Primary Bone Marrow Lymphoma Presenting with Cold-Type Autoimmune Hemolytic Anemia

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Abstract We report a rare case of primary bone marrow lymphoma with cold-type autoimmune hemolytic anemia (AIHA). A 70-year-old Japanese woman with suspected liver disorder presented to our hospital with palpitation. On physical examination, she had jaundice and signs of anemia. No lymphadenopathy or hepatosplenomegaly was noted. A direct antiglobulin test was positive for complement C3b and C3d. Anti-IgG testing was negative. Cold agglutinin was positive with a titer of $1:\geq 8,192$, and haptoglobin was absent. A diagnosis of cold-type AIHA was made. Bone marrow biopsy revealed involvement with a population of lymphocytes that were positive for CD20 (L-26), CD79a, and Bcl-2. No lymphoma lesion was detected on computerized tomography or on upper and lower endoscopy. The patient was diagnosed with diffuse large B cell lymphoma (DLBCL) presenting with cold-type AIHA. She was treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone, resulting in complete remission after six cycles. As of 22 months after presentation, no signs of cold-type AIHA or lymphoma were present.

Keywords Primary bone marrow lymphoma · Cold-type autoimmune hemolytic anemia · Diffuse large B-cell lymphoma

Introduction

Cold agglutinin disease (CAD) is a subgroup of autoimmune hemolytic anemia (AIHA). CAD is estimated to account for 13–15 % of all cases of AIHA [1]. In a retrospective follow-up study of patients with chronic CAD, 76 % of evaluable patients were found to have underlying lymphoma, of which 50 % had lymphoplasmacytic lymphoma [2–4]. Although malignant lymphomas have been associated with immunologic disorders, there have only been three reported cases of primary bone marrow DLBCL presenting with AIHA [5, 6]. In the present report, we describe the case here, followed by a discussion of the biological characteristics and treatment of the present cases and similar cases.

Case Report

A 70-year-old Japanese woman with suspected liver disorder presented with jaundice and was found to be anemic. The patient did not have any known history of chronic anemia. Physical examination revealed jaundice and signs of anemia but no lymphadenopathy or hepatosplenomegaly. Hematological examination on admission showed a white blood cell count of $5,900/\text{mm}^3$ with 53.5 % neutrophils, 33.5 % lymphocytes, and 11.0 % monocytes, hemoglobin level of 4.6 g/dl, hematocrit of 13.4 %, reticulocyte count of $70,200/\text{mm}^3$, and platelet count of $334,000/\text{mm}^3$. Blood smear examination revealed the presence of red blood cell agglutinates that resolved upon warming, and no abnormalities were seen in the differential white blood cell count. Blood biochemistry revealed elevated lactate dehydrogenase (LDH) at 802 IU/l (normal upper limit: 230 IU/l). Conjugated and unconjugated

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Fig. 1 The bone marrow aspirate smears reveal a hypercellular marrow with erythroid hyperplasia. (Wright-Giemsa stain, $\times 400$)

bilirubin were 0.1 and 5.9 mg/dl (normal upper limit: 1.0 mg/dl). Aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were 68, 23, and 272 IU/l respectively. Serum immunofixation electrophoresis detected a slight monoclonal protein defined as IgG κ . Direct antiglobulin test (DAT) was positive for complement C3b and C3d. Anti-IgG testing was negative. A test for cold agglutinin was positive with a titer of $1 \geq 8,192$. Haptoglobin was absent. A diagnosis of cold-type AIHA was made. The bone marrow was hypercellular with erythroid hyperplasia (Fig. 1). Analysis of the immunophenotype in bone marrow demonstrated the proliferation of lymphocytes expressing CD19, CD20, IgM, IgD, and κ . Chromosomal analysis of the bone marrow showed a normal karyotype. The split signals of *IGH*, *BCL1*, *BCL2*, and *BCL6* were not identified in bone marrow by fluorescent in situ hybridization (FISH). Bone marrow biopsy from the posterior iliac spine revealed involvement of medium-sized atypical lymphocytes that expressed CD20 (L-26), CD79a, Bcl-2, Pax-5 (Fig. 2), but not CD3, CD5, CD10, CyclinD1, CD138, or terminal deoxynucleotidyl transferase (TdT), indicating diffuse large B-cell lymphoma (DLBCL). The MIB-1 index was 5 %. There was no selective growth of lymphoma cells within the lumina of vessels. Analysis of the immunophenotype in the peripheral blood did not show the proliferation of B-lymphocyte.

Contrast-enhanced computed tomography (CT) of the neck, chest, abdomen and pelvis showed no evidence of lymphoma lesions. This patient was diagnosed with primary bone marrow DLBCL presenting with cold-type AIHA.

The patient was treated with six intravenous infusions of 375 mg/m^2 of rituximab and six cycles of CHOP (R-CHOP), given every 3 weeks [7]. Each CHOP cycle consisted of cyclophosphamide 750 mg/m^2 , doxorubicin 50 mg/m^2 , and vincristine 1.4 mg/m^2 (maximum dose,

(A)

(B)

Fig. 2 Histological examination shows proliferation of medium-sized atypical lymphocytes (Panel A) (hematoxylin and eosin, $\times 400$). The medium-sized atypical lymphocytes are immunoreactive for CD20 (L-26) (Panel B) ($\times 400$). **a** Hematoxylin and Eosin stain $\times 400$, **b** CD20(L-26) $\times 400$

2.0 mg), given intravenously on day 1, and oral prednisolone 100 mg/body given on days 1 through 5. The commencement of resolution of hemolytic anemia was seen after one cycle of chemotherapy. After six cycles of R-CHOP, the hemoglobin level increased to 11.8 g/dl, direct antiglobulin test was negative, the cold agglutinin titer decreased to a level of 1:64, and no signs of clonal lymphoproliferation were detected by bone marrow histology, immunohistochemistry, or flow cytometry. As of 22 months after presentation, no signs of cold-type AIHA or lymphoma were present.

Table 1 Clinical data, and results of primary bone marrow DLBCL with AIHA

Case no.	Publication	Age (years)/sex	Type of AIHA	Immunophenotype/immunohistochemical analysis	Chromosome/FISH	Management	Follow-up and outcome
1	Sumi et al. 2007 [5]	75/F	AIHA with erythroid hypoplasia	CD19+, CD20+, CD22+, κ +, Bcl-2+/CD3–, CD5–, CD10–, CD20+, CD23–, Bcl-2+, Bcl-6–, Mum1+, EBER-ISH–	46, XX, dup(17)(q21q25) [2]/47, XX, idem, +mar1[5]/46, XX[13] FISH: NA	R-CHOP	Disease-free at 6 months after R-CHOP
2	Ni Áinle et al. 2008 [6]	69/M	CAD	NA/CD5–, CD10–, CD20+, CD23–, CD79a+, TdT–	NA/FISH: <i>IGH/BCL6</i> : (–)	R-CHOP	Complete response
3	Present case	70/F	Cold-type AIHA	CD5–, CD19+, CD20+, CD23+, CD25+, IgM+, IgD+, κ +/CD3–, CD5–, CD10–, CD20+, CD79a+, CD138–, Bcl-2+, Pax-5+, CyclinD1–, TdT–	46, XX[20] FISH: <i>IGH</i> (–)/ <i>BCL1</i> (–)/ <i>BCL2</i> (–)/ <i>BCL6</i> (–)	R-CHOP	Disease-free at 17 months after R-CHOP

AIHA autoimmune hemolytic anemia, CAD cold agglutinin disease, *EVER-ISH* EBV (Epstein-bar virus)-encoded RNA (ribonucleic acid) in situ hybridization, *TdT* terminal deoxyribonucleotidyl transferase, *FISH* fluorescent in situ hybridization, NA not applicable, *R-CHOP* rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone

Discussion

AIHA can be classified into warm-, cold-, or mixed-reactive antibody types. Cold-reactive immunoglobulins against erythrocyte surface antigens are essential to the pathogenesis of cold agglutinin disease (CAD). Cold agglutinins are monoclonal, usually IgM κ autoantibodies that are directed against the red cell I antigen, causing hemagglutination and complement-mediated hemolysis. In a population-based retrospective follow-up study [2], CAD was defined by the combination of chronic hemolysis, cold agglutinin titer at 4 °C of 64 or higher, and a typical pattern for DAT. Typical DAT findings included a positive test when performed with polyspecific antiserum and a strongly positive test with anti-complement protein C3d (anti-C3d). The present case fulfills the criteria for CAD except for the absence chronic hemolysis. These observations suggest cold-type AIHA rather than CAD.

The immunophenotype in bone marrow cells revealed IgM, IgD, and κ ; however, serum immunofixation electrophoresis showed a slight monoclonal protein defined as IgG κ . The reasons for the discrepancy between the bone marrow cell and serum monoclonal protein profile are not clear. Comparing patients who had monoclonal IgM in serum with those who had only IgG or IgA showed a median initial cold agglutinin titer of 4,096 and 96, respectively, and this difference was statistically significant [2]. As the results of DAT were negative for anti-IgG, we considered that the slight IgG κ in the serum might not contribute to hemolysis.

Chronic CAD is a clonal lymphoproliferative bone marrow disorder accounting for 13–15 % of all AIHA cases [1] with an incidence of 1 per million people per year [2–4]. In a retrospective follow-up study of patients with chronic CAD, 76 % of evaluable patients had underlying lymphoma, of which 50 % had lymphoplasmacytic lymphoma [2]. Transformation to diffuse large B-cell lymphoma (DLBCL) appears to be a rare event, occurring in 3–4 % of patients with primary CAD after a disease duration of 10 years [2]. The bone-marrow histology in this case at the initial presentation was already consistent with DLBCL; therefore, it is unclear whether transformation occurred.

Primary bone marrow lymphoma (PBML) is a rare entity whose real boundaries and clinicobiological significance are not defined. PBML is characterized by the combination of isolated bone marrow infiltration (regardless of peripheral blood involvement), no evidence of lymph node, spleen, liver, or other extra marrow involvement on physical examination or on imaging studies (thoracic, abdominal, and pelvic CT scan), absence of localized bone tumors, no evidence of bone trabeculae destruction in the bone marrow biopsy, and exclusion of leukemia/lymphoma cases that are considered to involve primarily the bone marrow [8]. The present case fulfills the criteria for PBML. Primary bone marrow DLBCL is extremely rare, as highlighted in a review of three cases [9]. Only one of three cases demonstrated peripheral blood involvement. Cytogenetic analysis revealed complex karyotypes in two cases. These cases serve to highlight the biological and cytogenetic heterogeneity of DLBCL [9]. In our case, the

examination, including immunophenotyping, at the initial presentation did not show the involvement of the peripheral blood. Cytogenetic analysis revealed normal karyotype, and there were no split signals of *IGH*, *BCL1*, *BCL2*, and *BCL6*. Immunophenotyping demonstrated IgM, IgD, and κ expression at the cell surface, and immunohistochemistry did not show TdT expression, suggesting that lymphoma cells in our case might be derived from naïve B cells. Naïve B cells, which are often CD5 positive, are small resting lymphocytes that circulate in the peripheral blood and also occupy primary lymphoid follicles and follicle mantle zones and are therefore referred to as recirculating B cells [10].

Including the present case, there have been only three reported cases of primary bone marrow DLBCL presenting with AIHA [5, 6]. Table 1 shows the clinical characteristics of three cases at the time of presentation. All three cases were treated with R-CHOP. Although interpretation of therapeutic effect is difficult because of the small number and the variability in the duration of follow-up, rituximab-containing chemotherapy seems to induce a good response.

In conclusion, cold-type AIHA in this case indicates the importance of bone marrow biopsy to detect underlying lymphoid malignancy. The mechanisms by which PBML cells originate from bone marrow without other lesions remain unclear. We suspect that the origin of PBML cells might be different from that of nodal lymphoma. Further studies, including immunophenotypic and cytogenetic analyses, for primary bone marrow DLBCL with AIHA are needed to clarify the clinical characteristics and pathophysiology of this disorder.

References

1. Genry I, Michel M, Hermine O et al (2002) Characteristics of autoimmune hemolytic anemia in adults: retrospective analysis of 83 cases. *Rev Med Intern* 23:901–909
2. Berentsen S, Ulvestad E, Langholm R et al (2006) Primary chronic cold agglutinin disease: a population based clinical study of 86 patients. *Haematologica* 91:460–466
3. Berentsen S, Beiske K, Tjønnfjeld GE (2007) Primary chronic cold agglutinin disease: an update on pathogenesis, clinical features and therapy. *Hematology* 12:361–370
4. Berentsen S (2011) How manage cold agglutinin disease. *British J Hematol* 153:309–317
5. Sumi M, Ichikawa N, Shimizu I et al (2007) Primary diffuse large B-cell lymphoma of the bone marrow complicated with autoimmune hemolytic anemia and erythroid hypoplasia. *Rinsho Ketsueki* 48(7):571–575
6. Ni Áinle F, Hamnvik OP, Glumann C et al (2008) Diffuse large B-cell lymphoma with isolated bone marrow involvement presenting with secondary cold agglutinin disease. *Int J Lab Hematol* 30(5):444–445
7. Czuczman MS, Grillo-López AJ, White CA et al (1999) Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. *J Clin Oncol* 17(1):268–276
8. Martinez A, Ponzoni M, Agostinelli C et al (2012) Primary bone marrow lymphoma: an uncommon extranodal presentation of aggressive non-Hodgkin lymphomas. *Am J Surg Pathol* 36(2):296–304
9. Alvares CL, Matutes E, Scully MA et al (2004) Isolated bone marrow involvement in diffuse large B cell lymphoma: a report of three cases with review of morphological, immunophenotypic and cytogenetic findings. *Leuk Lymphoma* 45(4):769–775
10. Jaffe ES, Harris NL, Stein H et al (2008) Introduction and overview of the classification of the lymphoid neoplasms. WHO classification of tumors of haematopoietic and lymphoid tissues. International Agency for Research on Cancer Press, Lyon