Hemolytic adverse effects of intravenous immunoglobulin: modeling predicts risk reduction with anti-A/B immunoaffinity chromatography and to a lesser extent with anti-A donor screening

Rajiv Mallick,1 Alphonse Hubsch,2 and David G. Barnes3

BACKGROUND: The risk of hemolytic events (HEs) with intravenous immunoglobulin (IVIG) therapy appears to be linked to isoagglutinins (anti-A and anti-B) in the product. Patient risk factors include high IVIG dose, blood group, and underlying inflammatory state.

STUDY DESIGN AND METHODS: Using published anti-A and anti-B titers for IVIG products and HE rates calculated from HEs spontaneously reported to EudraVigilance, regression models were developed to infer the relationship between HE risk and IVIG isoagglutinin levels for each blood group. Applying estimated model coefficients to isoagglutinin levels associated with an IVIG (Privigen; CSL Behring, King of Prussia, PA), manufactured with and without isoagglutinin reduction steps, predicted HE risk values were generated for each product: 1) without any isoagglutinin reduction, 2) anti-A donor screening, and 3) anti-A/anti-B specific immunoaffinity chromatography (IAC; Ig IsoLo).

RESULTS: Predicted HE risk was highest for blood group AB, followed by A and B; it was low for O. Projected population shares of HEs by blood group were similar to reported real-world data. Compared with the original process (no isoagglutinin reduction), the model predicts lower hemolytic risk with anti-A donor screening and even lower hemolytic risk with IAC isoagglutinin reduction.

CONCLUSION: IAC isoagglutinin reduction is predicted to reduce the HE risk with IVIG substantially. Physicians should be especially vigilant to HE risk in patients with blood group AB and, to a lesser extent, A when using IVIG products with high anti-A titers.

MATERIALS AND METHODS

We used a two-step approach to predict HE risk. As a first step, we used historic data on anti-A and anti-B titers for IVIG products produced with modified production processes, including isoagglutinin reduction steps.

From the 1CSL Behring LLC, King of Prussia, Pennsylvania; 2CSL Behring AG, Bern, Switzerland; and 3CSL Behring, Ottawa, Canada.

Address reprint requests to: Rajiv Mallick, CSL Behring LLC, King of Prussia, PA 19406; e-mail: rajiv.mallick@cslehring.com.

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TABLE 1. Predicted Hemolytic Risk

| Variable                        | IVIG Production Process | Chromatographic, Isoagglutinin Reduction (n = 260) | Chromatographic, Donor Screening (n = 416) | Chromatographic, Donor Screening + IAC (n = 28) | Cohn-Like (n = 211) |
|---------------------------------|-------------------------|--------------------------------------------------|------------------------------------------|-----------------------------------------------|-------------------|
| Isoagglutinin titer, log₂ mean  | Anti-A                  | 5.4                                              | 4.91                                    | 3.32                                          | 3.09              |
| Predicted                       | Blood group A           | 3.17                                             | 1.23                                    | 0.17                                          | 0.14              |
| population-level                | Blood group B           | 1.93                                             | 1.00                                    | 0.06                                          | 0.07              |
| HE risk, cases/1000 kg          | Blood group AB          | 8.45                                             | 3.87                                    | 0.32                                          | 0.39              |
| IVIG shipped                    | Blood group O           | 0.01                                             | 0.01                                    | 0.01                                          | 0.01              |
| Predicted                       | US population, %*       | 1.87                                             | 0.77                                    | 0.09                                          | 0.09              |
| **Patient-level CI**            |                         | (0.043–0.373)                                    | (0.003–0.024)                           | (0.017–0.144)                                  | (0.002–0.019)     |
| HE risk, % of patients          | Blood group B           | 0.077                                            | 0.040                                   | 0.002                                         | 0.003             |
| affected (95% CI)               | Blood group AB          | 0.336                                            | 0.154                                   | 0.013                                         | 0.016             |
| Blood group O                   | <0.001                  | (0.002–0.0048)                                   | (0.049–0.489)                           | (0.004–0.041)                                  | (0.005–0.05)      |
| US population*                  |                         | 0.074                                            | 0.031                                   | 0.004                                         | 0.003             |
|                                 |                         | (0.002–0.0299)                                   | (0.001–0.100)                           | (0.001–0.010)                                  | (0.001–0.010)     |

Cl indicates confidence interval; HE, hemolytic event; IAC, immunoaffinity chromatography; IVIG, intravenous immunoglobulin; log₂, log-base 2; NA, not applicable.

* Estimated as weighted average of blood group–specific predicted HE risk, weights given by US blood group distribution noted above.

**TABLE 1. Predicted Hemolytic Risk**

IVIG ISOAGGLUTININ HEMOLYSIS MODEL

seven IVIG products and corresponding HE rates (adjusted by distribution of HE rates by blood group) reported to the EudraVigilance database, to develop regression models for each blood group and infer the historic relationship between anti-A and anti-B titers and corresponding HE rates across various IVIG products.

Separate quadratic regressions of the general specification 1) were estimated separately for blood groups A, B, 3, and AB 4), where the independent variable x is the anti-A/B titer in IVIG, measured by the European Pharmacopoeia (PhEur) direct assay after log-base 2 (log₂) transformation:

1) \[ \ln[HE] = a_0 + a_1x + a_2x^2 \]
2) \[ \ln[HE]_A = a_{0A} + a_{1A} \times [\log_2(\text{anti-A})] + a_{2A} \times [\log_2(\text{anti-A})]^2 \]
3) \[ \ln[HE]_B = a_{0B} + a_{1B} \times [\log_2(\text{anti-B})] + a_{2B} \times [\log_2(\text{anti-B})]^2 \]
4) \[ \ln[HE]_{AB} = a_{0AB} + a_{1AB} \times [\log_2(\text{anti-A})] + a_{2AB} \times [\log_2(\text{anti-B})] + a_{3AB} \times [\log_2(\text{anti-A})]^2 + a_{4AB} \times [\log_2(\text{anti-B})]^2 \]

where \( \ln \) represents the natural log.

The risk of HE was considered independent of isoagglutinins for blood group O (i.e., this was estimated as a constant, 5):

5) \[ \ln[HE]_O = a_{0O} \]

Data transformations and model specification were guided by intrinsic data units and the expected form of the relationships. Thus, anti-A and anti-B titer levels were log₂ transformed to convert titer steps measured in two-fold dilution multiples (2, 4, 8, 16, and so on) to unit increments (i.e., 1, 2, 3, and so on). The quadratic polynomial regression form was used to reflect the nonlinearity of increased HE risk and was measured in natural log terms because of the noncontinuous changes and zero lower bound in reporting frequencies of HES.

As a second step, estimated model parameters (regression coefficients) from the inferred historic relationship between isoagglutinin titers and HE risk were applied to predict \( \ln[HE] \) rates for IVIGs produced by CSL Behring, with established and modified production processes, given their known anti-A and anti-B titers. In addition to point predictions, 95% confidence intervals (CIs) were generated, applying the estimated standard errors of regression residuals for each blood type. The isoagglutinin titers (measured by PhEur direct assay) in IVIGs produced with these processes were provided by Swissmedic (Bern, Switzerland). The following products were evaluated:

1) An IVIG produced using a chromatographic process (Privigen; CSL Behring)
   a) without any isoagglutinin reduction measures, as used from 2007 to 2013,
   b) with an anti-A donor screening program eliminating approximately 5% of donors with high anti-A titers, as used from 2013 to 2015,
   c) incorporating an anti-A/anti-B specific IAC step in the manufacturing process, as implemented in 2016 (Ig IsoLo);

2) An IVIG produced with a Cohn-like cold ethanol fractionation process (Carimune NF/Sandoglobulin; CSL Behring).

The IAC process uses synthetic blood group antigens (A and B trisaccharides) bound to a base bead matrix to form anti-A and anti-B specific resins that are then blended...
to allow chromatographic reduction of isoagglutinins in one dedicated step.\textsuperscript{10}

Predicted ln(HE) point (and 95% CI) values were exponentiated to natural unit point (95% CI) predicted HE cases per 1000 kg of product shipped. The predictions were reported separately for each blood group and overall. Blood group distribution in the population exposed to IVIG was assumed to be 42% A, 10% B, 4% AB, and 44% O (similar to the one in the US population). We calculated the predicted HE risk per patient by dividing the point (95% CI) risk per 1000 kg IVIG shipped by the estimated mean yearly dose per patient (approx. 400 g).\textsuperscript{1}

To validate our predictions by blood group, we also compared the predicted HE rate for an IVIG produced via a chromatographic process without isoagglutinin reduction with an independently collected real-world data set of reported HE risk by blood group.\textsuperscript{7}

**RESULTS**

The estimated regression models for the historic data yielded the following:

1) \(\ln[\text{HE}]_A = -0.68 + (-1.41) \times (\text{anti-A}) + (0.32) \times (\text{anti-A})^2\)

for blood group A;

2) \(\ln[\text{HE}]_B = -4.48 + (0.55) \times (\text{anti-B}) + (0.16) \times (\text{anti-B})^2\)

for blood group B;

3) \(\ln[\text{HE}]_{AB} = 0.64 + (-3.61) \times (\text{anti-A}) + (3.11) \times (\text{anti-B}) + (0.51) \times (\text{anti-A})^2 + (-0.39) \times (\text{anti-B})^2\)

for blood group AB and

4) \(\ln[\text{HE}]_O = -4.57\)

for blood group O.

The model fitted the observed historic data well, explaining almost all variance in HE rates (adjusted \(R^2 = 0.95\) for blood group A, 0.93 for blood group B, and 0.97 for blood group AB). Predicted HE risk is shown in Table 1 and Figure 1.

The model predicted a nonlinear increase in HE risk with increased anti-A and anti-B titers in patients expressing the corresponding blood group antigens (Figure 1). Across blood groups, the predicted HE risk was highest for blood group AB, followed by groups A and B, and was low for group O (Table 1 and Figure 1). Both anti-A and anti-B titers independently contributed to predicted HE risk for group AB (Figure 1).\textsuperscript{11}

The model predicted a lower hemolytic risk, in each of blood groups A, B, and AB, with anti-A donor screening and an even lower risk with IAC, compared with the process without any isoagglutinin reduction (Figure 1). The HE (point estimate) risk with IAC was comparable to that for an IVIG
produced using a Cohn-like fractionation process, which results in low isoagglutinin titers and a documented low HE risk. For all processes except donor screening alone, predicted risk reduction was statistically significant: 95% CIs for all these processes were mutually exclusive with the 95% CI for the process without any modification (i.e., the upper limit HE risk associated with these processes was lower than the lower limit HE risk without any isoagglutinin reduction) (Table 1).

For IVIG produced via a chromatographic process without isoagglutinin reduction, the projected population shares of HE by blood group were similar to the observed population share of HE reported by Berg et al. (Table 2).

### DISCUSSION

Our model predicted that the patient-level HE risk associated with IVIG administration is highest in blood group AB, followed by A, then B, and lowest in blood group O. However, although the patient-level risk is lower in blood group A compared with AB, there is a much higher prevalence of blood group A at the population level. Therefore, at an individual patient level, clinicians need to be most aware of HE risk in patients with blood group AB. However, on a population health level, surveillance of risk in blood group A would be appropriate.

Our model predictions of the distribution of HE events by blood group are remarkably similar to the rates in a previous report, confirming the concordance of model predictions with a real-world data set. More recent studies have confirmed the differences in IVIG-associated HE risk between blood groups and refined the risk estimate within blood groups. Mielke et al. found a correlation between blood group antigen density on erythrocytes and HE risk, with a higher risk in blood group A1 versus A2. A genotyping study found a trend toward HE risk mitigation by the O allele, with a lower risk in AO and BO versus AB, AA, and BB genotypes. However, information on blood group A subgroups and genotypes was not available in the data used to build the model reported herein.

Rarely, HEs after IVIG therapy can occur because of the presence of non-ABO antibodies, most often anti-Rh(D). Although the currently available IVIG products contain little or undetectable non-ABO antibodies, it cannot be excluded that HEs in blood group O patients attributable to non-ABO antibodies in IVIG are rare, an additive effect of such antibodies in HE mediated by anti-A and anti-B cannot be excluded.

The model generated during this study was novel in providing a tool to quantitatively predict HE risks on the basis of historic distribution of HE rates and anti-A and anti-B titers across different IVIG products. Using evidence on the distribution of IVIG by volume (kg shipped) and patients by blood group, we were also able to translate IVIG volume-based HE risk predictions to derive patient-level rates of HE risk and the corresponding population distribution of HE events by blood group.

Isoagglutinin reduction measures in IVIG manufacturing are expected to significantly reduce the risk of hemolysis. A stand-alone donor screening program, excluding donors with the highest anti-A titers (approx. 5% of the donor pool), is predicted to produce a modest reduction of HE risk compared with the risk from a product without any isoagglutinin reduction, consistent with the reduction demonstrated in an observational cohort study. This reduction of risk is expected not only in patients with blood groups A and AB, but also in those with blood group B, consistent with the correlation of anti-B with anti-A in blood donors. However, the projected risk remains higher than with IVIG produced with a Cohn-like process. The Cohn-like process removes isoagglutinin in a

### TABLE 2. IVIG Exposure and Projected vs. Actual Historic Number of HEs

| Variable                  | A          | B          | AB         | O          | Total      |
|---------------------------|------------|------------|------------|------------|------------|
| Estimated IVIG use, kg*   | 20,496     | 4880       | 1950       | 21,472     | 48,800     |
| Projected HEs, n†         | 65         | 9          | 16         | 0          | 91         |
| No. of patients‡          | 51,450     | 12,250     | 4900       | 53,900     | 122,500    |
| Projected HE risk per patient, % (95% CI)¶ | (0.043–0.373) | (0.020–0.296) | (0.107–1.066) | (0.000) | (0.024–0.229) |
| Predicted HEs, % (95% CI)¶ | 71.3       | 10.3       | 18.1       | 0.2        | 100.0      |
| (68.6–75.8)               | (6.9–12.8) | (17.2–18.6) | (0.2–0.2)  | (100)      |
| Reported (actual) HEs, %  | 72         | 7          | 18         | 3†         | 100        |

* Applying US blood group distribution to total amount of IVIG shipped.
† Applying estimated HE risk per 1000 kg by blood group (Table 1) to distribution of IVIG shipped by blood group (rounded off to higher whole number if first decimal >0.5 and to the lower whole number if first decimal ≤0.5).
‡ Applying US blood group distribution to total number of patients.
§ Projected number of events by blood group divided by number of patients in each blood group.
¶ Projected number of events (point estimates and 95% CIs) by blood group as a proportion of total projected events. Projected 95% CIs of events by blood group calculated by taking lower/upper bound of predicted events in blood group as proportion of lower/upper bound of overall predicted events; percentages corresponding to lower and upper bounds may not necessarily be lower or higher, respectively.
† Projected number of events by blood group divided by number of patients in each blood group.

CI indicates confidence interval; HE, hemolytic event; IVIG, intravenous immunoglobulin; n, number of events.
precipitation step (removal of factor III) and historically has the lowest risk of hemolysis.8

The newly added IAC step in the Privigen production process is expected to reduce the risk to a much greater extent than a donor screening program, with a predicted risk similar to that of an IVIG produced with a Cohn-like process. An IAC step is also preferable over a donor screening program because it achieves lower IVIG isoagglutinin levels while maintaining the complete donor population pool. Eliminating approximately 5% of plasma donors from IVIG production with a screening program would further reduce the availability of plasma for fractionation, which is already in short supply. A donor screening program can, however, be implemented rapidly and, therefore, can be used as a transient measure to reduce the risk of hemolysis at least modestly during the development and implementation of an IAC step, as was done for Privigen.

In agreement with the recent report by Branch et al.,12 our results also suggest that physicians should be especially vigilant to the risk of HE in patients with blood group AB and, to a lesser extent, with blood group A, when using IVIG products produced with a chromatographic production process with no isoagglutinin reduction measures.

LIMITATIONS

The IVIG products used to build the model had higher anti-A than anti-B titers, most likely because of the blood group distribution. IVIG products had anti-A titers ranging from 4 to 64 and anti-B titers ranging from 2 to 32, and the model should not be used to extrapolate HE risk beyond these titer limits. HE rates at a patient level are IVIG dose dependent. This dose effect could not be modeled, because patient-level dose data were not available. HE risk may also be influenced by non-ABO antibodies, such as anti-Rh(D), if present in the IVIG product. Within blood groups, the HE risk is influenced by other patient factors, such as blood group A subgroup and the presence of the O allele; the model can only predict the risk for the “average” subject by blood group. Finally, the rate of HE considered is based on spontaneous reports; the real rate is probably higher because of expected underreporting.

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CONFLICT OF INTEREST

R.M., A.H.U., and D.G.B. are employees of CSL Behring. A.H.U. and D.G.B. own shares in CSL Behring.

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