Utility of platelet associated antibodies in the diagnosis of children with thrombocytopenia

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Abstract

Thrombocytopenia is a common hematological disorder in both children and adults. Immune or idiopathic thrombocytopenia purpura (ITP) is the most comment causes of immunemediated thrombocytopenia in children. It can be caused by non-immune causes such as apalastic anemia. In majority of cases the diagnosis is made clinically with the aid of limited laboratory investigations. The purpose of this study was to evaluate whether measuring platelet associated immunoglobulins (PAIg's); IgG and IgM with complement 3 (C3) would make a useful tool in diagnosing the cause of thrombocytopenia. It was a retrospective study of children with a diagnosis of thrombocytopenia. Their charts were reviewed in regard to age, sex, platelet count at the time of the diagnosis, and the treatment received. They were classifieds into primary and secondary types. The primary type included those with acute ITP (AITP) (n= 7) and chronic ITP (CITP) (n= 25) whereas the secondary type included those with thrombocytopenia due to other causes (n= 31). Furthermore, the thrombocytopenic episodes were reclassified into immune and non-immune mediated thrombocytopenia (n= 48) and (n= 29) respectively. A total of 63 children with 77 thrombo-cytopenic episodes were studied. Direct flow cytometry technique was used to measure the platelet associated IgG (PAIgG), platelet associated IgM (PAIgM), and platelet associated complement 3 (PAC3). Results of PAIgm in AITPand CITP were positive for 80 % and 63 % of cases respectively, while in secondary it was positive for 40 % of cases only. In case of immune and non-immune thrombocytopenia, the positivity of PAIgM occurred in 63 % and 34 % of cases. The results of pAIgG and PAC3 were also found positive inAITP, CITP, immune and non-immune thrombocytopenia but nonconclusive. Conclusion: These results indicate that measurement of PAIg's and PAC3in children with thrombocytopenia is not accurate and often fails to discriminate between the different causes of thrombocytopenia. Diagnosis of thrombocytopenia remains mainly clinical, and measuring of platelet associated antibodies is not recommended in clinical practice.

Introduction

ITP is a plateletdestructive disorder which is being caused by antiplatelet antibodies [1]. These antibodies target specific glycoprotein (GP) on the platelet membrane i.e. GP IIb/ IIIa, GP Ia/ IIa and GP Ib/ IX [2]. In 75 % of patients, these autoantibodies are directed against platelet GP complexes and can be identified by using GP-specific assays [1,3-5]. In the remaining 25 % of ITP patients, it is believed that other platelet epitopes are involved or that the thrombocytopenia is caused by some other mechanism [1,5]. Clinical immune thrombocytopenia (IT) occurs as primary IT, also known as ITP and secondary IT, which occurs with autoimmune diseases like systemic lupus erythematosus (SLE) [2,6-8], the antiphospholipid syndrome [2,9], and lymphoproliferative disorders [2,3]. ITP still remains a clinical diagnosis and laboratory only assists in its diagnosis. Methods to measure antiplatelet antibodies have evolved over the years. Early assay detecting platelet associated IgG showed that many thrombocytopenic disorders, both immune and non-immune were associated with increased amount of IgG bound to platelet, and these assays had little diagnostic values [1,3,10]. One of the first tests was the measurement of PAIgG. Currently used

PAIgG assays are “Competitive Enzymelinked Immunoassays” (CELISA) a quantitative method developed by Kiefel and coworkers [11] and the “Platelet Immunofluorescence Test” (PIFT) which was introduced by von dem Borne and coworkers two decades ago [12]. The latter is regarded to be highly sensitive, especially if performed by flow cytometery [7,13-15]. The aim of this study was to evaluate the usefulness of qualitative PAIg’s detection from the point of view of differential diagnosis of thrombocytopenia by flow cytometeric analysis.

Patients and Methods

A retrospective study was conducted by reviewing the charts of patients with thrombocytopenia who had PAIg’s and C3 tests done for them at the time of thrombocytopenic episode. The charts were reviewed with regard to age, sex, and platelet counts as well as the treatment received by the patients within one month of the test or at the time of the test.

A total of 63 children were studied, having 77 episodes of thrombocytopenia. Patients were classified into two main groups:

1. primary thrombocytopenia including acute ITP (AITP) and chronic ITP (CITP),
2. secondary thrombocytopenia

AITP was defined as: spontaneous bleeding symptoms and isolated thrombocytopenia (platelet counts < 150 X 109/ L) for less than 6 months with normal or increased marrow megakaryocytosis but without clinically apparent other causes of thrombocytopenia.

Chronic ITP was defined as AITP with the exception of more than 6 months duration of thrombocytopenia. Secondary thrombocytopenia was defined as thrombocytopenia (platelet counts < 150 X 109/ L) with associated disorders presumed to be the cause of the thrombocytopenic state.

PAIg’s (PAIgG and PAIgM) and C3 were performed using direct flow cytometry. The assay directly measures the amount of immunoglobulin on the surface of washed platelets.

Data was analyzed and presented as median values, ranges, sensitivity and specificity.

Results

AITP: there were 7 children with thrombocytopenia, having 10 episodes. Their ages ranged between 3.78 to 15.2 years with median age of 5 years. Male to female ratio was 5: 2. Minimum platelet count (X 109/L) was 1 and maximum was 90 with a median count of 43. PAIgG and PAC3 were positive in 4 out of 10 episodes (40 %), while PAIgM were positive in (80 %) [Table 1]. Three of the patients with platelet counts < 20 X 109/L had received treatment (prednisolone; 4 mg/kg/day for 4 days with a good response).

CITP: 25 children with thrombocytopenia were studied having 32 episodes. Their ages ranged between 1.78 to 15.8 years with a median age of 9.7 years. Male to female ratio was 2: 3. Minimum platelet count (X 109/L) was 10 and maximum count was 114 with a median count of 51. PAIgG was found positive in 19 out of32 episodes (59 %), PAIgM in 63 %, and PAC3 in 38 % of episodes [Table 1]. Three of the patients received treatment for symptomatic thrombocytopenia (intravenous gamma globulin in 1 patient and Anti-D in 2 patients). Eight patients underwent splenectomy in the past, while 14 asymptomatic patients did not require any treatment.

Table 1: Characteristics of the patients.

AITP (%)	CITP (%)	Secondary (%)
No. of cases	7	25
Median age (year)	5	9.7

Male: female	5: 2	2: 3
No. of episodes	10	32
Median platelet #	43	51
Positive PAIgG	4/10 (40)	19/32 (59)
Positive PAIgM	8/10 (80)	20/32 (63)
Positive PAC 3	4/10 (40)	12/32 (38)

Table 2: Distribution of causes of secondary thrombocytopenia

Cause	No. of cases	Percentage
Aplastic anemia	6	19
Post bone marrow transplant	4	13
Autoimmune hemolytic anemia	2	6
Leukemias	4	13
Hypersplenism	4	13
Post EBV viral infection	3	10
SLE	3	10
Miscellaneous	5	16
Total	31	100

Table 3: Flow cytometry detection of plateletassociated IgG (PAIgG), IgM (PAIgM) and complement 3 (PAC3) in patients with immune thrombocytopenia and other thrombocytopenic disorders. By number of episodes.

CAUSES	No. of episodes	No. of patients with positive		
		PAIgG (%)	PAIgM (%)	PAC3 (%)
Immune thrombocytopenia				
AITP	10	4	8	4
CTP	32	19	20	12
SLE	4	4	2	2
Autoimmune hemolytic anemia	2	1	0	0
TOTAL	48	29 (60)	30 (63)	18 (38)
Non –immune thrombocytopenia:				
Aplastic anemia	7	2	2	0
Post bone marrow transplant	5	3	2	0
Leukemias	5	2	0	2
Hypersplenism	4	4	1	1
Post EBV infection	3	3	2	1
Miscellaneous	5	2	3	2
Total	29	16 (55)	10 (34)	6 (21)
Grand Total	**77**			

(For larger image of table, click here)

Secondary thrombocytopenia: there were 31 children with thrombocytopenia who had 35 episodes. Their ages ranged between 2.78 to 17.6 years with a median age of 10.8 years. Male to female ratio was 1: 1. Minimum platelet count (X 109/L) was 13 and maximum count was 120 with a median count of 76. PAIgG were found positive in 22 out of 35 episodes (63 %), PAIgM in 40 %, and PAC3 in 20 % of the episodes [Table 1]. Thrombocytopenia was secondary to variety of conditions including aplastic anemia, post bone marrow transplant, autoimmune hemolytic anemia, leukemias, hypersplenism, post Epstein Barr virus (EBV), systemic lupus erythematosus (SLE), and secondary to five other causes. The miscellaneous causes included one case each of the following diagnoses; hemophilia, immune deficiency, myelodysplastic syndrome (MDS), von Willebrand's disease, and intestinal pseudoobstruction [Table 2].

Furthermore, thrombocytopenic episodes were also classified as having classical immunologic disorders i.e. AITP, CITP, autoimmune hemolytic anemia and SLE versus thrombocytopenia of nonimmunologic etiology i.e. leukemias, and aplastic anemia. 48 children with immune thrombocytopenic episodes were studied; 29 of them (60 %) had positive PAIgG, 30 (63 %) had positive PAIgM, and 18 (38 %) had positive PAC3. On the other hand, 29 nonimmune thrombocytopenic episodes were studied; 16 of them (55 %) had positive PAIgG, 10 (34 %) had positive PAIgM and only 6 (21 %) had PAC3 [Table 3].

Discussion

The mechanism of thrombocytopenia in these patients seems to be a peripheral destruction in the primary thrombocytopenia category and a combination of peripheral destruction and bone marrow suppression in the secondary category. ITP is caused by antibody-mediated platelet destruction. The diagnosis of ITP is based on the finding of thrombocytopenia with normal to increased bone marrow megakartocytes and the exclusion of other causes of the thrombocytopenia. Assays that measure PAIg's are not useful diagnostic tests because patients with immune or nonimmune thrombocytopenia have elevated levels of PAIgG [16,17]. According to the American Society of Hematology guidelines, bone marrow evaluation and PAIgG assays are unnecessary [18]. Some investigators have suggested that techniques capable of measuring immunoglobulins bound to individual's platelet glycoproteins can be used to diagnose ITP [19,21]. In vitro detection of platelet reactive antibodies remains difficult, despite numerous available assays for the measurement of platelet associated IgG and glycoprotein specific antibodies. The assays available for detection of PAIgG are regarded to be highly sensitive, especially if performed by flow cytometry [7,13-15], but their specificity is considered low, since elevated levels of PAIgG are found in numerous thrombocytopenic conditions other than ITP [3,6-9]. In contrast, GP-specific antibodies are mostly found in blood samples of patients with ITP [2,8, 9,22,23]. However, the assays for detection of GP-specific antibodies such as monoclonal antibody-specific immobilization of platelet antigen (MAIPA) are highly specific but less sensitive than the PAIgG-assays [17]. They are also laborious and require a certain amount of platelet, which is not always available in thrombocytopenia. Therefore, this method is actually not convenient for use in the routine hematology laboratories

[24]. The elevated levels of IgG, IgM and C3 are interpreted as an expression of certain types of hyperimmune state rather than as an impairment of immunoregulatory mechanism [25].

Flow cytometric approach appears to be at least as good as other techniques and better in many ways compared to MAIPA. IT identifies plateletassociated immunoglobulins which makes it a potentially useful diagnostic test for ITP [26].

In vivo complement activation occurs in most CITP patients with binding of C3 to the platelet surface. This in vivo complement activation may promote more efficient phagocytosis and possibly platelet lysis in some cases of ITP [6].

In this study, by the first classification of thrombocytopenia as primary type (AITP, CITP) versus secondary type; PAIgG, PAIgM and PAC3 were positive in both types in approximately half of the patients. Of note, PAIgM were the highest positive in AITP whereas PAC3 were the least positive in secondary type. Similarly, by the second classification of thrombocytopenia based on immune versus nonimmune mediated type; PAIgG, PAIgM, and PAC3 were positive in both conditions. Once again, PAC3 were the least positive in a nonimmune type. These data suggest that positive PAIg's could be detected in different thrombocytopenic conditions as they are not confined to the immune over the nonimmune types. This could be attributed to the cross reactivity of these antibodies among the different conditions of thrombocytopenia. It was anticipated to have the highest levels of PAIgG in either primary and/ or immune thrombocytopenia based on the pathogenesis of antigenantibody reaction in these diseases, but it was not the case as it occurred in all types of thrombocytopenia. The cause of the very high level of PAIgM in AITP is not clear, but could be explained by the relatively small number of cases in AITP compared to CITP or secondary thrombocytopenia. PAC3 were consistently low (less than quarter of cases) in both secondary and nonimmune thrombocytopenia. This is likely due to the fact that complement activation occurs more often in immune rather than nonimmune mediated conditions. Kayser et al. reported that PAC3 values are not restricted to immune thrombocytopenia [27]. Quantitative differences of PAC3 between immune and nonimmune thrombocytopenia patients suggest that part of the PAC3 is immunologically mediated and has a role in the pathogenesis of autoimmune thrombocytopenia.

Conclusion

The diagnosis of ITP is mainly clinical; it requires a detailed history, clinical examination and careful review of blood count and peripheral blood smear. Bone marrow examination is a useful tool in discriminating destructive causes of thrombocytopenia i.e. ITP from other causes like aplastic anemia. Apparently, platelet associated autoantibodies are of less value in determining the etiology of thrombocytopenia. They lack accuracy, costly and often cause more confusion regarding the diagnosis than help. Therefore, the author does not recommend routine search for platelet associated autoantibodies in children with thrombocytopenia in the clinical practice either by flow cytometric or other techniques like MAIPA. It would be appropriate to measure these antibodies for academic and research purposes. Further researches in this field are required aiming to find a novel test with high level of reliability in detecting platelet associated autoantibodies.

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