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Case Report Open Access

A Fatal Case of Cefazolin-Induced Immune Hemolytic Anemia in Iran

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Abstract

Introduction: Drug-induced immune hemolytic anemia (DIIHA) is known as an unpredictable and in some cases serious complication occurring upon pharmacotherapy. Cefazolin, as a first generation cephalosporin used against gram-negative bacteria, has rarely been reported to cause hemolytic anemia. Here, we report the first fatal case of hemolytic anemia associated with cefazolin treatment.

Materials and methods: A 24-year-old woman undergoing operative hysteroscopy for resection of the uterine septum developed hemolytic anemia upon receiving cefazolin immediately before and after the operation. A few hours after surgery, clinical signs of disseminated intravascular coagulation (DIC) appeared. Despite all supportive medical measures that were taken, the patient died of multiple organ failure about 24 hours after admission to ICU. The patient had no history of transfusion or allergies to any types of drugs including antibiotics. Direct anti-globulin test (DAT) was conducted, and the presence of antibody and complement on patient's RBCs was investigated. In addition, the reaction between patient's RBC eluate and cefazolin-coated control RBCs was explored.

Results: DAT was strongly positive (4+); in addition, the presence of IgG4 and C3d on the patient's RBCs was shown. Moreover, the eluate from patient's RBCs reacted (3+) with RBCs pre-coated with cefazolin.

Discussion: The results confirmed the presence of cefazolin-dependent antibodies. This case reveals the importance of taking precaution before using cefazolin even in cases where there is no previous evidence of drug sensitization.

Keywords: Cefazolin; Direct anti-globulin test; Disseminated intravascular coagulation; Immune-mediated hemolytic anemia

Introduction

Drug-induced immune hemolytic anemia (DIIHA) is a rare and unpredictable complication of pharmacotherapy. Accurate estimates of the current incidence of DIIHA are not available because of the difficulty in capturing all cases and estimating the true number of individuals exposed to a drug. The pharmacopeia associated with DIIHA has changed over the recent decades, because the prototypical drugs (e.g., high-dose penicillin and α-methyldopa) have declined in use [1].

However, about 130 new agents have been documented to cause immune system-mediated hemolysis [2]. Approximately 3-4% of patients receiving high doses (e.g., millions of units per day) of intravenous penicillin or first or second-generation cephalosporins develop a positive direct antiglobulin test (DAT). In contrast, in only a small fraction of these patients hemolytic anemia begins to manifest itself. The second and third generation cephalosporins are responsible for most cases of DIIHA today [1,3]. The first useful clue to the presence of an antibody directed against a drug may be that an eluate from the DAT positive red blood cells (RBCs) fails to react with normal RBCs. If the patient is being treated with penicillin or cephalothin, the next step is to test the eluate against drug-coated RBCs [4].

In present case, a 24-year-old woman underwent operative hysteroscopy for resection of the uterine septum. For prophylaxis against bacterial infection, due to a history of cervicitis and pelvic infection, she received 1 gr intravenous cefazolin immediately before and after the operation. Prior to admission she had taken an oral course of doxycycline. Her presenting complaint was primary infertility for six years and her past medical history was ordinary, with no history of transfusion or drug allergies. A complete blood count, few days before admission documented hemoglobin of 12.5 g/dl. About three hours after the operation (at 2:30 pm), she complained of dizziness and urinary retention. Her vital signs indicated collapse with a PR above 130 per min and a BP of 80/50 mmHg. CBC revealed hemoglobin of 7 g/dl, WBCs 20,000, and Platelets: 180000. Multiple blood samples exhibited severe intravascular hemolysis with schistocytes in a peripheral blood smear. Two units of red blood cells were ordered. Severe hemoglobinuria was noticed after urethral catheterization. Primary supportive measures did not make significant improvement in vital conditions. Shortly after, clinical signs of disseminated intravascular coagulation (DIC) with persistent oozing of blood from every needle puncture site were added to the scenario. Elevated values of PT: 36.8, PTT: 69, and INR: 5.7, D-dimer>10000 µg/l (reference value<500), Fibrinogen<50 mg/dl (reference value:

200-400), and Fibrin Degradation Products>20 µg/mL (reference value<5) confirmed the clinical diagnosis of DIC. At 4:30, her O_2 saturation dropped to 85 and clinical signs of pulmonary edema started to manifest. Due to the progressive courses of respiratory failure and pulmonary edema (ARDS), the patient was transferred to ICU. At 18:00, she was incubated. From this time on, complete anuria developed. Despite receiving 6 units of RBCs, 12 units of FFP and 18 units of cryoprecipitates, the patient died about 24 hours after admission to ICU with multiple organ failure. Immunohematologic testing was performed at the blood center reference laboratory. Specific laboratory parameters for the patient are listed (Tables 1-5). Through reporting this case, we aim at drawing the attention of patients' clinical teams (e.g. physicians and nurses) to the importance of close monitoring of patient status and recognizing DIIHA upon utilizing cefazolin.

Patient and Methods

The Iranian Blood Transfusion Organization's Immunohematology Reference Laboratory (IBTO - IRL) performed immunohematologic work-up for possible drug-related antibody analyses. All serological tests were performed on the post cefazolin blood samples. ABO and Rh (D) typing was conducted manually using standard tube methods (Anti-A, Anti-B and Anti-D blend, Diagast/Loos, France). In addition, the patient's RBCs were treated with chloroquine diphosphate solution (Sigma-Aldrich, UK) and subsequently ABO and Rh (D) tests were repeated using these cells. The antibody screening and auto-control tests were performed under three conditions including immediate spin, 37°C incubation, and in the presence of anti-human globulin (AHG; Bio-Rad Dreieich, Germany) according to standard low ionic strength saline (LISS) method (DiaLISS; Bio-Rad ,Cressier FR Switzerland).

The conventional direct anti-globulin test (DAT) was conducted using two different sources of poly-specific anti-human globulin (clone 1; Bio-Rad Dreieich, Germany and clone 2; IBRF holding co. Tehran, Iran); also differential DAT was performed using anti-IgG (Bio-Rad Dreieich, Germany), and anti-C3d (Diagast Loos, France) antibodies. DATs were carried out as per standard internationally accepted serologic methods [4]. An acid elution was performed to prepare an eluate solution from the patient's RBCs using a commercial kit (Red Cell–Elute Kit CE, Lorne Laboratories LTD, Lower Early, RG6 4UT, UK) according to the manufacturer's recommendation. The eluate was then tested for reactivity with a panel of 11 antibody identification cells (IBTO-IRL homemade 11 cell ID panel Kit).

To evaluate the cause of DIIHA and also due to the patient's physician request, cefazolin (antibiotic) and provive MCT-LCT 1% (propofol 10 mg/ml; a short acting general anesthetic agent), which were suspected of causing the fatal immune hemolysis were included in drug investigations. Both drugs were provided for the reference laboratory by the patient's physician; the drugs used in performing the laboratory experiments were the same as those given to the patient. Three separate sets of normal O RBCs were prepared (IBTO homemade 3 cell mini-panel). The first set was RBCs washed but not coated with a drug. The second and third sets of RBCs were those pre-incubated with cefazolin (0.04 g/ml in PBS) and provive (undiluted), respectively according to the method previously described by JUDD [5]. Three test tubes were allocated to each RBC set (Table 5). Assays were performed in three conditions: RT, 37°C and in the presence of AHG. Normal serum was tested against the untreated and drug-treated RBCs as the negative control.

Results

As for the patient's blood group typing, the records showed that four days prior to the surgery ABO and Rh (D) tests had been performed by Baghyat-Allah hospital laboratory and reported as A Rh (D) positive with no indications of discrepancy between forward and reverse reactions (Table 1).

a. Pre-Cefazolin treatment								
ABO & Rh	**Forward type**		**Reverse type**		**ABO Inter pretation**	**Rh(D) type**		**Rh Interpretation**
	anti-A	anti-B	A1 cells	B cells	anti-D	Rh control		
	4+	0	0	4+	A	4+	0	Positive
b. Post-Cefazolin treatment								
ABO & Rh	**Forward type**		**Reverse type**		**ABO Interpretation**	**Rh(D) type**		**Rh Interpretation**
	anti-A	anti-B	A1 cells	B cells	anti-D	Rh control		
	4+	W +	0	4+	Unresolved†	4+	W+	Unresolved
ABO & Rh repeated with chloroquine treated cells	4+	0	0	4+	A	4+	0	Positive
Weak Inconclusive result Strength of positive reactions from (W+,1+,2+,3+,4+) weakest to strongest Negative								

Table 1: ABO and Rh results.

However, upon performing blood group typing tests on post-operative samples we found out unexpected reactions (weak+) to anti-B and Rh (D) control (Table 1); this made ABO and Rh (D) interpretations inconclusive.

Hence, we repeated ABO and Rh (D) tests using both warm saline method and chloroquine-treated cells to disassociate potentially existing IgM/IgG from RBCs membranes [4]. Warm saline technique failed to resolve the discrepancy; however, the chloroquine results were consistent with the pre-operative results so that they confirmed the patient's ABO and Rh (D) blood group to be A Rh (D) positive.

The results for antibody screening test were negative and showed no alloantibody presence in patient's serum. In contrast, the auto-control was strongly reactive (4+) at all conditions (Table 2).

Incubation Phases	IS	37ºC	AHG	CCC
Cell 1 §	0	0	0	
Cell 2	0	0	0	
Cell 3	0	0	0	
AC	4+	4+	4+	

* Immediate Spin
AHG: Anti-Human Globulin
CCC: Coombs Control Cells
Numbers indicate antibody Screening Cells Positive reaction (1+- 2+)

Table 2: Antibody screening test results post-cefazolin specimen (patient serum).

DAT was strongly positive (4+) showing agglutination of patient's RBCs with two different clones of polyspecific anti-human globulin. In addition, differential DAT revealed the presence of IgG (4+) and C3d (4+) on the surface of patient's RBCs (Table 3). As control, 6% albumin control was used.

PS1*	CCC	PS2	CCC	Anti IgG	CCC	Anti C3d	CCC
4+	NT	4+	NT	4+	NT	4+	NT

* Poly specific anti-human globulin
Coombs Control Cells
Not Tested

Table 3: Direct antiglobulin test results (Post-Cefazolin Specimen).

The eluate from the patient's RBCs did not react with any the panel RBCs (Table 4). The patient's serum and the eluate did not react with either the untreated or provive-treated RBCs (Table 5). Also, the patient serum did not react with cefazolin-treated RBCs, while the eluate from the patient's RBCs reacted strongly (3+) at AHG phase with cefazolin-coated RBCs. The normal serum control in all three sets was non-reactive. Accordingly, a suspected drug-induced warm autoimmune hemolytic antibody was indicated (Tables 4 and 5).

	AHG	CCC	Control (last wash)	CCC
Cell 1*	0		0	
Cell 2	0		0	
Cell 3	0		0	
Cell 4	0		0	
Cell 5	0		0	
Cell 6	0		0	
Cell 7	0		0	
Cell 8	0		0	
Cell 9	0		0	
Cell 10	0		0	
Cell 11	0		0	

* Numbers indicate antibody Screening Cells
Anti-Human Globulin
Coombs Control Cells
Positive reaction (1+ - 2+)

Table 4: Acid elution results post cefazolin specimen (patient eluate).

Discussion

Although the field of drug-induced hemolytic anemia abounds with cases of reactions to second and/or third generation cephalosporins, such complications have been less commonly observed upon utilization of first generation cephalosporins [1,2]. In particular, among the latter group, only a few reports of cefazolin–associated hemolysis could be found in the literature [6-8]. Here, we described the first fatal case of cefazolin-associated hemolytic anemia.

To diagnose a case of antibiotic-induced hemolytic anemia one needs to exclude all other possible causes [9]. Having suspected that a drug might be the cause of hemolytic reactions, we performed a set of laboratory experiments to find the underlying mechanisms. Due to the short interval between patient's surgery (drug intake) and her death (which was quite unpredictable) very limited amount of blood sample was available for complete blood bank serology testing. Furthermore, no pre-operative (pre-drug intake) blood sample was available. Nonetheless, the Immunohematology Reference laboratory was able to perform the most necessary laboratory experiments on post-operative samples. These tests included resolving patient's ABO and Rh (D) discrepancies by pre-treating the patient's RBCs with chloroquin in order to separate any unwanted antibodies attached to the RBCs membrane. It is important to rule out any misidentification of patient's blood sample received by the laboratory. Since the patient received multiple blood transfusions at the onset of DIC event, correct identification of patient blood sample by comparing the ABO and Rh (D) blood group results, at hand, with previous records of patient's blood group was very important. As it was seen, auto control test on patient's blood sample was strongly reactive on all phases including AHG phase (Table 2). DAT test followed by a special immunohematology technique (acid elution process) were two other important procedures needed to be considered. Therefore, utmost care was applied to perform these tests (Table 5).

a. **Untreated Cells**			
Incubation phase	**Untreated cells + Patient serum**	**Untreated cells + Normal sera complement source**	**Untreated cells + Patient eluate**
RT	0	0	0
37ºC	0	0	0
AHG	0	0	0
CCC			
b. Cefazolin-coated cells			
Incubation phase	**Cefazolin-coated RBCs + Patient serum**	**Cefazolin-coated RBCs + normal serum**	**Cefazolin-coated RBCs + Patient eluate**
RT	0	0	0
37ºC	0	0	0
AHG	0	0	3+
CCC			NT
c. Provive-coated cells			
Incubation phase	**Cells treated with provive + Patient serum**	**Cells treated with provive + normal serum**	**Cells treated with provive + Patient eluate**
RT	0	0	0
37ºC	0	0	0
AHG	0	0	0
CCC			

Table 5: Reaction of normal sera, patient's serum and patient's RBC eluate with untreated RBCs, cefazolin-coated RBCs and provive-coated RBCs under different experimental conditions.

The result of one DAT is an important and basic requirement for suggesting a case as being drug-induced hemolytic anemia. In the present case, strongly positive DAT results on post-drug treatment specimens pointed to immune-mediated hemolytic anemia following drug injection. In addition, according to differential DAT results, IgG and C3d were present on the patient's RBCs, proposing that antibody attachment together with complement pathway, rather than non-specific binding of serum proteins, was responsible for RBC lysis. Here, the duration between drug administration and antibody-dependent drug-induced hemolytic anemia was surprisingly short suggesting the possibility that high titers of pre-existing anti-drug antibodies might have been responsible for the observed drug-induced anemia. Also, the clinical signs of DIC, observed in this case, are in favor of intravascular rather than extracellular hemolysis.

To find the main drug responsible for the observed DIIHA, normal O erythrocytes were coated with either cefazolin (prophylactically used antibiotic) or provive (general anesthetic agent) and examined for reaction with either patient's RBC eluate or her serum. As control groups, uncoated RBCs were tested accordingly. The eluate showed positive results in presence of cefazolin-treated RBSs, while no provive-related reactions were observed; these finding introduce cefazolin as the main cause of RBC hemolysis in the current case. Nonetheless, the specificity of eluate reaction with the drug could have been further corroborated through addition of a cefazolin solution for inhibition of the reaction; however, as mentioned earlier in this section, we had very limited patient's blood sample. In line with previous eluate results, no reaction was detected when the eluate was mixed with uncoated RBCs suggesting that the antibodies existing in the eluate function in a drug-specific manner. Notably, the patient's serum did not react with neither of the two groups of drug-coated RBCs. This raises the possibility that most of serum IgG specific for cefazolin-coated RBCs had been adsorbed to patient's erythrocytes leading to this fatal case of drug-associated hemolytic anemia.

Commonly, patients with drug-induced hemolytic anemia have a history of previous drug intake with no major problem; this could be the reason for generation anti-drug antibodies causing DIIHA following next treatments. The first case of cefazolin-induced DHIIA goes back to 1977 when a patient admitted for renal artery bypass surgery was prophylactically treated with cefazolin and showed signs of hemolytic anemia [8]. In that case, the patient had a history of penicillin sensitivity although had never received cephalosporins; hence, the authors assumed the structural similarities between penicillin and cefazolin as the reason for development of cross-reactive antibodies. In addition, it has been demonstrated that anti-penicillin antibodies cross-reactively bind to cephalothin-coated RBCs and also that such erythrocytes have shorter survivals in patients with

penicillin-induced hemolytic anemia. In our case, a noticeable drop in hemoglobin level only a few hours after drug injection suggests that preexisting antibodies, rather than newly developed ones, have been involved in erythrocyte lysis. However, our patient had no history of being treated with cefazolin, other cephalosporins, or penicillin. Similar to our case, a patient with no history of drug sensitivity developed hemolytic anemia after a total knee arthroplastic surgery [7]; multiple prior blood transfusions were assumed as the potential cause for the presence of preexisting in that case. However, our case had never experienced any kind of transplantation or blood transfusion according to her medical history making the clinical picture complicated. In fact, this phenomenon has always been difficult to explain; in this regard, it has been shown that a single dose of cefotetan can cause severe hemolytic anemia in patients with no history of cefotetan intake. It is of note that, it commonly happens that people have drugs not prescribed by their physicians and do not mention or recall it at the time of admission. In addition, a wide range of antibiotics are prophylactically given to cattle and chicken. Although meat and dairy products are used by a large portion of Iranian population, sensitivity to cefazolin is not commonly observed; nonetheless, it is still plausible that there have been epitopic similarities between the antibiotics given to cattle and those taken by our patient without a doctor's prescription; this could have led to the development of cross-reactive antibodies responsible for the observed clinical complications following cefazolin intake in the present case. However, whether or not our patient had developed cross-reactive B- or T- cell clones activated against a type of antibiotic is currently unclear to us.

Taken together, it seems that the attachment of preexisting antibodies to the surface of cefazolin-coated RBCs in patient's blood has caused severe hemolysis of erythrocytes leading to the patient's death despite all the standard supportive measures that were taken. Post-mortem pathological examination revealed the uterus and cervix within normal limits without significant pathological findings. Local hematoma identified in right pelvic and femur region, possibly due to multiple blood sampling from femoral route. There have been a few previous reports regarding sensitivity to cefazolin in the past and here we describe the first case of death following cefazolin-related hemolytic anemia. We wish to alert the physicians to the probability of facing fatal cases of cefazolin even when there is no previous history of drug sensitivity.

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