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ORIGINAL RESEARCH

Isoagglutinin Reduction in Human Immunoglobulin Products by Donor Screening

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ABSTRACT

Introduction: Hemolysis is considered a class effect and a rare adverse event that can occur following therapy with human normal immunoglobulin for intravenous administration [i.e., intravenous immunoglobulin (IVIG)]. Anti-A/B isoagglutinins (also referred to as isohemagglutinins) originating from donor plasma are present in polyvalent immunoglobulin G (IgG) products and are considered a probable risk factor for hemolysis. We hypothesized that, by excluding plasma from donors with high isoagglutinin titers, the final IVIG product would have a meaningful reduction in anti-A/B isoagglutinin titers.

Methods: A method for screening donor plasma for anti-A isoagglutinins using an automated indirect agglutination test (IAT) was developed. A cut-off for donor plasma exclusion was defined. Industry-scale donor plasma pools and final IVIG product were prepared according to the manufacturing process of Privigen® (CSL Behring, Berne, Switzerland; human 10% liquid IVIG). Anti-A/B isoagglutinin content in pooled plasma and final IVIG product was measured by IAT, direct agglutination test, and a flow cytometry-based assay [fluorescence-activated cell sorting (FACS) anti-A].

Results: Screening of plasma from 705 donors identified 48 (6.8%) donors with high anti-A isoagglutinin titers in plasma (IAT agglutination score ≥2+ in a 1:200 pre-dilution). Exclusion of plasma from these donors resulted in a one-titer-step reduction of anti-A isoagglutinin in pooled plasma, confirmed by a twofold anti-A isoagglutinin concentration reduction measured by FACS anti-A (1,352 vs. 2,467 μg/g IgG). When the same screening and exclusion were applied to industrial-scale plasma pools (resulting in the exclusion of plasma from 5% of donors), anti-A isoagglutinins were reduced by one titer step in the final IVIG product. Anti-B isoagglutinins were also reduced by one titer step, as many donors with high anti-A isoagglutinins also have high anti-B.

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Conclusion: Reduction of anti-A/B isoagglutinin titers in IVIG products on an industrial scale is feasible through implementation of anti-A donor screening, which may reduce the risk of hemolysis following IVIG therapy.

Keywords: Anti-A/B isoagglutinin; Hematology; Hemolysis; Hemolytic risk; Isoagglutinin; Isohemagglutinins; Intravenous immunoglobulin (IVIG); Plasma donor screening; Privigen

INTRODUCTION

Antibody-mediated hemolytic reaction is a known, rare class effect of immunoglobulin (Ig) G therapy administered intravenously [i.e., intravenous immunoglobulin (IVIG)]. Most of the reported IVIG-related cases are subclinical and self-limited; however, severe and/or serious cases have been reported [1, 2]. Known risk factors for IVIG-related hemolysis are: non-O blood group, high doses of IVIG therapy, and an underlying inflammatory state [1–5]. Characterization of risk factors and development of prevention methods are currently complicated by the absence of a commonly accepted definition of *clinically relevant* hemolysis.

In the majority of IVIG-related cases of hemolysis, anti-A/B isoagglutinins (also referred to as isohemagglutinins) have been suspected or shown to be involved [5–7]. Anti-A/B isoagglutinins originating from donor plasma are passively transferred during administration of IVIG. The role of anti-A/B isoagglutinins is supported by the very low occurrence of hemolysis in patients with O blood group. Patients with A blood group tend to have higher incidence of IVIG-related hemolytic reactions than B blood group patients [4], which suggests greater relevance of anti-A isoagglutinin. Isoagglutinin concentrations are lower in some IVIG products prepared by ethanol fractionation, as that process leads to significant reduction of isoagglutinins [6]. Modern Ig purification processes are designed to keep the full antibody repertoire of the donated plasma and thus do not reduce isoagglutinins. An increase in the frequency of reported hemolytic events with IVIG in recent years [4] may be in part due to the introduction of these modern Ig purification processes, leading to higher isoagglutinin titers, in addition to the generally increased use of IVIG products.

Screening and exclusion of donors with high anti-A/B isoagglutinin titers proved to be effective in preventing hemolytic reaction after ABO-incompatible platelet transfusion [7]. Screening for either anti-A or anti-B isoagglutinin alone may be sufficient to identify candidate donors for exclusion, as high titers of anti-A/B isoagglutinins tend to correlate in individual donors [8, 9]. To develop a screening program that can be used on an industrial scale, it is necessary to define a cut-off anti-A/B isoagglutinin level that produces isoagglutinin titer reduction in the final IVIG product with exclusion of a relatively small proportion of donors.

Versions of the indirect agglutination tests (IAT) and direct agglutination tests (DAT) [10] have been used to detect anti-A/B isoagglutinins in donor plasma [5, 7, 9] and IVIG products [8]. All the methods have limitations, and the inter-assay variability remains high [8, 11]; therefore, most results are not directly comparable, particularly when obtained with different methods. International reference material [specified in the European Pharmacopoeia

(PhEur)] has been established for DAT in IVIG products only [10]. The lack of overall standardization adds to the difficulty of developing measures of isoagglutinin reduction and of evaluating their effectiveness.

Privigen® (CSL Behring, Berne, Switzerland) is a 10% liquid preparation of polyclonal human IgG for intravenous administration, stabilized with L-proline [12]. Privigen is indicated for replacement therapy in patients with primary immunodeficiency and for immunomodulation in patients with immune thrombocytopenic purpura in the United States (US) [13]. In Europe, the list of indications also includes secondary immunodeficiency and selected immune-mediated diseases, such as chronic inflammatory demyelinating polyneuropathy, Guillain–Barré syndrome, and Kawasaki disease [14].

We hypothesized that the titer of anti-A/B isoagglutinins in IVIG products can be reduced by donor screening and exclusion of plasma with high anti-A isoagglutinin titers. Here, we report the effect of such anti-A isoagglutinin donor screening on anti-A/B isoagglutinin titers in industry-scale donor plasma pools and the final IVIG product (i.e., Privigen).

METHODS

This article does not contain any new studies with human or animal subjects performed by any of the authors.

Method Development: Anti-A Isoagglutinin Donor Screening

Donors were randomly selected for screening from regular plasmapheresis donors at CSL Plasma centers in the US. Plasma donors gave written consent that their plasma may be used for research purposes.

Indirect Isoagglutinin Test in Gel Cards

Anti-A isoagglutinin titer in plasma of individual donors was measured by IAT in gel cards (IAT-GC) containing microcolumns filled with dextran acrylamide gel and rabbit anti-human-IgG antibodies [ID-Micro Typing System™ (ID-MTS); Ortho Clinical Diagnostics (OCD), Rochester, NY, USA]. The testing was set up as a high-throughput, automated assay, using a Freedom EVO® programmable unit (Tecan Group Ltd., Männedorf, Switzerland) for sample predilution and a ProVue™ instrument (OCD) for sample processing. Plasma samples were prediluted 1:200 with 0.9% sodium chloride from primary tubes into barcoded secondary tubes. Prediluted samples were transferred into the ID-MTS gel cards and incubated at 37 °C with 0.8% ready-to-use suspension of A_1 blood group red blood cells (RBC; Affirmagen®; OCD) according to the manufacturer's instructions, followed by centrifugation. Slower sedimentation of RBC in the gel due to agglutination caused by reaction with the anti-human IgG allowed discriminating between samples with different anti-A isoagglutinin titers. RBC agglutination strength was detected automatically for each sample, according to a predefined RBC localization pattern: a well-defined pellet indicating no agglutination (agglutination score 0), predominantly localized in the lower half of the tube (1+), dispersed throughout the tube (2+), predominantly localized in the upper half of the tube (3+), solid band of RBC on the top of the gel (4+; Fig. 1). The endpoint anti-A isoagglutinin titer was defined as the reciprocal of the highest dilution with agglutination score ≥1+ in a 1:200 predilution. Samples with mixed readout were re-analyzed.

Donor plasma minipools were prepared by mixing 20 μL of plasma from each donor

Fig. 1 Indirect agglutination test in gel cards. Red blood cell localization patterns resulting from the indirect agglutination test in ID-Micro Typing System™ gel cards (Ortho Clinical Diagnostics, Rochester, NY, USA). Agglutination scores below the tubes indicate agglutination strength, from 0 (no agglutination) to 4+ (strongest agglutination). The *rightmost column* shows an example of a mixed readout (MF)

included in the development study (thus mimicking plasma pool preparation during Privigen production), with exclusion of donor plasma with agglutination scores ≥1+, ≥2+, or ≥3+. Anti-A isoagglutinin titer in minipools was measured by IAT-GC and an optimized IAT method in microtiter plates (IAT-PhEur/MTP) [10]. Double serial dilutions were used for titration; therefore, one titer step corresponded to a twofold change in isoagglutinin titer. Non-anti-A-screened plasma (a minipool prepared from plasma of all tested donors) was used for comparison in all tests.

Flow Cytometry-Based Anti-A Isoagglutinin Binding Assay

The concentration of anti-A isoagglutinin in donor plasma minipools was measured with a flow cytometry-based anti-A isoagglutinin binding assay [fluorescence-activated cell sorting (FACS) anti-A]. Prediluted plasma samples were incubated for 60 min at room temperature with A blood group RBC (a standard RBC preparation that was obtained from a single donor and was used in all tests). RBC-bound anti-A isoagglutinin was detected by subsequent incubations with biotinylated anti-human-IgG antibodies (no cross-reaction with IgM; Nordic Immunological Laboratories, Tilburg, the Netherlands) and with fluorescently labeled streptavidin. The median fluorescence intensity was quantified with a FACSCanto™ II cell analyzer (Becton-Dickinson AG, Allschwil, Switzerland). Anti-A isoagglutinin concentration was calculated based on a standard curve prepared with affinity-purified polyclonal human anti-A antibodies and was expressed in μg/g of total IgG.

Implementation on a Production Scale: Donor Plasma Pools and the Final IVIG Product

All plasma pools and final IVIG product lots were prepared according to the Privigen manufacturing process. Plasma pool lots were prepared by mixing up to 12,000 plasma

donations per lot using normal US source plasma obtained from CSL Plasma. Each final IVIG product lot was prepared from 3–5 plasma pool lots (16,000–60,000 plasma donations).

The anti-A-screened and non-anti-A-screened lots of both plasma pools and final IVIG product were prepared with or without implementation of the anti-A donor screening, respectively. The anti-A-screened plasma pool lots used for anti-A/B isoagglutinin testing were drawn randomly and do not necessarily match the plasma pool lots (also anti-A-screened) that were used for production of the anti-A-screened final IVIG product.

Anti-A/B Isoagglutinin Detection in Plasma Pools

Titers of anti-A/B isoagglutinins were measured in 120 anti-A-screened and 72 non-anti-A-screened plasma pool lots by IAT-GC (ID-Card Coombs Anti-IgG; Bio-Rad Laboratories-DiaMed GmbH, Cressier, Switzerland), according to the manufacturer's instructions. Undiluted plasma pool samples were titrated by double serial dilution below the anticipated detection limit of isoagglutinin. The dilutions were transferred into microcolumns attached to gel cards and incubated for 15 min at 37 °C with A_1 or B blood group RBC (ID-DiaCell ABO; Bio-Rad Laboratories–DiaMed GmbH), followed by centrifugation. The agglutination strength in each dilution was scored according to the RBC localization patterns as described above. The highest dilution with agglutination score $\geq$1+ was considered the endpoint titer.

Anti-A/B Isoagglutinin Detection in the Final IVIG Product

The concentration of anti-A/B isoagglutinins in the final IVIG product was measured according to PhEur 2.6.20, methods A and B [10]. A total of 30 anti-A-screened Privigen lots, 651 historical non-anti-A-screened Privigen lots and additional 30 non-anti-A-screened lots of final IgG products [Privigen or Hizentra® (CSL Behring, Berne, Switzerland), IgG purified by the same process, but formulated as 20% solution for subcutaneous use] were analyzed.

Method A: indirect agglutination assay in microtiter plates (IAT-PhEur/MTP) Twofold serial dilutions of the final IVIG product (starting from 30 g/L of total Ig) were incubated for 30 min at 37 °C with either A_1 or B blood group RBC in a microtiter plate. After removing non-specific IgG in a washing step (3 washes with centrifugation for 1 min at 300*g* at room temperature), anti-human globulin reagent Pelikloon™ (Sanquin Reagents, Amsterdam, the Netherlands) was added and mixed well with the RBC. Agglutination of the sedimented RBC was assessed macroscopically by inspection of the microtiter plate well. The highest dilution with visible RBC agglutination was considered the endpoint titer.

Method B: direct agglutination test Twofold serial dilutions of the final IVIG product (starting from 50 g/L of total Ig) were mixed with papain-treated A_1 or B blood group RBC in a microtiter plate, followed by centrifugation for 1 min at 80*g* at room temperature. After centrifugation the microtiter plate was placed at 70° angle, and agglutination strength was assessed by RBC localization pattern: streaming vs. non-streaming. The highest dilution with visible RBC agglutination (non-streaming pattern) was considered the endpoint titer.

FACS anti-A Anti-A isoagglutinin concentration in 10 anti-A-screened Privigen lots and 10 non-anti-A-screened lots of final IgG products (Privigen or Hizentra) was measured by the FACS anti-A assay as described above.

RESULTS

Method Development: Anti-A Isoagglutinin Donor Screening

A total of 705 plasma samples collected from randomly selected individual donors were tested for anti-A isoagglutinin titers using IAT-GC. All reported agglutination scores for single-donor plasma are based on a 1:200 pre-dilution. A small proportion of donors ($n = 48$; 6.8%; Table 1, excluded donors) were identified as having high anti-A isoagglutinin levels [agglutination score 2+ (5.4%) or 3+ (1.4%); Fig. 2]. The rest of the donors were either negative or weakly positive for anti-A isoagglutinin [agglutination score 0 (81%) or 1+ (12.2%)]. No donors with agglutination score 4+ were identified (Fig. 2).

Plasma-donor minipools were prepared based on results of the anti-A donor screening. Exclusion of donor plasma with agglutination score 3+ did not result in a measurable anti-A reduction (as determined by IAT-GC; Fig. 3), confirmed by a decrease of only about 20% in anti-A isoagglutinin concentration measured by FACS anti-A. Exclusion of donor plasma with agglutination scores ≥2+ resulted in reduction

Table 1 Plasma minipools based on the anti-A donor screening

	Minipool 1	Minipool 2	Minipool 3	Minipool 4
Minipool composition	All donors	Medium, low or negative scores	Low or negative scores	Negative scores
Included donors, *n*	705	695	657	571
Excluded donors, *n*	0	10	48	134
% of all donors	0.0	1.4	6.8	19.0
Anti-A agglutination score(s)	N/A	3+, 4+	2+, 3+, 4+	1+, 2+, 3+, 4+
Minipool titration: serial double dilutions tested by IAT-GC (OCD)				
Anti-A score(s)				
Titer 1:8	3+	3+	2+	1+
Titer 1:16	2+	2+	2+	1+
Titer 1:32	2+	1+	1+	(–)
Titer 1:64	1+	1+	(–)	(–)
Titer 1:128	(–)	(–)	(–)	(–)
Minipools tested by FACS for anti-A (μg/g IgG)				
Mean (SD)	2,467 (134)	2,006 (207)	1,352 (110)	673 (241)
Ratio vs. non-anti-A-screened plasma, %	100.0	81.3	54.8	27.3

Minipools were prepared by mixing equal amounts (20 μL) of each donor plasma thus mimicking a manufacturing pool (Minipool 1). Donors with anti-A agglutination scores 1+, 2+, 3+ (at 1:200 pre-dilution) were gradually excluded (Minipools 2–4), and the effect of reduction in anti-A content was shown by titrating each minipool in serial double dilutions

FACS anti-A Flow cytometry-based anti-A isoagglutinin binding assay, *IAT-GC* indirect agglutination test in gel cards, *IgG* Immunoglobulin G, *n* number of donors, *N/A* not applicable, *OCD* Ortho Clinical Diagnostics (Rochester, NY, USA), *SD* standard deviation

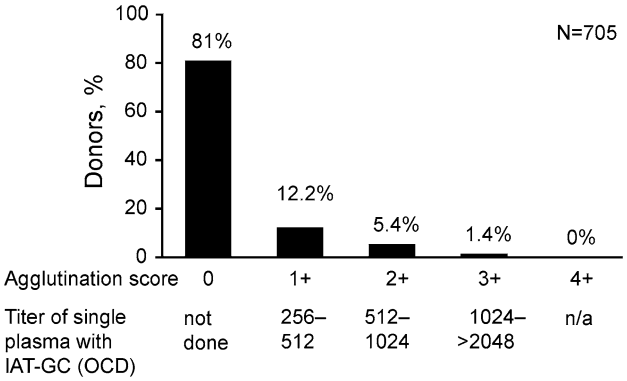

Fig. 2 Distribution of anti-A in donor population (method development). Anti-A isoagglutinin titers in plasma of a representative number of randomly selected donors ($N = 705$) were assessed by the indirect agglutination test in gel cards [Ortho Clinical Diagnostics (OCD), Rochester, NY, USA]. Distribution of donors with agglutination scores from 0 to 4+ (in a 1:200 pre-dilution) are shown as columns. No donors with agglutination score 4+ were found. The corresponding endpoint titers from serial double dilutions of individual plasma samples are shown below the graph. *IAT-GC* Indirect agglutination test in gel cards

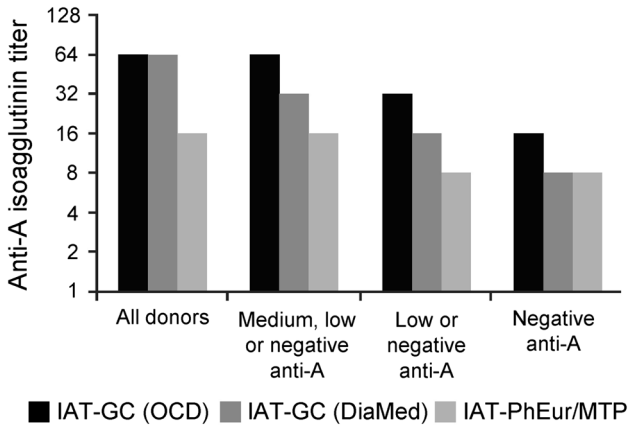

Fig. 3 Anti-A isoagglutinin titers in donor plasma minipools (method development). Plasma minipools were prepared from plasma samples of all screened donors ($N = 705$), donors with medium, low or negative anti-A (agglutination score $\leq 2+$; $n = 695$), donors with low or negative anti-A (agglutination score $\leq 1+$; $n = 657$), or from plasma of anti-A-negative donors only ($n = 571$). Anti-A isoagglutinin titers of each minipool were determined by the indirect agglutination test in gel cards (IAT-GC) obtained from Ortho Clinical Diagnostics (OCD, Rochester, NY, USA; *black columns*) or Bio-Rad Laboratories-DiaMed GmbH (DiaMed; *gray columns*), or in microtiter plates (IAT-PhEur/MTP; *light-gray columns*)

of the anti-A isoagglutinin titer compared with non-anti-A-screened plasma by one titer step. The decrease in anti-A isoagglutinin titer was confirmed by an approximately twofold reduction of anti-A isoagglutinin concentration measured by FACS anti-A ($1{,}352 \pm 110$ vs. $2{,}467 \pm 134$ μg/g IgG). Exclusion of all anti-A-positive donor plasma reduced the anti-A isoagglutinin titer by 2 titer steps and anti-A isoagglutinin concentration measured by FACS anti-A by approximately fourfold.

Exclusion of donor plasma with agglutination scores $\geq 2+$ resulted in a relevant reduction in anti-A/B isoagglutinins by one titer step, with an

acceptable rate of donor exclusions (6.8%). Exclusion of donors with plasma agglutination score 3+ would not result in a meaningful reduction of anti-A/B isoagglutinins. Exclusion of all anti-A positive donors with scores ≥1+ would lead to exclusion of approximately 20% of donor plasma and was considered not feasible at production scale. Thus, the cut-off agglutination score of ≥2+ was selected for further investigation on a production scale.

Implementation on a Production Scale: Donor Plasma Pools and the Final IVIG Product

The anti-A isoagglutinin donor screening was applied to the Privigen manufacturing process to test whether anti-A/B isoagglutinin reduction could be achieved in plasma pools and/or in the final IVIG product. Testing of a large number of donors confirmed the isoagglutinin distribution in the donor population seen in the experimental study. Of 188,337 screened donors, 94.9% had low anti-A isoagglutinin titers (agglutination score 1+ or 0) and 5.1% had either medium (2+; 3.6%) or high (3+; 1.5%) anti-A isoagglutinin titers.

Anti-A/B Isoagglutinin Concentration in Plasma Pools

Both the highest and most frequent anti-A isoagglutinin titer observed in 120 anti-A-screened plasma pool lots was 1 titration step lower than in 72 non-anti-A-screened lots (1:32 vs. 1:64; Fig. 4). The highest anti-B isoagglutinin titer observed in the same set of anti-A-screened plasma pool lots was one titration step lower than in the non-anti-A-screened lots (1:16 vs. 1:32; Fig. 4).

Anti-A/B Isoagglutinin Concentration in the Final IVIG Product

In 30 anti-A-screened Privigen lots analyzed by IAT-PhEur/MTP, both the highest (1:16 in 10% of lots) and the most frequent (1:8 in 90% of lots) anti-A isoagglutinin titers were reduced by 1 titer step compared with 651 historical, non-anti-A-screened lots (Fig. 5). Similarly, a two-step reduction of the highest anti-B isoagglutinin titer was observed in the anti-A-screened set (1:8 in 16.7% of lots) versus non-anti-A-screened set (1:32 in 0.2% of lots). The

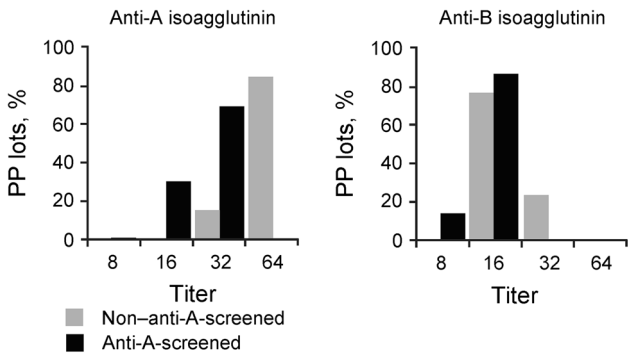

Fig. 4 Anti-A/B isoagglutinin titers in anti-A-screened and non-anti-A-screened donor plasma pool lots (production scale). Donor plasma pool lots were prepared either with implementation of the anti-A donor screening (anti-A-screened; *black columns*; 120 lots) or from plasma of all available donors (non-anti-A-screened; *gray columns*; 72 lots). Each final intravenous immunoglobulin product lot was prepared from 16,000 to 60,000 plasma donations. Anti-A/B isoagglutinin titers of each lot were determined by the indirect agglutination test in gel cards (DiaMed GmbH, Cressier, Switzerland). *PP* Plasma pool

geometric mean of anti-A (1:8.6 vs. 1:13.8) and anti-B isoagglutinin titers (1:4.5 vs. 1:7.3) was reduced by approximately one titer step.

The highest anti-A and anti-B isoagglutinin titers measured by DAT in 30 anti-A-screened Privigen lots (1:16–1:32 for anti-A; 1:8–1:16 for anti-B) were reduced by 1 titer step vs. 30 "non-screened" Privigen lots (1:16–1:64 for anti-A; 1:8–1:32 for anti-B), with the same median titers in both sets (1:32 and 1:16 for anti-A and anti-B isoagglutinin, respectively). The reference preparations established for the DAT in PhEur were assayed in each test run and complied with the PhEur specifications (1:32–1:64; data not shown). All IVIG product lots met the specified titers for anti-A and anti-B.

Reduction of anti-A isoagglutinin concentration upon implementation of the anti-A donor screening was confirmed by FACS anti-A in 10 anti-A-screened and 10 non-anti-A-screened Privigen lots. The mean anti-A isoagglutinin concentration was reduced 1.37-fold (1,220.3 μg/g IgG in the non-anti-A-screened lots vs. 893.2 μg/g IgG in anti-A-screened lots), which corresponds to approximately 0.5 titer steps.

DISCUSSION

We have developed an anti-A donor screening assay based on measurement of anti-A

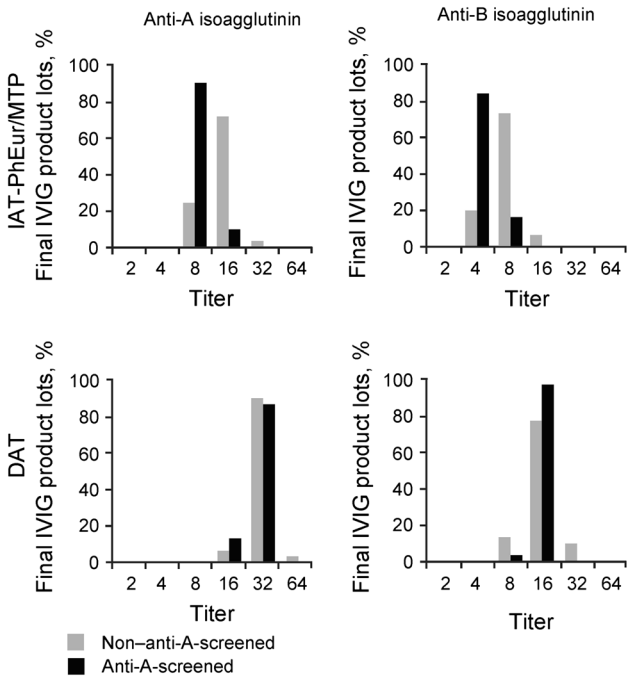

Fig. 5 Anti-A/B isoagglutinin titers in anti-A-screened and non-anti-A-screened final intravenous immunoglobulin (IVIG) product lots (production scale). Final IVIG product lots were prepared either with implementation of the anti-A donor screening (anti-A-screened; *black columns*) or from plasma of all available donors (non-anti-A-screened; *gray columns*). Each final IVIG product lot was prepared from 16,000 to 60,000 plasma donations. Anti-A/B isoagglutinin endpoint titers of 30 anti-A-screened and 651 historical, non-anti-A-screened Privigen lots were determined by the indirect agglutination test in microtiter plates (IAT-PhEur/MTP). The 30 anti-A-screened Privigen lots and additional 30 non-anti-A-screened lots of the final immunoglobulin G products (Privigen® or Hizentra®; both CSL Behring, Berne, Switzerland) were analyzed by the direct agglutination test (DAT)

isoagglutinin titers in donor plasma with IAT. A cut-off agglutination score ≥2+ in a 1:200 pre-dilution of plasma allowed exclusion of a small proportion of donated plasma (approximately 5%) with the highest anti-A isoagglutinin titers. The anti-A donor screening resulted in anti-A isoagglutinin titer reduction by one titer step both in industry-scale plasma pool lots and in the final IVIG product (i.e., Privigen). A similar one-titer-step reduction was observed for anti-B isoagglutinin titers, consistent with the known correlation of high anti-A and anti-B isoagglutinin titers in O blood group donors [8, 9]. These results strongly suggest that testing donors for anti-A isoagglutinin alone is sufficient for reduction of both anti-A and anti-B isoagglutinin in final IVIG products.

We presumed that a single test would be sufficient for each donor, rather than testing each donation, as isoagglutinin titers are not expected to change much over time [15]. All subsequent plasma donations from a donor with isoagglutinin titers above the cut-off would then be excluded from the production of IVIG, but not necessarily from the production of other plasma products, such as albumin or coagulation factors. The anti-A donor screening assay proved to be compatible with the Privigen production process on an industrial scale, suggesting that donor screening can be routinely applied to IVIG production. Comparison of the anti-A-screened and non-anti-A-screened plasma pool and final IVIG product lots was purely descriptive due to the very different numbers of lots, time of plasma collection and homogeneity of the donor groups. Nonetheless, we consider this comparison to be valid, as the same standardized plasma collection and storage conditions, as well as isoagglutinin detection methods, were used for all samples. We concluded that by donor screening and exclusion of plasma from donors with high anti-A isoagglutinin titers, both anti-A and anti-B isoagglutinins can be reduced by one titer step (i.e., the concentration can be reduced by 50%).

Privigen and most other IVIG products consist of purified IgG with only trace amounts of IgM, which excludes the majority of anti-A/B isoagglutinin molecules. The screening assay used here may have led to exclusion of plasma from donors with high concentrations of IgM isoagglutinins only. By excluding these donors without further screening, we adopted a conservative approach, even though the anti-A isoagglutinin titer of the final IVIG product would not have been affected by inclusion of plasma from such donors.

The clinical relevance of a one-titer-step anti-A/B reduction in IVIG is currently unknown and can be determined only in large trials. Hemolytic events tend to occur during high-dose IVIG therapy (1–2 g/kg), whereas very few cases have been reported in patients with primary immunodeficiency, who are usually treated with lower IgG doses (0.4–0.8 g/kg) [4]. This observation supports our hypothesis that a reduction of isoagglutinins by 1 titer step (or to 50% of the initial concentration) may be clinically important. A one-titer-step reduction was detected by IAT, while DAT and FACS anti-A results suggested a lesser reduction. All these methods have their limitations; the reason for the discrepancy is currently unknown. A prospective, observational clinical study is warranted to evaluate the efficacy of donor plasma screening on IVIG hemolytic risk.

Implementation of isoagglutinin screening as described here is possible for plasma collectors operating large plasmapheresis centers. This screening has been implemented by CSL Plasma in all its plasma collection

centers. However, multiple plasma sources are typically used for IVIG production, which may include recovered plasma obtained from local organizations. Implementation of a new screening program for these plasma sources may be difficult and is not entirely within the reach of IVIG manufacturers. Therefore, new methods for anti-A/B isoagglutinin quantification are currently in development (such as FACS anti-A).

CONCLUSION

This study confirms, using the example of Privigen, that reduction of anti-A/B isoagglutinin titers in IVIG products through anti-A donor plasma screening is feasible on an industrial scale. Implementation of isoagglutinin donor plasma screening with exclusion of donations with high titers may reduce the risk of hemolysis in patients treated with IVIG.

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Conflict of interest. Brigitte Siani is an employee of CSL Behring. Katharina Willimann is an employee of CSL Behring. Sandra Wymann is an employee of CSL Behring. Adriano A. Marques is an employee of CSL Behring. Eleonora Widmer is an employee of CSL Behring.

Compliance with ethics guidelines. This article does not contain any new studies with human or animal subjects performed by any of the authors. Plasma donors gave written consent that their plasma may be used for research purposes.

REFERENCES

1. 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.

2. Divan H, Menis M, Sridhar G, et al. Occurrence of hemolytic reactions (HRs) on the same day as immune globulin (IG) product administrations during 2008–2012 (Poster presented at 29th ICPE congress). 2013.

3. Padmore RF. Hemolysis upon intravenous immunoglobulin transfusion. Transfus Apher Sci. 2012;46:93–6.

4. Desborough MJ, Miller J, Thorpe SJ, Murphy MF, Misbah SA. Intravenous immunoglobulin-induced haemolysis: a case report and review of the

literature. Transfus Med. 2013 (Epub ahead of print).

5. MacLennan S. High titre anti-A/B testing of donors within the National Blood Service (NBS). Information document INF/MED/MA/004/02. 2006.

6. Kahwaji J, Barker E, Pepkowitz S, et al. Acute hemolysis after high-dose intravenous immunoglobulin therapy in highly HLA sensitized patients. Clin J Am Soc Nephrol. 2009;4:1993–7.

7. Quillen K, Sheldon SL, Daniel-Johnson JA, Lee-Stroka AH, Flegel WA. A practical strategy to reduce the risk of passive hemolysis by screening plateletpheresis donors for high-titer ABO antibodies. Transfusion. 2011;51:92–6.

8. Thorpe SJ, Fox BJ, Dolman CD, Thorpe R. Anti-A and anti-B activity in batches of different intravenous immunoglobulin products determined using a direct haemagglutination method. Biologicals. 2005;33:111–6.

9. Josephson CD, Mullis NC, Van DC, Hillyer CD. Significant numbers of apheresis-derived group O platelet units have "high-titer" anti-A/A, B: implications for transfusion policy. Transfusion. 2004;44:805–8.

10. Anti-A and anti-B hemagglutinins (indirect and direct methods). Monograph 07/2011:20620. European Pharmacopoeia. 7th ed. (7.2). Strasbourg: Directorate for the Quality of Medicines of the Council of Europe. 2011.

11. Bellac CL, Polatti D, Hottiger T, et al. Anti-A and anti-B haemagglutinin levels in intravenous immunoglobulins: are they on the rise? A comparison of four different analysis methods and six products. Biologicals. 2014;42:57–64.

12. Cramer M, Frei R, Sebald A, Mazzoletti P, Maeder W. Stability over 36 months of a new liquid 10% polyclonal immunoglobulin product (IgPro10, Privigen) stabilized with L-proline. Vox Sang. 2009;96:219–25.

13. Immune Globulin Intravenous [Human] 10% Privigen, Prescribing Information. 2010. http://www.privigen.com/Professional/Prescribing-Information.aspx. Accessed 13 Sep 2012.

14. EMEA. Privigen: EPAR—Product information (summary of product characteristics). 2013. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000831/WC500043077.pdf. Accessed 31 Jan 2014.

15. Thomson O. Die Stärken der menschlichen Isoagglutinine und entsprechende Blutkörperchernrezeptoren in verschiedenen Lebensaltern. Z Immunitätsforsch. 1929;63:67–93.