Platelet-specific antibodies are unrelated to the bleeding severities in children with newly diagnosed ITP and a severe decline of platelets

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Abstract

Background and objective: Immune thrombocytopenia (ITP) is an autoimmune-mediated hemorrhagic disease, which is characterized by thrombocytopenia and bleeding manifestation. The treatment options of ITP in children are selected based on bleeding severities and the treatment selection is critical in children with a serious decline in platelet count (platelet count of $<10\times10^{9}$/L). Although it is well known that platelet-specific antibodies play a key role in ITP, the relationship between different platelet-specific antibodies and bleeding severities is unclear. This study aimed to analyze the relationship between platelet-specific antibodies (anti-GPIIb/IIIa and anti-GPIb/IX antibodies) and bleeding severities in children with newly diagnosed ITP and a serious decline in platelet count. **Method:** This study was a single-center prospective observational study that analyzed children with newly diagnosed ITP and platelet count of less than 10×10^{9}/L from June 2018 to September 2021 in our Hospital. They were classified into mild-moderate and severe groups based on the treatments. Platelet-specific antibodies and titers were detected using a kit, PAKAUTO. We analyzed the relationship of bleeding severities with platelet-specific antibodies/titers . **Results:** A total of 86 cases were enrolled including 57 males and 29 females with a median age of 35 months (range 1 month to 198 months). And 11 cases were categorized as the mild-moderate group and 75 cases were categorized as the severe group based on bleeding severity score. The positive rates were 68.6% for anti-GPIIb/IIIa and 65.1% for anti-GPIb/IX. There was no significant difference in anti-GPIIb/IIIa and anti-GPIb/IX antibodies between the two bleeding severity groups (χ^2=0.530, P=0.467; χ^2<0.001, P=1.000), and also no difference was found between the two groups when two antibodies were analyzed together (χ^2=2.071, P=0.558). The antibody titers in plasma and eluent were also detected, but no significant difference was found in the antibody titer ratios between the two bleeding severity groups (P<0.05 in four plasma and eluent groups). **Conclusion:** Platelet-specific antibodies (anti-GPIIb/IIIa and anti-GPIb/IX antibodies) were not related to the bleeding severities in children with newly diagnosed ITP and serious decline of platelet count.

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Children; primary immune thrombocytopenia; platelet-specific antibodies; bleeding severities

Abbreviate table

ITP	Immune thrombocytopenia
GP	glycoprotein
IVIG	intravenous immunoglobulin
ELISA	enzyme-linked immunosorbent assay
vWF	von Willebrand Factor
rhTPO	recombinant human thrombopoietin

Abstract

Background and objective:

Immune thrombocytopenia (ITP) is an autoimmune-mediated hemorrhagic disease, which is characterized by thrombocytopenia and bleeding manifestation. The treatment options of ITP in children are selected based on bleeding severities and the treatment selection is critical in children with a serious decline in platelet count (platelet count of $<10\times10^9$/L). Although it is well known that platelet-specific antibodies play a key role in ITP, the relationship between different platelet-specific antibodies and bleeding severities is unclear. This study aimed to analyze the relationship between platelet-specific antibodies (anti-GPIIb/IIIa and anti-GPIb/IX antibodies) and bleeding severities in children with newly diagnosed ITP and a serious decline in platelet count.

Method:

This study was a single-center prospective observational study that analyzed children with newly diagnosed ITP and platelet count of less than 10×10^9/L from June 2018 to September 2021 in our Hospital. They were classified into mild-moderate and severe groups based on the treatments. Platelet-specific antibodies and titers were detected using a kit, PAKAUTO. We analyzed the relationship of bleeding severities with platelet-specific antibodies/titers.

Results:

A total of 86 cases were enrolled including 57 males and 29 females with a median age of 35 months (range 1 month to 198 months). And 11 cases were categorized as the mild-moderate group and 75 cases were categorized as the severe group based on bleeding severity score. The positive rates were 68.6% for anti-GPIIb/IIIa and 65.1% for anti-GPIb/IX. There was no significant difference in anti-GPIIb/IIIa and anti-GPIb/IX antibodies between the two bleeding severity groups (χ^2 =0.530, P =0.467;χ^2 <0.001, P =1.000), and also no difference was found between the two groups when two antibodies were analyzed together (χ^2 =2.071, P =0.558). The antibody titers in plasma and eluent were also detected, but no significant difference was found in the antibody titer ratios between the two bleeding severity groups (P <0.05 in four plasma and eluent groups).

Conclusion:

Platelet-specific antibodies (anti-GPIIb/IIIa and anti-GPIb/IX antibodies) were not related to the bleeding severities in children with newly diagnosed ITP and serious decline of platelet count.

Keywords: Children; primary immune thrombocytopenia; platelet-specific antibodies; bleeding severities

Background

Immune thrombocytopenia (ITP) is an autoimmune-mediated hemorrhagic disorder that is characterized by thrombocytopenia (peripheral blood platelet count of $<100\times10^9$/L), and the incidence ranges from 1.1 to 5.8 per 100,000 person-years among children[1]. The primary clinical manifestation is bleeding that varies from mild skin mucosal hemorrhage to severe intracranial hemorrhage [2,3]. The bleeding scores are graded from 0 to 4 according to the latest guidelines for children with ITP. The disorder not only affects the quality of life but also impacts the treatment planning for children with ITP and severe decline of platelet count (platelet count of $<10\times10^9$/L)[4,5].

The primary pathogenesis of ITP is loss of immune tolerance to the platelet autologous antigen, which causes immune destruction of platelets and reduction of megakaryocyte and platelet production[6]. Platelet-specific antibodies that target platelet membrane glycoprotein (GP) destroy the platelets. Two glycoproteins play important roles in hemostasis. GPIIb/IIIa, an integrin complex, mediates platelet aggregation by binding to divalent fibrinogen or multivalent von Willebrand Factor (vWF), and GPIb/IX, a membrane receptor complex, promotes the adhesion of activated platelets to endothelial cells and subendothelial structures of the injured vascular walls by binding to its most important ligand vWF[7]. And these glycoproteins could theoretically vary with different bleeding symptoms in patients with ITP. However, the relationship between different platelet-specific antibodies and severities of bleeding is unclear[8-11]. Therefore, our study aimed to analyze the relationship between platelet-specific antibodies (anti-GPIIb/IIIa and anti-GPIb/IX antibodies) and severities of bleeding in newly diagnosed ITP with severe platelet decline in children.

Materials and methods

Ethical approval

This was a prospective, single-center, observational cohort study. We enrolled the cases from June 2018 to September 2021 in the Beijing Children's Hospital. The guardians of all children signed informed consent forms. This study was approved by the local ethical committee of Capital Medical University.

Study patients

Inclusion criteria: The enrolled cases were all hospitalized newly diagnosed ITP patients who had platelet-specific antibodies tests before the onset of treatment. The diagnosis of ITP was accorded to the international consensus guidelines [12]. The baseline platelet counts in all cases were less than 10×10^9/L. The children were aged from 1 month to 198 months. The diagnosis of ITP was done in less than one month. All patients were without a history of taking special platelet elevation drugs.

Exclusion criteria: Thrombocytopenia caused by other diseases, such as infection-related thrombocytopenia, congenital immune thrombocytopenia, connective tissue disease, aplastic anemia, and others was excluded.

Patients having a history of taking special platelet elevation drugs were excluded. Patients who requested a withdrawal from the study were excluded from the analysis.

Bleeding severity classification

We classified the bleeding severities according to the treatments. Children who used only glucocorticoids or intravenous immunoglobulin (IVIG) during hospitalization were placed in the mild-moderate bleeding group and children who used glucocorticoids and IVIG with/without recombinant human thrombopoietin (rhTPO) were placed in the severe bleeding group.

Detection of platelet-specific antibodies

Peripheral blood was collected before the onset of treatment to detect platelet-specific antibodies. Antibodies, anti-GPIIb/IIIa and anti-GPIb/IX, were detected using a commercial kit(PAKAUTO; GTI Diagnostics Inc, Waukesha, WI, USA). The solid-phase modified antigen-capture enzyme-linked immunosorbent assay (ELISA) was employed to detect platelet-specific antibodies [13]. The ratio of the patient's antibody titer to the control's antibody titer was defined as the antibody titer ratio. We defined the ratio as positive when the antibody titer ratio was >1 in plasma or eluent and defined the ratio as negative when the antibody titer ratio was <1 in both plasma and eluent.

Statistical analysis

All statistical analyses were performed using SPSS version 26.0 software. The quantitative data, if normally distributed, were compared by the t -test. If they did not follow a normal distribution, the Mann-Whitney U test was used. Classification data were compared by the Chi-square test or Fisher's exact test. P -values of < 0.05 were considered statistically significant.

Results

A total of 86 patients were enrolled, 12.8% (11/86 cases) patients were classified into the mild-moderate group, and 87.2% (75/86 cases) patients were classified into the severe group. There was no statistical difference in gender (χ^2 =0.292, P =0.589) and age (Z =-0.770, P =0.442) between the two bleeding severity groups. The positive rate of platelet-specific antibodies was 73.3% (63/86). The positive rates of anti-GPIIb/IIIa and anti-GPIb/IX antibodies were 68.6% (59/86) and 65.1% (56/86), respectively. Details of baseline characteristics and different platelet-specific antibody types are shown in TABLE 1.

The respective distribution of anti-GPIIb/IIIa and anti-GPIb/IX antibodies in the two bleeding severity groups are shown in TABLE 2. There was no significant difference in the distribution of anti-GPIIb/IIIa (χ^2 =0.530, P =0.467) and anti-GPIb/IX antibodies (χ^2 <0.001,P =1.000) between the two bleeding severity groups.

The comprehensive distribution of anti-GPIIb/IIIa and anti-GPIb/IX antibodies in the two bleeding severity groups is shown in TABLE 3 and Fig. 1. There was no significant difference in the distribution of four different antibody combinations between the two bleeding severity groups (χ^2 =2.071, P =0.558). We also analyzed the differences between any two of four different antibody combinations with the Chi-square test or Fisher's exact test but did not find any significant difference (Fig. 1).

The antibody titer ratios of anti-GPIIb/IIIa and anti-GPIb/IX antibodies in plasma and eluent between the two bleeding severity groups are shown in Fig. 2. We found that the anti-GPIIb/IIIa antibody titer ratios in eluent were significantly higher than those in plasma (Z =-5.556,P <0.001), and the same was with anti-GPIb/IX antibody (Z =-5.862, P <0.001). Al-Samkari et al.[11] reported that direct assays for platelet-specific antibodies on platelets, as opposed to indirect assays, which measured free antibodies in plasma, had higher sensitivity and specificity and were considered optimal for platelet-specific antibodies testing. Our results were consistent with this study result. However, we found no significant difference in antibody titer ratios between the two bleeding severity groups in both plasma and eluent (anti-GPIIb/IIIa antibody in plasma: Z =-0.601, P =0.548; anti-GPIIb/IIIa antibody in eluent: Z =-0.556, P =0.578; anti-GPIb/IX antibody in plasma: Z =-0.388, P =0.698; anti-GPIb/IX antibody in eluent: Z =-0.175, P =0.861).

Discussion

Bleeding is the only clinical manifestation of ITP and varies from mild skin mucosal hemorrhage to severe intracranial hemorrhage. Severe bleeding not only affects the quality of life but also threatens life in children with ITP. Therefore, it is important to explore the influencing factors of bleeding in ITP. GPIIb/IIIa and GPIb/IX mediate platelet aggregation and adhesion and play an important role in hemostasis. The antibodies could theoretically aggravate bleeding symptoms in ITP patients. However, previous studies that elucidated the relationship between the platelet-specific antibodies and bleeding severities, reached different conclusions. Nomura et al.[9] reported that patients with anti-GPIb/IX antibodies had more severe bleeding manifestations than patients without anti-GPIb/IX antibodies. Mehta et al.[10]found that patients with both anti-GPIIb/IIIa and anti-GPIb/IX antibodies were more prone to develop moderate or severe bleeding symptoms than patients with only one antibody or without antibodies. Al-Samkari et al.[11]found a statistically significant predictive relationship between the increasing number of positive autoantibodies and disease severity. Fu et al.[8] from our research group found no significant difference in the degree of bleeding between different types of platelet-specific antibodies. However, that study focused on the relationship between platelet-specific antibodies and glucocorticoid response, but less on bleeding, and did not exclude the influence of platelet count and chronic ITP on bleeding. Therefore, whether platelet-specific antibodies result in severe bleeding manifestation requires more evidence.

Our study was a prospective and observational cohort study. The enrolled cases were all diagnosed according to the international consensus guidelines and did not have a history of taking special platelet elevation drugs before the detection of platelet-specific antibodies. The baseline platelet counts in all cases were less than 10×10^9/L to eliminate the influence of platelet count on bleeding. We detected platelet-specific antibodies in both plasma and eluent and analyzed antibodies' levels qualitatively and quantitatively to ensure a more accurate and credible conclusion of the relationship between platelet-specific antibodies and bleeding severities.

Our data demonstrated that there was no significant difference in the distribution of platelet-specific antibodies between the two bleeding severity groups, and also displayed no difference in antibody titer ratios between the two bleeding severity groups in both plasma and eluent. Finally, we concluded that platelet-specific antibodies (anti-GPIIb/IIIa and anti-GPIb/IX antibodies) were not related to the bleeding severities in children with newly diagnosed ITP with serious decline of platelet count.

Authorship Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Shuyue Dong, Linging Fu, Xingjuan Xie and Hao Gu; Hao Gu and Xingjuan Xie did the sample collection and antibody test;Lingling Fu, JingyaoMa and Jie Ma recruited all the subjects and collected the written informed consents;The first draft of the manuscript was written by Shuyue Dong and all authors commented on previous versions of the manuscript. Runhui Wu and Zhenping Chen read and approved the final manuscript.

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TABLE LEGENDS

TABLE 1 Characteristics of ITP patients

TABLE 2 Respective distribution of platelet-specific antibodies and bleeding severities

TABLE 3 Comprehensive distribution of platelet-specific antibodies and bleeding severities

FIGURE LEGENDS

FIGURE 1 Comprehensive distribution of platelet-specific antibodies and bleeding severities

FIGURE 2 Antibody titer ratios and bleeding severities. (a) The antibody titer ratios of anti-GPIIb/IIIa antibodies in plasma. (b) The antibody titer ratios of anti-GPIIb/IIIa antibodies in eluent. (c)The antibody titer ratios of anti-GPIb/IX antibodies in plasma. (d)The antibody titer ratios of anti-GPIb/IX antibodies in eluent.

TABLE 1 Characteristics of ITP patients

	Total	Mild-moderate group	Severe group
Number of patients	86	11	75
Gender (male/female)	57/29	6/5	51/24
Median (range) age, month	35 (1-198)	14 (3-109)	35 (1-198)
Median (range) platelet count, $\times 10^9$/L	5 (0-10)	7 (1-10)	5 (0-10)
Median (range) bleeding scores	2 (0-3)	1 (0-2)	2 (0-3)
Platelet antibodies			
anti-GPIIb/IIIa (+), anti-GPIb/IX (+)	52	6	46
anti-GPIIb/IIIa (+), anti-GPIb/IX (-)	7	0	7
anti-GPIIb/IIIa (-), anti-GPIb/IX (+)	4	1	3
anti-GPIIb/IIIa (-), anti-GPIb/IX (-)	23	4	19

ITP, Immune thrombocytopenia; GP, glycoprotein

TABLE 2 Respective distribution of platelet-specific antibodies and bleeding severities

		Mild-moderate group	Severe group	χ^2	P
Anti-GPIIb/IIIa	Positive	6 (10.2%)	53 (89.8%)	0.530	0.467
	Negative	5 (18.5%)	22 (81.5%)		
Anti-GPIb/IX	Positive	7 (12.5%)	49 (87.5%)	<0.001	1.000
	Negative	4 (13.3%)	26 (86.7%)		

GP, glycoprotein

TABLE 3 Comprehensive distribution of platelet-specific antibodies and bleeding severities

	Mild-moderate group	Severe group	χ^2	P
anti-GPIIb/IIIa (+), anti-GPIb/IX (+)	6 (11.5%)	46 (88.5%)	2.071	0.558
anti-GPIIb/IIIa (+), anti-GPIb/IX (-)	0 (0.0%)	7 (100.0%)		
anti-GPIIb/IIIa (-), anti-GPIb/IX (+)	1 (25.0%)	3 (75.0%)		
anti-GPIIb/IIIa (-), anti-GPIb/IX (-)	4 (17.4%)	19 (82.6%)		

GP, glycoprotein

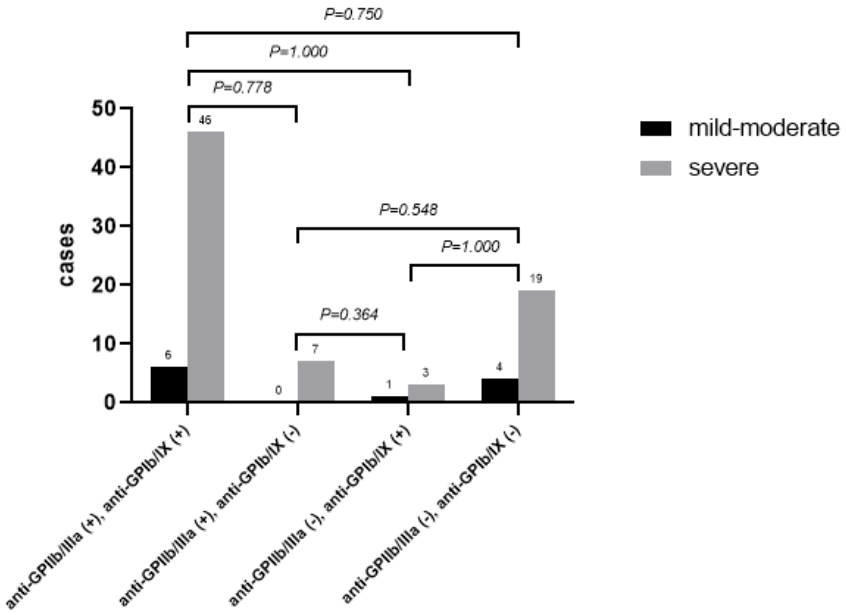


FIGURE 1 Comprehensive distribution of platelet-specific antibodies and bleeding severities

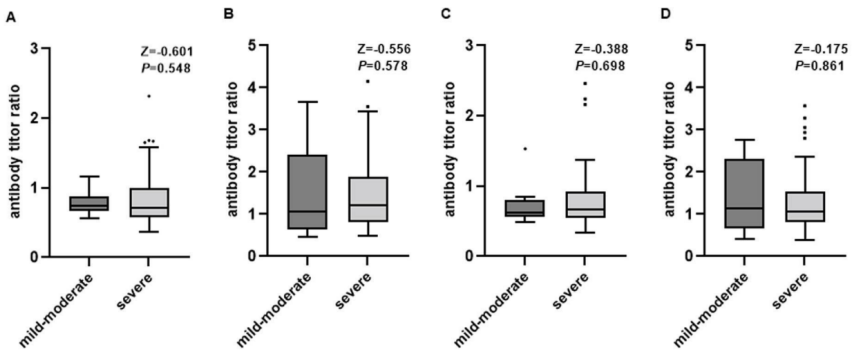
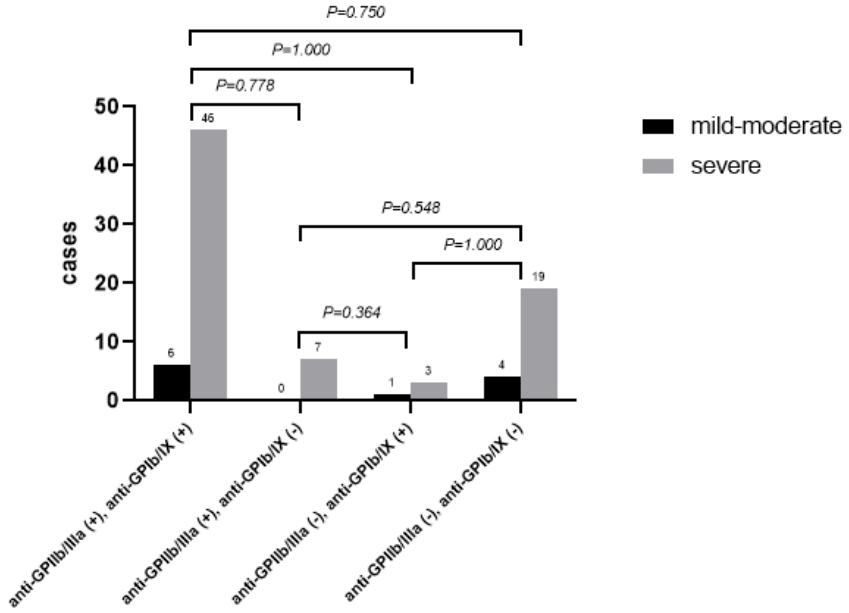
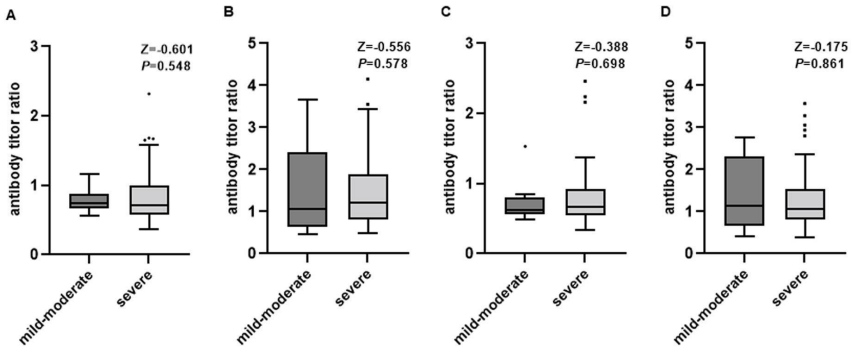


FIGURE 2 Antibody titer ratios and bleeding severities. (a) The antibody titer ratios of anti-GPIIb/IIIa antibodies in plasma. (b) The antibody titer ratios of anti-GPIIb/IIIa antibodies in eluent. (c)The antibody titer ratios of anti-GPIb/IX antibodies in plasma. (d)The antibody titer ratios of anti-GPIb/IX antibodies in eluent.





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