Anti-platelet antibodies in childhood immune thrombocytopenia: Prevalence and prognostic implications

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Abstract
Background: Anti-platelet antibody testing may be useful for the diagnosis and management of childhood immune thrombocytopenia (ITP).

Objectives: Here we aimed to assess the prevalence and prognostic significance of anti-platelet glycoprotein-specific IgM and IgG antibodies.

Methods: Children with newly diagnosed ITP were included at diagnosis and randomized to an intravenous immunoglobulins (IVIg) or careful observation group (TIKI trial). In this well-defined and longitudinally followed cohort (N = 179), anti-platelet glycoprotein-specific IgM and IgG antibodies were determined by monoclonal antibody-immobilization of platelet antigens.

Results: The dominant circulating anti-platelet antibody class in childhood ITP was IgM (62% of patients); but IgG antibodies were also found (10%). Children without IgM platelet antibodies were older and more often female. There was weak evidence for an association between IgM anti-GP IIb/IIIa antibodies and an increased bleeding severity (P = .03). The IgM and IgG anti-platelet responses partially overlapped, and reactivity was frequently directed against multiple glycoproteins. During 1-year follow-up, children with IgM antibodies in the observation group displayed a faster platelet recovery compared to children without, also after adjustment for age and preceding infections (P = 7.1 × 10⁻⁵). The small group of patients with detectable IgG anti-platelet antibodies exhibited an almost complete response to IVIg treatment (N = 12; P = .02), suggesting that IVIg was particularly efficacious in these children.

Conclusions: Testing for circulating anti-platelet antibodies may be helpful for the clinical prognostication and the guidance of treatment decisions in newly diagnosed childhood ITP. Our data suggest that the development of even more sensitive tests may further improve the clinical value of antibody testing.
INTRODUCTION

Childhood immune thrombocytopenia (ITP) is a rare autoimmune bleeding disorder with an incidence of 2 to 6 children per 100,000 annually, characterized by a platelet count below \(10^9/L\). About 50% of children experience an infectious episode before the onset of bleeding symptoms and thrombocytopenia, and it is hypothesized that epitope spreading or molecular mimicry may play a role in the development of self-directed anti-platelet immunity. The pathophysiology of ITP features both humoral and cellular immune responses against platelet self-antigens. Thus, anti-platelet antibody testing could potentially be used in the diagnosis and determination of prognosis of ITP.

A pathophysiological role of anti-platelet antibodies in ITP has been demonstrated by the identification of a transferable factor in the plasma of adult ITP patients, which induced thrombocytopenia in healthy individuals. This factor was located in the 7S fraction of human gamma globulin and could be adsorbed to platelets. Studies using platelets from patients with Glanzmann disease have identified the first platelet antigen targeted by ITP anti-platelet antibodies as the fibrinogen receptor glycoprotein (GP) IIb/IIIa. It is now known that multiple platelet glycoproteins can be involved, including GP V and the GP Ib/IX complex. In childhood ITP, IgM anti-platelet antibodies have been suggested to be at least as prevalent as anti-platelet IgG antibodies. Apart from affecting platelet clearance, anti-glycoprotein antibodies can functionally impair platelet aggregation by blocking the fibrinogen receptor interactions, or may potentially inhibit effective thrombopoiesis through GPIbα-mediated hepatocyte thrombopoietin production.

While early antibody tests have focused on the detection of antibodies bound to whole platelets, this approach has been dismissed because of the unspecific adsorption of protein to platelets, and false-positive results in non-immune mediated thrombocytopenia patients. Improved antigen-specific antibody tests were subsequently developed, for example by monoclonal antibody-immobilization of platelet antigens (MAIPA) or immunobead technology, and these have today largely replaced whole-platelet tests with improved specificity. The first glycoprotein-specific study amongst 15 children with acute ITP identified IgG anti-GP IIb/IIIa in 27% of patients, and detected no anti-GP Ib/IX antibodies. More recently, IgG glycoprotein-specific antibodies were investigated in 74 children with newly diagnosed and treatment-naïve ITP. IgG anti-GP IIb/IIIa and anti-GP Ib/IX antibodies were detected among 36% and 30% of children, respectively. This study found no association with transient versus chronic disease courses. Unfortunately, inclusion criteria, controls, clinical characteristics, and follow-up of included patients were not described, making it difficult to put these data in clinical perspective. In contrast, a Chinese study suggested that anti-GP Ib/IX antibodies in children with newly diagnosed ITP might be associated with the development of chronic ITP, whereas anti-GP IIb/IIIa antibodies may be associated with transient disease. Although these data are suggestive of a potential use of anti-platelet antibody testing, the diagnostic and prognostic value of such testing remains unclear.

In the present study, we sought to provide definitive evidence by evaluating the prevalence and prognostic significance of glycoprotein-specific anti-platelet antibody testing performed in our national reference laboratory in a clinically well-described, large cohort of children with newly diagnosed ITP, who were followed longitudinally for 1 year.

METHODS

2.1 Study participants

We report data from the phase 3 randomized controlled, open-label trial Therapy with or without Intravenous Immunoglobulins for Newly Diagnosed Immune Thrombocytopenia in Kids in the Netherlands (TIKI). The study protocol was approved by the ethical review board of University Medical Center Utrecht and all participating sites, and conducted in accordance with the second Declaration of Helsinki and Good Clinical Practice guidelines. Parents or patients aged ≥12 years gave written informed consent for study participation. Children with newly diagnosed ITP (N = 200) were allocated in a 1:1 ratio to be administered 0.8 g/kg intravenous immunoglobulin or followed by careful observation. The randomization into the trial arms was stratified by a platelet count below or above \(10^9/L\). To be eligible for inclusion, patients had to be 3 months to 16 years of age, with newly diagnosed ITP in the past 72 hours, according to guidelines, a platelet count \(\leq 20 \times 10^9/L\) and bleeding symptoms (Buchanan score 1-3).
criteria were severe bleeding with an indication for IVIg therapy (Buchanan score 4-5), immune modulatory treatment within the past month, or insufficient command of language. The primary outcome of the trial was development of chronic ITP. Secondary outcomes included bleeding symptoms, resolution of thrombocytopenia, and biological factors related to the pathophysiology of ITP and response to IVIg. Blood samples were collected at study sites and transferred to a central laboratory facility (Laboratory for Platelet and Leukocyte Serology; Sanquin Diagnostics).

Samples from children with chronic ITP were obtained from the multicenter cross-sectional CINKID study (Chronic ITP in the Netherlands in Kids). Inclusion criteria of the study were an age between 6 months to 17 years. Children were excluded for presence of autoimmune phenomena, other cytopenia, or features suggestive of hereditary thrombocytopenia. Parents or patients aged ≥12 years gave written informed consent for study participation. The study was approved by the ethical review board of University Medical Center Utrecht.

Control samples were obtained from healthy volunteers (Sanquin), adult refractory ITP patients included in the HOVON64 study, juvenile idiopathic arthritis (JIA) patients who were in remission, and patients with autoimmune neutropenia sent for anti-neutrophil antibody evaluation (Sanquin). The study was conducted in agreement with Dutch national guidelines (Human Tissue and Medical Research: Code of conduct for responsible use; https://www.federa.org/codes-conduct) and the Declaration of Helsinki. All analyses were performed on coded, de-identified data. Serum and plasma samples were stored at -20°C.

2.2 Clinical outcome definition

Bleeding symptoms were recorded on a modified Buchanan score. The recovery from thrombocytopenia was defined according to recommendations by the International Working Group on ITP. A complete response was defined as a platelet count ≥100 × 10^9/L.

2.3 Glycoprotein-specific IgM and IgG anti-platelet antibody profiling

The platelet antigen specificity and semi-quantitative levels of anti-platelet antibodies of the IgM and IgG classes were determined using the monoclonal antibody immobilization of platelet antigens (MAIPA) assay, as follows. Goat anti-mouse IgG-Fc (Jackson ImmunoResearch) was coated overnight in 96-well microplates (Nunc Maxisorb) at 3 µg/mL in 50 µL coating buffer at 4°C and blocked afterward with 0.2% BSA in 0.9% NaCl (w/v). Cryopreserved healthy human donor platelets from three individuals typed for human platelet antigens were washed and pelleted in U-bottom 96-well plates (Greiner). Washing of platelets was performed at 550 g for 5 minutes. The cells were then suspended in 50 µL PBS/2% BSA and incubated with 120 µL test serum or plasma for 30 minutes at 37°C. Subsequently, the plate was washed three times and incubated with 50 µL mouse anti-human antibody against glycoprotein IIb/IIIa (CD61; CLB-Thromb/1, C17, Sanquin; Y2.51 distributed in the Fourth International Workshop and Conference on Human Leukocyte Differentiation Antigens; Dr Cordell, John Radcliffe Hospital, Oxford, UK), glycoprotein Ibα (CD42b; CLB-MB45, Sanquin) or glycoprotein V (CD42d; CLB-SW16, Sanquin) for 30 minutes at 37°C. After three washing steps the cells were lysed with NP40 in 100 µL TRIS solubilization buffer in a fresh V-bottom 96-well plate (Nunc) for 30 minutes at 4°C. The plate was then centrifuged at 1400 g for 15 minutes. Supernatant (80 µL) was transferred into fresh reagent tubes, and mixed with 110 µL TRIS washing buffer containing 2% bovine serum albumin (BSA). The goat-anti-mouse IgG coated plate was washed five times with phosphate buffered solution (PBS)/Tween 0.05%. After this, 50 µL of the diluted supernatant was added to each well and the plate was incubated overnight at 4°C. The plate was then washed again and 50 µL goat anti-human IgG (Fcγ)-HRP or anti-human IgM (Fcμ)-HRP (Jackson ImmunoResearch) was added and incubated for 2 hours at 4°C. After five washing steps, the color reaction was started by addition of 50 µL substrate solution (o-phenylenediamine dihydrochloride [DAKO] supplemented with H2O2 in H2O) and subsequently stopped by addition of 50 µL 4 N H2SO4. The optical density was measured on an Anthos-HTII plate reader at 492 nm. All samples were tested in duplicate. The concentrations of mouse anti-human, goat anti-mouse, goat-anti human-HRP antibodies were determined against existing stocks and varied per batch. The monoclonal antibodies C17, Y2, MB45 and SW16 were produced and affinity-purified from hybridomas (Department of Experimental Immunohematology; Sanquin Research) and validated against commercially available antibodies (Sanquin Reagents).

2.4 Statistical analyses

Data was analyzed in R 3.6.0 (R Core Team). Continuous variables were tested with a non-parametric Wilcoxon rank test or Welch’s t test, as appropriate. Multiple groups were compared by Kruskal-Wallis test with a post-hoc Nemenyi test. Frequencies were compared with a Chi-squared test or Fisher’s exact test. Repeated measurements of platelet counts were analyzed using linear mixed effects models with random effects for the patient and fixed effects for platelet antibody status (positive/negative) and any covariates, as reported. A two-sided P-value below .05 was considered significant.

3 RESULTS

3.1 IgM anti-platelet antibodies are directed against multiple glycoproteins in childhood ITP

We evaluated the presence of glycoprotein-specific anti-platelet antibodies in 179 children with newly diagnosed ITP. The median age was 4 years and 53% had experienced a preceding infection (Table 1); 96 children were randomized to treatment with IVIg and
TABLE 1  Baseline characteristics of the study population

| Variable                        | TIKI study (N = 179) |
|---------------------------------|----------------------|
| Randomized to IVlg, n (%)       | 96 (53.6)            |
| Age, y (median [IQR])           | 4.10 [2.49, 7.62]    |
| Age, n (%)                      |                      |
| 0-1                             | 7 (3.9)              |
| >1-7                            | 123 (68.7)           |
| 7-18                            | 49 (27.4)            |
| Female (%)                      | 83 (46.4)            |
| Platelet count, ×10^9/L         | 6.00 (3.00, 9.50)    |
| Buchanan score, n (%)           |                      |
| 0                               | 3 (1.7)              |
| 1                               | 27 (15.2)            |
| 2                               | 75 (42.1)            |
| 3                               | 73 (41.0)            |
| Preceding infection, n (%)      | 95 (53.7)            |
| Leukocytes, ×10^9/L             | 8.20 [6.50, 10.70]   |
| Lymphocytes, ×10^9/L            | 3.80 [2.70, 5.00]    |
| Symptom duration, d             | 3.00 (2.00, 7.00)    |
| IgG MAIPA available, n (%)      | 176 (98.3)           |
| IgM MAIPA available, n (%)      | 167 (93.3)           |

Note: Data are median [interquartile range] unless otherwise specified. Abbreviations: MAIPA, monoclonal antibody-immobilization of platelet antigens; TIKI, Therapy with or without Intravenous Immunoglobulins for Newly Diagnosed Immune Thrombocytopenia in Kids

We next investigated if circulating IgG or IgM anti-platelet antibodies were associated with clinical characteristics among the children with newly diagnosed ITP. The children with IgM anti-platelet antibodies were younger (Figure 2A; P < .001), showed prolonged duration of symptoms (Figure 2B; P = .009), were more often male (Figure 2C; P = .006), and had higher leukocyte and lymphocyte counts (P < .001; P = .008). Of the patients with a preceding infection, 62% (55/89) had detectable IgM anti-platelet antibodies, compared to 62% (47/76) in the group without a preceding infection (P = 1.0). We found no association with the presence of the human Fc-gamma receptor FCGR2C open reading frame (ORF) variant (P = .81), which is associated with transient ITP.36  Thrombopoietin (TPO) levels were similar between children with and without IgM anti-platelet antibodies (median [interquartile range (IQR)]; 37 [24-72] versus 38 [27-54] IU/mL, respectively; P = .73). There were no detectable differences in clinical characteristics of the small group of patients with IgG anti-platelet antibodies, compared to patients without. Like for IgM platelet antibodies, no differences in circulating TPO levels were observed between IgG anti-platelet antibody-positive and antibody-negative patients (median [IQR]; 33 [23-68] versus 38 [26-60] IU/mL, respectively; P = .47). Taken together, these data suggested that children with...
**IGM MAIPA**

- GP IIb/IIIa: 64/167
- GP Ib/IX: 69/165
- GP V: 93/167

**IGG MAIPA**

- GP IIb/IIIa: 6/176
- GP Ib/IX: 11/176
- GP V: 11/176

**Correlation (Pearson r)**

- ns

**Figure E**

- IgM
  - GPIIb/IIIa: 28
  - GPIb/I: 7
  - GPV: 2
- IgG
  - GPIIb/IIIa: 5
  - GPIb/I: 3
  - GPV: 1
- Negative
  - IgM: 62
  - IgG: 159

**Figure G**

- Correlation (Pearson r)
  - 1.0
  - 0.5
  - 0.0
  - -0.1
  - 1.0

**Figure F**

- PC1
- PC2
- IgM
- IgG
IgM anti-platelet antibodies might represent a subgroup amongst a spectrum of clinically diagnosed ITP. In terms of observed bleeding at diagnosis, five children with isolated IgG anti-GPIIb/IIIa antibodies showed no more severe bleeding tendency than children without IgG anti-platelet antibodies (Buchanan scores of 1, 1, 2, 2, 3). In the whole cohort, 17% of children exhibited mild bleeding (Buchanan score ≤2). Compared to this, mild bleeding was observed in 1/28 (4%) of children with isolated IgM anti-GPIIb/IIIa antibodies, and 14/62 (20%) without any IgM autoantibody (Fisher’s exact test; \( P = .03 \)).

3.3 | Assessment of the anti-platelet antibody response during the course of disease and treatment

Next, the presence and evolution of platelet antibodies was evaluated during 1-year follow-up. We first assessed if IgM anti-platelet antibody responses were stable over time. Strikingly, the anti-GP IIb/IIIa IgM anti-platelet antibody response was short-lived, as samples obtained 1 week after diagnosis showed no more anti-platelet antibodies (Figure 3A). At this time point, the majority of patients in the observation group were still thrombocytopenic, suggesting that thrombocytopenia is maintained by mechanisms which are not detected by this assay. In addition, 1 year after diagnosis, we observed no new formation of IgM anti-platelet antibodies. This suggested that IgM anti-platelet antibodies mark a biological phenomenon that is present in a confined time period around the diagnosis of ITP, where delayed assessment of IgM anti-platelet antibodies may miss early antibody responses.

A key question was if patients with IgM antibodies at diagnosis would show class-switching to IgG antibodies during follow-up. Interestingly, we found no evidence of a preferential occurrence of new circulating anti-GP IIb/IIIa antibodies in patients with IgM anti-platelet antibodies at diagnosis for the same antigen (Figure 3B) for both treated and untreated patients.

Finally, of the patients who initially showed an IgG anti-GP IIb/IIIa antibody, ~50% kept this antibody response over at least 1 year (Figure 3C), even after the patients showed resolution of thrombocytopenia and recovered from ITP. A small proportion of patients showed de novo increases of anti-GP IIb/IIIa antibody levels during follow-up (Figure 3C). Amongst the treated patients, 1 week after
IVIg administration there was a statistically significant increase in anti-platelet IgG responses against all three assessed glycoproteins (data not shown), which was not shown in the observation group, and was lost again at 1-month follow-up. However, we could replicate a similar increase in MAIPA signals in vitro by incubating healthy donor platelets with IVIg (data not shown).

Together, these data indicated that the IgM anti-platelet antibody response was present only short term and downregulated after 1 week, even when no treatment was given and many patients were still thrombocytopenic. There was no observable class-switching. On the other hand, a large proportion of IgG anti-platelet antibody responses were present long term, even after resolution of thrombocytopenia, suggesting differential effector functions of these antibodies.

3.4 | Presence of anti-platelet antibodies is associated with favorable disease courses

Finally, we assessed the prognostic significance of anti-platelet antibodies. After 1 month follow-up was reached, we observed that patients with IgM anti-platelet antibodies at diagnosis in the observation cohort recovered faster than patients without (Figure 4A,B). This difference remained statistically significant (mixed effects model; $P = 7.1 \times 10^{-5}$) when we performed multivariate analyses with age and preceding infections as covariates, indicating that IgM anti-platelet antibodies independently determined prognosis in this group. In contrast, IVIg-treated patients positive for IgM anti-platelet antibodies at diagnosis showed no statistically significant differences in platelet count or response rates during follow-up (Figure 4A).

With regard to IgG anti-platelet antibodies, only few patients showed positive test results at diagnosis. We did not test differences in the observation group due to low sample numbers (Figure 4C). In IVIg-treated patients, we observed that patients with IgG anti-platelet antibodies showed increased platelet counts over time and a very high complete response rate to IVIg, much higher than the ~70% average observed amongst the whole group randomized to IVIg treatment\(^{31}\) (Figure 4C,D). Although the IgG antibody-positive group was small, this finding was statistically significant. The most prevalent IgG anti-platelet antibody was anti-GP IIb/IIIa, and focusing only on this group of patients showed the same result.

These data indicate that patients with IgM anti-platelet antibodies had a more favorable natural course of disease, compared to patients without. The data further suggested that when IgG anti-platelet antibodies were present, a favorable response to IVIg could be expected.

4 | DISCUSSION

4.1 | Main findings

We describe the presence, longitudinal evolution, and prognostic significance of antigen-specific platelet antibodies in newly diagnosed immune thrombocytopenia. The key findings of our study are that circulating anti-platelet antibodies are predominantly of the IgM class, and these antibody responses are present short term, without evidence of class-switching to IgG. Detectable anti-platelet IgG responses are apparently rare and occur separately from IgM anti-platelet antibody responses. No increases in circulating TPO levels among the antibody-positive patients were observed, suggesting...
that megakaryocytopoiesis is not suppressed. The presence of IgM anti-platelet antibodies is associated with a favorable spontaneous recovery from disease, also after taking into account age and preceding infection status. The patients with IgG anti-platelet antibodies against one of the major platelet glycoprotein complexes show higher longitudinal platelet counts and complete response rates after IVIg treatment. Finally, patients with IgM anti-GP IIb/IIIa antibodies exhibited more severe bleeding, suggesting functional impairment of platelet aggregation. Notably, this observation was made on a subset of patients and should be further investigated. Altogether, this modern reassessment of glycoprotein-specific antibody testing in a well-characterized and longitudinally followed patient population provides answers to the long-standing debate pertaining to the relevance of platelet anti-platelet antibody testing in newly diagnosed childhood ITP.

4.2 Findings of others

In a systematic review, we have identified 40 studies that investigated direct and indirect tests to detect anti-platelet antibodies in childhood ITP. However, due to heterogeneous and selective patient populations, varying sampling times from diagnosis and inference of treatments, most studies did not allow assessment of the diagnostic or prognostic value of anti-platelet antibody assessment. The studies that allowed assessment of sensitivity and specificity against healthy controls and non-immune thrombocytopenic controls showed moderate sensitivity and good specificity, indicating that antibodies were present in a proportion of childhood ITP patients (Schmidt et al; in press). These findings are in agreement with our results. Moreover, our finding of a high proportion of IgM anti-platelet antibodies by a newly developed glycoprotein-specific IgM
MAIPA agrees with and extends data from three previous studies that assessed IgM anti-platelet antibodies, but not in an antigen-specific manner.13-15

In contrast to our study, Biglino et al27 assessed glycoprotein-specific IgG antibodies in 74 children with transient or chronic disease courses and found no association with prognosis. Remarkably, the prevalence of circulating IgG antibodies in their study was above 30%, indicating differences in the patient population (selection bias) or technical performance of MAIPA.

One recent study in 134 Chinese children with ITP has suggested that isolated anti-GP Ib/IX IgG antibodies could be associated with chronic ITP.28 However, the observed predictive association of isolated anti-GP Ib/IX antibodies with chronic disease was based on only four patients. Moreover, the association did not persist when both anti-GP Ib/IIa and anti-GP Ib/IX antibodies were present, in contrast to data observed in adult ITP.39,40 Importantly, our data show that antigen-specific antibodies in childhood ITP do not occur isolated from, but responses for the different platelet antigens correlate strongly with, distinct IgG and IgM response patterns (Figure 1). The presence of multiple platelet antibodies at the same time could indicate epitope spreading in childhood ITP, and provides serological evidence for a previously described correlation between anti-GPIIb/IIa and anti-GPIb IgG producing B cells in circulation in ITP.41 While we interpret this phenomenon as the presence of antibodies against multiple antigens, it remains possible that platelet antibodies of ITP patients have a different avidity or propensity to complex formation or promotion of macromolecular interactions toward co-precipitation.

The serological presence of IgM anti-platelet antibodies may implicate a role for complement-mediated clearance of platelets, which has been suggested in immune thrombocytopenia.42,43 We could not show this experimentally when we tested a group of sera on healthy human donor platelets (data not shown).

A proposed working mechanism of IVIg is the clearance of platelet anti-platelet antibodies through saturation of the human neonatal Fc receptor (FcRn).44,45 The low number of patients with detectable circulating IgG anti-platelet antibodies in our study made it impossible to test this hypothesis. However, in the long term, treatment with IVIg did not completely abolish anti-platelet antibodies; in fact, we could detect IgG anti-platelet antibodies for at least 1 year in about half of patients with platelet antibodies at diagnosis. This is in full agreement with the antigen-specific assessment of anti-platelet IgG antibodies in patients with ITP in the past, from which they have recovered.46 In adult ITP patients, the response rate to IVIg has been suggested to be reduced when GPIb-specific IgG anti-platelet antibodies39,40 or anti-GPIb IgG producing B cells are found in circulation.41 Moreover, the response rate to rituximab was reduced in adult ITP patients without IgG anti-platelet antibodies47,48 although conflicting data have been published.49 Our data indicate that amongst children with ITP, the absence of detectable IgG anti-platelet antibodies is associated with lower response rates to IVIg, compared to children who do show IgG anti-platelet antibodies.

4.3 | Strengths and limitations

An important limitation of circulating anti-platelet antibody testing in autoimmune thrombocytopenia is the problem that antibodies with a high affinity and excellent effector functions are likely to have bound to platelets and may have been cleared preferentially in vivo. This is emphasized by a generally higher sensitivity of direct cell-bound anti-platelet antibody tests in ITP.11,26,50,51 In juvenile patients, due to the limited sample volume and the low platelet counts in ITP, we were not able to perform direct anti-platelet antibody tests on patients’ cells. Nonetheless, we show that indirect platelet antibody testing can still be of clinical benefit, as results correlated with clinical follow-up.

Many previous publications calculated sensitivity and specificity of anti-platelet antibody tests with the clinical diagnosis of ITP as gold standard (reference). With the known heterogeneity due to case-mix of the clinical diagnosis, and the absence of a reference group of children investigated for suspected ITP, such accuracy estimates can be misleading. A main strength of our study to circumvent this problem was to perform analyses directly related to longitudinal patient platelet counts and clinical response.

4.4 | Implications

Despite a long history of research, the role of antiplatelet antibody testing in childhood ITP remains under debate.29,30 Our study suggests several clinical implications. First, the assessment of circulating glycoprotein-specific anti-platelet antibodies may be helpful in determining the chance of spontaneous recovery. Second, the presence of IgG glycoprotein-specific anti-platelet antibodies was significantly associated with a complete response to IVIg. In such patients, treatment could be considered with a high chance of response, and IVIg treatment may be a useful rescue therapy in case of severe bleeding. These results may aid in the clinical counseling of patients and their families. Importantly, the study’s results should be confirmed in a secondary validation study before clinical implementation.

Because of the potential ongoing clearance of anti-platelet antibodies in vivo, it is possible that the detection of circulating anti-platelet IgG antibodies reflect merely the “tip of an iceberg.” We suggest that more sensitive (direct) laboratory techniques, which can be employed with low cell numbers, should be evaluated in respect to prognosis in childhood ITP. For instance, a direct MAIPA has a sensitivity of 80% to 90% in adult ITP,51 but children often have insufficient platelets available for this assay. Surface-plasmon resonance (SPR), which measures antigen-antibody interactions on a chip, can detect circulating low-avidity platelet antibodies that would be missed in an indirect MAIPA,10 but currently not all relevant antigens can be immobilized to the chip. Cellular SPR, in which sensitized cells are perfused over a microchip coated with Fc receptors that detect cell-bound antibodies,39,54 could be a sensitive alternative that should be explored for ITP.
5 | CONCLUSIONS

In newly diagnosed childhood ITP, glycoprotein-specific anti-platelet antibody testing may be useful to determine the prognosis and potential response to IV Ig. IgM anti-platelet antibodies are the dominant circulating antibody class. IgM antibodies are short-lived, without evidence of class-switching to IgG antibodies. Finally, our data suggest more sensitive techniques should be developed and evaluated to determine anti-platelet antibodies for prognosis in childhood ITP.

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CONFLICTS OF INTEREST

DES coordinated laboratory analyses, collected data, and discussed results. CEvdS and GV coordinated and supervised laboratory analyses. CEvdS and GV interpreted and discussed data. LP co-ordinated laboratory analyses, analyzed and interpreted the clinical studies, collected data, and discussed results. LP co-ordinated and supervised laboratory analyses. CEvdS and GV interpreted and discussed data. MdH designed and supervised the study. All co-authors reviewed, revised, and approved the manuscript.

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