Research Article

Comparing IgG Binding to Megakaryocytes in Thrombocytopenic Patients

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Abstract
Background and objectives: The aim of the present study is to evaluate and compare IgG-binding to megakaryocytes in bone marrow of ITP and MDS patients to determine megakaryocytes targeting by autoantibodies in vivo as a mechanism of platelet underproduction in these disorders. **Subjects and methods:** The study was carried out on 20 ITP (group I) patients and 20 thrombocytopenic patients with myelodysplastic syndrome (group II) who were admitted to minia university hospital. Serial histological sections from bone marrow biopsies were stained for IgG. **Results**: high IgG binding was found in ITP and MDS patients (group I & II) and there was no statistically significant difference between both groups ((14/20 (70%) vs. 13/20 (65%),), (P value =0.736). **Conclusion**: Antibody binding to megakaryocytes in a proportion of MDS patients suggests that immune mediated mechanism underlies PLT underproduction in those patients
Key Words: ITP, MDS, megakaryocytes, antibody specificity.

Introduction
Autoimmune thrombocytopenia (ITP) is an acquired immune-mediated disorder characterized by isolated thrombocytopenia, defined as a peripheral blood platelet count less than 100 $x10^3/\mu l$, in the absence of any obvious initiating and/or underlying cause of the thrombocytopenia (Ishida et al., 2017).

Platelet destruction is mediated by autoantibodies against platelet surface glycoproteins (GP), particularly GPIIb/IIIa and GPIb/IX. Those autoantibodies cause thrombocytopenia by accelerated Fcγ receptor-mediated platelet clearance in the reticuloendothelial system (Behzad et al., 2018)

One possible mechanism for platelet underpropduction is autoantibody-mediated megakaryocyte inhibition. As megakaryocytes also express CD41/CD61 and CD42b/CD42a on their surfaces, it has been proposed that antiplatelet antibodies might bind MK and cause their destruction, impair their function or delay their maturation and consequently interfere with platelet production. (Perdomo et al., 2013).

In support of this hypothesis, previous studies have demonstrated that autoantibodies and isolated immunoglobulin G (IgG) fractions from some ITP patients can inhibit megakaryocyte growth and maturation in vitro; and that antibodies from some ITP patients bind to target bone marrow megakaryocytes ex vivo (Iraqi et al., 2015).

The myelodysplastic syndromes (MDS) are clonal hematopoietic disorders characterized by ineffective hematopoiesis, peripheral blood cytopenia, dysplastic cell morphology and risk of progression to acute myeloid leukemia (AML) (Gibson et al., 2018).

Several mechanisms underlie thrombocytopenia in MDS including ineffective platelet production secondary to disordered maturation and proliferation of megakaryocytes or their precursors, increased megakaryocyte programmed cell death, increased peripheral destruction of platelet (e.g.in the spleen) and autoantibody mediated destruction of platelet and megakaryocytes (Brierley and Steensma, 2015).

In vivo studies investigating antibody binding in the bone marrow microenvironment are lacking. (Arnold et al., 2015).

Subjects and Methods

Study design:

he current study was carried out at Minia University Hospital in the period from December 2016 to December 2017. The study was performed on 20 patients diagnosed as primary ITP based on established clinical Criteria, platelet count levels below 100 x109/L at the time of bone marrow sampling and no other pathology identified on bone marrow examination as group I and20 thrombocytopenic patients whose clinical presentation and bone marrow features were consistent with MDS. as group II who were admitted to internal medicine department. Patients were excluded if they had Autoimmune disease; human immunodeficiency virus (HIV); hepatitis B or hepatitis C or if they had received treatment with a TPO receptor

Both groups were subjected to Complete history taking then Clinical examination: Including: pallor, purpura, liver, spleen and lymph nodes enlargement. In addition to the laboratory investigations; bone marrow biopsy samples were collected, processed and fixed.

Formalin fixed bone marrow tissue blocks were deparaffinized, washed in xylene and rehydrated with graded washes of ethanol in water. Serial sections (2 - 4μm) were pretreated with target retrieval in a water bath for 40 minutes. Slides were washed and blocked in peroxidase blocking reagant for 20 minutes at room temperature. After washing, slides were incubated with IgG for 60 minutes. Following incubation, slides were washed and incubated with Envision™ FLEX Substrate for 20 minutes and counterstained with hematoxylin.

Statistical analysis

Data were coded and entered using the statistical package SPSS version 21. Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparison of non-parametric quantitative variables was done using Mann Whitney test. Chi square test used for comparison of qualitative data between the two groups.

Results

Megakaryocytes associated IgG was increased in both ITP and MDS patients. (fig 1, 2). and There was no statistically significant difference between both groups (Mean ± SD (55.2± 18.9% versus 53 ± 15.6%), (P value =0.683). (Table1). Median number of bone marrow megakaryocytes was increased in ITP and MDS patients (group I & II) and there was no statistically significant difference between both groups (Mean ± SD, 8.1 ±5.1, vs. 6 ±3.1, cells per HPF; p=0.271). (Table 2).

Table (I): Comparison between group I & II regarding the proportion of megakaryocytes associated with IgG, IgG binding and megagakaryocytes bound to CD61

		ITP N=20	MDS N=20	P value
Megakaryocytes associated IgG(%)	Range Mean ± SD	**(10-80)** **55.2±18.9**	**(20-70)** **53±15.6**	**0.683**
IgG binding	Low binding n(%) High binding n(%)	**6(30%)** **14(70%)**	**7(35%)** **13(65%)**	**0.736**
Megakaryocytes bound CD61(%)	Range Mean ± SD	**(40-100)** **73.5±14.6**	**(40-90)** **66.5±11.8**	**0.104**

Table (2): Comparison between studied groups regarding Hb,TLC,platelet count and number of megakaryocytes(per HPF):

		ITP N=20	MDS N=20	P value
Hb (1)	Range Mean ± SD	(7-13.8) 11.3±1.6	(5.5-14) 10±2	***0.034****
TLC (1)	Range Mean ± SD	(4.4-21.4) 8.8±4.1	(2.9-10.8) 6.4±2.4	***0.023****
Platelets (2)	Range Mean ± SD Median	(5-61) 22±14.8 17.5	(8-64) 38.3±14.4 38.5	***0.001****
Megs (2)	Range Mean ± SD Median	(2-18) 8.1±5.1 7	(2-12) 6±3.1 6	*0.271*

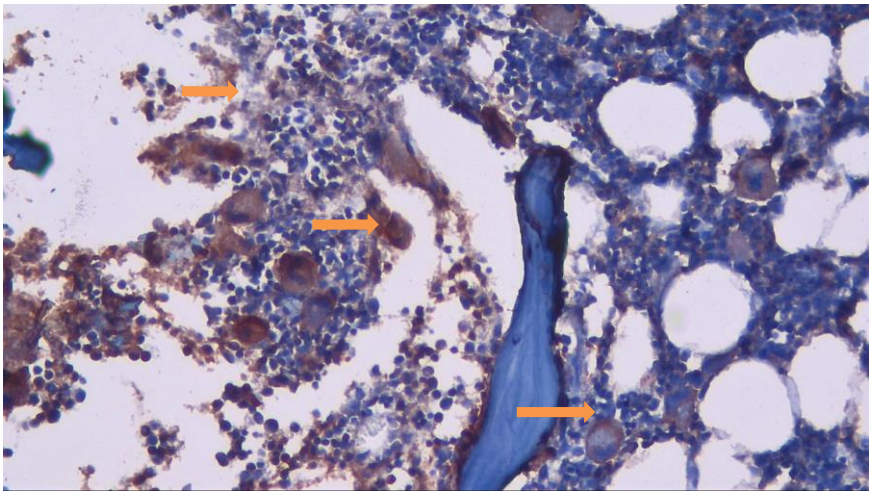

Figure 1: immunohistochemical stains for IgG of BM biopsy specimens from a patient with ITP. Arrows indicate megakaryocytes. Representative images are shown at 200X magnification

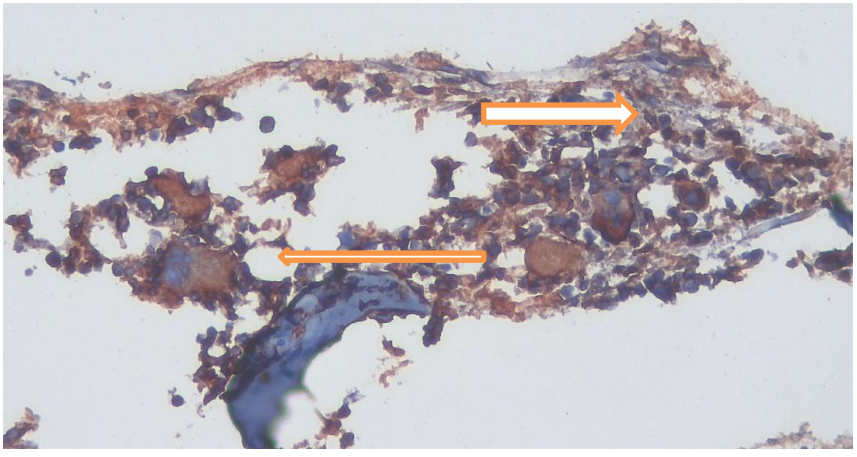

Figure 2: immunohistochemical stains for IgG of BM biopsy specimens from MDS. Arrows indicate megakaryocytes. Representative images are shown at 200X magnification.

Discussion

In the present study we found negative correlation between number of megakaryocytes and platelet count in ITP patients. This was in accordance with Barsam et al., 2011 who suggested that increasing MK production in ITP patients does not always correlate with a recovery of platelet counts, indicating that additional factors may prevent platelet release from MK.

On the other hand. Lev et al., 2014 noted that Purified IgG inhibited proplatelet formation, suggesting the involvement of auto-antibodies in the inhibition of thrombopoiesis, he concluded that reduced platelet production is caused, not only by autoimmune-mediated inhibition of megakaryopoiesis, but also, by impaired PPF from mature MKs. This may explain why the number of bone marrow megakaryocytes is normal or increased in patients with ITP despite severe thrombocy-topenia.

In this study high IgG-binding on megakaryo-cytes was found in immune (ITP), and also in non-immune thrombocytopenic conditions (MDS). These results were similar with Arnold et al., (2015) who found that proportion of ITP patients with high megakaryocyte-associated IgG was increased compared with normal controls, and the proportion of ITP patients with high IgG binding was no different than thrombocytopenic patients with MDS.

Yang et al., 2010 reported that several possible mechanisms may contribute to the suppression of megakaryocyte production by autoanti-bodies. First, megakaryocytes express GPIIb/ IIIa or GPIb/IX on their surfaces during maturation as well as platelets, autoantibodies binding to megakaryocytes, and platelets could mediate megakaryocyte and platelets destruc-tion by phagocytic cells. Second, autoantibody induced activation of complement may play a role in megakaryocyte apoptosis.

In this study we suggested that megakaryocytes are targeted by autoantibodies in vivo causing its suppression in both ITP and MDS patients while Houwerzijl et al., 2006 documented that In MDS, megakaryocytes show features of necrosis-like PCD, whereas ITP megakaryo-cytes demonstrate predominantly characteristics of apoptosis-like PCD (para-apoptosis). In MDS, the interaction of Fas/Fas-ligand might be of importance, whereas in ITP antiplatelet autoantibodies recognizing common antigens on megakaryocytes and platelets might be involved.

The present study showed significant positive correlations between IgG binding to megaka-ryocytes and number of megakaryocytes in both groups (ITP and MDS). Similarly, Arnold et al., 2015 found that IgG binding was associated with increased megakaryocyte numbers in ITP and MDS groups.

The results of this study were in agreement with Sloand et al., 2010 who demonstrated that a subset of patients with MDS respond to immunosuppressive therapy suggesting that the immune system plays a role in the pathogenesis of MDS, at least in some cases. He reported that immunological abnormalities are frequently observed in patients with MDS and revealed that about 10% of MDS patients have clinical autoimmune disorders.

So binding of IgG to megakaryocytes occurs in thrombocytopenic conditions, whether due to immune or non-immune causes, suggesting that Ab binding is not specific for ITP and the immune system plays a role in the pathogenesis of thrombocytopenia in MDS patients.

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