Original Article

Severe hemolytic disease of a newborn due to anti-E combined with anti-Mi^a, anti-Mur and anti-Hil

Ling Wei[1], Yew-Wah Liew[2], Brett Wilson[2], Aiqun Huang[3], Jizhi Wen[1], Zhen Wang[1], Guangping Luo[1], Yanli Ji[1]

[1]Institute of Clinical Blood Transfusion, Guangzhou Blood Center, Guangzhou, China; [2]Red Cell Reference Laboratory, Australian Red Cross Lifeblood, Queensland, Australia; [3]Blood Bank, the Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

Contributions: (I) Conception and design: L Wei, G Luo, Y Ji; (II) Administrative support: None; (III) Provision of study materials or patients: A Huang; (IV) Collection and assembly of data: L Wei, J Wen, Z Wang; (V) Data analysis and interpretation: Y Liew, B Wilson; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Yanli Ji; Guangping Luo. 31 Luyuan Road, Yuexiu District, Guangzhou 510095, China.
Email: jiyanli2013@163.com; luoguangping2016@163.com.

Background: Common alloantibodies leading to severe hemolytic disease of the fetus and newborn (HDFN) could vary among different ethnic groups. The MNS blood group hybrid glycophorin GP.Mur distributes with a high frequency in the regions of Southeast Asia. Alloantibodies against GP.Mur (anti-'Mi^a') often present as mixture of antibodies against several low frequency antigens. In this study, we first described a case of severe HDFN in Guangzhou, China, which was caused by alloantibodies of anti-E in combination with specificities to the GP.Mur including Mi^a, Mur and Hil.

Methods: Blood samples from the newborn boy and parents have been subjected to antibody screening and identification analysis followed by *GYP*Mur* genotyping. The direct antiglobulin test (DAT) and the eluate technique were also performed for the newborn.

Results: The mother was group B, CCDee, Mur–, the father was group B, ccDEE, Mur+, and the newborn was group B, CcDEe, Mur+. Genotyping results showed the mother was absent for *GYP*Mur*, while the father and the newborn carried heterozygous *GYP*Mur* allele. DAT test of the newborn was strongly positive with anti-IgG. Anti-E and anti-'Mi^a' were detected in the maternal serum and the newborn's eluate, whereas anti-E alone was detected in the newborn's serum. The anti-'Mi^a' specificity was further identified as combination of anti-Mi^a, anti-Mur and anti-Hil.

Conclusions: Because alloantibodies to GP.Mur could cause severe HDFN, it is highly recommended to include GP.Mur red cells in antibody screening cells to avoid miss detection of the alloantibodies in the populations of Southeast Asia.

Keywords: Anti-E; anti-'Mi^a'; GP.Mur; hemolytic disease of the fetus and newborn (HDFN)

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Introduction

Hemolytic disease of the fetus and newborn (HDFN) is caused by maternal alloantibodies directed against red blood cell (RBC) antigens of the fetus and newborn, that can lead to severe clinical course. The alloantibodies related to severe HDFN distribute with some differences between Chinese and Caucasian populations. Anti-D is the most important alloantibody contributing to HDFN followed by anti-K in Caucasians (1). In China, as the anti-D immunoglobulin prophylaxis has not been introduced for routine use in D negative pregnant women, allo anti-D is still the most common cause of severe HDFN, followed by alloantibodies against other Rh antigens and anti-M (2). GP.Mur, a MNS

hybrid glycophorin, distributes with a high frequency (6.3–9.5%) in Southern Chinese Han population (3,4), which expresses five low incidence antigens involving Mi^a, Mur, Hil, MUT and MINY. Antibodies against GP.Mur [colloquially referred as anti-'Mi^a' (5)] usually present as a mixture of specificities against one or several of those low incidence antigens. It is very hard to detect and identify the specificity of anti-'Mi^a' in majority of laboratories because of lacking rare red cells with different MNS hybrid glycophorins (obsolete Miltenberger subsystem) phenotype. Anti-'Mi^a' is reported to be the second most common alloantibody in MNS blood group system identified in the Chinese patients, but only several HDFN cases related to anti-'Mi^a' have been reported (2). Here we report one case of HDFN caused by anti-E combined with the mixed antibodies against GP.Mur (anti-'Mi^a') including anti-Mi^a, anti-Mur and anti-Hil. We present the following article in accordance with the MDAR reporting checklist (available at https://aob.amegroups.com/article/view/10.21037/aob-21-35/rc).

Methods

Case

A male newborn with a weight of 3,400 g was born at a gestational age of 38 weeks and 5 days to a gravida 4 mother by cesarean section. The mother had no history of transfusion. Her first baby was born healthy without any clinical problems, the other two pregnancies were induced abortions. The baby developed jaundice at 5 hours after delivery, and the total serum bilirubin (TSB) level was measured at 251.6 μmol L^{-1}. Laboratory testing showed his white blood cell (WBC) count was 52.82×10^9 L^{-1}, red blood cell (RBC) count was 1.47×10^{12} L^{-1}, hemoglobin (Hb) was 61g L^{-1}, platelet was 90×10^9 L^{-1}, the reticulocyte count was 347.8×10^9 L^{-1}, and reticulocyte percentage was 23.66%. The baby was group B, RhD positive, anti-E and anti-'Mi^a' were eluted from his RBCs, suggesting the presence of HDFN due to blood group incompatibility between mother and newborn. Phototherapy and intravenous immunoglobulin (IVIG) therapy were started immediately after the admission. Since the newborn suffered from severe anemia, he also received transfusion with 50 mL of B RhD(+) E(–) GP.Mur(–) RBCs on days 1, 2 and 3, respectively, and his Hb level increased to 168 g L^{-1} on day 6. Despite taken phototherapy and IVIG therapy, his TSB continued to rise to a peak of 361 μmol L^{-1} on day 5. The newborn recovered and was discharged after being treated for 17 days in hospital, when the TSB and Hb level were 132.2 μmol L^{-1} and 114 g L^{-1}, respectively. No anemia or pathologic hyperbilirubinemia was found in the further follow-up visit to the doctor.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Guangzhou blood center ethics board (No.2019005). Written informed consent was obtained from the patient's parent.

Blood group typing

The blood samples of the newborn and his parents were serologically typed for ABO and Mur antigen using a standard tube method in saline with monoclonal anti-A, anti-B (Shanghai Hemo-Pharmaceutical & Biological Co., Ltd., Shanghai) and anti-Mur (gift from M. Uchikawa of the Japanese Red Cross). Rh blood group phenotyping was performed using Rh blood grouping gel cards (Jiangsu LIBO Medicine Biotechnology Co., Ltd., Jiangsu, China) according to manufacturer's instructions. GP.Mur genotyping analysis for the newborn and his parents was performed using the high resolution melting (HRM) method described previously (4).

Antibody identification and titer testing

A set of in-house antibody screening cells and panel cells were used for alloantibody screening and identification in saline using a tube method and an indirect antiglobulin test (IAT) with DG Gel Coombs card (Grifols, Spain). Another set of selected rare RBCs with different MNS hybrid glycophorin phenotypes and the adsorption test using RBCs with GP.HF phenotype were used to identify the specificity of anti-'Mi^a'. Dithiothreitol (0.01 mol L^{-1}) (DTT) treatment was used to destroy IgM antibody in the maternal serum and then the titration of IgG antibody was performed. The titer of the alloantibodies was determined using reagent cells (group O, E+ and GP.Mur– for anti-E titration, and group O, E– and GP.Mur+ for anti-'Mi^a' titration) in saline for IgM and in a Coombs card for IgG antibody.

Direct antiglobulin test (DAT) and elution analysis of the newborn's RBCs

DAT testing of the newborn was conducted using DG Gel Newborn card (Grifols, Spain). Elution of antibodies from the DAT positive RBCs of the newborn was performed by

Table 1 Identification of antibodies against GP.Mur for the maternal serum

Phenotype	Antigens											Results	
	Mi^a	Vw	Hut	Mur	MUT	Hil	TSEN	MINY	Hop	Nob	DANE	Serum	Serum after adsorbed by GP.HF
GP.Vw (Mil)	+	+	0	0	0	0	0	0	0	0	0	2+	0
GP.Hut (MiII)	+	0	+	0	+	0	0	0	0	0	0	2+	nt
GP.Mur (MiIII)	+	0	0	+	+	+	0	+	0	0	0	4+	4+
GP.Hop (MiIV)	+	0	0	+	+	0	+	+	+	0	0	4+	nt
GP.Hil (MiV)	0	0	0	0	0	+	0	+	0	0	0	2+	nt
GP.Bun (MiVI)	+	0	0	+	+	+	0	+	+	0	0	4+	nt
GP.HF (MiX)	+	0	0	0	+	+	0	+	0	0	0	4+	0
GP.JL (MiXI)	0	0	0	0	0	0	+	+	0	0	0	0	nt

nt, not tested.

the acid elution technique using a commercial acid elution kit (Zhanquan Biological Technology Co., Ltd. Guangzhou, China) according to the manufacturers' recommendation. The eluate was tested using the in-house panel cells for antibody identification.

Results

Blood group typing of the newborn and his parents

The blood group phenotype of the mother was group B, CCDee, Mur–, the father was group B, ccDEE, Mur+, and the newborn was group B, CcDEe, Mur+. GP.Mur genotyping results showed that both the father and the newborn carry a heterozygous *GYP*Mur* allele, the mother was negative for *GYP*Mur* with a wild-type *GYPB/GYPB* genotype, which was consistent with the phenotyping results.

Serological testing results for the mother and newborn

The DAT of the newborn's RBCs was strongly positive (3+) with both polyspecific antihuman globulin (AHG) and monospecific anti-IgG. The eluate from his red cells by acid elution technique was collected for further antibody identification.

Antibody screening tests for the maternal serum and newborn's serum were both positive, indicating the existence of unexpected alloantibodies. Antibody identification using the panel cells included two GP.Mur+ cells was conducted. The serum of the mother and the eluate of the newborn's RBCs reacted positively with all E+ cells, while still positively with one E– but GP.Mur+ cell, which indicating the existence of anti-E combined with anti-'Mi^a'. However, the newborn's serum only reacted positively with all E+ cells, but negatively with the E– but GP.Mur+ cell indicating the existence of anti-E alone in the newborn's serum. Another set of rare RBCs with different MNS hybrid glycophorin phenotypes were used to further clarify the specificity of the anti-'Mi^a' in the maternal serum, the reaction profile primarily revealed anti-Hil was present in the serum, but the mixed antibodies may also contain anti-Mi^a, anti-Mur, and anti-MUT (*Table 1*). Then, GP.HF RBCs was used for adsorption test to separate the mixed antibodies. After adsorption, serum can react with GP.Mur cells but not GP.Vw cells that suggesting the existence of both anti-Mi^a and anti-Mur in the serum (*Table 1*). The identification results indicated the maternal serum contained anti-E, anti-Mi^a, anti-Mur and anti-Hil. The eluate from the newborn's RBCs was only tested against the in-house panel cells rather than the rare cells with different MNS hybrid glycophorin phenotypes due to insufficient volume.

DTT treatment of the maternal sera showed that both anti-E and anti-'Mi^a' were a mixture of IgM and IgG antibodies, the titer of anti-E was 64 for IgM and 256 for IgG, the titer of anti-'Mi^a' was 256 for IgM and 64 for IgG. For the newborn's serum the anti-E titer was 8 for IgG and anti-'Mi^a' was not detected (*Table 2*).

Discussion

HDFN caused by blood group incompatibility between the

Table 2 The results of antibody identification and titration for the mother and newborn

	Alloantibodies	Titer			
		IgM		IgG	
		Anti-E	Anti-'Mi^a'	Anti-E	Anti-'Mi^a'
Maternal serum	Anti-E+ anti-'Mi^a'	64	256	256	64
Newborn's serum	Anti-E	0	0	8	0
Newborn's eluate	Anti-E+ anti-'Mi^a'	nt	nt	nt	nt

nt, not tested.

mother and the newborn is the most common reason of pathologic hyperbilirubinemia in the newborns. In this case, although only anti-E was detected in the newborn's serum, considering the strongly positive DAT of the newborn's RBCs, and the presence of anti-E combined with anti-'Mi^a' in the maternal serum and eluate from the newborn's RBCs, in addition to the phenotype (E+, Mur+) of the newborn, we can draw a conclusion that anti-E and anti-'Mi^a' were both the cause of pathologic hyperbilirubinemia in this patient. The anti-'Mi^a' was not detected in the newborn's serum probably due to very low titer in the serum that is under the detection limit, as most of the antibodies were adsorbed by the newborn's RBCs. It is better to use maternal serum or eluate from the newborn's red cells instead of newborn's serum to do blood cross-matching. Otherwise, in this case, it is possible to transfuse GP.Mur positive cells to the newborn if the newborns' serum is used for the cross-match as they do not contain any detectable anti-'Mi^a', which could result in adverse hemolytic reaction and shortening of the life span of the transfused RBCs, as well as aggravation of jaundice in the patient.

HDFN caused by ABO incompatibility is common in China. However, it usually causes mild clinical symptom. Non-ABO HDFN caused by maternal unexpected antibodies seems more important, since it sometimes results in severe anemia and hyperbilirubinemia. In China, non-ABO HDFN is mainly due to Rh and MNS blood group incompatibilities (2). Anti-E was reported to be the most common unexpected antibody in the Chinese patients, but it was the second most common cause for non-ABO HDFN after anti-D in China as anti-D prophylaxis has not been routinely applied for D negative pregnant women (2).

Due to a high prevalence of GP.Mur in southern China, the alloantibodies against GP.Mur can be clinically significant besides anti-M in MNS blood group system (2). Anti-M was reported to cause severe HDFN with hyporegenerative and late-onset anemia (6,7). Li and colleagues (7) reported that the titer of allo anti-M was not related to the severity of HDFN, even very low titer of anti-M could result in fetal severe anemia and stillbirth. They also noted that DAT was negative or very weak in the fetus or neonate suffering from anti-M related HDFN. Anti-Vw, an alloantibody against Vw antigen which expressed on MNS hybrid glycophorins, was also reported to cause severe neonatal hyporegenerative anemia (8). Whether anti-'Mi^a' is similar to anti-M and anti-Vw which can lead to hyporegenerative anemia still needs further investigation, but the strong positive DAT was always observed in the HDFN due to anti-'Mi^a' (9-11). Although the strength of positive DAT was not related with severity of HDFN, it was an indication of HDFN due to unexpected antibodies. The severity of HDFN due to unexpected antibodies may vary from mild hyperbilirubinaemia to hydrops fetalis, which require exchange transfusion or intrauterine transfusion. In this case, it is the first report of HDFN due to anti-E combined with anti-'Mi^a', and the newborn suffered from severe anemia and received three transfusions within three days of delivery. Which antibody played the dominant role in the HDFN, and whether the clinical pictures of anti-E immunization was alleviated or aggravated by anti-'Mi^a' was not clear.

Anti-'Mi^a' is commonly present as mixed antibodies, which is very hard to identify the specificities, because some low frequency antigens are shared among different hybrid glycophorins, thus, a series of rare red cells with different hybrid glycophorin phenotypes are needed for the identification. In this case, from the reaction profile of the panel cells with rare hybrid glycophorin phenotypes, we only can confirm anti-Hil was present in the serum, but the mixed antibodies may also contain anti-Mi^a, anti-Mur, and anti-MUT. Then, we used the adsorption test with GP.HF phenotype to separate the mixed antibodies for further identification, and finally anti-Mi^a, anti-Mur

and anti-Hil were identified. The specificity of antibodies against GP.Mur may be associated with the variable severity of HDFN due to anti-'Mi^a".

The newborn in this case was born from the fourth pregnancy of his mother. His mother had no transfusion history, and her first child was born without any pathologic jaundice. The IgG unexpected antibodies in her serum probably produced from her several previous pregnancies. This case highlights the importance of maternal unexpected antibody screening in females with alloimmunization history such as multiple pregnancies or blood transfusion. Also, GP.Mur positive red cells should be recommended to be included in the screening cells to avoid the missing detection of anti-'Mi^a' in the Southeast Asia including the southern China.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://aob.amegroups.com/article/view/10.21037/aob-21-35/rc

Data Sharing Statement: Available at https://aob.amegroups.com/article/view/10.21037/aob-21-35/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://aob.amegroups.com/article/view/10.21037/aob-21-35/coif). LW and YJ serve as an unpaid Section Editors of the *Annals of Blood*; YWL serves as an unpaid editorial board member of the *Annals of Blood* March 2018 to March 2020. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Guangzhou blood center ethics board (No.2019005). Written informed consent was obtained from the patient's parent.

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