Drug-induced immune hemolytic anemia

George Garratty[1]

[1]*American Red Cross Blood Services—Southern California Region, Pomona, CA*

Drug-induced immune hemolytic anemia (DIIHA) is rare, and a specialized laboratory is often required to provide the optimal serological tests to confirm the diagnosis. The most common drugs associated with DIIHA and the hypotheses for the mechanisms thought to be involved have changed during the last few decades. The drugs most frequently associated with DIIHA at this time are cefotetan, ceftriaxone, and piperacillin. DIIHA is attributed most commonly to drug-dependent antibodies that can only be detected in the presence of drug (eg, cephalosporin antibodies). DIIHA can also be associated with drug-independent antibodies; such antibodies do not need drug to be present to obtain *in vitro* reactions (eg, fludarabine). In these latter cases, the drug affects the immune system, causing production of red cell (RBC) autoantibodies; the clinical and laboratory findings are identical to autoimmune hemolytic anemia (AIHA), other than the remission associated with discontinuing the drug. Some of the mechanisms involved in DIIHA are controversial. The most acceptable one involves drugs, like penicillin, that covalently bind to proteins (eg, RBC membrane proteins); RBCs become coated with drug *in vivo* and, a drug antibody (usually IgG) attaches to the drug-coated RBCs that are subsequently cleared by macrophages. The most controversial is the so-called immune complex mechanism, which has been revised to suggest that most drugs are capable of binding to RBC membrane proteins, but not covalently like penicillins. The combined membrane plus drug can create an immunogen; the antibodies formed can be IgM or IgG and often activate complement, leading to acute intravascular lysis and sometimes renal failure; fatalities are more common in this group. It is still unknown why and how some drugs induce RBC autoantibodies, sometimes causing AIHA.

Drug-induced immune hemolytic anemia (DIIHA) is rare. The incidence of drug-induced immune thrombocytopenia and neutropenia is quite well documented (10 to 18 and 2 to 15 cases per million, respectively),[1-3] but there are no good data for DIIHA. We have roughly estimated it to be around 1 in 1 million of the population, by comparing it to autoimmune hemolytic anemia (AIHA), which has been reported to occur in 1 in 80,000 of the population.[4] Over a 40-year experience we have encountered AIHA about ten times more often than DIIHA.[4] I believe this is a low estimate for DIIHA as only the more dramatic hemolysis leads to the appropriate investigations to prove that a drug is causing an immune hemolytic anemia (HA).

The number of drugs and the suggested mechanisms associated with DIIHA have changed over the last 40 years.[5] In 1967, only 13 drugs were implicated; in 1980, 32 drugs were reported; in 2007, we reviewed published reports of DIIHA and concluded that there were reasonable data to support DIIHAs caused by 125 drugs.[6] Three groups of drugs predominated: 42% were antimicrobials; 15% were anti-inflammatory; 11% were anti-neoplastics.[6] The specific drugs mainly implicated have changed dramatically. **Table 1** shows the drugs causing DIIHAs that were studied by our laboratory over a 40-year period. Another group reviewed DIIHAs they had encountered over a 20-year period with similar results.[7] In the 1970s the most common drug, by far, to cause DIIHA was methyldopa, which caused a true AIHA with no drug antibody involved and accounted for 67% of all DIIHA. High-dose intravenous penicillin accounted for 25% of DIIHA. When these therapies became less commonly used, the most common causative group of drugs became the cephalosporins, which, from the 1990s, account for 70% of the DIIHA, we encounter; most of these were due to one cephalosporin (cefotetan). There was a brief respite, when cefotetan was discontinued by Astra Zeneca (Wilmington, DE) in early 2006, but a generic version (Abraxis Pharmaceutical, Schaumburg, IL) became available in August 2007, and we have begun to see cases again.

Suggested Mechanisms Involved in DIIHA

There are two types of drug-related antibodies. Drug-independent antibodies are those antibodies that can be detected in vitro without adding any drug; thus, in vitro and in vivo characteristics are identical to cell red blood

Table 1. Number of cases of drug-induced immune hemolytic anemia (DIIHA) encountered by us over a 40-year period (10 years in San Francisco and 30 years in Southern California).

	San Francisco (Garratty and Petz)	Southern California (Garratty, Arndt, and Leger)			
Drug*	1969-1978 (10 yrs)	1979-1988 (10 yrs)	1989-1998 (10 yrs)	1999-2008 (10 yrs)	1979-2008 (30 yrs)
Methyldopa	29 (67%)	0	0	0	0 (0%)
Penicillin	10 (23%)	2 (15%)	0	0	2 (1.3%)
Cefotetan	0	0	36 (69%)	45 (53%)	81 (54%)
Ceftriaxone	0	1 (8%)	5 (10%)	14 (17%)	20 (13%)
Other cephalosporins	0	2 (15%)	0	0	2 (1.3%)
β-lactamase inhibitors	0	0	4 (8%)	6 (7%)	10 (7%)
Piperacillin	0	0	1 (2%)	12 (14%)	13 (9%)
Others	4** (9.3%)	8† (62%)	6‡ (12%)	8§ (9%)	22 (15%)
TOTAL	43	13	52	85	150

*Columns contain number (percentage) of cases associated with each drug
**Quinine (2), hydrochlorothiazide, rifampin
†Probenicid (2), chlorpropamide, phenacetin, nafcillin, rifampin, procainamide, erythromycin
‡Fludarabine (2), probenecid, tolectin, mefloquine, ticarcillin
§Oxaliplatin (2), carboplatin, rifampin, diclofenac, cimetidine, trimethoprim, sulfamethoxazole

cell (RBC) autoantibodies. Drug-dependent antibodies are those antibodies that will only react in vitro in the presence of drug (eg, bound to RBCs or added to the patient's serum in test systems to detect drug antibodies); these are antibodies directed at epitopes on the drug and/or its metabolites, or a combination of drug plus RBC membrane protein. The mechanisms involved in the serological and clinical findings are controversial. It is still unknown why or how some drugs can affect the immune system to cause RBC autoantibody formation, with or without HA.[4,8] The prototype drug is methyldopa, which causes the production of RBC autoantibodies in about 15% of the patients receiving the drug, but only about 0.5% to 1% develop an HA. Fludarabine is now the most common drug to induce RBC autoantibodies.[4]

With regard to the drug-dependent mechanisms, one mechanism is universally accepted. Some drugs bind covalently to proteins on the RBC membrane; thus, if conditions are optimal (eg, high enough drug concentration), circulating RBCs will be coated with drug; this does no harm to the RBCs, but if the patient makes an IgG antibody to the drug the antibody will bind to the drug on the RBC and the macrophages can interact, leading to Fc-mediated extravascular RBC destruction; complement may, on occasion, also be involved. These antibodies are easily detectable in vitro by testing the patient's serum or an eluate from the RBCs against drug-coated RBCs (prepared in vitro). The prototype drug is penicillin; cefotetan, but not ceftriaxone, can react by this mechanism. Unfortunately, most of the drugs that cause acute, severe intravascular hemolysis and sometimes renal failure, disseminated intravascular coagulation, and death seem to work by another mechanism, and usually involve drug-dependent antibodies that activate complement. Often, it is not possible to make drug-coated RBCs in vitro with drugs in this group, and the antibodies are only detectable by mixing the drug with the patient's serum (containing drug antibody) and RBCs. In studying drug-induced thrombocytopenia, it was suggested that the patient's platelets did not become coated with drug, but that the drug would combine with the drug antibody, forming immune complexes that could attach to "innocent bystander" platelets and activate complement. This concept reigned supreme from the early 1960s.[9,10] This so-called immune complex mechanism was also applied to DIIHA in the 1970s.[11,12] In the late 1970s evidence from various sources suggested that the concept was not valid (discussed in detail in Petz and Garratty[4]).

More recently, another concept was suggested for DIIHA that provided one model for all three types of drug antibodies. This model, known as the "unifying hypothesis," was originally suggested by Habibi[13] and later extended by Salama and Muller-Eckardt[14] and Garratty.[15] This hypothesis is based on early experiments,[16,17] showing that when animals were injected with simple low-molecular-weight chemicals, they made no antibodies but if the chemical was bound to protein (eg, albumin), three populations of antibodies might be found: one population reacting with the chemical (the hapten), one population reacting with a combination of hapten plus carrier protein epitopes, and one population possibly reacting with only the carrier

protein. As drugs are small molecules (haptens), one would have to suggest that they all must bind to protein, however loosely, to evoke an immune response; a patient may make one or more of the three populations of antibody. Penicillin-type antibodies bind strongly to protein and can evoke antibodies that appear to be directed mainly to the drug as one can inhibit the antibodies with penicillin alone, in vitro, using hapten-inhibition tests. Many other drugs appear to evoke antibodies that mainly appear to be directed against a combination of drug plus RBC membrane protein. Such antibodies are not inhibited by the drugs in vitro and the drugs do not bind well to RBCs. Such antibodies are those that were originally thought to be reacting by the immune complex mechanism. Shulman eventually came up with a concept that combined his original concept with the unifying hypothesis.[18]

A Possible New Mechanism for DIIHA

There is one further concept that has been of increasing interest to us in the last decade. Some drugs appear to be capable of modifying the RBC membrane so that proteins become non-immunologically adsorbed onto the RBC membrane. The first drug shown to cause non-immune protein adsorption (NIPA) was the first cephalosporin (cephalothin). In 1971, Spath et al[19] showed that cephalothin-treated RBCs adsorbed IgG, C3, albumin, fibrinogen, etc, after incubation in normal plasma; the proteins could be detected by the antiglobulin test. The drug appeared to modify the RBC membrane, causing proteins to attach nonimmunologically to the RBC membrane.[4] **Table 2** shows nine other drugs that cause the same effect. Up to about 1996, we believed that this was an in vitro phenomenon that caused us in vitro problems (eg, RBC-bound IgG detectable by the antiglobulin test), but that it had no clinical significance. We now believe NIPA can cause hemolytic anemia.[20-22] RBCs having IgG on their membrane caused by nonimmunologic adsorption yield positive monocyte monolayer assays (MMA), suggesting that the macrophages would interact with these coated RBCs, leading to decreased RBC survival.[21,22] We have published data suggesting that the NIPA mechanism can be the cause of decreased RBC survival in patients taking drugs that contain β-lactamase inhibitors (clavulanate, sulbactam, tazobactam)[20-22] and drugs in the platinum family[23] without any drug antibodies being involved.

Table 2. Drugs associated with nonimmunologic adsorption of proteins onto RBCs.

Cefotetan
Cephalothin
Cisplatin/oxaliplatin/carboplatin
Diglycoaldehyde (INOX)
Suramin
Sulbactam (contained in Unasyn)
Clavulanate (contained in Augmentin and Timentin)
Tazobactam (contained in Zosyn)

Drugs containing β-lactamase inhibitors also contain antibiotics (penicillins), which can cause DIIHA through well-described mechanisms.[24,25] Examples of these drugs include tazobactam plus piperacillin [Zosyn (Wyeth, Madison, NJ)]; sulbactam plus ampicillin [Unasyn (Pfizer, New York, NY)]; clavulanate plus ticarcillin [Timentin (GlaxcoSmithKline, Philadelphia, PA)]; and clavulanate plus amoxicillin [Augmentin (GlaxcoSmithKline, Philadelphia, PA)]. Thus, one or both mechanisms can be involved in the HA associated with these drugs. Certain chemotherapeutic agents such as cisplatin, carboplatin, and oxaliplatin have all caused DIIHA and/or positive DATs.[4,6] The mechanisms involved have been controversial over the years, but it is now clear that patients may make antibodies to the drug and/or NIPA may be involved in the hemolytic process.[4,23]

DIIHA Facts of Special Interest to Hematologists

Cephalosporins

Table 3 shows the number of individual cases of DIIHA caused by cephalosporins reported from 1971 through 2008.

Table 3. Cephalosporins reported to cause immune hemolytic anemia (IHA)* (1971-2008).

Drug	Number of cases (fatalities)
Cefotetan	47 (5)
Ceftriaxone	29 (10)
Cephalothin	5 (1)
Ceftizoxime	4 (2)
Cefotaxime	3
Ceftazidime	3
Cefoxitin	2 (1)
Cefazolin	2
Cephalexin	1
Cefamandole	1
Cefuroxime	1
Cefixime	1
Total	99 (19)

*Individual case reports; many other cases are reported, without individual case reports.[26,27]

Cefotetan represents more than 50% of the DIIHA you are likely to encounter (**Table 1**). Patients can present with acute intravascular hemolysis. Cefotetan is used commonly, prophylactially, for some surgeries (eg, Cesarean sections). We have seen healthy young women with hemoglobinuria and very low hemoglobin (eg, 4 g/dL) one week after receiving one dose of prophylactic cefotetan for surgery.[28] The drug is often not suspected, and we know of cases where post-surgical infection was suspected, the patient given more cefotetan leading to fatal HA (most of these were never published). The direct antiglobulin test (DAT) is usually positive and high titer cefotetan antibodies can be detected in the serum and in an eluate from the patient's RBCs.

The FDA reported on 85 cases of cefotetan-induced HA from approval of cefotetan in 1985 to 1997; 15/85 (18%) were fatalities.[27] Mean fall in hemoglobin (Hb) level was 6.7 g/dL, with mean final Hb of 5.2 g/dL. About half of the patients were transfused. Renal dysfunction was noted in 8% of patients. Fifty-nine percent of the patients received cefotetan for prophylaxis; 50% of the HAs were associated with surgery. Only 18% had a history of receiving cefotetan previously.

When a drug is discontinued, the HA usually resolves soon afterwards. Steroids are usually not required, and there are limited data to suggest that steroids have any effect when the HA is not autoimmune (ie, when it is caused by drug-dependent antibodies); most case reports where steroids appeared to help are confounded by simultaneous discontinuation of the drug.[29] A notable exception is cefotetan as the HA often continues for longer than expected after the drug is stopped. Davenport et al[30] found that cefotetan bound firmly to the RBCs and this RBC-bound cefotetan could be detected for up to 98 days after the last dose of the drug; 8% of the 65 patients receiving cefotetan made antibodies. These patients had only a mild HA. It seems possible that patients with powerful anti-cefotetan could still have cefotetan-coated RBCs to attack for a considerable period after stopping the drug.

Ceftriaxone is the second most common drug to cause DIIHA in our series (**Table 1**). Some children have dramatic HA; 50% are reported as fatal HA.[4,31] Analysis of 21 patients (15 children and 6 adults) we studied showed that 40% of the children started hemolyzing ≤ 1 hr after receiving ceftriaxone. Hemoglobin levels fell to ≤ 5g/dL in 62% and to ≤ 1 g/dL in 20% of the patients. Fatal HA occurred in 38% of the patients. The children have always received ceftriaxone previously, the DAT is usually positive (all have RBC-bound complement and most have IgG in addition), and ceftriaxone antibodies are detectable in the patient's serum. The HA is usually not as dramatic in adults. The fall in hemoglobin is much less and does not occur in a few hours; fatalities are less common.

Recently, Quillen et al[32] reported on the incidence of ceftriaxone-induced RBC antibodies in sickle cell disease (SCD) and HIV-infected pediatric patients. They found 8 of 64 (12.5%) had antibodies; 2 of these patients had drug-induced HA; 1 of these patients (11 years old) had catastrophic fatal hemolysis.

The HA associated with the cephalosporins, especially when given in association with surgery, and when the patient was transfused can cause misdiagnosis. We have seen several cases where a hemolytic transfusion reaction was the prime suspect because of posttransfusion hemolysis associated with a positive DAT. It is not uncommon for the attending physician not to know that pertinent drugs (eg, cefotetan) were given at surgery; we recommend that the surgical notes are reviewed in unexplained (eg, no RBC alloantibodies detected) hemolytic transfusion reactions, especially if signs of intravascular hemolysis are present. The presentation and serology can also mimic AIHA (ie, strongly positive DAT and sometimes antibodies in the serum reacting without the presence of drug[26]).

I am often asked whether it is safe to give other cephalosporins to a patient who had DIIHA due to cefotetan or ceftriaxone. The standard answer has been to recommend avoiding all cephalosporins as it is well documented that if a patient receives the same drug causing the DIIHA, the second bout of HA is much worse; there are also examples in the literature associated with drugs other than cephalosporins, where closely related drugs have caused a second more severe episode of HA. We studied in vitro cross-reactivity of the cephalosporins, using powerful anti-cefotetan and anti-ceftriaxone.[33] **Table 4** shows the results. There was a surprising low level of crossreactivity. Hapten inhibition tests showed that only cefotetan and cephalothin inhibited the anti-cefotetan in vitro. It should be emphasized that these are results from in vitro serological data; we have no in vivo data. Also, the results relate to IgG and IgM antibodies only; thus, allergic/anaphylactic reactions do not relate to these data.

Piperacillin

Piperacillin can cause DIIHA and/or positive DATs[24]; it is the third most common drug to cause DIIHA in our series (**Table 1**). Although a semi-synthetic penicillin, unlike other semi-synthetic penicillins (eg, ampicillin), it reacts differently than penicillin G. In contrast to penicillin, the in vivo RBC destruction can be complement-mediated; most of the DATs are positive due to RBC-bound complement and IgG.[24] Unlike penicillin antibodies, piperacillin

Table 4. Cross-reactivity of cefotetan and ceftriaxone antibodies with various cephalosporins and penicillin.[33] Positive results are shown in bold.

Drug	Cefotetan antibody A*	Cefotetan antibody B**	Ceftriaxone antibody B**
Cefotetan	**200,000**	**20/10,240** †	0/0/0
Ceftriaxone	<10	0/0	**80/5,120/640**†
Cefamandole	**40**	0/0	0/**1/1**
Cefazolin	<10	0/0	0/0/0
Cefepime	**20**	0/0	0/0/0
Cefoperazone	<10	0/0	0/**1**/0
Cefotaxime	**20**	0/0	**1/1/1**
Cefoxitin	**5120**	0/0	0/0/0
Ceftazidime	<10	0/0	0/0/0
Cephalothin	**5120**	0/**1**	0/0/0
Penicillin	**320**	0/0	0/0/0

*A: Serum (anti-cefotetan) tested against drug-coated RBCs
**B: Serum (anti-cefotetan and anti-ceftriaxone) tested against enzyme-treated group O RBCs in the presence of drugs (1 mg/mL)
†Titer for cefotetan antibody results are given as hemolysis/antiglobulin test titer; for ceftriaxone antibody, hemolysis/37°C agglutination/antiglobulin test titers

antibodies are best detected by the "immune complex" method.[24] Another unusual aspect of piperacillin is that most normal donor and patient sera appear to have piperacillin antibodies that react with piperacillin-coated RBCs, and this is not caused by nonimmunologic adsorption of protein. Normal sera also contain antibodies that react with penicillin- and cefotetan-coated RBCs,[25,26] but fewer sera react with these than with piperacillin-coated RBCs. Thus, to confirm the presence of piperacillin antibodies, patients' sera should be tested by the "immune complex" approach, which does not yield positive reactions using random patients and donors.[24] It seems that we are all exposed to antigens identical, or very similar, to some β-lactam antibiotics in our environment. This may be related to the common practice of adding antibiotics (eg, cephalosporins) to animal (eg, cattle) feed and treatment of animals used for food.[34,35]

Purine Analogues, Especially Fludarabine

Several groups reported that about 20% of patients with chronic lymphocytic leukemia (CLL) treated with fludarabine developed AIHA.[36,37] These conclusions could be challenged as up to 35% of CLL have been reported to form RBC autoantibodies. But further reports showed that rechallenge with the drug caused recurrence of the AIHA,[38] and a retrospective randomized trial comparing fludarabine with other drugs showed that 5% of the fludarabine arm developed autoantibodies (2% AIHA) compared to 0% in the other arm.[39] Cladribine has also caused DIIHA.[4] Borthakur et al[40] recently reported that 5.8% of 300 patients with CLL who received fludarabine, cyclophosphamide, and rituximab (FCR) developed HA, but 82.4% of these patients had a negative DAT. They reported that by combining cyclosphosphamide and rituximab with fludarabine, the incidence/severity of fludarabine-induced AIHA was reduced. They suggested FCR treatment may mask the DAT reactivity, leading to a DAT-negative AIHA.

Hydrocortisone

Hydrocortisone antibodies have been detected in individuals without HA.[41] A recent finding should be of interest to hematologists: the first case of a DIIHA due to hydrocortisone has been described.[42] This adds another possible explanation for poor responses to steroid therapy in some cases of AIHA where steroid-induced DIIHA may be masked by the autoimmune process.

Antibodies to Epitopes on Drug Metabolites

If the clinical and hematological findings strongly suggest a DIIHA, but no drug antibodies are detected, one can pursue confirmation of the diagnosis by testing the patient's serum with drug metabolites; the most convenient source is urine from a patient (or volunteer) taking the drug. DIIHA has been associated with antibodies that will react with metabolites, but not the parent drug.[43]

Disclosures

Conflict-of-interest disclosure: The author declares no competing financial interests.
Off-label drug use: None disclosed.

Correspondence

George Garratty, PhD, FRCPath, Scientific Director, American Red Cross Blood Services, Southern California Region, 100 Red Cross Circle, Pomona, CA 91768; Phone: 909-859-7406; Fax: 909-859-7680; e-mail: garratty@usa.redcross.org

References

1. Kaufman DW, Kelley JP, Johannes CB, et al. Acute thrombocytopenic purpura in relation to the use of drugs. Blood. 1993;82:2714-2718.
2. van den Bemt PMLA, Meyboom RHB, Egberts ACG. Drug-induced immune thrombocytopenia. Drug Safety. 2004;27:1243-1252.
3. Andrés E, Maloisel F. Idiosyncratic drug-induced agranulocytosis or acute neutropenia. Curr Opin Hematol. 2008;15:15-21.
4. Petz LD, Garratty G. Immune Hemolytic Anemias. 2nd ed. Philadelphia: Churchill Livingstone; 2004:261-

317.
5. Arndt PA, Garratty G. The changing spectrum of drug-induced immune hemolytic anemia. Semin Hematol. 2005;42:137-144.
6. Garratty G, Arndt PA. An update on drug-induced immune hemolytic anemia. Immunohematology. 2007;23:105-119.
7. Johnson ST, Fueger JT, Gottschall JL. One center's experience: the serology and drugs associated with drug-induced immune hemolytic anemia – a new paradigm. Transfusion. 2007;47:697-702.
8. Garratty G, Arndt P, Prince HE, Shulman IA. The effect of methyldopa and procainamide on suppressor cell activity in relation to red cell autoantibody production. Br J Haematol. 1993;84:310-315.
9. Miescher PA, Gorstein F. Mechanisms of immunogenic platelet damage. In: Johnson SA, Monto RW, Rebuck JW, et al, eds. Blood Platelets. London: Churchill; 1961:671.
10. Shulman NR. A mechanism of cell destruction in individuals sensitized to foreign antigens and its implications in auto-immunity. Ann Int Med 1964;60:506-20.
11. Worlledge SM. Immune drug-induced hemolytic anemias. Semin Hematol. 1973;10:327-344.
12. Garratty G, Petz LD. Drug-induced immune hemolytic anemia. Am J Med. 1975;58:398.
13. Habibi B. Drug induced red blood cell autoantibodies co-developed with drug specific antibodies causing haemolytic anaemias. Br J Haematol. 1984;61:139-143.
14. Mueller-Eckhardt C, Salama A. Drug-induced immune cytopenias: a unifying pathogenetic concept with special emphasis on the role of drug metabolites. Transfus Med Rev. 1990;4:69-77.
15. Garratty G. Target antigens for red-cell-bound autoantibodies. In: Nance SJ, ed. Clinical and Basic Science Aspects of Immunohematology. Arlington, VA: American Association of Blood Banks: 1991;33-72.
16. Landsteiner K. The Specificity of Serological Reactions. Springfield, IL: Thomas; 1936.
17. Kitagawa M, Yagi Y, Pressman D. The heterogeneity of combining sites of antibodies as determined by specific immunoadsorbents. II. Comparison of elution patterns obtained with anti-P-azobenzoate antibodies by different kinds of immunoadsorbent and eluting hapten. J Immunol. 1965;95:455-465.
18. Shulman NR, Reid DM. Mechanisms of drug-induced immunologically mediated cytopenias. Transfus Med Rev. 1993;VII:215-229.
19. Spath P, Garratty G, Petz L. Studies on the immune response to penicillin and cephalothin in humans. II. Immunohematologic reactions to cephalothin administration. J Immunol. 1971;107:860-689.
20. Garratty G, Arndt PA. Positive direct antiglobulin tests and haemolytic anaemia following therapy with beta-lactamase inhibitor containing drugs may be associated with nonimmunologic adsorption of protein onto red blood cells. Br J Haematol. 1998;100:777-783.
21. Broadberry RE, Farren TW, Bevin SV, et al. Tazobactam-induced haemolytic anaemia, possibly caused by non-immunological adsorption of IgG onto patient's red cells. Transfus Med. 2004;14:53-57.
22. Arndt PA, Leger RM, Garratty G. Positive direct antiglobulin tests and haemolytic anaemia following therapy with the beta-lactamase inhibitor, tazobactam, may also be associated with non-immunologic adsorption of protein onto red blood cells (letter). Vox Sang. 2003;85:53.
23. Arndt P, Garratty G, Isaak E. Bolger M, Lu Q. Positive direct and indirect antiglobulin tests associated with oxaliplatin can be due to drug antibody and/or drug-induced nonimmunologic protein adsorption. Transfusion. 2009;49:711-718.
24. Leger RM, Arndt PA, Garratty G. Serological studies of piperacillin antibodies. Transfusion. 2008;48:2429-2434.
25. Garratty G. Immune cytopenia associated with antibiotics. Transfus Med Rev. 1993;VII:255-267.
26. Arndt PA, Leger RM, Garratty G. Serology of antibodies to second- and third-generation cephalosporins associated with immune hemolytic anemia and/or positive direct antiglobulin tests. Transfusion. 1999;39:1239-1246.
27. Viraghavan R, Chakravarty AG, Soreth J. Cefotetan-induced haemolytic anaemia. A review of 85 cases. Adv Drug React Toxicol Rev. 2002;21:101-107.
28. Garratty G, Leger RM, Arndt PA. Severe immune hemolytic anemia associated with prophylactic use of cefotetan in obstetric and gynecologic procedures. Am J Obstet Gynecol. 1999;181:103-104.
29. Salama A. Drug-induced immune hemolytic anemia. Expert Opin Drug Saf. 2009;8:73-79.
30. Davenport RD, Judd WJ, Dake LR. Persistence of cefotetan on red blood cells. Transfusion. 2004;44:849-852.
31. Kapur G, Valentini RP, Mattoo TK, Warrier I, Imam AA. Ceftriaxone induced hemolysis complicated by acute renal failure. Pediatr Blood Cancer. 2008;50:139-142.
32. Quillen K, Lane C, Hu E, Pelton S, Bateman S. Prevalence of ceftriaxone-induced red blood cell antibodies in pediatric patients with sickle cell disease and human immunodeficiency virus infection. Pediatr Infect Dis J. 2008;27:357-358.
33. Arndt PA, Garratty G. Cross-reactivity of cefotetan and ceftriaxone antibodies, associated with hemolytic anemia, with other cephalosporins and penicillin. Am J

Clin Pathol. 2002;118:256-262.
34. Anderson AD, Nelson JM, Rossiter S, Angulo FJ. Public health consequences of use of antimicrobial agents in food animals in the United States. Microb Drug Resist. 2003;9:373-379.
35. Shea KM. Nontherapeutic use of antimicrobial agents in animal agriculture: implications for pediatrics. Pediatrics. 2004;114:862-868.
36. Longo G. Fludarabine and autoimmune hemolytic anemia in chronic lymphocytic leukemia. Eur J Haematol. 1997;59:124-125.
37. Hamblin TJ, Oscier DG, Young BJ. Autoimmunity in chronic lymphocytic leukemia. J Clin Pathol. 1986;39:713-716.
38. Diehl LF, Ketchum LH. Autoimmune disease and chronic lymphocytic leukemia: autoimmune hemolytic anemia, pure red cell aplasia, and autoimmune thrombocytopenia. Semin Oncol. 1998;25:80-97.
39. Johnson S, Smith AG, Löffler H, et al. Multicenter prospective randomized trial of fludarabine versus cyclophosphamide, doxorubicin, and prednisone (CAP) for treatment of advanced-stage chronic lymphocytic leukemia. The French Cooperative Group on CLL. Lancet. 1996;347:1432-1438.
40. Borthakur G, O'Brien S, Wierda W, et al. Immune leukemias in patients with chronic lymphocytic leukaemia treated with fludarabine, cyclophosphamide and rituximab - incidence and predictors. Br J Haematol. 2007;136:800-805.
41. Umlas J, Turner LA. Antibodies to hydrocortisone in reagent red cells causing positive antibody screening tests. Transfusion. 1993;33:686-688.
42. Martinengo M, Ardenghi DF, Triopdi G, Reali G. The first case of drug-induced immune hemolytic anemia due to hydrocortisone. Transfusion. 2008;48:1925-1929.
43. Salama A, Mueller-Eckhardt C, Kissel K. Ex vivo antigen preparation for the serological detection of drug-dependent antibodies in immune haemolytic anaemias. Br J Haematol. 1984;58:525-531