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Plenary Paper

CLINICAL TRIALS AND OBSERVATIONS

Intravenous immunoglobulin vs observation in childhood immune thrombocytopenia: a randomized controlled trial

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KEY POINTS

- **In children with newly diagnosed ITP, IVIg treatment at diagnosis does not result in a lower rate of chronic ITP.**
- **Upfront treatment with IVIg led to faster recovery and less severe bleeding events.**

Management of children with newly diagnosed immune thrombocytopenia (ITP) consists of careful observation or immunomodulatory treatment. Observational studies suggest a lower risk for chronic ITP in children after intravenous immunoglobulin (IVIg) treatment. In this multicenter randomized trial, children aged 3 months to 16 years with newly diagnosed ITP, platelet counts 20 × 10⁹/L or less, and mild to moderate bleeding were randomly assigned to receive either a single infusion of 0.8 g/kg IVIg or careful observation. Primary outcome was development of chronic ITP, which at the time of study initiation was defined as a platelet count lower than 150 × 10⁹/L after 6 months. Two hundred six children were allocated to receive IVIg (n = 102) or careful observation (n = 104). Chronic ITP occurred in 18.6% of the patients in the IVIg group and 28.9% in the observation group (relative risk [RR], 0.64; 95% confidence interval [CI], 0.38-1.08). Platelet counts lower than 100 × 10⁹/L at 12 months (current definition of chronic ITP) were observed in 10% of children in the IVIg group and 12% in the observation group (RR, 0.83; 95% CI, 0.38-1.84). Complete response rates in the first 3 months were significantly higher in the IVIg group. Immunoglobulin G Fc receptor IIb genetic variations were associated with early complete response in both groups. Grade 4 to 5 bleeding occurred in 9% of the patients in the observation group vs 1% in the IVIg group. This trial was registered at www.trialregister.nl as NTR 1563. (*Blood*. 2018;132(9):883-891)

Introduction

Childhood immune thrombocytopenia (ITP) is an immune-mediated disease characterized by an isolated low platelet count (peripheral blood platelet count <100 × 10⁹/L) in the absence of other causes that are associated with thrombocytopenia.[1] The majority of patients present with acute development of purpura and bruising, often after a mild viral infection and with platelet counts below 20 × 10⁹/L[2-5] Most children recover within 3 to 12 months. Chronic ITP, which until 2009 was defined as a platelet count lower than 150 × 10⁹/L at 6 months after diagnosis, is reported to occur in about 20% to 25% of children with ITP.[1-5] Since 2009, chronic ITP has been defined as a platelet count lower than 100 × 10⁹/L at 12 months after diagnosis,[1] and the rate of occurrence is not yet fully determined, but is reported as 28%, based on the data in the Intercontinental Cooperative ITP Study Group.[6]

Pathogenesis of ITP is complex, but many patients have platelet-specific autoantibodies leading to accelerated clearance of opsonized platelets by Fc-γ receptor (FcγR) bearing phagocytes, particularly in the spleen.[7,8] Both activating FcγRs as well as the inhibitory FcγRIIb are subject to genetic variation that affects their function. Genetic variation of *FCGR2B* is associated with response to IVIg treatment in patients with Kawasaki disease,[9] but this has not yet been assessed in children with ITP.[10] *FCGR2B* polymorphism has also been reported to be involved in the course of ITP.[10]

Management of children with newly diagnosed ITP consists of careful observation regardless of platelet count in case of skin bleeding only. Severe bleeding, occurring in only 3% to 5% of children,[11] requires treatment with corticosteroids, intravenous immunoglobulin (IVIg), or anti-Rh-D immunoglobulin, either alone or in combination.[12,13]

Treatment with IVIg has proved to be safe and effective in children with ITP. It is generally well tolerated with transient headaches as the most common adverse effect. Several dosing regimens have been used in the treatment of ITP, varying

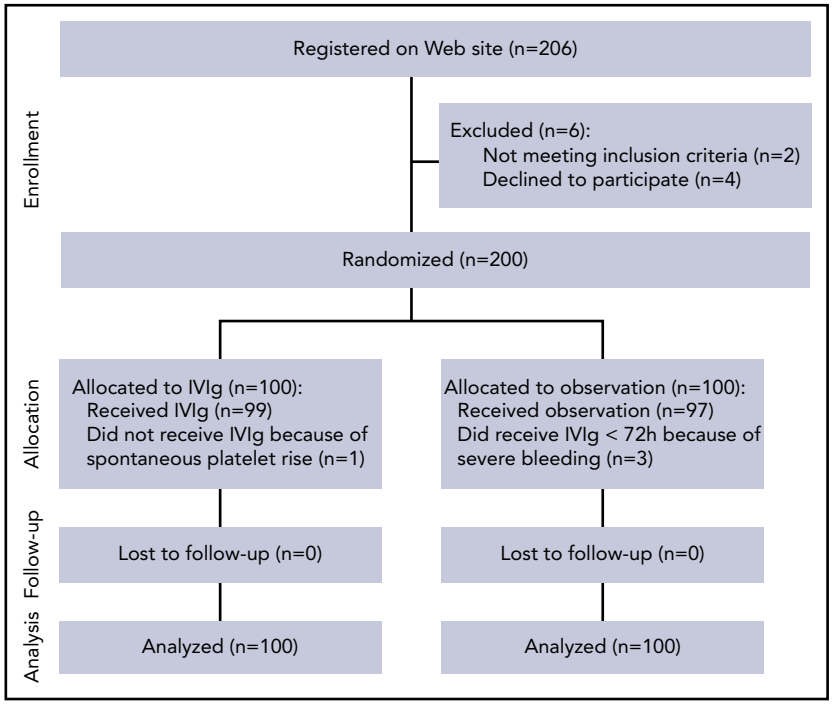

Figure 1. Flow diagram. Randomization and follow-up of the TIKI trial patients. Two hundred six patients were registered at the Web site. Four patients declined to participate, 1 patient appeared to have chronic ITP, and 1 patient appeared to have Evans' syndrome. Of 200 eligible patients, 100 patients were randomly assigned to receive IVIg and 100 to observation. One patient did not receive IVIg because of quick spontaneous recovery of platelet counts, and 3 patients in the observation group did receive IVIg within 72 hours after inclusion because of grade 4 mucosal bleeding. No patients were lost to follow-up before they had recovered.

between 0.4 and 1.0 g/kg body weight per day for 1 to 5 days, but in the most recent American Society of Hematology guideline, a single dose of 0.8 to 1.0 g/kg is recommended.[13] Platelet counts rise within 48 hours in 70% to 80% of patients, and this effect lasts for 2 to 4 weeks.[14-18] Observational studies suggested a lower incidence of chronic ITP in children who were treated with IVIg.[10,19] This was confirmed by a recent meta-analysis,[20] but randomized studies are lacking. As chronic ITP may have a huge effect on children and their families, because of the bleeding risk, fatigue, and other issues hampering health-related quality of life, being able to prevent chronic disease would be of major importance.

To address the question whether IVIg treatment at onset can prevent a chronic course of the disease in children with ITP, the multicenter randomized controlled trial Treatment With or Without IVIg for Kids With ITP (TIKI) was performed.

Methods

Trial design

The TIKI trial was a phase 3 multicenter, stratified, open, parallel-group study and was open in 60 of 90 hospitals (pediatric departments of 7 university hospitals and of 53 general hospitals) in The Netherlands. The incidence of pediatric ITP in The Netherlands is approximately 100 to 150 new patients each year.

The primary end point was the efficacy of a single dose of IVIg in reducing the rate of chronic ITP in children with newly diagnosed ITP. According to Dutch legislation for multicenter trials, the accredited Institutional Review Board of the University Medical Center Utrecht provided central Institutional Review Board approval. After this approval, the boards of all participating centers gave permission for execution of the study in their own institutions. The principal investigators had full access to the data. Data analyses were performed by K.M.J.H.-P., M.C.A.B., and M.d.H. and were independently verified by the Julius Support for Research and Trials Centre for both accuracy and adherence to the protocol. The study was registered in the Dutch Trial register (NTR 1563) and conducted in accordance with Good Clinical Practice guidelines.

Participants

Children aged 3 months to 16 years with newly diagnosed ITP, a platelet count of 20 $\times$ 10^9/L or less and with mild to moderate bleeding (grade 1-3 on the adapted Buchanan bleeding score)[21] were eligible for inclusion in the TIKI trial. Diagnosis of ITP was made according to internationally accepted guidelines.[12,13] Patients were excluded if they had severe bleeding at diagnosis (grade 4-5), received immunomodulatory drugs within 1 month of diagnosis, or suffered from conditions that formed a contraindication for IVIg (such as renal failure or IgA deficiency), or if comprehension of the Dutch language was insufficient to give informed consent. All parents and patients aged 12 years and older gave written informed consent before inclusion.

Interventions

Within 72 hours after diagnosis, patients were randomly assigned to receive either a single dose of 0.8 g/kg IVIg (Nanogam, Sanquin, The Netherlands) or careful observation and immunomodulatory treatment only in case of severe bleeding.

In some centers, it is customary to give steroids along with IVIg to prevent headaches. This is not the case in The Netherlands, so no premedication was administered in our trial.

Data collection

At diagnosis, demographic data were collected. At diagnosis, after 1 week, 1 month, 3 months, 6 months, and 12 months, health-related quality of life (HRQoL) questionnaires (PedsQL

Table 1. Baseline characteristics

	IVIg (n = 100)	Observation (n = 100)
Male	54 (54.0)	55 (55.0)
Age, y	3.6 (0.3-16.1)	4.5 (0.5-16.6)
0-1 y	3 (3)	5 (5)
1-10 y	83 (83)	71 (71)
>10 y	14 (14)	24 (24)
Duration of symptoms, d	3 (1-60)*	3 (0-60)*
Platelet count at diagnosis, $\times 10^9$/L	6 (0-20)	5 (0-20)
Preceding infection	56 (56.6)	51 (52.0)
Preceding vaccination	3 (3.0)	5 (5.1)
Mucosal bleeding, grade 3	38 (38.4)	42 (42.4)
Leukocyte count, $\times 10^9$/L	8.7 (4.5-17.5)	8.0 (4.0-18.6)
Lymphocyte count, $\times 10^9$/L	4.0 (1.3-12.6)	3.7 (0.9-14.0)
FCGR2B†		
232II	74	64
232IT	22	17
232TT	3	0

Data are numbers (%) or medians (minimum-maximum) unless otherwise specified. Duration of symptoms denotes the duration of symptoms at the moment of diagnosis of ITP. Preceding infection: an infection within 28 days before diagnosis. Preceding vaccination: a vaccination within 28 days before diagnosis. Because of a small amount of missing data, percentages do not always correspond with the total number of patients.

FCGR, IgG-Fc receptor.

*In both groups there was 1 patient with a longer duration of bleeding symptoms, 60 d, before the diagnosis of ITP was made. Because symptoms were mild and not necessarily explained by ITP, combined with the fact that according to current definitions they were still categorized as newly diagnosed ITP, both patients were not excluded from the study.

†Blood samples for multiplex ligation probe amplification were available for 99/100 patients in the IVIg group and for 81/100 patients in the observation group.

and KIT) were filled out and laboratory studies were performed. At diagnosis, blood samples were taken for DNA isolation to perform multiplex ligation-dependent probe amplification to detect genetic variations in FcγR genes *FCGR2A*, *FCGR2B*, *FCGR2C*, *FCGR3A*, and *FCGR3B*, as previously described.[22] Data regarding bleeding severity, treatment, laboratory results, and HRQoL were entered in a web-based case record form.

Outcomes

The primary end point was the development of chronic ITP. At the start of the study, chronic disease was defined as a platelet count lower than 150 × 10^9/L at 6 months after diagnosis. With the international accepted change in definition of chronic ITP published by Rodeghiero in 2009,[1] we decided to report platelet counts lower than 100 × 10^9/L at 12 months as well. Because the study protocol already included study visits at 6 and 12 months, study procedures were not changed.

Secondary outcome parameters were safety and efficacy of both treatment groups, recovery rates, the influence of treatment on bleeding score, platelet counts, and HRQoL, as well as biological factors involved in response to IVIg and recovery. HRQoL data of this study have been published elsewhere,[23] and hence will not be reported in detail here. In summary, HRQoL was not associated with bleeding severity or treatment modality, but only with clinical course of the disease.

Definitions of complete response (platelet count >100 × 10^9/L) and partial response (platelet count ≥30 × 10^9/L and at least 2-fold increase of the baseline count) are used as published by Rodeghiero et al.[1] Because recurrence of childhood ITP is rare, patients who showed complete response on 2 or more consecutive occasions were considered to remain complete responders at later points if platelet counts were not available.

Sample size

A sample size of 100 patients per group was calculated to detect a difference of 15% in development of chronic disease (10% vs 25%, defined as a platelet count <150 × 10^9/L at 6 months after diagnosis),[10] with a 2-sided 5% significance and a power of 80%.

Interim analyses and stopping rules

An independent data and safety monitoring board reviewed the efficacy and safety data yearly. Criteria for temporarily putting the study on hold were excess of occurrence of grade 4 and 5 bleedings in any of the treatment groups, as well as any unexpected severe adverse effect that could be related to the use of IVIg.

Randomization

After receiving informed consent, pediatricians registered eligible patients on the study Web site. Web-based randomization was performed using a computer-generated randomization list ensuring allocation concealment, and was stratified by platelet counts at diagnosis (<10 × 10^9/L and 10-20 × 10^9/L), to ensure equal distribution within both treatment groups. The allocated group was subsequently shown on the study Web site.

Blinding

Because there was no placebo, patients and physicians were aware of the allocated treatment.

Statistical methods

We used χ^2 tests to compare categorical variables. In case of expected cell count below 5, Fisher Exact Tests were used. To compare nonparametric continuous variables, Mann-Whitney *U* tests were performed. For the analysis of the primary and secondary outcome parameters, we calculated relative risks with 95% confidence intervals (95% CIs) and corresponding *P* values. Analyses were performed by the intention-to-treat principle. Logistic regression was used to perform multivariable analysis of predictors of response. SPSS version 21 for Macintosh was used to perform analyses.

Results

Enrolment and follow-up

Randomization and follow-up of participants are shown in Figure 1. From May 2009 through April 2015, 206 patients from 48 different sites were registered on the Web site. Two were ineligible and 4 declined participation. Twelve sites did not enroll patients. Two hundred patients were included in the intention-to-treat analysis: 100 patients were allocated to receive

Table 2. Response rates at different time points

	IVIg (n = 100)	Observation (n = 100)	RR (95% CI)	*P* value
Chronic ITP former definition	n = 89 [97]	n = 87 [97]		
Platelet count <150 × 10^9/L at 6 mo	18 (18.6)	28 (28.9)	0.64 (0.38-1.08)	.09
Chronic ITP current definition	n = 87 [100]	n = 85 [100]		
Platelet count <100 × 10^9/L at 12 mo	10 (10)	12 (12)	0.83 (0.38-1.84)	.65
Response at 1 wk	n = 99 [99]	n = 99 [99]		
Overall response	77 (77.8)	40 (40.4)	1.93 (1.48-2.50)	<.001
Complete response	68 (68.7)	23 (23.0)	2.99 (2.04-4.39)	<.001
Response at 1 mo	n = 99 [99]	n = 98*[99]		
Overall response	82 (82.8)	61 (61.6)	1.34 (1.12-1.61)	.001
Complete response	64 (64.6)	41 (41.4)	1.56 (1.18-2.06)	.001
Response at 3 mo	n = 98 [99]	n = 94 [99]		
Overall response	87 (87.0)	77 (78.6)	1.11 (0.97-1.26)	.12
Complete response	81 (81.0)	64 (65.3)	1.24 (1.04-1.47)	.01
Response at 6 mo	n = 89 [100]	n = 87 [100]		
Overall response	92 (92.0)	88 (88.0)	1.04 (0.95-1.15)	.35
Complete response	84 (84.0)	78 (78.0)	1.07 (0.94-1.23)	.28
Response at 12 mo	n = 87 [100]	n = 85 [100]		
Overall response	96 (96.0)	96 (96.0)	1.00 (0.95-1.06)	1.00
Complete response	90 (90.0)	88 (88.0)	1.02 (0.93-1.13)	.65

Response data are numbers (%). Overall response is defined as platelet counts 30 × 10^9/L or more and more than 2-fold increase of baseline counts. Complete response is defined as platelet counts 100 × 10^9/L or more. In every gray row, the first number (n) is the number of patients with available platelet numbers; the second number (in brackets) represents the number of patients with available response data when former platelet counts were also taken into account in the way described in Methods. At 6 mo, for 3 patients in both groups, earlier platelet counts were between 100 and 150 × 10^9/L, so for these patients, only response data could be reported and no data regarding chronic ITP according to the former definition.

RR reported as the risk for the outcome in the IVIg group compared with the risk in the observation group.

*For 1 patient only a description of the platelet count ("fully recovered") could be retrieved from the patient file.

IVIg and 100 to receive careful observation. No participants were lost to follow-up before they had reached complete response.

Baseline characteristics of participants

Baseline clinical and laboratory characteristics of the participants are shown in Table 1. Distributions of all characteristics were similar in both groups.

End points

Development of chronic ITP Chronic ITP, defined as a platelet count lower than 150 × 10^9/L 6 months after diagnosis, occurred in 18 (18.6%) of 97 children with available data in the IVIg group and in 28 (28.9%) of 97 children with available data in the observation group (relative risk [RR], 0.64; 95% CI, 0.38-1.08; Table 2).

When using the current definition, a platelet count lower than 100 × 10^9/L at 12 months, chronic ITP occurred in 10 (10%) of 100 children in the IVIg group and in 12 (12%) of 100 children in the observation group (RR, 0.83; 95% CI, 0.38-1.84; Table 2).

Subgroup analyses All infants (n = 8) showed complete response at 6 months. In children aged 1 to 10 years (n = 154), complete response rates were 82.5% and 89.0% at 6 and 12 months, respectively. In children aged 10 years and older (n = 38), complete response rates at 6 and 12 months were 71.1% and 86.8%, respectively. Differences in the response rates between the IVIg group and observation group in all age groups were not statistically significant.

Secondary end points Response rates at all different points are displayed in Table 2. At 1 week and 1 month, both overall and complete response rates in the IVIg group were significantly higher than in the observation group. At 3 months, only the complete response rate was significantly different. From 6 months on, there was no statistically significant difference

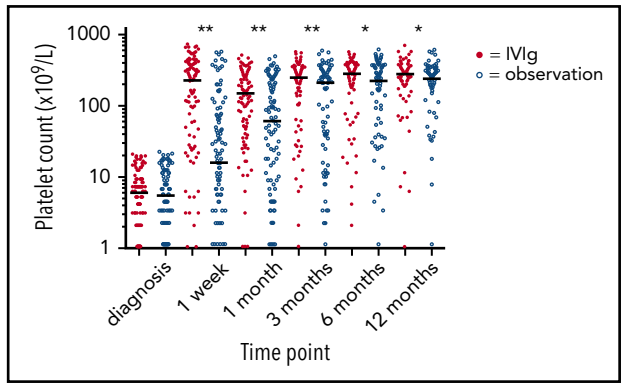

Figure 2. Platelet counts at several points for both study groups. Platelet counts are represented as dots on a logarithmic scale. The horizontal lines represent median platelet counts. **$P < .01$; *$P < .05$.

Table 3. Baseline characteristics and complete response at 1 week in both groups

	IVIg group			Observation group		
	Complete response at 1 wk (n = 68)	No complete response at 1 wk (n = 31)	*P* value	Complete response at 1 wk (n = 23)	No complete response at 1 wk (n = 77*)	*P* value
Male	35 (51.5)	18 (58.1)	.54	16 (69.6)	39 (50.6)	.11
Age at diagnosis, y	4.3 (0.3-16.1)	3.5 (1.1-14.2)	.44	3.9 (0.9-16.6)	4.8 (0.5-16.5)	.44
Duration symptoms at diagnosis, d	3 (1-60)	4 (1-30)	.17	1.5 (1-14)	5 (0-60)	<.001
Platelet count at diagnosis, $\times 10^9$/L	6 (0-20)	5 (1-19)	.13	7 (1-20)	5 (0-19)	.74
Preceding infection	42 (62.7)	13 (41.9)	.05	18 (81.8)	33 (43.4)	.002
Preceding vaccination	1 (1.5)	2 (6.5)	.23	1 (4.5)	4 (5.3)	1.00
Mucosal bleeding, grade 3	26 (38.8)	12 (38.7)	.99	10 (45.5)	32 (41.6)	.74
Leukocyte count, $\times 10^9$/L	8.7 (4.5-17.5)	8.1 (4.9-15.5)	.49	7.9 (4.4-18.3)	8.0 (4.0-18.6)	.86
Lymphocyte count, $\times 10^9$/L	3.9 (1.3-12.6)	4.5 (1.4-9.1)	.80	3.9 (1.3-14.0)	3.5 (0.9-11.0)	.74
FCGR2B†			.03			.02
232II	52 (77.6)	21 (67.7)		17 (100)	47 (73.4)	
232IT	15 (22.4)	7 (22.6)		0 (0)	17 (26.6)	
232TT	0	3 (9.7)		0 (0)	0 (0)	

Data are numbers (%) or medians (lower-upper limit) unless otherwise specified. Duration of symptoms denotes the duration of symptoms at the moment of diagnosis of ITP. Preceding infection: an infection within 28 d before diagnosis. Preceding vaccination: a vaccination within 28 d before diagnosis.

FCGR, IgG-Fc receptor.

*For 1 patient in the observation group at 1 wk no exact platelet count was available but based on a platelet count of 38 $\times$ 10^9/L at 1 mo a count <100 $\times$ 10^9/L was assumed.

†Blood samples for multiplex ligation probe amplification were available for 98/99 patients in the IVIg group with available response data and for 81/100 patients in the observation group.

between the 2 groups. Median platelet counts at all points for both study groups are depicted in Figure 2.

We compared baseline characteristics between patients who did and did not have a complete response after 1 week for both groups (Table 3). The rationale for reporting data for the observation group, as well, is that some baseline characteristics may be associated with early recovery anyhow, regardless of IVIg treatment. Complete response to IVIg at 1 week was seen in 68/99 patients (68.7%). All complete responders to IVIg displayed the *FCGR2B-232I* allele. None of the 3 patients homozygous for the *FCGR2B-232T* allele responded. No other statistically significant differences were found. In the observation group, a higher rate of preceding infection and a shorter duration of symptoms before diagnosis were observed in children who showed complete response after 1 week. All complete responders in the observation group possessed a homozygous *FCGR2B-232II* genotype.

Baseline clinical and laboratory parameters in patients with and without complete response at 12 months are shown in Table 4. Because complete response rates at 12 months did not differ between the IVIg group and observation group, data from all patients are reported together. Patients who showed complete response at 12 months were younger and had a shorter duration of symptoms before diagnosis, a higher lymphocyte count at diagnosis, and more often mucosal bleeding at diagnosis. In multivariable analysis with inclusion of the 2 most clinically relevant statistically different parameters, duration of symptoms, and presence of mucosal bleeding, only duration of symptoms remained statistically significant (data not shown).

In patients randomly assigned to IVIg who showed complete response at 1 week (n = 68), the rate of chronic ITP was significantly lower than in those children who did not have a complete response at 1 week (4/68 [5.9%] vs 6/31 [19.4%]; $P = .04$). This difference remained statistically significant after correction for duration of symptoms and bleeding score, both associated with complete recovery at 12 months (data not shown)

Safety and adverse events Severe adverse events are displayed in Table 5. In the observation group, 18 admissions resulting from bleeding were reported in 13 different patients. Ten of these admissions in 9 different patients were because of grade 4 to 5 bleeding, of which 8 admissions occurred within the first month of diagnosis. All grade 4 to 5 bleeding events, mainly epistaxis and menorrhagia, were transient and patients recovered completely after medical intervention within a few days. One child in the observation group developed a spontaneous intracranial hemorrhage in the left parietal lobe 16 days after diagnosis. She recovered quickly and completely after medical intervention with methylprednisolone, IVIg, and platelet transfusions. Seven of 8 patients who were admitted because of grade 4 to 5 bleeding in the first month after diagnosis, including the patient with intracranial hemorrhage, experienced grade 3 bleeding at diagnosis (1 of 42 patients with grade 3 bleeding at diagnosis and 1 of 58 without grade 3 bleeding). In the IVIg group, there were 2 reported severe adverse events because of

Table 4. Baseline characteristics and complete response at 12 mo

	Complete response 12 mo (n = 178)	No complete response 12 mo (n = 22)	*P* value
Male	98 (55.1)	11 (50.0)	.65
Age at diagnosis, y	3.8 (0.3-16.5)	6.6 (1.3-16.6)	.04
Duration of symptoms, d	3 (0-60)	7 (1-60)	<.001
Platelet count at diagnosis, $\times 10^9$/L	6 (0-20)	6 (1-19)	.73
Preceding infection	97 (55.1)	10 (47.6)	.51
Preceding vaccination	7 (4.0)	1 (4.8)	.60
Mucosal bleeding, grade 3	76 (43.2)	4 (18.2)	.02
Leukocyte count, $\times 10^9$/L	8.4 (4.4-18.6)	7.3 (4.0-11.0)	.12
Lymphocyte count, $\times 10^9$/L	4.0 (1.1-14.0)	2.9 (0.9-7.8)	.04
IVIg treatment	90 (50.6)	10 (45.5)	.65
FCGR2B*			.52
232II	123 (77.4)	15 (71.4)	
232IT	33 (20.8)	6 (28.6)	
232TT	3 (1.9)	0 (0)	

Data are numbers (%) or medians (lower-upper limit) unless otherwise specified. Duration of symptoms denotes the duration of symptoms at the moment of diagnosis of ITP. Preceding infection: an infection within 28 days before diagnosis. Preceding vaccination: a vaccination within 28 days before diagnosis.

*Blood samples for multiplex ligation probe amplification were available for 159/178 with complete response at 12 months and for 21/22 patients without complete response.

bleeding between 3 and 6 months after diagnosis concerning the same patient. There were no deaths during the study period.

In addition to the reported severe adverse events, no patients in the IVIg group and 3 patients in the observation group received rescue medication during the first month after diagnosis (prednisone in 2 patients and IVIg in 1 patient) because of bleeding symptoms. After the first month, 2 patients in the IVIg group received additional IVIg and 1 received prednisone. In the observation group, 3 patients received IVIg and 1 received prednisone after the first month.

Four patients in the IVIg group had a readmission or prolonged admission because of nausea and vomiting (n = 4) with (n = 2) or without (n = 2) headache after IVIg administration; none of them needed computer tomography of the brain to rule out bleeding. One patient in the IVIg group suffered from an allergic reaction during IVIg administration that warranted prolonged admission. One or more milder adverse effects of IVIg were reported in 14 patients, of whom 8 reported headache, 6 nausea, 4 fever, 2 malaise, and 1 skin rash.

Discussion

In this phase 3 multicenter randomized controlled trial evaluating the efficacy of IVIg treatment vs careful observation in children aged 3 months to 16 years with newly diagnosed ITP, the primary end point (the rate of chronic ITP) did not differ statistically significantly between the IVIg group and the observation group. We observed a rate of chronic ITP after 1 year of 11%, which is substantially lower than the 20% to 25% of patients assumed until now.[1-6] Genetic variations of *FCGR2B* were associated with response to IVIg within 1 week, as well as with recovery of platelet counts above 100 $\times$ 10^9/L within 1 week in untreated patients.

Although there was a trend toward a lower rate of chronic ITP in the IVIg group when applying the former definition of chronic ITP (a platelet count <150 $\times$ 10^9/L at 6 months), we did not find a difference in the occurrence of chronic ITP at 12 months. This finding is in contrast with the studies of Bruin,[10] Tamminga,[19] and our own systematic review.[20] Previous studies were all nonrandomized, and confounders may have influenced these results. For example, younger children and children with mucosal bleeding will generally more frequently be treated with IVIg than children with skin bleeding only or with an older age. However, mucosal bleeding and a younger age are also known as characteristics associated with a lower frequency of developing chronic ITP.

The observed rate of chronic ITP after 1 year of 11% is substantially lower than historical data,[1-5] and lower compared with the 28% reported by the Intercontinental Cooperative ITP Study Group.[6] In accordance with the literature,[24] also in our trial, a high recovery rate between 6 and 12 months after diagnosis was observed: 54% of children with a platelet count lower than 150 $\times$ 10^9/L and 45% of children with a platelet count lower than 100 $\times$ 10^9/L at 6 months showed complete response (platelet count >100 $\times$ 10^9/L) at 12 months, partly explaining the lower rate of chronic ITP observed in our trial when applying the current definition of chronic ITP. Another explanation can be that our study setting allowed for enrollment of a wide range of pediatric patients with ITP, whereas other studies were limited to patients in academic hospitals.

Table 5. Severe adverse events

	IVIg (n = 100)	Observation (n = 100)
Bleeding events	n = 1	n = 13
Grade 2/3 bleeding, observation only	0	5
Grade 3 bleeding, treatment instituted*	1	3†
Grade 4/5 bleeding, necessitating treatment	1‡	10¶
Adverse reactions treatment	n = 5	n = 0
Allergic reactions	1	0
Other (nausea, vomiting, headache)	4	0
Other severe adverse events	n = 4	n = 6
Observation after mild traumatic head injury	3	7§
Infections	1	3‖

Severe adverse events included events that occurred from the day of enrollment until the last study visit at 12 months.

The numbers in gray rows are numbers of patients; the numbers in white rows are numbers of reported events. A patient could have more than 1 event, and each patient could be counted in more than 1 category.

*Treatment not mandatory but based on wish of parents or patients/decision of treating pediatrician.

†One patient is also listed in this table for clinical observation because of grade 3 bleeding.

‡This patient was also treated with methylprednisolone for grade 3 bleeding, as listed in this table.

¶Two times in 1 patient; 3 other patients in this group are also listed for grade 2/3 bleeding for which clinical observation was instituted.

§Four times in 1 patient.

‖Two times in 1 patient.

We observed a high rate of early complete response in both groups in case of the homozygous *FCGR2B*232I* genotype and a lack of response to IVIg in the small number of patients with the homozygous *FCGR2B*232T* genotype. It has been demonstrated that the *FCGR2B*232I* variant enhances inhibition of activating IgG-Fc receptors, possibly influencing clearance of antibody-coated platelets and modulates activation of B cells.[25] Our findings may indicate a possible mechanism of action of IVIg via FcγR IIb signaling,[26,27] as well as a genotype associated with quick recovery in general. These findings may help in guiding management decisions, provided that results of *FCGR* genotyping are available soon after diagnosis, which is, at least in The Netherlands, feasible.

The remarkably higher rate of chronic ITP in children who did not show complete response to IVIg after 1 week, formerly also reported by Imbach et al,[28] may indicate that children at risk of development of chronic disease already carry this risk early on in the course of the disease. In these children, rebalancing the immune system may be more difficult, perhaps because of a strong T-cell response already present at diagnosis, as suggested by some authors.[7] The absence of response to IVIg may serve as a diagnostic tool to identify this subgroup with a higher risk of developing chronic disease.

Our study is the first randomized trial focusing on the effect of treatment with IVIg on development of chronic ITP and the largest randomized controlled trial in children with ITP, including an observation group conducted so far. There are some limitations. On the basis of the incidence of pediatric ITP in The Netherlands (100-150 patients/y), we enrolled approximately 25% to 30% of all children with newly diagnosed ITP during the study period. One explanation is that the TIKI trial was finally conducted in 60 of 90 Dutch hospitals, and it took more than 3 years before all centers were able to participate. Twelve participating centers did not enroll any patients. However, most of these centers stated that they did not encounter any eligible patients. In addition, approximately 15% to 25% of children with ITP present with a platelet count higher than 20×10^9/L,[2,3,10] whereas our study only included children with a platelet count 20×10^9/L or lower. Patients with bleeding grade 4 to 5 at diagnosis could not be included as well. When taking these aspects into account, we included 60% of approximately 310 to 350 eligible patients. On the basis of our inclusion criteria, our results may not be applicable for selected groups, such as patients with a high bleeding tendency or with an insidious clinical course and higher platelet counts at diagnosis.

According to our national guidelines that allow for careful observation in case of mucosal bleeding, we also enrolled children presenting with grade 3 bleeding in our study. The inclusion of children with grade 3 bleeding at diagnosis might explain the higher rate of grade 4 to 5 bleeding in the observation group compared with the rate previously reported by Neunert et al,[11] as 7 of 8 patients who developed grade 4 to 5 bleeding events during the first month were noted to have grade 3 bleeding at diagnosis. In contrast, 35 of 42 children in the observation group with grade 3 bleeding at diagnosis did not develop severe bleeding during the first month.

Although IVIg treatment may increase platelet counts in the majority of children with newly diagnosed ITP, risks, costs, and benefits of treatment should be carefully weighed. The vast majority of children in the observation group did not have serious bleeding events. One hundred patients would have to be treated with IVIg to prevent 8 severe bleeding events (including 1 intracranial hemorrhage that is life-threatening) in the first month after diagnosis (number needed to treat, 13; 95% CI, 7.5-37.3), but by doing so, 5 admissions would be caused as a result of the severe adverse effects of IVIg. Furthermore, several reports of children with ITP show that intracranial hemorrhage often develops despite therapy.[11,29-32] In our opinion, management with careful observation is safe in the vast majority of children with newly diagnosed ITP with bleeding grades below 4, provided that emergency care as well as rescue medication is available 24/7 and within acceptable traveling distance. For patients with grade 3 bleeding score (mild mucosal bleeding), the results of our study provide valuable information to counsel parents and patients to make an informed decision regarding treatment. For patients who may benefit from higher platelet counts during the first weeks after diagnosis, for example, toddlers with a higher tendency to fall and bump or pubertal girls with a risk for menorrhagia, treatment with IVIg can be offered to temporarily increase platelet counts.

The risk of bleeding is not only determined by platelet counts and clinical characteristics such as age, sex, and behavior, but also by platelet function. There is some evidence that platelet function tests that can be performed in patients with low platelet counts correlate well with bleeding tendency in children with ITP.[33,34] To tailor IVIg treatment to children with a higher bleeding tendency, further research is needed.

The results of our randomized TIKI trial can be used to update international guidelines on management of pediatric ITP, but can also be used to counsel patients and parents regarding risks and benefits of therapy and to offer support for communities and countries that cannot afford expensive treatments such as IVIg.

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Authorship

Contribution: K.M.J.H.-P. substantially contributed to the conception and design of the study, the acquisition of data, and interpretation of data and wrote the manuscript; C.S.P.M.U. was involved in the design of the study, interpreted data, and revised the manuscript critically; L.P. was involved in the conception of the study and revised the manuscript critically; R.Y.J.T., F.J.S., N.L.v.W., J.W., G.V., and A.G.L. substantially contributed to the acquisition of data and critically revised the manuscript; M.d.H. substantially contributed to the conception and design of the study, the acquisition of data, and interpretation of data and wrote the manuscript; and M.C.A.B. substantially contributed to the conception and design of the study, the acquisition of data, and interpretation of data and wrote the manuscript.

Conflict-of-interest disclosure: L.P. and M.d.H. are employed at Sanquin Blood Supply, manufacturer of IVIg. The remaining authors declare no competing financial interests.

A complete list of investigators in the Therapy with or without IVIg for Kids with acute ITP (TIKI) trial appears in the online appendix.

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Footnotes

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The online version of this article contains a data supplement.

There is a *Blood* Commentary on this article in this issue.

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REFERENCES

1. Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. 2009;113(11):2386-2393.
2. Kühne T, Buchanan GR, Zimmerman S, et al; Intercontinental Childhood ITP Study Group. A prospective comparative study of 2540 infants and children with newly diagnosed idiopathic thrombocytopenic purpura (ITP) from the Intercontinental Childhood ITP Study Group. *J Pediatr*. 2003;143(5):605-608.
3. Rosthøj S, Hedlund-Treutiger I, Rajantie J, et al; NOPHO ITP Working Group. Duration and morbidity of newly diagnosed idiopathic thrombocytopenic purpura in children: A prospective Nordic study of an unselected cohort. *J Pediatr*. 2003;143(3):302-307.
4. Watts RG. Idiopathic thrombocytopenic purpura: a 10-year natural history study at the childrens hospital of alabama. *Clin Pediatr (Phila)*. 2004;43(8):691-702.
5. Donato H, Picón A, Martinez M, et al. Demographic data, natural history, and prognostic factors of idiopathic thrombocytopenic purpura in children: a multicentered study from Argentina. *Pediatr Blood Cancer*. 2009;52(4):491-496.
6. Neunert CE, Buchanan GR, Imbach P, et al; Intercontinental Cooperative ITP Study Group Registry II Participants. Bleeding manifestations and management of children with persistent and chronic immune thrombocytopenia: data from the Intercontinental Cooperative ITP Study Group (ICIS). *Blood*. 2013;121(22):4457-4462.
7. Cines DB, Cuker A, Semple JW. Pathogenesis of immune thrombocytopenia. *Presse Med*. 2014;43(4 Pt 2):e49-e59.
8. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol*. 2011;11(4):264-274.
9. Shrestha S, Wiener HW, Olson AK, et al. Functional FCGR2B gene variants influence intravenous immunoglobulin response in patients with Kawasaki disease. *J Allergy Clin Immunol*. 2011;128(3):677-680.
10. Bruin M, Bierings M, Uiterwaal C, et al. Platelet count, previous infection and FCGR2B genotype predict development of chronic disease in newly diagnosed idiopathic thrombocytopenia in childhood: results of a prospective study. *Br J Haematol*. 2004;127(5):561-567.
11. Neunert CE, Buchanan GR, Imbach P, et al; Intercontinental Childhood ITP Study Group Registry II Participants. Severe hemorrhage in children with newly diagnosed immune thrombocytopenic purpura. *Blood*. 2008;112(10):4003-4008.
12. Provan D, Stasi R, Newland AC, et al. International consensus report on the investigation and management of primary immune thrombocytopenia. *Blood*. 2010;115(2):168-186.
13. Neunert C, Lim W, Crowther M, Cohen A, Solberg L Jr, Crowther MA; American Society of Hematology. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood*. 2011;117(16):4190-4207.
14. Blanchette VS, Luke B, Andrew M, et al. A prospective, randomized trial of high-dose intravenous immune globulin G therapy, oral prednisone therapy, and no therapy in childhood acute immune thrombocytopenic purpura. *J Pediatr*. 1993;123(6):989-995.
15. Blanchette V, Imbach P, Andrew M, et al. Randomised trial of intravenous immunoglobulin G, intravenous anti-D, and oral prednisone in childhood acute immune thrombocytopenic purpura. *Lancet*. 1994;344(8924):703-707.
16. Fujisawa K, Iyori H, Ohkawa H, et al; Japanese Study Group on Childhood ITP. A prospective, randomized trial of conventional, dose-accelerated corticosteroids and intravenous immunoglobulin in children with newly diagnosed idiopathic thrombocytopenic purpura. *Int J Hematol*. 2000;72(3):376-383.
17. Duru F, Fisgin T, Yarali N, Kara A. Clinical course of children with immune thrombocytopenic purpura treated with intravenous immunoglobulin G or megadose methylprednisolone or observed without therapy. *Pediatr Hematol Oncol*. 2002;19(4):219-225.
18. Ancona KG, Parker RI, Atlas MP, Prakash D. Randomized trial of high-dose methylprednisolone versus intravenous immunoglobulin for the treatment of acute idiopathic thrombocytopenic purpura in children. *J Pediatr Hematol Oncol*. 2002;24(7):540-544.
19. Tamminga R, Berchtold W, Bruin M, Buchanan GR, Kühne T. Possible lower rate of chronic ITP after IVIG for acute childhood ITP an analysis from registry I of the Intercontinental Cooperative ITP Study Group (ICIS). *Br J Haematol*. 2009;146(2):180-184.
20. Heitink-Pollé KM, Nijsten J, Boonacker CW, de Haas M, Bruin MC. Clinical and laboratory predictors of chronic immune thrombocytopenia in children: a systematic review and meta-analysis. *Blood*. 2014;124(22):3295-3307.
21. Buchanan GR, Adix L. Grading of hemorrhage in children with idiopathic thrombocytopenic purpura. *J Pediatr*. 2002;141(5):683-688.

22. Breunis WB, van Mirre E, Bruin M, et al. Copy number variation of the activating FCGR2C gene predisposes to idiopathic thrombocytopenic purpura. *Blood*. 2008;111(3):1029-1038.

23. Heitink-Pollé KM, Haverman L, Annink KV, Schep SJ, de Haas M, Bruin MC. Health-related quality of life in children with newly diagnosed immune thrombocytopenia. *Haematologica*. 2014;99(9):1525-1531.

24. Imbach P, Kühne T, Müller D, et al. Childhood ITP: 12 months follow-up data from the prospective registry I of the Intercontinental Childhood ITP Study Group (ICIS). *Pediatr Blood Cancer*. 2006;46(3):351-356.

25. Willcocks LC, Lyons PA, Clatworthy MR, et al. Copy number of FCGR3B, which is associated with systemic lupus erythematosus, correlates with protein expression and immune complex uptake. *J Exp Med*. 2008;205(7):1573-1582.

26. Crow AR, Song S, Freedman J, et al. IVIg-mediated amelioration of murine ITP via FcgammaRIIB is independent of SHIP1, SHP-1, and Btk activity. *Blood*. 2003;102(2):558-560.

27. Samuelsson A, Towers TL, Ravetch JV. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science*. 2001;291(5503):484-486.

28. Imbach P, Wagner HP, Berchtold W, et al. Intravenous immunoglobulin versus oral corticosteroids in acute immune thrombocytopenic purpura in childhood. *Lancet*. 1985;2(8453):464-468.

29. Medeiros D, Buchanan GR. Major hemorrhage in children with idiopathic thrombocytopenic purpura: immediate response to therapy and long-term outcome. *J Pediatr*. 1998;133(3):334-339.

30. Lilleyman JS; Paediatric Haematology Forum of the British Society for Haematology. Intracranial haemorrhage in idiopathic thrombocytopenic purpura. *Arch Dis Child*. 1994;71(3):251-253.

31. Psaila B, Petrovic A, Page LK, Menell J, Schonholz M, Bussel JB. Intracranial hemorrhage (ICH) in children with immune thrombocytopenia (ITP): study of 40 cases. *Blood*. 2009;114(23):4777-4783.

32. Iyori H, Bessho F, Ookawa H, et al; Japanese Study Group on childhood ITP. Intracranial hemorrhage in children with immune thrombocytopenic purpura. *Ann Hematol*. 2000;79(12):691-695.

33. van Bladel ER, Laarhoven AG, van der Heijden LB, et al. Functional platelet defects in children with severe chronic ITP as tested with 2 novel assays applicable for low platelet counts. *Blood*. 2014;123(10):1556-1563.

34. Frelinger AL III, Grace RF, Gerrits AJ, et al. Platelet function tests, independent of platelet count, are associated with bleeding severity in ITP. *Blood*. 2015;126(7):873-879.