



## Case Report

# Cold agglutinin syndrome secondary to splenic marginal zone lymphoma: a case report

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## Introduction

Cold-antibody autoimmune hemolytic anemias (cAIHAs) are a type of anemia in which autoantibodies can cause symptoms related to the agglutination of red blood cells (RBC) at low temperatures. The so-called cold agglutinins (CA) are antibodies that recognize antigens on RBCs at temperatures below normal core body temperature. Typically, the antibodies are immunoglobulin M (IgM), and the antigens are “I” or “i”.<sup>1</sup> IgM being a pentameric antibody allows a multiple antigen-binding, resulting in numerous RBCs bound on the same IgM. This explains why a small amount of antibodies can produce obvious agglutination.<sup>2</sup> The temperature range at which the antibodies are active is called thermal amplitude, and activity peaks usually occurs at 4°C. Thermal amplitude it's generally more clinically significant than the titles; however, pathogenic antibodies usually shows higher titles (> 64).<sup>3</sup> CA with thermal amplitude may not react at 37°C, but

temperatures from 28-30°C may be sufficient for agglutination on body extremities.

Recently, there has been a change in the subdivision of cAIHA: in cold agglutinin disease (CAD), patients may have a B-cell clonal lymphoproliferative disorder (LPD) detectable in blood or marrow, but no clinical or radiological evidence of malignancy.<sup>1</sup> On the other hand, cold agglutinin syndrome (CAS) is associated with the presence of a clinical disease, such as infection, autoimmune disease or lymphoma. Paroxysmal cold hemoglobinuria is also a cAIHA, but with distinctive mechanisms.

Hemolysis in cAIHA is primarily extravascular and mediated by complement.<sup>3</sup> The bound IgM recruits components of the classical pathway, such as C1, C4, and C2.<sup>4</sup> The C3b-coated RBCs are phagocytosed by macrophages in the reticuloendothelial system, predominantly Kupffer cells in the liver.<sup>4</sup> In cAIHA, the phagocytes tend to engulf the entire cell rather than a portion, like occurs in the IgG-mediated. This may explain the absence of spherocytosis in cAIHA compared with warm autoimmune hemolytic anemia. On the non-phagocytosed RBCs, IgM dissociates upon warming, but C3b remains attached. Surface C3b undergoes cleavage to C3d that can be detected by the DAT. A positive DAT for complement is, therefore, one of the initial findings that suggest cAIHA.

**Abbreviations:** CAD, Cold agglutinin disease; AIHA, Autoimmune hemolytic anemias; CA, Cold agglutinins

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The diagnosis of a cAIHA should always trigger the broad investigation of infections (specially *Mycoplasma pneumoniae* and Epstein-barr virus), autoimmune diseases and LPD. Older individuals (above 60 years) with cAIHA show a higher prevalence of lymphoid malignancy. In a series of 78 patients with persistent cold agglutinins, associated LPD were present in 65%. The most common was lymphoma (40%) and Waldenström macroglobulinemia in second place (17%).<sup>5</sup> Patients with cAIHA usually develop symptoms when exposed to cold temperatures, mainly in extremities.

Splenic marginal zona lymphoma (SMZL) is a rare disease, mainly characterized by massive splenomegaly and no other extranodal involvement other than bone marrow and liver.<sup>6</sup> Cytopenias and lymphocytosis are frequent.<sup>6</sup> cAIHA is likewise an infrequent condition and it should always warrant investigation for hematological malignancy in older adults. Here, we aim to describe the case of a CAS caused by marginal splenic lymphoma, with remarkable high titers of cold agglutinins, as opposed to only mild clinical manifestations.

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## Case report

A 61 years-old male was referred to consultation after an incidental finding of splenomegaly on a preoperative evaluation. He had a previous history of vasectomy and an orthopedic surgery on the left knee. Preoperative evaluation for hernia repair surgery paved the way for identifying asymptomatic massive splenomegaly, palpable on physical examination. He was then referred to a hematologist to proceed with the investigation.

He had experienced an 8 kilograms weight loss on the past 3 months, but was following a diet. He had no other B symptoms or abdominal pain. On physical examination, he had a palpable spleen until the lower left flank. He did not have lymph nodes enlargements.

His first complete blood count (CBC) showed a hemoglobin of 9.8 g/dL, but the erythrocyte count and other hematimetric levels were impaired by erythrocyte agglutination. He had a white blood count of 36,080 total leukocytes/ $\mu$ L, with 29,590 lymphocytes/ $\mu$ L, and atypical lymphocytes and cellular debris on peripheral blood film. Remarkably, he also had a monocytosis of 2,520 monocytes/ $\mu$ L. He had aggregated platelets, but they appeared morphologically normal on microscopic evaluation.

Liver (including bilirubins) and renal function tests were normal, and screening for hepatitis B and C, and HIV was negative. Anemia investigation yielded an elevated lactate dehydrogenase and reticulocyte count. He had no presence of hemoglobinuria. The monospecific direct antiglobulin test for C3d and IgM 3+ were strongly positive. Two samples were collected at 37°C to perform cold agglutinin screening (an EDTA and a no-anticoagulant tube) and kept at this temperature until tests were performed. After separation, by pre-warmed centrifugation at 3000 rpm for 5 min (Metafuge 16R – Thermo Scientific®), sera were extracted to a new tube and serially diluted. RBCs were washed with pre-warmed saline and resuspended in a 3% solution, then added to each dilution tube. After overnight incubation at 4°C and centrifugation for 30s at 3000rpm (Centrifuge 5702 – Eppendorf®), hemagglutination

reactions were evaluated, categorized as positive (range from 1 to 4+) or negative. The final title was defined as the last tube with a reaction of at least 1+. In this case, 8,192 reinforcing the diagnosis of cAIHA.

Antibody identification was performed through the tube hemagglutination method using patient sera incubated with a red blood cell panel (BioRad®) and one cord blood sample. Tubes were incubated at room temperature and 4°C for 15 min and centrifuged for 30 seconds at 3000 rpm (Centrifuge 5702 – Eppendorf®). Hemagglutination reactions were evaluated and classified as positive (range from 1 to 4+) or negative. Sera reacted with all adult, including autologous, red blood cells at room temperature, and positive reactions were increased at 4°C. Umbilical red blood cells tested negative at both room temperature and 4°C. Tests at 37°C were not performed, since no transfusion was necessary. The antibody was, therefore, identified as auto-anti-I.

He had a 0.4 g/dL monoclonal gamma globulin peak seen on protein electrophoresis, identified as IgM/kappa on immunofixation. Only after the laboratory diagnosis of cAIHA and the direct inquiry about symptoms, the patient remembered observing a Raynaud's phenomenon in hands and feet during the previous year, especially associated with lower temperatures.

The patient continued clinical investigation with a bone marrow biopsy and aspiration. Bone marrow aspiration revealed a hypercellular marrow at the expense of medium lymphocytes (43%) with a high nucleus-cytoplasm ratio. Flow cytometry of bone marrow reported 31% of B lymphoid monoclonal cells expressing CD19, CD20strong, CD22strong, CD79b, CD43weak, CD31weak, CD49d, CD27 partial, HLA-DR, Kappa, and CD45. Those cells were negative for: CD38, CD5, CD10, CD23, CD200, CD11c, CD103, CD95, LAIR1, CD39 and lambda. Bone marrow biopsy also displayed a hypercellular marrow with lymphocyte infiltration. Immunohistochemistry showed infiltration of cells positive for CD20, PAX5, BCL2, Ki67 of 1%, and negative for CD3, CD10, CD5, CD23, cyclin, and CD43, indicating the diagnosis of marginal zone lymphoma. PET-scan revealed a diffuse increase in the spleen's volume and metabolic activity (31 cm and SUVmax of 4), with no other areas with increased metabolic activity.

The proposed treatment was six cycles of R-CVP (Rituximab plus cyclophosphamide, vincristine, and prednisone). After the six cycles, a new PET-scan revealed a partial reduction in volume and normalization of the spleen's metabolic activity, measuring 20.8 cm with SUVmax of 2.9 (Deauville 1). The patient was asymptomatic, and anemia was resolved; thus, he was put off therapy and started clinical follow-up.

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## Discussion

cAIHA is an uncommon disease that, when diagnosed, should always trigger the investigation for hematological malignancies. The recent division between CAD and CAS, as distinct clinical entities, was due to better understanding of the primary pathogenesis. CAD, previously described as idiopathic, has now its etiology associated with low-grade LPD, detectable in blood or marrow. Frequently, the aspects of the neoplastic cells can be interpreted as MZL; however, in CAD, patients do not have an extramedullary MZL. Thereby, in the

described case, because of the presence of splenic disease, the case was ruled out as CAS.

The remarkable peculiarity, in this case, was the abyss between laboratory and clinical findings. The title threshold for CAD diagnosis is usually > 64, although there is no consensus that titer is related to the severity of hemolysis or clinical manifestations. In fact, some *in vitro* studies have demonstrated that other properties of the antibody (such as avidity for the RBC membrane, thermal amplitude, and the ability to fix complement) could be more closely related to hemolysis rather than the titles.<sup>4</sup> The patient in question had a high titer (8,192) but, interestingly, the Raynaud's phenomenon was not a remarkable symptom for him, as he just reminded it after directed inquiry. It is known that clinical manifestations in the extremities (such as acrocyanosis and Raynaud's phenomenon) are closely related to the local climate and to the patient's exposure. In the reported case, the high titer was not associated with high activity of the autoantibody, possibly related to the patient's behavior. Thermal amplitude was not evaluated, therefore low thermal amplitude could be the reason for the mild antibody activity.

In SMZL, cytopenias are frequent and most commonly secondary to hypersplenism. However, they can be immune-mediated, such as the case of cAIHA.<sup>7</sup> A large portion of SMZL patients present with a serum monoclonal component, usually IgM, reported in as many as 45% of patients in a reported cohort.<sup>7</sup> This monoclonal component is broadly associated with immune issues, such as hemolytic anemia, immune thrombocytopenia and coagulation disorders,<sup>7</sup> as in the reported case.

There is no consensual treatment for SMZL since there are no prospective randomized trials. Treatment options include active surveillance, splenectomy, or chemotherapy (rituximab alone or combined with cytotoxic agents). Concerning the treatment of CAS, the underlying cause should be the focus of treatment. In some cases of B-cell lymphomas, rituximab appears to be an effective therapy. A recent review approaching the paper of rituximab on the treatment on AIHA secondary to non-Hodgkin lymphoma concluded that it represents a valid therapeutic option, although a significant proportion of patients will relapse after an initial response.<sup>8</sup> The treatment's choice for our patient was based on staff experience and availability.

CAD's treatment is another point of discussion. Patients with mild anemia or compensated hemolysis, and absence of clinical symptoms, have no benefit from treatment. The basic rule in CAD's treatment is to avoid cold exposure. Otherwise,

patients with troubling symptoms should be considered for specific therapeutic care.<sup>1</sup> Treatment options rely on B-cell directed approaches, such as a combination of rituximab and bendamustine,<sup>9</sup> or, complement inhibitors as an emerging option.<sup>1</sup> It's important to note that, in case of complement inhibition, ischemic symptoms will not be relieved, as they are not complement mediated.

Besides its rarity and anecdotal clinical and laboratory findings, this case emphasizes the importance of the mutual exchange between the clinical and the immunohematology laboratory teams, providing a correct, fast and efficient diagnosis to support the patient's need.

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## Conflicts of interest

The authors have no conflicts of interest to declare.

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