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Case Study

B-cell Chronic Lymphocytic Leukemia is Complicated by Autoimmune Hemolytic Anemia and Anti-phospholipid Syndrome

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We describe a rare case of a 68-year-old man with B-cell chronic lymphocytic leukemia (B-CLL) who developed autoimmune hemolytic anemia (AIHA) and anti-phospholipid syndrome (APS). On admission, he was diagnosed as having B-CLL and AIHA on the basis of CD5-positive B-lymphocytes infiltrated into his bone marrow and a positive Coombs test, respectively. Although the symptoms of B-CLL and AIHA were improved by chemotherapy, he developed deep-vein thrombosis, which was probably caused by a lupus anticoagulant. It was interesting that the thrombosis due to APS occurred following a decrease of hemolysis from AIHA after intensive chemotherapy.

Key words B-cell chronic lymphocytic leukemia, autoimmune hemolytic anemia, anti-phospholipid syndrome, lupus anticoagulant, deep-vein thrombosis

INTRODUCTION

Autoimmune diseases are closely associated with B-cell chronic lymphocytic leukemia (B-CLL)[1−3] and other lymphoproliferative diseases[4,5]. Patients with B-CLL often develop autoimmune hemolytic anemia (AIHA)[1−3,6−9]. However, there are only a few reported cases of B-CLL or other lymphoproliferative diseases in which the patients have both Coombs and anti-phospholipid antibodies[10,11]. Here, we describe a case of B-CLL with AIHA and deep-vein thrombosis, probably due to lupus anticoagulant.

CASE REPORT

A 68-year-old man, with no history of thromboembolism or heart disease, was admitted to our hospital because of palpitations on March 25, 1999. On admission, no surface lymphadenopathy was seen, but a computed tomography (CT) scan examination of his abdomen showed hepatosplenomegaly and multiple para-aortic lymphadenopathy. Although premature ventricular contractions were observed on an electrocardiogram, findings by ultrasonic cardiography were within normal limits.

Hematological examination revealed a Hb of 6.1 g/dl, RBC of $148\times10^4/\mu$l, hematocrit 18.4%, MCV 124.1 fl, and reticulocyte count $4.3\times10^4/\mu$l (n: $3.0-10.0\times10^4/\mu$l), indicating macrocytic anemia. His WBC was $5.5\times10^3/\mu$l with a lymphocyte count of $3.2\times10^3/\mu$l, platelet count $17.1\times10^4/\mu$l, prothrombin time 71%, thrombin time 54%, and activated partial thromboplastin time of 39.6 seconds. A biochemical examination showed serum lactate dehydrogenase at 1,035 IU/l, indirect bilirubin 3.50 mg/dl, haptoglobin 0 g/l, and a serum protein concentration of 5.5 g/dl with hypogammaglobulinemia 0.56 g/dl.

The result of immunoelectrophoresis of urine, but not that of serum, revealed κ-type Bence Jones proteins. Renal function test results,

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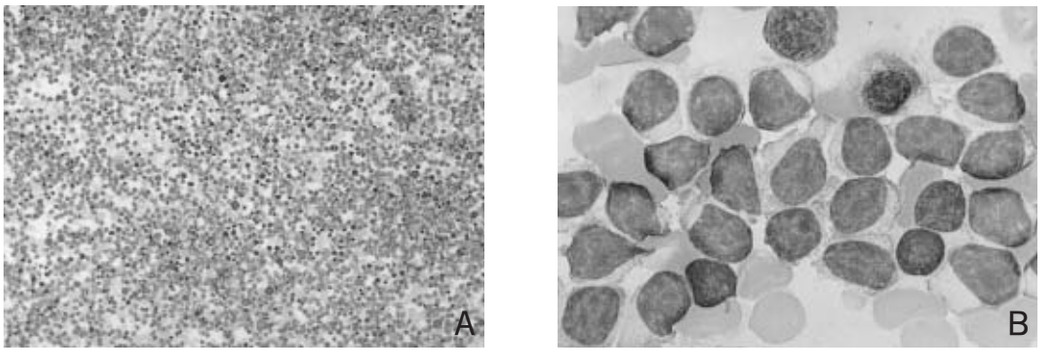

Fig. 1. Morphological appearance of these cells is compatible with B-cell chronic lymphocytic leukemia. A : May-Giemsa-stained film from bone marrow (100×) indicates a numerous mononuclear cells in the aspiration smear of bone marrow. B : May-Giemsa-stained film from bone marrow (1000×) indicates a monotonous proliferation of small mature lymphocytes with condensed chromatin. Nuclear irregularities and nucleoli are inconspicuous.

including serum creatinine and urea nitrogen, were normal. C-reactive protein was 0.9 mg/dl. IgG antibody in a direct Coombs test was strongly positive (1 : 64) and his cold agglutinin titer was low. Antinuclear antibody, anti-DNA antibody, anti-Sm antibody, anti-neutrophilic cytoplasmic antibody, rheumatoid factor, and antiplatelet antibody were negative. Bone marrow aspirate demonstrated hypercellularity with 76.8% small lymphoid cells (Fig. 1A and B), which was also found in a bone marrow biopsy. Flow cytometry of bone marrow and peripheral blood mononuclear cells showed, respectively, that 53.1% and 50.5% were $CD5^+$, 93.7% and 60.2% $CD19^+$, 47.4% and 36.3% $CD21^+$, 51.8% and 44.3% $CD23^+$, 93.1% and 95.7% $CD43^+$, and 95.3% and 83% HLA-DR^+. Also, surface IgG^+, IgA^+, IgM^+, and κ- and λ-light $chain^+$ cells were 12%, 0%, 1%, 14% and 1%, respectively, of bone marrow mononuclear cells.

A chromosomal analysis, without the addition of mitogen to the bone marrow cells, showed 46, XY (20/20 cells). A rearranged band in the immunoglobulin heavy-chain gene was detected by Southern blot analysis of the bone marrow specimen, while expression of cyclin D1 measured by fluorescence *in situ* hybridization was not increased in this patient compared to healthy volunteers. Based on the findings, the patient was diagnosed as having B-CLL, according to the World Health Organization classification of neoplastic diseases of hematopoietic and lymphoid tissues[12], along with AIHA. The clinical stage was C in accordance with the criteria of the clinical staging system of CLL developed by Binet[13].

The patient's clinical course is summarized in Fig. 2. Although his anemia improved slightly from 60 mg/day of prednisolone starting in April, 1999, he developed a fever higher than 39°C and developed pancytopenia. On May 12, his Hb was 7.6 g/dl, WBC $1.2\times10^3/\mu$l with 6% granulocytes and 94% lymphocytes, and his platelet count was $13.0\times10^4/\mu$l. Bone marrow aspirate taken on May 20 showed remarkable infiltration, with 85.4% leukemic cells, 52% of which were $CD5^+$, 90% $CD19^+$, and 65.3% $CD23^+$. A CT scan showed systemic progression of lymphadenopathy and hepatosplenomegaly.

Starting on May 24, 1999, the patient was treated with two courses of pirarubicin, cyclophosphamide, vincristine, and prednisolone. After the chemotherapy, his lymphadenopathy and hepatosplenomegaly improved markedly. On June 18, 1999, hematological parameters were : WBC of $6.9\times10^3/\mu$l, Hb of 8.4 g/dl, and a platelet count of $18.0\times10^4/\mu$l. A bone marrow aspirate showed 67.4% leukemic cells, 23.4% myeloid cells, and 8.0% erythroblasts with no morphological abnormalities of myeloid, erythroid and megakaryocytic lineages or hemophagocytosis. A chromosomal analysis of bone marrow cells showed 46, XY (20/20 cells). The titer of Coombs antibody (IgG) also dropped (1 : 2). However, further chemotherapy could not be administered because of interstitial pneumonia following candidiasis.

On June 23, 1999, the patient suddenly com-

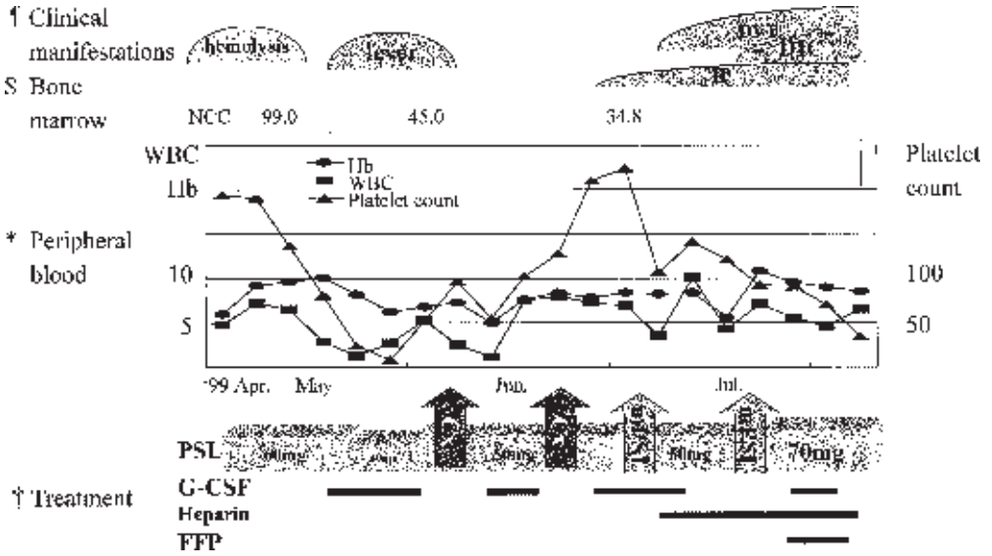

Fig. 2. Course of clinical manifestations, hematological findings, and treatment. ¶ Clinical manifestations: IP, interstitial pneumonia; DVT, deep-vein thrombosis; and DIC, disseminated intravascular coagulation. $ Bone marrow: NCC, nucleated-cell count ($10^4/\mu$l). *Peripheral blood: WBC ($10^3/\mu$l); concentration of Hb (g/dl); platelet count ($10^4/\mu$l). †Treatment: PSL, prednisolone (mg/day); mPSL, methylprednisolone (1000 mg/day); Cx, chemotherapy with cyclophosphamide, pirarubicin, vincristine and PSL; G-CSF, granulocyte colony-stimulating factor; FFP, frozen fresh plasma.

plained of swelling, edema and pain in his right leg, and his platelet count had decreased to $11.7\times10^4/\mu$l. As a cause of thrombocytopenia there were no findings that suggested myelodysplastic syndrome, aplastic anemia, or immune thrombocytopenia in his bone marrow. In addition, the concentrations of plasma fibrinogen degradation products, thrombin-antithrombin III complex, and D-dimer increased to 80 μg/ml ($n<10$), 60 μg/ml ($n<3.0$), and 470 μg/ml ($n<0.5$), respectively. He was diagnosed with deep-vein thrombosis due to anti-phospholipid syndrome (APS) because of the positive finding of lupus anticoagulant, although the concentration of anti-cardiolipin β2 GP I antibody was 3.2 U/ml ($n<3.5$). The autoantibodies described above were assessed again and were also negative.

Based on the diagnosis of APS, 15,000 units/day of heparin were administered for one month with no improvement of deep-vein thrombosis in his leg following disseminated intravascular coagulation. On July 23, 1999, he died of respiratory failure due to progression of interstitial pneumonia. No autopsy was performed.

DISCUSSION

Although the peripheral blood lymphocyte count in the our patient was below $5.0\times10^3/\mu$l, a criterion of the National Cancer Institution Working Group[8], he was diagnosed with B-CLL based on peripheral blood lymphocytes that had the same surface phenotype, as determined by flow cytometry, with cells infiltrating the bone marrow, suggesting B-CLL or B-cell small lymphocytic lymphoma (B-SLL). In fact, it is thought that B-CLL is the same disorder as B-SLL as reflected in some recent classifications[12,14].

A normal counterpart of the abnormal cells in B-CLL is a subpopulation of mature CD5-positive B-lymphocytes, differentiated to producing autoantibodies and located in the mantle zone of the lymphoid follicle and in the peripheral blood in small numbers[1,2]. The number of these CD5-positive B-lymphocytes increases in autoimmune diseases although it is not defined whether autoantibody-producing cells belong to the CD5-positive lymphocytes[1]. In the present patient, CD5-positive B-lymphocytes increased at the time of onset of AIHA and decreased as AIHA

improved. This fact suggests the possibility that CD5-positive B-lymphocytes contributed to the occurrence of AIHA.

Patients with lymphoproliferative disorders often develop autoimmune diseases[4,5]. 7 to 25% of B-CLL patients[3,6,7] and 8% of APS patients[15] develop AIHA, but reports of lymphoproliferative diseases with anti-phospholipid antibodies are rare. To our knowledge, 19 cases of lymphoproliferative diseases, including B-CLL with antiphospholipid antibodies, have been reported[10,11,16–18]. Coombs tests were positive in three of the 19 cases reported : one with B-CLL[10] and two with splenic marginal zone cell lymphoma (SMZCL)[11]. One case with B-CLL developed hemolytic anemia and phlebitis[10], and two cases with SMZCL had AIHA without thrombosis[11]. One reported case with B-CLL and another with SMZCL had lupus anticoagulant and IgM anticardiolipin antibody, and a further case with SMZCL had only lupus anticoagulant. The leukemic-B cells of the patient with B-CLL produced IgM λ, which reacts with anticardiolipin antibody, but not with anti-human IgG[10]. In patients with SMZCL, not only antiphospholipid antibodies but also Coombs antibodies disappeared after treatments for lymphoma, suggesting that lymphoma cells probably produced these autoantibodies[11].

It is not clear why the hemolytic anemia improved slightly, but thrombosis progressed, after intensive chemotherapies in the present patient. Unfortunately, we did not examine for lupus anticoagulant before the intensive chemotherapy or the improvement of AIHA because there were no clinical signs suggesting APS. However, from his age and various coagulation tests, no genesis of thrombosis other than from APS was found in this patient. In addition, although antibodies to red cells from our patient belonged to the IgG subclass, we could not determine the Ig subclasses of lupus anticoagulants because the antibodies are generally found by coagulation tests[19,20], suggesting that the relationship between AIHA antibodies, anti-lupus antibodies, and B-CLL cells is unclear.

On the other hand, only two cases with lymphoproliferative diseases who developed thrombosis due to APS without AIHA have been reported[16,17]. Lymphoplasmacytic lymphoma of the spleen was diagnosed in one of them[16], who had lupus anticoagulant and developed a pulmonary embolism. The titer of lupus anticoagulant in this patient decreased after splenectomy. In the other patient, with diffuse large B-cell lymphoma (DLBCL) who had lupus anticoagulant but not anti-cardiolipin antibodies, thrombosis progressed despite intensive chemotherapies, but improved by administration of methylprednisolone and plasma exchange[17]. Our case also had only the lupus anticoagulant without anti-cardiolipin antibodies. It is possible that thromboses in patients with lymphoproliferative disorders and APS tend to be caused mainly by lupus anticoagulant. We should consider splenectomy and/or plasma exchange to treat thrombosis from APS-resistant to intensive chemotherapies against leukemia.

In conclusion, we described a rare case of B-CLL complicated by AIHA and APS with deep-vein thrombosis probably due to lupus anticoagulant. It was interesting that the thrombosis due to APS occurred following a decrease in hemolysis from AIHA after intensive chemotherapy.

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