Case Report

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Severe drug-induced immune hemolysis due to ceftriaxone

Ravi C. Dara, Rakesh Sharma, Himanshu Bhardwaj

Abstract:

Drug-induced immune hemolytic anemia (DIIHA) is a rare condition that results primarily due to drug-induced antibodies, either drug dependent or drug independent. For its diagnosis, specialized immunohematology laboratory is often required for performing complex serological tests. The exact incidence of DIIHA is not known, but as per data published by Garratty, the incidence of DIIHA is estimated to be one in million population.[1] There are many drugs which are implicated in causing DIIHA ranging from antimicrobials, antineoplastics to anti-inflammatory drugs. Among antimicrobials, cephalosporins are commonly reported to cause hemolytic anemia.[2] In this report, we present a life-threatening hemolytic reaction to cephalosporin (ceftriaxone) in a 15-year-old child, which was diagnosed and managed in a timely manner. Our patient was suddenly deteriorated after two doses of intravenous ceftriaxone, with increase in pallor, fatigue, and frank hematuria. Repeat laboratory investigations showed signs of hemolysis, presence of schistocytes, raised lactic dehydrogenase, and indirect bilirubin. Reticulocyte count was 3.4%. Direct antiglobulin test was strong positive (4+) with IgG and C3d positive. Testing for drug-dependent antibody confirmed the presence of ceftriaxone-dependent antibody. Drug was stopped immediately. There was a rapid improvement in patient's general condition after discontinuation of drug. Laboratory parameters were improved after 48 h, and the patient was stable with no further drop in hemoglobin and hemolytic episodes. We suggest the need for proper immunohematological services to diagnose and solve such complex cases promptly.

Keywords:

Autoimmune hemolytic anemia, drug-induced hemolytic anemia, ceftriaxone

Introduction

Hemolytic anemia can be defined as anemic state primarily due to a shortened red-cell survival because of premature destruction. The hemolytic anemia is caused either due to the intrinsic abnormalities arising from within the red blood cell (RBC) and/or its membrane (intracorpuscular defects) or extrinsic abnormalities in the environment of the RBC. Extrinsic abnormalities are mainly because of the autoimmune mechanisms as in warm and cold hemolytic anemia.

Drugs are also responsible for hemolytic anemia called as drug-induced immune hemolytic anemia (DIIHA). It is a rare condition that results primarily due to drug-induced antibodies, either drug dependent or drug independent. For its diagnosis, specialized immunohematology laboratory is often required for performing complex serological tests. The exact incidence of DIIHA is not known, but as per data published by Garratty, the incidence of DIIHA is estimated to be one in million population.[1] There are many drugs which are implicated in causing DIIHA ranging from antimicrobials, antineoplastics to anti-inflammatory drugs. Among antimicrobials, cephalosporins are commonly reported to cause hemolytic anemia.[2] We hereby report a life-threatening hemolytic reaction to cephalosporin (ceftriaxone) in a 15-year-old child.

Department of Transfusion Medicine, Manipal Hospitals, Jaipur, Rajasthan, India

Address for correspondence:
Dr. Ravi C. Dara, Department of Transfusion Medicine, Manipal Hospitals, Sikar Road, Jaipur - 302 013, Rajasthan, India.
E-mail: rcdara@gmail.com

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Case Report

Clinical picture

A 15-year-old male child, with no relevant medical and family history, presented with the complaints of malaise, cough with expectoration, and fever. On laboratory tests, his white blood cell (WBC) counts were high (15,500/µl), and X-ray findings were suggestive of lobar pneumonia. He was started with ceftriaxone (intramuscularly 1 g/day) for 7 days, salbutamol, and paracetamol for fever as an outpatient because of refusal of admission by his parents. After 3 days of treatment, fever was persistent and his general condition got deteriorated. He was hospitalized, blood tests were repeated, and ceftriaxone was continued intravenously. The complete blood counts were hemoglobin 9.1 g/dl, hematocrit 24%, WBC count 10,500/µl, and platelet count 313,000/µl. After two doses of intravenous (IV) ceftriaxone, in the night, condition worsened further. His vital signs were unstable, heart rate 170/min, respiratory rate 30/min, temperature 37.8°C, and blood pressure 90/60 mmHg. There was increase in pallor and fatigue with pale conjunctivas. Urine was red indicating frank hematuria. Repeat laboratory investigations showed signs of hemolysis as hemoglobin of 6.3g/dl, hematocrit of 17%, presence of schistocytes on peripheral blood smear, LDH and indirect bilirubin were also raised. Reticulocyte count was 3.4%. Renal and liver function tests were normal. Direct antiglobulin test (DAT) was strong positive (4+).

In the view of hemolysis with strong positive DAT, blood sample was sent to our immunohematology laboratory for evaluation of DAT positivity and hemolysis.

Immunohematological workup

Blood grouping was B positive with no discrepancy in forward and reverse typing. Antibody screen and auto control were negative while DAT was 3+ positive. This picture of anemia with positive DAT was suggestive of possible hemolytic anemia. Blood grouping was done by conventional tube technology (CTT), and DAT was on polyspecific antihuman globulin (AHG) column agglutination card (Ortho Clinical Diagnostics; Mumbai, Maharashtra, India).

Direct antiglobulin test monospecific

DAT was repeated using monospecific DAT card (IgG, IgM, IgA, C3c, C3d, and Control; Bio-Rad laboratories; Mumbai, Maharashtra, India) to identify the type of antibody sensitization. Results showed DAT positivity due to IgG and complement (C3d3+ and C3c1+ positive). Control was negative which validated the results.

Elution

Acid elution (Bag Systems; Germany) was performed to free the IgG antibody and recover it in usable form. Positive and negative controls were used in parallel to validate the results. Eluate showed no reactivity with three reagent cell screening panel (Ortho Clinical Diagnostics; Mumbai, Maharashtra, India). Eluate was also negative with three different A cells and B cells. Last wash was negative which validating the elution and wash process.

Elution was repeated to confirm the negative results, but the results were same.

Workup for DIHA

With the hemolytic clinical picture, DAT positivity and negative eluate possibility of DIIHA were considered.

Drug history

Drug history was evaluated asking:

- What drug(s) is (are) the patient taking now or recently?
- Have any ongoing drugs been implicated in causing DIIHA?
- Is there a temporal relationship between the drug administration and the hemolytic anemia?

Drug, ceftriaxone, was identified and found implicated in causing DIIHA based on various published studies.[2] History showed a strong temporal relationship between ceftriaxone administration and hemolysis which warrants drug testing.

Drug-induced immune hemolytic anemia testing

As the ceftriaxone antibody testing was well-studied, we have used previously described method for drug antibody testing.

Preparation of drug for testing

Many antibodies against drugs are detected by testing untreated RBCs in the presence of a solution of the drug. Cephalosporins and piperacillin antibiotics come under this group. Ceftriaxone is the third-generation cephalosporin which is available as 250 mg powder to be mixed with distilled water before administration. We used 1 mg/ml solution of drug in phosphate-buffered saline (PBS) for testing a patient's sample in the presence of soluble drug. A volume of 10 mg of powder was dissolved in 10 ml of PBS. The mixture was centrifuged at 3000 rpm for 3 min and the supernatant is transferred to a clean test tube. pH was checked and found to be 6.7 (target pH was between 6 and 8, to be compatible with RBCs).

Complement source

Pooled fresh normal serum collected from volunteers was used as a source of complement along with serum and drug testing.

Controls

Positive controls were not available. Negative controls included combination of serum + PBS, serum + complement + PBS, complement + drug, and complement + PBS.

Reagent red cells

R1R1, R2R2, and rr cells were used in 10% suspension instead of routine 3%–5% suspension because heavier RBC suspension enhances the detection of hemolysis and aliquot of it was treated with enzyme.[3]

Procedure

All testings were done by CTT. Twelve tubes in set of two were used (six tubes for untreated RBCs and six tubes for enzyme-treated RBCs).

- Tubes were labeled as follows:
  - Serum + drug
  - Serum + PBS
  - Serum + complement + drug
  - Serum + complement + PBS
  - Complement + drug
  - Complement + PBS.
- Two drops of serum, complement source, drug, and PBS were added in the appropriate test tubes
- One drop of untreated or enzyme-treated RBCs (10% suspension) in appropriate sets of test tubes
- Mixed and incubated at 37°C for 1 h
- Centrifuged and examined for hemolysis and agglutination
- RBCs were washed 4 times and after washing, polyspecific AHG were added to the cell button
- Results were noted
- IgG-coated RBCs (Check cells) were added to the nonreactive tests.[3]

Results

Hemolysis or agglutination was considered as positive results. Results of drug antibody in the presence of soluble drug are shown in Table 1.

Interpretation

Positive results or reactivity in the tests with patient's serum plus drug with or without complement indicate the presence of antibody against drug (ceftriaxone). There is no reactivity or hemolysis in the control tests of patient's serum plus PBS or with complement source with no patient's serum.

Patient status

Offending drug (ceftriaxone) was suspected in causing hemolysis and DAT positivity. Ceftriaxone-induced DIIHA was diagnosed and drug was stopped immediately. The patient was kept under observation on supportive care with oxygen and nutritional support. IV immunoglobulin and packed red cells were given without any antibiotic support. There was a rapid improvement in patient's general condition after discontinuation of drug. Laboratory parameters were improved after 48 h, and the patient was stable with no further drop in hemoglobin and hemolytic episodes. Drug-dependent antibody testing was repeated after 4th day. The patient was discharged with hemoglobin of 11.2 g/dl with normal laboratory parameters on 5th day.

Follow-up testing

The same procedure listed above was repeated with the new fresh serum sample 4 days after discontinuation of drug. There was no reactivity in any of the tests indicating drug antibody has been resolved [Table 2].

Discussion

Ceftriaxone is a commonly used and trusted broad-spectrum antibiotic to treat Gram-positive and Gram-negative bacterial infections in every third patient in India. Cephalosporins are well known for causing drug-induced hemolytic anemia specially ceftriaxone which accounts for >20% cases.[4] As per published reports, it has been observed that children are more susceptible

Table 1: Results when patient's serum was tested with treated and untreated red blood cells with controls

Sample type	Samples tested	Untreated RBCs		Treated RBCs	
		37°C	IAT	37°C	IAT
Test serum sample	Serum + drug	2+	2+	3+	3+
	Serum + complement + drug	+/hemolysis	2+	+/hemolysis	3+
Controls	Serum + PBS	0	0✓	0	0✓
	Serum + complement + PBS	0	0✓	0	0✓
	Complement + drug	0	0✓	0	0✓
	Complement + PBS	0	0✓	0	0✓

0✓ = Negative IAT reading with check cells added and reactive as expected. PBS = Phosphate buffered saline, IAT = Indirect antiglobulin test, RBCs=Red blood cells

Table 2: Results when patient's serum (after discontinuation of drug) was tested with treated and untreated red blood cells with controls

Sample type	Samples tested	Untreated RBCs		Treated RBCs	
		37°C	IAT	37°C	IAT
Test serum sample	Serum + drug	0	0✓	0	0✓
	Serum + complement+drug	0	0✓	0	0✓
Controls	Serum + PBS	0	0✓	0	0✓
	Serum + complement + PBS	0	0✓	0	0✓
	Complement + drug	0	0✓	0	0✓
	Complement + PBS	0	0✓	0	0✓

0✓ = Negative IAT reading with check cells added and reactive as expected. PBS = Phosphate buffered saline, IAT = Indirect antiglobulin test, RBCs = Red blood cells

to ceftriaxone-induced hemolytic anemia causing severe cardiovascular compromise with renal failure leading to even mortality.[5] In our case also, a 15-year-old male child was diagnosed with ceftriaxone-induced hemolytic anemia presented in cardiovascular decompensation and severe hemolysis. There are many drugs which are attributed in causing DIIHA. These drugs lead to formation of drug-dependent (immune complex type) or drug-independent antibodies (autoantibodies). Drug-independent antibodies are similar to warm autoantibodies which can be detected *in vitro* without adding any drug, while drug-dependent antibodies are detected only in the presence of drug either by treating red cells with drug or adding drug in solution with patient's serum. Drug-dependent antibodies are immune complex type either IgG or IgM subtype. These immune complexes bind nonspecifically to RBC membranes and activate complement and destroys RBCs. Ceftriaxone belongs to this group and antibody against ceftriaxone rarely causes hemolysis. In this case, hemolysis was present with no history of receiving drug previously. Similar serological characteristics were reported by Arndt *et al*. in his series of 25 DIIHA cases, in which, all were reactive with antiC3 and 47% were reactive with due to ceftriaxone.[6] Vehapoğlu *et al*. also reported ceftriaxone-induced hemolytic anemia in a child with DAT positive for IgG (3+) and for C3d (4+).[5] Tasch and Gonzalez-Zayaz reported ceftriaxone-induced hemolytic anemia in a case of a 65-year-old woman on ceftriaxone infusions after being diagnosed with acute mitral valve endocarditis, which presented with severe anemia and bilateral transient vision loss. The patient being a Jehovah's Witness refused blood transfusions and was managed with alternative therapies. The etiology of the symptoms was suspected to be a hemolytic anemia directly related to her ceftriaxone infusions.[7] De Wilde *et al*. reported life-threatening ceftriaxone-induced immune hemolytic anemia with an acute kidney injury in a 76-year-old woman,[8] while Mulkens *et al*. reported ceftriaxone-induced severe hemolytic anemia in a 57-year-old female who was diagnosed with neuroborreliosis and treated with ceftriaxone. The patient developed severe massive intravascular hemolysis led to shock and acute renal failure, necessitating mechanical ventilation, and dialysis.[9]

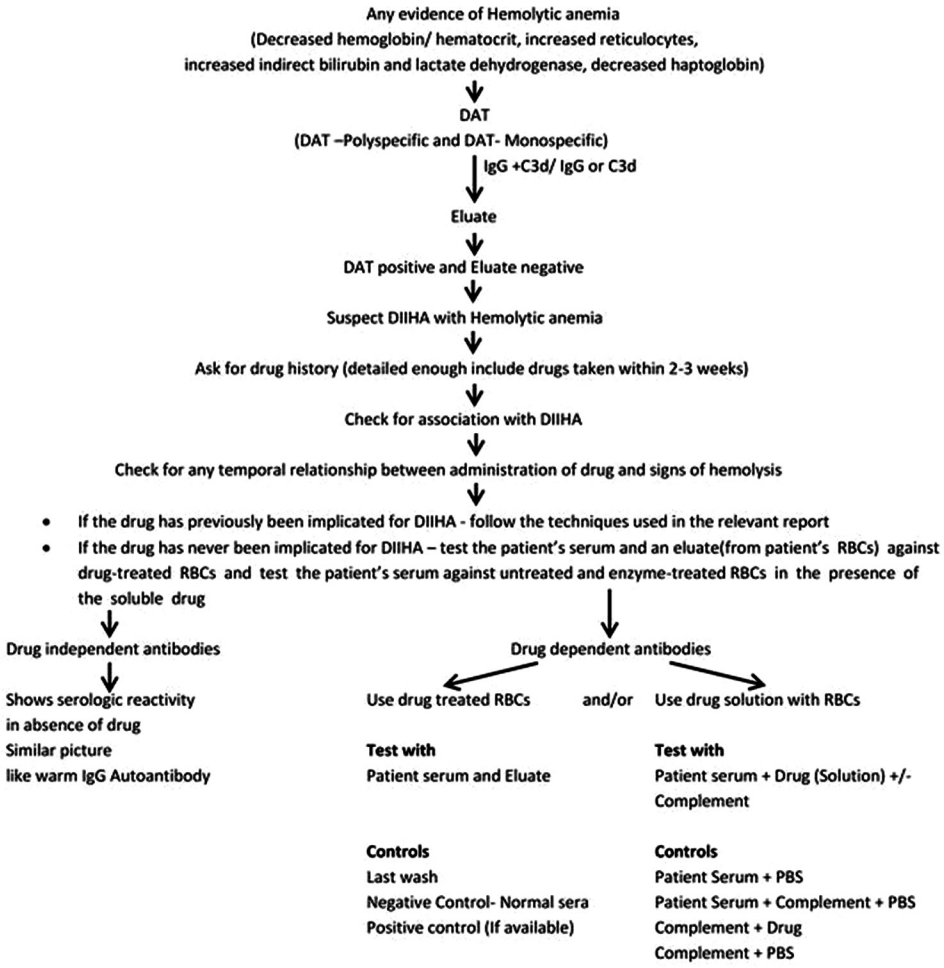

Figure 1: Protocol for investigating drug-induced immune hemolytic anemia

Diagnosing or suspecting DIIHA, without available drug-dependent antibody testing, is one of the main challenges that restrict proper management of these patients. This case highlights the importance of availability of proper immunohematological services which at present are lacking in various regions of country.

In this case report, we have used an approach to diagnose DIIHA given by AABB technical manual and Leger *et al*.[3] The authors would like to summarize the approach for investigating DIIHA for easy understanding [Figure 1].

Conclusion

DIIHA is a very rare event and investigation needs a good effort to diagnose DIIHA, which should only be undertaken when the patient has definite evidence of a hemolysis. If the investigation confirms DIIHA, the physician should be informed to stop the drug immediately. Drug-dependent antibodies react with drug-treated red cells and enzyme-treated or untreated RBCs in the presence of drug solution. DIIHA sometimes mimics with AIHA or hemolytic transfusion reaction which makes the picture more confusing. This case report reemphasizes the need for proper immunohematological services to diagnose and solve such complex cases promptly to save the life of these patients.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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