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Platelet-specific antibodies and differences in their expression in childhood immune thrombocytopenic purpura predicts clinical progression

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ABSTRACT

Importance: Immune thrombocytopenic purpura (ITP) is the most common bleeding disorder in children. Despite the highly spontaneously remission, still almost 20% of cases progress into chronic or refractory ITP, which seriously affects children's quality of life. Currently there is no method to predict the initial stage of childhood ITP.

Objectives: To evaluate platelet-specific antibodies and compare differences in their expression in childhood ITP to predict clinical progression.

Methods: This is a single-center prospective cohort study from April 2014 to October 2015. We enrolled children initially diagnosed as ITP. Anti-GPIIb/IIIa and GPIb/IX antibodies were assayed by enzyme-linked immunoadsorbent assay (ELISA) and patients were followed up for 1 year. We also analyzed the relationship between the expression of the platelet-specific antibodies GPIIb/IIIa and GPIb/IX and their respective clinical prognoses.

Results: Overall, 134 cases were enrolled including 77 boys and 57 girls with a median age of 19 months (range: 1 to 159). Positive rates of anti-platelet antibodies were 79.8%. After a 1-year observation period, 84.3% were diagnosed as newly diagnosed ITP and 13.4% were diagnosed as chronic ITP. Patients with anti-GPIIb/IIIa antibody had a higher risk for newly diagnosed ITP compared with patients who were anti-GPIb/IX antibody positive only (93% vs 25%, $P = 0.005$; 87% vs 25%, $P = 0.014$, respectively). There were more anti-GPIb/IX antibody positive only cases, diagnosed as chronic ITP, compared with anti-GPIIb/IIIa antibody positive only cases and double GPIIb/IIIa and GPIb/IX antibody positive cases (75% vs 7%, $P = 0.005$; 75% vs 13%, $P = 0.014$, respectively).

Interpretation: Patients with anti-GPIIb/IIIa antibody (either single or double) were predicted to have a good prognosis, whereas anti-GPIb/IX antibody only predicted a poor prognosis. These results should be confirmed via a larger cohort multicenter study.

KEYWORDS

Childhood ITP, Platelet-specific antibodies, Prognosis

INTRODUCTION

Primary immune thrombocytopenia (ITP) is an acquired immune bleeding disorder characterized by isolated thrombocytopenia (peripheral blood platelet count $< 100 \times 10^9/L)^1$ caused by pathogenic anti-platelet autoantibodies.[2,3] Clinical manifestations include petechiae, purpura, bruising, and overt bleeding. The current estimate of the incidence of acute ITP in children is between 1.9 and 6.4 per 10^5 children per year.[4] However, most cases have an acute course and more than 80% cases recover spontaneously.[5-7] Furthermore, 10%–20% of children with ITP progress to chronic ITP.[4] Because of the high impact of ITP on their quality of life, factors that predict the disease course are important, but few factors have been identified.

ITP is an antibody-mediated destructive disease, and autoreactive antibodies that target platelet antigen complexes are considered responsible for the accelerated destruction of platelets by the reticuloendothelial system and for the inhibition of megakaryopoiesis, in which platelet glycoprotein GPIIb/IIIa and GPIb/IX are the two most frequently targeted autoantigens.[8-10] Studies on adult ITP report that different platelet-specific antibodies may affect clinical prognosis and that the presence of anti-GPIb/IX autoantibodies is a predictive factor for poor prognosis risk.[11-13] In accordance with data from animal models,[14] a recent study suggested ITP patients who were anti-GPIbα antibody positive were insensitive to dexamethasone treatment.[15] However, there has been no relevant study in children.

The aim of this study was to evaluate whether platelet-specific antibodies can predict the progression of childhood ITP.

METHODS

Ethical approval of the study

This was a prospective study. The clinical data of children initially diagnosed as ITP were collected from our hospital from April 2014 to October 2015. The parents or guardians of all the enrol children signed informed consent forms. This study was approved by the local ethical committee of Capital Medical University.

Study inclusion/exclusion criteria

Inclusion criteria: the diagnosis of ITP was based on the presence of isolated thrombocytopenia and the absence of any obvious initiating and/or underlying cause of the thrombocytopenia in accordance with the recently released international consensus guidelines[16]; age between 1 month to 14 years old; a platelet count of $\leq 30 \times 10^9/L$, without any previous treatment; duration less than 1 month; and voluntary principles and signing of informed consents.

Exclusion criteria: we excluded patients with a history of any ITP-specific treatment administered prior to first-line therapy. We also excluded other thrombocytopenias including congenital immune thrombocytopenia, systemic lupus erythematosus and/or infection-related thrombocytopenia; with abnormal diseases, such as aplastic anemia or neoplastic diseases; and abnormal distribution, including hypersensitivity.

Definitions and responses

ITP diagnostic criteria used were according to the International Working Group (IWG)[16]: at least two blood tests PLT $< 100 \times 10^9/L$, no abnormal blood cell morphology; skin bleeding, ecchymosis and/or mucous membranes, organ bleeding and other clinical manifestations; generally no splenomegaly; exclusion of other secondary thrombocytopenias such as low proliferative leukemia, thrombocytopenia as the first hematologic abnormality of aplastic anemia, hereditary platelets that reduce the disease, secondary to other immune diseases, as well as infection and drug factors.

The response was evaluated according to the IWG criteria[16]: complete response (CR) was defined as any platelet count of at least $100 \times 10^9/L$; Response (R) was defined as any platelet count between 30 and $100 \times 10^9/L$ and at least double the baseline count; and no response (NR) was defined as any platelet count below $30 \times 10^9/L$ or less than double the baseline count.

Bleeding symptoms were classified from grade 0 to 5 according to the WHO scale.[17]

Prognoses were divided into three groups according to the final duration: newly diagnosed (from diagnosis until 3 months); persistent ITP (3–12 months); and chronic ITP (ITP lasting for more than 12 months).

Detection of anti-GP autoantibodies

Platelet-specific antibodies were detected prior to treatment initiation. Anti-GPIIb/IIIa and anti-GPIb/IX autoantibodies were tested routinely in our hospitals using a PAKAUTO kit (GTI Diagnostics Inc, Waukesha, WI, USA), as previously described.[18] Specificity of autoantibodies was evaluated with EDTA-anticoagulated plasma and, in some cases, by using a platelet-adsorbed plasma processing PAKAUTO kit according to the manufacturer's instructions. This kit is a qualitative solid phase ELISA designed to detect platelet autoantibodies, including those directed against immobilized GPs complexes - GPIIb/IIIa and GPIb-IX.

Analysis methods

We analyzed differences in the clinical manifestations of different platelet-specific antibodies including age, sex, pretreatment platelet count, and degree of bleeding. All

patients received first-line treatment, including intravenous immunoglobulin and/or glucocorticoid treatment. Peripheral blood counts and conditions of bleeding were obtained at diagnosis and at 1, 3, 6, and 12 months after first-line treatment. Follow-up registration was done in the outpatient clinic or by telephone communication. Patients diagnosed as persistent ITP were excluded from the analysis. To evaluate whether platelet-specific antibodies predicted the progression of the condition of children with ITP, we compared the differences in the expression of platelet-specific antibodies at the time of the initial diagnosis between newly diagnosed patients and chronic ITP patients.

Statistical analysis

All statistical analyses were performed with SPSS software. Quantitative data, if given as a normal distribution, are described by the mean (standard deviation), and the two groups were compared using the *t*-test. If they did not follow a normal distribution, they were described by the median (upper and lower quartiles) and the Wilcoxon rank-sum test was used. Classification data was described by frequency (percentage), and the Chi-square test was used for comparisons between groups. We used logistic regression analysis to explore prognostic factors differences. $P < 0.05$ were considered statistically significant.

RESULTS

Demographic features of patients

From April 2014 to October 2015, 144 patients were screened for eligibility. Seven patients were excluded because of a diagnosis of secondary thrombocytopenia, including four with aplastic anemia, two with acute myeloid leukemia, and one with systemic lupus erythematosus. All the other patients were followed up for at least 12 months after first-line treatment. Three patients were lost to follow-up, 113 patients were newly diagnosed as ITP, three patients were diagnosed as persistent ITP, and eighteen patients were diagnosed as chronic ITP. Overall, 134 patients (77 boys and 57 girls), who were followed up for at least 12 months, were included in this analysis (Table 1). The male: female ratio was 1.35:1; the median age was 19 months (range, 1 to 159); and the platelet count baseline was $(10.68 \pm 10.54) \times 10^9$/L. Regarding bleeding conditions, 10 cases were absent of any bleeding, 111 cases were associated with petechiae, bleeding of the oral mucosa and/or epistaxis were found in 11 cases, and urinary tract and gastrointestinal bleeding occurred in 2 cases. Positive rates of platelet-specific antibodies were identified in 79.8% (107/134) of cases, and antibody positive against GPIIb/IIIa was identified in 76.9% of cases (103/134). Forty-two cases were anti-GPIIb/IIIa antibody positive only, 4 cases were anti-GPIb/IX antibody positive only, and 61 cases were double GPIIb/

IIIa and GPIb/IX antibody positive.

TABLE 1 Patient characteristics ($n = 134$)

Characteristics	Number of patients, *n* (%)
Gender	
Male	77 (57.5)
Female	57 (42.5)
Age (months, range)	19 (1–159)
Platelet count before treatment ($\times 10^9$/L, mean ± SD)	10.68 ± 10.54
Bleeding condition	
No bleeding	10 (7.5)
Petechiae	111 (82.8)
Oral mucosa bleeding and/or epistaxis	11 (8.2)
Urinary tract and gastrointestinal bleeding	2 (1.5)
Platelet-specific antibody	
anti-GPIIb/IIIa antibody positive only	42 (31.3)
anti-GPIb/IX antibody positive only	4 (3.0)
double antibody positive	61 (45.5)
double antibody negative	27 (20.2)

We investigated gender, age, platelet count and bleeding grade to determine whether different platelet-specific antibodies influenced clinical manifestations. Differences in gender, age, platelet count, and bleeding between 103 patients positive for anti-GPIIb/IIIa antibodies and 65 patients positive for anti-GPIb/IX antibodies were similar ($P = 0.450$, $P = 0.733$, $P = 0.668$, $P = 0.930$, respectively) (Table 2). Therefore, gender, age, platelet count, and bleeding status were not significantly related to the type of antibody, and the type of antibody could not be determined by the degree of bleeding.

Different platelet-specific antibody types and clinical prognosis are shown in Figure 1 and Table 3. Significantly more anti-GPIIb/IIIa antibody positive only patients were newly diagnosed ITP compared with anti-GPIb/IX antibody positive patients (93% vs 25%, $P = 0.005$). The proportion of newly diagnosed ITP patients with anti-GPIIb/IIIa antibody only was similar to double antibody positive ITP patients (93% vs 87%, $P = 0.522$). The proportion of newly diagnosed ITP double antibody positive patients was significantly higher than that of anti-GPIb/IX antibody positive only patients (87% vs 25%, $P = 0.014$). The proportion of positive anti-GPIb/IX antibody only cases who eventually became chronic ITP was higher than the anti-GPIIb/IIIa antibody positive only and the double antibody positive cases (75% vs 7%, $P = 0.005$; 75% vs 13%, $P = 0.014$), suggesting that anti-GPIIb/IIIa

TABLE 2 Clinical manifestations related to different antibody types

Items		anti-GPIIb/IIIa antibody positive (n = 103)	anti-GPIb/IX antibody positive (n = 65)	F/χ2 value	P
Gender				0.571	0.450
	Male	60	34		
	Female	43	31		
Age (months, range)		18 (1–159)	18 (2–159)	−0.342	0.733
Platelet count before treatment (×10^9/L, mean ± SD)		10.19 ± 9.35	9.60 ± 8.23	0.429	0.668
Bleeding grade[†]				0.008	0.930
	Mild bleeding	97	61		
	Moderate bleeding	6	4		

[†]Patients with no bleeding and petechiae were combined into mild bleeding. Oral mucosa, epistaxis, digestive tract, urinary tract bleeding were added as moderate bleeding.

TABLE 3 Antibodies and prognosis of primary ITP

Items		Newly diagnosed ITP (n = 113)	Chronic ITP (n = 18)	χ^2	P
Gender				0.181	0.670
	Male	63	11		
	Female	50	7		
Age(months, range)		18 (1–159)	31 (5–118)	−0.250	0.803
Platelet count before treatment (×10^9/L, mean ± SD)		10.09 ± 37.46	12.06 ± 10.12	−0.806	0.422
Platelet-specific antibody				9.714	0.021
anti-GPIIb/IIIa antibody positive only		39	3		
anti-GPIb/IX antibody positive only		1	3		
double antibody positive		53	8		
double antibody negative		20	4		

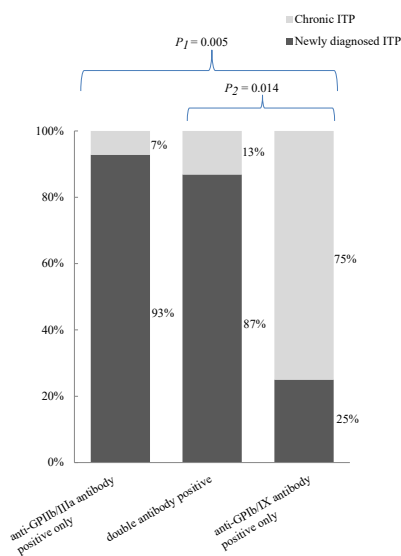ITP, Immune thrombocytopenic purpura.

FIGURE 1 Platelet-specific antibody types and clinical prognosis. P_1, anti-GPIIb/IIIa antibody positive only compared with anti-GPIb/IX antibody positive only. P_2, double antibody positive compared with anti-GPIb/IX antibody positive only. ITP, immune thrombocytopenic purpura.

antibody positive is an indicator of a good prognosis and anti-GPIb/IX antibody might predict a poor prognosis. The above data suggest that anti-GPIIb/IIIa antibody positive cases may achieve a better prognosis than anti-GPIb/IX antibody only cases in children with ITP, which implies a better response to first-line treatment. Anti-GPIIb/IIIa antibody positive patients may be associated with a good prognosis.

DISCUSSION

Children with chronic or refractory ITP face the risk of bleeding and long-term use of drugs, which seriously affects their quality of life.[19,20] It is important to identify reliable predictors for the outcome of childhood ITP at the time of diagnosis, as well as after the initial therapy. This helps clinicians to provide patients and their parents with specific information about the expected clinical course. Furthermore, it can guide decisions relating to the therapeutic management of the disease.

In our study, the positive rate of ITP platelet-specific

antibodies in children was 79.8%. Most patients were positive for anti-GPIIb/GPIIIa antibodies. The results observed in children were very similar to that found in adults: approximately 70%–80% of patients had autoantibodies against GPIIb/GPIIIa and 20%–40% had autoantibodies against the GPIb complex. This study found no significant difference in the degree of bleeding between different types of platelet-specific antibodies. Fabrizio[21] and others reported that patients with antibodies against GPIb/IX or multiple antigens suffered from more severe bleeding symptoms. Autoantibodies reacting with glycoprotein (GP)IIb/IIIa may affect platelet aggregation and these are the most common autoantibodies reported in primary ITP.[22] Antibodies against GPIb/IX may undermine platelet adhesion to the sub-endothelial matrix, which result in severe bleeding. Because these two kinds of autoantibodies both influence the normal function of platelets, the clinical manifestation of bleeding showed no difference, indicating more data is required.

The specificity of platelet autoantibodies may play an important role in determining the therapeutic outcome in childhood ITP. In our study, we evaluated the treatment response with respect to platelet autoantibody specificities in children with initial ITP. The data clearly demonstrates that patients with autoantibodies specific for the GPIIb/IIIa antigens, either alone or in combination with anti-GPIb/IX autoantibodies, showed a good response to treatment. Moreover, the presence of anti-GPIIb/IIIa autoantibodies is a good marker for assessing responsiveness to first-line therapy. However, patients with anti-GPIb/IX autoantibodies only may have a poor prognosis. In our study, 3 of 4 patients with anti-GPIb/IX became chronic ITP. We conclude that for children with ITP and with anti-GPIb/IX may be less responsive to first-line treatment. A study of ITP in adults found that most patients with antibodies against GPIIb/IIIa (31/43 = 72.1%) were sensitive to treatment.[12] Their response rate was 2–3 times higher than the response rate of patients with antibodies against GPIbα (9/34 = 26.5%, $P < 0.01$). Chen et al[23] reported high-dose dexamethasone in 185 adults with primary immune thrombocytopenia. Patients who were positive for anti-GPIbα-IgG antibody had a worse response to treatment compared with those who were positive for anti-GPIIb/IIIa antibody (50% vs 87.5%). Zhu et al[12] found that in an anti-GPIIb/IIIa antibody positive only group ($n = 43$), 12 cases failed to respond to treatment. Furthermore, in an anti-GPIbα antibody positive only group ($n = 34$), 25 cases had no treatment effect. The difference was significant ($P < 0.05$). The research results of Chen et al[23] were similar to other studies, which suggests that patients with anti-GPIbα antibody only are poorly responsive to hormone therapy. The results of the current study are similar to those of adult studies, suggesting anti-GPIIb/IIIa antibody positive children have better clinical outcomes than patients with anti-GPIb/IX antibody only. The reason might be that the destruction

of platelets is mediated by different mechanisms and that anti-GPIIb/IIIa antibodies are dependent on the Fc segment.[13] However, data from other studies[24] suggest that anti-GPIbα antibodies may induce platelet activation, aggregation, and apoptosis, which may lead to Fc-independent platelet clearance in the reticuloendothelial system. In contrast to anti-GPIIb/IIIa, F(ab)$_2$ fragments of anti-GPIbα induced thrombocytopenia with the same intensity as intact antibodies. These data support an Fc-independent mechanism of platelet destruction by anti-GPIbα, which produces different responses to first-line therapy.

Our study provides evidence that the specificity of platelet autoantibodies might play an important role in determining the therapeutic outcome of first-line therapy, and that the presence of anti-GPIIb/IIIa autoantibodies is a predictive factor for a good response to first-line therapy in childhood ITP. However, children with ITP and with anti-GPIb/IX may be less responsive to first-line therapy. This study had some limitations, such as insufficient sample size. Furthermore, platelet-specific antibodies were not detected in the same patient at different disease stages. Therefore, larger sample sizes should be used for future studies.

CONFLICT OF INTEREST

The authors state that they have no conflicts of interest.

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