Anti-A/B isoagglutinin reduction in an intravenous immunoglobulin product and risk of hemolytic anemia: a hospital-based cohort study

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BACKGROUND: Intravenous immunoglobulins (IVIG) are derived from large human plasma pools. IVIG-associated hemolytic anemia (HA) is a known class effect, likely attributed to dose-dependent passive transfer of anti-A/B isoagglutinins. Two isoagglutinin reduction steps were implemented in the manufacturing process of Privigen (human 10% liquid IVIG): exclusion of high–anti-A–titer donors in 2013, replaced by specific immunoaffinity chromatography in 2015. We aim to estimate the clinical effectiveness of both measures.

STUDY DESIGN AND METHODS: Using the US hospital-based Premier Healthcare Database, three Privigen cohorts were generated based on calendar periods indicative of manufacturing changes: Period 1 (baseline) January 2008 to December 2012, Period 2 (high–anti-A–titer donor exclusion) October 2013 to December 2015, and Period 3 (immunoaffinity chromatography) October 2016 to April 2019. HA within a 10-day at-risk period after Privigen administrations was identified from review of patient record summaries. Incidence rate ratios (IRRs) were estimated from Poisson regression (Period 1 reference) adjusting for hospital setting, sex, age, Privigen indication, dose, and first use.

RESULTS: Crude incidence rates of HA were 1.49 per 10,000 person-days in Period 1 (38 HA, 9439 patients), 1.01 in Period 2 (20 HA, 7710 patients), and 0.14 in Period 3 (3 HA, 7759 patients). Adjusted IRR for HA in Period 2 was 0.71 (95% confidence interval [CI], 0.41-1.23), and in Period 3 was 0.10 (0.03-0.33) compared with Period 1. The IRR for HA in Period 3 compared with Period 2 was 0.14 (95% CI, 0.04-0.47).

CONCLUSION: Implementation of immunoaffinity chromatography in Privigen manufacturing resulted in a significant 90% reduction of HA risk. HA has become a rare event in association with Privigen use.

Intravenous immunoglobulin (IVIG) products are derived from large human plasma pools. IVIG was developed to treat patients of all ages with primary immune deficiency. IVIG has increasingly been used for the treatment of secondary immune deficiency and in higher immunomodulatory doses for the treatment of various autoimmune and inflammatory diseases, such as immune thrombocytopenia, chronic inflammatory demyelinating polyneuropathy, Guillain-Barré syndrome and Kawasaki disease.[1]

Hemolytic anemia (HA), presenting as acute or delayed HA, is a known adverse event associated with IVIG use, mainly seen in those with an underlying inflammatory disease receiving high cumulative IVIG doses.[2–4] Acute HA develops within 24 hours and delayed reactions within 3 to 30 days after the IVIG transfusion.[5]

ABBREVIATIONS: CI = Confidence interval; DAT = Direct antiglobulin test; HA = hemolytic anemia; IAT = Indirect antiglobulin test; IVIG = Intravenous immunoglobulin; IRR(s) = incidence rate ratio(s); PHD = Premier Healthcare Database.

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Hypothesized mechanisms for HA occurrence after IVIG administration are the dose-dependent passive transfer of A/B isoagglutinins to non-O blood group patients, and the enhanced activity of the immune system in patients with an underlying inflammatory state, with accelerated removal of sensitized red blood cells from the circulation. The latter mechanism has been supported by the observation of IVIG-associated hemolytic reactions in patients with serologic evidence of inflammatory conditions including pneumonia, Kawasaki disease, and juvenile dermatomyositis.[4,6–11] Some studies have reported the incidence of HA per number of patients treated with IVIG,[8,11,12] but none have provided the rate of IVIG-associated HA per administered IVIG. IVIG-associated crude hemolysis incidence rates derived from published literature range between 2.1 and 2.8 per 1000 IVIG administrations depending on IVIG product.[8,12] Crude incidence rates of HA and of hemolysis may depend on the patient's background risk of HA, due to the presence of other independent predictors of HA, such as lymphoproliferative disorders, solid organ transplantation, concomitant transfusions of blood, and blood products.[13] Higher doses are more likely to be associated with hemolysis as is non-O blood group.[8,13] After the hypothesized mechanisms of HA, the incidence rate of HA attributed to IVIG use could be primarily decreased by reducing the amount of anti-A/B isoagglutinins in the IVIG product.

Privigen (IgPro10, CSL Behring) is an IVIG 10% liquid stabilized with proline. The Privigen production process includes cold ethanol fractionation, octanoic acid fractionation, and anion-exchange chromatography.[14] It was first approved in the United States in 2007 and marketed since 2008. Initially, the Privigen manufacturing process did not include an isoagglutinin reduction step. Between 2013 and 2016 two independent isoagglutinin reduction measures were implemented in the manufacturing of Privigen to decrease the quantity of isoagglutinin in the product and thereby to decrease the risk of HA. A temporary measure to screen for and exclude high–anti-A–titer donors from pooled plasma, implemented from 2013 to 2015, which was found to have some clinical effectiveness.[15,16] This measure was progressively replaced by a specific immunoaffinity chromatography step.[17]

In vitro assays have shown a not more than one-titer step reduction of median anti-A and anti-B titers in lots produced after the exclusion of plasma from donors, and a two-titer step reduction (from median anti-A of 32 and anti-B of 16 to 8 and 4, respectively) in lots manufactured after the implementation of the immunoaffinity chromatography, compared with lots produced before the implementation of the two isoagglutinin reduction measures.[15,17] The immunoaffinity chromatography step did not change other product characteristics[17] and efficacy was similar in animal models.[18] The objective of this study was to measure the clinical effectiveness of both HA risk minimization measures in the manufacturing of Privigen.

MATERIALS AND METHODS

Setting and data source

The study patients' data were provided from the US Premier Healthcare Database (PHD). It includes approximately one-sixth of all hospital discharges in the United States. Patients can be tracked across the inpatient and hospital outpatient settings at the same facility, hospital-owned clinics, and emergency rooms using a unique person identifier. Patient demographic characteristics (age, sex, ethnicity, and region), discharge diagnoses, and discharge status (including death, but not its cause) are available for all PHD hospitals. Service-level data include date-stamped logs for all billed items for each patient, including medications and laboratory, diagnostic, and therapeutic services. Laboratory results are not available. Hospital discharge diagnoses are coded with ICD-9 CM up to September 2015 and subsequently with ICD-10 CM codes, while medications prescribed at the visit are identified using a text description of the product and dose from the hospital encounter charge details file. All procedures and diagnoses are captured for each patient as well as all drug utilization information.

Study design and population

This is a retrospective cohort study based on data extracted in September 2019 for patients of any age with at least one administration of Privigen in the inpatient or outpatient setting between January 1, 2008, and April 30, 2019. Three study periods were defined based on the implementation status of manufacturing changes to Privigen: Period 1 (baseline period), January 1, 2008, to December 31, 2012; Period 2 (after exclusion of high–anti-A–titer donors), October 1, 2013, to December 31, 2015, and Period 3 (after implementation of the immunoaffinity chromatography), October 1, 2016, to April 30, 2019.

The date of the first Privigen administration whether as inpatient or outpatient defined the patient's cohort entry date in each study period (and the start date of the first treatment episode). The observational period for each treatment episode started with the first administration of Privigen and ended with the earliest of: 1) the end of the at-risk period, defined as 10 days after the last administration of Privigen; 2) the start of treatment with another IVIG; 3) the occurrence of an HA event; 4) the end of the respective study period; or 5) death. Patients with a history of HA any time before the first administration of Privigen were excluded.

Exposure of interest

The exposure of interest was treatment with Privigen, determined for each study subject and from all records in the database during the entire study period. Privigen treatment episodes were defined by consecutive daily administrations

of Privigen. A gap of more than 1 day between Privigen administrations constituted a new treatment episode.

The amount of each Privigen administration was calculated from billing data. The first step consisted of the manual extraction of the vial size and amount of administered Privigen available in structured and unstructured data fields and the assessment of the quantity of ordered Privigen. Then, the extracted vial size/amount was multiplied with the respective quantity variable. All Privigen administrations received during a single Privigen treatment episode were summed to yield a total cumulative quantity received for that episode.

Because body weight is not captured in the database, the cumulative Privigen dose in g/kg body weight was estimated for each treatment episode separately by dividing the cumulative Privigen quantity administered during that episode with the corresponding age- and sex-specific median body weight estimate from the US population.[19] Privigen dose was set to missing if the amount of Privigen administered was missing or when the calculated dose was not plausible defined as outside the range of 0.05 to 6.0 g/kg body weight.

Outcomes

The outcome of interest was HA. Potential HA events were identified from ICD-9 CM and ICD-10 CM hospital discharge codes for HAs and from medical codes for unspecified transfusion reactions together with one of the following laboratory tests performed in the workup of HA and in temporal association with Privigen: haptoglobin test, a direct antiglobulin test (DAT), or indirect antiglobulin test (IAT).

Database record summaries of each potential HA consisted of a chronological listing of hospital admissions, discharge diagnoses, hospital charge descriptions, in-hospital dispensed medications, ordered laboratory tests, surgical procedures, and medical and diagnostic interventions in the 120 days before and 90 days after the date of onset of potential HA. Database record summaries with masked IVIG product information were reviewed by a hematologist (TLS) and an epidemiologist (CM) who developed an algorithm to classify potential HA events as probable or possible HA and to determine the date of onset of the HA event. Probable HAs were identified from hospital discharge codes indicative of HA only. Possible HAs consisted of unspecified transfusion reaction in temporal association with a haptoglobin, a DAT or IAT performed in the workup of HA. Because ICD-9 CM and ICD-10 CM codes describe hospital discharge diagnoses only, the algorithm used the information on whether the discharge diagnosis was present on admission. The onset of a probable or possible HA was also derived from the recorded day of a laboratory test in the workup of HA (see Fig. S1a and b, available as supporting information in the online version of this paper, for details). The algorithm for the HA assessment and for the determination of the index day was applied to the entire database. Potential HA events within 10 days of IVIG use were manually reviewed; the remaining were used for censoring purposes. The 10-day at-risk period for the recording of probable or possible HA after administration of Privigen, derived from a case series analysis,[8] was applied to assess whether there was a temporal relationship with Privigen use.

Covariates

Indications for Privigen use were identified from ICD-9 CM and ICD-10 CM codes and consisted of low-dose indications, that is, immunodeficiency and malignant neoplasm of lymphatic and hematopoietic tissue, and high-dose indications, that is, immune thrombocytopenia, Kawasaki disease, chronic inflammatory demyelinating polyneuropathy, Guillain-Barré syndrome, and myasthenia gravis. Immunodeficiency was defined if recorded at any time, and all other diagnoses if recorded during the respective hospitalization with Privigen exposure. For Privigen administrations with unknown indication the indication of the previous Privigen administration was imputed. If none of the prespecified indications were recorded, the indication was considered "unknown." Patients with a low-dose and a high-dose indication were allocated to the respective high-dose indication.

Comorbidities of interest included the updated Charlson Comorbidity Index[20] based on the history of congestive heart failure, dementia, chronic pulmonary disease, rheumatologic disease, liver disease, diabetes with chronic complications, hemiplegia or paraplegia, renal disease, any malignancy, leukemia and lymphoma, metastatic solid tumor, AIDS, and HIV. Other comorbidities were renal transplant rejection, hereditary spherocytosis, history of incompatible blood transfusions, and history of autoimmune disorders.

Other covariates were transfusions of blood products consisting of blood transfusions and transfusion of blood components, such as plasma, platelets, and antihemophylic factors and use of systemic corticosteroids, cephalosporins, levofloxacin, penicillin and its derivatives, nonsteroidal anti-inflammatory drugs, mycophenolate mofetil, and methotrexate.

Statistical analysis

Descriptive summary statistics of demographic and clinical characteristics are presented as of the cohort entry day for each of the three study periods separately. Treatment episodes with administration of Privigen, the total dose (categorized as <1.0, ≥1.0 to < 1.5, or ≥1.5 g/kg body weight or unknown) and the average dose over different treatment episodes were summarized overall and stratified by Privigen indication.

For each study period, crude incidence rates of probable HA cases per 10,000 Privigen person-days at risk were calculated with respective 95% confidence intervals (CIs)

based on a Poisson distribution. Rate numerators consisted of the number of probable HA cases, and the denominators of person-days of at-risk time after Privigen administration using a 10-day at-risk period after Privigen use. Incidence rates were also provided separately for inpatient or outpatient setting, age group, sex, Privigen indication, dose, and first or subsequent administration of Privigen.

Overall incidence rates of probable HA in association with Privigen use in Period 3 and separately in Period 2 were compared with the respective rate in Period 1 by calculating the incidence rate ratio (IRR) from Poisson regression. IRRs were adjusted for inpatient and outpatient setting, age, sex, Privigen dose (<1.0, ≥1.0 to < 1.5, or ≥1.5 g/kg body weight or unknown), indication for Privigen use, and first or subsequent administration of Privigen.

Four sensitivity analyses were performed. First, by extending the at-risk period for the occurrence of HA after administration of Privigen from 10 to 30 days to allow for the inclusion of clinically significant delayed hemolytic reactions. For this purpose, all potential HA events within 11 and 30 days of IVIG

TABLE 1. Baseline characteristics of Privigen user cohorts by study period*

	Period 1, Jan 2008 to Dec 2012	Period 2, Oct 2013 to Dec 2015	Period 3, Oct 2016 to Apr 2019
Total	9439	7710	7759
Age† (years)			
Mean (±SD)	50.8 (±25.9)	46.7 (±27.3)	47.5 (±27.0)
Median (IQR)	57 (34-71)	54 (23-69)	55 (23-69)
<18	1478 (15.8)	1738 (22.5)	1658 (21.4)
18 to <65	4419 (47.2)	3412 (44.3)	3378 (43.5)
≥65	3460 (37.0)	2560 (33.2)	2723 (35.1)
Unknown age	82 (0.9)	0 (0.0)	0 (0.0)
Male	4452 (47.2)	3717 (48.2)	3809 (49.1)
Race			
White	6820 (73.5)	5505 (72.8)	5790 (76.2)
Black	1066 (11.5)	903 (11.9)	849 (11.2)
Hispanic	136 (1.5)	29 (0.4)	10 (0.1)
Other	1256 (13.5)	1122 (14.8)	949 (12.5)
Unstated	161 (1.7)	151 (2.0)	161 (2.1)
Updated Charlson Comorbidity Index‡			
Mean (±SD)	1.8 (±2.1)	1.8 (±2.2)	1.8 (±2.2)
Median (IQR)	1 (0-3)	1 (0-3)	1 (0-3)
Medical condition for Privigen use§			
Prespecified indication	7473 (79.2)	6177 (80.1)	6225 (80.2)
Low-dose indication	2713 (36.3)	2009 (32.5)	2354 (37.8)
Immunodeficiency‖	1445 (19.3)	1112 (18.0)	1272 (20.4)
Malignant neoplasm of lymphatic or hematopoietic tissue	1268 (17.0)	897 (14.5)	1082 (17.4)
High-dose indication	4760 (63.7)	4168 (67.5)	3871 (62.2)
Immune thrombocytopenia	2890 (38.7)	2240 (36.3)	1972 (31.7)
Kawasaki disease	376 (5.0)	393 (6.4)	334 (5.4)
Immune-mediated neuropathies	1606 (21.5)	1642 (26.6)	1674 (26.9)
Chronic inflammatory demyelinating polyneuropathy	470 (6.3)	468 (7.6)	518 (8.3)
Guillain-Barré syndrome	675 (9.0)	676 (10.9)	624 (10.0)
Myasthenia gravis	533 (7.1)	585 (9.5)	608 (9.8)
No prespecified indication recorded	1966 (20.8)	1533 (19.9)	1534 (19.8)
History of comorbidities¶			
Autoimmune disorder	1445 (15.3)	1298 (16.8)	1243 (16.0)
Hereditary spherocytosis	2 (0.0)	3 (0.0)	5 (0.1)
Incompatible blood transfusion	44 (0.5)	47 (0.6)	75 (1.0)
Renal transplant rejection	122 (1.3)	147 (1.9)	253 (3.3)
Transfusions administered**			
Blood transfusion	2298 (24.3)	1681 (21.8)	1387 (17.9)
Blood component transfusion††	1346 (14.3)	881 (11.4)	641 (8.3)
History of other IVIG use	1449 (15.4)	1025 (13.3)	1298 (16.7)

* Data are reported as number (%) unless otherwise specified.
† On admission day of hospitalization with first Privigen administration.
‡ As of first Privigen administration.
§ Any Privigen use for respective indication in study Period. Diagnoses not mutually exclusive; patients may have more than one specified indication.
‖ Recorded any time.
¶ Any history before first Privigen administration, excluding indications for Privigen treatment.
** Any history in 180 days before first Privigen administration.
†† Excluding transfusions of IVIGs and blood transfusions.
IQR = interquartile range.

TABLE 2. Privigen dose per episode by indication and study period*

Privigen indication†	Period 1		Period 2		Period 3	
	Total episodes‡	Privigen dose§	Total episodes‡	Privigen dose§	Total episodes‡	Privigen dose§
All Privigen users	28,754	0.76 (±0.71)	23,249	0.85 (±0.74)	25,471	0.85 (±0.71)
Prespecified indication	25,278	0.72 (±0.68)	20,375	0.83 (±0.73)	21,900	0.82 (±0.69)
Low-dose indication	14,087	0.46 (±0.34)	10,261	0.54 (±0.40)	11,586	0.54 (±0.39)
Immunodeficiency	9,357	0.47 (±0.32)	7,426	0.56 (±0.41)	7,290	0.56 (±0.41)
Malignant neoplasm	4,730	0.46 (±0.37)	2,835	0.49 (±0.36)	4,296	0.51 (±0.36)
High-dose indication	11,191	1.04 (±0.83)	10,114	1.12 (±0.86)	10,314	1.11 (±0.81)
Immune thrombocytopenia	5,581	0.97 (±0.81)	4,185	1.02 (±0.81)	4,132	1.02 (±0.77)
Kawasaki disease	438	2.09 (±0.81)	453	2.06 (±0.76)	372	2.02 (±0.69)
Chronic inflammatory demyelinating polyneuropathy	2,966	0.88 (±0.67)	3,192	0.95 (±0.75)	3,267	1.04 (±0.76)
Guillain-Barré syndrome	1,270	1.50 (±0.87)	1,209	1.48 (±0.93)	1,111	1.48 (±0.87)
Myasthenia gravis	1,728	0.96 (±0.79)	1,864	1.14 (±0.92)	2,140	1.13 (±0.84)
No prespecified indication recorded	3,476	1.03 (±0.86)	2,874	1.00 (±0.80)	3,571	1.05 (±0.81)

* Data are reported as number or mean (±SD).
† Diagnoses are not mutually exclusive and patients may have more than one specified indication.
‡ One episode is defined as consecutive daily administrations of Privigen. A gap of more than 1 day between Privigen administrations constituted a new episode.
§ Dose of Privigen administered in g/kg body weight.

use were also manually reviewed;[5] second, by removing subsequent Privigen episodes and Privigen users with a history of any IVIG use before the first Privigen episode to restrict the study cohorts to first-ever IVIG users; third, by combining HA cases assessed as probable and possible HA, to include potential HA events identified from unspecific recording of HA; and fourth, by stratifying Privigen dose in two categories (<0.75 or ≥0.75 g/kg body weight).

All statistical procedures were performed with computer software (Stata MP Version 14.2, StataCorp LLC). The study protocol was approved by the Pharmacovigilance Risk Assessment Committee (EUPAS Registration Number 6040).

RESULTS

The study cohorts in the three study periods consisted of 9439 patients in Period 1 (January 2008 to December 2012), 7710 in Period 2 (October 2013 to December 2015), and 7759 in Period 3 (October 2016 to April 2019). Patients in Period 1 were older than patients in Period 2 and Period 3 (mean, 50.8 years compared with 46.7 and 47.5 years, respectively). While the mean updated Charlson Comorbidity Index was 1.8 in all study periods, there were some trends in the indications for Privigen use: increasing proportion of patients with immunodeficiency and immune-mediated neuropathies and decreasing proportion of those with immune thrombocytopenia over time (Table 1). Transfusions of blood and of other blood components in the 180 days before the first Privigen administration decreased in Periods 2 and 3.

The mean dose of Privigen administered per treatment episode was 0.8 g/kg body weight and varied by indication ranging from 2.0 g/kg body weight for Kawasaki disease and 1.5 g/kg body weight for Guillain-Barré syndrome to 0.6 g/kg body weight for immunodeficiency in Period 3. Mean indication-specific doses of Privigen were similar in all study periods (Table 2). The number of probable HA cases decreased from 38 in Period 1 to 20 in Period 2 and only three in Period 3 (Fig. 1).

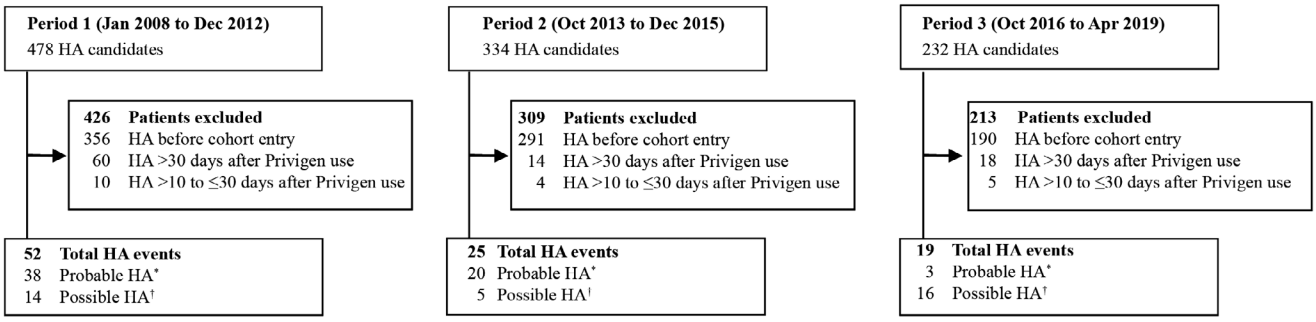

Fig. 1. Ascertainment of probable and possible HA events recorded within 10 days after Privigen use by study period. *Probable HA: specific HA code recorded. †Possible HA: unspecified transfusion reaction recorded in association with laboratory test indicative of HA workup.

TABLE 3. Incidence rates of probable HA within 10 days of Privigen use by study period

	Period 1			Period 2			Period 3		
	HA cases	Person-days at risk	Crude IR (95% CI)*	HA cases	Person-days at risk	Crude IR (95% CI)*	HA cases	Person-days at risk	Crude IR (95% CI)*
Total	38	254,437	1.49 (1.06-2.05)	20	197,327	1.01 (0.62-1.57)	3	215,698	0.14 (0.03-0.41)
Hospital setting									
Inpatient	28	84,077	3.33 (2.21-4.81)	14	63,326	2.21 (1.21-3.71)	2	56,648	0.35 (0.04-1.28)
Outpatient	10	170,360	0.59 (0.28-1.08)	6	134,001	0.45 (0.16-0.97)	1	159,050	0.06 (0.00-0.35)
Sex									
Male	18	109,990	1.64 (0.97-2.59)	5	85,747	0.58 (0.19-1.36)	3	96,312	0.31 (0.06-0.91)
Female	20	144,447	1.38 (0.85-2.14)	15	111,580	1.34 (0.75-2.22)	0	119,386	0.00 (0.00-0.31)
Age (years)									
<18	11	23,344	4.71 (2.35-8.43)	1	32,557	0.31 (0.01-1.71)	1	28,747	0.35 (0.01-1.94)
18 to <65	18	120,551	1.49 (0.88-2.36)	10	90,648	1.10 (0.53-2.03)	1	93,417	0.11 (0.00-0.60)
≥65	9	107,228	0.84 (0.38-1.59)	9	74,122	1.21 (0.56-2.30)	1	93,534	0.11 (0.00-0.60)
Unknown age	0	3,314	0.00 (0.00-11.13)	0	0	NA	0	0	NA
Prespecified indication†									
Low-dose indication	6	142,082	0.42 (0.15-0.92)	4	98,225	0.41 (0.11-1.04)	1	109,444	0.09 (0.00-0.51)
Immunodeficiency	2	104,056	0.19 (0.02-0.69)	3	75,358	0.40 (0.08-1.16)	1	76,057	0.13 (0.00-0.73)
Malignant neoplasm	4	38,026	1.05 (0.29-2.69)	1	22,867	0.44 (0.01-2.44)	0	33,387	0.00 (0.00-1.10)
High-dose indication	23	84,350	2.73 (1.73-4.09)	7	75,538	0.93 (0.37-1.91)	1	77,968	0.13 (0.00-0.71)
Immune thrombocytopenia	14	35,770	3.91 (2.14-6.57)	2	24,611	0.81 (0.10-2.94)	1	23,994	0.42 (0.01-2.32)
Guillain-Barré syndrome	3	9,511	3.15 (0.65-9.22)	1	9,835	1.02 (0.03-5.67)	0	7,900	0.00 (0.00-4.67)
Kawasaki disease	4	2,983	13.41 (3.65-34.33)	0	2,717	0.00 (0.00-13.58)	0	2,580	0.00 (0.00-14.30)
Myasthenia gravis	1	15,425	0.65 (0.02-3.61)	3	15,597	1.92 (0.40-5.62)	0	19,026	0.00 (0.00-1.94)
Chronic inflammatory demyelinating polyneuropathy	1	23,564	0.42 (0.01-2.36)	1	25,487	0.39 (0.01-2.19)	0	26,948	0.00 (0.00-1.37)
Indication not stated	9	28,005	3.21 (1.47-6.10)	9	23,564	3.82 (1.75-7.25)	1	28,286	0.35 (0.01-1.97)
Privigen dose (g/kg body weight)									
<1.0	18	181,759	0.99 (0.59-1.57)	9	134,847	0.67 (0.31-1.27)	1	149,629	0.07 (0.00-0.37)
≥1.0 to <1.5	5	18,911	2.64 (0.86-6.17)	0	19,234	0.00 (0.00-1.92)	0	24,144	0.00 (0.00-1.53)
≥1.5	13	35,128	3.70 (1.97-6.33)	10	33,826	2.96 (1.42-5.44)	2	36,572	0.55 (0.07-1.98)
Unknown dose	2	18,639	1.07 (0.13-3.88)	1	9,420	1.06 (0.03-5.91)	0	5,353	0.00 (0.00-6.89)
Sequence of Privigen‡									
First-ever use	30	73,162	4.10 (2.77-5.85)	16	51,964	3.08 (1.76-5.00)	3	51,754	0.58 (0.12-1.69)
Subsequent use	4	79,962	0.50 (0.14-1.28)	4	74,193	0.54 (0.15-1.38)	0	90,920	0.00 (0.00-0.41)

* Incidence rate of HA per 10,000 person-days at risk after Privigen use.
† More than one indication per Privigen episode possible.
‡ Restricted to IVIG-naïve patients.
IR = incidence rate; NA = not applicable.

TABLE 4. IRR of HA in temporal association with Privigen use by study period

	HA cases	Person-days at risk	Crude incidence rate (95% CI)*	Crude IRR (95% CI)†	Adjusted IRR (95% CI)†‡	p value‡§
Main analysis: probable HA						
Period 1	38	254,437	1.49 (1.06-2.05)	1	1	
Period 2 (reference: Period 1)	20	197,327	1.01 (0.62-1.57)	0.68 (0.37-1.20)	0.71 (0.41-1.23)	0.11
Period 3 (reference: Period 1)	3	215,698	0.14 (0.03-0.41)	0.09 (0.02-0.29)	0.10 (0.03-0.33)	<0.01
Period 3 (reference: Period 2)				0.14 (0.03-0.46)	0.14 (0.04-0.47)	<0.01
Sensitivity analysis: first Privigen use only‖						
Period 1	30	73,162	4.10 (2.77-5.85)	1	1	
Period 2 (reference: Period 1)	16	51,964	3.08 (1.76-5.00)	0.75 (0.38-1.42)	0.74 (0.40-1.37)	0.17
Period 3 (reference: Period 1)	3	51,754	0.58 (0.12-1.69)	0.14 (0.03-0.45)	0.14 (0.04-0.5)	<0.01
Period 3 (reference: Period 2)				0.19 (0.04-0.66)	0.18 (0.05-0.62)	<0.01
Sensitivity analysis: probable and possible HA						
Period 1	52	254,437	2.04 (1.53-2.68)	1	1	
Period 2 (reference: Period 1)	25	197,327	1.27 (0.82-1.87)	0.62 (0.37-1.02)	0.67 (0.41-1.08)	<0.05
Period 3 (reference: Period 1)	19	215,698	0.88 (0.53-1.38)	0.43 (0.24-0.74)	0.51 (0.30-0.87)	0.01
Period 3 (reference: Period 2)				0.70 (0.36-1.31)	0.78 (0.43-1.42)	0.21
Sensitivity analysis: 30-day at-risk period						
Period 1	47	635,702	0.74 (0.54-0.98)	1	1	
Period 2 (reference: Period 1)	22	469,255	0.47 (0.29-0.71)	0.63 (0.36-1.07)	0.68 (0.41-1.13)	0.07
Period 3 (reference: Period 1)	4	522,056	0.08 (0.02-0.20)	0.10 (0.03-0.28)	0.11 (0.04-0.31)	<0.01
Period 3 (reference: Period 2)				0.16 (0.04-0.48)	0.18 (0.06-0.51)	<0.01

* Incidence rate of HA per 10,000 person-days at risk after Privigen use.
† Estimated from Poisson regression.
‡ Adjusting for hospital setting, sex, age, Privigen indication, Privigen dose, and first/subsequent Privigen administration.
§ Using one-sided Wald test for Poisson regression.
‖ Analysis restricted to first Privigen at-risk period and exclusion of patients with history of other IVIG use.

TABLE 5. IRRs of probable HA in temporal association with Privigen use in Period 1 (reference) and Period 3

	Crude IRR (95% CI)*	Adjusted IRR (95% CI)*†	p-value*†‡
Total	0.09 (0.02-0.29)	0.10 (0.03-0.33)	<0.01
Hospital setting			
Inpatient	0.11 (0.01-0.42)	0.10 (0.02-0.44)	<0.01
Outpatient	0.11 (0.00-0.75)	0.09 (0.01-0.73)	0.01
Sex			
Male	0.19 (0.04-0.65)	0.24 (0.07-0.81)	0.01
Female	0.00 (0.00-0.25)	NA	NA
Age (years)			
<18	0.07 (0.00-0.51)	0.11 (0.01-0.84)	0.02
18 to <65	0.07 (0.00-0.45)	0.07 (0.01-0.51)	<0.01
≥65	0.13 (0.00-0.92)	0.17 (0.02-1.40)	0.054
Prespecified indication			
Low-dose indication**	0.22 (0.03-1.80)	0.21 (0.03-1.76)	0.08
Immunodeficiency	0.68 (0.01-13.14)	0.50 (0.04-5.88)	0.29
Malignant neoplasm	0.00 (0.00-1.73)	NA	NA
High-dose indication	0.05 (0.01-0.35)	0.06 (0.01-0.42)	<0.01
Immune thrombocytopenia	0.11 (0.00-0.70)	0.12 (0.02-0.89)	0.02
Guillain-Barré syndrome	0.00 (0.00-2.91)	NA	NA
Kawasaki disease	0.00 (0.00-1.75)	NA	NA
Myasthenia gravis	0.00 (0.00-31.62)	NA	NA
Chronic inflammatory demyelinating polyneuropathy	0.00 (0.00-34.10)	NA	NA
Privigen dose (g/kg body weight)			
<1.0	0.07 (0.00-0.43)	0.08 (0.01-0.57)	0.01
≥1.0 to < 1.5	0.00 (0.00-0.85)	NA	NA
≥1.5	0.15 (0.02-0.65)	0.18 (0.04-0.81)	0.01
Sequence of Privigen**			
First-ever use	0.14 (0.03-0.45)	0.14 (0.04-0.45)	<0.01
Subsequent use	0.00 (0.00-1.33)	NA	NA
Sensitivity analysis			
Privigen dose (g/kg body weight)			
<0.75	0.09 (0.00-0.61)	0.10 (0.01-0.77)	0.01
≥0.75	0.08 (0.01-0.31)	0.10 (0.02-0.43)	<0.01

* Estimated from Poisson regression using Period 1 as reference.
† Adjusted for hospital setting, sex, age, Privigen indication, Privigen dose, and first or subsequent Privigen administration.
‡ Using one-sided Wald test for Poisson regression.
§ Not adjusted for Privigen dose.
** Restricted to patients without any other IVIG history.
NA = not applicable.

Overall crude incidence rates of probable HA of 1.49 (95% CI, 1.06-2.05) per 10,000 person-days in Period 1 dropped to 1.01 (95% CI, 0.62-1.57) in Period 2 and to 0.14 (95% CI, 0.03-0.41) in Period 3. Crude incidence rates were higher in the in-hospital setting, age less than 18, and high-dose indications for Privigen dose of at least 1 g/kg body weight and first-ever Privigen use in Period 1. The incidence rates in all subgroups with at least three HA cases in Period 1 decreased in Period 2 and Period 3 (Table 3).

The overall adjusted IRR estimate for HA in Period 2 versus Period 1 was 0.71 (95% CI, 0.41-1.23) and 0.10 (95% CI, 0.03-0.33) for Period 3 versus Period 1, reflecting a 90% reduction in HA incidence in Period 3. The IRR for HA in Period 3 was also significantly reduced when compared with Period 2 (IRR, 0.14 [95% CI, 0.04-0.47]; Table 4).

Subgroup analyses by hospital setting, sex, age group, high-dose indications, and sequence of administration showed IRRs of not more than 0.24 with significant reductions of the incidence rate of HA in all subgroups, except age 65 and older and low-dose indications. Adjusted IRR estimates comparing Period 3 with Period 1 were also significantly decreased when Privigen doses of less than 1.0 and 1.5 g/kg body weight or more were administered and in first-ever Privigen use (Table 5). Because five HA cases were observed in Period 1 but none in Periods 2 and 3 in association with Privigen dose 1.0 or more to less than 1.5 g/kg body weight, Privigen dose was stratified into less than 0.75 and 0.75 g/kg body weight or more. This sensitivity analysis yielded a significant IRR reduction of 90% in both dose strata (Table 5).

Further sensitivity analyses restricting the study population to first-time Privigen users showed that 88.2% (30 of 34) of HA cases in Period 1, 80% (16 of 20) of HA cases in Period 2, and all 100% (three of three) of HA cases in Period 3 had occurred in temporal relationship with the first Privigen use. The reduction of the adjusted IRR was 86% after the implementation the immunoaffinity chromatography step (Table 4).

The sensitivity analysis using an at-risk period of 30 days instead of 10 days after Privigen administrations included 47, 22, and four HA cases in Periods 1 to 3, respectively (Table S1, available as supporting information in the online version of this paper). This analysis showed consistent findings (89% reduction in overall HA incidence in Period 3 vs. Period 1) because at least 75% of all HA were recorded within 10 days after the IVIG administration: 38 of 47 (80.9%) in Period 1, 20 of 22 (90.9%) in Period 2, and three of four (75.0%) in Period 3 (Fig. S2a, available as supporting information in the online version of this paper). The sensitivity analysis combining probable and possible HA events showed less pronounced but significant risk reductions comparing Period 3 with Period 1 and a nonsignificant risk reduction for Period 3 versus Period 2 (Table 4; Fig. S2b, available as supporting information in the online version of this paper).

DISCUSSION

To our knowledge, this is the first population-based study that has investigated and compared the risk of HA before and after the implementation of manufacturing process changes to reduce the amount of anti-A and anti-B isoagglutinin in an IVIG. Measured in rate of HA per 10,000 person-days of Privigen use, the HA incidence rate decreased from 1.49 before any intervention to 1.01 after implementation of donor screening to 0.14 after the implementation of immunoaffinity chromatography. This is equivalent to a 29% reduction of the IRR of HA after the implementation of the donor screening and a 90% reduction after the implementation of the immunoaffinity chromatography step in the manufacturing of Privigen. High Privigen dose indications, which appeared strongly associated with HA until 2016, are not found to be associated with an increased HA risk anymore. This finding is consistent with the hypothesis that the lowering of anti-A and anti-B titers in Privigen is associated with a decreased risk of HA, particularly at high doses.

Strengths and limitations

The estimated risk of HA after Privigen administration from this study is likely to only reflect the risk of HA requiring hospitalization and/or treatment, because patients with mild HA may not be diagnosed or not readmitted to hospital and therefore may not have been captured in the database. This may lead to nondifferential underestimation of HA events in the different study periods but is unlikely to affect IRR estimates.

The possible HA events included in one of the sensitivity analyses may include transfusion reactions other than HA, especially because no hemoglobin test results are available in the database. This may have led to overestimation of HA events but is unlikely to have affected the IRR estimates in this sensitivity analysis. Because the objective of the study was to investigate the effectiveness of two risk minimization measures, it was fundamental to compare the risk of HA after each of the two independent risk minimization measures with the background risk of HA before the implementation of manufacturing changes. The proportion of potential known and unknown independent risk factors of HA, for example, Privigen dose, first and subsequent Privigen use, and transfusions of blood or of other blood components, could have changed in the three study periods. This could have confounded the incidence rate of HA in the different study periods. Therefore, we provided adjusted IRR estimates of HA by controlling for all measured potential confounders in our study including first and subsequent Privigen use. In addition, the sensitivity analysis based on first-time Privigen use only in all study periods showed consistent findings. Furthermore, residual confounding to unknown or unmeasured risk factors of HA with differential distribution in the three study periods and within 10 days of the IVIG administration is possible. One example for an unmeasured risk factor of HA is blood

group. PHD does not capture blood group information and hence adjustment for blood group was not possible. This may have led to bias if the distribution of blood groups in Privigen users had changed over time, for example, due to increased awareness for HA in non-O blood group patients.

A washout period of 9 months between the study periods were chosen to allow for the lots produced after screening for and exclusion of high–anti-A–titer donors and for lots manufactured with immunoaffinity chromatography to have replaced the previous product. Any lots not replaced would have carried the risk of HA associated with the previous product and resulted in underestimation of the risk reduction in this study. Any other residual confounding is unlikely to explain the decrease of 90% of the HA risk observed in our study.

Despite the efforts to impute the onset of the HA from the date of an ordered antiglobulin test or a code indicative of a transfusion reaction, there may be some inaccuracies in assessing the date of onset of HA affecting the assumed temporal relationship of Privigen use and the HA event. Furthermore, HA events may be recorded more than 10 days after Privigen administration. The sensitivity analysis expanding the IVIG at-risk period to 30 days confirmed the main findings (Table 4).

The risk of first-time HA is likely to vary from the risk of a subsequent HA episode. To enable the comparison of the HA risk in temporal association with IVIG use in all study periods, we restricted all study cohorts to patients who had no history of HA before the first Privigen use and censored patients on the day of an incident HA.

The coding system for hospital discharge diagnoses and of in-hospital procedures changed in 2014 from ICD-9 CM to ICD-10 CM codes. The higher granularity of ICD-10 CM could have resulted in different proportions of incomplete and/or inaccurate recording of medical diagnoses and procedures in the three study periods.

With the limitations noted, the study results are generalizable as to the impact of the risk minimization measures, on the incidence of HA among patients treated with Privigen. To the extent that the risk minimization measures are adopted in other countries, the results should be generalizable beyond the US.

In conclusion, the manufacturing changes to reduce the quantity of isoagglutinin in Privigen have resulted in a progressive reduction of the risk of HA. HA has become a rare event in association with Privigen use. The screening for and exclusion of high–anti-A–titer donors from pooled plasma, implemented from 2013 to 2015, resulted in a 29% reduction of the incidence rate of HA. The implementation of the immunoaffinity chromatography step in the manufacturing of Privigen in 2015 has been more effective resulting in a 90% reduction of the risk of HA.

CONFLICT OF INTEREST

CW and CM are employees of the Institute for Epidemiology, Statistics and Informatics GmbH. The Institute has received grants from Bayer, Bristol-Myers Squibb, CSL Behring, and Merz Pharma outside the submitted work and from CSL Behring for the conduct of this study. AP, AS, AH, and TLS are employees of CSL Behring and own shares in CSL Behring AG. CW and CM have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

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

Appendix S1. Supporting information.