Abstract

Drug induced immune anemia is a rare disease and some of the mechanisms involved in its pathogenesis are controversial. Specialized laboratories often must provide optimal serological testing to confirm the diagnosis.

Most of the drugs that cause acute, severe intravascular hemolysis and sometimes renal failure, disseminated intravascular coagulation (DIC), and even death, seem to work directly on erythrocytes, with consequence of chemical hemolysis or by mechanisms which usually involve drug dependent antibodies that activate serum complement in peripheral blood.

In this work, a case of drug induced hemolytic anemia, in a 58-year old woman, as discovered via laboratory testingis, is presented.

Keywords: antiglobulin test, epitopes, hemolytic anemia, hemolysis, drug induced hemolytic anemia (DIIHA)

Introduction

Anemia is often discovered through laboratory tests, but medical history and physical examination of the patients can provide important clues about the type of anemia with the intra-vascular mechanism of hemolysis and its underlying causes. Hemolysis involves premature destruction of erythrocytes (RBC) and hence results a shortened RBC life span (<120 days). Many diseases, conditions, and factors can cause the body to destroy its own red blood cells.

Hemolysis may be acute, chronic, or episodic. Chronic hemolysis may be complicated by aplastic crisis (temporary failure of erythropoiesis). Intravascular hemolysis is an important reason for premature RBC destruction and usually occurs when the cell membrane has been severely damaged by any of a number of different mechanisms, including autoimmune phenomena, direct trauma, (i.e. defective mechanical heart valves), bacterial toxins and drug administration.

Certain medications can cause a reaction that develops into drug induced hemolytic anemia (DIIHA), which encompasses three mechanisms of action: drug-absorption hapten-induced, (i.e. high-dose penicillin therapy), immune complex (drug-antibodies complex on erythrocytes membrane) or autoantibody (anti-erythrocytes antibodies induction) [1].

Hemoglobin is an iron rich protein that carries oxygen from the lungs to the rest of the body. At the molecular level, also, oxidative stress related changes in the context of the erythrocytes damage in peripheral blood cells. With aging, oxidative stress increases in the erythrocytes whereas elevates concentrations of hydrogen peroxide and organic hydroperoxides decreases.
Glutathione/glutathione disulfide ratio and glutathione transferase activity can be detected in lower values. These oxidative disturbances are also accompanied by reductions in ATP levels and into cells energy charge [2]. Reductions in the ATP levels and adenylate energy charge accompanied these oxidative disturbances and progressive destruction of RBC, which become more sensitive to oxidant agents stress [3,4].

Case report

A 58-year-old woman, with a history of 8 years treatment with the drugs, Perindopril and Chinidin for hypertensive disease, was admitted to the Department of Cardiology at Emergency County Hospital Târgu Jiu, with symptoms of dyspnea, fatigue, occasionally back pain and jaundice. In a resting state, tachycardia with a flow murmur were observed. On ultrasound CT imaging, the patient’s enlarged liver and spleen displayed hepatic steatosis and hypersplenism.

The treating physician from the Department of Cardiology recommended special analyses of the patient’s serum in response to signs and symptoms of anemia.

Laboratory Results, complete blood count (CBC)

Hemoglobin (HGB) = 8.3g/dL (reference range = 12.6-14.0g/dL); Hematocrit (HCT) = 24.8% (37.0%-47.0%); RBC count = 1.85µL×10³ (3.8µL-5.8µL×10³); Platelet (PLT) count = 166µL×10³ (150µL-450µL×10³); White blood cell (WBC) count = 4.3µL×10³ (4µL-10µL×10³); Erythrocyte sedimentation rate (ESR) = 55mm/h; suspect flags on Coulter HmX Analyzer (Beckman Coulter Inc): No signal.

Peripheral blood-smear findings

As registered on blood smear via optic microscopy, the differential count showed: granulocytes unsegmented = 0; granulocytes segmented = 55.2%; eosinophiles = 4.5%; basophiles = 0.5%; lymphocytes = 36.6%; monocytes = 3.2%; Erythrocyte sedimentation rate (ESR) = 55mm/h; suspect flags on Coulter HmX Analyzer (Beckman Coulter Inc): No signal.

The results of other laboratory biochemical tests revealed the following results: Lactate dehydrogenase (LDH) = 710U/L, (reference range = 313U/L-618U/L); Glucose = 151mg/dL. (75mg/dL-110mg/dL); Urea = 50.5mg/dL, (17mg/dL-43mg/dL); Creatinine = 1.26mg/dL, (0.6mg/dL-1.1mg/dL); Triglycerides = 575mg/dL, (20mg/dL-150mg/dL); Iron = 139µg/dL (49µg/dL-181µg/dL); Serum ferritin = 15ng/mL, (8ng/mL-10ng/mL); Total bilirubin = 2.4mg/dL, (0.20mg/dL-1.30mg/dL); Conjugate bilirubin = 0.6mg/dL, (0.0mg/dL-0.30mg/dL); Indirect bilirubin = 1.8mg/dL, (0.0mg/dL-1.10mg/dL); Delta bilirubin = 0.6mg/dL, (0.0mg/dL-0.5mg/dL); Alanine aminotransferase (ALT) = 33.6U/L (5U/L-31U/L), Aspartate aminotransferase (AST) = 61.0 U/L (5U/L-32U/L); Activated partial thromboplastin time (APTT) = 36 seconds, (normal results = 26-35 sec); Prothrombin time (PT), INR = 1.02, (normal results = 0.90-1.2).

Patient Diagnosis

Based on the clinical symptoms (pallor, sclera jaundice, fatigue, dizziness, history of drugs administration) and her laboratory test results (CBC with differential count and peripheral smear, reticulocyte count, serum bilirubin, LDH, and AST), the diagnosis of Chronic Hemolytic drugs induced Anemia, probably induced by antihypertensive drugs.

Confirmatory diagnosis will follow by measurement of urinary serum haptoglobin and measurement of RBC survival using a radioactive label, such as radiochromium (51Cr), after the specific drug administration (Perindopril and Chinidin) in the specialized array laboratories of toxicology.

Differential diagnosis

The differential diagnosis for the patient include: Glucose-6-phosphate dehydrogenase deficiency (G6PD) which can lead to hemolysis, poikilocytosis, and Heinz bodies in erythrocytes (hemolytic anemia) in the presence of oxidative stress. G6PD deficiency is one of the most prevalent disease-causing mutations worldwide. However, most of the G6PD isoenzymes with decreased activity are associated with only moderate health risks without a significant effect on longevity. G6PD is a housekeeping enzyme essential for basic cellular functions, including protecting red cell proteins from oxidative damage. Oxidant damage of hemoglobin leads to the precipitation of hemoglobin, which may be morphologically recognized as Heinz bodies. The enzymatic activity of G6PD generates NADPH that is utilized for glutathione reduction. Reduced glutathione restores hemoglobin to the soluble form. Thus, maintaining a high ratio of reduced-to-oxidized glutathione represents the major defense against oxidative damage of hemoglobin. Reticulocytes have five times higher G6PD enzyme activity than the oldest erythrocyte subpopulation [5].
Hereditary Spherocytosis, which is characterized by spherocytes, usually is diagnosed via a family history of the condition and a negative direct antiglobulin test result [6].

Sickle-cell anemia [7] and Thalassemia [8], which are hemoglobinopathic manifestations, are characterized by chronic hemolysis. When iron deficiency is severe, anemia is hypochromic and microcytic; however, in milder degrees of iron deficiency, anemia is normocytic.

In Megaloblastic Anemia, the type of anemia is macrocytic and associated with leucopenia and thrombocytopenia in peripheral blood. Macrocytes and ovalocytes can be also present on peripheral blood film. Deficiency of vitamin B12 or folic acid also leads to the production of giant metamyelocytes and multisegmented macropolicitcs [9].

Abnormalities in the granulocytic series do not disappear as promptly as megaloblasts do after specific therapy. The presence of such abnormalities may be helpful in the diagnosis. Via our microscopic slide examination of bone marrow from our patient, we observed a hyperplasic series of erythrocytes of approximately 45%, deficiency of erythropoiesis, polychromatophil and acidophil erythroblasts with giant band forms, on smear, in peripheral blood.

Other laboratory tests that can help discern the causes of hemolysis include the following: Quantitative Hb electrophoresis, RBC enzyme assays, Flow cytometry, Cold agglutinins and Osmotic fragility.

Discussion

Along with anemia, a characteristic laboratory feature of hemolysis is reticulocytosis, the normal response of the bone marrow to the peripheral loss of RBCs. In the absence of concomitant bone-marrow disease, brisk reticulocytosis should be observed within 3 to 5 days after a decline in hemoglobin. In a minority of patients, the bone marrow is able to chronically compensate, leading to a normal and stable hemoglobin concentration. Anemia resulting from hemolysis usually is normocytic, although a marked reticulocytosis can lead to an elevated measurement of MCV because the average MCV of a reticulocyte is 150 Fl (N = 80-96Fl) [10].

Also hemolysis is accentuated by oxidative stress inside erythrocytes by drug-induced immune hemolytic anemia [11]. Perindopril and Chinidin have the potential adverse effect of hemolytic anemia. These drugs can cause serious adverse effects, including headache, dizziness, fatigue, tickling cough, weakness, numbness or tingling in the arms, legs, or feet, nausea, taste disturbances, itching, rash or increased sweating, and hemolytic anemia after a number of years of drugs ingestion.

RBC is the oxygen carrier, so its metabolic status is important for the regulation of oxygen affinity of hemoglobin (Hb). The ability of Hb to release O2 is determined by a variety of metabolites in RBCs, especially 2,3-diphosphoglycerate (2,3-DPG), a by-product of glycolysis unique to RBCs. 2,3-DPG is synthesized in the Rapoport-Luebering glycolytic shunt, which bypasses the phosphoglycerate kinase (PGK) step [12].

RBCs are the only oxygen carrier and generate energy to perform their own function almost exclusively through the anaerobic glycolysis. A decrease in RBC concentration of 2,3-DPG is known to be the result and a predictor of disturbance of the RBC glycolytic pathway, defects in the distal glycolytic enzymes, and lack of sufficient energy. Secondly, it results in the increase in Hb affinity to O2 a decrease in the ability of Hb to unload O2, so that O2 is transferred to the brain less efficiently [13].

Chronically, e.g. during human aging from 40 to 70-80 years, the above events may occur very slowly but permanently in all cells including those having mitochondria (i.e. all cells other than RBC). The glycolysis pathway and shunt hexozo-mono-phosphate into erythrocytes are not only primarily ATP generators but also are primary consumers of oxygen [14].

The more common type of extra-vascular hemolysis is the removal and destruction of RBCs with membrane alterations by the macrophages of the spleen and liver. Circulating blood is filtered continuously through thin-walled splenic cords into the splenic sinusoids with hypersplenism because of hemolytic anemia that involves RBC destruction [15].

Recently, the involvement of the heme oxygenase (HO) pathway in anti-degenerative mechanisms, has received considerable attention, as it has been demonstrated that the expression of HO is closely related to that of amyloid precursor protein. HO induction, which occurs during various physio-pathological conditions, by generating the vasoactive molecule carbon monoxide and the potent antioxidant bilirubin, represents a protective system potentially active against brain oxidative injury [16].

The algorithm for evaluation of hemolytic anemia

For establishment of hemolytic anemia diagnosis, the following laboratory tests are described in the medical specialty literature: CBC = complete blood count; LDH = lactate dehydrogenase; DAT = direct antiglobulin test;
**G6PD** = glucose-6-phosphate dehydrogenase; **PT/PTT** = prothrombin time/partial thromboplastin time; **TTP** = thrombotic thrombocytopenic purpura; **HUS** = hemolytic uremic syndrome; **DIC** = disseminated intravascular coagulation (Figure 1).

Increased breakdown of HGB has as results increased bilirubin level (mainly indirect-reacting) with clinical jaundice, presence of urinary urobilinogen, hemoglobinemia, hemoglobinuria, and hemosiderinuria. The level of LDH in the blood can be elevated; haptoglobin levels are decreased. Peripheral blood-smear microscopy emphasizes the presence of RBC fragments (schistocytes) and that some RBCs can be appeared smaller and rounder than usual (spherocytes). The result of direct Coombs testing is positive, with hemolysis being caused by an immune-system process if the anemia has an autoimmune mechanism.

The balance between RBC destruction and bone-marrow compensation can be determined with the severity of anemia. Hemosiderin in urine indicates chronic intravascular hemolysis; also, the level of urobilinogen in the urine is increased as a biological effect of increased indirect bilirubin in peripheral blood.

Among treatment options in infectious diseases, the most acceptable one is to prescribe drugs such as penicillin that covalently bind to proteins (e.g. RBC membrane proteins). In this type of treatment, the RBCs become coated with the prescribed drug(s) in vivo, and a drug antibody (usually immunoglobulin, (IgG)) attaches to the drug-coated RBCs that are subsequently cleared by macrophages [17].

The most controversial mechanism is the immune complex mechanism, which has been revised to suggest that most drugs are capable of binding to RBC membrane proteins but not covalently, as penicillin. RBC and test agglutination with anti-IgG serum reflects warm autoimmune hemolytic anemia (AIHA), whereas a positive anti-C3 DAT result occurs in cold AIHA. DAT results demonstrate the presence of auto-antibodies (shown in our patient’s serum) or complement on the surface of RBCs.

There are 2 types of drug-related antibodies. Drug-independent antibodies can be detected in vitro without adding any drug in vitro and in vivo characteristics are identical to those of RBC auto-antibodies. The type of drug, as drug-dependent antibodies, is methyldopa, which causes the production of RBC antibodies in approximately 15% of the patients receiving the drug; however, only 0.5% to 1% of patients develop hemolytic anemia disease (HA) [18].

The United States Food and Drug Administration (FDA) reported on 85 cases of Cefotetan-induced HA since the approval of cefotetan; 18% of those cases were fatal [14]. The mean decrease in HGB level was 6.7g per dL, with mean final Hb of 5.2g per dL. Also, hydrocortisone antibodies have been detected in individuals without HA [19]. A recent novel finding should be of interest to hematologists, namely, the first case of DIIHA occurring due to hydrocortisone treatment [20,21]. This finding adds another possible explanation for poor responses to corticosteroid therapy in some cases of AIHA in which hcorticosteroid-induced DIIHA may be masked by the autoimmune process (Table I).

### Conclusions

Perindopril and Chinidin, drugs which the patient had previously taken, had side effects of hypersensitivity reactions. Increased breakdown of HGB had as results increased bilirubin level (mainly indirectly-reacting) with clinical jaundice. The combination of RBC membranes and an appropriate drug can create an immunogen, leading to an intravascular lysis of erythrocytes, in long antihypertensive treatment, in this case.
References

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Table I. Drugs that cause Immune-mediated hemolysis

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<th>Immune Complex</th>
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<td>Positive anti-IgG</td>
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