

Long-Term Remission of Paroxysmal Nocturnal Hemoglobinuria Following Chemoimmunotherapy for Non-Hodgkin Lymphoma

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Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare disorder caused by a somatic mutation of the *PIG-A* gene, resulting in a deficiency or absence of the glycosylphosphatidylinositol (GPI)-linked proteins and hematopoietic cells lacking several important GPI-linked proteins such as CD55 and CD59. CD55 and CD59 are complement regulatory proteins. The absence of these proteins renders cells more sensitive to complement-mediated cell lysis,¹ leading to intravascular hemolysis, pancytopenia, and thrombosis.

Historically, treatment has largely been supportive, until the recent development of the monoclonal antibody eculizumab (Soliris, Alexion Pharmaceuticals). However, this therapy has significant limitations. Here, we report a patient diagnosed with PNH and concurrent diffuse large B-cell lymphoma (DLBCL) who achieved long-term remission of her PNH following chemoimmunotherapy with rituximab (Rituxan, Genentech), cyclophosphamide, doxorubicin, vincristine, and prednisone, suggesting another potential option of therapy for PNH.

Case Report

A 49-year-old woman presented in December 2006 with severe anemia. Work-up for gastrointestinal bleeding was negative. The patient also had fevers, night sweats, and a 20-pound weight loss with persistent anemia. Her hemoglobin remained low despite multiple blood transfusions. A computed tomography (CT) scan of the abdomen/pelvis was unremarkable. A biopsy of an enlarged cervical lymph node showed intermediate-grade large B-cell lymphoma.

The patient had generalized lymphadenopathy. Her hemoglobin level was 8.1 g/dL, and mean cell volume

was 85 fL. The white blood cell (WBC) count was 9,350 μ L, with 67% neutrophils, 8% lymphocytes, and 25% monocytes; the platelet count was 270,000/ mm^3 . Serum chemistry was significant for total protein of 5 g/dL, albumin of 1.3 g/dL, and uric acid of 2.5 mg/dL. Lactate dehydrogenase (LDH) was 1,105 U/L, and the direct antiglobulin test was negative.

The bone marrow was hypercellular without lymphoma, dysplasia, or an increase in the number of blasts seen. The peripheral blood smear showed a normochromic-normocytic anemia with polychromasia, a relative monocytosis, and unremarkable granulocytes and platelets. A repeat CT scan showed a large mediastinal mass extending into the right superior neck, enlarged lymph nodes in the abdomen and in both axilla, innumerable pulmonary nodules, and splenic infiltration (Figure 1). A positron emission tomography (PET) scan showed uptake above and below the diaphragm and spleen.

The patient was enrolled in a phase II clinical trial with dose-dense chemoimmunotherapy using rituximab [Rituxan, Genentech] plus cyclophosphamide, vincristine, doxorubicin, and prednisone (R-CHOP), followed by radioimmunotherapy with ibritumomab tiuxetan (Zevalin, Spectrum Pharmaceuticals) consolidation with pegfilgrastim (Neulasta, Amgen) support. Meanwhile, CD55 and CD59 came back abnormally low, consistent with a diagnosis of PNH. The patient tolerated the immunochemotherapy well.

The patient completed chemotherapy in May 2007. A bone marrow biopsy in April 2007 showed a normocellular bone marrow with no lymphoma. Her CT in February 2007 and a PET scan confirmed complete remission. A CT scan in June 2010 confirmed continued complete remission. The striking finding in this patient was that her hemoglobin level also improved with administration of R-CHOP; it trended up progressively from 10, in January 2007, to 14, in May 2007 (Table 1). The patient's PNH resolved at the same time she completed chemotherapy, and she became

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Table 1. Progressive Improvement in Hemoglobin Level With R-CHOP

Dec 2006	Jan 2007	May 2007	June 2009	Sept 2010
7.8 g/dL	10.4 g/dL	14.3 g/dL	13.6 g/dL	14.5 g/dL

Note: Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) was completed in May 2007, with subsequent resolution of anemia.

transfusion-independent. The repeat CD55/59 study on both red blood cells (RBCs) and WBCs in September 2010 was normal with no evidence of a PNH clone, and her latest hemoglobin level was 14.5 g/dL.

Discussion

We present a case of a patient with PNH and concurrent DLBCL. We have not found a reported case of PNH occurring simultaneously with DLBCL. Even more notable is the resolution of the PNH along with DLBCL following R-CHOP therapy.

Historically, the treatment of PNH has been mainly supportive. Therapeutic interventions included supplementation with iron and folic acid, and the administration of RBC transfusions. In addition, steroids have been administered to help suppress hemolysis and stimulate hematopoiesis, but they have resulted in short-term remissions. The only curative option is allogeneic stem cell transplantation (SCT). However, SCT is not a very attractive option for several reasons: scant literature has been published on the indications for SCT and these indications are not always clear, and graft failure and relapse have been reported after SCT. The European Blood and Marrow Transplant group reported a 5-year survival rate of 70% with allogeneic SCT for PNH in patients with a median age of 30 years. However, only 54% of those met criteria for classic PNH.² One review of 17 patients found that relapse of PNH occurred in all 5 recipients of syngeneic transplants without conditioning.³ SCT may eliminate PNH clones, but it may also select out PNH clones that could lead to relapse of PNH. This has been documented via polymerase chain reaction (PCR) analysis of the *PIG-A* gene in 1 patient, where relapse of PNH was due to the emergence of a new clone, rather than persistence of the original clone. This is also the same hypothesis explaining PNH development after chemotherapy, where chemotherapy may select out PNH clones or create elective conditions that favor the emergence of PNH clones.

Eculizumab is the only US Food and Drug Administration–approved drug for the treatment of PNH. It is a monoclonal antibody that inhibits terminal complement activation, thus preventing the lysis of cells. Eculizumab has proven its efficacy in 2 phase III clinical trials, and has



Figure 1. Diffuse metastatic infiltration of the spleen. Innumerable low-density lesions are seen involving the entire splenic parenchyma. The overall size of the spleen is enlarged.

been shown to be highly effective in reducing intravascular hemolysis, the need for blood transfusions, and the risk of thrombosis.^{4,5} However, the disadvantages of eculizumab include cost, long-term administration, loss of response after discontinuation of therapy, and the possibility of extravascular hemolysis (based on a recent report).⁶

There are very limited data on the role of chemotherapy for treating PNH. Only 2 studies have used chemotherapy to manage PNH, and both were conducted in China. In 1 study, 8 refractory patients with PNH were treated with low doses of chemotherapy (melphalan 2–6 mg/day, prednisone 0.5 mg/kg/day, 1 course lasting about 14–21 days), which resulted in a response rate of 62.5% (5/8 patients). Those 5 patients did not relapse during follow-up for 2.5 years, and there was no bone marrow failure or serious side effects manifested.⁷ In the other study, 8 patients with refractory and relapsed PNH were treated with 2 types of chemotherapy regimens. Three patients were treated with daunomycin 40 mg/day intravenous infusion (IV) on the first and second day, 20 mg/day IV infusion on the third day, and Ara-C 100 mg/day IV infusion for 5 days. Five patients were treated with homoharringtonine 2–3 mg/day IV infusion for 5 days and Ara-C 100 mg/day IV infusion for 5 days. All 8 patients responded well. The PNH clone was diminished in 5 patients, and hemolysis stopped in 6 cases.⁸

In our case, the patient's hemoglobin improved steadily while she received R-CHOP for DLBCL, and she became transfusion-independent. We concluded that the R-CHOP eradicated the PNH clones. The role of rituximab therapy in treating PNH has not yet been studied, although there

have been studies documenting its success in treating pure red cell aplasia and autoimmune cytopenias.^{9,10}

Our patient remains in long-term remission (>3 years) for her PNH after receiving R-CHOP for her DLBCL, concluding that it is possible that chemoimmunotherapy with R-CHOP can lead to long-term remission of PNH. Considering the limitations of current therapeutic options and the potential life-threatening complications of PNH, chemoimmunotherapy should be considered in patients who do not have other reasonable options for therapy.

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Review

Chemoimmunotherapy's Potential to Impact Paroxysmal Nocturnal Hemoglobinuria

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The case presented by Lai and colleagues¹ describes a patient with paroxysmal nocturnal hemoglobinuria (PNH) that resolved following chemoimmunotherapy (rituximab [Rituxan, Genentech], cyclophosphamide, vincristine, doxorubicin, and prednisone [R-CHOP]) administered for treatment of diffuse large B-cell lymphoma (DLBCL). In addition to achieving a complete remission of her stage IV DLBCL, the patient had a resolution of her anemia. This case report proposes further consideration of chemoimmunotherapy for PNH, prompting this commentary and review.

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Pathophysiology

PNH is an acquired disease derived from a defect in glycosylphosphatidylinositol (GPI)-linked proteins affecting the stem cells. The red cells that evade apoptosis in PNH are deficient in GPI due to an underlying genetic defect in the *PIG-A* gene. The normal function of the *PIG-A* gene product is an enzyme active in the formation of GPI. The genetic defect can arise via a number of somatic mutations including missense and nonsense mutations. Two GPI-linked proteins are CD55 (also referred to as decay accelerating factor) and CD59 (also referred to as membrane inhibitor of reactive lysis). Both of these molecules control the status of the complement cascade.²

Historically, PNH was diagnosed by the Hams test or the sugar water test, though today the diagnosis is made almost exclusively using flow cytometry techniques where CD55 and CD59 expression levels can be quantified.

Clinical Diagnosis

The clinical presentation varies significantly. A minority of patients note hemoglobinuria, and the anemia present at diagnosis can range from minor to profound. Patients most often present with pain either in the abdomen or back, or headache and fever, which are symptoms attributed to the underlying hemolysis. Flares can be provoked by a number of stimuli: infection, blood transfusions, or stresses such as surgeries.

The type of anemia is commonly macrocytic, though microcytic anemia can be seen if iron deficiency is also present. Blood tests typically reveal a reticulocyte count that is not appropriately elevated, and elevated lactate dehydrogenase with detectable urine hemosiderin. Additional cell lines can be involved as well. Other hematologic disorders are often diagnosed concurrently with PNH. Twenty percent of both aplastic anemia and myelodysplasia patients have detectable PNH clones.³ In addition, there are a group of patients with PNH clones who do not experience symptoms.⁴

Prognosis

Patients at risk for increased mortality typically present with involvement of another cell line, evidence of thrombosis, or progression to myelodysplasia (MDS) or acute myeloid leukemia (AML). For those patients with a reduced white blood cell (WBC) count, higher rates of infection and the risk of more severe infection increases the mortality risk. For patients with thrombocytopenia, the increased bleeding risk raises the mortality rate. The median overall survival is 10–15 years.⁵ Risk factors that indicate poor prognosis are age (≥ 55 years at the time of diagnosis), progression to a secondary malignancy (MDS or AML), development of pancytopenia, or an episode of thrombosis. If the patient has a normal WBC, normal platelet count, and no sign of complications from PNH, then he or she will likely have a normal life span.

Treatment

In general, treatment is aimed at controlling the hemolytic anemia or treating thrombotic complications. For anemia, options include steroids, iron and folic acid supplementation, epoetin injections, and eculizumab (Soliris, Alexion Pharmaceuticals). The mechanism of activity for steroids is likely via lowering the amount of active complement and thereby lowering the rate of hemolysis. Large doses are often required, and accompanying side effects of elevated glucose and Cushingoid symptoms can be seen. Eculizumab as described in the accompanying case report is a promising, effective agent in reducing hemolysis by blocking C5 in the complement cascade. If needed, blood transfusions are given to maintain adequate blood stores while treating PNH.

For thrombotic complications, the sites can vary from common presentations such as the extremities to atypical sites such as the hepatic vasculature or intracerebral veins. Prophylactic anticoagulation is recommended in high-risk settings, and once a clot has occurred, lifelong anticoagulation is recommended. Thrombolytics and heparin infusion as a bridge to warfarin are commonly employed.⁶

Bone marrow failure can occur before or after diagnosis, prompting the recommendation that patients with a diagnosis of aplastic anemia have an annual test for PNH. Treatment approaches for this presentation include antithymocyte globulin (ATG), cyclosporine, supportive care, or even

allogeneic transplant. The goal of immunosuppressive treatment with ATG/cyclosporine is control of the hemolysis via T-cell suppression. Supportive care includes epoetin and/or granulocyte-stimulating agents for red cell and granulocyte support, respectively. Allogeneic transplant offers the potential for cure; however, it carries high risks from treatment-related morbidity and mortality and has potential for relapse, as outlined in the case reported by Lai and colleagues.¹ In our practice, attempts are made to balance the timing of the transplant procedure by managing the risk/benefit measure; not all patients are able to have this modality offered. We focus this approach on younger patients who have risk factors conferring bad response to long-term supportive care or who already manifest an adverse event from the disease. With this caveat, this modality must be performed before the patient develops multi-organ failure.

To our knowledge, this is the first report of chemoimmunotherapy for treatment of PNH. In the 2 Chinese case series described in the report by Lai and colleagues, there were responses to cytotoxic chemotherapy. There are also existing reports of PNH occurring after myeloablative chemotherapy,⁷ but this case opens the discussion for why R-CHOP may have provided benefit. One possibility is that a direct effect of rituximab on the humoral immune system may affect complement activation. Alternatively, the chemotherapy, including cyclophosphamide, may have been the key to eradicating the PNH clone and hemolysis via the cytotoxic and anti-T-cell actions. A third possibility is that the combination of rituximab and chemotherapy was synergistic. Accordingly, further investigation into the mechanistic reasons for the chemoimmunotherapeutic effect is warranted.

Conclusion

This case presents a novel observation of a patient who had durable resolution of PNH while being treated with R-CHOP for concurrent DLBCL. This presents an additional option for treatment for PNH, which is a disease in need of new treatment strategies.

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