ABO Discrepancy in a Multiple Myeloma Patient: A Case Study

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ABBREVIATIONS: AHG = anti-human globulin; LISS = low-ionic strength solution.

INDEX TERMS: ABO discrepancies; antibody identification; hematology; immunohematology; multiple myeloma; rouleaux.

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The hospital transfusion services received 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 of 7.0 g/dL (Table 1). Blood was obtained from the patient and the clinical laboratory scientist (CLS) 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_1 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).

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The CLS requested additional history on the patient. The diagnosis was multiple myeloma; there had been no previous transfusions.

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.[1] Production of normal immunoglobulins is impaired with a significant increase in the number of abnormal plasma cells.[2] 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.[3,4] 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.[5,6]

Etiology and epidemiology

The etiology of multiple myeloma is unknown; however, genetics, radiation exposure and chronic antigenic stimulation have been suggested as predisposing factors.[7] 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.[8] Atomic bomb survivors and individuals exposed to radiation in the workplace have demonstrated an increased incidence of mul-

Table 1: Hematological profile

Analyte	Patient Results	Reference Range
RBC	2.14	4.7 - 6.1 X 10^6/µL
WBC	4.5	4.8 – 10.8 X 10^3/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 10^3/µL
Peripheral smear	marked rouleaux	no rouleaux

tiple myeloma. Studies of workers at nuclear power plants also suggest that chronic exposure to low levels of radiation may lead to increased risk.[1] 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.[9] 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.[10]

The incidence of myeloma is five cases per 100,000 persons each year and males have approximately 50% greater risk than females. There is greater incidence in black individuals than white individuals; persons of Japanese and Chinese descent experience the least incidence. Age increases the risk of multiple myeloma as the disease is rarely seen in persons less than 40 years of age with the mean onset at age 60.[1,11]

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.[8]

Table 2. Initial patient ABO and Rh phenotyping reactions

Anti-A	4+
Anti-B	0
Anti-D	3+
Rh Control	0
A_1 cells	1+
B cells	4+

Patients may present with persistent unexplained skeletal pain (usually in the back or thorax), weakness and fatigue, confusion, and/or recurrent bacterial infections.[2] 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.[3] 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.[2]

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.[3]

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.[11] The destruction of bone tissue results in an increase in blood calcium levels (hypercalcemia).[5] Diffuse osteoporosis or discrete osteolytic lesions develop, usually in the pelvis, spine, ribs, and skull. Lesions are due to bone replacement by expanding plasmocytomas 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.[2]

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.[5] If hypercalcemia is untreated, renal insufficiency develops as well.

Table 3. Antibody screening and initial crossmatch results

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

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.[2]

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.[9] The peripheral smear shows rouleaux formation as the result of elevated globulins or fibrinogen in the plasma.[12] 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.[13] These stacks appear evenly dispersed throughout the smear. Rouleaux formation correlates with a high erythrocyte sedimentation rate and occurs as a direct result of protein deposition on the erythrocyte membrane.[14]

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.[12] 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.[12]

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 more than 10% and often more than 30% of total bone marrow cells are present. 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.[3] Laboratory findings that may occur in multiple myeloma are provided in Table 4.

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 two to three 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.[2]

Chemotherapy is helpful in prolonging survival. Median survival of chemotherapy nonresponders is less than one year and responders three to four years.[7] A cure is not presently attainable with standard chemotherapy, interferon, or high-dose chemotherapy followed by autologous transplantation regimens with bone marrow

Table 4. Possible laboratory findings that may occur in multiple myeloma

Analyte	Possible findings
Serum chemistries	
Protein	elevated
Calcium	elevated
BUN	elevated
Creatinine	elevated
LDH	elevated
Uric acid	elevated
Immunological studies	
Immunoglobulins	decreased
SPE	monoclonal gammopathy
C-Reactive protein	positive
Urine chemistries	
Bence-Jones Protein	present
Protein	present
Hematological studies	
ESR	elevated
WBC	decreased
RBC	decreased
Platelets	decreased
Peripheral smear	
Plasma cells	present in advanced disease
Rouleaux	present
Hemostasis	
APTT	prolonged
Prothrombin time	prolonged

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.[15] Treatment-related mortality for patients treated with this approach still exceeds 50%.[16] 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.[9] Chemotherapy decreases serum or urine monoclonal protein and increases median survival time three to sevenfold.[2]

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.[9,17]

ABO discrepancies

ABO discrepancies occur when the red cell testing does not agree with the expected serum testing.[13] 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:[14]

- 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.[18] The cells are then resuspended in saline and recentrifuged. This saline is removed from the cell button, 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 six months prior to his admission to the hospital and the 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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