

# A Novel Approach for Treatment of Cold Agglutinin Syndrome-Related Severe Hemolysis

Anteneh Tesfaye<sup>a, b</sup>, Catherine Broome<sup>a</sup>

## Abstract

Intravascular hemolysis related to cold agglutinin syndrome results from the activation of the classical complement pathway by red blood cell (RBC) surface I/i antigen bound autoantibodies. Despite built-in mechanisms that limit continued downstream complement activation, some patients may develop life-threatening intravascular hemolysis due to the formation of membrane attack complexes. We present a case of severe intravascular hemolysis due to cold agglutinin syndrome that was treated successfully using proximal complement inhibition with commercial C1 esterase inhibitor. The role of complement inhibition in controlling the intravascular hemolysis and resolving the immune dysfunction that leads to the syndrome is discussed.

**Keywords:** Cold agglutinin syndrome; Intravascular hemolytic anemia; Complement pathway; Complement inhibition; C1 esterase inhibitor

## Introduction

Hemolysis related to cold agglutinin syndrome is the result of red blood cell (RBC) surface I/i antigen bound antibodies activating the classical complement pathway. This culminates in the deposition of C3b on the surface of RBCs. The RBCs are subsequently cleared by the reticuloendothelial system, the primary mechanism of the low-grade chronic hemolysis observed in most patients [1, 2].

RBC membrane-bound CD55 and CD59 normally limit downstream complement activation. In some patients, especially during heightened immune stimulation, complement activation may proceed beyond C3b formation, leading to formation of C5a and C5b-9 (the membrane attack complex) and intravascular hemolysis which can be severe and life-threatening [1, 2].

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We report a case of cold agglutinin syndrome presenting with acute intravascular hemolysis. We hypothesized that immediate proximal inhibition of the complement cascade with a commercially available C1 esterase inhibitor would be an effective strategy to halt intravascular hemolysis.

## Case Report

A 61-year-old African American male with metastatic adenocarcinoma of the lung, receiving second-line pemetrexed, presented with severe anemia 2 weeks after his last treatment. Although complete blood count (CBC) could not be performed due to autoagglutination of the anticoagulated blood specimen, even at room temperature, his hemoglobin was measured at 3.8 g/dL on blood gas analysis. Haptoglobin was undetectable and lactate dehydrogenase was 1,034 IU/L, consistent with a significant intravascular hemolysis. His ABO blood group was A+D+. Both indirect Coombs and DAT were positive for C3d (3+) and negative for IgG. His peripheral smear at room temperature revealed severe agglutination of RBCs, which improved after incubation at 37°. Cold agglutinin titers using I+ve/i-ve reagent RBCs were 1:1,024 at room temperature (22 °C), 1:1,024 at 4 °C, and 1:32 at 37 °C. With I-ve/i+ve reagent RBCs, the titers were 1:256 at 22 °C, 1:1,024 at 4 °C, and 1:1:8 at 37 °C.

Daily treatment with the commercially available C1 esterase inhibitor, Berinert<sup>®</sup>, at a dose of 20 units/kg/day intravenously, was initiated along with prednisone 1 mg/kg/day and four weekly doses of rituximab 375 mg/m<sup>2</sup>. Transfusions were avoided due to difficulty with cross-matching and concerns of additional hemolysis of transfused RBCs. CBC, haptoglobin, and LDH were evaluated prior to and 2 h after the administration of Berinert<sup>®</sup> daily. Following the administration of each dose of C1 esterase inhibitor, there was an immediate improvement in the hemolysis parameters and a modest rise in hemoglobin (Fig. 1, 2).

Patient's hemoglobin increased to baseline level at day 14 after a total of eight doses of Berinert<sup>®</sup> and two doses of rituximab without any packed RBC transfusion (Fig. 1). We were able to accurately perform CBC analysis on day 7 after he received six daily doses of Berinert<sup>®</sup>, daily prednisone and one dose of rituximab. On day 14, after eight doses of Berinert<sup>®</sup>, 2 weeks of steroids, and two doses of rituximab, the cold agglutinin titers using I+ve/i-ve reagent RBCs were 1:4

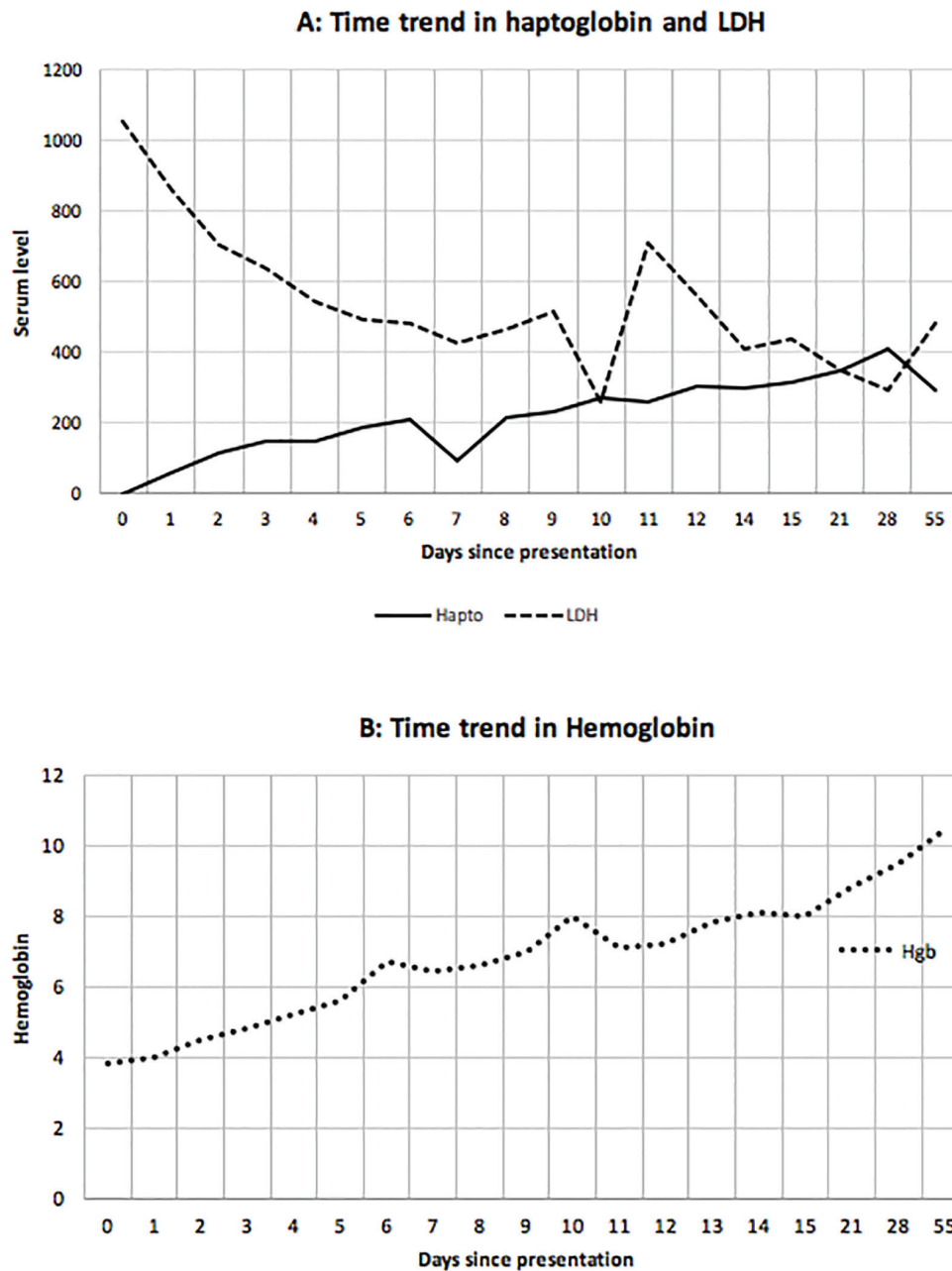
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**Figure 1.** Time trend in haptoglobin and lactate dehydrogenase (LDH) (A) and hemoglobin (B). Day 0 was when the consult was received. Berinert was administered on day 0 to day 8 (except day 6). Rituximab was given on days 4 and 11. The other two doses were given as outpatient. Prednisone 1 mg/kg/day was started on day 0 and was continued till tapered off as outpatient.

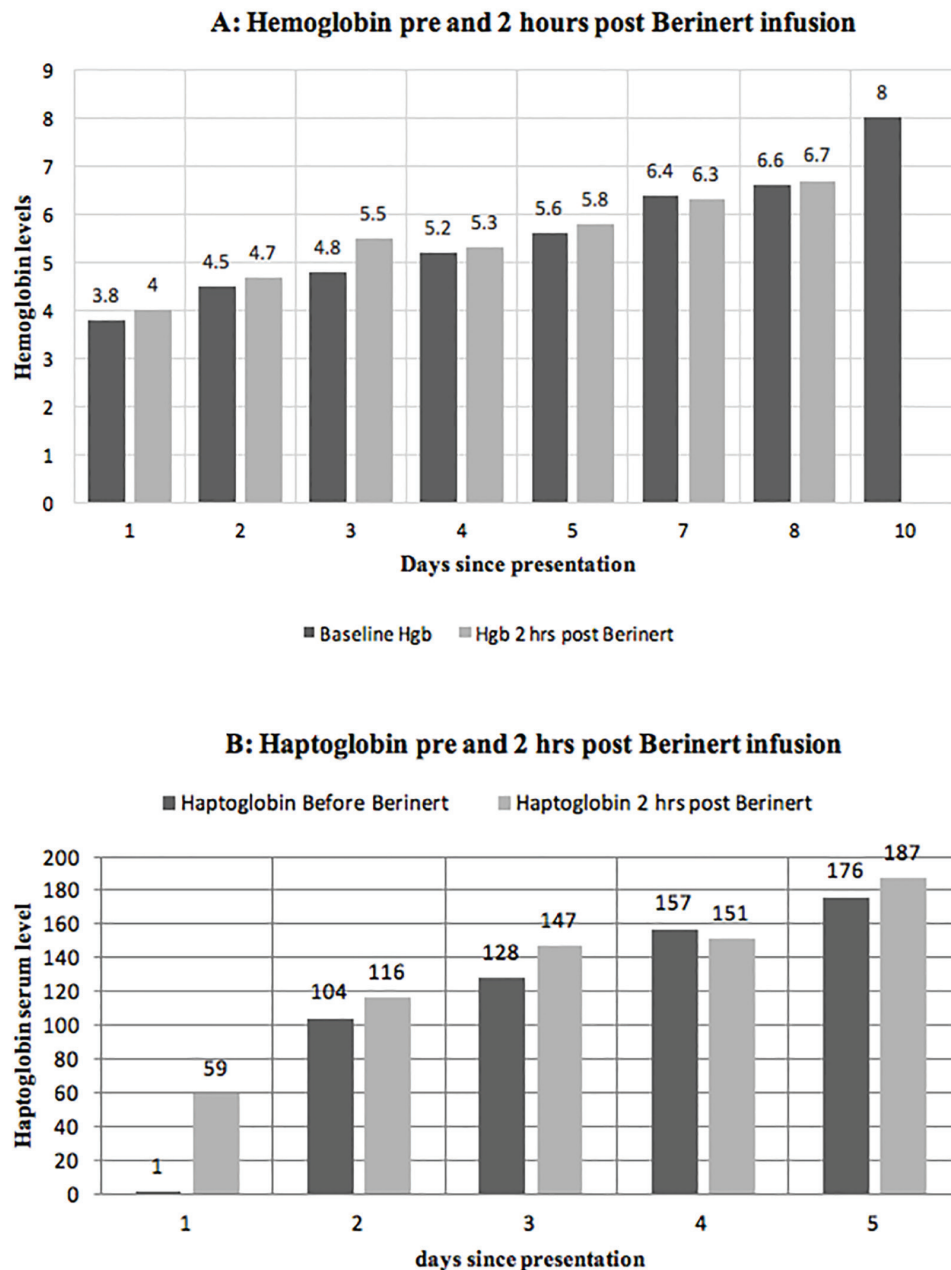
at room temperature (22 °C), 1:64 at 4 °C, and 1:1 at 37 °C, indicating a significant response to treatment. The hemoglobin has continued to slowly improve with no evidence of ongoing hemolysis after completion of the planned therapy including a taper-off of prednisone.

**Discussion**

Patients with cold agglutinin syndrome usually present with

chronic low-grade hemolytic anemia. There may be prominent autoagglutination of their red cells *in vivo* and/or *in vitro*, especially at cold temperatures. Presentation with acute, severe intravascular hemolysis is rare [1, 3].

Cold agglutinin syndrome has been linked most often to underlying lymphoproliferative disorders [4]; however, rare cases of paraneoplastic cold agglutinin syndrome associated with solid tumors have been reported [5-7]. In the absence of any other findings, we hypothesize that the cold agglutinin syndrome may be a paraneoplastic manifestation of active



**Figure 2.** Levels of hemoglobin (A) and haptoglobin (B) before and 2 h after the administration of Berinert showing an immediate improvement in hemolysis.

lung adenocarcinoma.

In *in vitro* models, the proximal inhibition of complement using a monoclonal antibody against C1 esterase has resulted in the inhibition of C3b formation, RBC phagocytosis, and downstream complement mediated intravascular hemolysis [2]. The use of plasma derived C1 esterase inhibitor in a patient with hemolytic crisis caused by warm antibody autoimmune hemolytic anemia has been shown to improve response to packed red cell transfusion [8].

Reports indicate rituximab may have a response rate as high as 60% in reducing cold agglutinin titers. Almost all of

the responses were partial responses and there was high rate of relapse [1, 4]. Berentsen and colleagues showed that the median time to response in patients treated with rituximab was 1.5 months. The median duration of response observed was 11 months [4]. The utility of steroids in treating cold agglutinin syndrome is reported to be very limited [1, 3, 4].

There is a growing body of evidence indicating an integral role of the complement system in immune regulation, i.e. antibody production by B lymphocytes, activity of effector T cells and activity of regulatory T cells.

The binding of complement protein fragment C3dg to the

B-cell complement receptor, (CR2, CD21) facilitates antibody production by B cells. It has been demonstrated that complement depletion leads to impaired antibody production [9, 10].

Downstream complement activation products have been implicated in the generation of effector T cells that help maintain immune response by enhancing T-cell proliferation and diminishing T-cell apoptosis [11]. The complement proteins have also been shown to function as paracrine and autocrine stimulators of effector T cells through their G protein coupled receptors. Disabling this interaction resulted in diminished effector T-cell responses [11]. T cells deficient in complement interaction undergo accelerated cell death [12].

Regulatory T cells play an indispensable role in maintaining immunologic unresponsiveness to self-antigens and in suppressing excessive immune responses deleterious to the host [13]. Regulatory T-cell generation, stability, and suppressive function are decreased by C3a and C5a signaling [14]. The binding of C3a and C5a to receptors on regulatory T cells leads to uninhibited autoreactivity and possibly to immune-related organ injury and transplant organ rejection [15].

There has been speculation that inhibition of the complement cascade may help suppress the activation of antibody production and cell-mediated immunity, including in the post-transplant setting [10, 15, 16].

Beriner<sup>®</sup> is a plasma-derived parenteral C1 esterase inhibitor concentrate that is commercially available in the United States for the treatment of hereditary angioedema. When used for the treatment of hereditary angioedema, the drug is well tolerated with few adverse effects. A possible adverse effect of this therapy is an increased risk of venous thromboembolism, although this has not been demonstrated consistently [17].

Utilizing a novel approach of proximal complement blockade with a C1 esterase inhibitor, we successfully treated a life-threatening autoimmune hemolytic crisis associated with cold agglutinin syndrome. In addition, we hypothesize that the use of proximal complement inhibition may have augmented the resolution of antibody production by dampening B-cell activity and enhancing regulatory T-cell function.

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