

A Thorough Diagnostic Approach to Discriminate Idiopathic from Drug-Induced Immune Hemolytic Anemia

Eliassen Astrid*; Overgaard Ulrik; Dziegiel Morten Hanefeld; Holst Helle

*Eliassen Astrid

Department of Clinical Pharmacology, Bispebjerg University Hospital, Denmark

Tel: +45 23 37 97 17; Email: astrid.eliasen@regionh.dk

Abstract

Autoimmune hemolytic anemia can be idiopathic or accompanying and complicating an underlying disease. We present a 66-year old woman, who developed acute onset of severe hemolytic anemia associated with thrombocytosis and leukocytosis sixteen days after antibiotic treatment of pneumonia. Blood transfusion, glucocorticoids, and rituximab were initiated, and the patient survived with no sequelae. Follow-up blood tests revealed no signs of a drug-induced reaction, and re-exposure to ciprofloxacin and cefuroxime did not result in hemolysis. The diagnosis of drug-induced immune hemolytic anemia is mainly clinical, but an investigation for a drug antibody may be reasonable if there is a temporal relationship between drug administration and the immune hemolytic anemia.

Keywords

Hemolytic anemia; haemolytic anaemia; autoimmune; idiopathic; ciprofloxacin

Abbreviations

AIHA: Autoimmune hemolytic anemia; DAT: Direct antiglobulin test; DIIHA: Drug-induced immune hemolytic anemia; Hb: Hemoglobin; RBCs: Red blood cells; PBS: Phosphate-buffered saline; CRP: C-reactive protein; LDH: Lactate Dehydrogenase

Introduction

Autoimmune hemolytic anemia (AIHA) is a heterogeneous condition defined as the increased destruction of erythrocytes in the presence of anti-erythrocyte auto antibodies. Several immunologic mechanisms are involved in the pathogenesis of AIHA, herein anti-erythrocyte autoantibodies, B and T lymphocytes, with or without complement activation.

AIHA can be classified as warm AIHA or cold AIHA based on the characteristics of the autoantibodies involved, and is usually demonstrated by a positive direct antiglobulin test (DAT). AIHA may be primary (idiopathic) or secondary to underlying disease, such as infection, autoimmune disease, lymphoproliferative disease, neoplastic disease, or drug-induced [1]. The latter cause of AIHA is termed drug-induced immune hemolytic anemia (DIIHA) [2].

DIIHA is a rare, but potentially fatal, complication of drug administration. The incidence of DIIHA has been roughly estimated to be one in one million of the population, but is likely under diagnosed [2], and more than 130 drugs have been implicated [2]. A number of factors can lead to misdiagnosis. Many physicians are not familiar with DIIHA. Because of its rarity and the significant overlap with symptoms of

the underlying condition, it remains unrecognized. DIIHA usually presents with a sudden drop in hemoglobin (Hb) following treatment with the causative drug. If the drug is administered for the first time, the immune response becomes clinically evident following a period of approximately one week. The serological testing is often inadequate or lacking, as there remain no standard tests or guidelines to confirm the diagnosis [3]. The pathogenesis of DIIHA is not fully elucidated, and may involve more than one mechanism for some drugs. Thus, hemolytic anemia can occur with or without drug antibodies involved. There are two types of drug-related antibodies:

1. Drug-dependent antibodies react only *in vitro* in the presence of drug. These are classified into two subtypes by their *in vitro* activity:

a. React with drug-treated red blood cells (RBCs). The drug binds covalently to the RBC membrane. The antibodies will react with RBCs that are coated with the drug, and these antibodies are detectable *in vitro* by testing the patient's serum or an eluate from the RBCs against drug-coated RBCs (prepared *in vitro*).

b. React with untreated RBCs in the presence of a solution of the drug. The drug does not bind covalently to the RBC membrane. It elicits an immune response, usually by drug-dependent antibodies that activate complement. Antibodies can be detected by incubating patient's serum (containing drug antibody), drug and RBCs.

2. Drug-independent antibodies, which can be detected *in vitro* without adding any drug. They are indistinguishable from idiopathic warm auto antibodies.

Drugs can also induce a positive antiglobulin test and hemolytic anemia, but with no drug antibodies involved. The drugs modify the RBC membrane, so that proteins attach to the RBCs leading to positive direct or indirect antiglobulin test. RBCs are destroyed in the spleen and liver [2,4].

This report describes a case of severe autoimmune hemolytic anemia, which was first thought to be a complication of ciprofloxacin-treatment. However, a *thorough diagnostic approach revealed no signs of drug allergy or underlying disease.*

Case Presentation

A 66-year old Caucasian woman was readmitted to hospital. Prior to admission she reported dyspnea, fatigue, and palpitations during the last four days. She had been discharged sixteen days earlier after one month of hospitalization with pneumonia, positive for pneumococcal urinary antigen test, but blood culture and PCR negative in respiratory samples for viral pneumonia, *L. pneumophila*, *C. pneumoniae*, *C. psittaci* and *M. pneumoniae*. Here, she also received treatment for pulmonary embolism, later disconfirmed, atrial fibrillation, and pulmonary edema. Treatment included antibiotics (cefuroxime, clarithromycin, metronidazole, meropenem, and ciprofloxacin, (figure 1)), tinzaparin, apixaban, digoxin, amiodarone, metoprolol, furosemide, and spironolactone. Previous medical history included well controlled hypertension, with daily administration of amlodipine 5 mg and bendroflumethiazid 1.25 mg / kaliumchlorid 573 mg, hypercholesterolemia, treated with simvastatin 10 mg daily. She had childhood allergic reactions to penicillin with symptoms of skin reactions.

On admission she was pale, but showed no clinical signs of bleeding. Blood analysis revealed signs

of severe hemolytic anemia associated with thrombocytosis and leukocytosis (table 1). Hb level was 3.7 g/dL, and dropped to 2.7 g/dL after five hours. Only sixteen days before it was 10.3 g/dL (table 1 and figure 1). The anemia was normocytic. The patient was transferred to the Haematology Department. Peripheral blood smear showed neutrophilia and thrombocytosis. The erythrocytes were characterized by reticulocytosis, anisocytosis, polychromasia, and poikilocytosis, as can be seen, when hemolytic anemia is present. Standard DAT was positive for IgG and C3d, and *warm* autoantibodies were found. Bone marrow biopsy was hypercellular dominated by erythropoiesis with no signs of underlying malignant hematological disease. CT of thorax and abdomen revealed no signs of malignant or other underlying disease. Antinuclear antibody and antineutrophil cytoplasmic antibody blood tests for autoimmune disease were negative. Repeated blood cultures and blood tests for viruses (cytomegalovirus, Epstein-Barr virus, hepatitis B and C virus and human immunodeficiency virus) were all negative. *E. coli* was found with mixed flora in urine.

The patient was treated with two units of packed RBCs, prednisolone (125 mg/day), folic acid, insulin for hyperglycemia, cefuroxime, metronidazole, and fluconazole for suspected infection, and IV fluids. One week after admission her Hb was 6.9 g/dL, and treatment with rituximab (600 mg/week for four weeks) was initiated (figure 1). The patient's Hb level stabilized at 7.3 g/dL before discharge, and six weeks after admission Hb reached normal values of 12.3 g/dL, and the patient reported well-being.

Standard DAT was repeated on day 129 after cessation of ciprofloxacin, and the test was still positive, though significantly weaker. At this time, an attempt to detect ciprofloxacin-dependent antibodies was also made in the immunohematology laboratory. Briefly, one volume of 2 mg ciprofloxacin per mL phosphate-buffered saline (PBS) was added to one volume of patient's plasma. The mix was added to normal donor RBCs and to papain-treated donor RBCs. A parallel incubation with PBS served as the negative control. After 60 minutes incubation the cells were examined in ID-cards with anti-IgG and -C3d (BioRadID-Card (Id-number: 50531, Denmark)) according to standard procedures. The reactions were identical for PBS with ciprofloxacin, as well as for PBS. Thus, we conclude that no ciprofloxacin-dependent antibodies were detected. The patient was subsequently re-exposed to ciprofloxacin without ensuing hemolysis.

Discussion

The present report describes a case of hyperhemolysis, thrombocytosis, and leukocytosis sixteen days after antibiotic treatment of pneumonia. The hemolysis was diagnosed by increased bilirubin, serum LDH elevation, a low serum haptoglobin and reticulocytosis.

An algorithm for the assessment of hemolytic anemia can be seen in figure 2.

In the present case, standard DAT was positive with warm autoantibodies and complement (C3d), which are the main serological findings in DIIHA (figure 2), and we searched for a drug as a causal agent. During the first admission the patient received cephalosporins (e.g. cefuroxime), which is one of the drug classes most frequently implicated in DIIHA. Further, β -lactamase inhibitors (e.g. meropenem), and fluoroquinolones (e.g. ciprofloxacin) are also known to cause DIIHA in rare cases [2,4]. Past medical history did not report of intake of any of these drugs. There was a significant drop in Hb during treatment with cefuroxime at the first week of the first admission, and Hb level increased when this treatment was

stopped (figure 1). However, there was no biochemical evidence of hemolysis, as both total bilirubin and LDH were in the normal age related range. Also, there was no drop in Hb during reexposure with cefuroxime at the first week of the second admission. The drop in Hb at the first admission could be explained by anemia of chronic disease, or rehydration after dehydration at admission. The treatment was changed to meropenem for four days, in addition to ciprofloxacin for fourteen days. At first, this caused a rise in Hb, which could be explained by treatment of the infection. During treatment with ciprofloxacin, Hb dropped dramatically. The temporal relationship suggested a causal association between ciprofloxacin intake and the adverse event (figure 1). To confirm the diagnosis, an attempt to identify ciprofloxacin-dependent antibodies was made, but the test was negative. This does not exclude ciprofloxacin as the causative agent. The test for ciprofloxacin-dependent antibodies was performed 129 days after cessation of ciprofloxacin, and it is possible that with the successful immunosuppressive treatment of the patient, no antibodies were left in the plasma of the patient. It is preferable to have a sample collected during active in vivo hemolysis. According to Abdulgabar Salama and George Garratty, many drugs supposed to be involved in severe AIHA are detectable in laboratory investigations only when their degradation products, either in serum/plasma or urine, are available [6]. The drug antibodies can also be drug-independent. Drug-independent antibodies cannot be defined or excluded by serological testing [7]. In other reported cases, fluoroquinolone-dependent antibodies have been detected [4]. In our case, standard DAT was still positive 129 days after cessation of ciprofloxacin, and the test for enhancement by the addition of ciprofloxacin was negative. Re-exposure to ciprofloxacin did not result in hemolysis, which makes the diagnosis of DIIHA unlikely.

More common causes of hemolysis need to be excluded (figure 2). DIIHA is difficult to distinguish from other types of AIHA. A differential diagnosis includes AIHA secondary to infections. In the present case, an infectious disease cannot be excluded as the possible cause of anemia. The negative findings obtained from blood cultures and other microbiology laboratory tests could be explained by the use of prior antibiotics. However, the fact that AIHA secondary to infections is primarily cold type AIHA (figure 2), makes infectious disease an unlikely etiology.

There was no evidence of other autoimmune, lymphoproliferative, or neoplastic disease. The present case had leukocytosis and thrombocytosis with no evidence of hematological malignancy, so this could be explained by reactive thrombocytosis caused by hemolytic anemia or infection.

As no underlying disease was found, the condition is termed idiopathic AIHA.

Treatment of warm AIHA is primarily based on immunosuppression. The first line treatment is corticosteroids. Following this, other immunosuppressants (e.g. rituximab) are to be considered. Rituximab as first line treatment combined with corticosteroids lower the risk of recurrence of hemolysis [8]. Blood transfusion may be indicated in patients with low Hb levels. The ideal therapy would be the identification and elimination of the causative origin of autoimmunization, why the patients need to be diagnosed properly [9,10].

Conclusion

AIHA is a heterogeneous condition, where hemolysis or an underlying disease can dominate the clinical picture. The idiopathic form can easily be interpreted as a drug-induced reaction, as clinical and

initial serology can mimic each other. An investigation for a drug antibody may still be reasonable if a patient has immune hemolytic anemia, and there is a temporal relationship between drug administration and the hemolytic event. Clinicians should cooperate with clinical immunology departments to insure correct testing and interpretation of serological findings. Though, DIIHA still remains a clinical diagnosis, where discontinuation of drug administration leads to rapid resolution of the hemolysis.

What is already known

Autoimmune hemolytic anemia is a rare complication of drug administration

The diagnosis of drug-induced immune hemolytic anemia often rely solely on authors suspicion that a drug was the cause of a hemolytic anemia

What this study adds

The diagnosis of drug-induced immune hemolytic anemia is mainly clinical, but should include serological testing supporting an immune etiology

An investigation for a drug antibody may be reasonable if immune hemolytic anemia is confirmed, and there is a temporal relationship between drug administration and the hemolysis

Figures

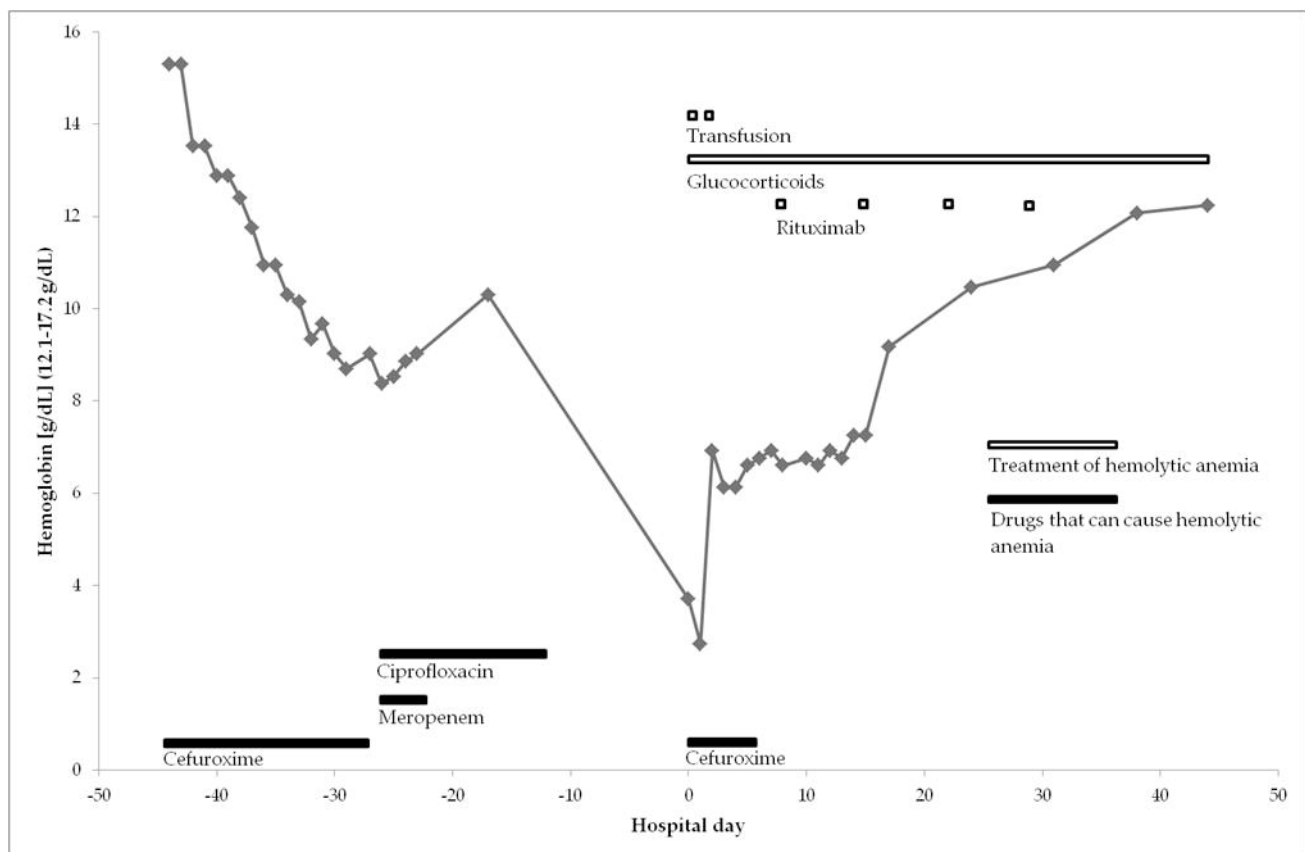


Figure 1: Hospital course: Hemoglobin and medications

The day the patient was admitted for the second time was considered as day 0.

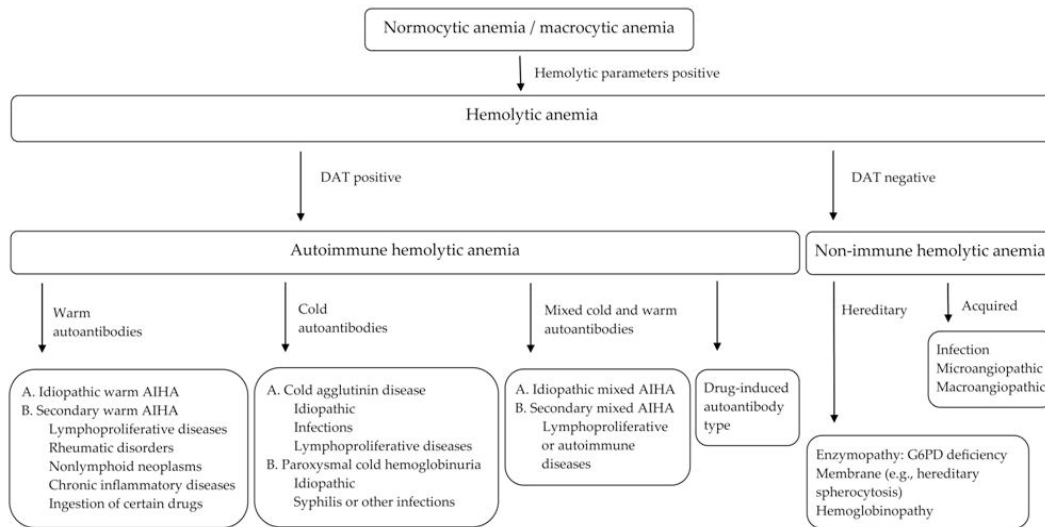


Figure 2: Assessment of causes of hemolytic anemia (modified from [5,6])
DAT, Direct antiglobulin test; G6PD, Glucose-6-phosphate dehydrogenase

Table

Parameters (normal values)	27 days before admission 1	17 days before admission 2	Day 0 (admission 2)	5 hours	Day 1	1 week	2 weeks (discharge)	6 weeks
Hemoglobin (12.1-17.2 g/dL)	9.0	10.3	3.7	2.7	5.5	6.9	7.3	12.3
Leukocytes (4-10 x 10 ⁹ /L)	10.9	12.3	45.6	29.1	32.7	17.9	8.0	10.9
Neutrophil (2.5-7.5 x 10 ⁹ /L)	-	9.1	-	-	28.1	13.8	5.9	7.0
Platelets (150-400 x 10 ⁹ /L)	283	569	862	708	598	329	463	316
Total bilirubin (0.3-1.5 mg/dL)	0.5	-	4.3	3.3	-	1.6	1.3	0.5
Reticulocytes (0.5-2.2 %)	-	-	-	-	6.6	-	22.2	-
Creatinine (0.6-1.1 mg/dL)	0.59	0.53	0.90	0.78	0.67	0.54	0.55	0.64
Glucose (70-130 mg/dLpreprandial)	-	-	580	472	595	-	-	108
CRP (< 10 mg/L)	89	75	65	78	73	<3	14	16
Haptoglobin (47-205 mg/dL)	-	-	-	<0.08	-	<0.08	97	-
LDH (105-205 U/L)	-	-	1450	1320	1140	402	220	213

Table 1: Time course of laboratory parameters

The day the patient was admitted for the second time was considered as day 0.

CRP, C-reactive protein; LDH, Lactate Dehydrogenase

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Manuscript Information: Received: August 06, 2016; Accepted: December 07, 2016; Published: December 09, 2016

Authors Information: Eliassen Astrid^{1*}; Overgaard Ulrik²; Dziegiel Morten Hanefeld³; Holst Helle¹

¹Department of Clinical Pharmacology, Bispebjerg University Hospital, Denmark

²Department of Hematology, Copenhagen University Hospital, Denmark

³Department of Clinical Immunology, Copenhagen University Hospital, Denmark

Citation: Astrid E, Ulrik O, Hanefeld DM, Helle H. A thorough diagnostic approach to discriminate idiopathic from drug-induced immune hemolytic anemia. *Open J Clin Med Case Rep*. 2016; 1195

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