Pathology Consultation on Drug-Induced Hemolytic Anemia

Arand Pierce, MD,1 and Theresa Nester, MD1,2; for the Education Committee of the Academy of Clinical Laboratory Physicians and Scientists

Key Words: Pathology consultation; Drug-induced immune hemolytic anemia; Hemolytic anemia; Cephalosporins

DOI: 10.1309/AJCPBVLZD6W6RQMX

Abstract

Drug-induced immune hemolytic anemia is considered to be rare but is likely underrecognized. The consulting pathologist plays a critical role in integrating serologic findings with the clinical history, as drug-induced antibodies should be distinguished as either drug-dependent or drug-independent for appropriate clinical management. Drug-dependent antibodies (DDABs) are most commonly associated with cefotetan, ceftriaxone, and piperacillin, whereas fludarabine, methyldopa, β-lactamase inhibitors, and platinum-based chemotherapeutics are frequent causes of drug-independent antibodies (DIABs). DDABs usually demonstrate a positive direct antiglobulin test and a negative elution, while DIABs are serologically indistinguishable from warm autoantibodies and are similarly steroid-responsive. Drug cessation is always recommended.

Consult

Drug-induced immune hemolytic anemia (DIIHA) is an uncommon entity that is frequently mistaken for warm autoimmune hemolytic anemia (WAIHA). Providing diagnosis and guidance to the inpatient care team is paramount because failure to recognize DIIHA may result in death. Once recognized, treatment options differ based on the nature of the antibody.

The condition of a 28-year-old man with cystic fibrosis (CF) and bilateral lung transplants who was hospitalized for fever, productive cough, and dyspnea progressed to respiratory failure requiring mechanical ventilation. Further investigations identified drug-resistant H1N1 viral infection, gram-negative bacteremia and pneumonia, fungal pneumonia, and systemic reactivation of cytomegalovirus. Therapy was immediately started with broad-spectrum antiviral and antimicrobial therapy, including piperacillin-tazobactam. Soon after initiation of therapy, a steady decrease in hemoglobin and hematocrit values (nadir, 5.2 g/dL [52 g/L] and 16% [0.16], respectively) was accompanied by elevations in lactate dehydrogenase and total bilirubin levels. Coagulation study results were normal. A WAIHA was suspected, and RBC transfusion was ordered.

An antibody screen of patient plasma revealed 2+ reactions with both screening cells; an antibody identification panel disclosed positive agglutination of all cells with variable reactivity (2-3+) and a positive autologous control (2+). A direct antiglobulin test (DAT) was strongly (3+) reactive using polyspecific and anti-IgG antibody reagents but non-reactive with anti-C3. All controls reacted appropriately. However, acid elution showed no reactivity of the patient’s serum against any cells of the RBC test panel, in contrast with what is expected of a pan-agglutinating warm autoantibody.

Upon completion of this activity you will be able to:

• list the drugs most commonly implicated in drug-induced immune hemolytic anemia (DIIHA).
• describe the mechanisms of DIIHA.
• direct the appropriate laboratory and clinical evaluation of DIIHA.
• provide clinically relevant recommendations for DIIHA management based on specific laboratory and clinical findings.

The ASCP is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians. The ASCP designates this journal-based CME activity for a maximum of 1 AMA PRA Category 1 Credit™ per article. Physicians should claim only the credit commensurate with the extent of their participation in the activity. This activity qualifies as an American Board of Pathology Maintenance of Certification Part II Self-Assessment Module.

The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose. Questions appear on p 152. Exam is located at www.ascp.org/ajcpcme.
An underlying alloantibody was ruled out by autologous adsorptions. Compatible RBC units were allocated, and the transfusion physician made the inpatient care team aware of the possibility of DIIHA. The team then requested guidance on how best to support the patient given these findings.

Questions

DIIHA is a rare but serious complication that is often underdiagnosed. This consult will outline the current state of knowledge regarding DIIHA with the aim of answering the following:

• What drugs are most commonly implicated in DIIHA?
• What are the mechanisms of DIIHA?
• How is DIIHA diagnosed?
• What is the recommended management of DIIHA?

Background

DIIHA occurs in approximately 1 in 1 million people but is likely underdiagnosed despite its potential lethality. Reports of DIIHA first began in the 1950s. As the drug armamentarium has evolved, so have the drugs most commonly associated with DIIHA. Forty years ago, methyldopa and high-dose intravenous penicillin were most commonly associated with hemolytic anemias. Today, second- and third-generation cephalosporins are implicated in the vast majority of DIIHA cases. Other drugs commonly implicated are listed in Table 1. However, myriad other drugs have been implicated, mostly in case reports, so any drug is a potential culprit.

DIIHA antibodies should be classed as drug-dependent or drug-independent for the purposes of diagnosis and management. An antibody is drug-dependent if it demonstrates reactivity only in the presence of drug (in serum or added for in vitro testing). An antibody is drug-independent if it is capable of in vitro reactivity in the absence of drug. However, the in vivo mechanisms of most drug antibody-antigen interactions are poorly understood, and drug-associated hemolysis is likely mediated by more than 1 mechanism. Despite these limitations, a consistent serologic investigation coupled with a thorough clinical history is usually sufficient to arrive at the correct diagnosis.

Discussion

Drug-Dependent Antibodies

Most drugs that cause hemolysis are mediated by DDABs. In the 30-year experience of 1 reference laboratory, cefotetan, ceftriaxone, and piperacillin were, in aggregate, responsible for 76% of all cases of DIIHA, with cefotetan accounting for the majority of cases. Hemolysis typically appears within 2 weeks after starting the drug, and the patient has progressive anemia or other evidence of hemolysis. There is often no clinical suspicion of drug-mediated hemolysis because a routine request for RBC transfusion or preoperative screen typically initiates the evaluation. A positive DAT is the most reliable laboratory finding in DIIHA; if the DAT is negative, the likelihood of DIIHA is exceedingly small.

Following a positive DAT, an elution is performed to characterize the antibody coating the patient’s RBCs. The

| Table 1 | Overview of Drug-Induced Hemolytic Anemia Based on Mechanism of Hemolysis |
|-----------------|-----------------|-----------------|
| **Drug-Dependent Antibody** | **Drug-Membrane Interaction** | **Drug-Independent Antibody** |
| **RBC Coating** | **Drug-Membrane Interaction** | **True Autoimmune** |
| Drugs | Penicillin (high-dose); cefotetan | Ceftriaxone; piperacillin; NSAIDs; quinine/quinidine; probenecid | Fludarabine; cladribine; methyldopa; levodopa; procainamide |
| Mechanism | RBCs coated covalently with bound drug Drug Ab directed against drug only; drug-coated RBCs experience extravascular immune hemolysis via RES macrophage Fc receptor recognition | Drug covalently or noncovalently bound to RBC, creating neoantigen; antibody may react predominantly with drug, drug + membrane, or membrane portion of neoantigen; abrupt onset; primarily intravascular hemolysis; fatalities more common | Drug may stimulate autoantibody via molecular mimicry, drug adsorption causing altered RBC membrane antigens, immune dysregulation, or other mechanisms. |
| Diagnosis | + DAT; ± IAT; – elution highly suggestive but not pathognomonic; drug-dependent Ab detection | + DAT; ± IAT; – elution highly suggestive but not pathognomonic; drug-dependent Ab detection | + DAT; ± IAT; + elution (indistinguishable from WAIHA) |
| Management | Drug cessation | Drug cessation | Drug cessation; steroids |

Ab, antibody; DAT, direct antiglobulin test; IAT, indirect antiglobulin test; NIPA, nonimmunologic protein adsorption; NSAIDs, nonsteroidal anti-inflammatory drugs; RES, reticuloendothelial system; WAIHA, warm autoimmune hemolytic anemia.

* Clavulanate (found in Augmentin and Timentin), sulbactam (found in Unasyn), and tazobactam (found in Zosyn).

† Carboplatin, cisplatin, and oxaliplatin.

‡ Elution may be positive in some cases.
RBCs are washed free of unbound antibody, followed by chemical modification (usually acid) to remove the attached antibodies; the resultant eluate is then characterized by testing against reagent RBCs. The eluate fails to react because the drug is not present in this in vitro testing. A careful clinical history, including medications and their temporal relationship with the onset of hemolysis, is critical because a positive DAT with a negative eluate is commonly associated with the passive transfer of ABO antibodies, such as anti-A or anti-B from a prior out-of-group plasma or platelet transfusion, intravenous immunoglobulin (IVIG) therapy, or hemolytic disease of the newborn and fetus. Nonimmunologic RBC adsorption of IgG due to hypergamma-globulinemia is another cause to consider besides DDABs.

Because significant concentrations of residual drug or drug-antibody complexes may be present in the patient’s serum, DDABs will frequently react against non–drug-treated RBCs. A broadly reactive antibody, including against the autocontrol, is found in half of cases, suggestive of an autoantibody. Anticefotetan and anticeftriaxone antibodies may exhibit this reactivity. This also occurs with piperacillin antibodies, which may further mimic an autoantibody by showing an apparent preference for Rh(e)+ reagent cells. These findings may be inconstant because they are highly contingent on the plasma drug concentration; thus, relating the laboratory results to the dosage and timing of administration is critical.

If the autocontrol is negative and all other panel RBCs are positive, an antibody against a high-prevalence antigen should alternatively be considered. A subsequent elution may help distinguish between WAIHA and DIIHA, but unfortunately, rare cases of DDABs cause positive elutions. This is more common with cefotetan and ceftriaxone because they are most frequently positive in initial indirect antiglobulin test (IAT) testing, as described. When positive, the eluate in DIIHA typically reacts weakly compared with serum at initial antibody testing, a clue to consider DIIHA rather than WAIHA. The clinical risk for a patient with DIIHA is that the finding of reactive antibodies in the serum or eluate will lead to an incorrect diagnosis of WAIHA and improper treatment with steroids but worse, continued administration of the offending drug that may prolong or worsen hemolysis. The only reliable confirmation of DDAB-mediated DIIHA over WAIHA requires testing patient serum after drug cessation and clearance of any circulating drug or drug-antibody complexes, indicated by a clear decrease in serologic reactivity. Figure 1 depicts the basic testing algorithm for diagnosing DIIHA, always keeping in mind that the drug history is key to this investigation.

Antibodies may be directed against the drug, its metabolites, a neoantigen formed by the drug and RBC membrane proteins, or any combination thereof. The best-understood mechanisms of hemolysis involve penicillin and cefotetan, which covalently bind to RBC membranes, provoking IgG antibodies directed only to drug epitopes. The coated RBCs undergo extravascular destruction via Fc-receptor recognition by splenic macrophages. Because drug-RBC binding is covalent, the drug may be bound firmly enough to RBCs to prevent clearance from the circulation. Extravascular hemolysis (albeit mild) may persist for months after drug cessation in this situation due to DDABs acting against the “stabilized” antigen. In vitro testing of these drug-specific antibodies is characterized by serum reactivity against drug-treated RBCs. It is interesting that many case reports of cefotetan and DIIHA involve a single surgical prophylactic dose in a patient who has never received the drug. It is speculated that this is a sequela of widespread antibiotic use in US livestock and supported by the high prevalence of anticefotetan antibody in US blood donors. A single dose of prophylactic antibiotic is often overlooked when investigating a patient’s drug history (especially when given in the operating room).

Most other drugs, including ceftriaxone and piperacillin, do not bind as strongly to RBC membranes, such that drug-coated RBCs are difficult to create for in vitro testing. These DDABs may interact noncovalently with the RBC membrane, forming a neoantigen variably composed of RBC membrane proteins and drug epitopes. These antibodies are associated with higher morbidity and mortality because they can fix complement and provoke precipitous intravascular hemolysis.

Ceftriaxone causes robust hemolysis with a disproportionately high fatality rate in pediatric patients (50%-100%), whereas adults are not as severely affected and fatality rarely occurs. Piperacillin is frequently prescribed for patients

© American Society for Clinical Pathology

Figure 1

Drugs

<table>
<thead>
<tr>
<th>NIPA</th>
<th>β-lactamase inhibitors; platinum-based chemotherapeutics; cefotetan; cephalothin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterogeneous, controversial</td>
<td>Drug alters RBC membrane so that serum proteins (including immunoglobulins) are nonimmunologically adsorbed onto the RBC membrane. In vitro and rare in vivo examples of decreased RBC survival have been reported.</td>
</tr>
</tbody>
</table>

Consider drug cessation if there is evidence of decreased RBC survival.

DOI: 10.1309/AJCPBVLJZH6W6RQM Am J Clin Pathol 2011;136:7-12
with CF, and the associated DIIHA disproportionately affects this patient population; in fact, the only reported drugs associated with DIIHA in patients with CF are piperacillin and piperacillin-tazobactam. Unlike cefotetan, all cases of piperacillin-associated DIIHA have been associated with prior drug exposure.

The treatment of DDABs DIIHA is straightforward: stop the drug. Hematologic recovery should occur within 2 weeks, although the DAT may remain positive for months. If WAIHA is presumed by the clinicians and treated with steroids, the patient is being placed at unnecessary risk because there is no compelling evidence that steroids are effective in treating DIIHA with DDABs. If the patient is immunosuppressed or infected, this is an especially important point for discussion with clinicians to avoid the potential harm of unnecessary steroid administration.

Drug-Independent Antibodies

DIIHA is less commonly associated with DIABs. Most DIABs are associated with the generation of a true autoantibody-mediated WAIHA, often against a Rhesus antigen if any specificity is discernible. Metyldopa, which is now used to treat gestational hypertension, was the historic cause of drug-induced WAIHA; fludarabine now accounts for the majority of drug-induced WAIHA among all drugs (Table 1). The mechanisms of DIAB formation are poorly understood and include molecular mimicry, drug adsorption causing altered RBC membrane protein antigens, and immune dysregulation. Because these drug-independent immunologic processes are essentially identical to WAIHA, in vitro testing cannot discriminate this type of DIIHA from WAIHA. The workup will show a positive DAT, positive antibody screen, and positive elution. Only hematologic improvement after stopping the drug can confirm the diagnosis of DIAB-mediated DIIHA. However, steroids are indicated for this type of DIIHA, as for any WAIHA; if hemolysis is persistent or severe, treatment with IVIG or immunosuppression should be considered, as for any non–drug-associated WAIHA.

Fludarabine is frequently used to treat chronic lymphocytic leukemia (CLL); thus, it may be impossible to differentiate a primary WAIHA associated with CLL from a DIIHA secondary to fludarabine. There is good evidence that fludarabine is independently causative of WAIHA in CLL, and such cases should be scrutinized carefully because specific medical management may control or prevent hemolysis.

A second drug-independent mechanism is gaining recognition: Nonimmunologic protein adsorption has been implicated in hemolysis associated with β-lactamase inhibitors, platinum-based chemotherapeutic agents, and cephalosporins, including cefotetan (Table 1). These drugs may alter the RBC membrane such that serum proteins (including immunoglobulins) are nonspecifically adherent, causing “spurious” positive DATs due to “bystander” antibodies present on the RBC membranes. This effect was previously considered to cause false-positive in vitro testing results, but there is now evidence that these adherent proteins may truly mediate RBC destruction in certain instances. Of course, because β-lactamase inhibitors are commonly administered with antimicrobials and other medications, it is essential to consider these mechanisms when evaluating the cause of drug-related hemolysis.
such as piperacillin (eg, piperacillin-tazobactam), multiple hemolytic mechanisms may be operative.

Conclusions

In the case described herein, the consultant pathologist advised the inpatient care team that DIIHA was likely based on the negative elution, the temporal relationship of hemolysis with piperacillin, and the clinical scenario of a patient with CF. Piperacillin was immediately stopped, but the inpatient team was simultaneously considering administration of steroids, despite ongoing immunosuppression and severe polymicrobial pneumonia. The pathologist advised that because the antibody was likely drug-dependent, steroids would not be useful in reversing the hemolysis. The inpatient team followed this advice, which was confirmed by rapid hematologic recovery after piperacillin cessation. A reference immunohematology laboratory specializing in DDAB detection confirmed the antipiperacillin antibody. Methods for in-house DDAB testing are available,1,33 but the current complexity of this testing and its interpretation persuades most to send such cases to experienced reference laboratories.

For clinical management of DIIHA, it is useful to categorize the antibody as drug-dependent or drug-independent. This distinction can be made in the laboratory, but it is the pathologist’s role to provide clinical guidance based on this knowledge and the clinical circumstances. For drug-dependent hemolysis, cessation of the drug (usually cefotetan, ceftriaxone, or piperacillin) is critical. This is equally true for drug-independent hemolysis (usually fludarabine or methyl-dopa), but because of the true autoantibody, patients should additionally be treated as for WAIHA (ie, with steroids and also IVIG if there is intravascular hemolysis). The pathologist will need to follow the clinical course and response because more than 1 mechanism of hemolysis may be in play for any given drug.

A positive DAT and negative eluate are classic for DDABs mediating DIIHA once the common causes of passive transfer of anti-A or anti-B antibodies have been ruled out. Still, many DDABs can behave like an autoantibody with IAT methods (eg, positive serum or eluate reactivity) when drug or drug-antibody complexes are still present in blood. A thorough drug history is the key to any case of hemolysis with a positive DAT, regardless of the IAT findings. If an autoantibody is suspected on routine testing but the eluate reaction is comparatively weak against the reagent cells, consider DIIHA. Retesting several days after drug cessation is the only way to truly differentiate between a DDAB behaving as an autoantibody and an independent true autoantibody.

Extra vigilance is required in cases of drug-independent DIIHA because they are impossible to differentiate in the laboratory from WAIHA. As in the more complex DDAB cases, the evaluation is empirical: Drug cessation with added steroids should provide hematologic recovery within 2 weeks. The DAT may remain positive for weeks or months despite full recovery.

References