Hemolysis related to intravenous immunoglobulins is dependent on the presence of anti-blood group A and B antibodies and individual susceptibility

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BACKGROUND: Patients treated with intravenous immunoglobulins (IVIG) rarely experience symptomatic hemolysis. Although anti-A and anti-B isoagglutinins from the product are involved in most cases, the actual mechanisms triggering hemolysis are unclear.

STUDY DESIGN AND METHODS: A prospective, open-label, multicenter, single-arm clinical trial in 57 patients with immune thrombocytopenia treated with IVIG (Privigen, CSL Behring) was conducted.

RESULTS: Twenty-one patients received one infusion (1 g/kg) and 36 received two infusions (2 × 1 g/kg) of IVIG. After a study duration of more than 2 years, no cases of clinically significant hemolysis as defined in the protocol were identified. Data of patients with mild hematologic and biochemical changes were analyzed in more detail. Twelve cases (10/23 patients with blood group A1 and 2/11 patients with blood group B, all having received 2 g/kg IVIG) were adjudicated as mild hemolysis (median hemoglobin [Hb] decrease, −3.0 g/dL); Hb decreases were transient, with partial or full recovery achieved by last visit. Eighteen patients (31.6%), all with non-O blood group, of whom 16 (88.9%) received 2 g/kg IVIG, fulfilled post hoc criteria for hemolytic laboratory reactions. Red blood cell (RBC) eluates of all direct antiglobulin test–positive samples were negative for non-ABO blood group antibodies. Blood groups A and B antigen density on RBCs appeared to be a risk factor for hemolytic laboratory reactions. Platelet response to treatment was observed in 42 patients (74%); eight of 12 patients with complete response had blood group A1.

CONCLUSION: Isoagglutinins are involved in clinically nonsignificant hemolysis after treatment with IVIG, but individual susceptibility varies greatly.

Immunoglobulin therapy is the cornerstone of prophylactic therapy for primary antibody deficiency. In addition, high-dose intravenous immunoglobulins (IVIG) are used increasingly as immunomodulatory therapy in the management of a number of autoimmune diseases.1-3

The use of IVIG and the demand for high-quality, safe products has remained evident since the first attempts at IV administration in 1962.4-6 Most products are now manufactured as 10% liquids to minimize the volume

ABBREVIATIONS: AE(s) = adverse event(s); bw = body weight; EMA = European Medicines Agency; ITP = immune thrombocytopenia; LASSO = least absolute shrinkage and selection operator; LLN = lower limit of normal; SAE(s) = serious adverse event(s); ULN = upper limit of normal.

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administered. Available products are well tolerated, and their administration has rarely been associated with serious systemic adverse events (AEs) such as thromboembolic complications, anaphylactic reactions, or renal failure. 

A potential association between IVIG and hemolysis has been evaluated in previous studies. Most patients experiencing hemolysis express the A blood group antigen (i.e., blood group A or AB). Clinically significant hemolysis is reported less frequently among patients with blood group B and only very rarely in those with blood group O, suggesting a mechanism involving anti-A and anti-B isoagglutinins in the IVIG product. In addition, hemolysis occurs more frequently in patients who receive a high dose of IVIG, for example, 2 g/kg body weight (bw) IVIG. IVIG preparations produced using chromatography have a higher concentration of anti-A and anti-B isoagglutinins compared with preparations produced by Cohn-like ethanol fractionation, and with these preparations, a higher incidence of hemolysis has been reported. The association between hemolysis and IVIG has not been confirmed in all published cases. The true incidence of hemolysis is difficult to estimate because data on the number of patients receiving any particular dose of any individual product are not available, and reporting of AEs is voluntary. Additionally, most patients with minor laboratory findings may remain undiagnosed, as they rarely develop clinically significant hemolysis.

Most available IVIG preparations, including Privigen (CSL Behring), a 10% L-proline stabilized IVIG, have been shown to contain isoagglutinins and have been associated with hemolysis, including severe hemolysis in rare cases. In a previous study of Privigen in patients with primary immune thrombocytopenia (ITP), none of the 57 patients studied developed severe hemolysis. To further investigate the mechanism of hemolysis associated with IVIG administration, we evaluated the incidence and potential causes of hemolytic events related to treatment with IVIG (Privigen) in patients with ITP.

**MATERIALS AND METHODS**

**Study design**

This study was a prospective, open-label, multicenter, single-arm exploratory safety study (NCT01390649) in patients with chronic ITP treated with IVIG (Privigen), conducted after approval of the drug by the US Food and Drug Administration (FDA) in 2007. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008. Informed consent was obtained from all patients before inclusion in the study. The study protocol and all amendments were approved by the independent ethics committee/institutional review board of the participating centers.

**Patient population**

Eligible patients had a diagnosis of chronic ITP (disease duration of at least 6 months), a platelet (PLT) count of not more than $30 \times 10^9$/L at screening and an age of 18 to 65 years. Patients were excluded from study participation if any of the following criteria applied: planned splenectomy throughout the study period, treatment with IVIG or RhIG within 3 weeks before screening, use of drugs that had any pharmacologic effect on the blood clotting system within 3 weeks before screening, known allergic or severe reaction to blood products, hyperprolactinemia, abnormal laboratory measures at screening (hemoglobin $<11$ g/dL, positive direct antiglobulin test [DAT], indirect bilirubin $>$ the upper limit of normal [ULN], serum free haptoglobin $<0.2$ g/L, lactate dehydrogenase $>$ ULN, alanine aminotransferase $>$ 2.5 $\times$ ULN, aspartate aminotransferase $>2.5$ $\times$ ULN, creatinine $>$ 1.5 $\times$ ULN, reticulocyte count $>$ 1.5 $\times$ ULN, iron $<$ 50% of the lower limit of normal [LLN], ferritin $<$ 50% of LLN, vitamin B12 $<$ 50% of LLN), immunoglobulin (Ig)A below the detection limit, or red blood cell (RBC) transfusion or erythropoietin treatment within the past 14 days before screening. Patient blood groups (ABO and D factor) were determined before the IVIG infusion. Blood groups A1 and A2 (assessed as non-A1) were serotypically evaluated along with blood group antigen density determination using preinfusion RBCs stored in liquid nitrogen.

**Study drug administration**

All patients received IVIG (Privigen) 1 g/kg bw on Day 1. Depending on their PLT count on Day 3, patients received a second infusion on Day 3. The second infusion was mandatory if the PLT count was less than $50 \times 10^9$/L or optional (at the discretion of the investigator) if the PLT count was at least $50 \times 10^9$/L.

Four different Privigen lots were used. Isoagglutinin titers in all lots were 16 and 32 for anti-A and 8 and 16 for anti-B (measured by the European Pharmacopoeia direct method). Non-ABO RBC antibodies were detected in any of the Privigen lots, except for weakly positive results for anti-Wr(a) by indirect antiglobulin test (IAT).

**Study schedule**

Patients underwent clinical and laboratory evaluation before and after each administration of IVIG on Days 1, 2, 3, 5, 7, 15, and 29 during the observation period (30-35 days). Bleeding status, blood cell count, hematocrit, bilirubin, Hb, LDH, haptoglobin, blood biochemistry analyses, and microscopic urine analysis were performed.
Study objectives and endpoints

The primary objective of the study was to characterize the specificity of antibodies binding to RBCs in vivo and to analyze other factors that might be present in patients treated with IVIG who experienced clinically significant hemolysis. The secondary objective was to further confirm the efficacy of Privigen in ITP. The main efficacy endpoint was the proportion of patients with a PLT response, defined as a PLT count increase to at least 50 × 10^9/L at least once within 6 days after the first infusion (i.e., up to and including Day 7). Further efficacy endpoints included PLT responses as defined in the European Medicines Agency (EMA) guideline: 1) response, defined as a PLT count increase to at least 30 × 10^9/L and at least twofold increase from baseline; and 2) complete response, defined as an increase to at least 100 × 10^9/L.31 Both response and complete response required PLT counts to reach the aforementioned thresholds on at least two separate occasions, at least 7 days apart, and in the absence of bleeding.

In vitro analyses performed on patient’s RBCs collected before administration of IVIG included blood groups A and B antigen density, amount of IgG bound to RBCs after incubation in vitro with the Privigen lot administered to the respective patient and expression of RBC surface proteins involved in the control of complement activation and complement-mediated RBC lysis (CD35, CD55, and CD59).

AEs and serious AEs (SAEs, including hemorrhages) were recorded continuously during the study and were assessed for frequency, severity, and causal relationship to the study drug.

The trial was originally planned to continue until 10 patients with clinically significant hemolysis were detected or, for reasons of feasibility, until 150 patients were enrolled. For determination of clinically significant hemolysis, data of patients showing any clinical signs of hemolysis (presumptive hemolysis) were evaluated by an independent adjudication committee. These data included decrease of Hb of at least 2 g/dL, positive DAT after infusion, haptoglobin lower than the LLN, LDH higher than the ULN, and indirect bilirubin higher than the ULN. Detailed clinical and laboratory data of subjects fulfilling at least three criteria were evaluated by the adjudication committee based on clinical judgment.

After study duration of more than 2 years and an enrollment of 57 patients, no case of clinically significant hemolysis was detected and the study was terminated. To increase the sensitivity of the safety data analysis, the patients’ data were then grouped into patients with and without evidence of hemolytic laboratory reactions and analyzed accordingly. This post hoc definition of hemolytic laboratory reactions was based on the following criteria: a Hb decrease greater than 1 g/dL with positive DAT and one or more laboratory findings indicative of hemolysis (haptoglobin lower than the LLN, LDH higher than the ULN, bilirubin higher than the ULN, hemoglobinuria, hemoglobinemia, and spherocytosis).23 The presence or absence of hepatosplenomegaly, jaundice, or dark urine was also considered. Results presented here are based on this definition. Methods of assessment are presented in the online supplementary material (available as supporting information in the online version of this paper).

Statistical analyses

Analyses were based on the safety data set (all patients who received any amount of study medication) and the full-analysis set (all patients who received study medication and had at least one measurement for a specific variable during the treatment period). As all patients had measurements during the treatment period, the safety data set and the full-analysis set were identical. Baseline values were the last measurements taken before IVIG infusion on Day 1 of the study.

Safety and efficacy data were analyzed descriptively. Potential risk factors for hemolysis (IVIG dose [1 g/kg bw vs. 2 g/kg bw], ABO blood group, ABO blood group antigen density on RBCs [%], D factor, positive vs. negative, antibodies eluted from RBC after positive DAT [yes vs. no], IgG binding to patients’ RBCs, complement regulatory protein expression on RBCs [%], age [≤44 years vs. >44 years], sex) were analyzed using a logistic model with least absolute shrinkage and selection operator (LASSO)-based selection. LASSO selection is based on a constrained form of ordinary least-squares regression, where the sum of the absolute values of the regression coefficients is constrained to be smaller than a specified variable. By increasing this variable in discrete steps, a sequence of regression coefficients is obtained, with the nonzero coefficients at each step corresponding to selected variables. This is based on the algorithm developed by Efron and colleagues,32 which can be viewed as a stepwise procedure with a single addition to, or deletion from, the set of nonzero regression coefficients at any step. The corrected Akaike’s information criterion was used to choose among the models at each step of the selection process. Further logistic models were also applied. All statistical analyses were conducted with computer software (SAS, Version 9.3, SAS Institute, Inc.).

RESULTS

Patient disposition

The study was conducted from 2011 until 2014 at 18 hospitals in Bulgaria, Romania, and Serbia. Of 100 screened patients, 58 were eligible for inclusion in the study. One patient withdrew consent before receiving study drug, leaving 57 patients who formed the safety data set;
one patient who received treatment discontinued the study owing to physician decision and 56 patients completed the study (Fig. 1). Table 1 shows the patient demographics.

**Hemolysis results**

Fifteen patients, all of whom had received a total IVIG dose of 2 g/kg bw, had a decrease in Hb of at least 2 g/dL along with other signs of hemolysis. These cases were evaluated by the independent adjudication committee. Three patients were judged to have no hemolysis, and 12 patients (21%) were judged to have mild hemolysis. Ten of these 12 patients had blood group A1 (incidence 10/23, 43.5%) and two had blood group B (incidence 2/11, 18.2%). In these 12 patients, there was a median decrease of Hb of 2.3 g/dL (range, 2.0 to 2.5 g/dL) on Day 9 (nadir), with Hb values ranging from 9.9 to 13.2 g/dL. These Hb decreases were transient and followed by recovery or partial recovery with median Hb decrease of 2.1 g/dL (range, 2.0 to 2.2 g/dL) at Day 29 (end of study) and Hb values ranging from 11.8 to 15.8 g/dL. One of the 12 patients experienced mild dyspnea between Day 9 and Day 16, and one patient experienced mild dizziness on Day 4. No patient was judged as having experienced clinically significant hemolysis. None of the 12 patients required blood transfusion.

When applying the post hoc criteria to increase the sensitivity of the safety data analysis (Hb decrease of >1 g/dL with positive DAT and at least one additional laboratory sign of hemolysis), hemolytic laboratory reactions were detected in 18 patients (31.6%). None of these patients showed evidence of hepatosplenomegaly or other clinical signs of hemolysis, except for one patient with dark urine on Day 5. Two of these 18 patients received 1 g/kg bw IVIG, and 16 received 2 g/kg bw IVIG (Table 2).

All 18 patients had non-O blood groups: blood groups A1 (14/23, 60.9%), A2 (2/7, 28.6%), and B (2/11, 18.2%). Both sexes appeared similarly affected. Hemolytic laboratory reactions were reported with all lots of Privigen administered, and no apparent clustering pattern was observed.

Hemolytic laboratory reactions and mild hemolysis as arbitrated by the adjudication committee overlapped: 11 of 12 cases adjudicated as mild hemolysis also met the post hoc criteria for hemolytic laboratory reactions. Seven cases that met the post hoc definition were either adjudicated as no hemolysis (n = 3) or were not reviewed by the adjudication committee (n = 4), as they did not qualify for presumptive hemolysis (i.e., a Hb decrease of >2 g/dL). One case adjudicated as mild hemolysis did not meet the post hoc criteria.

The relevant factors for hemolytic laboratory reactions, based on the variable selection using LASSO, were A and B blood group antigen density on RBCs (%) and Privigen dose (1 g/kg vs. 2 g/kg). Both were also significant in a logistic model including only these two factors (p = 0.0006 for A and B blood group antigen density on RBCs and p = 0.0049 for Privigen dose). A trend for a higher hemolysis risk was seen for D+ individuals (not significant, p = 0.0701) in a model including the factors D, ABO blood group antigen density on RBCs, and Privigen dose. Analysis of hemolysis cases as defined by the adjudication committee confirmed these findings. Figure 2 shows the impact of ABO blood group antigen density and Privigen dose on the risk of hemolytic laboratory reactions.

**Time dynamics of Hb levels and other laboratory changes**

After the IVIG infusions on Days 1 and 3, blood Hb decreased by more than 1 g/dL in the majority of patients (Fig. 3A). This decrease occurred regardless of patient blood group (9/16 patients with blood group O and 36/41 with non-O blood group) and was not always associated with other signs of hemolysis. After Day 5, blood Hb decreases of more than 1 g/dL were seen in 35 patients who had predominantly non-O blood group (31/35, 88.6%). Nine patients had no Hb decreases of more than

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**Fig. 1. Patient disposition. The number of patients participating in the study is shown.**

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**TABLE 1. Patient demographics**

| Demographic            | All patients (n = 57) |
|------------------------|----------------------|
| Females                | 37 (64.9)            |
| Age (years)            | 43.5 (±13.1)         |
| Blood group A†         |                      |
| A1                     | 23 (40.4)            |
| A2                     | 7 (12.3)             |
| Blood group B          | 11 (19.3)            |
| Blood group O          | 16 (28.1)            |

* Data are reported as number (%) or mean (±SD).
† There were no patients with blood group AB in the study.
1 g/dL at any time point in the study, and six (66.6%) of these had blood group O.

Haptoglobin levels showed decreases and increases after IVIG administration similar to Hb. The decrease in non-O blood group patients persisted from Day 5 to Day 7 or Day 9 for IVIG doses of 1 or 2 g/kg, respectively (Fig. 3B). By the end of the study, haptoglobin values in all patients were approaching the baseline levels.

A marked increase in reticulocyte count was observed only in patients with non-O blood group receiving a total IVIG dose of 2 g/kg, with a maximum on Day 15 (Fig. 3C). By Day 29, reticulocyte counts in these patients had normalized.

### Antibody binding to RBCs

A positive DAT was observed in 27 patients at least once during the study, most frequently in patients with blood group A: four of nine patients with one infusion and 19 of 21 patients with two infusions. In contrast, the DAT remained negative in all five blood group B patients with one infusion and in three of the six blood group B patients with two infusions. One of 16 patients with blood group O who received two infusions developed a positive DAT on Day 3 that remained positive until the end of the study.

RBC eluates from patients with positive DAT (31 samples from 24 patients, collected at different time points of the study) were tested for regular (isoagglutinins) and non-ABO RBC antibodies by IAT. All RBC eluates were found negative for non-ABO RBC antibodies. Eleven eluates from nine patients were IAT positive; nine samples were tested for isoagglutinins of which six eluates from five patients were positive for anti-A. These five patients had blood group A and showed hemolytic laboratory reactions. The other IAT-positive eluates were either negative for anti-A (two samples of one patient with blood group O) or not tested (three samples from three patients with blood group A).

### Blood group antigen density and IgG binding

For RBCs with blood group A, two subgroups could be distinguished (Fig. 4): higher antigen A density (>70%...
compared with control from a normal volunteer) and lower antigen A density (<25% compared with control from a normal volunteer). These two groups were serologically confirmed to correspond to blood groups A1 (high antigen A density) and A2 (low antigen A density). A total of 23 patients belonged to blood group A1 and seven to blood group A2. Among the 11 patients with blood group B, seven had antigen B densities between 25 and 70% and four had high antigen B density (>70% compared with control from a normal volunteer); no blood group B patient had an antigen density of less than 25%. In patients with blood group O, no blood group A antigen was detected on RBCs (antigen density < 0.1%), as expected. IgG binding to RBCs was higher in patients with a high antigen A density (blood group A1) than in patients with blood group B (Fig. 4). IgG binding was lowest in patients with a low antigen A density (blood group A2). There was no IgG binding to RBCs of patients with blood group O. Blood group antigen density on RBCs was a significant risk factor for hemolysis (Fig. 2).

Complement regulatory protein expression on RBC surface

The mean densities of CD35, CD55, and CD59 on RBCs compared to control were 65, 76, and 117%, respectively. No relevant differences in expression were found between patients with laboratory signs of hemolysis and those without. Based on statistical analyses, these factors appear not to be indicative of hemolytic laboratory reactions.

Fig. 3. Median change from baseline in Hb, haptoglobin, and reticulocytes over time. Median change from baseline by blood group and total IVIG dose is shown. (A) Hb. The following time points include only measurements in patients with two infusions: Day 3, 30 minutes and 5 hours after infusion; Day 4; and Day 9. (B) Haptoglobin. The following time points include only measurements in patients with two infusions: Day 3, 30 minutes and 5 hours after infusion; Day 4; and Day 9. (C) Reticulocytes. The following time points include only measurements in patients with two infusions: Day 3, 30 minutes after infusion, and Day 9. BL = baseline.
Efficacy analyses

Platelet response defined as a PLT count increase to at least $50 \times 10^9$/L within 6 days after the first infusion was observed in 42 patients (74%; 95% confidence interval [CI], 61%-83%). Response according to EMA guidelines was observed in 35 patients (61%) and complete response in 12 (21%; Table 3). The response rate (EMA) was between 43 and 73% (lowest in blood group A2, highest in blood group B). Complete response rate was 35% in blood group A1 and 14, 18, and 6% in blood groups A2, B, and O, respectively.

AEs

Overall, 29 patients (50.9%) reported at least one AE; 14 patients (24.6%) had AEs considered at least possibly related to the study IVIG and 21 (36.8%) had temporally associated AEs that occurred during an infusion or within 72 hours after the end of infusion. The most common AE was headache (17 patients [29.8%]), followed by pyrexia (three patients [5.3%]). All other AEs were reported by at least two patients. All AEs reported during the study were mild or moderate in intensity. One SAE of ITP exacerbation was reported as unrelated to the study IVIG and resolved by end of study. There were no deaths during the study.

**DISCUSSION**

The aim of this study was to investigate potential risk factors and mechanisms for the development of clinically significant hemolysis during treatment with IVIG. However, no cases of clinically significant hemolysis occurred according to the assessment of an independent adjudication committee in this study after more than 2 years and 57 enrolled patients. The study was thus terminated in agreement with the FDA.

A post hoc definition of hemolytic laboratory reactions was then applied to increase the sensitivity of the safety data analysis; it used lower thresholds for decrease in Hb ($>1 \text{ g/dL}$ vs. $\geq 2 \text{ g/dL}$ in the original study protocol) with positive DAT and at least one other laboratory marker of hemolysis. Clinical signs including hepatosplenomegaly, jaundice, or chromaturia were also considered as markers of hemolysis. However, of these symptoms, only chromaturia (dark urine) was reported in one patient with mild hemolysis in this trial. Applying this post hoc definition, hemolytic laboratory reactions were observed in 18 patients (31.6%).

The definition used in the post hoc analysis reported here, based on the criteria proposed by Health Canada, includes a Hb decrease of more than 1 g/dL at any time point after IVIG administration. This low threshold makes the analysis very sensitive but also possibly prone to artefacts, as changes in Hb of this magnitude may occur with phlebotomy (blood draws) and hemodilution resulting from the large volume of IVIG infused. A positive DAT was included as a mandatory criterion, as hemolysis due to IVIG involves binding of an antibody to RBCs. The concordance of the hemolytic laboratory reactions and the diagnosis reached by experts confirms the validity of the selected definition: 11 of the 12 cases classified as mild hemolysis by the adjudication committee met the criteria for hemolytic laboratory reaction.
The results of this trial support the hypothesis that anti-A/B antibodies are associated with development of hemolysis after treatment with IVIG but that clinically significant hemolysis requires additional patient-specific features. Hemolytic laboratory reactions and mild hemolysis occurred only in patients with non-O blood groups. All six RBC eluates that tested positive for anti-A were from patients with hemolytic laboratory reactions. Furthermore, in patients with blood group A, risk of hemolysis appeared to be dependent on the subgroup. Density of blood group antigens on the RBC may largely account for the difference between blood groups A1 (high antigen density) and A2 (low antigen density). Finally, all patients with mild hemolysis and most patients with hemolytic laboratory reactions received the high dose of 2 g/kg bw (16/18 patients, 88.9%), suggesting an antibody-dose effect. Within blood groups A1, A2, and B a clear effect of IVIG dose was observed, resulting in higher risk with higher dose. The risk was particularly high with blood group A1 compared to blood groups A2 and B, suggesting a role for both antigen density and IVIG dose.

Our results showed no risk of hemolytic laboratory reactions associated with blood group O. However, hemolytic reactions after IVIG administration have been reported in blood group O subjects, albeit very rarely. These cases are often associated with anti-D. In some cases, the causative antibodies in hemolysis cases in blood group O subjects could not be identified because of the lack of suitable reagents or because of their low concentration in preparations, patients’ plasma samples, or patients’ RBCs. None of the available, routinely used serologic tests (including antibody screening, DAT, and RBC eluates) is universally capable of detecting all causative antibodies in patients with immune hemolytic anemia. We noted a nonsignificant trend for a higher hemolysis risk in D+ individuals. However, this result is difficult to interpret, as only 10 of 57 patients in the study were D+.

Although antibody classes and subclasses, soluble A and/or B substances, and the presence of underlying inflammatory diseases have been shown to play a role in triggering hemolysis after IVIG administration, the role of complement as an additional factor remains open. Our results did not show any conclusive findings in this respect. The complement regulatory proteins CD35, CD55, and CD59 had a normal level of expression on RBCs and were not indicative of hemolytic laboratory reactions. Thus, the occurrence of clinically significant hemolysis appears to depend on the presence of individual factors that cannot be identified yet. This is supported by the fact that hemolysis may occur in children and adults, after infusion of preparations with a low concentration of isoagglutinins and/or low IVIG doses. Ultimately, hemolysis is usually unpredictable, even in patients with a disposition, that is, blood group A1 and/or inflammatory diseases. Recently, activation of the mononuclear phagocyte system along with up regulation of high-affinity Fcγ receptor 1 were hypothesized to be components of the underlying inflammatory condition contributing to development of hemolysis after high-dose IVIG administration and should be investigated in future studies.

Overall, IVIG-related RBC sensitization and destruction, whether it is considered clinically significant hemolysis or not, seems primarily related to anti-A and anti-B isoagglutinins in the product. The clinical significance is clearly influenced by additional, largely still poorly understood, factors. The most promising tools for reducing the incidence of hemolysis appear to be measures to reduce isoagglutinins in IVIG products, such as specific immunoaffinity chromatography, which has now been instituted by CSL Behring and some other manufacturers.

Finally, in this study, 61% of patients reached the threshold PLT count required for a response according to the EMA guideline from 2010, supporting the efficacy of Privigen in patients with ITP. It is worth mentioning that, although the response rate appears similar across blood groups, complete response occurred most frequently in patients with blood group A1. This raises the interesting possibility that coating of patients’ RBCs with antibodies is involved in the mechanisms of action of IVIG in ITP.

The majority of AEs reported were mild in intensity, none were severe, and the only reported SAE (ITP exacerbation) resolved by the end of the reporting period and was assessed as being not related to treatment.

A limitation of the current study is that no clinically significant hemolysis was observed throughout the duration of the trial, and the analyses are based only on mild cases and laboratory evidence of hemolysis without any clinical sequelae. The relevance of the conclusions for rare cases of severe hemolysis therefore remains unknown.

In conclusion, our findings suggest that anti-A/B isoagglutinins and IVIG dose play the most prominent role in triggering hemolysis observed in patients treated with high-dose IVIG. Measures for reducing isoagglutinins in Privigen have been implemented, and their effectiveness in reducing clinically significant hemolysis is currently being investigated.

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CONFICT OF INTEREST
OM, AS, MOS, SW, BLD, JPL, and AH are employed by CSL Behring. OM, AS, and AH own CSL Behring stock. SF is a member of the adjudication committee of the study. VGM and AS have disclosed no conflicts of interest.

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