



Universiteit  
Leiden  
The Netherlands

## Individualized prognosis in childhood immune thrombocytopenia

Schmidt, D.E.

### Citation

Schmidt, D. E. (2022, April 7). *Individualized prognosis in childhood immune thrombocytopenia*. Retrieved from <https://hdl.handle.net/1887/3281832>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3281832>

**Note:** To cite this publication please use the final published version (if applicable).

PLATELET AUTOANTIBODY IMMUNOASSAYS IN  
CHILDHOOD ITP: A SYSTEMATIC REVIEW.

---

Vox Sanguinis, 2020.

# Anti-platelet antibody immunoassays in childhood immune thrombocytopenia: a systematic review

David E. Schmidt,<sup>1,2</sup>  Anke J. Lakerveld,<sup>1,2</sup> Katja M. J. Heitink-Pollé,<sup>3</sup> Marrie C. A. Bruin,<sup>3,4</sup> Gestur Vidarsson,<sup>1,2</sup> Leendert Porcelijn<sup>5</sup> & Masja de Haas<sup>5,6,7,8</sup>

<sup>1</sup>Sanquin Research, Department of Experimental Immunohematology, Amsterdam, The Netherlands

<sup>2</sup>Landsteiner Laboratory, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

<sup>3</sup>Department of Pediatric Hematology, University Medical Center Utrecht, Utrecht, The Netherlands

<sup>4</sup>Princess Maxima Center for Pediatric Oncology, Utrecht, The Netherlands

<sup>5</sup>Department of Immunohematology Diagnostics, Sanquin Diagnostic Services, Amsterdam, The Netherlands

<sup>6</sup>Sanquin Research, Center for Clinical Transfusion Research, Leiden, The Netherlands

<sup>7</sup>Jon J van Rood Center for Clinical Transfusion Science, Leiden University Medical Center, Leiden, The Netherlands

<sup>8</sup>Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, The Netherlands

## Vox Sanguinis

**Background** In adult immune thrombocytopenia (ITP), an acquired autoimmune bleeding disorder, anti-platelet autoantibody testing may be useful as a rule-in test. Childhood ITP has different disease characteristics, and the diagnostic and prognostic value of anti-platelet antibody testing remains uncertain.

**Objective** To systematically review the diagnostic accuracy of anti-platelet autoantibody testing in childhood ITP.

**Methods** PubMed and EMBASE were searched for studies evaluating immunoassays in childhood ITP. Study quality was assessed (QUADAS2), and evidence was synthesized descriptively.

**Results** In total, 40 studies (1606 patients) were identified. Nine studies reported sufficient data to determine diagnostic accuracy measures. Anti-platelet IgG antibody testing showed a moderate sensitivity (0.36–0.80 platelet-associated IgG [direct test]; 0.19–0.39 circulating IgG [indirect test]). In studies that reported control data, including patients with non-immune thrombocytopenia, specificity was very good (0.80–1.00). Glycoprotein-specific immunoassays showed comparable sensitivity (three studies) and predominantly identified IgG anti-GP IIb/IIIa antibodies, with few IgG anti-GP Ib/IX antibodies. Anti-platelet IgM antibodies were identified in a substantial proportion of children (sensitivity 0.62–0.64 for direct and indirect tests).

**Conclusion** The diagnostic evaluation of IgG and IgM anti-platelet antibodies may be useful as a rule-in test for ITP. In children with insufficient platelets for a direct test, indirect tests may be performed instead. A negative test does not rule out the diagnosis of ITP. Future studies should evaluate the value of anti-platelet antibody tests in thrombocytopenic children with suspected ITP.

**Key words:** immune thrombocytopenia, paediatrics, autoantibodies, clinical laboratory techniques, systematic review.

Received: 15 November 2019,  
accepted 17 January 2020,  
published online 20 February 2020

Correspondence: Masja de Haas, Department of Immunohaematology Diagnostic Services, Sanquin, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands.  
E-mail: m.dehaas@sanquin.nl

## Introduction

Childhood immune thrombocytopenia (ITP) is an autoimmune bleeding disorder [1]. Given a lack of laboratory tests to rule-in or rule-out the diagnosis of ITP, the

disease is diagnosed clinically by exclusion of alternative causes of thrombocytopenia [2,3]. This may lead to misdiagnoses, as much as 12% in adult ITP [4], for example for patients with hereditary platelet and immune disorders [5,6]. Misdiagnosed patients are not only exposed to unsuitable ITP-specific treatments, but also the treatment-associated side-effects and costs. Misdiagnoses also lead to delayed diagnosis and adequate management of the actual underlying disorder. On the other hand, with new treatment options, for example the early administration of TPO agonists that are currently being investigated [7], clinicians may wish to ascertain an ITP diagnosis when considering such treatments. Thus, there is an unmet clinical need for laboratory tests to support the diagnosis of ITP [2].

A pathophysiological hallmark of ITP are anti-platelet autoantibodies specific to platelet glycoproteins [8,9]. Soon after the discovery of these platelet autoantibodies, assays to measure anti-platelet antibodies were developed [10,11] and several studies identified anti-platelet antibodies in childhood ITP [11–13]. Nonetheless, anti-platelet antibody testing currently remains of unclear clinical benefit [2,3], for several reasons. The results of early studies were dismissed by the observation of non-specific adsorption of plasma protein to platelets and potential false-positive results in healthy controls and patients with non-immune thrombocytopenia [14–20]. Later, to overcome these challenges, antigen-specific assays were developed that are now considered standard for assessment of anti-platelet antibodies in ITP [16,21,22]. A recent systematic review suggested that such antigen-specific anti-platelet antibody testing may be a useful rule-in test for adult ITP [23]. However, it is unknown whether this finding is transferable for childhood ITP. In contrast to adult ITP, childhood ITP has a large proportion of patients with preceding infections and mostly self-limiting disease courses, and the clinical context of suspected ITP in children is clearly different. A further reason for uncertainty is the heterogeneous background of ITP, which represents a mix of cases with distinct underlying pathophysiology. For instance, ITP may also be caused by T-cell-mediated immunity directed towards platelet autoantigens and megakaryocytes [24]. It is unknown how this influences the diagnostic and prognostic value of anti-platelet antibody testing. In sum, the role of anti-platelet antibody testing in the diagnosis of childhood ITP remains uncertain.

In the present study, we aimed to synthesize the available evidence to determine the potential diagnostic accuracy of anti-platelet antibody testing in childhood ITP. In addition, we describe some data on the prognostic significance of anti-platelet antibody testing.

## Methods

### Study identification and quality assessment

Reporting standards of the PRISMA guidelines were followed. PubMed and EMBASE were searched from inception until 4 April 2019 to determine the diagnostic accuracy of various immunoassays measuring platelet autoantibodies in childhood ITP (Table S1). The search string contained three elements: domain (children, 3 months to 18 years), disease (ITP) and diagnostic tests for comparison (platelet autoantibody tests). The sensitivity of the search strategy was assessed by the inclusion of pre-determined index publications. Screening of abstracts, full-text assessment and data extraction was performed independently by two investigators (D.S. and A.L.; Fig. 1). Studies were included if they were published in English language and evaluated a platelet autoantibody assay in children with immune thrombocytopenia and fully described the anti-platelet antibody immunoassay in the manuscript or a previous publication. Studies were excluded if they did not separate adult and childhood ITP data, assessed neonates or used anti-platelet antibodies as criteria for the diagnosis of ITP. Case series (less than 10 patients) were excluded. Data collection was validated independently by a third investigator (L.P.). The methodological quality of included articles was assessed using a standardized protocol for quality assessment of diagnostic accuracy studies (QUADAS2) [25].

Methodological details are described in the Supplementary Methods.

### Synthesis of results

Given substantive heterogeneity in study populations and methodological quality, study results were not pooled for meta-analysis of diagnostic accuracy.

## Results

### Study identification and characteristics

After screening and full-text assessment, 40 studies (1606 patients) were identified for this systematic review (Fig. 1; Table S2). The studies assessed either platelet-associated antibodies on autologous patient platelets (direct test) or circulating anti-platelet antibodies (indirect test).

### Assessment of risk of bias and concerns for applicability

We first assessed the methodological quality of included studies across four domains: (1) recruitment and

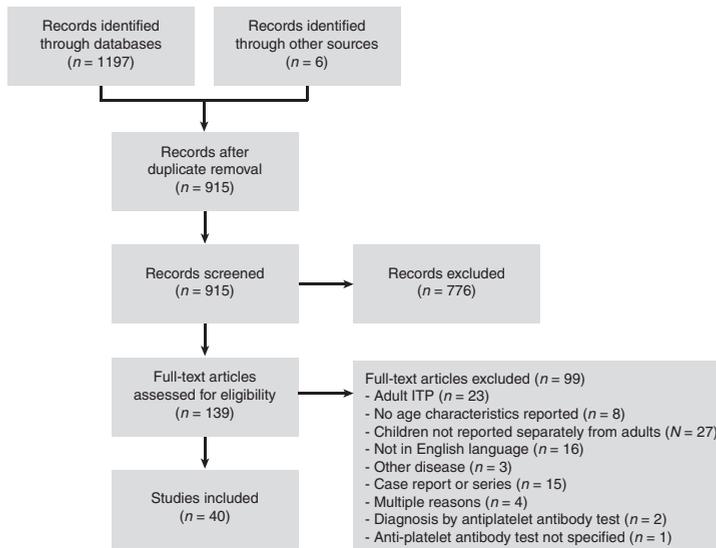


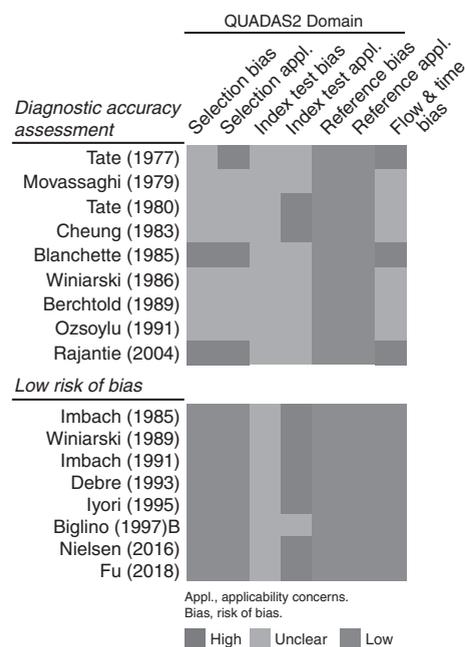
Fig. 1 Flowchart of study identification and assessment.

inclusion of patients (patient selection), (2) the evaluated anti-platelet antibody test (index test), (3) the criteria for diagnosis of ITP (reference standard) and (4) included disease stages and treatments (flow and timing). Overall, this assessment indicated that the risk of bias was unclear or high for a large proportion of studies (Fig. S1). Three factors were primarily responsible for this. First, although studies usually assessed the assay in at least some controls (either in the same study or historically), a substantial analysis was often lacking for control subjects, or this was incompletely reported. Second, studies did not report sensitivity and specificity or dichotomized test results from which these could be derived, that is true-positive, true-negative, false-negative and false-positive results. Third, many studies included heterogeneous disease stages with varying time from diagnosis, as well as mixing patients with prior or current treatments, both of which are known to influence anti-platelet antibody testing [11,13,26]. Nine of the 40 studies reported sufficient data to determine diagnostic accuracy of anti-platelet antibody testing; all nine were of unclear or high risk of bias (Fig. 2; top panel) [27–35]. Eight of the 40 included studies were judged to be of low risk of bias regarding the inclusion of ITP patients, but none of them explicitly reported control data (Fig. 2; lower panel) [36–43]. We subsequently focused our analysis on the nine studies which allowed assessment of diagnostic accuracy data and describe data of the eight

studies with low risk of bias and the remaining studies, as applicable.

### Diagnostic accuracy of anti-platelet antibody testing in childhood ITP

Nine studies allowed an assessment of the diagnostic accuracy of anti-platelet antibody testing (Table 1). The studies had a median sample size for cases and controls of 21 and 20, respectively. When we assessed these studies together, the primary finding was that most of the nine studies showed moderate sensitivity of immunoassays, with very good to excellent specificity (Table 2). These results were consistent across small study cohorts ( $N < 20$ ) and larger studies, as well as amongst studies that included vs. did not include non-immune thrombocytopenic controls. Indirect tests potentially have a lower sensitivity than direct tests, but a direct comparison was lacking (Table 2). Specificity was high across the studies (Table 2). Importantly, although false-positive results may be missed by small studies, specificity results were the same in studies which included small or large control groups. Next, we assessed some of the methodological concerns of these studies with unclear or high risk of bias. Treatment with steroids (two studies) may have lowered the sensitivity of the tests; one small study included IVIg-treated patients that may lead to false-positive test results [33],



**Fig. 2** Quality assessment of studies with available data for diagnostic accuracy assessment, as well as studies including patients with a low risk of bias, with judgement presented per study (full assessment of 40 studies in Fig. S1).

and another study measured anti-platelet antibody levels in IVIg-treated patients, but by an antigen-specific immunobead assay [35]. The inclusion of patients later in the disease course, as opposed to assessment at diagnosis, may skew results, but the studies at low risk of bias which included patients within a week of diagnosis showed a similar sensitivity (discussed below).

We calculated positive likelihood ratios that indicate how much more likely it is that a positive test result occurs in a patient, compared to controls, where ratios of  $\geq 10$  are considered clinically useful to rule-in disease [44]. Amongst four of the nine studies (92 patients) where positive likelihood ratios (LR) could be calculated, the positive LR was at least 8.8 in three studies, indicating that the assays could be used as rule-in tests for childhood ITP. In the other studies, only patients showed positive test results. On the other hand, with the given sensitivity and negative LR ranging between 0.14 and 0.73 (170 patients), the assays were insufficient as a rule-out criterion for ITP, for which a negative LR of  $\leq 0.1$  would be considered useful. In sum, there was weak-to-moderate evidence that anti-platelet antibody

testing could potentially be useful as a rule-in test of ITP.

## Supporting evidence

We next compared these data to results of the other studies that were identified by our systematic review. First, amongst the eight studies with low risk of bias (Table 1), six studies assessed anti-platelet IgG in patients with newly diagnosed ITP within a week of diagnosis, and two studies included patients with chronic ITP (Table 3; results of all 40 identified studies are shown in Table S5). We observed a similar moderate sensitivity as for the studies discussed above. Moreover, sensitivity was the same for newly diagnosed, as well as chronic ITP, and for glycoprotein-specific assays [39,40,42,43]. The results were unchanged when only the largest studies were considered [37,42,43].

For the remainder of the studies, a further eight reported quantitative results (i.e. mean or median and distribution) of platelet antibody immunoassays in cases and controls (Table S3). These eight studies unanimously indicated distinct anti-platelet antibody levels in patients with ITP compared to controls (Table S3). Thus, even though it was not possible to calculate the diagnostic accuracy, these studies confirmed that anti-platelet antibody levels are elevated in ITP patients compared to controls.

Together, these findings provide support that IgG anti-platelet antibodies can be detected in children with ITP and that results could be relatively specific for ITP.

## Detection of anti-platelet IgM and IgA antibodies

Three of the eight studies with low risk of bias assessed immunoassays for evaluation of anti-platelet IgM antibodies, two in newly diagnosed ITP and one in chronic ITP (Table 3; full results, Table S6). The sensitivity for detection of IgM autoantibodies was 0.62–0.69, indicating that IgM class autoantibodies could potentially be as prevalent as anti-platelet IgG antibodies [37,39,41]. Due to a lack of control data, the specificity could not be determined. Nonetheless, these findings in studies with low risk of bias suggest that IgM anti-platelet antibodies could be of importance in childhood ITP.

Three studies assessed the presence of anti-platelet IgA antibodies using direct ELISA or direct and indirect PIFT, identifying such antibodies in 5–24% of patients (Table S7). In comparison with IgM and IgG autoantibodies assessed in the same study by the same assay, IgA was detected at a 2- to 10-fold lower frequency [12,45,46]. None of these studies was of low risk of bias. Thus, IgA antibodies might be implicated in ITP, but a

Table 1 Characteristics of included studies (selection)

Author	Year	Region	Study design	Immunoassay class	Sample size	Age (years)	Female (%)	Chronic ITP (%)	Prior and current treatments
Studies for assessment of diagnostic accuracy									
Tate	1977	USA	Unclear	PA-Ig	10	6.8 (mean)	40	70	Steroids (4/10)
Movassaghi	1979	USA	Unclear	Functional	42	5.3 (1.25–14; median, range)	61	nd	Treatment naive
Tate	1980	USA	Unclear	PA-Ig	12 <sup>a</sup>	1–14 (range)	25	nd	nd
Cheung	1983	USA	Prospective cohort	ELISA	48	0.25–16 (range)	nd	46	nd
Blanchette	1985	Canada	Prospective cohort	PA-Ig	29	acute: 3 (0.25–16; median, range) chronic: 9 (3–17; median, range)	55	28	Steroids (11 of acute; 2 of chronic)
Winiarski	1986	Sweden	Prospective cohort	ELISA	18 <sup>c</sup>	nd	39	0	nd
Berchtold	1989	USA	Prospective cohort	Immunobead	39	acute: 5 (0.6–11; median, range) chronic: 10 (3–19; median, range)	46	62	IgG and corticosteroids
Ozoylu	1991	Turkey	Unclear	Functional	149	0.25–15 (range)	nd	31	nd
Rajantie	2004	Finland	Prospective cohort	PIFT	13/14 <sup>b</sup>	6.2 ± 1.1 (mean ± SE)	57	64	IgG and steroids (6/14), steroids (2/14)
Studies with homogeneous patient populations and low risk of bias									
At diagnosis									
Imbach	1985	Switzerland, Germany	Clinical trial	PA-Ig	57/94 <sup>b</sup>	6 (mean)	52	37	Treatment naive
Winiarski	1989	Sweden	Prospective cohort	Immunoblot	21	1.5–15 (range)	29	nd	Treatment naive
Debre	1993	France	Clinical trial	PA-Ig	10/12 <sup>b</sup>	7.2 (3–13; mean, range)	42	nd	Treatment naive
Biglino	1997b	Italy	Unclear	MAIPA	74	5.5 (0.25–13; mean, range)	nd	34	Treatment naive
Nielsen	2016	Denmark	Retrospective cohort	PA-Ig	68	44% is up to 3 years	56	18	Treatment naive
Fu	2018	China	Prospective cohort	ELISA	134	1.6 (0.1–13.25; mean, range)	43	13	Treatment naive
Chronic ITP									
Imbach	1991	Multinational	Prospective cohort	Immunobead	36	1–16 (range)	nd	100	No treatment 3 weeks before inclusion
Iyori	1995	Japan	Prospective cohort	PIFT, PA-Ig	29	11 (1.8–21.2; mean, range)	38	100	Splenectomy (2/29); rest untreated

Full data of all 40 identified studies are given in the Supplementary Tables.

nd, not described.

<sup>a</sup>Two adults originally included in the study were excluded.

<sup>b</sup>Only r/N patients had anti-platelet antibodies tested; characteristics are reported for the whole cohort.

<sup>c</sup>Children with chronic disease were not included as they were not distinguishable from adults.

Table 2 Diagnostic accuracy of anti-platelet antibody testing

Author	Year	Immunoassay class	Test type	IgG class	Type ITP	Type Controls	ITP (n)	Positive (n)	Negative (n)	Controls (n)	Positive (n)	Negative (n)	Sensitivity (95% CI)	Specificity (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
Tate	1977	PA-Ig	Indirect	Total Ig		Healthy controls; non-immune thrombocytopenia	10	10	0	19	1	18	1.00 (nd)	0.95 (0.85; 1.00)	19.0 (2.8; 128)	nd
Movassaghi	1979	Functional	Indirect	Total Ig		Healthy controls; non-immune thrombocytopenia	42	23	19	155	7	148	0.55 (0.40; 0.70)	0.96 (0.92; 0.99)	12.1 (5.6; 26.3)	0.47 (0.34; 0.66)
Tate	1980	PA-Ig	Indirect	IgG		Healthy controls; non-immune thrombocytopenia	12	12	0	27	0	27	1.00 (nd)	1.00 (nd)	nd	nd
Chung	1983	ELISA <sup>b</sup>	Direct	IgG	Acute	Healthy controls	26	22	4	17 <sup>a</sup>	0	17	0.85 (0.71; 0.98)	1.00 (nd)	nd	0.15 (0.06; 0.38)
Chung	1983	ELISA <sup>b</sup>	Direct	IgG	Chronic	Healthy controls	22	22	0	17 <sup>a</sup>	0	17	1.00 (nd)	1.00 (nd)	nd	nd
Blanchette	1985	PA-Ig	Direct	IgG	Acute	Healthy children; non-immune thrombocytopenia; thrombocytopenia; non-immune thrombocytopenia; non-immune thrombocytopenia; haematological disorders	21	16	5	46	4 <sup>c</sup>	42	0.76 (0.58; 0.94)	0.91 (0.82; 0.99)	8.8 (3.3; 23.0)	0.26 (0.12; 0.56)
Blanchette	1985	PA-Ig	Direct	IgG	Chronic	Healthy children; non-immune thrombocytopenia; haematological disorders	8	7	1	46	4 <sup>c</sup>	42	0.88 (0.65; 1.00)	0.91 (0.83; 0.99)	10.1 (3.8; 26.6)	0.14 (0.02; 0.86)
Winiarski	1986	ELISA	Indirect	IgG	Acute	Healthy blood donors	21 <sup>c</sup>	10	11	25	1	24	0.48 (0.26; 0.69)	0.96 (0.88; 1.00)	11.9 (1.7; 85.5)	0.55 (0.36; 0.83)
Berchtold	1989	Immunobead, GP IIb/IIIa	Indirect	IgG	Acute	Healthy children	15	4	11	10	0	10	0.27 (0.05; 0.48)	1.00 (nd)	nd	0.73 (0.54; 0.99)
Berchtold	1989	Immunobead, GP IIb/IIIa	Indirect	IgG	Chronic	Healthy children	24	14	10	10	0	10	0.58 (0.38; 0.78)	1.00 (nd)	nd	0.42 (0.26; 0.67)
Berchtold	1989	Immunobead, GP IIb/IIIa	Indirect	IgG	Acute	Healthy children	15	0	15	10	0	10	0.00 (nd)	1.00 (nd)	nd	nd
Berchtold	1989	Immunobead, GP IIb/IIIa	Indirect	IgG	Chronic	Healthy children	24	0	24	10	0	10	0.00 (nd)	1.00 (nd)	nd	nd
Ozsoylu	1991	Functional	Indirect	Total Ig		Healthy children; non-immune thrombocytopenia	146	146	0	126	0	126	1.00 (nd)	1.00 (nd)	nd	nd
Rajantie	2004	PIIT	Direct	Total Ig		Healthy children; familial thrombocytopenia	13	7	6	10	2	8	0.54 (0.27; 0.81)	0.80 (0.55; 1.00)	2.7 (0.7; 10.3)	0.58 (0.30; 1.12)

Only studies were included that presented dichotomized results or quantitative data in a graph.

CI, confidence interval; LR, likelihood ratio.

<sup>a</sup>Number abstracted from graph.

<sup>b</sup>The employed assay involved radiochemistry instead of enzyme, but the test principle falls under the ELISA category.

<sup>c</sup>21 sera tested from 18 patients; nd, not determined.



Table 3 Sensitivity of anti-platelet antibody testing in ITP (studies with low risk of bias)

Author	Year	Immunoassay class	Type test	Subclass	Antigen	Prognosis	ITP (N)	Positive (n)	Negative (n)	Sensitivity	95% CI
At diagnosis											
Imbach	1985	PA-Ig	Direct	IgG			57	43	14	0.75	0.64; 0.87
Debre	1993	PA-Ig	Direct	IgG			10	8	2	0.80	0.55; 1.00
Nielsen	2016	PA-Ig	Direct	IgG			68	30	38	0.44	0.32; 0.56
Nielsen	2016	PA-Ig	Direct	IgM			68	43	25	0.63	0.52; 0.75
Winiarski	1989	Immunoblot	Indirect	IgG			21	4	17	0.19	0.02; 0.36
Winiarski	1989	Immunoblot	Indirect	IgM			21	13	8	0.62	0.41; 0.83
Biglino	1997b	MAIPA	Indirect	IgG	GP IIb/IIIa	Transient	49	19	30	0.39	0.25; 0.52
Biglino	1997b	MAIPA	Indirect	IgG	GP IIb/IIIa	Chronic	25	8	17	0.32	0.14; 0.50
Biglino	1997b	MAIPA	Indirect	IgG	GP Ib/IX	Transient	49	15	34	0.31	0.18; 0.44
Biglino	1997b	MAIPA	Indirect	IgG	GP Ib/IX	Chronic	25	7	18	0.28	0.10; 0.46
Fu	2018	ELISA	Indirect	IgG	GP IIb/IIIa	Transient	113	92	21	0.81	0.74; 0.89
Fu	2018	ELISA	Indirect	IgG	GP IIb/IIIa	Chronic	18	11	7	0.61	0.39; 0.84
Fu	2018	ELISA	Indirect	IgG	GP Ib/IX	Transient	113	54	59	0.48	0.39; 0.57
Fu	2018	ELISA	Indirect	IgG	GP Ib/IX	Chronic	18	11	7	0.61	0.39; 0.84
Chronic ITP											
Imbach	1991	Immunobead	Direct	IgG	GP IIb/IIIa, GP Ib <sup>b</sup>		36	26	10	0.72	0.58; 0.87
Iyori	1995	PA-Ig	Direct	IgG	GP IIb/IIIa <sup>a</sup>		25	9	16	0.36	0.17; 0.55
Iyori	1995	PIFT	Direct	IgG			29	22	7	0.76	0.60; 0.91
Iyori	1995	PIFT	Direct	IgM			29	20	9	0.69	0.52; 0.86

<sup>a</sup>By immunoprecipitation of the antigen in platelet lysates.<sup>b</sup>Results were reported only in aggregate.

more extensive assessment is required to determine their role for diagnostic testing.

### Association of anti-platelet antibodies with prognosis

Some of the included studies assessed the association of anti-platelet antibodies in early disease with future disease outcomes. Nielsen *et al.* observed that only 7% of patients with chronic disease course showed glycoprotein-specific IgM or IgG antibodies, compared to 41% of patients with a transient ITP course (N = 15 vs. N = 37) [47]. Moreover, when measuring antibodies at diagnosis, Fu *et al.* found that patients with a prognosis of chronic ITP showed enrichment of anti-GP Ib/IX antibodies, compared to those with transient disease courses (N = 18 vs. N = 113) [43]. Findings of these two studies are contrasted by Biglino *et al.* who observed equal rates of anti-GPIIb/IIIa or anti-GPIb/IX antibodies in patients with transient or chronic disease courses (N = 49 vs. N = 25)[42] and Nielsen *et al.* who showed a similar rate of anti-platelet antibodies by PA-IgG/IgM amongst patients with transient and chronic diseases courses (N = 46 vs. N = 12)[37]. Taken together, some studies indicate a role of anti-platelet antibody testing to determine prognosis, but in the presence of conflicting evidence further data are required.

### Discussion

In this study, we systematically reviewed 40 studies and summarized the current knowledge regarding the diagnostic accuracy of anti-platelet antibody testing in childhood ITP. Although many studies suffered from insufficient reporting and a lack of controls, we carefully analysed and weighted the available data. The main finding of this review is the overall good diagnostic accuracy of anti-platelet antibody testing, based on multiple case-control and prospective cohort studies, supported by evidence from low risk of bias studies. Sensitivity was moderate in multiple studies, and this could be due to low levels of circulating antibodies during active clearance of platelet-antibody immune complexes, low avidity of the antibodies or insensitivity of the used tests or heterogeneity in the involved pathomechanisms, such as antibody-mediated or T-cell-mediated anti-platelet clearance [24]. Even though evidence has been published that indicated potential false-positive results in patients with thrombocytopenia by other causes than ITP, particularly with PA-Ig assays [14–16], studies included in this review showed good specificity in healthy children and patients with non-immune thrombocytopenia (multiple case-control studies), also in the largest included studies [28,31,32]. It might be argued that studies that did not report control

data showed a high rate of false-positive results. Regardless, studies which used the more recently developed antigen-specific assays [16] showed similar sensitivity as whole-platelet testing (three studies; two with low risk of bias), which provides a compelling argument against skewing to false-positive results. Thus, when balancing the available data on diagnostic accuracy, anti-platelet antibody assays may potentially be used as a rule-in test, as also indicated by a high positive likelihood ratio. This is in line with results of a recent systematic review of anti-platelet antibody testing in adult ITP [23]. On the other hand, given the limited sensitivity, the data show that anti-platelet antibody assays cannot be used as a rule-out test to exclude a diagnosis of ITP, which is also in agreement with the conclusions of the systematic review in adult ITP [23].

A secondary finding of our analysis in childhood ITP was that IgM anti-platelet antibodies may be as prevalent as IgG anti-platelet antibodies (three studies), which should be assessed in future studies. Moreover, tests for circulating anti-platelet antibodies are interesting alternatives when children have insufficient platelets available for a direct test. We found that although indirect tests might have a reduced sensitivity, they could still be of diagnostic value. Several studies described lower anti-platelet antibody levels when patients were assessed later in their disease course or after treatment [11,13,28,32,48], indicating that delayed testing may affect the diagnostic accuracy. Finally, with regard to relevant platelet antigens, in two studies, anti-GPIb/IX antibodies were not detected at all, and GP IIb/IIIa was the predominant anti-platelet antibody [35,49]. Conversely, antibodies directed against GP IIb/IIIa and GP Ib/IX were found at about the same rate by one study [43]. Thus, the role of antibodies against specific platelet glycoproteins for the diagnosis and prognosis of childhood ITP remains elusive.

The primary limitation of this review was the reference test of our study (gold standard), which was a clinical diagnosis of ITP. As surrogates of patient heterogeneity between studies, we extracted data on age, sex and preceding infection, which are associated with ITP prognosis [50]. Moreover, the reported diagnostic accuracy may be optimistic estimates, since none of the studies investigated anti-platelet antibody assays in children with suspected ITP. However, in the context of a rare disease and a paediatric study population, such studies are notoriously difficult to conduct. Two further shortcomings regarding the methodological quality of included studies limited our review: (1) insufficient reporting of control data to determine specificity for the majority of studies and (2) the inclusion of heterogeneous patient populations at various disease stages and divergent treatments. Nonetheless, we recognized that the identified studies

represent the best evidence to date in a disease setting that is challenging to investigate, and carefully assessed and compared the available data. We suggest that to improve the quality of data in the field, future studies published in childhood ITP should be explicitly required to disclose clinical characteristics, inclusion criteria, time from diagnosis and previous and current treatments.

Finally, during routine diagnostic testing, not all childhood patients have sufficient platelet numbers to perform direct tests; thus, results could be biased to older children, for whom more material is available. None of the studies disclosed the number of patients that could not be evaluated.

For future directions, direct tests should be developed that allow the use of low number of platelets. The assessment of anti-platelet antibodies may be of prognostic significance, potentially indicating a subgroup amongst heterogeneous ITP patients, and this should be investigated further. A key current area of uncertainty is the evaluation of anti-platelet antibody assays in children with suspected ITP.

In conclusion, this systematic review indicates that anti-platelet antibody testing could potentially be used as

a rule-in test for childhood ITP, although anti-platelet antibody testing cannot be used to exclude a diagnosis of ITP.

## Acknowledgements

This work was supported by a research grant from the Landsteiner Foundation for Blood Transfusion Research (LSBR) and a doctoral stipend to David E. Schmidt by the Studienstiftung des Deutschen Volkes.

## Conflict of interest

The authors declare no competing financial interests.

## Author contributions

D.E.S. and A.J.L. screened and assessed articles. A.J.L. wrote an initial draft. D.E.S. wrote the manuscript. L.P. assessed articles, validated and interpreted results. K.M.J.H.-P., M.C.A.B. and G.V. interpreted results. M.d.H. supervised the study. All authors revised and approved the manuscript.

## References

- 1 Terrell DR, Beebe LA, Vesely SK, *et al.*: The incidence of immune thrombocytopenic purpura in children and adults: A critical review of published reports. *Am J Hematol.* 2010; 85:174–80
- 2 Provan D, Stasi R, Newland AC, *et al.*: International consensus report on the investigation and management of primary immune thrombocytopenia. *Blood.* 2010; 115:168–86
- 3 Neunert C, Lim W, Crowther M, *et al.*: The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood* 2011; 117:4190–4207
- 4 Arnold DM, Nazy I, Clare R, *et al.*: Misdiagnosis of primary immune thrombocytopenia and frequency of bleeding: lessons from the McMaster ITP Registry. *Blood Advances* 2017; 1:2414–2420
- 5 Althaus K, Najm J, Greinacher A: MYH9 related platelet disorders - often unknown and misdiagnosed. *Klin Padiatr* 2011; 223:120–5
- 6 Bader-Meunier B, Proulle V, Trichet C, *et al.*: Misdiagnosis of Chronic Thrombocytopenia in Childhood. *J Pediatr Hematol Oncol* 2003; 25:548–552. B. Bader-Meunier, Department of Pediatrics, Bicetre Hospital, 94270 Le Kremlin Bicetre, France. E-mail: brigitte.bader-meunier@bct.ap-hop-paris.fr
- 7 Lippincott Williams and Wilkins (530 Walnut Street, P O Box 327, Philadelphia PA 19106–3621, United States).
- 7 Eltrombopag vs Standard Front Line Management for Newly Diagnosed Immune Thrombocytopenia (ITP) in Children [Internet]. Clinicaltrials.gov. [cited 2019 Oct 25]. Available from: <https://clinicaltrials.gov/ct2/show/NCT03939637>.
- 8 van Leeuwen EF, van der Ven JT, Engelfriet CP, *et al.*: Specificity of autoantibodies in autoimmune thrombocytopenia. *Blood* 1982; 59:23–6
- 9 Shulman NR, Marder VJ, Weinrach RS: Similarities between known anti-platelet antibodies and the factor responsible for thrombocytopenia in idiopathic purpura. physiologic, serologic and isotopic studies. *Ann N Y Acad Sci* 1965; 124:499–542
- 10 McMillan R, Smith RS, Longmire RL, *et al.*: Immunoglobulins associated with human platelets. *Blood* 1971; 37:316–22
- 11 Cines DB, Schreiber AD: Immune Thrombocytopenia. *N Engl J Med* 1979; 300:106–111.
- 12 van Leeuwen EF, von dem Borne AE, van der Plas-van Dalen C, *et al.*: Idiopathic thrombocytopenic purpura in children; detection of platelet autoantibodies by immunofluorescence. *Scand J Haematol.* 1981; 26:285–91
- 13 Lightsey ALJ, Koenig HM, McMillan R, *et al.*: Platelet-associated immunoglobulin G in childhood idiopathic thrombocytopenic purpura. *J Pediatr* 1979; 94:201–4
- 14 Mueller-Eckhardt C, Kayser W, Mersch-Baumert K, *et al.*: The clinical significance of platelet-associated IgG: a study on 298 patients with various disorders. *Br J Haematol* 1980; 46:123–31
- 15 Kelton JG, Steeves K: The amount of platelet-bound albumin parallels the amount of IgG on washed platelets from patients with immune thrombocytopenia. *Blood* 1983; 62:924–7
- 16 Warner MN, Moore JC, Warkentin TE, *et al.*: A prospective study of protein-

- specific assays used to investigate idiopathic thrombocytopenic purpura. *Br J Haematol* 1999; 104:442–7
- 17 Borne AEGK, Verheugt FWA, Oosterhof F, *et al.*: A simple immunofluorescence test for the detection of platelet antibodies. *Br J Haematol* 1978; 39:195–207
  - 18 Bizzaro N, Brandalise M: EDTA-dependent pseudothrombocytopenia. Association with antiplatelet and antiphospholipid antibodies. *Am J Clin Pathol*. 1995; 103:103–7
  - 19 Muniz-Diaz E, Madoz P, Pujol-Moix N, *et al.*: Characterization of antibodies directed against platelet cryptantigens detected during the immunological study of 356 consecutive patients with presumed autoimmune thrombocytopenia (AITP). *Transfus Med (Oxford, England)*. 1995; 5:185–91
  - 20 Kelton JG, Murphy WG, Lucarelli A, *et al.*: A prospective comparison of four techniques for measuring platelet-associated IgG. *Br J Haematol* 1989; 71:97–105
  - 21 Kiefel V, Santoso S, Weisheit M, *et al.*: Monoclonal antibody-specific immobilization of platelet antigens (MAIPA): a new tool for the identification of platelet-reactive antibodies. *Blood* 1987; 70:1722–6
  - 22 Arnold DM, Santoso S, Greinacher A, *et al.*: Recommendations for the implementation of platelet autoantibody testing in clinical trials of immune thrombocytopenia. *J Thromb Haemost* 2012; 10:695–697
  - 23 Vrbensky JR, Moore JE, Arnold DM, *et al.*: The sensitivity and specificity of platelet autoantibody testing in immune thrombocytopenia: a systematic review and meta-analysis of a diagnostic test. *J Thromb Haemost* 2019; 17:787–94
  - 24 Zufferey A, Kapur R, Semple JW: Pathogenesis and therapeutic mechanisms in immune thrombocytopenia (ITP). *J Clin Med* 2017; 6:16
  - 25 Whiting PF, Rutjes AWS, Westwood ME, *et al.*: QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011; 155:529–36
  - 26 Ljung R, Nilsson IM, Frohm B, *et al.*: Platelet-associated IgG in childhood idiopathic thrombocytopenic purpura: measurements on intact and solubilized platelets and after gammaglobulin treatment. *Scand J Haematol* 1986; 36:402–7
  - 27 Tate DY, Sorenson RL, Gerrard JM, *et al.*: An immunoenzyme histochemical technique for the detection of platelet antibodies from the serum of patients with idiopathic (autoimmune) thrombocytopenic purpura (ITP). *Br J Haematol* 1977; 37:265–75
  - 28 Movassaghi N, Moorhead J, Leikin S: Antiplatelet antibodies in childhood idiopathic thrombocytopenic purpura. *Am J Dis Child* 1979; 133:257–9
  - 29 Tate DY, Carlton GT, Nesbit ME, *et al.*: Detection of platelet associated IgG in immune thrombocytopenia: a new assay employing protein A and peroxidase anti-peroxidase (PROA-PAP). *Am J Hematol* 1980; 9:349–61
  - 30 Cheung NK, Hilgartner MW, Schulman I, *et al.*: Platelet-associated immunoglobulin G in childhood idiopathic thrombocytopenic purpura. *J Pediatr* 1983; 102:366–70
  - 31 Blanchette V, Hogan V, Esseltine D, *et al.*: Evaluation of a simple immunodiffusion technique for quantitation of platelet-associated immunoglobulin G in childhood immune thrombocytopenias. *Am J Pediatr Hematol Oncol* 1985; 7:125–31
  - 32 Ozsoylu S, Karabent A, Irken G, *et al.*: Antiplatelet antibodies in childhood idiopathic thrombocytopenic purpura. *Am J Hematol* 1991; 36:82–5
  - 33 Rajantie J, Javela K, Joutsu-Korhonen L, *et al.*: Chronic thrombocytopenia of childhood: use of non-invasive methods in clinical evaluation. *Eur J Haematol* 2004; 72:268–72
  - 34 Winiarski J, Ekelund E: Antibody binding to platelet antigens in acute and chronic idiopathic thrombocytopenic purpura: a platelet membrane ELISA for the detection of antiplatelet antibodies in serum. *Clin Exp Immunol* 1986; 63:459–65
  - 35 Berchtold P, McMillan R, Tani P, *et al.*: Autoantibodies against platelet membrane glycoproteins in children with acute and chronic immune thrombocytopenic purpura. *Blood* 1989; 74:1600–2
  - 36 Imbach P, Wagner HP, Berchtold W, *et al.*: Intravenous immunoglobulin versus oral corticosteroids in acute immune thrombocytopenic purpura in childhood. *Lancet* 1985; 2:464–8
  - 37 Nielsen OH, Tuckuviene R, Nielsen KR, *et al.*: Flow cytometric measurement of platelet-associated immunoglobulin in children with newly diagnosed Immune Thrombocytopenia. *Eur J Haematol* 2016; 96:397–403
  - 38 Debré M, Bonnet MC, Fridman WH, *et al.*: Infusion of Fc gamma fragments for treatment of children with acute immune thrombocytopenic purpura. *Lancet* 1993; 342:945–9
  - 39 Iyori H, Fujisawa K, Akatsuka J: Autoantibodies and CD5+ B cells in childhood onset immune thrombocytopenic purpura. *Acta Paediatr Jpn* 1995; 37:325–30
  - 40 Imbach P, Tani P, Berchtold W, *et al.*: Different forms of chronic childhood thrombocytopenic purpura defined by antiplatelet autoantibodies. *J Pediatr* 1991; 118(4 Pt 1):535–9
  - 41 Winiarski J: IgG and IgM antibodies to platelet membrane glycoprotein antigens in acute childhood idiopathic thrombocytopenic purpura. *Br J Haematol* 1989; 73:88–92
  - 42 Biglino P, Perutelli P, Mori PG: Circulating antiplatelet antibody specificity in children with immune thrombocytopenic purpura at onset. *Haematologica* 1997; 82:127
  - 43 Fu L, Cheng Z, Gu H, *et al.*: Platelet-specific antibodies and differences in their expression in childhood immune thrombocytopenic purpura predicts clinical prognosis. *Pediatr Invest* 2018; 2:230–5
  - 44 Deeks JJ, Altman DG: Diagnostic tests 4: likelihood ratios. *BMJ* 2004; 329:168–9
  - 45 Taaning E, Petersen S: Pattern of platelet-associated immunoglobulin (classes and IgG subclasses) in childhood immune thrombocytopenic purpura. *Eur J Haematol* 1988; 41:449–53
  - 46 Brink S, Hesselting PB, Amadhila S, *et al.*: Platelet antibodies in immune thrombocytopenic purpura and onyala. *S Afr Med J* 1981; 59:855–8
  - 47 Nielsen HE, Andersen EA, Carlsen N, *et al.*: Presence of platelet antibodies in idiopathic thrombocytopenic purpura may discriminate acute from chronic disease. *Acta Paediatr* 2003; 92:1208–10

- 48 Biglino P, Perutelli P, Mori PG: Platelet antibody detection in pediatric immune thrombocytopenic purpura: evaluation of three screening methods. *Vox Sang* 1997; 72:242–6
- 49 Nieminen U, Peltola H, Syrjala MT, *et al.*: Acute thrombocytopenic purpura following measles, mumps and rubella vaccination. A report on 23 patients. *Acta Paediatr* 1993; 82: 267–70
- 50 Heitink-Polle KMJ, Nijsten J, Boonacker CWB, *et al.*: Clinical and laboratory predictors of chronic immune thrombocytopenia in children: a systematic review and meta-analysis. *Blood* 2014; 124:3295–307

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Quality assessment of all 40 included studies, with judgement presented per study.

Table S1 Search strategy for Pubmed and EMBASE searching.

Table S2 Study characteristics of 40 included studies.

Table S3 Quantitative differences of anti-platelet antibody levels in ITP versus controls.

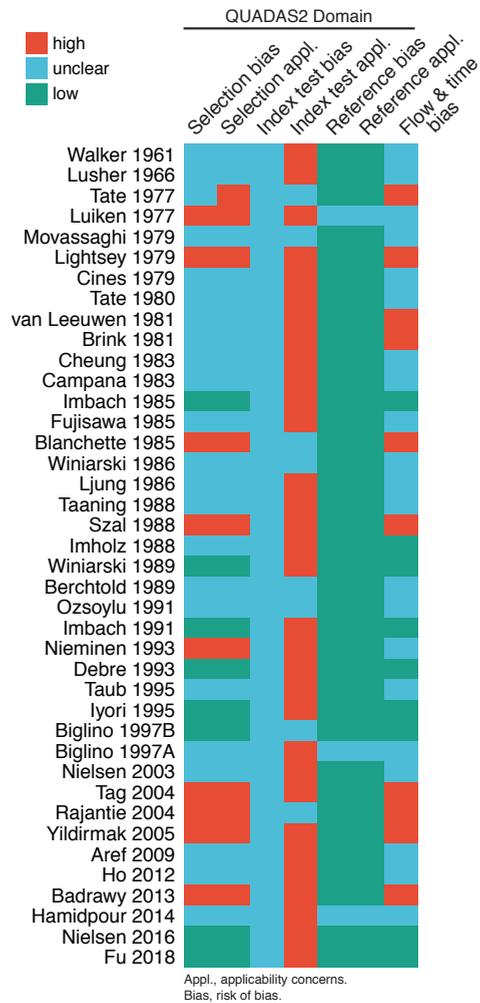
Table S4 Sensitivity of anti-platelet antibody testing in ITP for total Ig.

Table S5 Sensitivity of anti-platelet antibody testing in ITP for IgG isotype.

Table S6 Sensitivity of anti-platelet antibody testing in ITP for IgM isotype.

Table S7 Sensitivity of anti-platelet antibody testing in ITP for IgA and unclear Ig target.

**Supplementary Figure S1.** Quality assessment of all 40 included studies, with judgement presented per study. Assessment was performed using the Quality Assessment for Diagnostic Accuracy Studies (QUADAS2) tool.



## **Supplementary Methods**

### *Study identification and screening*

This systematic review follows reporting standards outlined in the PRISMA guidelines. It has not been pre-registered. A search strategy was developed for the PubMed and EMBASE databases to determine the diagnostic accuracy of various immunoassays measuring platelet autoantibodies in childhood ITP (Supplementary Table S1). In brief, the search string contained three elements: domain (children between 3 months and 18 years old), disease (ITP), and diagnostic tests for comparison (platelet autoantibody tests). Databases were searched for all articles indexed from inception until April 4, 2019. Studies assessing all various disease states (newly diagnosed, chronic) were included. Congress abstracts were excluded in EMBASE. The sensitivity of the search strategy was assessed by the inclusion of pre-determined index publications. Screening of abstracts was performed independently by two investigators (D.S. and A.L.). All selected studies were reviewed in full text.

### *Study eligibility, data extraction and validation*

After initial screening, articles were assessed for eligibility in full-text by the same two investigators. Studies were included if they were published in English language and evaluated a platelet autoantibody assay in children with immune thrombocytopenia, irrespective of the definition of diagnosis or the duration of disease. The anti-platelet antibody immunoassay needed to be previously established in a peer-reviewed publication, or sufficiently described in the manuscript. Studies were excluded if they did not separate adult and childhood ITP data, assessed neonates or used anti-platelet antibodies as criteria for the diagnosis of ITP. Review articles, case reports, and series (less than ten patients) were excluded. The following data was extracted from included studies using standardized data collection forms:

characteristics of study population (setting, sample size, age, gender, chronic ITP rate, preceding infection rate, bleeding symptoms, treatments, type and number of controls), and characteristics of tests under evaluation (assay, number of positives and negatives for ITP patients and controls). Data was extracted separately per disease stage if it was presented in such a way. When data for heterogeneous populations (concerning disease stage or treatment) was given, but a subset was specified for a group of `acute onset` or `untreated`, the data of this subset of patients were extracted (as indicated). Data collection was validated independently by a third investigator (L.P.). For all included studies we calculated sensitivity and specificity as well as positive and negative likelihood ratios [1,2]. These measures of diagnostic accuracy are not influenced by the ratio of number of controls and cases, making them useful in studies where investigators determined this ratio.

### *Definitions*

The original terminology of the authors was kept for `acute` and `chronic` ITP. We used the term `at diagnosis` exclusively for patients that had recently been diagnosed, i.e. within one week, and referred to `transient ITP` when patients had resolution of disease within three months [3]. `Direct` and `indirect` tests were defined as the measurement of antibodies bound to autologous patient platelets (direct) or measurement of circulating autoantibodies in either serum or plasma (indirect).

Immunoassay classes were defined by assay principle into enzyme-linked immunosorbent assay (ELISA), platelet-associated immunoglobulin (PA-Ig) [4-6], platelet immunofluorescence technique (PIFT) [7], monoclonal antibody-specific immobilization of platelet antigens (MAIPA) [8], immunoblot and immunobead or functional assays.



### *Assessment of methodological quality*

The methodological quality of included articles was assessed using a standardized protocol for quality assessment of diagnostic accuracy studies (QUADAS2) [9], after tailoring to the specific research question of this systematic review and developing review-specific guidance for judgment. The questionnaire was initially tested on a random sample of four articles, subsequently refined and then applied to all articles. Publication bias was not assessed.

### **Supplementary References**

1. Knottnerus AJ, editor. Evidence Base of Clinical Diagnosis. London : BMJ Books; 2002.
2. Deeks JJ, Altman DG. Diagnostic tests 4: likelihood ratios. *BMJ*. 2004 Jul 17;329(7458):168–9.
3. Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. American Society of Hematology; 2009 Mar 12;113(11):2386–93.
4. Dixon R, Rosse W, Ebbert L. Quantitative determination of antibody in idiopathic thrombocytopenic purpura. Correlation of serum and platelet-bound antibody with clinical response. *New England Journal of Medicine*. 1975 Jan 30;292(5):230–6.
5. McMillan R, Smith RS, Longmire RL, Yelenosky R, Reid RT, Craddock CG. Immunoglobulins associated with human platelets. *Blood*. 1971 Mar;37(3):316–22.
6. Luiken GA, McMillan R, Lightsey AL, Gordon P, Zevely S, Schulman I, et al. Platelet-associated IgG in immune thrombocytopenic purpura. *Blood*. 1977 Aug;50(2):317–25.
7. Borne von dem AEGK, Verheugt FWA, Oosterhof F, Riesz E, Rivière AB, Engelfriet CP. A Simple Immunofluorescence Test for the Detection of Platelet Antibodies. *British Journal of Haematology*. Blackwell Publishing Ltd; 1978 Jun;39(2):195–207.
8. Kiefel V, Santoso S, Weisheit M, Mueller-Eckhardt C. Monoclonal antibody--specific immobilization of platelet antigens (MAIPA): a new tool for the identification of platelet-reactive antibodies. *Blood*. 1987 Dec;70(6):1722–6.
9. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011 Oct 18;155(8):529–36.

## Full references to the included studies

Walker, J. H., & Walker, W. (1961). Idiopathic thrombocytopenic purpura in childhood. *Archives of Disease in Childhood*, 36, 649–657.

Lusher, J. M., & Zuelzer, W. W. (1966). Idiopathic thrombocytopenic purpura in childhood. *The Journal of Pediatrics*, 68(6), 971–979.

Luiken, G. A., McMillan, R., Lightsey, A. L., Gordon, P., Zevely, S., Schulman, I., et al. (1977). Platelet-associated IgG in immune thrombocytopenic purpura. *Blood*, 50(2), 317–325.

Tate, D. Y., Sorenson, R. L., Gerrard, J. M., White, J. G., & Krivit, W. (1977). An immunoenzyme histochemical technique for the detection of platelet antibodies from the serum of patients with idiopathic (autoimmune) thrombocytopenic purpura (ITP). *British Journal of Haematology*, 37(2), 265–275.

Cines, D. B., & Schreiber, A. D. (1979). Immune thrombocytopenia. Use of a Coombs antiglobulin test to detect IgG and C3 on platelets. *New England Journal of Medicine*, 300(3), 106–111. <http://doi.org/10.1056/NEJM197901183000302>

Lightsey, A. L. J., Koenig, H. M., McMillan, R., & Stone, J. R. J. (1979). Platelet-associated immunoglobulin G in childhood idiopathic thrombocytopenic purpura. *The Journal of Pediatrics*, 94(2), 201–204.

Movassaghi, N., Moorhead, J., & Leikin, S. (1979). Anti-platelet antibodies in childhood idiopathic thrombocytopenic purpura. *American Journal of Diseases of Children* (1960), 133(3), 257–259.

Tate, D. Y., Carlton, G. T., Nesbit, M. E., White, J. G., Krivit, W., & Sorenson, R. L. (1980). Detection of platelet associated IgG in immune thrombocytopenia: a new assay employing protein A and peroxidase anti-peroxidase (PROA-PAP). *American Journal of Hematology*, 9(4), 349–361.

Brink, S., Hesseling, P. B., Amadhila, S., & Visser, H. S. (1981). Platelet antibodies in immune thrombocytopenic purpura and onyalai. *South African Medical Journal = Suid-Afrikaanse Tydskrif Vir Geneeskunde*, 59(24), 855–858.

van Leeuwen, E. F., Borne, von dem, A. E., van der Plas-van Dalen, C., & Engelfriet, C. P. (1981). Idiopathic thrombocytopenic purpura in children; detection of platelet autoantibodies by immunofluorescence. *Scandinavian Journal of Haematology*, 26(4), 285–291. <http://doi.org/10.1111/j.1600-0609.1981.tb01663.x>

Campana, D., Bergui, L., Camussi, G., Miniero, R., Morgando, M. P., Sardi, A., et al. (1983). Immune-complexes and anti-platelet antibodies in idiopathic thrombocytopenic purpura. *Haematologica*, 68(2), 157–166.

Cheung, N. K., Hilgartner, M. W., Schulman, I., McFall, P., Glader, B. E., N K, C., V, et al. (1983). Platelet-associated immunoglobulin G in childhood idiopathic thrombocytopenic purpura. *Journal of Pediatrics*, 102(3), 366–370.

Blanchette, V., Hogan, V., Esseltine, D., Hsu, E., Luke, B., & Rock, G. (1985). Evaluation of a simple immunodiffusion technique for quantitation of platelet-associated immunoglobulin G in childhood immune thrombocytopenias. *The American Journal of Pediatric Hematology/Oncology*, 7(2), 125–131.

Fujisawa, K., & Akatsuka, I. J. (1985). Platelet-associated IgG in children. Values and evaluation of PAIgG in various thrombocytopenias. *Acta Paediatrica Japonica : Overseas Edition*, 27(3), 445–454.

Imbach, P., Wagner, H. P., Berchtold, W., Gaedicke, G., Hirt, A., Joller, P., et al. (1985). Intravenous immunoglobulin versus oral corticosteroids in acute immune thrombocytopenic purpura in childhood. *Lancet (London, England)*, 2(8453), 464–468.

Ljung, R., Nilsson, I. M., Frohm, B., & Holmberg, L. (1986). Platelet-associated IgG in childhood idiopathic thrombocytopenic purpura: measurements on intact and solubilized platelets and after gammaglobulin treatment. *Scandinavian Journal of Haematology*, 36(4), 402–407.

Winiarski, J., & Ekelund, E. (1986). Antibody binding to platelet antigens in acute and chronic idiopathic thrombocytopenic purpura: a platelet membrane ELISA for the detection of anti-platelet antibodies in serum. *Clinical and Experimental Immunology*, 63(2), 459–465.

Imholz, B., Imbach, P., Baumgartner, C., Berchtold, W., Gaedicke, G., Gugler, E., et al. (1988). Intravenous immunoglobulin (i.v. IgG) for previously treated acute or for chronic idiopathic thrombocytopenic purpura (ITP) in childhood: A prospective multicenter study. *Blut*, 56(2), 63–68.

Szal, M., & Blumberg, N. (1988). Clinical correlates in patients with elevated platelet-associated immunoglobulins. *Annals of Clinical and Laboratory Science*, 18(1), 24–33.

Taaning, E., & Petersen, S. (1988). Pattern of platelet-associated immunoglobulin (classes and IgG subclasses) in childhood immune thrombocytopenic purpura. *European Journal of Haematology*, 41(5), 449–453.

Berchtold, P., McMillan, R., Tani, P., Sommerville-Nielsen, S., & Blanchette, V. S. (1989). Autoantibodies against platelet membrane glycoproteins in children with acute and chronic immune thrombocytopenic purpura. *Blood*, 74(5), 1600–1602.

Winiarski, J. (1989). IgG and IgM antibodies to platelet membrane glycoprotein antigens in acute childhood idiopathic thrombocytopenic purpura. *British Journal of Haematology*, 73(1), 88–92.

Imbach, P., Tani, P., Berchtold, W., Blanchette, V., Burek-Kozłowska, A., Gerber, H., et al. (1991). Different forms of chronic childhood thrombocytopenic purpura defined by anti-platelet autoantibodies. *Journal of Pediatrics*, 118(4 Pt 1), 535–539.

Ozsoylu, S., Karabent, A., Irken, G., & Tuncer, M. (1991). Anti-platelet antibodies in childhood idiopathic thrombocytopenic purpura. *American Journal of Hematology*, 36(2), 82–85.

Debré, M., Bonnet, M. C., Fridman, W. H., Carosella, E., Philippe, N., Reinert, P., et al. (1993). Infusion of Fc gamma fragments for treatment of children with acute immune thrombocytopenic purpura. *Lancet (London, England)*, 342(8877), 945–949.

Nieminen, U., Peltola, H., Syrjala, M. T., Makiperna, A., & Kekomäki, R. (1993). Acute thrombocytopenic purpura following measles, mumps and rubella vaccination. A report on 23 patients. *Acta Paediatrica*, 82(3), 267–270.

Iyori, H., Fujisawa, K., & Akatsuka, J. (1995). Autoantibodies and CD5+ B cells in childhood onset immune thrombocytopenic purpura. *Acta Paediatrica Japonica : Overseas Edition*, 37(3), 325–330.

Taub, J. W., Warrier, I., Holtkamp, C., Beardsley, D. S., & Lusher, J. M. (1995). Characterization of autoantibodies against the platelet glycoprotein antigens IIb/IIIa in childhood idiopathic thrombocytopenia purpura. *American Journal of Hematology*, 48(2), 104–107.

Biglino, P., Perutelli, P., & Mori, P. G. (1997<sup>A</sup>). Platelet antibody detection in pediatric immune thrombocytopenic purpura: evaluation of three screening methods. *Vox Sanguinis*, 72(4), 242–246.

Biglino, P., Perutelli, P., & Mori, P. G. (1997<sup>B</sup>). Circulating anti-platelet antibody specificity in children with immune thrombocytopenic purpura at onset. *Haematologica*, 82(1), 127.

Nielsen, H. E., Andersen, E. A., Carlsen, N., Nir, M., & Taaning, E. (2003). Presence of platelet antibodies in idiopathic thrombocytopenic purpura may discriminate acute from chronic disease. *Acta Paediatrica*, 92(10), 1208–1210.

Tag, L. M., Ezz-Eldeen, A. M., Mahmoud, M. S., Rashed, H.-A. G., Noaman, H. A., L M, T., et al. (2004). Serum IL-2 and platelet-associated immunoglobulins are good prognostic markers in immune thrombocytopenic purpura. *The Egyptian Journal of Immunology*, 11(2), 121–132.

Rajantie, J., Javela, K., Joutsu-Korhonen, L., & Kekomäki, R. (2004). Chronic thrombocytopenia of childhood: use of non-invasive methods in clinical evaluation. *European Journal of Haematology*, 72(4), 268–272. <http://doi.org/10.1111/j.1600-0609.2004.00215.x>

Yildirmak, Y., Yanikkaya-Demirel, G., Palanduz, A., & Kayaalp, N. (2005). Antiplatelet Antibodies and Their Correlation with Clinical Findings in Childhood Immune Thrombocytopenic Purpura. *Acta Haematologica*, 113(2), 109–112. <http://doi.org/10.1159/000083448>

Aref, S., Selim, T., Ibrahim, L., Abd-Elghaffar, H., & Ashery, R. E. (2009). Flow cytometry detection of platelets autoantibodies in children with idiopathic thrombocytopenic purpura. *Indian Journal of Hematology & Blood Transfusion : an Official Journal of Indian Society of Hematology and Blood Transfusion*, 25(3), 96–103. <http://doi.org/10.1007/s12288-009-0028-0>

Ho, W.-L., Lee, C.-C., Chen, C.-J., Lu, M.-Y., Hu, F.-C., Jou, S.-T., et al. (2012). Clinical features, prognostic factors, and their relationship with anti-platelet antibodies in children with immune thrombocytopenia. *Journal of Pediatric Hematology/Oncology*, 34(1), 6–12. <http://doi.org/10.1097/MPH.0b013e3182282548>

Badrawy, H., Elsayh, K. I., Zahran, A. M., El-Ghazali, M. H., H, B., K I, E., et al. (2013). Platelet antibodies, activated platelets and serum leptin in childhood immune thrombocytopenic purpura. *Acta Haematologica*, 130(4), 312–318. <http://doi.org/http://dx.doi.org/10.1159/000353384>

Hamidpour, M., Khalili, G., Tajic, N., Shamsian, B. B. S., Hamidpour, R., M, H., et al. (2014). Comparative of three methods (ELIZA, MAIPA and flow cytometry) to determine anti-platelet antibody in children with ITP. *American Journal of Blood Research*, 4(2), 86–92.

Nielsen, O. H., Tuckuviene, R., Nielsen, K. R., & Rosthøj, S. (2016). Flow cytometric measurement of platelet-associated immunoglobulin in children with newly diagnosed Immune Thrombocytopenia. *European Journal of Haematology*, 96(4), 397–403. <http://doi.org/10.1111/ejh.12605>

Fu, L., Cheng, Z., Gu, H., & Wu, R. (2018). Platelet-specific antibodies and differences in their expression in childhood immune thrombocytopenic purpura predicts clinical prognosis. *Pediatric Investigation*, 2(4), 230–235.

*Available tables in Online Supplement*

**Supplementary Table S1.** Search strategy for Pubmed and EMBASE searching.

**Supplementary Table S2.** Study characteristics of 40 included studies.

**Table S3.** Quantitative differences of anti-platelet antibody levels in ITP versus controls.

Author	Year	Immunoassay class	Diagnostic test	IgG class targeted	ITP Group	ITP (N)	Controls (N)	Average ITP	Average controls	Type controls	Measurement
<i>Direct immunoassays</i>											
Badrawy	2013	PA-Ig	flow PAIgG	IgG		40	40	41.8 ± 4.0**	9.5 ± 1.0	Healthy children	% IgG positive cells
Badrawy	2013	PA-Ig	PAIgM	IgM	Acute	40	40	32.5 ± 4.2**	7.2 ± 0.7	Healthy controls	% IgM positive cells
Fujisawa	1985	PA-Ig	Fab anti-Fab	IgG	Chronic	14	30	8675 ± 3298	1224 ± 476		ng Ig / 10 <sup>9</sup> platelets
Fujisawa	1985	PA-Ig	Fab anti-Fab	IgG		34		4380 ± 2216			
Fujisawa	1985	PA-Ig	Fab anti-Fab	IgG	Splenectomized, in remission	8		2096 ± 609			
Fujisawa	1985	PA-Ig	Fab anti-Fab	IgG	Splenectomized, no remission	4		4940 ± 2748			
Lightsey	1979	PA-Ig	Fab anti-Fab	IgG	Acute	7	11	12 552 ± 8875	1446 ± 580	Healthy children	ng Ig / 10 <sup>9</sup> platelets
Lightsey	1979	PA-Ig	Fab anti-Fab	IgG	Chronic	13		3956 ± 1090			
Luilken	1977	PA-Ig	Fab anti-Fab	IgG	Acute	6	70	range 5588 - 56 250	1231 ± 424	Healthy controls	ng Ig / 10 <sup>9</sup> platelets
Luilken	1977	PA-Ig	Fab anti-Fab	IgG	Chronic	12		4923 ± 3955			
Aref	2009	PA-Ig	PAIg total Ig	Total Ig	Acute	6	5	82.8 (71-92)	3.7 (0.9-6.2)	Matched controls	% Ig positive cells
Aref	2009	PA-Ig	PAIg total Ig	Total Ig	Chronic	7		45.2 (33.5 - 69.2)			
Aref	2009	PA-Ig	PAIgM (flow)	IgM	Acute	6	5	8.7 (2.1 - 71)	0.5 (0 - 2.9)	Matched controls	% IgM positive cells
Aref	2009	PA-Ig	PAIgM (flow)	IgM	Chronic	7		5 (3 - 19.1)			
Aref	2009	PA-Ig	PAIgA flow	IgA	Acute	6	5	2 (1 - 3.5)	0 (0-1.3)	Matched controls	% IgA positive cells
Aref	2009	PA-Ig	PAIgA flow	IgA	Chronic	7		1.5 (0.9 - 3.1)			
Aref	2009	PA-Ig	PAIgG flow	IgG	Acute	6	5	68 (19 - 80)	0.7 (0.9 - 4.5)	Matched controls	% IgG positive cells
Aref	2009	PA-Ig	PAIgG flow	IgG	Chronic	7		35.2 (25 - 63)			
Yildirmak	2005	PA-Ig	PAIgG flow	IgG	Acute	39	10	22.8 ± 27.9	2.8 ± 1.2	Healthy children	% IgG positive cells
Yildirmak	2005	PA-Ig	PAIgG flow	IgG	Chronic	31		24.8 ± 28.2			
Yildirmak	2005	PA-Ig	PAIgM flow	IgM	Acute	39	10	23.0 ± 23.4	2.2 ± 1.0	Healthy children	% IgM positive cells
Yildirmak	2005	PA-Ig	PAIgM flow	IgM	Chronic	31		8.6 ± 11.2			
<i>Indirect immunoassays</i>											
Fujisawa	1985	PA-Ig	PBIgG (indirect PAIgG)	IgG		14	6	7678 ± 2298	4124 ± 1124	Healthy controls	ng Ig / 10 <sup>9</sup> platelets

Average data are mean ± standard error or median (range).

\* Data abstracted from Graph.

\*\* Badrawy 2013 also presented results split by a prognostic score for spontaneous recovery (Edslev BJH 2007) but observed no differences. nd, not described.

**Supplementary Table S4.** Sensitivity of anti-platelet antibody testing in ITP for total Ig.

Author	Year	Immunoassay class	Test specification	Diagnostic test	Group	ITP (N)	Positive (n)	Negative (n)	Apparent sensitivity	95% CI
<i>Direct and indirect immunoassays for total Ig</i>										
van Leeuwen	1981	PIFT	direct	PIFT total-Ig	Acute	22	19	3	0.86	0.72; 1.00
van Leeuwen	1981	PIFT	direct		Chronic	48	41	7	0.85	0.75; 0.95
van Leeuwen	1981	PIFT	direct		Recurrent	10	5	5	0.50	0.19; 0.81
van Leeuwen	1981	PIFT	indirect	PIFT total Ig	Acute	22	11	11	0.50	0.29; 0.71
van Leeuwen	1981	PIFT	indirect		Chronic	48	17	31	0.35	0.22; 0.49
van Leeuwen	1981	PIFT	indirect		Recurrent	10	4	6	0.40	0.09; 0.70
Nieminen	1993	PIFT	unclear	PIFT		15	10	5	0.67	0.43; 0.91
Fu	2018	ELISA	indirect, GP IIb/IIIa	PakAuto	Acute	113	92	21	0.81	0.74; 0.89
Fu	2018	ELISA	indirect, GP IIb/IIIa		Chronic	18	11	7	0.61	0.39; 0.84
Fu	2018	ELISA	indirect, GP Ib/IX		Acute	113	54	59	0.48	0.39; 0.57
Fu	2018	ELISA	indirect, GP Ib/IX		Chronic	18	11	7	0.61	0.39; 0.84
Rejantie	2004	PIFT	direct	PIFT IgG/IgM/IgA		13	7	6	0.54	0.27; 0.81
Nielsen	2003	MAIPA	indirect****	MAIPA IgG/IgM (reported in aggregate)	Acute	37	15	22	0.41	0.25; 0.56
Nielsen	2003	MAIPA	indirect****		Chronic	16	1	15	0.06	0.00; 0.18
Ozsoylu	1991	Functional	indirect	Leukocyte phagocytosis		146	146	0	1.00	nd
Tate	1977	PA-Ig	indirect	PA-IgG/IgM/IgA Immunohistochemistry		10	10	0	1.00	nd
Movassaghi	1979	Functional	indirect	Platelet serotonin release		42	23	19	0.55	0.40; 0.70
Ho	2012	ELISA	indirect, GP IIb/IIIa	PAKPlus		25	9	16	0.36	0.17; 0.55
Ho	2012	ELISA	indirect, GP Ib/IX			25	3	22	0.12	0.00; 0.25
Ho	2012	ELISA	indirect, GP Ia/IIa			25	4	21	0.16	0.02; 0.30
Ho	2012	ELISA	indirect, GP IV			25	4	21	0.16	0.02; 0.30

PA-Ig, platelet associated immunoglobulin test. PIFT, platelet immunofluorescence test. MAIPA, monoclonal antibody immobilisation of platelet antigen assay. ELISA, enzyme-linked immunosorbent assay.

\*\*\*\* IgG and IgM were performed for GP IIb/IIIa, GP Ib/IX, GP Ia/IIa; but reported in aggregate.

nd, not determined.



**Supplementary Table S5.** Sensitivity of anti-platelet antibody testing in ITP for IgG isotype.

Author	Year	Immunoassay class	Test specification	Diagnostic test	Group	ITP (N)	Positive (n)	Negative (n)	Apparent sensitivity	95% CI
<i>Direct immunoassays</i>										
Taaning	1988	ELISA		PAIgG (micro-ELISA)	Acute	11	10	1	0.91	0.74; 1.00
Taaning	1988	ELISA			Chronic	5	5	0	1.00	nd
Cheung	1983	ELISA*		MSPIRA	Acute	26	22	4	0.85	0.71; 0.98
Cheung	1983	ELISA*			Chronic	22	22	0	1.00	nd
Imbach	1991	Immunobead	GP IIb/IIIa, GP Ib**	Immunobead		36	26	10	0.72	0.58; 0.87
Debre	1993	PA-Ig		PAIgG by RAI		10	8	2	0.8	0.55; 1.00
Ljung	1986	PA-Ig	Whole platelet	PAIgG on whole platelet		12	11	1	0.92	0.76; 1.00
Ljung	1986	PA-Ig	Lysate	PAIgG (lysed)		13	13	0	1.00	nd
Szal	1988	PA-Ig		PAIgG		13	9	4	0.69	0.44; 0.94
Blanchette	1985	PA-Ig		PAIgG by radial immunodiffusion	Acute	21	16	5	0.76	0.58; 0.94
Blanchette	1985	PA-Ig		PAIgG by radial immunodiffusion	Chronic	8	7	1	0.88	0.65; 1.00
Imbach	1985	PA-Ig		PAIgG by anti-IgG radioactivity		57	43	14	0.75	0.64; 0.87
Nielsen	2016	PA-Ig		flow PAIgG		68	30	38	0.44	0.32; 0.56
Aref	2009	PA-Ig			Acute	6	6	0	1.00	nd
Aref	2009	PA-Ig			Chronic	7	7	0	1.00	nd
Aref	2009	MAIPA	not specified	MAIPA IgG (Diamed kit)	Acute	6	5	1	0.83	0.54; 1.00
Aref	2009	MAIPA	not specified	MAIPA IgG (Diamed kit)	Chronic	7	7	0	1.00	nd
Iyori	1995	PIFT		PIFT IgG		29	22	7	0.76	0.60; 0.91
van Leeuwen	1981	PIFT		PIFT anti-IgG	Acute	16	9	7	0.56	0.32; 0.81
van Leeuwen	1981	PIFT			Chronic	38	38	0	1.00	nd
van Leeuwen	1981	PIFT			Recurrent	5	3	2	0.60	0.17; 1.00
Iyori	1995	PA-Ig	GP IIb/IIIa***			25	9	16	0.36	0.17; 0.55

Author	Year	Immunoassay class	Test specification	Diagnostic test	Group	ITP (N)	Positive (n)	Negative (n)	Apparent sensitivity	95% CI
<i>Indirect immunoassays</i>										
Biglino	1997 <sup>A</sup>	ELISA		EIA	Acute	32	11	21	0.34	0.18; 0.51
Biglino	1997 <sup>A</sup>	ELISA			Chronic	9	3	6	0.33	0.03; 0.64
Berchtold	1989	Immunoassay	GP IIb/IIIa	immunobead GP IIb/IIIa	Acute	15	4	11	0.27	0.04; 0.49
Berchtold	1989	Immunoassay	GP IIb/IIIa	immunobead GP IIb/IIIa	Chronic	24	14	10	0.58	0.39; 0.78
Berchtold	1989	Immunoassay	GP IIb/IIIa	immunobead GP IIb/IIIa	Acute	15	0	15	0.00	nd
Berchtold	1989	Immunoassay	GP IIb/IIIa	immunobead GP IIb/IIIa	Chronic	24	0	24	0.00	nd
Winiarski	1989	Immunoblot		IgG immunoblot		21	4	17	0.19	0.02; 0.36
Nieminen	1993	MAIPA	GP IIb/IIIa	MAIPA IgG GP IIb/IIIa		15	5	10	0.33	0.10; 0.57
Nieminen	1993	MAIPA	GP IIb/IIIa	MAIPA IgG GP IIb/IIIa		15	0	15	0.00	nd
Taub	1995	MAIPA	GP IIb/IIIa	MAIPA IgG GP IIb/IIIa	Acute	40	27	13	0.68	0.53; 0.82
Taub	1995	MAIPA	GP IIb/IIIa	MAIPA IgG GP IIb/IIIa	Chronic	21	13	8	0.62	0.41; 0.83
Aref	2009	MAIPA	not specified	MAIPA IgG GP IIb/IIIa	Acute	6	5	1	0.83	0.54; 1.00
Aref	2009	MAIPA	not specified		Chronic	7	7	0	1.00	nd
Tate	1980	PA-Ig				12	12	0	1.00	nd
Biglino	1997 <sup>A</sup>	PA-Ig		Platelet bound Ig reactive index (PAIgG by ProA-PAP)	Acute	33	13	20	0.39	0.23; 0.56
Biglino	1997 <sup>A</sup>	PA-Ig		SPRCA	Chronic	9	3	6	0.33	0.03; 0.64
Brink	1981	PIFT		PIFT-IgG		12	10	2	0.83	0.62; 1.00
Campana	1983	PIFT		PIFT		18	15	3	0.83	0.66; 1.00
Campana	1983	PIFT		Fab assay in PIFT		14	8	6	0.57	0.31; 0.83
Biglino	1997 <sup>A</sup>	PIFT	Eluate Fab	PIFT-IgG	Acute	41	14	27	0.34	0.20; 0.49
Biglino	1997 <sup>A</sup>	PIFT			Chronic	10	7	3	0.70	0.42; 0.98
Biglino	1997 <sup>B</sup>	MAIPA	GP IIb/IIIa	MAIPA IgG GP IIb/IIIa	Acute	49	19	30	0.39	0.25; 0.52
Biglino	1997 <sup>B</sup>	MAIPA	GP IIb/IIIa	MAIPA IgG GP IIb/IIIa	Chronic	25	8	17	0.32	0.14; 0.50
Biglino	1997 <sup>B</sup>	MAIPA	GP IIb/IIIa	MAIPA IgG GP IIb/IIIa	Acute	49	15	34	0.31	0.18; 0.44
Biglino	1997 <sup>B</sup>	MAIPA	GP IIb/IIIa	MAIPA IgG GP IIb/IIIa	Chronic	25	7	18	0.28	0.10; 0.46
Winiarski	1986	ELISA		ELISA IgG		21	10	11	0.48	0.26; 0.69
Walker	1961	PA-Ig		Agglutination platelets, sheep RBC	Acute	11	6	5	0.55	0.25; 0.84
Walker	1961	PA-Ig		Agglutination platelets, sheep RBC	Chronic	23	18	5	0.78	0.61; 0.95
Lusher	1966	PA-Ig		Platelet agglutination	Acute	43	15	28	0.35	0.21; 0.49
Lusher	1966	PA-Ig		Platelet agglutination	Chronic	11	5	6	0.45	0.16; 0.75
<i>Immunoassays of unclear type</i>										
Cines	1979	PA-Ig		PA-IgG		19	16	3	0.84	0.68; 1.00
Tag	2004	PIFT		PIFT IgG	Acute	23	18	5	0.78	0.61; 0.95
Tag	2004	PIFT		PIFT IgG	Chronic	26	21	5	0.81	0.66; 0.96

PA-Ig, platelet associated immunoglobulin test. PIFT, platelet immunofluorescence test. MAIPA, monoclonal antibody immobilisation of platelet antigen assay. ELISA, enzyme-linked immunosorbent assay. \* The employed assay involved radiochemistry instead of enzyme, but the test principle falls under the ELISA category. \*\*\* Results were reported only in aggregate. \*\*\*\*\* Results were also presented separately for patients who had acute or chronic disease courses (all were positive). nd, not determined.

**Supplementary Table S6.** Sensitivity of anti-platelet antibody testing in ITP for IgM isotype.

Author	Year	Immunoassay class	Diagnostic test	Group	ITP (N)	Positive (n)	Negative (n)	Apparent sensitivity	95% CI
<i>Direct immunoassays</i>									
Taaning	1988	ELISA	PAIgM ELISA	Acute	11	5	6	0.45	0.16; 0.75
Taaning	1988	ELISA		Chronic	5	4	1	0.80	0.45; 1.00
Szal	1988	PA-Ig	PAIgM		13	5	8	0.38	0.12; 0.65
Nielsen	2016	PA-Ig	PaIgM (flow)		68	43	25	0.63	0.52; 0.75
Iyori	1995	PIFT	PIFT - IgM		29	20	9	0.69	0.52; 0.86
van Leeuwen	1981	PIFT	PIFT anti-IgM	Acute	16	11	5	0.69	0.46; 0.91
van Leeuwen	1981	PIFT		Chronic	38	17	21	0.45	0.29; 0.61
van Leeuwen	1981	PIFT		Recurrent	5	3	2	0.60	0.17; 1.00
<i>Indirect immunoassays</i>									
Winiarski	1989	Immunoblot	IgM immunoblot		21	13	8	0.62	0.41; 0.83
Brink	1981	PIFT	PIFT-IgM		12	2	10	0.17	0.00; 0.38
Biglino	1997	PIFT	PIFT IgM	Acute	41	11	30	0.27	0.13; 0.40
Biglino	1997	PIFT		Chronic	10	2	8	0.20	0.00; 0.45
<i>Immunoassays of unclear type</i>									
Tag	2004	PIFT	PIFT IgM	Acute	23	9	14	0.39	0.19; 0.59
Tag	2004	PIFT	PIFT IgM	Chronic	26	4	22	0.15	0.02; 0.29

**Supplementary Table S7.** Sensitivity of anti-platelet antibody testing in ITP for IgA and unclear Ig target

Author	Year	Immunoassay class	Test specification	Diagnostic test	Type Test	Ig class targeted	ITP (N)	Positive (n)	Negative (n)	Apparent sensitivity
Hamidpour	2014	MAIPA	GP IIb/IIIa	MAIPA GP IIb/IIIa	indirect	unclear	38	18	20	0,47
Hamidpour	2015	MAIPA	GP Ib/IX	MAIPA GP Ib/IX	indirect	unclear	38	21	17	0,55
Hamidpour	2016	MAIPA	GP Ia/IIa	MAIPA GP Ia/IIa	indirect	unclear	38	10	28	0,26
Hamidpour	2017	PA-Ig	Whole platelet	Flow cytometry	indirect	unclear	38	24	14	0,63
Hamidpour	2018	ELISA	Lysate	ELISA whole platelet	direct	unclear	38	24	14	0,63
Hamidpour	2019	ELISA		ELISA platelet lysate	direct	unclear	38	20	18	0,53
Taaning	1988	ELISA		PAIgA ELISA	direct	IgA	17	4	13	0,24
van Leeuwen	1981	PIFT		PIFT IgA	direct	IgA	59	3	56	0,05
Brink	1981	PIFT		PIFT-IgA	indirect	IgA	12	1	11	0,08

PA-Ig, platelet associated immunoglobulin test. PIFT, platelet immunofluorescence test. PIFT-IgA, monoclonal antibody immobilisation of platelet antigen assay. ELISA, enzyme-linked im

