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ORIGINAL ARTICLE

Intravenous Immunoglobulin Administration Induces Severe Anemia and Cross-Matching Incompatibility in Neonates

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SUMMARY

Background: **This study aimed to explore the severe anemia and cross-matching incompatibility caused by intravenous immunoglobulin (IVIG) administration and the selection of accurate red blood cells (RBC) for transfusion.**
Methods: **Cross-matching, direct antiglobulin test (DAT), free antibody test (FAT), and elution test (ET) were performed with the neonatal samples before and after IVIG administration simultaneously. The blood samples after IVIG administration were performed by cross-matching with antihuman globulin (AHG) microcolumn gel card (AHG-card) and AHG tube method (AHG-tube) with the same packed RBC suspension simultaneously. The laboratory results before and after IVIG administration and after packed RBC suspension transfusion were collected. The incidence of RBC transfusion adverse reactions was collected as well.**
Results: **Out of 30 neonates enrolled in this study, the indication of IVIG was sepsis in 18, necrotizing enterocolitis (NEC) in 10, and undetermined reason in 2. The IVIG administration ranges of dose, frequency, and dose/weight were 1.8 - 7.5 g, 1 - 3 times, and 0.8 - 2.9 g/kg. Cross-matching tests by AHG-card, DAT, FAT, and ET were all positive in 30 neonates after administration of IVIG, the elevation of lactic dehydrogenase (LDH) and unconjugated bilirubin and reduction of RBC and HGB were statistically significant after IVIG administration. The incompatibility cases of AHG-card and AHG-tube were 30 and 6, respectively. Compared with laboratory test results before IVIG administration, after packed RBC suspension transfusion, the RBC and HGB of 30 neonates increased significantly. No RBC transfusion adverse reaction was observed.**
Conclusions: **IVIG administration can lead to severe anemia and cross-matching incompatibility using AGH-card. Safe and cautious IVIG administration is necessary for neonates. AGH-tube can be a solution for cross-matching incompatibility using AGH-card.**
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intravenous immunoglobulin, neonatology, cross-matching test, anti human globulin microcolumn gel card, retrospective analysis

INTRODUCTION

Intravenous immunoglobulin (IVIG) is a fractionated blood product prepared from the plasma of a large number of healthy donors, mainly consisting of pooled poly specific immunoglobulin G (IgG) [1]. IVIG administration in neonates has increased significantly since the first use of IVIG to treat an infant with Rh-incompati-

bility hemolytic anemia. Now, IVIG is used to treat hemolytic disease of newborns (HDN), neonatal alloimmune thrombocytopenic and bacterial sepsis in preterms [2]. Generally, IVIG therapy is considered an effective and safe treatment. However, many adverse effects including severe hemolysis cases requiring blood transfusion have been reported [3,4]. In neonates, the main adverse effects, necrotizing enterocolitis (NEC), thrombosis, and anaphylaxis, have been well researched [5]. Because hemolysis has always been neglected, studies concerning hemolysis of IVIG administration in neonates are relatively poor.

Moreover, the product of IVIG contains a great diversity of IgG anti-A and/or anti-B antibodies, which can cause interference for the pre-transfusion recipient tests including ABO blood group, DAT, irregular antibody detection test, and cross-matching test, which remain the requisite to ensure patient safety [6]. Cross-matching incompatibility may ultimately cause delay of blood transfusion for patients. In addition, ways to resolve the potential cross-matching incompatibility associated with administration of IVIG have not been researched well. Therefore, in this study, we conducted a retrospective analysis of 10 neonates from our institute. Both hemolytic anemia and cross-matching incompatibility were caused by IVIG administration. Resolution of IVIG-induced cross-matching incompatibility and selection of accurate RBCs for transfusion are reported as well.

MATERIALS AND METHODS

Study subjects

From January through December 2024, 38 neonates with severe hemolytic anemia and cross-matching incompatibility after IVIG administration in our institute were selected. Exclusion criteria: 1) with hemolytic disease, including HDN, autoimmune hemolytic anemia (AIDH), glucose-6-phosphate dehydrogenase (G6PD) deficiency, thalassemia, and paroxysmal nocturnal hemoglobinuria (PNH), 2) received operations, and 3) with history of blood transfusion. Written informed consent was obtained from the parents or legal guardians of all neonates included in the study. This study was approved by the Ethics Committee of Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology.

IVIG-associated severe hemolytic anemia

Hemolysis occurred within 10 days of IVIG administration with a decrease in HGB of more than 10 g/L, requiring RBC transfusion, positive DAT, elevated lactate dehydrogenase (LDH), and unconjugated bilirubin [7].

Measure technique

GRIFOLS Ltd. provided AHG-card, reagent cells, solution, incubator, and centrifuge. Shanghai Blood Biomedicine Ltd. provided AHG reagent. KUBOTA Ltd. provided Centrifuge KA-2200.

AHG-card cross-matching test

Major side: 25 μL of neonatal plasma and 50 μL of the 1% packed RBC suspension are added to AHG-card. Minor side: 25 μL of plasma from packed RBC and 50 μL of the 0.8 - 1% neonatal RBC suspension are added to the AHG-card. Next, incubation at 37℃ for 15 minutes. Finally, centrifugation with g-force 128.1 ± 1.0 g for 9 minutes.

AGH-tube cross-matching test

Major side: 2 drops of neonatal plasma and 1 drop of the 5% packed RBC suspension are added to the test tube. Minor side: 2 drops of plasma from packed RBC and 1 drop of the 5% neonatal RBC suspension are added to the test tube and mixed. Next, the test tubes are incubated at 37℃ for 60 minutes. Then, the RBCs are washed three times with saline, and the final wash is completely decanted. Finally, AHG is added to the dry RBC button and mixed, centrifuged with g-force 1,000 g for 15 seconds.

DAT

For DAT, 50 μL of neonatal RBC suspension with a concentration of 1% is added to AHG-card. Next, centrifugation with g-force 128 g for 9 minutes.

FAT

For FAT, 50 μL of neonatal plasma and 50 μL of the reagent cells are added to the AHG-card. Next, incubation at 37℃ for 15 minutes. Finally, centrifugation with g-force 128 g for 9 minutes.

ET

Neonatal RBC washed three times is mixed evenly with saline (the volume ratio of neonatal RBC to saline is based on the HCT of the neonates). Then, it is centrifuged immediately after incubation at 56℃ for 15 minutes. The upper liquid, which is the elution liquid, is taken out, and 50 μL elution liquid and 50 μL of the reagent cells are added to AHG-card. Next, incubation at 37℃ for 15 minutes. Finally, centrifugation with g-force 128 g for 9 minutes.

Testing process

Neonatal blood samples before and after IVIG administration were performed by AHG-card cross-matching test, DAT, FAT, and ET simultaneously. Neonatal blood samples after IVIG administration were performed by AHG-card and AHG-tube cross-matching test with the same packed RBC suspension simultaneously. Blood group antibody titer of IVIG products were analyzed by AHG-card.

Data collection

The basic conditions of all infants have been collected anonymously, including gender, age of hospitalization, diagnosis, body weight, dose and frequency of IVIG ad-

Table 1. Baseline characteristics of study population.

Demographics	n (%)
Gender	
Male	12 (40)
Female	18 (60)
Mode of delivery	
Caesarean section	24 (80)
Spontaneous labor	6 (20)
Fetus number	
Singleton	27 (90)
Twin	3 (10)
Maternal history	
Primipara	12 (40)
Multipara	18 (60)
Gestational age	
Full term	15 (50)
Premature	15 (50)
Birth weight	
Less than 1,500 g	6 (20)
More than 1,500 g	24 (80)
Blood group	
A	15 (50)
B	9 (30)
AB	6 (20)
Rh positive	30 (100)
Rh negative	0 (0)
Frequency of IVIG administration	
One	12 (40)
Two	15 (50)
Three	3 (10)
Hospitalization age: median (range), days	3.7 (0 - 10)
IVIG administration age: median (range), days	8.2 (2 - 30)
Dose of IVIG administration: median (range), g	2.5 (1.8 - 5.0)
IVIG dose/weight: median (range), g/kg	1.4 (0.8 - 2.9)
RBC transfusion volume: median (range), mL	34.5 (21 - 120)

ministration, dose and frequency of RBC transfusion, and incidence of RBC transfusion adverse reactions. Results of laboratory tests, including ABO blood type, HGB, RBC, LDH, and unconjugated bilirubin, were collected.

Statistical analysis

Normally distributed data were expressed by median (range). Paired sample *t*-test was used to compare the differences of measurement data before and after IVIG administration and before and after RBC transfusion. Chi-squared test was used to compare the differences of count data before and after IVIG administration and of cross-matching tests with AGH-card and AGH-tube. The difference of $p < 0.05$ was considered significant. These analyses were performed using SPSS 20.0 statistical software (IBM Corp., Armonk, NY, USA).

RESULTS

During the study period, 38 neonates with cross-matching incompatibility after IVIG administration were selected. Eight cases were excluded, 4 with hemolytic disease, 2 that had undergone operations, and 2 with history for blood transfusion in other hospitals. Finally, 30 cases were enrolled in this study, 12 were male and 18 were female. The indication of IVIG was sepsis in 18, NEC in 10, and undetermined reason in 2. The IVIG administration ranges of dose, frequency, and dose/weight were 1.8 - 7.5 g, 1 - 3 times, and 0.8 - 2.9 g/kg, as seen in Table 1. As shown in Table 2, the cross-matching tests by AHG-card, DAT, FAT, and ET were all negative before and positive after administration of IVIG in 30 neonates. Compared with laboratory test results before IVIG administration, the elevation of LDH and unconjugated bilirubin and reduction of RBC and HGB were statistically significant after IVIG administration. After RBC transfusion, RBC and HGB of 30 neonates increased. The elevation was statistically significant compared with those before RBC transfusion. No RBC transfusion adverse reaction was observed. Table 3 shows incompatibility cases of AHG-card and AHG-tube were 30 and 6, respectively. The difference is statistically significant. The blood group antibody titers of IVIG: batch 1 (anti-A: 128; anti-B: 16; anti-O: 4), batch 2 (anti-A: 256; anti-B: 32; anti-O: 4).

DISCUSSION

The incidence rate of IVIG-induced hemolysis occurs in approximately 1.6% of patients and ranges from 2.1 - 2.8 per 1,000 IVIG administrations [8,9]. Most cases do not show obvious clinical symptoms; hemolysis has not been usually recognized. Therefore, severe hemolysis after IVIG administration could lead to life threatening anemia, even acute renal failure and thrombosis [10] without appropriate management. In the present study, all 30 neonates showed severe anemia with HGB of 88.5 (39 - 110) g/L and HGB drop of 36.5 (16 - 92) g/L after IVIG administration. A multi-center prospective study [11] in 2017 showed a median decrease of HGB of 30 (9 - 58) g/L, with HGB of 99 - 132 g/L after IVIG administration in 12 adult patients. A prospective obser-

Table 2. Laboratory results before and after IVIG administration.

Test	IVIG administration		After RBC transfusion
	Before	After	
Cross-matching incompatibility with AHG-card: n (%)	0 (0)	30 (100)	-
Major side	0 (0)	9 (30)	-
Minor side	0 (0)	9 (30)	-
Both	0 (0)	12 (40)	-
Positive DAT: n (%)	0 (0)	30 (100)	-
1+	0 (0)	12 (40)	-
2+	0 (0)	15 (50)	-
3+	0 (0)	3 (10)	-
Positive FAT: n (%)	0 (0)	30 (100)	-
Positive ET: n (%)	0 (0)	30 (100)	-
RBC: median (range), 10^{12}/L	3.62 (2.89 - 4.85)	2.58 (1.09 - 3.42)	3.84 (3.17 - 5.02)
HGB: median (range), g/L	122 (100 - 191)	88.5 (39 - 110)	121.5 (100 - 161)
LDH: median (range), U/L	482.6 (236 - 843)	702.0 (380 - 1,058)	-
Unconjugated bilirubin: median (range), µmol/L	47.1 (4.2 - 138.5)	108.6 (21.6 - 154.3)	-

[a] Comparison of results before and after IVIG administration.
[b] Comparison of RBC and HGB before and after RBC transfusion.

Table 3. Incompatibility cases in cross-matching tests by AGH-card and AHG-tube.

Cross-matching test	AGH-card	AHG-tube	p
Incompatibility: n (%)	30 (100)	6 (20)	0.000
Compatibility: n (%)	0 (0)	24 (80)	

vational study [12] on the incidence and risk factors for hemolysis after IVIG administration enrolled 78 patients with a mean age of 48.9 (2 - 82) years and showed HGB decrease of 14.9 ± 7.6 g/L. Contrary to previous prospective studies, our study cohort exhibited significant differences in both HGB reduction levels and post-IVIG values. Generally, IVIG-induced hemolysis is considered to associate with high-dose and -frequency IVIG administration. Previously, Van Anh [13] and colleagues' observational study enrolled 661 acute KD pediatric patients treated with IVIG, and the multi-center prospective study [11] in patients with chronic ITP treated with IVIG demonstrated that the IVIG dose ≥ 2 g/kg was an independent risk factor for severe IVIG-induced hemolysis. The IVIG dose in our study was 1.4 (0.8 - 2.9) g/kg. Among 30 neonates, there were only 2 cases with IVIG doses exceeding 2 g/kg. Meanwhile, previous case reports [14,15] showed 3 adult patients with severe anemia after IVIG administration for five consecutive days. In our study, the main frequency of IVIG administration was 1 - 2 times, only 3 neonates underwent IVIG administration three times. In our study, lower dose and frequency of IVIG administration in neonates could lead to severer anemia, compared with adults and children. For neonates, the immature immune and blood systems, poor tolerance to hemolysis, low blood volume, and physiological anemia make them more likely to undergo severe anemia, even with low dose and frequency of IVIG administration. Most of IVIG administration is based on expert guidelines and clinical experience. Irrational use of IVIG may cause severe adverse effects and great economic burden to neonates. Therefore, reasonable and safe IVIG administration is crucial, and neonatologists need to consider both the benefits and the risks of IVIG administration.

The passive transfer of anti-A and anti-B hemagglutinins containing in IVIG products play a major role in the pathogenesis of hemolysis after IVIG administration [16]. Thus, non-O blood type was associated with IVIG-induced hemolysis. In our study, the neonatal blood type distribution of A, B, and AB was 15, 9, and 6, re-

spectively. The previous studies showed that patients with blood group A and AB have been observed with greater hemolysis risk [16,17], and recent studies report similar findings for individuals with blood group A, stating they have the highest risk of hemolytic events occurring [14,18] after IVIG administration. In our study, the DAT, FAT, and ET of all 30 neonates were negative before IVIG administration and positive after IVIG administration. The three tests were performed to detect hemolytic anemia caused by hemagglutinins [19]. Therefore, the IVIG administration was the cause of severe anemia in the 30 neonates. Although some manufacturers have removed the majority of ABO hemagglutinins from IVIG by a chromatographic step in their processing, the high antibody titer (anti-A: 128 and 256; anti-B: 16 and 32, respectively) may be one of the reasons for anemia after IVIG administration in our study. Therefore, strict management of IVIG products to reduce hemagglutinin can limit the risk of hemolytic anemia [20,21].

Cross-matching tests are a crucial step for safety and efficacy of RBC transfusion. At present, the commonly used methods for cross-matching tests are polybrene, AHG by tube, and AHG by microcolumn gel card. Microcolumn gel card is a combination of immune reaction technology and gel technology and uses particle filter to separate free and condensed RBC. It has been used in most transfusion departments because of the characteristics of convenient, fast, standardized operation [22]. However, the incidence of microcolumn gel card cross-matching incompatibility is 12.2%, with a false positive rate of 60.9%. In our clinical practice, the performance of cross-matching test and selection of appropriate RBC suspension for patients after IVIG administration has always been the problem that troubles us. Firstly, IVIG is a blood product containing anti-A and anti-B hemagglutinins. And according to Technical Manual (Aabb) 2014, as a macromolecular attachment protein, IVIG can lead to cross-matching incompatibility in AHG-card. Then, when IVIG-induced cross-matching incompatibility occurs, O-type washed RBC suspension is recommended by current expert consensus. However, the washing process increases risk of bacterial contamination, increases hemolysis, and reduces the cell mass, which may affect clinical outcomes in transfused recipients [23,24]. The severe shortage of O-type RBC resulted from the frequency of blood type distribution in mainland China, and lack of small packaging O-type washed RBC can led to delay in RBC transfusion and increased economic burden for patients. Compared to AHG-card, although the AHG-tube has a longer operation time, more complicated steps, and slightly lower sensitivity, the antigen-antibody reaction of the tube test is carried out in an independent system. Moreover, the step of washing the reaction system three times before adding AHG can eliminate the interference of nonspecific agglutination and macromolecular proteins. Therefore, as a classic cross-matching method, the AHG-tube is still widely used.

In our study, the AHG-card cross-matching test using samples after IVIG administration were all incompatible. Whereas, before IVIG administration, all samples tested negative with the same packed RBC suspension. By combining the results of DAT, FAT, and ET of blood samples collected before and after IVIG administration, it can be confirmed that IVIG was the cause of the AHG-card cross-matching incompatibility. To address this issue, we attempted to use AHG-tube to the perform cross-matching test. In our study, the number of positive cases by AHG-tube cross-matching was significantly lower compared with that by AHG-card cross-matching. Possible reasons for this are the following: Firstly, all neonates received a relatively small dose of IVIG, resulting in low levels of ABO hemagglutinins entering the bloodstream, as most of these bind to RBCs. Comparing the results of FAT and ET for all 30 neonates after IVIG administration, the distribution of positive FAT was 1+ in 18 cases and 2+ in 12 cases, while the distribution of positive ET was 1+ in 6 cases, 2+ in 18 cases, and 3+ in 6 cases. The intensity of the ET result was significantly stronger than that of the FAT. Additionally, the positive intensity of the major side cross-matching incompatibility in 21 cases was mainly 1+ (1+ in 16 cases and 2+ in 5 cases).

Secondly, the AHG-tube can eliminate the interference of macromolecular proteins, and the longer incubation time allows for a more adequate antigen-antibody reaction, resulting in a lower false positive rate. Based on the results of the AHG-tube cross-matching, we selected the packed RBC with the same blood type as the 24 neonates, due to limited supply of O-type washed RBC during the study period, coupled with the urgent situation of the neonates. As a result, for 6 infants with incompatible AGH-tube cross-matching on the major side, we still administered the packed RBC with the same blood type of neonates. During the transfusion process, no adverse transfusion reactions occurred. Furthermore, the levels of RBC and HGB significantly increased after transfusion, and the clinical symptoms improved remarkably, indicating the effectiveness of RBC transfusion.

In summary, for neonates, a relatively small dose of IVIG administration can lead to severe anemia. Therefore, careful consideration of the pros and cons is required when administering IVIG to neonates. When encountering cross-matching incompatibility caused by IVIG administration using the AGH-card, considering switching to the AGH-tube for cross-matching can be a solution.

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Declaration of Interest:

The authors have no competing interests to disclose.

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