 female, 15 male, mean age 52±19). c-mpl staining was carried out retrospectively. Immunohistochemical (IHC) staining using Avidin-Biotin complex system (ABC) was conducted. For IHC, dissections prepared from blocks were taken onto poly-L-lysine coated slides (MicroSlides

Snowcoat X-tra, Surgipath, Richmond, IL, USA) and kept in an incubator at 37°C overnight. Dissections were treated with IHC c-mpl (Santa Cruz/sc-13187) stain. Cytoplasmic and nuclear staining was observed in megakaryocytes using IHC c-MPL. Immunohistochemical results of c-MPL were evaluated at 4 grade (0-3+) as negative, weak, medium, and strength according to staining intensity. The evaluation was made based on the intensity of the staining; i.e. negative (0), weak (1+), moderate (2+) and strong (3+) (3) (Figure 1). The grade of 1 and above was accepted as positive in Table 1. All patients who had splenectomy were in the chronic phase of the disease. **fiser exact test** was used for statistical analyses. The present study was supported as a Scientific Research Project by Adnan Menderes University (TPF-15027). This study was approved by the local ethical committee of Adnan Menderes University Medical Faculty. Signed informed consent was obtained from all participants (number: 2014/425).

Result: Splenectomy was not performed in the second group due to stable platelet count and response to the first line treatment such as the corticosteroid. There were no statistical differences for the platelet levels in the staining group, and also in patients with and without splenectomy. Also, There was no correlation between c-mpl positivity on megakaryocytes and platelet count in patients performing the splenectomy. c-mpl negativity was higher in splenectomized patients compared to patients without splenectomy. A significant difference was found for platelet counts before and after splenectomy. Our study show that, c-mpl positivity was statistically significant in patient group who did not have splenectomy (Table 1). In the patient group who had the splenectomy, c-mpl was not associated with refractory status.

Discussion: Status of c-mpl in ITP is ambiguous. C-mpl type 1 is a member of the hematopoietic growth factor receptor (HER) family (5). In a study of Gurney et al. on c-mpl deficient mice, it was determined that platelets and megakaryocyte counts reduced at a rate of 85% whereas other hematopoietic cell types were in normal range (6). TPO / c-mpl interaction does not affect only maturation and differentiation of megakaryocytes but also regulates serum levels of TPO and surface expression of c-mpl (7). In a study conducted on thrombocytopenia due to various etiologies including ITP, the levels of c-mpl and thrombopoietin were compared with normal individuals. A significant increase was detected in the levels of thrombopoietin and c-mpl compared to the control groups, which was statistically significant in the patients with ITP (3). In a study conducted by Ichikawa et al., serum levels of TPO and platelets were measured before and after splenectomy in a group of patients with liver cirrhosis, gastric cancer, and lymphoid malignancy. The levels of TPO and platelets showed an increase after splenectomy (8). In a study of Navez et al. site of platelet sequestration was not significantly associated with recurrence-free survival. Younger patients with platelet count $>100 \times 10^9/\mu\text{L}$ should have a better response to splenectomy (2). Retrospective design and the small sample size were the limitations of the study. The limitation of the study is that we could not evaluate the correlation between serum TPO level and c-mpl expression on megakaryocytes.

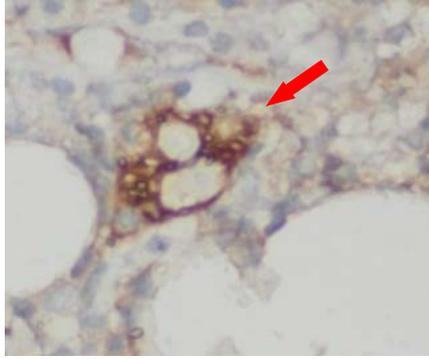
Conclusion :

In conclusion, the significant level of c-mpl negativity might the useful parameter for splenectomy indication in patients with immune thrombocytopenia.

Table 1: c-mpl positivity in patients with and without splenectomy

	with splenectomy (n)	without splenectomy (n)	p-value
c-mpl positivitiy (n)	1	12	<0,001
c-mpl negativity (n)	23	18	
Total (n)	24	30	

Figure 1: Megakaryocyte and c-mpl positivity (2+), c-mplx400

**Conflict of Interest Statement:**

We did not receive any financial support. And this study has not been published in any journal. We have not conflict of interest.

Concent :

Informed and written consent was obtained from all individual participants included in the study.

Ethical :

As per international standard ,written ethical approval has been collected and preserved by the author(s).

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