Antibody Identification in a Patient with Chronic Lymphocytic Leukemia: A Case Study

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ABBREVIATIONS: CLL = chronic lymphocytic leukemia; DAT = direct antiglobulin test; RBC = red blood cells; WAIHA = warm autoimmune hemolytic anemia.

INDEX TERMS: antibody identification; chronic lymphocytic leukemia; CLL; hematology; immunohematology; lymphoproliferative disorder; WAIHA; warm autoimmune hemolytic anemia.

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CASE PRESENTATION

A 77-year old man from Tennessee was on vacation in southwest Florida. He presented to the emergency room (ER) complaining of a sudden onset of weakness, fatigue, chest pain, and shortness of breath. Pertinent clinical history included diagnosis and chemotherapeutic treatment of chronic lymphocytic leukemia (CLL) ten years ago resulting in remission. This past year, the patient had a recurring episode of CLL and subsequent to treatment went back into remission. The patient had a routine physical with laboratory testing including a complete blood count (CBC) and a chemistry panel one month previous to this ER episode with all results reported within the reference range. The ER attending physician ordered hematology, coagulation, and chemistry laboratory tests. The results were reported to the physician with attention to the 'critical values' (Table 1).

Subsequent to the receipt of the initial laboratory results the ER attending physician ordered four units of packed red blood cells (RBCs) and admitted the patient to the hospital's intensive care unit. The results of the type and crossmatch are seen in Table 2.

The patient had no previous transfusion history. The immediacy of need was recognized with the resulting low RBC count and hemoglobin. Hospital transfusion services processed several units with no compatible units identified. A request from the hospital transfusion services to the regional blood bank for assistance was initiated. Leukocyte-reduced RBCs were obtained from the regional blood bank supply and administered to the patient.

DISCUSSION

CLL is categorized in the general lymphoproliferative disorders which include acute lymphoblastic leukemia, prolymphocytic leukemia, non-Hodgkin's lymphoma, and hairy cell leukemia, among others.[1,2] Chronic lymphocytic leukemia is most often the malignant overproduction of B-lymphocytes and less commonly T-lymphocytes. Chronic lymphocytic leukemia is recognized as the most common type of leukemia in older adults in the western hemisphere.[1,3]

Chronic lymphocytic leukemia is primarily a disease of the male adult with 90% of patients being older than 50 years of age and men being affected more than twice as frequently as women.[2] Inherited or acquired immunologic defects may predispose individuals to susceptibility to leukemogenic agents.[2] Genetic factors may play an important role in the etiology of CLL as there are reports of multiple incidences of CLL in families.[4] A genetic basis is highly likely as seen in the differences in the incidence of CLL in different countries with 40 % of all CLL occurring in the Western countries.[3]

CLINICAL PRESENTATION AND PATHOPHYSIOLOGY

Symptoms of CLL may develop slowly and often diagnosis is made unexpectedly during routine laboratory testing. Most often patients complain of fatigue, which is frequently attributed to age, and thus, diagnosis may be postponed. Signs and symptoms develop gradually and the duration of a relatively asymptomatic phase of CLL is variable. Fatigue is followed by neutropenia, bruising, pallor, weakness, jaundice associated with anemia, fever, recurrent or persistent infection, bone and muscle tenderness, and weight loss. [1,2]

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Table 1. Laboratory results of patient during emergency room admission

TEST	RESULT	REFERENCE RANGE
Hematology and Coagulation		
White blood cell count	52.9 x 10^9/L *	4.1 - 9.0 x 10^9/L
Differential		
Granulocytes	11%	31 - 76%
Bands	0	0 - 6%
Lymphocytes	80%	24 - 44%
Monocytes	7%	2 - 14%
Eosinophils	1%	0 - 6%
Basophils	1%	0 - 2%
Red blood cell count	1.13 x 10^{12}/L*	4.10 - 5.80 x 10^{12}/L
Hemoglobin	5.1 g/dL*	12.8 - 17.2 g/dL
Hematocrit	14.1%*	38.3 - 50.0%
Mean corpuscular volume	115.2 fL^3	80.0 - 100.0 fL^3
Mean corpuscular hemoglobin	45.0 pg	26.0 - 34.1 pg
Mean corpuscular hemoglobin concentration	35.9 g/dL	32.8 - 36.0 g/dL
Red cell distribution width	20.4%	11.4 - 14.7%
Platelet count	173 x 10^9/L	140 - 350 x 10^9/L
Mean platelet volume	6.5 fL	6.6 - 9.7 fL
Prothrombin time	13.2 sec	11.7 - 13.4 sec
International normalized ratio	1.09	0.85 - 1.24
Activated partial thromboplastin time	25.7 sec	23.0 - 35.0 sec
Chemistry		
Total bilirubin	3.2 mg/dL	0.20-1.30 mg/dL
Direct bilirubin	0.4 mg/dL	0.0 - 0.5 mg/dL
Aspartate aminotransferase	53 U/L	9 - 40 U/L
Alanine aminotransferase	25 U/L	5 - 33 U/L
Alkaline Phosphatase	67 U/L	20-120 U/L
Calcium	8.9 mg/dL	8.4-10.2 mg/dL
Total protein	5.5 g/dL	6.0 - 8.0 g/dL
Albumin	3.9 g/dL	3.2 - 5.0 g/dL
Lactate dehydrogenase	381 U/L	85 - 200 U/L

* Critical value

The pathophysiology of CLL is attributed to the immunologically dysfunctional lymphocytes in the peripheral blood and bone marrow. The proliferation and accumulation of lymphocytes are unresponsive to antigenic stimuli resulting in a dormant stage with accumulation in the peripheral blood, bone marrow, lymph nodes, and spleen. According to the present data, CLL is a disorder in which a very small number of cells cycle at a normal rate. The CLL lymphocytes also have an increased relative cell production rate and can be termed proliferative as well.[5] As the bone marrow becomes extensively infiltrated by the leukemic lymphocytes, the production of other marrow components is stifled resulting in anemia, thrombocytopenia, and neutropenia. Organ infiltration can lead to massive adenopathy with splenomegaly, hypersplenism, and cytopenias. An increased tendency for hemorrhage further contributes to anemia and compromises hemostasis.[1]

The disease may manifest in dermatologic findings such as nodular and diffuse skin infiltrations, erythroderma, dermatitis, and secondary skin infections. Generalized herpes zoster with a demonstrated rash can also be present. Patients have significantly im-

Table 2. Laboratory results of patient type and crossmatch

TEST	RESULTS
ABO group typing	O
Rh typing	Positive
Indirect Coombs	Positive
Direct antiglobulin test	Positive
Antibody panel	Positive
Antibody identification	Warm autoantibody Positive anti-e

paired immunologic activity with approximately 50% of patients reporting hypogammaglobulinemia.[1] This deficiency in immunoglobulin leads to persistent or recurrent infections which are accountable for much of patient morbidity and mortality.

The clinical course of CLL shows a marked heterogeneity with an overall median survival ranging from 2 to 20 years.[6] Five years after diagnosis 50% of the patients are still living and 30% of patients have a ten year survival.[1] Patients with a normal karyotype have a median overall survival of more than 15 years in contrast to 7.7 years for patients with clonal changes.[7] The disease of CLL can be an inactive disease with an asymptomatic presentation which may not require any treatment until progressive lymphocytosis of the peripheral blood and marrow occur. This may be as late as 10 to 15 years from initial diagnosis. In contrast to those with a slow course of disease progression, approximately 20% of patients with CLL have a very aggressive clinical course resulting in death in one to two years.[1] The wide variation of disease progression seen among patients is not fully understood. Clinical and physical data have been used to try to predict the CLL patient's prognosis and identify various stages and risk groups.

When the signs and symptoms of progressive disease appear, therapeutic intervention is necessary. The goal of treatment is to reduce signs and symptoms of disease and the prevention of complications with minimal discomfort or risk to the patient. Conventional treatment for CLL is chemotherapy using combinations of chemotherapeutic agents. In addition to chemotherapeutic intervention, radiation and leukapheresis are employed. The use of high-dose intravenous gamma globulin therapy is used to prevent major bacterial infections. Experimental therapies are also being studied which include new drugs and the use of various monoclonal antibodies and biological mediators. Bone marrow transplantation is being explored as a possible curative therapy for patients with aggressive CLL.[3] Blood transfusions are commonly used to alleviate the anemia. CLL is not considered curable with current available therapy.

LABORATORY FINDINGS

Chronic lymphocytic leukemia is commonly diagnosed by the finding of a lymphocytosis in the peripheral blood. Absolute lymphocyte counts may be between 10 and 150 x 10^9/L, but may be as high as 1000 x 10^9/L.[2] The lymphocytes vary from small to slightly larger than the normal lymphocyte. These lymphocytes appear with a relatively mature morphology with a clumped or condensed nuclear chromatin often creating a 'soccer-ball' appearing pattern.[1,2] The lymphocytes are more fragile than normal and peripheral smears often contain 'smudge cells' as a distinguishing characteristic of CLL. Although CLL lymphocytes differ morphologically in patients, they are usually similar in any given patient attesting to the clonal origin of the disease.[2]

The lymphocytosis causes neutropenia, anemia, and thrombocytopenia as the lymphoid tissue fills 50% or more of the bone marrow space preventing other cell lines from reproducing.[1,2] As the disease progresses, infiltration of the marrow occurs. Thirty percent or more lymphocytes in the marrow, when accompanied by a sustained lymphocytosis in the peripheral blood, is considered diagnostic of CLL. Lymphocytes increase in number until normal marrow cells are crowded out.

Anemia occurs because of decreased red cell production, of splenic sequestration of red cells, shortened red cell survival, or autoimmune hemolysis. Patients with CLL have altered humoral immunity that suppresses all immunoglobulin classes. They may also develop autoimmune disease with the production of autoantibodies. These autoantibodies may lead to autoimmune hemolytic anemia.[1,8] Anemia may occur as normochromic and normocytic with a normal or low reticulocyte count.[1] However, macrocytosis may be present when acute blood loss is indicated. Autoimmune responses leading to autoimmune hemolytic anemia may precede, accompany, or follow CLL and be characterized by a bone marrow erythroid hyperplasia, secondary reticulocytosis, positive direct antiglobulin test, and an elevated indirect serum bilirubin level.[2] A decreased platelet count is not uncommon.

Chromosomal abnormalities are detected in more than 50% of patients with CLL. The most common abnormalities are seen in trisomy 12 and structural abnormalities of chromosomes 13 and 14.[7]

CHALLENGES IN TRANSFUSION SERVICES AND THE BLOOD BANK

Many patients develop a warm autoimmune hemolytic anemia (WAIHA) secondary to the CLL. WAIHA is characterized by an abnormality within the immune system whereby the ability for self-recognition of an individual's own red cell antigens is lost. WAIHA autoantibodies' serologic reactivity is optimal at 37 °C.[9] The onset of WAIHA is usually insidious and can be precipitated by many factors, such as trauma, infection, stress, or surgery.[8] Symptoms of WAIHA do not usually develop until the patient has sig-

nificant anemia. Symptoms include pallor, weakness, dizziness, jaundice, and unexplained fever.[8]

Patients with WAIHA usually demonstrate evidence of free autoantibody at low titer of IgG alone or with IgA, IgM, or C3. In 80 percent of cases of WAIHA, the antibody causing the hemolysis is an IgG immunoglobulin.[8] Complement proteins act synergistically with immunoglobulins to cause red cell hemolysis. Testing with a polyspecific antiglobulin reagent reveals a positive direct antiglobulin test (DAT).[9] As autoantibody is produced, it adsorbs onto the associated antigens present on the patient's own RBCs. A peripheral blood smear can demonstrate the signs of extravascular hemolysis, including red cell fragments and polychromasia.[7] Other indications of extravascular hemolysis are demonstrated in the laboratory data such as elevated bilirubin and lactate dehydrogenase (LD).[9]

Patients presenting with WAIHA present a perplexing problem for the blood bank because anemia of sufficient severity to require transfusion is not uncommon. Transfusion is avoided when possible because it has the potential of increasing the hemolysis.[8,9] Transfusion is reserved for situations that are life-threatening.

The serum of a patient with warm autoagglutinins may contain just autoantibody or a mixture of autoantibody and alloantibody if the patient has had a previous transfusion. If the patient is determined by history not to have alloantibody, testing may proceed to determine the specificity of the autoantibody. The specificity of a warm autoantibody is directed to the Rh system, especially to the e-antigen. The patient serum appears to be anti-e, although the patient's RBCs are e-positive and have a positive DAT. To identify the specificity of a warm-reactive autoantibody, an eluate prepared from the patient's RBCs is tested against a panel of reagent red cells. The eluate is usually reactive with all panel cells tested when working with a warm autoantibody.[10] Warm auto-antibodies may have an apparent autoanti-e specificity in the serum but may show panagglutination of RBCs when tested with the eluate because the concentration of antibody removed from the cells in the elution process may be greater than that in the serum.[8]

Specificity may also be helpful in selecting blood for transfusion. Some workers prefer to transfuse RBCs that are compatible with the autoantibody when, for example, the specificity is anti-e-like.[8] All donor blood is usually incompatible to the patient suffering from WAIHA. Therefore, any transfused blood that is given is called the 'least incompatible' in the crossmatch. Leukocyte-reduced RBCs is the blood product of choice because of the low number of white cells present in this blood component. This leukofiltered blood is transfused slowly in small volumes (100mL) and the patient is observed closely for any adverse reactions.[8-10]

CASE RESOLUTION

The patient presented with physical and laboratory findings consistent with an active stage of CLL. The production of the autoantibodies in the patient led to the development of a warm autoimmune hemolytic anemia (WAIHA) secondary to his CLL. This was characterized by a positive DAT and the macrocytic anemia. Furthermore, elevations of the total bilirubin and LD was an indication that the patient was experiencing extravascular hemolysis. Because of the critical findings of low RBCs, hemoglobin, and hematocrit, transfusion therapy was warranted. Four units of leukocyte-reduced RBCs were transfused in small volumes with no incident. The patient stabilized and was soon out of a life-threatening situation. The staff pathologist performed a bone marrow biopsy and aspiration with the following results: diffuse and patchy lymphocytic infiltrates consistent with CLL; markedly diminished iron stores; macrocytosis; and diminished megakaryocytes.

When the bone marrow preparation was compared with his current peripheral smear, it was determined that the patient was not in an active leukemic state. After five days of intravenous therapy and transfusion of leukocyte-reduced RBCs, the patient was discharged from the hospital and returned home.

The major physical and clinical signs and symptoms demonstrated by this patient when he presented at the ER identify advancing disease and are indications for future treatment. These indications include the abnormal bone marrow findings and the autoimmune hemolytic anemia. Because there is no cure for the disease, the goal of treatment is to reduce signs and symptoms. At this juncture, the patient's physician will need to determine if the conventional treatment of chemotherapy and/or radiation therapy is warranted.[3,11,12]

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