Practical aspects of investigating drug-induced immune hemolytic anemia due to cefotetan or ceftriaxone—a case study approach

P. A. ARNDT

In the 1970s, the most common causes of drug-induced immune hemolytic anemia were methyldopa and penicillin. Since 1990, the most common causes of drug-induced immune hemolytic anemia have been the second- and third-generation cephalosporins, cefotetan and ceftriaxone. Three case histories illustrate the common findings in the serologic investigation of immune hemolytic anemias due to these two drugs. *Immunohematology* 2002;18:27–32.

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Presently, most drug-induced immune hemolytic anemias (IHAs) that our laboratory investigates are due to cefotetan, a second-generation cephalosporin, or ceftriaxone, a third-generation cephalosporin. The first case of ceftriaxone-induced IHA was referred to our laboratory in November 1987; since then we have identified nine more cases (40% of which were fatal). The first case of cefotetan-induced IHA was referred to our laboratory in March 1990; since then we have investigated 66 more cases (16% were fatal). In contrast, since November 1987, we’ve investigated only seven other cases of drug-induced IHA, one each due to cefotaxime, mefloquine, ticarcillin, or tolmetin, and three due to piperacillin.

The detailed serology of our first eight ceftriaxone and 43 cefotetan cases was published in 1999. The following three case studies are representative of the types of drug-induced IHA workups that we currently see in our laboratory. They illustrate some important points about investigating IHA due to cefotetan or ceftriaxone.

**CASE 1**

The patient was a 31-year-old woman who delivered her third child by cesarean section on March 4. She was discharged from the hospital on March 7 with a hemoglobin of 8.9 g/dL, but was readmitted on March 10 with hemolytic anemia (hemoglobin 8.7 g/dL, reticulocytes 10.5%, total bilirubin 3.3 mg/dL, LDH 503 U/L, haptoglobin < 6 mg/dL) and a 3+ positive direct antiglobulin test (DAT) with anti-IgG. Her serum contained anti-C and -e that had been previously identified and she had a history of a previous hemolytic anemia. On March 13 the hemoglobin decreased further to 6.1 g/dL and the patient was transfused with two units of red blood cells (RBCs).

The hospital blood bank technologists suspected that this patient had an antibody to cefotetan (Cefotan, Zeneca Pharmaceuticals, Wilmington, DE), as she had received two doses of that drug at the time of her surgery. These blood bank technologists had seen a previous patient with IHA due to cefotetan just 2 months earlier, so they were aware of these types of cases. The blood bank still had samples from the time of this patient’s surgery. The DAT was negative the day before surgery, before she received her first dose of cefotetan. It was 2+ the day after the surgery, after she had received her second dose.

The hospital technologists checked into the patient’s previous hemolytic anemia history and found that 3 years earlier, she had a cesarean section when delivering her second child and she received one dose of cefotetan at that time. Thirteen days after that surgery she was readmitted with hemolytic anemia (hemoglobin 5 g/dL, reticulocytes 15%, haptoglobin < 6 mg/dL) and she received four units of RBCs. In retrospect, the cause of this previous hemolytic anemia could also have been an antibody to cefotetan.

Further studies with this patient’s current sample showed the presence of IgG (4+), C3 (2+), and IgA (3+)
on her RBCs. We have found RBCs from patients with cefotetan-induced IHA to be coated with IgG 100 percent of the time, C3 86 percent of the time, IgA 44 percent of the time, and IgM 7 percent of the time.7

Cefotetan-treated RBCs were prepared as previously described:8 a 40 mg/mL solution of cefotetan in pH 7.3 phosphate buffered saline (PBS) was incubated with one-tenth volume of packed, fresh group O, C-e- RBCs for 1 hour at 37°C and then washed × 4. We would advise against using 6% albumin to prepare the cefotetan solution, as has been suggested for some other drugs, as cephalosporins bond efficiently to albumin. When testing drug-treated RBCs, controls are important. A positive control (i.e., a sample from a previous patient with the same drug antibody) will show that the treated RBCs are coated with the drug in question. Without this control, a negative result with the patient's sample is difficult to interpret: is drug antibody not present or are the RBCs not coated with drug? A negative control is also important. For many drugs, a pool of normal “inert” serum can be used. But for some drugs, like cefotetan or cephalothin (Keflin), that cause nonimmunologic adsorption of serum proteins onto drug-coated RBCs,9-12 the normal serum control will be reactive. This can be overcome when testing cephalothin-treated RBCs, by diluting the normal serum and the patient's serum 1 in 20 (in PBS) to reduce the serum protein level before testing. Some normal sera diluted 1 in 20 are still reactive with cefotetan-treated RBCs (unpublished results), so a higher dilution of serum is recommended when testing cefotetan-treated RBCs (e.g., 1 in 100). Note: This low-titer reactivity of normal sera with cefotetan-treated RBCs has not been associated with hemolytic anemia, and in all cases of drug-induced IHA due to cefotetan that we have seen, the antibody titer has been greater than 100. Eluates can be tested without dilution as they have a low protein content. For drugs that are known to cause nonimmunologic protein adsorption, PBS can be used as a negative control.

The patient's March 3 (preshurgery, predrug) serum sample was shown to contain anti-cefotetan that reacted with cefotetan-treated RBCs to a titer of 512 by antiglobulin test (untreated RBCs tested in parallel were nonreactive). The presence of preformed anti-cefotetan in this sample thus confirmed that the hemolytic anemia seen 3 years previously was likely due to cefotetan. The patient's March 10 (postdrug) serum sample reacted more strongly: the anti-cefotetan hemolyzed, agglutinated, and sensitized cefotetan-treated RBCs to titers of 128, 1024, and 128,000, respectively. The March 10 serum also reacted weakly with untreated RBCs by antiglobulin test. The presence of an autoantibody, i.e., drug-independent antibody, is not an uncommon finding in cases of IHA due to cefotetan. We found autoantibodies in about one-third of the patients with cefotetan-induced IHA that we studied (and up to 44%, if PEG was used in the test system).

An acid eluate prepared from the patient's RBCs reacted with cefotetan-treated RBCs both when anti-IgG (American Red Cross, Washington, DC) and when anti-IgA (Tago, Burlingame, CA) were used, indicating that the anti-cefotetan had both IgG and IgA components. Anti-IgA is not routinely used to test eluates, but was used in this case to determine if the IgA coating on the patient's RBCs had anti-cefotetan specificity.

When eluates are tested against cefotetan-treated RBCs it is important to always test the last-wash supernatant control in parallel. Many times this last-wash control will react with cefotetan-treated RBCs (we found 74% to do so, 57% of these reacted ≥ 1+).7,13 Although this reactivity probably relates to the fact that serum cefotetan antibodies typically have very high titers (range 4000 to 262,000; median = 20,000), we found no direct relationship between the serum anti-cefotetan titer and the presence of a reactive last wash.13 There was a relationship seen between the presence of a reactive last wash and the strength of the patient's DAT and/or the presence of autoantibody in the patient's serum or eluate.15 Although increasing the number of times the patient's RBCs are washed before eluate preparation is not always helpful, the type of wash solution that is used can be important. In general, we have found 4°C low-ionic-strength saline (LISS; Löw and Messeter formulation)14 to be better than PBS as a wash solution when performing acid eluates using commercial kits (we stopped using the commercial kit wash solution because of the problem of falsely positive eluates in the presence of high-titer serum antibodies).15 When LISS was used as a wash solution in cases of cefotetan-induced IHA, less strongly reactive last washes were seen.15 This may be because low-ionic solutions help keep more low-affinity antibodies on the RBCs during the washes.

Anti-cefotetan will also react when the patient's serum is tested by the "immune complex" method.7 Briefly, 2 drops of the patient's serum were incubated without or with 2 drops of fresh normal serum (as a source of complement) + 2 drops of a 1 mg/mL
solution of drug + 1 drop of C-e- untreated or enzyme-treated RBCs for 1 to 2 hours at 37°C. Tests were examined for hemolysis and agglutination and the antiglobulin test was performed. This patient’s March 10 serum reacted to a titer of 512 (antiglobulin test) versus untreated RBCs in the presence of cefotetan; enzyme-treated RBCs were strongly agglutinated; no hemolysis of test RBCs was observed.

In conclusion, this patient had a high-titer anti-cefotetan present in her serum, and her RBCs were also coated with anti-cefotetan. This antibody most likely caused the current hemolytic episode in addition to the hemolysis noted 3 years previously. This patient should be warned not to receive cefotetan again, as that may lead to a fatal hemolytic anemia.16

Some important points about cefotetan-induced IHA
- Cefotetan is a commonly used antibiotic, especially prophylactically with surgeries, e.g., cesarean sections.
- The hemolytic anemia usually becomes clinically apparent about 1 to 2 weeks after receiving cefotetan. Unfortunately, patients sometimes get more cefotetan at readmission (e.g., if an infection is suspected).
- A single dose of cefotetan can result in dramatic hemolytic anemia. In some cases, the hemolysis may take several weeks to subside.
- The DAT is positive; this can range from strongly (4+) to only weakly positive.
- Autoantibody (drug-independent antibody) may be present in the patient’s serum and/or eluate. Thus, this drug-induced IHA can be confused with warm autoimmune hemolytic anemia, or if the patient was transfused (e.g., during surgery when cefotetan was given), a delayed hemolytic transfusion reaction may initially be suspected.
- The serum antibody (anti-cefotetan) typically reacts to a very high titer with, and may completely hemolyze, cefotetan-treated RBCs.
- The serum antibody (anti-cefotetan) usually also reacts by the “immune complex” method, but more weakly.
- Eluates prepared from the patient’s DAT-positive RBCs will react strongly with cefotetan-treated RBCs.
- In a large percentage of cases, the last-wash eluate control will also react with cefotetan-treated RBCs, although usually weakly so. LISS appears to be a better wash solution than PBS for trying to reduce this problem.

CASE 2
The patient was a 76-year-old male who was admitted with a diagnosis of pneumonia. On day 1, his hemoglobin was 11.8 g/dL, creatinine 0.4 mg/dL, and serum and urine were clear. He was started on the antibiotic ceftriaxone (Rocephin; Hoffman-LaRoche, Nutley, NJ). On day 2, his creatinine was 1.0 mg/dL and hemoglobinemia was noted. On day 3, his hemoglobin/hematocrit were 8.8 g/dL/23%, total bilirubin was 1.5 mg/dL and hemoglobinuria, and oliguria were noted. The ceftriaxone was stopped.

On day 4, his hematocrit was 21.2%, LDH 2858 U/L, serum hemoglobin 92.9 mg/dL, haptoglobin 16 mg/dL, and creatinine 4.3 mg/dL. He was transfused with two units of RBCs. Over the next 4 days, plasmapheresis was performed three times. The patient was discharged on day 11 with a creatinine of 8.9 mg/dL and no recovery of his renal function.

The patient’s DAT results were negative on day 1 (before drug administration) 2½+ with anti-C3 only on day 2, and then 1+ with anti-IgG and 3+ with anti-C3 on days 3 and 4. This patient’s hemolytic anemia was suspected to be due to anti-ceftriaxone. He had a history of multiple previous ceftriaxone treatments. Typically, patients with IHA due to ceftriaxone have received multiple doses of the drug.17 In some cases, patients have had previously unrecognized hemolytic episodes.

All of the ceftriaxone antibodies that we have worked with and those that have been reported have only reacted by the “immune complex” method (i.e., patient’s serum + drug + RBCs). When this patient’s sera were tested by the “immune complex” method, e.g., in the presence of ceftriaxone (1 mg/mL) against untreated RBCs, agglutination was noted (titers = 4 to 16); the antiglobulin test was negative to only very weakly positive. Controls of patient’s sera + PBS instead of drug were nonreactive, thus this patient’s serum contained anti-ceftriaxone.

In other cases, we have seen dramatic differences in reactivity when testing enzyme-treated RBCs by the “immune complex” method.7 For example, one patient’s anti-ceftriaxone reacted to titers of 4/0 (agglutination/antiglobulin test) against untreated RBCs but reacted to titers of 256/1024 against enzyme-treated RBCs. Unfortunately, titrations of the sera from case 2 against enzyme-treated RBCs were not performed. The agglutinin in case 2 was inhibited by treatment with 0.01M dithiothreitol (DTT), and therefore appeared to
be an IgM antibody. And, as in other cases of IHA due to ceftriaxone, the eluate was nonreactive.

Despite the fact that none of the previously identified ceftriaxone antibodies have reacted when tested against ceftriaxone-treated RBCs, if we have enough sample we sometimes attempt that method. We have tried coating RBCs with ceftriaxone dissolved in PBS (pH 7.3) or barbital buffer (pH 9.8), or by a chemical-coupling method, e.g., using carbodiimide. We tested this patient's sera against RBCs that had been treated with ceftriaxone in PBS or barbital buffer. Sera from day 3 and day 4 agglutinated (2+ and 1+, respectively) not only drug-treated but also untreated RBCs; serum from day 5 was nonreactive. As the patient received his last dose of ceftriaxone on day 3, we believe that the positive results seen on days 3 and 4 were due to circulating drug-antibody immune complexes that were still present in the samples from those days (the half-life of ceftriaxone is about 9 hours in elderly subjects, and about 15 hours in patients with impaired renal function). These immune complexes had cleared by day 5. If autoantibody had been present it would have still been detectable in the day 5 sample. If the anti-ceftriaxone had indeed been reacting with these ceftriaxone-treated RBCs, it should also have been detected in the day 5 sample. Note: Since no anti-ceftriaxone has ever been shown to react with ceftriaxone-treated RBCs, we had no positive control to prove that these treated RBCs were indeed coated with ceftriaxone.

In conclusion, this patient developed an anti-ceftriaxone that caused intravascular hemolysis and renal failure.

Some important points about ceftriaxone-induced IHA

- The patients have typically received multiple doses of ceftriaxone previously.
- In adults, the reaction tends to become apparent after the patient has received the drug for a day or two. In children, the reactions tend to be very dramatic, occurring within minutes of receiving ceftriaxone.
- The DAT is positive due to C3 or C3 + IgG coating. The eluate is usually nonreactive.
- Ceftriaxone antibodies have only been demonstrated by the “immune complex” method (enzyme-treated RBCs react better than untreated RBCs); drug-treated RBCs are nonreactive. In two cases, antibody was only demonstrable in the presence of ex vivo drug (urine from patients receiving ceftriaxone).
- Reactivity of the patient’s serum against untreated RBCs without drug being present can be due to circulating drug-antibody immune complexes (if transient) or due to autoantibody (if persistent). This is true of any drug, not just ceftriaxone.

**CASE 3**

We received a telephone call from a pathologist at a commercial reference laboratory about a ceftriaxone antibody workup on a postsurgical patient who had a positive DAT and a hemoglobin of 5 g/dL. The sample arrived a few days later with more information. The patient, a 59-year-old woman, had surgery a couple of weeks earlier and then developed a postoperative infection. On December 20, her hemoglobin was 10.3 g/dL and she received 1 g of ceftriaxone. On December 21, her hemoglobin had decreased to 8.2 g/dL and she received another 1 g of ceftriaxone. On December 22, her hemoglobin had decreased further to 5 g/dL and the ceftriaxone was discontinued. Her DAT was positive, her reticulocyte count was 3.7%, and she was transfused with four units of RBCs.

When the hospital blood bank was called to verify the patient’s identification (she had the same name as another patient we had previously worked up with an IHA due to anti-cefotetan), we were told that the doctor remembered that this patient had received ceftriaxone with her bowel surgery a few weeks earlier. Thus, this patient’s history was what we might expect with a ceftriaxone antibody, i.e., the patient had received the drug before and the reaction had taken a day or two to become apparent (as seen in adults).

The patient’s DAT was positive (anti-IgG 1+, anti-C3 3+), but the “immune complex” testing in the presence of ceftriaxone was negative! Thus, this patient did not have a ceftriaxone antibody. We wondered, what if we had been given an incorrect history? What if the patient had received another drug (e.g., cefotetan) during the surgery a few weeks previously and not ceftriaxone? The time frame of hemolysis a couple of weeks after surgery is what would be expected in a case of cefotetan-induced IHA.

The patient’s serum and eluate were tested against cefotetan-treated RBCs and found to contain anti-cefotetan. The undiluted serum hemolyzed cefotetan-treated RBCs and when diluted reacted to titers of 320 and 10,240 (agglutination and antiglobulin test,
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respectively). Untreated RBCs were nonreactive. The hospital blood bank was contacted and asked to determine if the patient had received cefotetan during the surgery. They checked the records and discovered that the patient had received 2 g of cefotetan with her surgery on December 10; she had been readmitted on December 20, exactly 10 days later. Luckily, she did not receive more cefotetan at the time of readmission.

In conclusion, this patient had an IHA due to anti-cefotetan, not anti-ceftriaxone.

An important point illustrated by this case

• A good history is important and may be difficult to obtain. When a drug-induced IHA is suspected, it is important to find out not only what drug(s) the patient is currently taking, but also what the patient received (e.g., in surgery) a few weeks back. This information often is “hidden” in the anesthesiologist’s notes and can take some detective work to discover.

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References


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