Case Report

A case of megaloblastic anemia simulating a cold autoimmune hemolytic anemia

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We report a case of pernicious anemia in which the first diagnosis suspicion was cold autoimmune hemolytic anemia (cAIHA) due to the presence of cold autoantibodies. A 47-year-old woman with a medical history of autoimmune thyroid disease came to the hospital with a clinical and serologic presentation of AIHA. However, because of determination of vitamin B12 (VB12) deficiency, she was finally diagnosed with megaloblastic anemia. In the acute period, the patient received short-term corticosteroid therapy and later VB12. The patient's hemoglobin level and general condition showed improvement. ***Immunohematology* 2020;36:89–92.**

Key Words: pernicious anemia, megaloblastic anemia, cobalamin, autoimmune anemia, cold agglutinins

Cold autoimmune hemolytic anemia (cAIHA) is an autoimmune hemolytic anemia mediated by cold agglutinins, which are mostly specific for the I antigen. Autoanti-I may be detected in patients with cAIHA as well as in healthy individuals. Cold agglutinins belong to the immunoglobulin M (IgM) subclass and, in the majority of patients with primary cAIHA, are monoclonal IgM-kappa antibodies. Primary cAIHA is most often seen in elderly patients (median age at onset is 67 years [range 30–92 years]), and the incidence rate is 1 per 1 million people per year.[1] Primary cAIHA may develop in association with various hematologic and immunologic diseases, including pernicious anemia (PA) and common variable immunodeficiency.

PA is an autoimmune gastritis that results from the destruction of gastric parietal cells and the associated lack of an intrinsic factor to bind ingested vitamin B12 (VB12). VB12 deficiency leading to megaloblastic anemia (MA) and demyelinating central nervous system disease is well known; rare individuals with presentations of VB12 deficiency have also been described.[2]

Here we report a case of a middle-aged Spanish woman with MA due to decreased VB12 levels in whom the first suspicious diagnosis was cAIHA because of clinical features and first findings in the immunohematologic study.

Case Report

A 47-year-old woman, who had well-controlled autoimmune hypothyroidism as her only medical antecedent, was hospitalized because of acute hemolytic anemic syndrome with primary diagnostic suspicion of AIHA. The patient did not present with fever, cough, dyspnea, weight loss, or other AIHA-associated symptoms except for a few days of asthenia. There was no history of drug intake and no recent ongoing alcohol abuse, but she explained inappropriate dietary intake. She had no family history of PA.

On admission, the patient (height 160 cm and body weight 75 kg) was faintly icteric and pale. Initial hemoglobin (Hb) value was 6.9 g/dL (normal 12–16 g/dL) but worsened in the following 24 hours to 5.9 g/dL. Macrocytosis was present (mean corpuscular volume [MCV] = 128.3 fL, normal 78–100 fL). Leukocyte and platelet counts were normal. Direct antiglobulin test (DAT) was positive. High levels of lactate dehydrogenase (LDH) and indirect bilirubin, haptoglobin <10 (normal >10), and reticulocytes of 1.92 percent (normal 0.5–2%) were the most relevant laboratory findings. There were no schistocytes in the peripheral blood smear, but some plasma cell and lymphoplasmacytic lymphocytes were observed by morphology, so the sample was sent for study and to rule out myeloma or a lymphoproliferative process.

Material and Methods

The patient's red blood cells (RBCs) typed as group O, D+ with the C+E–c–e+; K– phenotype. Immunohematology study showed a positive DAT (2+ reactivity) with polyspecific antihuman globulin (AHG). DAT using monospecific AHG reagents was strongly positive (3+ reactivity) with C3d and negative with IgG. Eluate carried out with an acid elution (ELU-KIT; Immucor, Dreieich, Germany) was negative.

The patient's serum was tested by low-ionic-strength solution-indirect antiglobulin test (LISS-IAT) against a three-

cell antibody screening panel (Ortho Clinical Diagnostics, Raritan, NJ), and all results were positive at AHG. Because of these findings and DAT positivity with C3d, another sample was collected and incubated at 37°C before removing the serum for cold autoantibody study. Thermal amplitude test was 3+ reactive at 4°C, 22°C, and 30°C but was negative at 37°C. The serum and RBCs used in the thermal amplitude test were brought to the appropriate temperature (4°C, 22°C, 30°C, and 37°C) before they were mixed together for the test. An 11-cell identification panel was tested using the LISS-IAT to guide the identification of the antibody and to rule out cold alloantibodies, such as P1, M, N, and Lewis and other clinically significant alloantibodies. Results were negative for all cells indicating that antibodies to common antigens were ruled out. Anti-I specificity was identified by testing the patient's serum against adult OI RBCs, OI RBCs treated with ficin, Oi cord RBCs, autologous RBCs, and group O RBCs. Cold autoantibodies reacted more strongly with OI adult RBCs than with Oi cord RBCs. Anti-I titer was less than 64 at 4°C.

Serum protein electrophoresis and immunoglobulin levels (IgM/IgG/IgA) were normal. T-receptor cell analysis of bone marrow (BM) was polyclonal. Lymphocyte immunophenotype assessed by flow cytometry was normal. Total body computed tomography scan revealed no lymph adenopathy, hepatosplenomegaly, or other abnormalities except uterine myoma.

BM aspiration was compatible with MA, and BM biopsy evidenced erythroid hyperplasia and megaloblastic traits without morphologic signs of lymphoproliferative infiltration. Biochemical parameters proved undetectable VB12 and low folic acid levels with a gastric parietal cell antibody titer of 160.

Results

Diagnosis of MA was concluded with the digestive tract biopsy by esophagogastroduodenoscopy showing chronic atrophic gastritis.

The patient required one RBC transfusion at diagnosis and received treatment with VB12 supplementation (cyanocobalamin 1000 μg/day for 1 week, then weekly, and finally once per month) and folic acid (one 10-mg tablet per day). Corticosteroids were administered for 1 day and stopped when the diagnosis of megaloblastic anemia was made. The patient recovered progressively: Hb levels increased from 5.9 to 12.4 g/dL, LDH serum levels decreased from 2395 to 162 U/L (normal 135–214 U/L), and the MCV was normalized from 128 fL to 80 fL over 2.5 months after VB12 treatment was started. Finally, DAT and antibody detection test by LISS-IAT (with a sample collected and maintained at room temperature) became negative after 2 months with recovering normal serologic and hematologic parameters. We have not repeated the thermal amplitude test; thus, we cannot completely rule out the presence of cold agglutinins.

Discussion

cAIHA may develop in association with Waldeström macroglobulinemia, type B lymphoma, systemic lupus erythematosus, Epstein-Barr virus, or infection with *Mycoplasma pneumoniae,*[3–7] although there are some cases in the literature that do not show such underlying diseases.[2,8] The diagnostic criteria for cAIHA are chronic hemolysis, positive polyspecific DAT, monospecific DAT with C3d strongly positive, cold agglutinin titer ≥64 at 4°C, and no underlying malignant disease.[8,9] Patients with cold agglutinin disease have signs and symptoms of hemolytic anemia. Patients with secondary agglutinin disease may also have an underlying disease, often autoimmune. The finding of hemolytic anemia plus a positive DAT (C3d) led to the diagnosis of AIHA due to cold autoantibodies. Not all cold agglutinins are associated with hemolysis, however. Benign cold autoantibodies are quite often detected in healthy people: low titers, no clinical or serologic disorders, and a short thermal amplitude range are critical findings to distinguish benign from pathologic cold autoantibodies.[7,10] The immunohematologic study of our patient showed a cold autoantibody with unclear diagnostic criteria: it was active at 30°C, but the titer was low. These characteristics should make one review the diagnosis of cAIHA and look for other causes of hemolytic anemia. Our patient was not studied before the onset of acute anemic syndrome, so we do not know if she had previous high titers of cold agglutinin, but she did not mention the typical clinical characteristics of chronic cAIHA.

PA is an autoimmune disorder due to the production of autoantibodies against parietal cells or intrinsic factor that causes an abnormal absorbance of VB12 from the terminal ileum. The final diagnosis is demonstrated by gastroscopy-mediated biopsy showing chronic atrophic gastritis. There are a large variety of clinical presentations or symptoms associated with the lack of VB12. The most common sign is MA (sometimes associated with other cytopenias), but it is not always easy to diagnose because PA can simulate many diseases; therefore, physicians should be aware of

different and challenging scenarios. The unusual clinical presentation of MA includes: spuriously normal or high cobalamin levels, normocytic or microcytic anemia, non-anemic macrocytosis, AIHA, pseudo-thrombotic microangiopathy, hyperhomocysteinemia-associated thromboembolism, pseudoleukemia, bone marrow failure, bone marrow ring sideroblasts, and neurologic chapters without anemia or macrocytosis.[11] Hemolysis findings are detected in 1.5 percent of patients affected with MA due to ineffective erythropoiesis and intramedullary RBC progenitor destruction.[11] Prompt detection and early treatment by supplementation is essential in prognosis and sequels of these patients, since hematologic disorders are reversible, but not all neurologic complications will be resolved.

In our case, the initial diagnosis presumption was AIHA due to high LDH, low haptoglobin, severe anemia with normal reticulocytes, and positive DAT; therefore, corticosteroid therapy was started in the emergency department. It is well known that AIHA usually induces an increase in bone marrow RBC progenitors, and the consequence is an increase in MCV. One thing that alerted us was the high MCV, which in our case was extremely high. It has to be taken into account that increased RBC production also may result in folic acid deficiency and MA. In addition, positive DAT with IgG only and with complement C3 was found in 10 of 32 patients diagnosed with MA and disappeared after treatment with VB12.[12] To date, 23 cases of associated diagnosis of MA plus AIHA have been published; in two of them, the AIHA was due to cold autoantibodies.[13,14] However, the presence of MA associated with the production of benign cold autoantibodies has not been reported. cAIHA was not present in our case, but there was a cold autoantibody without clinical activity. This association was thought to probably not be a coincidence. In particular, some studies have reported cases showing association between PA and cold agglutinin production.[14,15] A lesson we learned was that hemolysis parameters plus positive DATs do not always mean AIHA.

The role of VB12 in human immunity is still obscure; some suggest that VB12 has important immunomodulatory effects on cellular immunity and abnormalities in the immune system in PA.[11] An *in vitro* experimental study demonstrated that VB12 was involved in the production of concanavalin-dependent T-cells and in the synthesis of mitogen-dependent immunoglobulin pokeweed in B-cells and can significantly modify serum levels of IgG, IgA, and IgM.[16] In this sense, the literature reports that 1.5 percent of patients with cobalamin deficiency can be seen concomitantly with or preceded by AIHA.[10]

Making an accurate diagnosis is critical because the therapeutic approach is different for cAIHA and MA. Our patient received 1 day of corticosteroids, but this treatment was suspended, and VB12 was administered. The hematologic parameters and the clinical condition of the patient improved, and the DAT became negative after recovering normal hematologic and serologic parameters.

Clinically significant cold autoantibodies often do have a high titer—greater than 64 at 4°C. But clinical significance is related more to the thermal range of the antibody than the titer or specificity. The anti-I in this case report appeared to react 3+ at 30°C, which would indicate that it was potentially clinically significant. The fact that the patient's anemia resolved after folic acid and VB12 treatment would seem to indicate that the cold agglutinin was truly benign. Whether our patient will develop cAIHA is a future possibility that we cannot yet determine.

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