

# ***CLINICAL LAB INVESTIGATIONS: CASE STUDIES FOR THE LABORATORY PROFESSIONAL***

## ***CASE SET #1***

**Immunology/Immunoematology**

**Case Studies:**

**Hashimoto Thyroiditis**

**Antibody Identification in a Patient with CLL  
ABO Discrepancy in a Multiple Myeloma Patient**



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## HASHIMOTO THYROIDITIS\*

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\*Case adapted from Wilson JA, Ogedegbe H. *Hashimoto Thyroiditis. ASCLS Today.* August 2004. pp. 4-5.

### **CASE PRESENTATION**

A fifty-one year old woman presented to her internist with complaints of swelling in the front of her neck. Upon history and physical examination, her physician noted that she had a weight gain in the past year of 23 pounds, had shortness of breath, and was easily fatigued. She indicated she had attributed these symptoms to getting older. In her family history, it was noted that her mother, aged 78, was living and had been taking thyroid medication for many years for reasons unknown to the patient. The physician ordered a complete blood count, a basic metabolic panel, thyroid stimulating hormone (TSH) assay, and a free thyroxine (FT<sub>4</sub>) assay. All results were in reference range except for an elevation in total cholesterol, TSH, and FT<sub>4</sub>. Results are listed below.

### **Laboratory Results**

<b>Laboratory Parameters</b>	<b>Results</b>	<b>Reference Range</b>
Total Cholesterol mg/dL (mmol/L)	267 (6.9)	<200(<5.17)
FT <sub>4</sub> ng/dL (pmol/L)	0.4 (5.15)	0.9-2.3 (12-30)
TSH $\mu$ IU/mL ( $\mu$ IU/mL)	9.3 (9.3)	0.5-5.0 (0.5-5.0)

### **DISCUSSION**

Hashimoto thyroiditis was first described by Dr. Hakaru Hashimoto in 1912. He described patients with chronic endocrine gland disorder of the thyroid as having struma lymphomatosa because of the diffuse infiltration of the gland with lymphocytes.<sup>1,2</sup> The disease has been referred to as Hashimoto thyroiditis or Hashimoto disease since this original description. Other descriptions include chronic thyroiditis, lymphocytic thyroiditis, lymphadenoid goiter, and goitrous autoimmune thyroiditis.

Hashimoto thyroiditis is the most common cause of hypothyroidism and has been reported to be from 5 times to up to 20 times more common in women than in men.<sup>3,4,5</sup> It occurs most frequently at 40 to 60 years of age, but has been diagnosed in other age groups and even in young children.<sup>3,4,5</sup> It is considered an autoimmune disease and has demonstrated clustering in families as an apparently inherited dominant trait.<sup>2</sup> The incidence of Hashimoto thyroiditis in the general population is 1 out of 10,000 people.<sup>6</sup>

Hashimoto thyroiditis begins as a gradual enlargement of the thyroid gland followed by a usually slow development of hypothyroidism.<sup>1</sup> Patients typically present with an enlarged thyroid resulting in diffuse goiter in the anterior region of the neck. The goiter is usually asymptomatic, but occasionally patients have difficulty in swallowing and a feeling of local pressure.

Hypothyroidism is a common late sequela of Hashimoto thyroiditis and may result in patient fatigue, slowing of mental and physical performance, change in personality; intolerance to cold; exertional dyspnea; hoarseness; constipation; decreased sweating, easy bruising, muscle cramps, hair loss, and unintentional weight gain.<sup>5,7</sup> The hypothyroidism resulting in deficiency of thyroid hormones causes many metabolic processes to slow and has been associated with increased total cholesterol and an increase in risk of coronary heart disease.<sup>7,8</sup>

The thyroid gland follicular cells enclose an amorphous colloid material composed of thyroglobulin. Thyroglobulin consists of the two hormones, triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>).<sup>8,9</sup> Regulation of the thyroid begins with thyrotropin-releasing hormone (TRH) secreted by the hypothalamus and acts on the pituitary gland to stimulate synthesis and release of TSH. It is TSH that binds to receptors in the thyroid gland to initiate the breakdown of thyroglobulin into free T<sub>3</sub> and T<sub>4</sub>. TSH synthesis and release are regulated by a negative feedback mechanism by the level of free T<sub>3</sub> and T<sub>4</sub>.

The exact mechanism for Hashimoto thyroiditis is unknown, but it has been characterized by gradual thyroid failure because of an autoimmune destruction of the thyroid gland.<sup>3,5</sup> The disease is characterized by cellular and humoral responses, as demonstrated by serum antibodies to thyroid antigens and the lymphocytic infiltration of the gland. The thyroid autoantibodies present in patients include antithyroid peroxidase antibody (TPOAb), anti-thyroglobulin antibody (TgAb), and anti-TSH receptor autoantibodies (TSHRAb). A high titer of at least 1 of the 3 antibodies has been found in 97% of patients with Hashimoto thyroiditis.<sup>4</sup>

In Hashimoto thyroiditis, the immunologic attack is aggressive and destructive. TgAb are positive in about 60-80% of patients, and TPOAb in 95% of patients diagnosed with the disease. The positive titers for these antibodies are considered markers for the disease.<sup>1,2,8</sup> TPOAb appears to be incapable of initiating the autoimmune process, but rather may be the consequence of the initial immune damage. As a result, antibodies to TPO may in fact be better indicators of active thyroid inflammation than TgAb.<sup>10</sup>

The circulating antibodies against components of the thyroid follicles act as thyroid blockers. Production of autoantibodies interferes with the process of TSH binding to receptors and the release of T<sub>3</sub> and T<sub>4</sub> from thyroglobulin.<sup>10</sup> TgAb are targeted against thyroglobulin. TSHRAb in Hashimoto disease bind to receptors and block the binding and function of TSH and are referred to as thyroxine-binding inhibitory immunoglobulins. TPOAb bind with the microsomal antigens associated with the follicular cells lining the microsomal membrane. They are mainly IgG and fix complement and may be cytotoxic.<sup>8</sup> This immune response progressively destroys the thyroid gland. Progressive thyroid cell damage can change the apparent clinical picture from goitrous hypothyroidism to that of primary hypothyroidism and is considered to be the end stage of Hashimoto thyroiditis.<sup>1</sup>

### ***Diagnostic Testing***

Increased levels of TSH are the first detectable abnormality seen in laboratory testing for correctly diagnosing hypothyroidism. This may occur even before the patient is symptomatic. As hypothyroidism progresses, the FT<sub>4</sub> will decrease. The current recommendation for testing has made obsolete the need for thyroid profiles or panels and the recommendation is for the measurement of TSH complemented by FT<sub>4</sub>.

Evaluation of endocrine function has been difficult as the endocrine hormones circulate in extremely small amounts in the blood (nanograms per deciliter). Detection of the hormones requires sensitive analytical methods. The high sensitivity TSH (sTSH) assay is considered to be the first and best laboratory test for identification of thyroid abnormalities.<sup>8</sup> The sTSH assay is extremely useful in the detection and confirmation of suspected primary hypothyroidism. sTSH has replaced the traditional monoclonal RIA technique for the hormone. Whereas the traditional RIA techniques measured an antigen-antibody complex, the 3<sup>rd</sup> generation sTSH immunochemiluminometric assays (ICMA) use two or three separate monoclonal antibodies in the methodologies and provide a sensitivity of 0.01-0.02mU/L.<sup>1</sup> The requirement of binding to more than one site gives the sTSH its high specificity and sensitivity. The first antibody is in excess, bound to a solid phase support and extracts the TSH molecule from the serum and is usually specific for the beta subunit of the TSH molecule. The second antibody usually attaches to the alpha subunit of the TSH molecule.<sup>8</sup>

Total T<sub>4</sub> (TT<sub>4</sub>) assays detect both the T<sub>4</sub> bound to protein and the free, biologically active T<sub>4</sub>. Although FT<sub>4</sub> is present as a very small fraction of TT<sub>4</sub> (approximately 0.02% of TT<sub>4</sub>)<sup>8</sup>, FT<sub>4</sub> is the test of choice as an indicator of thyroid status. FT<sub>4</sub> determinations are most often performed using direct nonisotopic immunoassay such as microparticle enzyme immunoassay (MEIA) and chemiluminescence.

The diagnosis of autoimmune thyroiditis may be confirmed by the detection of thyroid autoantibodies. TgAb and TPOAb are the most commonly detected thyroid autoantibodies.<sup>10</sup> TPOAb assays are sensitive and specific and lend themselves to high-volume testing with RIA, ELISA, and most recently, chemiluminescence. TgAb can be assayed by hemagglutination, RIA, ELISA, and ICMA.<sup>1</sup>

### **CASE CONCLUSION**

Upon receipt of the laboratory reports of the elevated TSH and decreased FT<sub>4</sub>, the physician requested TPOAb and TgAb testing. The results of the antibody testing indicated a markedly increased presence. With these laboratory findings and given the clinical symptoms, the physician diagnosed Hashimoto thyroiditis. The patient was placed on L-thyroxine (levothyroxine) as a thyroid hormone replacement therapy to prevent further thyroid destruction.<sup>1,8</sup>

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## ANTIBODY IDENTIFICATION IN A PATIENT WITH CHRONIC LYMPHOCYtic LEUKEMIA \*

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\*Case adapted from Wilson JA, Chabot S. *Antibody identification in a patient with chronic lymphocytic leukemia: a case study. Clinical Laboratory Science*, Summer 2002. 15(3)136-139.

### 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) 10 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).

**Table 1.** Laboratory results of patient during emergency room admission

TEST	RESULT	REFERENCE RANGE
<b><u>Hematology and Coagulation</u></b>		
White blood cell count	52.9 x 10 <sup>3</sup> /μl *	4.1 - 9.0 x 10 <sup>3</sup> /μl
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 %

(Table 1. cont.)

<b>TEST</b>	<b>RESULT</b>	<b>REFERENCE RANGE</b>
Red blood cell count	1.13 x 10 <sup>6</sup> /μl *	4.10 - 5.80 x 10 <sup>6</sup> /μl
Hemoglobin	5.1 g/dL *	12.8 - 17.2 g/dL
Hematocrit	14.1 % *	38.3 - 50.0 %
Mean corpuscular volume	115.2 μm <sup>3</sup>	80.0 - 100.0 μm <sup>3</sup>
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 <sup>3</sup> /μl	140 - 350 x 10 <sup>3</sup> /μl
Mean platelet volume	6.5 μm <sup>3</sup>	6.6 - 9.7 μm <sup>3</sup>
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
<b><u>Chemistry</u></b>		
<b>Total bilirubin</b>	<b>3.2 mg/dL</b>	<b>0.20-1.30 mg/dL</b>
Direct bilirubin	0.4 mg/dL	0.0 - 0.5 mg/dL
<b>Aspartate aminotransferase</b>	<b>53 U/L</b>	<b>9 - 40 U/L</b>
<b>Alanine aminotransferase</b>	<b>25 U/L</b>	<b>5 - 33 U/L</b>
Alkaline Phosphatase	67 U/L	20-120 U/L
<b>Calcium</b>	<b>8.9 mg/dL</b>	<b>8.4-10.2 mg/dL</b>
<b>Total protein</b>	<b>5.5 g/dL</b>	<b>6.0 - 8.0 g/dL</b>
<b>Albumin</b>	<b>3.9 g/dL</b>	<b>3.2 - 5.0 g/dL</b>
<b>Lactate dehydrogenase</b>	<b>381 U/L</b>	<b>85 - 200 U/L</b>

\*Critical Value

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

**Table 2.** Laboratory results of patient type and crossmatch

<i>TEST</i>	<i>RESULTS</i>
ABO group typing	O
Rh typing	Positive
Indirect Coombs	Positive
Direct Antiglobulin Test	Positive
Antibody panel	Positive
Antibody identification	Warm autoantibody Positive anti-e

The patient had no previous transfusion history. The immediacy of need was recognized with the resulting low red cell 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**

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

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.<sup>2</sup> Inherited or acquired immunologic defects may predispose individuals to susceptibility to leukemogenic agents.<sup>2</sup> Genetic factors may play an important role in the etiology of CLL as there are reports of multiple incidences of CLL in families.<sup>4</sup> A genetic basis is highly likely as seen in the differences in the incidence of CLL in different countries with 40 percent of all CLL occurring in the Western countries.<sup>3</sup>

### ***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.<sup>1,2</sup>

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.<sup>5</sup> As the bone marrow becomes extensively infiltrated by the leukemic lymphocytes, other marrow components are stifled in production 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.<sup>1</sup>

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 impaired immunologic activity with approximately 50% of patients reporting

hypogammaglobulinemia.<sup>1</sup> 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.<sup>6</sup> Half of patients are alive 5 years after diagnosis and 30% of patients have a 10-year survival.<sup>1</sup> 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.<sup>7</sup> 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 1 to 2 years.<sup>1</sup> 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.<sup>3</sup> 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<sup>9</sup>/L, but may be as high as 1000 x 10<sup>9</sup>/L.<sup>2</sup> 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.<sup>1, 2</sup> 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.<sup>2</sup>

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.<sup>1,2</sup> 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, as the result of splenic sequestration of red cells, shortened red cell survival, or autoimmune hemolysis. The 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.<sup>1,8</sup> Anemia may occur as a normochromic and normocytic with a normal or low reticulocyte count.<sup>1</sup> 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.<sup>2</sup> 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.<sup>7</sup>

### ***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.<sup>9</sup> The onset of WAIHA is usually insidious and can be precipitated by many factors, such as trauma, infection, stress or surgery.<sup>8</sup> Symptoms of WAIHA do not usually develop until the patient has significant anemia. Symptoms include pallor, weakness, dizziness, jaundice and unexplained fever.<sup>8</sup>

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.<sup>8</sup> Complement proteins act synergistically with immunoglobulins to cause red cell hemolysis. Testing with a polyspecific antiglobulin reagent reveals a positive direct antiglobulin test (DAT).<sup>9</sup> 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.<sup>7</sup> Other indications of extravascular hemolysis are demonstrated in the laboratory data such as elevated bilirubin and lactate dehydrogenase (LDH).<sup>9</sup>

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.<sup>8,9</sup> 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.<sup>10</sup> Warm autoantibodies may have an apparent autoanti-e specificity in the serum but may show

panagglutination of RBCs 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.<sup>8</sup>

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.<sup>8</sup> 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 leuko-filtered blood is transfused slowly in small volumes (100mL) and the patient is observed closely for any adverse reactions.<sup>8, 9, 10</sup>

### **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 is characterized by a positive DAT and the macrocytic anemia. Furthermore, elevations of the total bilirubin and LDH are 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 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 5 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 emergency room identify advancing disease and are indications for future treatment. These indications include that 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.<sup>3,11,12</sup>

**\*\*editors note:** Some areas serviced by United Blood Services receive only leukoreduced units as their source of random RBC's. In these situations, there is no need to request specialized units. Antilogous units are usually whole blood, so can not be substituted in these situations.

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## ABO DISCREPANCY IN A MULTIPLE MYELOMA PATIENT\*

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### CASE PRESENTATION

The hospital transfusion services obtained an order for three units of red blood cells for a 45-year-old Caucasian male. His most recent hematological study revealed a hemoglobin level was 7.0 g/dL (**Table 1**). Blood was obtained from the patient and the new clinical laboratory scientist graduate proceeded with pre-transfusion testing of phenotype and antibody screening. The results of the forward typing of ABO and Rh indicated that the patient was phenotype A Rh positive (**Table 2**). However, a discrepancy was found in the reverse typing with A<sub>1</sub> cells and B cells producing agglutination. Using antibody screening cells, the patient's serum was tested. The results indicated agglutination upon immediate spin, but no agglutination in subsequent testing including 37°C with LISS enhancement (**Table 3**).

**Table 1: Hematological Profile**

<u>Analyte</u>	<u>Patient Results</u>	<u>Reference Range (Conventional units)</u>
RBC	2.14	4.7 - 6.1 X 10 <sup>6</sup> /μL
WBC	4.5	4.8 - 10.8 X 10 <sup>3</sup> /μL
HGB	7.0	14 - 18 g/dL
HCT	20.4	42 - 52%
MCV	95.3	80 - 94 fL
MCH	32.7	27 - 31 pg
MCHC	34.3	32 - 36%
Platelets	80	150 - 450 X 10 <sup>3</sup> /μL
Peripheral Smear	marked rouleaux	no rouleaux

**Table 2: Initial patient ABO and Rh phenotyping**

Reaction with Anti- A	Reaction with Anti-B	Reaction with Anti-D	Reaction with Rh Control	Reaction with A <sub>1</sub> cells	Reaction with B cells
4+	0	3+	0	1+	4+

**Table 3. Antibody Screening and initial crossmatch results**

Immediate Spin	37°C/ LISS enhancement	AHG	AHG control cells
Screening Cell I	1+	0	2+
Screening Cell II	1+	0	2+
Screening Cell III	1+	0	2+
Auto Control	1+	0	2+

The clinical laboratory scientist called for additional history of the patient and learned that he was diagnosed with multiple myeloma. He had no previous transfusion history.

## **DISCUSSION**

Multiple myeloma is a hematological malignant neoplasm of the bone marrow. It is a neoplastic disease characterized by the infiltration of bone and bone marrow by myeloma cells forming multiple tumor masses.<sup>1</sup> Production of normal immunoglobulins is impaired with a significant increase in the number of abnormal plasma cells.<sup>2</sup> The condition is usually progressive and generally fatal. The disease causes pain, fractures, anemia, hypercalcemia, kidney failure, bacterial infections, nerve compression with paralysis, skeletal deformities and changes in mental status ranging from mild to severe confusion.<sup>3, 4</sup> According to the American Cancer Society, about 14,400 new cases will be diagnosed and about 11,200 Americans are expected to die of multiple myeloma in 2001.<sup>5, 6</sup>

### ***Etiology and Epidemiology***

The etiology of multiple myeloma is unknown; however genetics, radiation exposure and chronic antigenic stimulation have been suggested as predisposing factors.<sup>7</sup> Increases in the incidence of multiple myeloma during this past century implicate environmental factors as important causal agents. A single insult is not thought to be sufficient to induce the disease. However, continued exposure results in the clonal expansion of an idiotypic plasma cell after cumulative mutational damage has altered its genetic makeup.<sup>8</sup> Atomic bomb survivors and individuals exposed to radiation in the workplace have demonstrated an increased incidence of multiple myeloma. Studies of workers at nuclear power plants also suggest that chronic exposure to low levels of radiation may lead to increased risk.<sup>1</sup> The molecular and cytogenetics of cells in multiple

myeloma are under investigation, but the precise causes of these abnormalities are largely unknown.

Chronic stimulation of the immune system has been a suspected trigger of multiple myeloma with certain medical conditions such as rheumatoid arthritis, chronic allergic conditions, and chronic infections as they are implicated in the stimulation of the aberrant production of plasma cells.<sup>9</sup> Anticipation, a phenomenon in which an inherited disease is diagnosed at an earlier age in each successive generation of a family, has been demonstrated in multiple myeloma.<sup>10</sup>

The incidence of myeloma is 5 cases per 100,000 persons each year and males have approximately 50 percent greater risk than females. There is greater incidence in black individuals than white individuals, with persons of Japanese and Chinese descent experiencing the least incidence. Age increases the risk of multiple myeloma as the disease is rarely seen in persons under 40 years of age with the mean onset at age 60.<sup>1, 11</sup>

### ***Clinical Presentation and Pathophysiology***

Multiple myeloma does not have the same biology in all patients; it is best viewed as a heterogeneous disease with different prognoses, clinical course, and response to therapeutic interventions in different subjects.<sup>8</sup>

Patients may present with persistent unexplained skeletal pain (usually in the back or thorax), weakness and fatigue, confusion, and/or recurrent bacterial infections.<sup>2</sup> This disease is diagnosed in some patients with no symptoms after a screening blood test reveals abnormally high serum protein levels or there is evidence of calcium loss from bones. In some cases, patients are diagnosed only after they have developed marked changes in their mental state with extensive bone destruction and kidney failure.<sup>3</sup> Pathological fractures and vertebral collapse are common. Renal failure may be caused by extensive cast formation in the renal tubules, atrophy of tubular epithelial cells, and interstitial fibrosis. Anemia predominates in some patients, and a few have manifestations of hyperviscosity syndrome.<sup>2</sup>

An expanding plasma cell mass in the bone marrow undergoes continued clonal replication. As the growth proceeds, normal bone marrow is gradually replaced by the steadily growing malignant plasma cell colonies. Normal circulating blood cells decrease in number resulting in anemia, thrombocytopenia and neutropenia. The resulting pancytopenia causes fatigue, delayed hemostasis and an increased susceptibility to bacterial infections.<sup>3</sup>

The expanding plasma cells infiltrate bone causing destruction of the surface cortex of the bone. Stretching of the overlying nerve-rich periosteum leads to pain that is present at diagnosis in more than two-thirds of patients.<sup>11</sup> The destruction of bone tissue results in an increase in blood calcium levels (hypercalcemia).<sup>5</sup> Diffuse osteoporosis or discrete osteolytic lesions develop, usually in the pelvis, spine, ribs, and skull. Lesions are due to bone replacement by expanding plasmacytomas or a factor secreted by malignant plasma cells (osteoclast-activating factor). The osteolytic lesions are usually multiple, but occasionally are solitary intramedullary masses. Extra-osseous plasmacytomas are unusual, but may occur in any organ, especially the upper respiratory tract.<sup>2</sup>

Hypercalcemia occurs in 15% of patients with multiple myeloma at diagnosis and should be suspected in the presence of anorexia, nausea, vomiting, polyuria, polydipsia,

increased constipation, weakness, confusion, or stupor. Because calcium affects nerve cell function, hypercalcemia can cause weakness and confusion.<sup>5</sup> If hypercalcemia is untreated, renal insufficiency develops as well.

The malignant plasma cells produce immunoglobulins resulting in an overproduction of intact immunoglobulins (IgG, IgA, IgD, or IgE) or Bence Jones protein. Plasmacytomas produce IgG in about 55% of myeloma patients and IgA in about 20%; of these IgG and IgA patients, 40% also have Bence Jones proteinuria. Light chain myeloma is found in 15 to 20% of patients; their plasma cells secrete only free monoclonal light chains, and a monoclonal spike is usually absent on serum electrophoresis.<sup>2</sup>

### ***Laboratory Findings and Diagnosis***

During the different stages of the disease, almost all patients develop anemia usually presenting with a normocytic normochromic anemia with hemoglobin levels between 7.0 and 12.0 g/dL.<sup>9</sup> The peripheral smear shows rouleaux formation as the result of elevated globulins or fibrinogen in the plasma.<sup>12</sup> Red cells that are constantly bathed in the abnormal plasma affect a spontaneous pseudo-agglutination which appears as stacks of coins in the peripheral smear.<sup>13</sup> These stacks appear evenly dispersed throughout the smear. Rouleaux formation correlates with high erythrocyte sedimentation rate and occurs as a direct result of protein deposition on the erythrocyte membrane.<sup>14</sup>

Large amounts of protein can cause the peripheral blood smear to have a bluish tinge macroscopically. A few abnormal plasma cells may be seen in later stages on the peripheral blood differential. The leukocyte and platelet counts usually are normal in early stages of the disease until overpopulation of the marrow with abnormal plasma cells occurs. This may produce pancytopenia and elicit a leukoerythroblastic response.<sup>12</sup> Serum creatinine, BUN, LDH, calcium, protein, and serum uric acid are frequently elevated.

Monoclonal peaks of immunoglobulin can be found in serum protein electrophoresis. The immunoglobulin type can be determined by immunoelectrophoresis or immunofixation electrophoresis. Bence-Jones protein or light chain proteins can be identified in urine in 80% of myeloma patients.<sup>12</sup>

Bone marrow aspiration and biopsy usually indicate increased numbers of plasma cells at various stages of maturation; rarely is the number of plasma cells normal and usually present with more than 10 % and often more than 30 % of total bone marrow cells. Diagnostic criteria for multiple myeloma is based on a combination of the presence of multiple criteria including plasmacytoma, greater than 30 % plasma cells in the bone marrow, multiple lytic bone lesions, monoclonal protein spike in serum protein electrophoresis, and depressed synthesis of normal immunoglobulins.<sup>3</sup> Laboratory findings that may occur in multiple myeloma are provided in **Table 4**.

**Table 4: Laboratory Findings That May Occur in Multiple Myeloma**

<b>Analyte</b>	<b>Possible laboratory findings in multiple myeloma</b>
<b>Serum Chemistries</b>	
Protein	elevated
Calcium	elevated
BUN	elevated
Creatinine	elevated
LDH	elevated
Uric Acid	elevated
<b>Immunological Studies</b>	
Immunoglobulins	decreased
SPE	Monoclonal gammopathy
C-Reactive Protein	positive
<b>Urine Chemistries</b>	
Bence-Jones Protein	present
Protein	present
<b>Hematological Studies</b>	
ESR	elevated
WBC	decreased
RBC	decreased
Platelets	decreased
<b>Peripheral Smear</b>	
Plasma cells	present in advanced disease
Rouleaux	present
<b>Hemostasis</b>	
APTT	prolonged
Prothrombin Time	prolonged

***Treatment and Prognosis***

The disease is progressive, but good management improves quality and duration of life. Prognosis varies and is dependent on the stage of the disease at diagnosis. The median survival rate is 2 to 3 years. The patient with multiple myeloma should be carefully evaluated from the standpoint of symptoms, physical findings, and laboratory data. If there are no symptoms or evidence of early or impending complications, treatment is often delayed until progression of the disease occurs. About 60% of patients treated show objective improvement. At diagnosis, high levels of monoclonal protein in serum or urine, elevated beta-2-microglobulin levels, diffuse bone lesions, hypercalcemia, anemia, and renal failure are unfavorable prognostic signs.<sup>2</sup>

Chemotherapy is helpful in prolonging survival. Median survival of chemotherapy nonresponders is less than 1 year and responders 3 to 4 years.<sup>7</sup> A cure is not presently attainable with standard chemotherapy, interferon, or high-dose chemotherapy followed by autologous transplantation regimens with bone marrow or peripheral blood stem cells. Cure or long-term disease-free survival is seen in less than 20% of patients under 55 years of age who have related-donor match and receive an

allogenic graft, either from bone marrow or peripheral blood stem cells.<sup>15</sup> Treatment-related mortality for patients treated with this approach still exceeds 50%.<sup>16</sup> If the patient is younger than 70 years, autologous peripheral blood stem cell transplantation is considered. If the patient is older than 70 years, chemotherapy is indicated.<sup>9</sup> Chemotherapy decreases serum or urine monoclonal protein and increases median survival time three to sevenfold.<sup>2</sup>

Because anemia occurs in almost all patients during the course of multiple myeloma, transfusion of packed RBCs is indicated for symptomatic anemia. In recent prospective, randomized, placebo-controlled blind clinical trials, it has been demonstrated that erythropoietin/epoetin alfa is beneficial in the treatment of anemia in multiple myeloma as well.<sup>9,17</sup>

### ***ABO Discrepancies***

ABO discrepancies occur when the red cell testing does not agree with the expected serum testing.<sup>13</sup> Washing the patient red blood cells with saline can usually resolve the ABO discrepancy if the initial test was performed using red blood cells suspended in serum or plasma. ABO discrepancies are divided into four groups:<sup>14</sup>

- Group I are discrepancies between forward and reverse groupings because of weakly reacting or missing antibodies.
- Group II are discrepancies between forward and reverse groupings resulting from weakly reacting or missing antigens.
- Group III discrepancies are found between forward and reverse groupings caused by protein or plasma abnormalities and result in rouleaux formation or pseudo-agglutination.
- Group IV discrepancies are between forward and reverse groupings encompassing miscellaneous problems such as warm or cold autoantibodies and polyagglutination.

Multiple myeloma elevates the globulin level resulting in rouleaux and Group III ABO discrepancies. Because rouleaux formation causes the red blood cells to adhere to one another as in stacked coins, it can be mistaken for agglutination by a new or inexperienced laboratorian. Phenotyping can usually be accomplished by washing the patient's red cells several times with a saline solution. Using washing techniques as outlined in the American Association of Blood Banks' *Technical Manual*, serum is removed from the centrifuged serum/cell mixture.<sup>18</sup> The cells are then resuspended in saline and recentrifuged. This saline is removed from the cell button and fresh saline is added and the cells are resuspended once again. This is repeated three times. The washed cells are used for testing. This washing technique rids the red cell membranes from the protein and frees the cells in the case of rouleaux formation in the reverse type. In true agglutination, red cells will continue to clump after the washing with saline.

### **CASE RESOLUTION**

This patient was diagnosed with multiple myeloma 6 months prior to his admission to the hospital and transfusion order. The positive patient control cells (**Table 3**) indicate that something is coating the patient's red cells. The patient's red cells were washed with normal saline after complete decanting of the serum. After repeating the forward and reverse typing with the washed cells, the patient's phenotype was confirmed to be A positive. The rouleaux disappeared from all phases of the testing. The CLS

completed the crossmatch and the three units of A positive blood were successfully transfused.

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