CASE REPORT

Resolution of Serologic Problems Due to Cold Agglutinins in Chronic Lymphocytic Leukemia

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Abstract

Introduction Autoimmune hemolytic anemia can be classified depending on presence of warm, cold or mixed type of autoantibodies that are directed against antigens on the red blood cell surface. Here we report a case of pathological cold agglutinin disease which was eventually detected due to blood group discrepancy.

Case details A request was sent to the blood bank for two units of packed red cells in a diagnosed case of CLL which showed type IV discrepancy during blood grouping. The discrepancy was subsequently resolved after warm saline washing of red cells along with repetition of reverse grouping with pre-warmed serum. The direct antiglobulin test was positive and revealed autoantibodies against C3b/C3d only. Indirect antiglobulin test was performed with 3-cell panel in a polyspecific gel card (IgG+C3d) showed a pan-reactive pattern along with a positive autocontrol. Subsequently a cold agglutinin titration was performed and titers of 1024 at 4 °C; titer of 2 at room temperature were detected. Dithiothreitol (DTT) treatment of serum was undertaken and IgM type of autoantibody was detected in this case confirming a case of secondary cold agglutinin disease in this patient. Two units of red cells were transfused to this patient after successfully performing cross-match with pre-warmed serum. It was advised from the

blood bank that the blood should be transfused slowly through a blood-warmer and patient should be kept in warm condition to avoid in-vivo hemolysis due to high titer of cold agglutinin. The transfusion was uneventful and patient is on regular follow-up till now.

Conclusion Thus we concluded that serological discrepancies observed in blood bank can successfully guide the bedside transfusion protocol in case of cold agglutinin disease.

Keywords Serologic problems · CLL · Cold agglutinins · Titer

Introduction

Autoimmune hemolytic anemias can be classified depending on presence of warm, cold or mixed type of autoantibodies that are directed against antigens on the red blood cell (RBC) surface [1]. The autoantibodies may be idiopathic (primary) or secondary to conditions such as infection, malignancy, or immune disease [2]. Cold-antibody mediated hemolytic anemias are further categorized into cold agglutinin disease and paroxysmal cold hemoglobinuria [3].

Such cases may present difficulties in blood grouping and cross matching which then have to be resolved by special techniques. Cold autoantibodies react more strongly at 0–4 °C than at warmer temperatures. Cold autoantibodies are often detected in healthy individuals, with the majority being benign. Pathological cold autoantibodies are characterized by wide thermal amplitude and/or high antibody titers. We report a case of pathological cold agglutinin disease which was eventually detected due to blood group discrepancy.

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Case Details

A request was received at the blood bank for two units of packed red cells in a 62 years old male patient who was a known case of chronic lymphocytic leukemia (CLL), diagnosed at a private hospital about 2 months back. There was no history of blood transfusion and chemotherapy was not started yet. Peripheral blood smear showed rouleaux formation with anisocytosis and poikilocytosis.

Significant medical history revealed that the patient was a diabetic and had a moderately enlarged spleen. There was no history of blood transfusion. Routine blood cell counts revealed hemoglobin 7.1 gm/dl, total leukocyte count of 19,450 cells/cu mm and platelet count of 72,000/cu mm. Since the patient was anemic the hematologist requested for two units packed red cells (RBC). Blood grouping was done by Column agglutination technique (CAT, Ortho Biovue System, UK) and type IV discrepancy was observed during blood grouping. There was no autoagglutination observed in blood sample after collection. Forward grouping by ABD reverse diluent card showed B positive blood group and reverse grouping (at 22 °C) showed 4+ agglutination with A reagent cells and 2+ agglutination with B and O reagent cells (Fig. 1). Reverse grouping after half an hour incubation at 4 °C, showed enhanced agglutination of B and O cells. Since these observations were suggestive of cold agglutinins; the reverse grouping was then repeated using pre-warmed plasma (plasma kept at 37 °C for 1 h). Now the agglutination with B and O cells disappeared.

The direct antiglobulin test (DAT) was performed by CAT method along with an autocontrol and both were positive (4+).

Fig. 1 Blood group discrepancy

Keeping in mind the possibility of cold agglutinins, we used patient's red cells washed with warm saline to remove residual plasma containing cold antibodies. Results using the monospecific card showed presence of only C3b/C3d (+4) and no IgG component (Fig. 2). It is our practice to perform antibody screen prior to all cross matches and so the indirect Coomb's test (ICT) was done using the 3 cell

Fig. 2 Direct antiglobulin test

Fig. 3 Antibody screening

panel polyspecific card (Ortho Biovue System, UK) which showed pan-reactivity (3+) (Fig. 3). Further, the ICT was repeated with pre-warmed serum which showed attenuation of the agglutination (1+).

To identify two compatible RBC units for transfusion, we performed the cross match by CAT method. Cross match with two B positive RBC units showed 2+ incompatibility. The cross match when repeated using pre-warmed patient plasma and the same two donor units, showed a compatible cross match and these units were safely transfused.

The cold agglutinin titer was performed using pooled O cells and phosphate buffer saline, and titers of 1024 at 4 °C; titer of 2 at room temperature and negative at 37 °C were obtained. Next dithiothreitol (DTT) treatment was undertaken to determine the nature of the antibody. On treating the serum with 0.01 (M) DTT and incubating at 37 °C for 1 h, agglutination disappeared at 4 °C and at room temperature, suggesting IgM nature of the antibody.

Discussion

In the twentieth century, Landsteiner [4] first described blood agglutination at cold temperatures and Schuboth named the disorder “cold agglutinin disease” [5]. ‘Physiological’ cold agglutinins develop because of change in expression of red cell antigens that occurs after birth. These antibodies react best at about 4 °C. Pathological cold agglutinins are maximally reactive at around 28–31 °C and tend to occur at very low titres. Though our case had a high titer of 1024, there was no agglutination at 37 °C.

The titer levels are considered less concordant with disease activity because hemolysis occurs even at titers as low as <1:32 [4]. However titer levels >1:512 are considered to be clinically significant [6]. Berentsen et al. [7] in a study from Norway, observed a median titer of 2048 among 86 patients, whereas Stone et al. [8] reported titers ranging from 512 to 65,536 among 172 patients.

In this case though the thermal amplitude showed no agglutination at 37 °C but the high titer (1024) at 4 °C suggestive of an antibody that could cause interference in blood grouping and cross matching in the blood bank, might increase the turn-around time during issue of a blood unit for transfusion along with a risk of in vivo hemolysis in cold temperature.

In 90 % of cases, cold agglutinins are IgM by nature. Rarely, monoclonal IgG, IgA, or λ light chain restriction, are responsible. Similarly, even our case had IgM cold agglutinins and hence the antibody reacted below 22 °C, with strong reaction at 4 °C. Warm autoimmune hemolytic anemia, where antibodies react at 37 °C, are predominantly IgG-driven [9–11].

IgM cold antibodies efficiently activate the complement cascade at lower temperatures, as in the peripheral parts of the body and extremities. The complement cascade leads to intravascular hemolysis. In our case the initial laboratory findings suggested mild hemolysis as the reticulocyte count was 2.27 % and there was an elevated LDH (659 U/l). The total bilirubin was within normal limit (1.5 mg/dl). Perhaps the patient’s free hemoglobin over a period of time caused injury to the kidneys, as evidenced by a mildly elevated serum creatinine (1.5 mg/dl).

According to literature 76 % of all hematological disorders are associated with CAD and among them only 6 % are associated with CLL [6].

Cold antibodies can interfere with blood grouping and cross-matching. It is necessary to use O reagent cells in the reverse grouping and also to carefully note the strength of the agglutination. In cases of severe disease, samples have to be collected and maintained at 37 °C until the plasma and red cells are separated or pre-warmed plasma (plasma kept at 37 °C for 10–15 min) may be used as was done in this case. For the DAT, the patient’s red cells were washed with warm saline to remove residual plasma containing cold antibodies. Warming reduces the adsorption of the cold antibodies onto the red cells. Minimizing adsorption during sample processing yields more accurate titers of cold antibodies, and avoids false-positive results [12].

Specificity of the cold antibodies can be determined by testing patient’s plasma against type O blood from adults and newborns. Adult red cells are I antigen positive, and infant red cells are i antigen positive. Therefore, agglutination of adult red cells, and cord red cells infers presence of anti-I and anti-i respectively. However, in our case we could not confirm the specificity of the cold antibody.

Patients usually respond well to rituximab or a combination of fludarabine and rituximab. Patients with a low-titer antibody and low thermal amplitude may not require any additional treatment, since the antibodies are not clinically significant. In case of high titer or high thermal amplitude adequate precautions are imperative and the patient must be kept warm. For surgery the management strategies involve peri-operative plasma exchange with fresh frozen plasma, use of warmed products (including bypass priming fluids, blood products, and anesthesia gases), and blankets to keep the body warm. Intra-operative monitoring must ensure that the core body temperature is above the reactive thermal amplitude of the cold agglutinin antibody to prevent hemolysis [6]. Our case was managed with rituximab 375 mg/m² once a week for 4 weeks and compatible warmed RBC were transfused slowly through blood warmer and patient was kept in warm condition in this case to improve the patient’s hemoglobin. The patient responded to two units of RBC and was discharged.

Conclusion

Pathological cold agglutinin disease can be associated with variable thermal amplitudes and titers. Depending on the thermal amplitude, they may interfere in routine serological tests in the blood bank. To correctly identify and resolve these problems it is necessary that standard procedures are followed for blood grouping including the use of O reagent cells for reverse grouping. In addition, we also conclude that serological discrepancies observed in a blood bank can successfully guide the bedside transfusion protocol in case of cold agglutinin disease.

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