Hemolysis in patients with antibody deficiencies on immunoglobulin replacement treatment

Isabella Quinti,[1] Federica Pulvirenti,[1]* Cinzia Milito,[1] Guido Granata,[1] Gianluca Giovannetti,[2] Fabiola La Marra,[1] Anna M. Pesce,[1] Albert Farrugia,[3,4,5] Serelina Coluzzi,[2] and Gabriella Girelli[2]*

BACKGROUND: Immunoglobulin (Ig)G replacement with intravenous or subcutaneous immunoglobulins is a lifelong substitutive therapy in patients with primary antibody deficiencies (PADs). Hemolysis after immunoglobulin therapy was described in patients receiving high immunoglobulin dosages. The issue of hemolysis after immunoglobulin administration at replacement doses has been considered of little clinical significance.
STUDY DESIGN AND METHODS: This was a single-center observational study over a 2-year period on immunoglobulin-induced hemolysis in a cohort of 162 patients with PADs treated with immunoglobulin administered at replacement dosages.
RESULTS: Six patients had signs and symptoms of immunoglobulin-induced hemolysis. Two additional asymptomatic patients were identified by a short-term study run on 16 randomly selected asymptomatic patients. Alloantibodies eluted from patients' red blood cells (RBCs) had anti-A and Rh specificities (anti-D and anti-C). The immunoglobulins contained alloantibodies with the same specificities of the antibodies eluted from patients' RBCs.
CONCLUSION: Hemolysis occurred in patients receiving immunoglobulin at replacement dosages. Polyvalent immunoglobulin preparations contained multiple clinically significant antibodies that could have unexpected hemolytic consequences, as anti-C whose research and titration are not required by the European Pharmacopoeia. The issue of hemolysis in long-term recipients of immunoglobulin treatment administered at replacement dosages should be more widely recognized.

Immunoglobulin (Ig)G replacement with intravenous (IVIG) or subcutaneous (SCIG) immunoglobulins is the standard therapy for primary antibody deficiencies (PADs) aiming to replace the missing antibodies and thereby to prevent recurrent infections.[1-3] Immunoglobulin administered at replacement dosages is considered universally a safe long-life therapy with a low rate of adverse events.

More often, immunoglobulin administration–related adverse events, including hemolysis, have been described in patients receiving high immunoglobulin dosages.[4-8]

Risk factors recognized for immunoglobulin-induced hemolysis include female sex of recipients; blood group A, B, or AB; and high doses of immunoglobulin. Thus, immunoglobulin-associated hemolysis rarely occurred in PAD patients receiving immunoglobulin at low dosages (http://www.adrreports.eu). In pivotal trials on immunoglobulin, none of the PAD patients enrolled developed evidence of hemolysis or anemia, despite 8.5% up to 47% of them becoming direct antiglobulin test (DAT) positive after 24 hours up to 10 days after the immunoglobulin

ABBREVIATIONS: CBC = complete blood count; PAD(s) = primary antibody deficiency(-ies); RCI = corrected reticulocyte index; SCIG(s) = subcutaneous immunoglobulin(s).

From the [1]Department of Molecular Medicine, [2]Unit of Immunohematology and Transfusion Medicine, Sapienza University of Rome, Rome, Italy; the [3]Plasma Protein Therapeutics Association, Annapolis, Maryland; the [4]School of Surgery, University of Western Australia, Crawley, Western Australia; and the [5]School of Medicine, Australian National University, Canberra, Australia.

Address reprint requests to: Isabella Quinti, Viale dell'Università 37, 00186 Rome, Italy; e-mail: isabella.quinti@uniroma1.it.

*IQ and FP equally contributed to this work.

Received for publication June 9, 2014; revision received September 29, 2014, and accepted October 3, 2014.

doi: 10.1111/trf.12939

TRANSFUSION 2015;55:1067–1074

infusion.[9] However, the positivity of DAT[9] due to the passive transfer by immunoglobulin preparations of isohemagglutinins, alloantibodies, and possibly immune complexes present in the immunoglobulin product that copurify with other IgG[10-12] is not sufficient per se to diagnose an hemolysis.

In 2009, the Canadian IVIG Hemolysis Pharmacovigilance Group elaborated criteria to define an "IVIG-induced hemolysis." They included a reduction of Hb levels of at least 1 g within 10 days after immunoglobulin administration, with appearance of positive DAT and at least two of the following criteria: increase in the reticulocyte count, elevation of lactate dehydrogenase (LDH) and unconjugated bilirubin serum levels, low haptoglobin level, hemoglobinuria, hemoglobinemia, presence of significant spherocytosis, in the absence of alternative causes of anemia.[13]

Nowadays, all commercial immunoglobulin products must undergo anti-A and anti-B testing and regulatory requirements assess that blood antibody titers should be less than or equal to 64 at 5% (wt/vol).[14,15] Nevertheless, hemolysis might occur even in recipients of immunoglobulin products that meet these specifications.[10] Consequently, it has been suggested that immunoglobulin recipients should be monitored for clinical signs and symptoms of hemolysis (report of the FDA meeting on Strategies to Address Hemolytic Complications of Immune Globulin Infusions, Washington, DC, January 2014). We showed here that immunoglobulin-induced hemolysis occurred in PAD patients treated at replacement dosages.

MATERIALS AND METHODS

Study design

A 2-year single-center observational study was conducted on 162 PAD patients receiving IVIG or SCIG as replacement therapy (Fig. 1). A total of 162 PAD patients receiving IVIG or SCIG were monitored over a 2-year period (2011-2013) for evidence of symptomatic immunoglobulin-induced hemolysis, following the Canadian IVIG Hemolysis Pharmacovigilance Group criteria,[13] applied also to patients receiving SCIG. Additionally, a short-term study was performed in 16 PAD patients to possibly detect an asymptomatic immunoglobulin-induced hemolysis. All participants provided written informed consent.

Patients

A total of 162 patients under regular follow-up at our Referral Center for Primary Immune Deficiencies had a diagnosis of PAD based on the current diagnostic criteria of the European Society for Immune Deficiencies/Pan-American Group for Immune Deficiencies.[16] No immunoglobulin-naïve newly diagnosed PAD patient was enrolled in the study and thus no pretreatment data were available for this study. Personal data, immunoglobulin administration dosages, blood group, Rh phenotype, and immunologic and clinical manifestations were available for patients enrolled in the study.

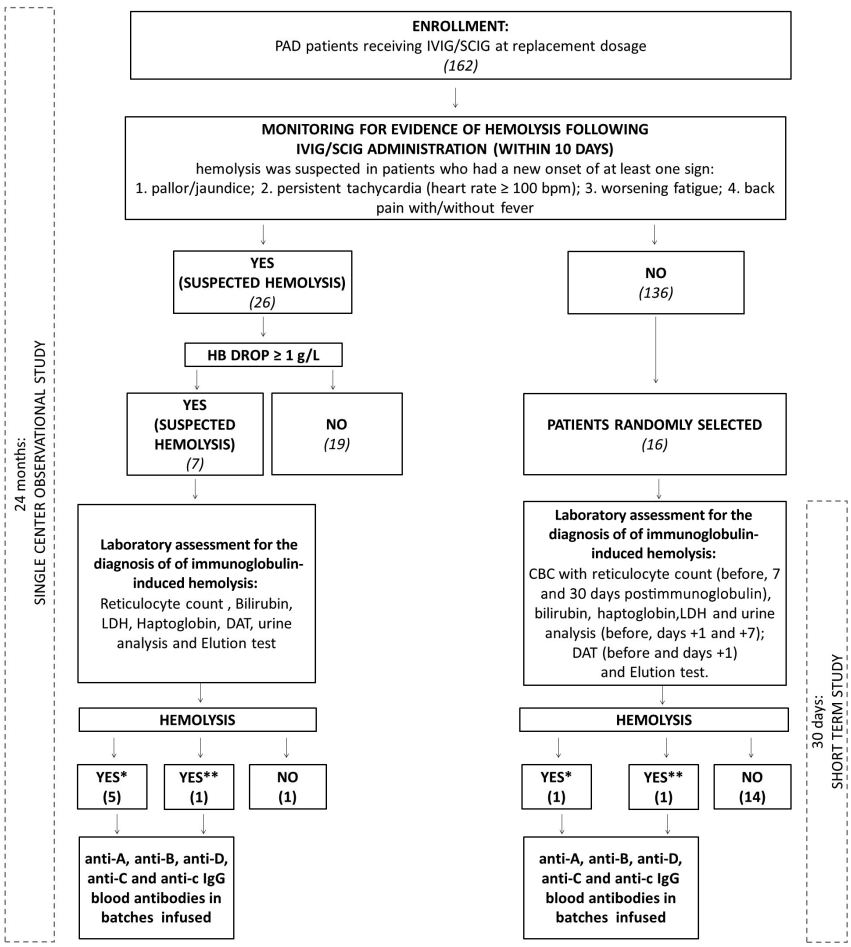

Fig. 1. Study design. A 2-year single-center observational study on 162 PAD patients receiving immunoglobulin replacement therapy monitored for evidence of symptomatic immunoglobulin-induced hemolysis. An additional short-term study on 16 PAD patients to detect an asymptomatic immunoglobulin-induced hemolysis was also performed. *Patients that fulfilled the criteria for the standardized case definition of immunoglobulin-induced hemolysis, as developed by the Canadian IVIG Hemolysis Pharmacovigilance Group.[13] **Patients with signs or symptoms of hemolysis that did not completely fulfill the Canadian criteria.[13]

Hemolysis was suspected if patients had a new onset of at least one of the following signs and symptoms: pallor or jaundice, persistent tachycardia (heart rate ≥ 100 bpm), worsening fatigue, or back pain with or without fever within 10 days from IVIG or SCIG administration. Patients who met these criteria underwent a complete blood count (CBC). Patients with a decrease in hemoglobin (Hb) of at least 1 g/dL underwent a laboratory assessment including reticulocytes counts, bilirubin, LDH and haptoglobin serum levels, urine analysis, and DAT.

In patients with hemolysis we performed the elution test from red blood cells (RBCs). Anti-A, anti-B, and other RBC IgG alloantibodies were investigated in the IVIG and SCIG batches infused at the time of hemolysis.

Additionally, at the time of immunoglobulin infusion, 16 PAD patients without signs or symptoms of immunoglobulin-induced hemolysis were randomly selected on a voluntary basis to participate in a short-term study. Since the majority of episodes of immunoglobulin-induced hemolysis have been detected between 12 hours and 10 days after immunoglobulin administration,[6] we analyzed the following variables at different time points: CBC immediately before and after immunoglobulin infusion (Day +7 and Day +30), reticulocyte count, and corrected reticulocyte index (RCI) immediately before and after immunoglobulin infusion (Day +7); DAT (immediately before immunoglobulin and Day +1 postimmunoglobulin); and total and unconjugated bilirubin serum level, haptoglobin serum level, LDH serum level, and urine analysis immediately before and after immunoglobulin (Day +1 and Day +7). In patients with an asymptomatic immunoglobulin-induced hemolysis we performed the elution test. In the IVIG or SCIG batches infused at the time of the short-term study we evaluated the presence of anti-A, anti-B, anti-D, anti-C, and anti-c IgG alloantibodies.

Blood group determination

We analyzed RBCs by liquid phase using reagents provided by Ortho Clinical Diagnostics (Raritan, NJ). All patients had their RBCs typed for A, B, D, C, c, E, and e blood group antigens.

DAT

DAT was performed with a broad-spectrum antiserum (Ortho Clinical Diagnostics). DAT-positive samples were tested with monospecific anti-IgG, -IgA, -IgM, -C3d, and -C3b reagents (Dia-Med, Cressier sur Morat, Switzerland).

Elution test

To confirm the positive DAT, an IgG elution was performed with a low-pH glycine buffer using a commercially available kit (Elu-Kit, Immucor, Norcross, GA). The eluate was incubated with A RBCs and/or O RBCs panel to check for its specificity on the basis of RBC patient phenotype.

Serologic tests

Serum was incubated with a three-cell O RBCs panel by microcolumn agglutination test. In the case of positivity for RBCs alloantibodies, we identified the specificity using O RBC extended panels on the basis of the pattern of reactivity and the knowledge of antigens to which the patient's serum was exposed (reagent from Ortho Clinical Diagnostics and Dia-Med). To identify the possible involvement of anti-A, anti-B, anti-D, anti-C, or anti-c in hemolytic reaction, the patient's serum was also tested with A, B, and O R_1R_1, r_1r, rr RBCs, on the basis of patient phenotype.

Immunoglobulin preparation tests

All the immunoglobulin commercial preparations were licensed by batch release from our National Health Institute (Istituto Superiore di Sanità).

Volumes of 100 μL of immunoglobulin serial twofold dilutions were mixed with 50-μL volumes of the diluted (5%) A *rr* cells (Ortho Clinical Diagnostics) in tubes incubated at 20 and 37°C for 1 hour. After three washes with the sodium chloride solution, the antiglobulin serum was added. The endpoint titer was taken as the highest dilution that results in weak agglutination (trace reaction: small agglutinates of 3-4 RBCs with many not agglutinated RBCs, according to *Judd's Methods in Immunohematology*, 3rd ed, Table 1-F-1 grading serologic reactions, p. 28). In addition, 40 μL of reconstituted 5% (wt/vol) immunoglobulin preparations were incubated at 37°C for 10 minutes with 10 μL of diluted (3%) O RBCs panels of known specificity by column agglutination test (Ortho Clinical Diagnostics) to identify any other antibody specificity. We incubated 1 mL of immunoglobulin preparations (5%) with 1 mL of O R_1R_1, r_1r, rr RBCs at 37°C for 1 hour. To define RBC antibody specificity 40 μL of eluates was incubated with 10 μL of diluted (3%) O R_1R_1, r_1r, rr RBCs by a microcolumn method; O RBCs without D, C, and c antigens were used as negative control.

Statistical analysis

The analysis of variance test and the Mann-Whitney U test were used for comparison of data. Comparison between single variables was assessed by simple linear regression analysis. Comparison between patient groups was analyzed by contingency tables—Fisher's exact test. Statistical analysis was performed with software (StatView, SAS Institute, Cary, NC). A p value equal or less than 0.05 was considered significant.

RESULTS

Hemolysis occurred in patients treated with immunoglobulin administered at replacement dosages

We enrolled in the study 162 PAD patients (97 females and 65 males): 142 patients regularly received IVIG in hospital setting at intervals of 2 or 3 weeks and 20 patients self-administered SCIG at home every week, at a cumulative monthly dosage of 301 ± 121 mg/kg. Immunoglobulin treatment times ranged between 2 and 35 years (mean, 18 ± 14 years). Sixty-six patients were of blood group A, 16 were B, 13 were AB, and 57 were O; 144 patients were D+ and 18 were D–.

Within 10 days from immunoglobulin administration, 26 patients had at least one of the signs or symptoms defined for a suspected hemolysis. According to the study design (Fig. 1), all patients underwent CBC. Nineteen patients maintained their Hb levels, while seven patients (six patients on IVIG treatment and one patient on SCIG treatment) had a decrease in Hb levels. Six of seven patients (Table 1) had a positive DAT. Three patients (Patients 1, 3, and 5) had new-onset symptoms after immunoglobulin administration including fatigue, tachycardia (hearth rate ≥ 100 bpm), pallor, and/or jaundice associated with a severe Hb decrease (range, 5.1-6.9 g/L). Two patients were on IVIG treatment and one patient was on SCIG treatment. Three additional patients (Patients 2, 4, and 6) had fever and back pain 6 to 12 hours after the IVIG infusion and a mild Hb decrease (range, 1.1-1.5 g/L). Three patients were blood group A D+, one patient was blood group A D–, and two patients were blood group O D+. Positive DAT results were due to IgG only in three patients and to IgG and complement in three patients. All DAT results showed a score of at least 2+.

The eluted antibodies had anti-A specificity in three patients of A phenotype; anti-C specificity in two patients of A CcDee and O CcDee phenotype, respectively; and anti-C and anti-D in one patient of O CcDee phenotype.

The eluted antibodies had the same specificity found in the batches of immunoglobulin infused at the time of the hemolytic episode (Table 2). Hb decrease and reticulocyte increase were more evident in patients who developed the acute hemolytic episode due to anti-D and/or anti-C alloantibodies than that observed in patients who received immunoglobulin products containing anti-A alloantibodies (Hb, 5.7 ± 1 g/L vs. 1.3 ± 0.2 g/L, p = 0.017; reticulocytes, 9.8 ± 2.5 % vs. 3.2 ± 1.1%, p = 0.0014). Serum haptoglobin levels were undetectable in all patients who received anti-D and/or anti-C alloantibodies.

The haptoglobin was undetectable also in one patient who received an anti-A from an immunoglobulin batch. However, in a further analysis, this patient had a mutation in the haptoglobin gene (manuscript in preparation). All patients met the criteria for the standardized case defini-

TABLE 1. Patient characteristics and outcome of the hemolytic episode in six patients with immunoglobulin-induced hemolysis*

Patient	Sex	Age (years)	Pregnancies (number)	Batch/ brand	Monthly immunoglobulin dosage (mg/kg)	Hb (g/L)†	Hb (g/L)‡	Blood group, Rh phenotype	DAT	Eluted antibody	Haptoglobin (mg/L)‡	LDH (U/L)‡	Unconjugated bilirubin (mg/dL)‡	Ret%‡	Treatment	Outcome	Shift to a different immunoglobulin batch/brand	New hemolytic episodes after immunoglobulin shifting
1	Female	38	No	D/1	380	11.5	5.1	O CcDee	Positive (IgG, C_{3d})	Anti-C and anti-D	<30	397	0.8	9.46	Prednisone	Recovery	Yes/yes	No
2	Female	34	Yes (2)	B	460	11.0	9.5	A CCDee	Positive (IgG)	Anti-A	250	245	1.8	3.70	Prednisone	Recovery	Yes/no	No
3	Female	42	Yes (2)	C	370	14.7	9.6	A CcDee	Positive (IgG, C_{3d})	Anti-C	<30	401	1.5	7.50	Prednisone	Recovery	Yes/yes	No
4	Male	33		A	420	11.0	9.6	A CCDee	Positive (IgG)	Anti-A	450	221	0.8	1.9	Prednisone	Recovery	Yes/no	No
5	Female	54	Yes (1)	D/2	460	11.0	4.1	O CcDee	Positive (IgG, C_{3d})	Anti-C	<30	2338	5.5	12.5	Prednisone	Death		
6	Female	15	No	A	390	13.0	11.9	A CcDee	Positive (IgG, C_{3d})	Anti-A	<30	221	1.8	3.86	Prednisone	Recovery	Yes/no	No

* Hb concentration, patient's blood phenotype, DAT, specificity of the eluted antibody, reticulocyte percentage (Ret%), serum haptoglobin, LDH, and unconjugated bilirubin are shown.
† Data collected before the hemolytic episode.
‡ Data collected at the time of DAT positivity.

TABLE 2. IgG anti-A, anti-B antibodies and unexpected IgG RBC alloantibodies in five batches of IVIG and SCIG of four different brands administered in patients at the time of hemolysis

Brand	Immunoglobulin	Anti-A (titer)	Anti-B (titer)	RBCs unexpected alloantibodies
A	IVIG	1:16	0	Not detected
B	IVIG	1:16	1:16	Not detected
C	SCIG	1:32	1:32	Not detected
D/1	IVIG	1:32	0	Anti-C and anti-D
D/2	IVIG	1:32	0	Anti-C and anti-D

Alphabetic letters indicate immunoglobulin brands.
Numbers indicate different batches of the same brand.

tion of immunoglobulin-induced hemolysis, as developed by the Canadian IVIG Hemolysis Pharmacovigilance Group,[13] except for Patient 4 (A phenotype) who had a Hb decrease of more than 1 g, positive DAT, and a mild increase in reticulocyte percentage (1.9%) but he did not have low haptoglobin level, high LDH levels, nor high unconjugated bilirubin. However, the antibody eluted from his RBCs (IgG anti-A) had the same specificity found in the batch of immunoglobulin infused at the time of Hb drop.

Alloantibodies in IVIG and SCIG

Anti-A, anti-B, anti-D, anti-C, and anti-c IgG RBC alloantibodies in four batches of IVIG and one batch of SCIG of four different brands infused in the patients who had hemolysis at the time of the study are shown in Table 2. The anti-A and anti-B titers in the immunoglobulin preparations were within those recommended by the European Pharmacopoeia, which recommends that no anti-A and anti-B isohemagglutinins should be detectable above 1:32 dilution at 5% (wt/vol).[17-19] Two batches from one brand were positive for anti-D and anti-C IgG RBCs alloantibodies; the European Pharmacopoeia does not recommend to test for the presence of the latter antibodies.[20]

Laboratory data monitored before the start and after the infusion of immunoglobulin

In a group of 16 patients, we ran a short-term study to possibly detect signs of asymptomatic hemolysis (Table S1, available as supporting information in the online version of this paper). On Day +1 postimmunoglobulin, two of 16 patients (one male and one female, both of them of blood group A D+) had a decrease in Hb level of 1 g/L and a positive DAT at Day +7 postimmunoglobulin. In one patient reticulocyte percentage increased from 1.5% to 2%, and RCI increased from 1.33 to 1.74; in the second patient, reticulocyte percentage increased from 2% to 2.4% and RCI from 2.0 to 2.35. Hb levels and RCI from patients without or with a suspected asymptomatic hemolysis were shown in Figure 2. Serum haptoglobin and LDH levels did not change. Only one patient showed an increase of unconjugated bilirubin

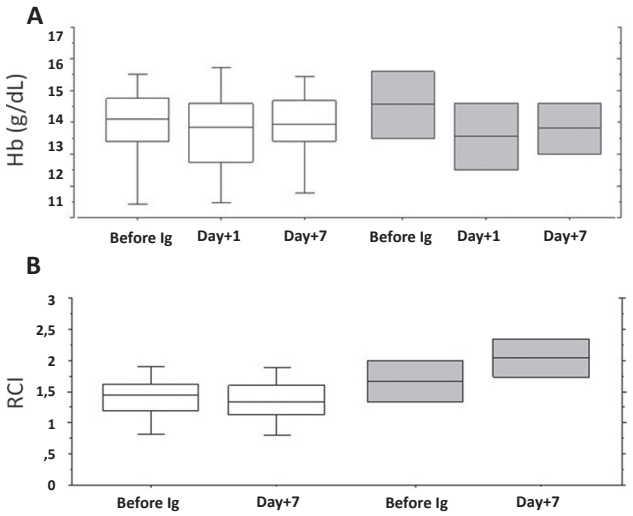

Fig. 2. Short-term study. Hb (A) and RCI (B) values in two patients with immunoglobulin-induced hemolysis (▭) and in 14 patients without it (▬). Hb levels were collected just before and at Day +1 and at Day +7 after immunoglobulin administration; RCI values were collected just before and at Day +7 after immunoglobulin administration.

from 0.59 to 1.76 mg/dL. Thus, one additional patient met the requirement for the diagnosis of immunoglobulin-induced hemolysis.[13] However, for both patients we considered the diagnosis of immunoglobulin-induced hemolysis as the antibodies eluted from patients' RBCs had the same specificity (IgG anti-A) found in the batches of immunoglobulin administered at the time of the study.

Surprisingly, from this short-term study we found an additional patient with undetectable haptoglobin levels before and after immunoglobulin administration. In a further analysis this patient had a mutation in the haptoglobin gene (manuscript in preparation).

Clinical outcome

All six symptomatic patients received treatment at the time of hemolysis: three patients with the mild decrease of Hb (Patients 2, 4, and 6) received a course of 5 days of oral

prednisone at a dosage of 1 mg/kg. All recovered their baseline Hb levels after 7 to 14 days. Two of three patients (Patients 1 and 5) who developed the hemolytic episode due to anti-D and/or anti-C alloantibodies required hospitalization, transfusion of RBCs, and steroid therapy. Patient 1 recovered her Hb levels after 30 days of intravenous steroid treatment, while Patient 5 died because of a sepsis. Patient 3 received a prolonged course of oral prednisone at a dosage of 1 mg/kg for 3 weeks and then tapered to 0.5 mg/kg for an additional 3 weeks. She recovered her Hb levels after 2 weeks. Moreover, three patients shifted to a different immunoglobulin commercial brand and two patients shifted to a different immunoglobulin batch of the same brand. The two asymptomatic patients, identified by the short-term study, did not receive specific treatment and both shifted to a different immunoglobulin brand.

DISCUSSION

Our study showed that immunoglobulin-induced hemolysis occurred in PAD patients receiving a longlife treatment with immunoglobulin at replacement dosages caused by RBC IgG alloantibodies passively transferred through immunoglobulin.

The passive transfer of RBC alloantibodies through IVIG is a well-described phenomenon, generally observed in patients treated with higher doses (reviewed in Desborough et al.[10]). However, in a recent letter Pintova and coworkers[21] described self-limiting acute hemolysis in two patients with Guillain Barré syndrome treated with low immunoglobulin dosages. On eluate from patients' RBCs, the only unexpected antibody detected was an IgG anti-A passively transferred through IVIG. In our series, hemolysis was due to IgG anti-A alloantibodies in three patients, to IgG anti-C and IgG anti-D alloantibodies in one patient and IgG anti-C alloantibodies in two patients. The latter antibodies caused severe hemolytic episodes, while anti-A caused a milder hemolysis. The finding of a severe hemolysis after passive transfer of anti-C and anti-D is a new observation in recipients of polyvalent immunoglobulin, even if anti-D IgG induced hemolytic episodes.[7,20,22] Moreover, we showed that polyvalent immunoglobulin preparations can contain clinically significant alloantibodies that could have unexpected hemolytic consequences, as anti-C whose research and titration are not required by the European Pharmacopoeia.[17-19]

The passive transfer of IgG alloantibodies through immunoglobulin is difficult to explain because they are prepared from plasma of thousands of donors and a dilution effect would be expected. Since immunization to RBC alloantigens can occur because of past transfusions or pregnancies, the hypothetical numbers of alloimmunized plasma donors should be very low. Recently, other mechanisms underlying alloimmunization related to molecular mimicry have been demonstrated.[23]

Whatever the cause of the presence of alloantibodies in immunoglobulin preparations, it should be underlined that a DAT positivity does not necessarily imply hemolysis. Thus, the Canadian IVIG Hemolysis Pharmacovigilance Group elaborated criteria to define an "IVIG-induced hemolysis."[13] According to these criteria, six of 162 PAD patients had immunoglobulin-induced hemolysis. Two additional patients had hemolysis due to alloantibodies, but did not completely fulfill the Canadian criteria. In the presence of a mild hemolysis signs such as increase of unconjugated bilirubin, LDH, low haptoglobin, hemoglobinuria, and hemoglobinemia, significant spherocytosis might not be evident. The overall figure of eight of 162 patients with immunoglobulin-induced hemolysis might appear to be very high, considering that PAD patients received low replacement immunoglobulin dosages. It should be mentioned that PAD patients have additional risk factors for hemolysis, in particular inflammatory conditions. It has been demonstrated that underlying inflammatory disorders might lead to enhanced immune responses to RBCs, similar to that described in patients with sickle cell disease.[24] Clinical infectious or inflammatory diseases characterizing the longlife course of PAD are a continuous inflammatory trigger in patients with a dysregulated immune system. PAD patients frequently experience infections and other clinical conditions including autoimmune manifestations.[25,26] Cytopenias might occur with a prevalence of 5% to 11%.[27] Here, we demonstrated that in PAD patients, beside autoimmune hemolytic anemias,[28] hemolysis might also be due to passive transfer of alloantibodies through immunoglobulin. RBC IgG alloantibodies present in the immunoglobulin can bind RBCs that might be removed by macrophages of the reticuloendothelial system with a subsequent extravascular hemolysis.[29]

Methods to improve IgG recovery and increase productivity have been implemented in the past few years as a response to growing clinical demand for immunoglobulin. In addition, approval has been granted for rapid administration and high concentration formulations of many immunoglobulin preparations and subcutaneous preparations. Hence, a significant shift in the immunoglobulin clinical landscape has been effected, and adverse effects, only detectable after market approval and large-level exposure to many patients, may result from these changes. In addition, most preparations now incorporate chromatographic purification in lieu of ethanol precipitation steps in the manufacture of immunoglobulin and the effect of omitting such steps, with their attendant potential of partitioning entities such as blood group substance–antibody complexes is uncertain. In a recent workshop convened by the US Food and Drug

Administration, manufacturers of immunoglobulin presented data suggesting that the anti-A reduction capacity of processes based on Cohn fractionation is at least two titer steps higher, as measured with the immune antiglobulin test, than processes not based on such traditional techniques[30] and that precipitation of Cohn Fraction III, a step commonly omitted in many modern processes, is particularly effective.[31] The effects of the recent changes in the immunoglobulin production and schedules of administration should be assessed in studies of drug surveillance, necessary to evaluate on large numbers of what it is initially reported on patients enrolled in the pivotal studies, usually in the absence of most of the main disease-associated clinical conditions, that affect the pharmacokinetics, efficacy, and tolerability of immunoglobulin treatments. It is likely that this will form part of a spectrum of measures requested by regulatory approval agencies to address this problem, along with suitable measures to screen out higher titer donors and to remove anti-A and anti-B through manufacture. Since this procedure has been suggested to prepare batches of IVIG to be used for immunomodulatory treatments, it would be possible that batches of IVIG with high RBC alloantibody high titers might remain in the market for patients under immunoglobulin at replacement dosages. According to our data and to our hypotheses, this topic should be better discussed. We conclude that in terms of safety, the issue of hemolysis in long-term recipients of immunoglobulin treatment administered at replacement dosages should be more widely recognized.

ACKNOWLEDGMENTS

The authors acknowledge Jeffrey Modell Foundation, Fondazione Lorillard Spencer Cenci, Progetto Ricerche Universitarie Sapienza, 2011, 2012. IQ takes primary responsibility for the paper. She contributed to obtaining funding, study design; analysis and interpretation of data; and writing, critical revision, review, and approval the manuscript. FP contributed to the collection of data and to performing the statistical analysis and analysis and interpretation of data; CM contributed to the collection of data and to performing the statistical analysis and analysis and interpretation of data; GG contributed to the collection of data; GG performed the laboratory work; FL contributed to the collection of data; AMP contributed to the collection of data; AF contributed to the critical revision of the paper, review, and approval the manuscript; SC analyzed and interpreted immunohematologic data and critically supervised laboratory results evaluation; and GG contributed to study concept and design and critical revision.

CONFLICT OF INTEREST

IQ declares the following conflict of interest: Baxter, Kedrion, CSL Behring; AF declares the following conflict of interest: PPTA. The other authors have disclosed no conflicts of interest.

REFERENCES

1. Orange JS, Hossny EM, Weiler CR, et al. Use of intravenous immunoglobulin in human disease: a review of evidence by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology. J Allergy Clin Immunol 2006;117:S525-53.
2. Yong PL, Boyle J, Ballow M, et al. Use of intravenous immunoglobulin and adjunctive therapies in the treatment of primary immunodeficiencies: a working group report of and study by the Primary Immunodeficiency Committee of the American Academy of Allergy Asthma and Immunology. Clin Immunol 2010;135:255-63.
3. Cunningham-Rundles C. Key aspects for successful immunoglobulin therapy of primary immunodeficiencies. Clin Exp Immunol 2011;164 Suppl 2:16-9.
4. Thorpe SJ, Fox BJ, Dolman CD, et al. Batches of intravenous immunoglobulin associated with adverse reactions in recipients contain atypically high anti-Rh D activity. Vox Sang 2003;85:80-4.
5. Baxley A, Akhtari M. Hematologic toxicities associated with intravenous immunoglobulin therapy. Int Immunopharmacol 2011;11:1663-7.
6. Daw Z, Padmore R, Neurath D, et al. Hemolytic transfusion reactions after administration of intravenous immune (gamma) globulin: a case series analysis. Transfusion 2008; 48:1598-601.
7. Rodeghiero F, Schiavotto C, Castaman G, et al. A follow-up study of 49 adult patients with idiopathic thrombocytopenic purpura treated with high-dose immunoglobulins and antiD immunoglobulins. Haematologica 1992;77: 248-52.
8. Michelis FV, Branch DR, Scovell I, et al. Acute hemolysis after intravenous immunoglobulin amid host factors of ABO-mismatched bone marrow transplantation, inflammation, and activated mononuclear phagocytes. Transfusion 2014;54:681-90.
9. Schroeder HW Jr, Dougherty CJ. Review of intravenous immunoglobulin replacement therapy trials for primary humoral immunodeficiency patients. Infection 2012;40: 601-11.
10. Desborough MJ, Miller J, Thorpe SJ, et al. Intravenous immunoglobulin-induced haemolysis: a case report and review of the literature. Transfus Med 2014;24:219-26.
11. Shoham-Kessary H, Naot Y, Gershon H. Immune complex-like moieties in immunoglobulin for intravenous use (i.v.Ig) bind complement and enhance phagocytosis of human erythrocytes. Clin Exp Immunol 1998;113: 77-84.
12. Kessary-Shoham H, Levy Y, Shoenfeld Y, et al. In vivo administration of intravenous immunoglobulin (IVIg) can lead to enhanced erythrocyte sequestration. J Autoimmun 1999;13:129-35.
13. Canadian Blood Services. Important information regarding IVIG associated hemolysis. 2009 [cited 2009 July 7].

Available from: http://www.bloodservices.ca/CentreApps/Internet/UW_V502_MainEngine.nsf/resources/CustomerLetters09/$file/CL_2009-02.pdf.
14. Thorpe SJ, Fox BJ, Dolman CD, et al. Anti-A and anti-B activity in batches of different intravenous immunoglobulin products determined using a direct haemagglutination method. Biologicals 2005;33:111-6.
15. Thorpe SJ, Fox B, Sharp G, et al. International collaborative study to evaluate candidate reference reagents to standardize haemagglutination testing for anti-A and anti-B in normal intravenous immunoglobulin products. Vox Sang 2009;97:160-8.
16. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol 1999;93:190-7.
17. European Directorate for the Quality of Medicines and Healthcare. Human normal immunoglobulin for intravenous administration. European pharmacopoeia. 7th Monograph 0918 ed. Strasbourg (France): Council of Europe; 2011. p. 4166-8.
18. European Directorate for the Quality of Medicines and Healthcare. Anti-A and anti-B haemagglutinins. European pharmacopoeia. 7th General chapter 2.6.20 ed. Strasbourg (France): Council of Europe; 2011. p. 151-99.
19. European Directorate for the Quality of Medicines and Healthcare. Test for anti-D antibodies in human immunoglobulin. European pharmacopoeia. 7th General chapter 2.6.26 ed. Strasbourg (France): Council of Europe; 2011. p. 3546.
20. Thorpe SJ, Fox B, Heath A, et al. International collaborative study to assess candidate reference preparations to control the level of anti-D in IVIG for use in Europe and the United States. Biologicals 2006;34:209-12.
21. Pintova S, Bhardwaj AS, Aledort LM. IVIG—a hemolytic culprit. N Engl J Med 2012;367:974-6.
22. Golla S, Horkan C, Dogaru G, et al. Rapid encephalopathy associated with anti-D immune globulin treatment for idiopathic thrombocytopenic purpura. Acta Haematol 2008;120:100-3.
23. Hudson KE, Lin E, Hendrickson JE, et al. Regulation of primary alloantibody response through antecedent exposure to a microbial T-cell epitope. Blood 2010;115:3989-96.
24. Brittain JE, Parise LV. Cytokines and plasma factors in sickle cell disease. Curr Opin Hematol 2007;14:438-43.
25. Chapel H, Lucas M, Lee M, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood 2008;112:277-86.
26. Ochtrop ML, Goldacker S, May AM, et al. T and B lymphocyte abnormalities in bone marrow biopsies of common variable immunodeficiency. Blood 2011;118:309-18.
27. Cunningham-Rundles C. The many faces of common variable immunodeficiency. Hematology Am Soc Hematol Educ Program 2012;2012:301-5.
28. Quinti I, Soresina A, Spadaro G, et al. Long-term follow-up and outcome of a large cohort of patients with common variable immunodeficiency. J Clin Immunol 2007;27:308-16.
29. Wong GK, Goldacker S, Winterhalter C, et al. Outcomes of splenectomy in patients with common variable immunodeficiency (CVID): a survey of 45 patients. Clin Exp Immunol 2013;172:63-72.
30. Romberg V. Effects of the manufacturing process on anti-A titers in IVIG. Presentation at Workshop "Strategies to Address Hemolytic Complications of Immune Globulin Infusions." US Food and Drug Administration 29 Jaunary 2014, Bethesda, MD, USA.
31. Nardini C. Anti-A and anti-B haemagglutinin tend analysis during manufacturing process of IVIG. Presentation at Workshop "Strategies to Address Hemolytic Complications of Immune Globulin Infusions." US Food and Drug Administration 29 January 2014, Bethesda, MD, USA.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's Web site:

Table S1. Individual data of patients enrolled in the short-term study.