A Case of Diffuse Large B Cell Lymphoma Presenting as Cold Agglutinin Disease

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Abstract
Patients with lymphoma have been known to present with autoimmune hemolytic anemia (AIHA). Most of the AIHA are caused by warm autoantibodies. Only a minority of patients with lymphoma present with cold agglutinin disease (CAD), with only a few reports on diffuse large B cell lymphoma (DLBCL) presenting as cold agglutinin disease. In this case report, we describe a patient with DLBCL presenting with agglutination of peripheral red blood cells at room temperature and cold autoantibody in the cross match blood sample. It further indicates the importance of including DLBCL and other lymphoma in the differential diagnosis of patients with CAD.

INTRODUCTION
Cold agglutinin disease (CAD) is a form of autoimmune hemolytic anemia (AIHA). It is caused by the presence of circulating cold antibodies, usually IgM, in the patient’s blood. These cold antibodies typically bind to red blood cells (RBC) at low temperature, cause RBC agglutination and initiate complement-mediated hemolysis.

Cold agglutinin disease can be idiopathic or secondary. Secondary CAD can arise due to infections, such as mycoplasma, influenza B, HIV, infectious mononucleosis etc. However, it can also be caused by neoplastic growth of a single B or T cell clone as in the case of lymphoma or leukemia. Some of the primary CAD cases have been found to have bone marrow disorders characterized by clonal expansion of B cells.1, 2, 3 In a retrospective study of a Norwegian population, Berentsen et al. found that 50 out of 66 patients with CAD had Non-Hodgkin’s lymphoma.4 Crisp et al. also reported that 31 out of 78 patients with CAD had lymphoma.5 Therefore, it is important to diagnose underlying cold agglutinin disease in patients with lymphoma for prompt and appropriate treatment.

Diffuse large B cell lymphoma (DLBCL) is the largest subgroup of non-Hodgkin’s lymphomas (NHL) and is characterized by relatively frequent extranodal presentation. However, diffuse large B cell lymphoma presenting with cold agglutinin disease has only been reported in a few cases. Economopoulos et al. reported that only 4 out of 370 patients with NHL presented with cold reacting autoantibodies.6 Most of the lymphoma cases associated with CAD were lymphoplasmacytic lymphoma, marginal zone lymphoma and small lymphocytic lymphoma/chronic lymphocytic leukemia.4 Eskazan et al. reported a case of primary gastrointestinal diffuse large B cell lymphoma presenting with cold agglutinin disease.7 Airaghi et al. described a patient with renal and adrenal DLBCL associated with cold agglutinin disease and hypercoagulable state.8 Sallah et al. reported 3 patients with cold agglutinin disease out of a cohort of 501 patients, with only 1 of the patients having a diagnosis of DLBCL.9 Finally, Niáinle et al. described a case of diffuse large B-cell lymphoma with isolated bone marrow involvement and presenting with secondary cold agglutinin disease.10

CASE REPORT
We hereby report a case of bone marrow DLBCL presenting with cold agglutinin disease. The patient was a 78-year-old Caucasian male admitted to the hospital for symptoms of heart failure and anemia. The patient’s past medical history included coronary artery disease, congestive heart failure, and chronic obstructive pulmonary disease. Physical examination of the patient revealed hepatosplenomegaly.

Routine complete blood cell count was performed. Interestingly, automated hematology analyzer results were flagged for hemoglobin (9.5 g/dL) and hematocrit (HCT) (9.9%) match failure. Further review of the results showed spuriously low red blood cell count (0.99 x million/µL; normal range: 4.7 – 6.1 million/µL) and HCT (9.9%; normal...
range: 42 – 52%), and spuriously high mean corpuscular hemoglobin (MCH) (96.3 pg; normal range: 27.0 – 31.0 pg) and mean corpuscular hemoglobin concentration (MCHC) (95.9%; normal range: 31.5 – 37.0%). Hence, a spun hematocrit was performed and was reported at 27.3% (normal range: 42 – 52%). The supernatant plasma showed hemolysis. In automated hematology analyzers if two or more cells enter the aperture simultaneously, they will be counted as one pulse, giving spuriously low cell count. In our case, because of the red cell agglutination, multiple agglutinated red cells being counted as a single cell resulted in spuriously low value. The hemoglobin result of 9.5 g/dL was inaccurate because free hemoglobin in the plasma was measured along with the hemoglobin in the red cells. Spuriously low red cell count resulted in spuriously low calculated HCT and spuriously high calculated value of MCH. For determining MCHC, hemoglobin value is divided by the HCT and expressed as percentage. In calculation for MCHC, hemoglobin value was divided by spuriously low HCT value of 9.9 resulting in an impossibly high MCHC result of 95.9%. Microscopic examination of peripheral blood smear revealed red blood cell agglutination, which was not seen in the smear prepared from post 37oC incubated blood sample (Figure 1). Therefore, a diagnosis of cold agglutinin disease was suspected.

Total bilirubin was 3.76 mg/dL (normal range: 0.1-1.5 mg/dL), while conjugated bilirubin was 0.67 mg/dL (0-0.3 mg/dL). Haptoglobin was < 6 mg/dL (normal range: 36-195 mg/dL). Reticulocyte count was 4.5% (normal range: 1.45 – 2.28%).

The Blood Bank also received the patient’s blood sample for type and screen. The red cells were group B, Rh positive on forward typing. However, reverse typing with the patient’s serum displayed 2+ to 3+ agglutination with A1 red cells as well as B reagent red blood cells at room temperature. After prewarming the patient’s serum, the reaction was negative with B reagent red blood cells. The cold agglutinin titer was 1:128 (normal range = not detected) at room temperature phase. There were no alloantibodies. Further work up was not ordered.

Bone marrow biopsy of the patient revealed a uniformly hypercellular bone marrow (60% - 70% cellularity) with erythroid hyperplasia (myeloid: erythroid ratio of 0.65:1). More importantly, small groups and scattered large cells with irregular nuclei and prominent nucleoli were seen (Figure 2). Immunohistochemical studies of the bone marrow showed these large cells to be positive for CD20 and PAX5, while negative for CD10 (Figure 3). CD20 also highlighted smaller reactive B cells, while CD3 highlighted the background small T lymphocytes. Therefore, the diagnosis of diffuse large B cell lymphoma involving bone marrow was made. Flow cytometry of the peripheral blood and bone marrow failed to reveal the presence of these B lymphoma cells, presumably due to low percentage of lymphoma cells and fragility of the large B cells. Cytogenetic study on the bone marrow revealed an abnormal male karyotype with loss of Y chromosome in 5 out of 16 metaphase cells. CAT scan examination of the patient revealed splenomegaly with a 3.5 cm soft tissue density, mediastinal lymphadenopathy, and bilateral hilar lymphadenopathy, suggestive of multi-organ involvement by the lymphoma.

The patient received transfusion of packed red blood cells. Chemotherapy was to start after the patient’s cardiac condition was stabilized. Unfortunately, the patient expired a few weeks later due to deterioration of heart condition.

**Figure 1**
Peripheral Blood smear before and after the blood sample was warmed at 37oC. A. The smear revealed diffuse agglutination of the red blood cells in the pre-warm sample. B. No agglutination of red blood cells noted after the sample was warmed at 37oC, indicating attenuation of cold agglutinin.

**Figure 2**
Bone marrow biopsy. A. Low magnification (10 x) view of a slightly hypercellular bone marrow with groups (circled) and scattered large lymphoma cells. B. High magnification (40 x) view of the bone marrow showing a cluster of large lymphoma cells with irregular nuclei and prominent nucleoli. Mitotic figure was also seen.
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Figure 3
Immunohistochemical studies of the bone marrow. The lymphoma cells are positive for CD20, PAX5, and negative for CD10 (not shown). CD3 immunostain highlighted the smaller T lymphocytes in the background (not shown). This immuno profile is consistent with the diagnosis of diffuse large B cell lymphoma involving the bone marrow.

Discussion
AIHA is an uncommon complication of non-Hodgkin’s lymphoma. It has been reported that most of the AIHA are caused by warm autoantibodies and are independent of the stage of the disease. Cold agglutinin disease makes only a minor group of AIHA. Cold agglutinin disease can be primary or secondary nature. While Primary CAD has been considered a stable or very slowly progressive disease with a fairly good prognosis, secondary CAD can be caused by lymphoproliferative disorders and therefore associated with a poor prognosis. DLBCL is the most common type of non-Hodgkin’s lymphoma. However, only a few cases of DLBCL presenting as cold agglutinin disease have been reported. DLBCL from the gastrointestinal tract, adrenal glands, kidney, and bone marrow, have been reported to be associated with cold agglutinin disease. In this case report, we describe a patient with DLBCL involving the bone marrow, probably spleen, mediastinum and hilum, and presenting with cold agglutinin disease, further demonstrating the importance of including diffuse large B cell lymphoma in the differential diagnosis of patients with persistent cold agglutinin disease.

It is well known that cold agglutinin disease is more difficult to treat than AIHA due to warm-reacting antibodies. Therapies with corticosteroids, alkylating agents, interferon, cladribine, and splenectomy have failed to show significant clinical impact. Sallah et al. reported that both lymphoma and the autoimmune hemolytic anemia were refractory to chemotherapy in two of the three patients with cold agglutinin disease. However, anti-CD20 antibody, rituximab, has been shown to induce remission in more than 50% of CAD patients in one study. This suggests the involvement of CD20 positive B cell lymphomas such as DLBCL, in cold agglutinin disease.

In addition, proliferation of monoclonal B cells in cold agglutinin disease has been found to be associated with aberrant karyotypes, including trisomy 3, trisomy 12, t(8;22), trisomy 18, and chromosome 17p abnormality. In our patient, loss of Y chromosome was the sole clonal aberration identified. Rare cases of loss of Y chromosome has been reported in testicular cancer and its role in the etiology of prostate cancer has not been established. Since loss of the Y chromosome can be a normal finding, its association with DLBCL and cold agglutinin disease is unknown and requires further study to clarify its potential role.

In summary, we report a case of diffuse large B cell lymphoma presenting as cold agglutinin disease.

References
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