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Brief Report

A hemolysis verified to be induced by anti-meropenem

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ABSTRACT

Incompatibility of blood groups or unexpected antibodies are primary considerations when acute hemolysis occurs during or after transfusion. However, less attention is paid to drug-induced immune hemolytic anemia (DIIHA), which is a rare but potentially life-threatening autoimmune disease. We present the case of a 34-year-old woman (group A, RhD+) who was treated with multiple antibiotics after meningioma resection. As her hemoglobin (Hb) decreased significantly from 109 g/L to 52 g/L without obvious bleeding, a blood transfusion was conducted soon after the medication, during which acute hemolysis occurred. An unexpected antibody, anti-M (MNS blood group system), was identified in the patient. It was confirmed that both the recipient and donor were group A, M antigen negative (M−) with CCDee phenotype, and no agglutination reactivity was observed in major crossmatch by testing the specimens before and after transfusion. Meanwhile, the results of the direct antiglobulin test (DAT) changed from negative to positive. Anti-meropenem, a drug-dependent antibody of meropenem, was detected, and hemolysis resolved after cessation. Anti-meropenem may mainly act through an "immune complex-type" reaction that induces intravascular hemolysis. Notably, the peculiarity of this case was dependent on the occurrence time of the acute hemolysis. Hence, it is necessary to raise awareness of DIIHA in clinical antibiotic treatments.

Keywords: transfusion, drug-induced immune hemolytic anemia, hemolysis, meropenem

INTRODUCTION

Blood transfusion is an essential treatment that can save patients' lives in emergency situations. However, it has the potential to bring about a series of adverse reactions, including hemolysis, anaphylaxis, fever, graft-versus-host disease, purpura, and so on[1]. As hemolysis is the most serious complication of blood transfusion, awareness for this adverse effect must be raised among physicians. If acute hemolysis happens during transfusion, a large amount of attention will be focused on the incompatibility of blood groups or unexpected antibodies. However, drug-induced immune hemolytic anemia (DIIHA) is under-recognized worldwide, especially in China, and although it is rare, it can be potentially life-threatening[2]. Its incidence is approximately 1 per million/year[3]. More than 100 drugs have been reported to induce DIIHA[4], of which 42% are antimicrobials, 16% are non-steroidal anti-inflammatory agents, 13% are anti-neoplastics, and 6% are anti-hypertensives/diuretics[5]. DIIHA is caused by drug-related antibodies that can be drug-dependent and drug-independent[6]. Many antibiotics

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(such as penicillin, cefotetan, and ceftriaxone) have been reported to induce drug-dependent antibodies associated with DIIHA, while a small number of drugs (such as fludarabine and a-methyldopa) can induce drug-independent antibodies[7]. Drug-dependent antibodies can be detected by using untreated red blood cells (RBCs) in the presence of soluble drugs or drug-treated RBCs. However, as not all drug-dependent antibodies act in the same way, there is still a risk of oversight due to unawareness or misdiagnosis.

We herein report a case of acute hemolytic reaction that was proven to be induced by anti-meropenem antibody during transfusion in a patient who had taken multiple antibiotics, including cefepime, meropenem, vancomycin, levofloxacin, and ceftriaxone. The process of finding the cause of this complex hemolysis was illustrated in this study.

MATERIALS AND METHODS

Patient and clinical information

A 34-year-old woman (group A, RhD+, G1P1, with prior transfusion history, hepatitis B virus carrier, with a history of penicillin allergy, Tujia nationality, China) was admitted to the hospital with meningioma. During her current hospital stay, the patient underwent meningioma resection. After the operation, there was subcutaneous hydrops around the operated portion of her head, and the results of bacterial culture showed to be serratia. Considering the intracranial abscess, cefepime was used on postoperative day (POD) 10 for 10 days. Due to poor anti-infective effect, meropenem was used instead on POD 20 for 20 days, and vancomycin was added on POD 22 for 1 day. However, the antibacterial effect was still poor. On POD 26, left frontal surgery area debridement and infected skull removal were performed. During treatment, the blood routine examination showed that hemoglobin (Hb) was significantly reduced from 109 g/L to 52 g/L without any obvious bleeding on POD 38 (***Fig. 1***). As a result, a transfusion was promptly conducted to improve oxygen capacity. However, during the transfusion of about 100 mL of packed RBCs, an acute hemolytic transfusion reaction was reported with symptoms of high fever, chills, nausea, dark red urine, and so on. The incompatibility of ABO group and unexpected antibodies were excluded timely by testing the specimens before and after the transfusion (***Table 1***). Two drug-dependent antibodies (anti-vancomycin and anti-meropenem) were detected. The patient's symptoms gradually relieved under a series of treatments, such as immediate meropenem cessation, immuno-suppression, and renal function protection. Considering the patient's urinary tract infection, levofloxacin was added on POD 40 for 5 days. Owing to poor anti-infective effects, ceftriaxone was used on POD 46 (8 days after transfusion) for 20 days and the infection was brought under control. The details of antibiotic administration are listed in ***Table 2***.

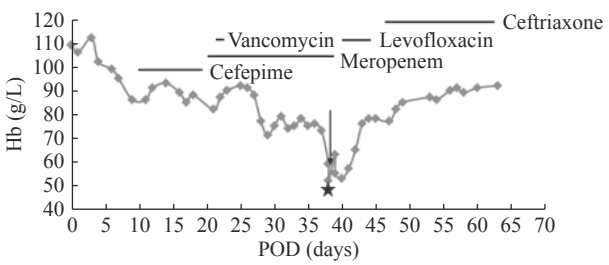

Fig. 1 **Medication during hospitalization and postoperative Hb fluctuation.** During hospitalization, five antibiotics were used at different time. After the operation, the patient's Hb decreased slowly from 109 g/L to 52 g/L on POD 38 without obvious bleeding, when the RBC transfusion was performed (arrow). Suspected acute hemolysis was initiated during transfusion (star). Hb: hemoglobin; POD: postoperative day.

Table 1 **Serologic testing on the patient and the donor**

Time	Patient		Donor*		Unexpected antibody	DAT	Eluent test of DAT
	ABO	Rh	ABO	Rh			
On admission	A	CCDee	/	/	Positive	Negative	Negative
1 DBT	A	CCDee	A	CCDee	Positive	1+	Negative
Hemolysis	A	CCDee	A	CCDee	Positive	3+	Negative

Once acute hemolysis occurred, the patient's and the donor's blood was collected and tested to rule out blood group incompatibility. Meanwhile, the unexpected antibody screening and direct antiglobulin test (DAT) were tested in representative time. *: the unexpected antibody screening and DAT of the donor were all negative; DBT: days before transfusion.

Serologic testing

ABO/Rh typing (Grifols, Barcelona, Spain) and Rh phenotype (Jiangsu LIBO, China) were tested with gel cards. Unexpected antibody detection was tested using

Table 2 **Antibiotic administration and drug-dependent antibody detection**

Antibiotic	Lasting days (POD)		Dosage	Drug antibody detection	
	Started	Stopped		Drug + untreated RBCs*	Drug-treated RBCs*
Cefepime	10	19	2 g, ivgtt, q12h	Negative	Negative
Meropenem	20	39	2 g, ivgtt, q8h	4+	1+
Vancomycin	22	22	1 g, ivgtt, q12h	1+	Negative
Levofloxacin	40	44	600 mg, po, qd	NT	NT
Ceftriaxone	46	63	4 g, ivgtt, qd	NT	NT

Drug-dependent antibodies were detected by drug-treated RBCs or in the presence of antibiotic solution. POD: postoperative day; *: RBCs tested were M−; NT: not tested.

a manual gel card method (Grifols and Jiangsu LIBO) with panel cells (Sanquin, Amsterdam, Netherlands). The direct antiglobulin test (DAT) was performed using micro-column gel antiglobulin card (mixed with anti-IgG and anti-C3d, Grifols and Jiangsu LIBO).

Drug-dependent antibody testing

The patient's blood sample was collected on POD 39. Cefepime sodium (Southwest Pharmaceutical Co., Ltd., Chongqing, China), meropenem sodium (Southwest Pharmaceutical Co., Ltd., Chongqing, China), and vancomycin hydrochloride (Pfizer Inc., New York, USA) powders dissolved in phosphate-buffered saline (PBS, pH 7.1–7.3). The drug-dependent antibodies were tested by drug-treated RBCs and untreated RBCs with soluble drug method, as described in the literature[5,8–9].

Method of drug-treated RBCs

Briefly, RBCs (M−) were used to prepare drug-treated RBCs. The cells were prepared by incubating 1/10 volume of RBCs with 40 mg/mL cefepime or 40 mg/mL meropenem or 1 mg/mL vancomycin in PBS for 1 hour at 37 ℃. The patient's serum was incubated with the antibiotic-treated or untreated RBCs at 37 ℃ for 1 hour and then centrifuged. Pools of normal sera were used as negative control in parallel with the patient's serum.

Method of untreated RBCs with soluble drugs

Briefly, the patient's serum was incubated with untreated RBCs (M−), with and without the addition of polled fresh normal sera as a source of complement in the presence of soluble antibiotics according to the literature[5,8–9]. After centrifugation, the results were judged in terms of hemolysis or agglutination.

Immunoglobulin class identification

To determine the immunoglobulin class, the patient's reactive sera were treated with dithiothreitol (DTT) according to standard protocols[10]. Briefly, the equal volume of sera and DTT were mixed and incubated at 37 ℃ for 2 hours before relative tests. PBS was used as control.

RESULTS

Hb changes on POD

After the meningioma resection, the Hb level decreased slowly without any obvious bleeding. On POD 38, the Hb dropped from 109 g/L to 52 g/L. To improve oxygen capacity, blood transfusion was carried out. However, acute hemolysis, which was reported with symptoms of hyperpyrexia, rigor, nausea, dark red urine, and so on, occurred after the transfusion of only 100 mL RBC suspension (***Fig. 1***).

Serologic testing

After admission to the hospital, the patient's unexpected antibody screening was positive and further investigation showed it to be anti-M. Then, a donor with M− was selected for transfusion. Since acute hemolysis was suspected, the blood of the patient and the donor was checked again to exclude the possibility of ABO group incompatibility and unexpected antibodies. Unsurprisingly, the recipient and donor were both group A and M− with CCDee phenotype. Moreover, no agglutination reactivity was observed in major crossmatch (patient's plasma and donor's RBCs). However, it did appear in minor crossmatch (patient's RBCs and donor's plasma) for both samples before and after transfusion. Meanwhile, the DAT results changed from negative (on admission) to positive (1 day before transfusion). No reaction was observed with the erythrocyte elution of DAT. The details of the serological tests are shown in ***Table 1***.

Antibiotic administration and drug-dependent antibody detection

After reviewing the patient's medical history, five antibiotics were used after the operation (***Table 2***). As the hemolysis occurred on POD 38 (levofloxacin

and ceftriaxone were used after that day), sera from POD 39 were tested to find out the antibodies induced by antibiotics (cefepime, meropenem, and vancomycin). There was no reactivity with M− RBCs in the presence of soluble cefepime or cefepime-treated RBCs. However, reactivity was observed both in the presence of meropenem and meropenem-treated RBCs. A reduction of agglutination reaction was noticed after treatment with DTT, indicating that the anti-meropenem belonged to the immunoglobulin M (IgM) class. What's more, although vancomycin was used for just one day on POD 22, the drug-dependent antibodies (anti-vancomycin) could still be detected by M− RBCs in the presence of vancomycin (***Table 2***).

Changes of biochemical parameters at representative time

To figure out the time of hemolysis, the biochemical parameters at different representative time were analyzed. As early as two days before blood transfusion, there were abnormal elevations in aspartate transaminase (AST), lactate dehydrogenase (LDH), total bilirubin (T-Bil), conjugated bilirubin (CB), and unconjugated bilirubin (UCB). These continued to rise for a certain time (***Table 3***), and returned to normal levels after immediate meropenem discontinuation on POD 39.

Table 3 **Changes of biochemical parameters before and after blood transfusion**

Time	POD	Urea	Cre	UA	ALT	AST	LDH	T-Bil	CB	UCB
OD	0	6.3	58	265	22	16	319	5.1	0.9	4.2
3 DBT	35	7.4	43	144*	39	27	452	12.6	2	10.6
2 DBT	36	7	57	110*	85#	109#	929#	39.3#	4.8	34.5#
1 DBT	37	6.7	49	105*	58	63#	1043#	48.1#	6.7#	41.4#
DT	39	6.3	57	110*	43	87#	1843#	60.8#	8.1#	52.7#
18 HAT	39	5.1	48	148*	38	79#	2133#	28#	3.1	24.9#
32 HAT	40	4.9	57	110*	46	31	1282#	4.8	1.8	4
58 HAT	41	4.2	44	165	42	23	1101#	4.8	1.8	3
6 DAT	45	3.4	49	151*	95#	54#	645#	8.5	0.8	7.7
13 DAT	50	3.1	46	232	89#	46	404	4.8	1.5	3.3
POD 63	64	2.8	47	254	27	23	699	3.8	0.1	2.7

OD: operation day; DBT: days before transfusion; DT: during transfusion; HAT: hours after transfusion; DAT: days after transfusion; POD: postoperative day; Urea: normal range 2.6–7.5 mmol/L; Cre: creatinine, normal range 41–73 μmol/L; UA: uric acid, normal range 155–357 μmol/L; ALT: alanine transaminase, normal range 13–69 U/L; AST: aspartate transaminase, normal range 15–46 U/L; LDH: lactate dehydrogenase, normal range 313–618 U/L; T-Bil: total bilirubin, normal range 3.0–22.0 μmol/L; CB: conjugated bilirubin, normal range 0–5.0 μmol/L; UCB: unconjugated bilirubin, normal range 0–19.0 μmol/L; *: below normal range; #: above normal range.

DISCUSSION

DIIHA is a rare but potentially life-threatening autoimmune disease. Its mechanism is complicated and still not fully understood by now[2,11–12]. Basically, DIIHA has been reported to occur *via* non-immunologic protein adsorption (NIPA) or drug-induced antibodies[13]. Generally, few hemolysis happens through NIPA while most of the hemolysis is associated with drug-induced antibodies. Furthermore, drug-induced antibodies can be divided into drug-independent and drug-dependent antibodies. The former antibodies are true autoantibodies which can bind erythrocytes in the absence of drugs and are hard to be serologically distinguished from warm autoimmune hemolytic anemia (WAIHA), which is mediated by autoantibodies.

Meanwhile, drug-dependent antibodies can lead to extra- or intra-vascular hemolysis. Hence, the diagnosis of DIIHA focuses on the detection of drug-dependent antibodies. These antibodies can be detected with drug-treated RBCs (drugs bind tightly to the RBC's surface, so-called "drug-adsorption mechanism" reaction) or with untreated RBCs while in the presence of the soluble drug (drugs cannot bind tightly to the RBC's surface, so-called "immune complex-type" reaction)[6]. The drug-adsorption mechanism can induce subacute extravascular hemolysis in the presence of more drugs than the immune complex-type reaction that is associated with worse intravascular hemolysis due to IgM-antibody formation and complement activation. However, as these drugs act in a number of different ways, both drug-treated and untreated RBSs are still needed for the detection of DIIHA. Our experiment confirmed that anti-meropenem may act primarily through the immune complex-type reaction, since a reduction of agglutination reaction was noticed after treated with

DTT, which indicated that the anti-meropenem belonged to the IgM class, while it also may act secondarily through the drug-adsorption mechanism. Hence, agglutination intensity was so different between the drug-treated group and drug + untreated group (***Table 2***).

In this case, acute hemolysis took place after the transfusion of 100 mL RBC suspension on POD 38. The transfusion was discontinued, and the blood samples from the recipient and donor were collected and tested. At first, it was supposed that the hemolysis might be induced by ABO group incompatibility or unexpected antibodies (anti-M, which might be induced by pregnancy or transfusion before). However, the blood of the recipient and donor was confirmed to be group A and M− with CCDee phenotype (***Table 1***). Meanwhile, the compatibility of major crossmatch could rule out our previous hypothesis. The main reason why there was no agglutination in the major crossmatch was that all the soluble meropenem in the vessel was binding tightly to the anti-meropenem (immune complex-type). However, as the result of the patient's DAT changing from negative to positive (1+, before transfusion), agglutination reactivity was observed in the minor crossmatch. Since the agglutination intensity was weaker than the self-control, the crossmatch test was cleared for use and blood was sent out for clinical treatment. If more attention had been paid on the transformation of DAT and weak agglutination reactivity in the minor crossmatch, the drug-associated antibody would have been detected earlier, and the drug treatment would have ceased earlier.

In this study, as the patient's Hb level decreased slowly from 109 g/L to 52 g/L without any obvious bleeding, a hemolytic anemia was suspected. After going through the patient's medical history, the possibility of DIIHA came into our consideration. After laboratory testing, drug-dependent antibodies were detected (anti-vancomycin and anti-meropenem). As vancomycin was only used on POD 22 for a single day, it was excluded. Meanwhile, the anti-meropenem may function through both complex-type immune and drug-adsorption mechanism. Hence, meropenem discontinuation was executed immediately, and the patient's symptoms were relieved gradually. In addition, more clues were found when checking over the changes in biochemical parameters at representative time points. Two days before transfusion, ALT, AST, LDH, T-Bil, and so on all increased significantly, and then dropped once meropenem ceased. Further investigation showed that meropenem was taken just an hour before the transfusion. A hemolytic reaction occurred in the presence of meropenem, anti-meropenem, and fresh blood. With an awareness of the Hb drop and the change of biochemical parameters, we should conclude that it may be caused by DIIHA, and then the hemolysis after transfusion may not have occurred.

In 2015, Oka *et al*. first reported a DIIHA case induced by anti-meropenem[5]. However, a main feature of our case was that acute hemolysis occurred during transfusion, soon after the administration of medication (meropenem). Hence, blood group incompatibility or unexpected antibodies were suspected. Through a careful and timely analysis, DIIHA induced by meropenem was detected. Once meropenem administration ceased, the patient gradually recovered. Apart from meropenem, DIIHA induced by cephalosporin, especially ceftriaxone, had the highest incidence among antibiotics[7,14–16]. To ensure patient safety during medication, drug-dependent antibodies should be monitored.

As less attention is paid to DIIHA in China, many DIIHA cases may be unappreciated or misdiagnosed. Thus, it is necessary to raise awareness of DIIHA among clinicians involved in antibiotic treatment.

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