

ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

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Drug-induced Immune Hemolytic Anemia

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H&O What is the incidence/prevalence of drug-induced immune hemolytic anemia (DIIHA)?

GG DIIHA is rare, but there are no good data on the exact incidence and prevalence. There are good data for drug-induced thrombocytopenia (10–18 cases per million)^{1,2} and neutropenia (2–15 cases per million),³ but only guesses for DIIHA. In 1980, we reported that in 347 cases of immune hemolytic anemia, approximately 12% were drug-induced.⁴ As autoimmune hemolytic anemia (AIHA) is said to occur in approximately 1 in 100,000 people,^{5,6} we estimated that DIIHA may have been occurring in approximately 1 per million of the population.⁵ Having said that, most reports are of severe hemolytic anemia; I think that there are many more less-severe cases that are not being correctly identified.

H&O How do drugs cause immune hemolytic anemia?

GG Drugs are small molecular weight chemicals (usually only several hundred kD) and thus need to bind to protein before evoking an immune response; this may be a protein that is free in the plasma or on cell membranes (eg, platelets, red blood cells [RBCs]). Some drugs (eg, penicillins) bind covalently to the protein and will bind so well to RBC membranes that they stay on the RBCs even after multiple washes *in vitro*. Such drug-coated RBCs are a useful tool for detecting drug-dependent antibodies. This interaction also occurs *in vivo*. Patients receiving large amounts of intravenous penicillin will have penicillin-coated RBCs circulating, which, by itself,

does not harm the RBC. If the patient makes a high titer IgG penicillin antibody, it will attach to the patient's penicillin-coated RBCs, and the now IgG-coated RBCs will lead to a positive direct antiglobulin test (DAT) and perhaps (as in approximately 3% of the patients) to a hemolytic anemia (HA) due to macrophage interactions with the IgG-coated RBCs.⁵

Many drugs that cause DIIHA do not covalently bind to RBCs but probably form a loose bond that does not stand up to the *in vitro* washing of drug-treated RBCs, but may provide, *in vivo*, a determinant (part drug, part RBC protein) that evokes an immune response.⁵ Drug-coated RBCs cannot be prepared *in vitro* to use for detecting antibodies to drugs in this group. The drug-dependent antibodies are detected by mixing the patient's serum (containing the antibody) with drug and untreated RBCs, under certain conditions, with appropriate controls. *In vitro* reactions may be RBC lysis, agglutination, or sensitization detected by the antiglobulin test. Drug antibodies of this type are often IgM, but can be IgG; they often activate complement. Acute intravascular hemolysis often occurs (50% of patients have renal failure). There are more fatalities in this group of patients.

A less common mechanism of DIIHA involves drug-independent antibodies. Such antibodies do not need drugs to be present for them to be detected *in vitro*; the antibodies react with untreated RBCs, giving identical reactions *in vitro* and *in vivo* to RBC autoantibodies associated with idiopathic "warm type" AIHA. There are no antibodies to the drug involved. It is thought that drugs in this group (eg, methyldopa, procainamide, and fludarabine) affect the immune system, causing a true autoimmune condition.⁵

The newest mechanism proposed for DIIHA also does not involve antibodies to the drug. Some drugs (eg, cefotetan, ceftriaxone, cisplatin, oxaliplatin, beta lactamase inhibitors [tazobactam, sulbactam, clavulanate]) appear to modify the RBC membrane, causing non-immunologic protein adsorption (NIPA).⁵ This can lead to a positive DAT and sometimes HA, as macrophages can react with RBC-bound IgG and complement (C3), even though the IgG is not directed at any RBC antigens or membrane-bound drug.⁷⁻⁹

H&O What are the most common drugs to cause DIIHA?

GG In 1980, we reviewed DIIHA in a series of 347 immune hemolytic anemias (IHA) we had studied in the previous 10 years.⁴ We had encountered 43 (12% of all IHA) cases in this period, of which 68% were due to a then commonly used drug: methyldopa; 23% were due to penicillin (high-dose intravenous therapy). These therapies were used less commonly over the following few decades, so the most common drugs to cause DIIHA during the last 4 decades are very different; we have not seen a methyldopa- or penicillin-induced IHA for more than 20 years.^{10,11} In the last decade, we encountered 81 cases of DIIHA; 54 (67%) were due to cephalosporins (of these, 67% were associated with cefotetan and 32% with ceftriaxone).¹¹ The next most common drug was piperacillin (17%). Other drugs causing DIIHA in this period included tazobactam (5 cases); oxaliplatin (2 cases); and sulbactam, carboplatin, rifampin, bactrim, diclofenac, and cimetidine (1 case each).¹¹

In 2007, we read almost all reports of DIIHA in the literature to see how many we felt provided enough evidence to support a diagnosis of DIIHA.¹² Unfortunately, many of them did not fit our criteria; most of these based their conclusions on a hemolytic anemia developing following therapy with a particular drug, followed by a hematologic improvement after the drug was stopped. This is not good enough for us to define the HA as immune; the data should include some evidence for an antibody-mediated mechanism (eg, positive direct antiglobulin test, the presence of drug-induced antibodies in the plasma, and/or an eluate from the RBCs). We finally concluded that there were 125 drugs with reasonable evidence of DIIHA; much of the evidence was based on single case reports, but some was represented by multiple reports, including some series. Forty-two percent were antimicrobials, 15% were anti-inflammatory drugs, 13% were antineoplastics, and 6% were antidiuretics or anti-hypertensives; many of the remaining 23% were often single examples of other drugs.

H&O Cefotetan, ceftriaxone, and piperacillin are the most common drugs to cause DIIHA. How many patients receiving these drugs make drug antibodies, and how many of those develop hemolytic anemia? How severe is the hemolytic anemia?

GG There are not much data to answer the first question. I know of no large-scale studies addressing this question, but recently there were 2 reports on small numbers of patients. Davenport and colleagues¹³ followed 60 patients who received cefotetan and found that 5 (8%) made cefo-

tetan antibodies 5–78 days after receiving the drug. All 5 patients had a slightly decreased hemoglobin (median 1.2 g/dL) but no signs of a HA. One interesting finding was that some of the patients' RBCs became coated with cefotetan, and these RBCs circulated for 16.5–92 days. The authors felt that this might explain why a hematologic response takes longer than expected in patients with cefotetan-induced HA compared with other DIIHAs. Quillen and colleagues¹⁴ followed pediatric sickle cell disease and HIV-infected patients; 8 (12.5%) made ceftriaxone antibodies, among whom 2 developed HA (1 was fatal).

All 3 of these drugs have been used on millions of patients without any ill effects; nevertheless, severe and even fatal HA can occur. In the workup of HA, a careful drug history must be taken, and if suspected, a search for drug-induced antibodies should be made by a reference laboratory with expertise in this area.

A U.S. Food and Drug Administration (FDA) group published data on 85 cases of cefotetan-induced HA.¹⁵ There was a mean hemoglobin decrease of 6.7 g/dL, with a final mean hemoglobin of 5.2 g/dL. There were 18% fatalities and 8% renal failures. Fifty-nine percent of the cases were associated with prophylactic use of cefotetan (60% associated with surgery). Only 18% of the patients had received cefotetan previously. This seemingly unusual finding may relate to it being common to find cefotetan antibodies in random patients and blood donors. This could be due to exposure of the American public to cephalosporins that are added to animal food and used prophylactically in chickens and cattle; this practice is banned in several European countries, and such bans have been suggested in the United States because of the increasing problems with antibiotic-resistant organisms.^{16,17} We have published in the obstetric literature because of our concern about the severe HA we were seeing in healthy young women following cesarean section. We published a report of 10 cases of cefotetan-induced HA in which a single dose of cefotetan was used prophylactically for gynecologic/obstetric surgery.¹⁸ The hemoglobin fell to 3.5–7.6 g/dL at 9–14 days following surgery. This was often intravascular lysis. We know of some cases where more cefotetan was given, leading to fatal HA; the correct diagnosis of DIIHA was made retrospectively.

Ceftriaxone-induced HA can be dramatic in children.^{5,11,19} There are 8 published cases of fatal HA in which the onset was usually 2–30 minutes following receipt of the drug, with hemoglobin/hematocrit nadirs of 0.9–5/1.5–10.

Piperacillin-induced HA (17% of our series) is very interesting, as one would expect it to cause HA by a mechanism similar to penicillin, but that is not so. The hemolysis is usually a complement-mediated intravascular hemolysis in contrast to that of penicillin or semi-synthetic

penicillins (eg, ampicillin), which do not involve complement activation and are associated with less dramatic IgG-associated extravascular lysis. One other interesting finding is that many patients/donors have antibodies that will react with piperacillin-coated RBCs in vitro, which complicates the serologic investigations.²⁰ This common occurrence of antibodies is probably explained by the same rationale discussed above for the cefotetan findings (ie, antibiotics in animal products).

H&O If I suspect that a hemolytic anemia was caused by a particular drug, how do I prove that it is a DIIHA?

GG First, you should get the result of a DAT. If you are dealing with an immune etiology, the DAT will usually be positive. In acute intravascular hemolysis, the targeted RBCs may have been destroyed and the DAT may be negative, or the DAT may be only weakly positive due to C3dg sensitization of the RBCs. To confirm DIIHA, you may have to contact a laboratory that has experience in this area; most hospital laboratories (ie, blood banks) can tell you if there are drug-independent antibodies (autoantibodies) present, but will have limited ability to prove that other drugs are involved (ie, detection of drug-dependent antibodies). Remember, if RBC autoantibodies are detected, one cannot prove, in the laboratory, that they were drug-induced. After stopping the drug, it may take months before the serology becomes negative, but a hematologic response may be seen within days/weeks. Once again, remember that the drug may be a “red hering”; idiopathic AIHA is far more common than DIIHA.

H&O What is known about the AIHA that can develop in patients with chronic lymphocytic leukemia (CLL) following treatment with fludarabine?

GG There are many single case reports, but there are only 4 reports describing a series of CLL patients. Hemolytic anemia following fludarabine therapy occurred in 14 of 66 (22%),²¹ 9 of 52 (17%),²² 5 of 36 (14%),²³ and 5 of 104 (5%)²⁴ patients. It is unclear why the largest series of 104 patients yielded far fewer DIIHA cases than the 3 smaller studies. The analyses on CLL patients are more complex, with many more confounding factors than the data accumulated in the 1970s on the prototype drug (methyl dopa) to induce RBC autoantibodies and AIHA in patients taking the drug for hypertension. CLLs comprises a very different group of patients, as CLL is known to be associated with positive DATs and AIHA without any drug involvement. Nevertheless, there are reports of exacerbation of AIHA that was present before fludarabine

therapy. There are fewer reports of AIHA due to fludarabine in de novo CLL patients receiving fludarabine for the first time compared with patients who have had multiple courses of alkylating agents, among whom the prevalence is approximately 20%. There are reports of catastrophic hemolysis (some fatalities) following fludarabine therapy.

In 2008, an interesting study of 777 CLL patients randomized to receive chlorambucil or fludarabine alone or with cyclophosphamide (FCy) was published.²⁵ Fourteen percent of the CLL patients had a pretreatment positive DAT. Only 28% of positive DATs had an associated hemolytic anemia. Of 249 patients, those treated with fludarabine were most likely to become DAT-positive. Patients treated with fludarabine or chlorambucil were twice as likely as those treated with FCy to have AIHA. The authors concluded that a positive DAT was a good prognostic indicator and that FCy combination therapy may protect against AIHA. There is an excellent review on AIHA in CLL by Hamblin.²⁶

H&O How should patients with DIIHA be managed?

GG If DIIHA has occurred, the balance between the severity of the hemolytic anemia and the value of therapy with this particular drug should be considered; the suspect drug should be discontinued if possible. Once the drug is discontinued, a hematologic response is usually seen relatively rapidly (eg, in the first week); the serology (eg, positive DAT) may remain positive for weeks to months. Although steroids are often used in addition, I (and others²⁷) know of no data showing that steroids have any effect on drug-dependent antibodies; they may have some effect on drug-induced autoantibodies. It is difficult to evaluate this, as steroids are often given close to when the drug is stopped. Publications that say “once steroids were given there was a rapid improvement” do not take into account that the improvement may be entirely due to the drug discontinuation. When severe acute hemolysis (eg, complement-mediated intravascular lysis) occurs, transfusions may be necessary, and there are several reports of success with plasma exchange (many of the antibodies involved were IgM).^{27,28}

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