Severe Hemolysis With Negative Direct Antiglobulin Test: A Case Report

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Case report

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Abstract

A 49-year-old woman with type 2 diabetes mellitus presented to the emergency department. Her examination showed marked pallor, exhaustion, lethargy, yellowish eyes, anorexia, nausea and vomiting.

Laboratory analysis revealed: Hemoglobin (Hb) 4.8 g/dl, MCV 91fl, platelet count 233 × 10^6 /L, Total bilirubin 7.0 mg/dl, Glucose 316 mg/dl, hematuria and normal G6PD. Hemolytic panel was unswerving with hemolysis. IV fluids and 2 units of packed cell were transfused. Despite transfusion, during the first 4 days of hospitalization the hemolysis continued so that immune hemolysis was suspected in spite of negative coomb's test. After 3 weeks of the patient refer to the hospital, she was discharged home with stable vital signs and Hb10 g/dl. Blood transfusion along with corticosteroids, IVIG and rituximab saved the life of this patient. We concluded in cases that presented with a severe drop in hemoglobin, even if there is a negative direct antiglobulin test (DAT) pay special attention to the immune mediated hemolysis and do not be misled with a negative coomb's test.

Introduction

Hemolytic anemia can be divided into immune and nonimmune types. Autoimmune hemolytic anemia (AIHA) is caused by increased erythrocyte destruction with autoantibodies directed against the person's own red blood cells and susceptible them to lyse and consequent insufficient number of red blood cells in the circulation. The disease may be primary, or secondary to another underlying illness such as drug induced, associated with lymphoproliferative neoplasms, solid tumors, or transplants, autoimmune and infectious diseases and immunodeficiencies(1–3). The primary form is idiopathic and accounts for approximately 50% of cases(4, 5).

Warm or cold antibody are responsible in AIHAs. The most common type of AIHA is related to immunoglobulin G (IgG) autoantibodies that may activate complement. Typically, these IgG antibodies normally can't fix complement, therefore induce extravascular hemolysis. Extravascular hemolysis occurs in antibody-dependent cell mediated cytotoxicity (ADCC) by macrophages and activated lymphocytes in the lymphoid organs and spleen (1).

The AIHA diagnosis can be made with a stepwise approach that used the laboratory and clinical evidence and determined the immune nature of hemolysis with the direct anti-globulin test (DAT) (5). Occasionally Warm AIHA can have IgM or IgA nature. Cold AIHA is typically caused by IgM antibodies that bind and agglutinate RBCs at low temperatures and they do bind complement and hemolysis can be occur intravascular(6).

Identifying hemolytic anemia with a positive DAT is made by clinical assessment of obvious causes such as delayed transfusion reaction subsequent to a recent transfusion, alloimmune hemolytic anemia following allogeneic stem cell or solid organ transplantation, drug-induced immune hemolysis, or hemolytic disease of the fetal or newborn (HDFN). AIHA identify unusually with a negative DAT by standard protocol. A diagnosis of DAT-negative AIHA can be made following careful exclusion of

alternative causes of hemolysis, and confirmation by a sensitive technique and by a response to steroid therapy(7, 8). DAT may be falsely positive in healthy individuals, after therapies such as IVIG, Rh immune globulins, anti-thymocyte globulins, daratumumab, in recently transfusion (alloantibodies), in paraproteinemia or elevated serum globulins, in patients with AIHA in remission So that AIHA cannot be diagnosed from positive DAT alone and it's necessary to search hemolysis evidence, and other congenital or acquired hemolytic disorders should be excluded in complex cases.(9, 10).

Case Presentation

A 49-year-old woman originated from Iran was admitted to the emergency department mid-august 2020 for marked pallor, exhaustion, lethargy, yellowish eyes, anorexia, nausea and vomiting. Past medical history revealed T2DM for over 10 years, hyperlipidemia and drug consumption of metformin 500, atorvastatin 20, pioglitazone 30, ASA 80, and Cetirizine. Laboratory analysis on admission showed: Hb 4.8 g/dl, MCV 91fl, platelet 233 × 106 /µl, WBC 9.8 × 106 /L, total bilirubin 7.0 mg/dl, Glucose 316 mg/dL, Ferritin 1490 ng/ml, G6PD normal, direct coomb's negative, hematuria and urine sugar + 3. The peripheral blood smear showed some spherocyte and micro-spherocyte and no schistocytes; 4% immature granulocytes and 3% nucleated red blood cells (NRBCs) were noted. The serum haptoglobin level was not determined. Due to the suspicion of hemolysis in the patient, initial measures including transfusion and hydration were performed. Serum normal saline and severe diuresis in the range of 1.5-2cc/kg/h with furosemide were used for the patient. In spite of receiving 4 units of packed cells in the first 3 days of hospitalization, on day 4 the patient’s condition worsened with Hb level 2.3g/dl (Table 1) and she was transferred to ICU. Despite the continuous transfusion to reach the hemoglobin level higher than 7g/dl, the patient's Hb was dramatically decreased, and this was strongly in favor of an immune process, so that in spite of the patient's coombs test was reported negative to confirm diagnosis of hemolytic anemia, methylprednisolone (1.5mg/kg), folic acid, chlorpheniramine, rituximab, IVIG and due to the high risk of the patient in terms of deep vein thrombosis (DVT) and pulmonary embolism (PE) prophylaxis with enoxaparin was started. According to hemoglobin equal to 4.7.0g/dl on the 5th day of hospitalization, 3 units of O- negative pack cell with corton pulse (1 g hydrocortisone) and chlorpheniramine were slowly infused. At the same time, the patient lost her consciousness and she was intubated, she suffered cardiac arrest during the intubation process, which was revived after 15 minutes of cardiopulmonary recovery. Gradually, the patient's level of consciousness returned to normal her general condition was improving and he had regained consciousness and treatment with O-negative packed cell, corton pulse, chlorpheniramine, rituximab and IVIG was continued. After one week the patient's hemoglobin reached near to 7 g/dl, but unfortunately on the same day despite a good response she suffered from sudden chest pain. We previously started prophylaxis of enoxaparin for the patient, but the symptoms of PE were obvious in the patient on the 7 day. Because the patient was hospitalized in the ICU and CT angiography was not possible but clinical signs, D-Dimer test and portable radiography confirmed the diagnosis of PE (Fig. 1). Enoxaparin was converted from a prophylactic dose to a therapeutic dose. On the 9 day of hospitalization, the patient was in good general condition and her vital signs were stable and O2

saturation was equal 100% and the patient was cared for one week later in general ward. After 3 weeks of patient referral, she was discharged home with stable vital signs and Hb10 g/dl.

Table 1
Laboratory data on the first 7 days of admission

Indices	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Red blood cells ($\times 10^{12}$ /L)	1.07	1.9	1.59	0.76	1.2	0.76	2.02
Hemoglobin (g/dl)	4.8	6.0	5.3	2.3	4.7	3.0	6.5
Total bilirubin (mg/dl)	7.0	-	-	4.2	18.1	8.3	11.0
LDH (U/L)	-	-	-	1907	1441	1242	1337
Hematocrit (%)	15.6	19.8	15.9	7.7	13.1	8.7	19.8
Platelets ($\times 10^{6}$ /L)	233	166	207	145	183	141	195

Discussion

When a patient presents with anemia, a stepwise approach should be followed. The diagnosis of AIHA can be made with laboratory and clinical evidence of hemolysis and then determine the immune nature of hemolysis with DAT. Decreased RBC count, Hb and Hematocrit normo-macrocytic anemia, increased reticulocyte count, raised indirect bilirubin and LDH, reduced serum haptoglobin, and blood smear features with polychromasia or spherocytes, schistocyte and agglutination may be helpful for the physician to diagnosis of hemolysis as the cause of the anemia(5, 11).

In this patient's medical history showed no hemolytic risk factors and her blood G6PD levels was reported normal. Laboratory findings is shown in this case report during the first 7 days of admission (Table 1). Increasing of serum LDH and decreasing of blood hemoglobin showed hemolytic anemia. Hemoglobin dropped consistently in spite of transfusion. In this patient, clinical evidence and laboratory findings indicate hemolytic anemia but DAT test was negative. Additionally, platelet count was normal and the peripheral blood smear on admission showed anisocytosis, normo-macrocytosis, polychromasia, some spherocytes and micro-spherocytes and no schistocytes that confirm extravascular immune hemolytic anemia in spite of negative DAT test.

Immune mediated hemolysis or G6PD deficiency have been known as two main causes of hemolytic anemia (12, 13). The precise incidence of presentation of patients that have an anemia compatible to warm-AIHA and a negative DAT is not known but has been estimated at 3–11% of all cases(14–16). Different causes for this finding included possible hemolysis by natural killer cells (NK cells) independent of antibody, presence of low affinity IgG that removed by preparatory washes protocols, sensitization below the threshold of detection of the commercial antiglobulin reagent (anti-human reagent potency), IgA or IgM autoantibodies, red cell sensitization by IgA alone, or rarely monomeric-IgM alone, that not

accompanied by complement fixation, and therefore not detectable by a commercial polyclonal antiglobulin reagent. Due to these different possibilities, a negative DAT must be interpreted in conjunction with clinical findings. If clinical suspicion is high and research into non- immune causes is not justified, in addition more sensitive than the standard DAT protocol (microcolumn, solid phase, washings with cold or low-ionic salt solutions) may be negative but the patient response to steroid therapy as first line or rituximab as second-line treatment (9, 13, 17–19).

In this situation additionally to initial measures for treatment and resuscitation of the suspected cases of AIHA patient, pay special attention to immune mediated hemolysis and do not be misled by a negative coombs test. In these situation negative DAT did not confirm the diagnosis and DAT may be positive after several weeks of illness.

Declarations

Ethics approval and consent to participate: The current case report was only a medical and laboratory data collection. Written informed consent for participation of the case report was obtained from the patient.

Consent for publication: Not applicable

Availability of data and material

Not applicable

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Figures

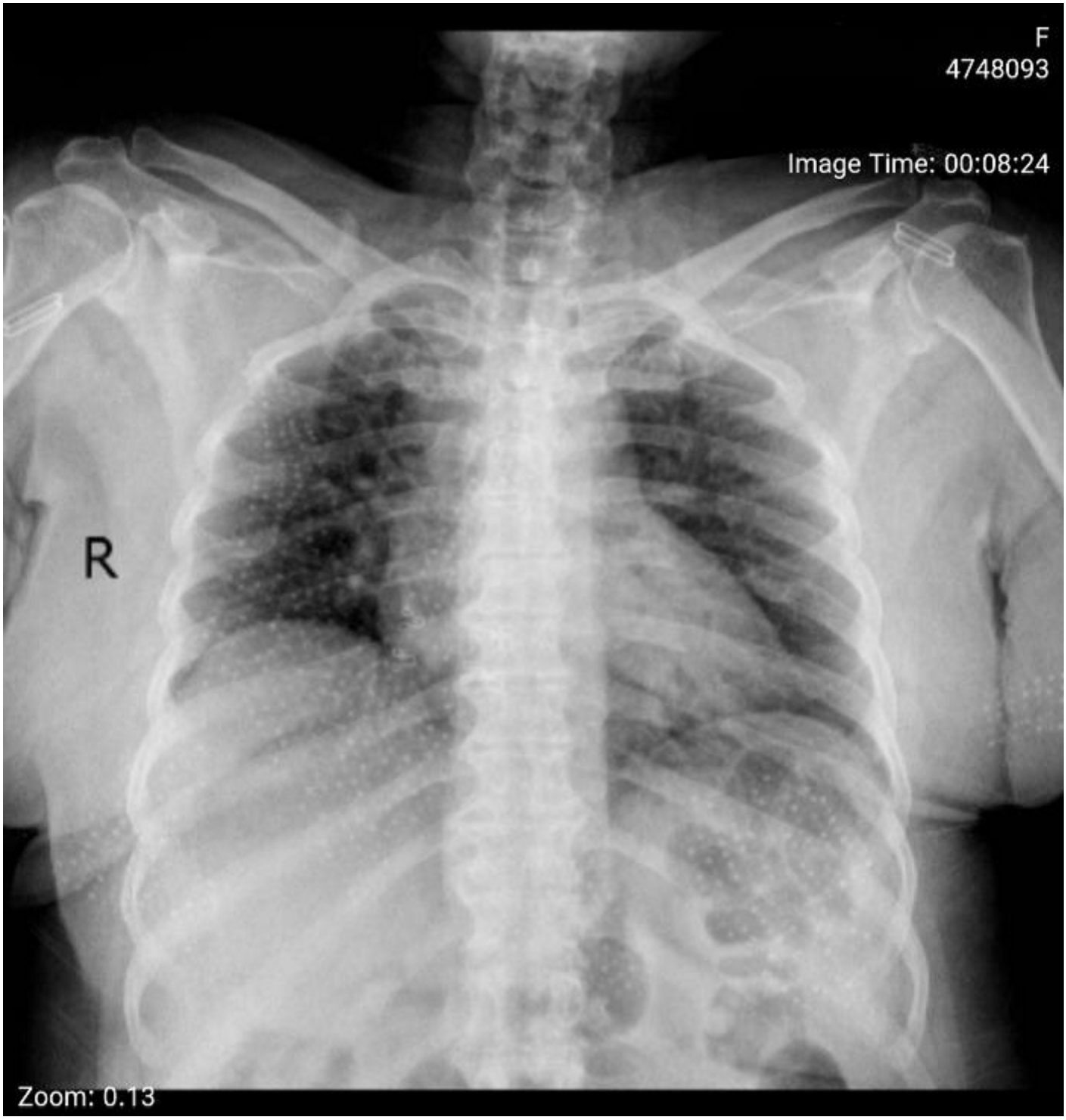

Figure 1

A plain X-ray of the patient in rotation-free expiration is seen in addition to a mild atelectasis in both lungs, a suspected PTE mass in the right lung.