

CLINICAL VIGNETTE

Pancytopenia and Coombs Negative Hemolytic Anemia

Alexander C. Black, M.D.

Case

This 53-year-old female had been in excellent health until approximately 1 ½ years ago when she began developing increasing fatigue and was found to be pancytopenic. Her initial hematologist performed blood tests and a bone marrow biopsy (BM BX) that was non-diagnostic but suggestive of myelodysplastic syndrome (MDS). She had a repeat BM BX one month later, which had similar findings. She was managed with red blood cell (RBC) and platelet transfusions.

After about 6 months, she sought a second opinion and underwent testing for possible paroxysmal nocturnal hemoglobinuria (PNH) as a rare cause of pancytopenia. Her peripheral blood flow cytometric analysis of CD 55 and CD 59 showed deficiencies in both, diagnostic of PNH.^{1,2} She was started on eculizumab^{3,4} intravenously with a weekly loading dose schedule and then every 2 weeks at 900 mg. Her haptoglobin, as a marker of intravascular hemolysis, went from undetectable to normal within the first few weeks. However, the patient remained pancytopenic and both platelet and RBC transfusion dependency. A repeat BM BX after 6 weeks of eculizumab showed a cellular marrow with normal hematopoiesis with increased iron stores and normal cytogenetics and no abnormalities on fluorescence in situ hybridization (FISH). There was no evidence of MDS or aplastic anemia (AA).

She was continued on eculizumab and transfusion of RBC and platelets for hemoglobin < 8 and platelets < 15. After 4 months of this therapy, she was started on deferasirox,⁵ an oral iron chelator, at 20 mg/kg/day to try to ameliorate RBC transfusion related iron overload. After about 9 months of therapy with eculizumab, her blood counts began to slowly improve with an increase in absolute neutrophil count (ANC) from 0.2 to 0.3 (x 10³/uL) to 0.8 to 1.0 and an increase in platelets to 25-35 (x 10³/uL); she no longer required platelet transfusions. Her RBC transfusion requirement also decreased from one unit per week to one unit approximately every 3 weeks. To see if a further improvement in her baseline blood counts could be obtained, she has recently increased to 1200 mg of eculizumab every 2 weeks. She is being considered for a possible allogeneic stem cell transplant (allo-SCT), if necessary in the future.

Discussion

Paroxysmal Nocturnal Hemoglobinuria (PNH) is an acquired hematopoietic stem cell disorder in which a mutation in the PIG A gene (phosphatidylinositol glycan anchor biosynthesis, class

A) causes a deficiency in glycosyl phosphatidyl inositol (GPI), which anchors several proteins to the cell membrane in hematopoietic cells.³ Although the PIG A gene is on the X chromosome, this is an acquired abnormality and so affects both sexes. Estimates of incidence vary from 1-several per million and the median age at diagnosis is in the early 30s.³

PNH is associated with the specific loss on RBCs of CD 55, or decay accelerating factor which converts the active membrane bound complement factor C3b to the inactive C3d,¹ and of CD 59, which inhibits the complement membrane attack complex (MAC), C 5-9 that causes cell lysis.² Decreased RBC CD 55 and CD 59 result in chronic intravascular hemolysis and detection of decreased levels of expression on RBCs by peripheral blood flow cytometry is diagnostic for PNH. The deficiency of CD 55 and CD 59 can be partial in type 2 PNH RBCs or complete in type 3 PNH RBCs. This intravascular hemolysis can cause both hemolytic anemia that is non-immune and so Direct Coombs negative and iron deficiency from urinary loss of hemoglobin with bound iron. Eculizumab is a humanized monoclonal antibody that binds to membrane bound complement factor C5 and prevents its cleavage to C5 b and subsequent assembly of the MAC to prevent cell lysis. In particular, eculizumab rapidly inhibits intravascular hemolysis as was seen with this patient. Continued inhibition requires chronic administration of eculizumab.

PNH is also associated with pancytopenia, which can develop into aplastic anemia (AA) and the preferential outgrowth of the PNH clone and other hematopoietic subclones that can develop into MDS. Our patient had persistent albeit recently improving pancytopenia without evidence of either AA or MDS. The mechanism underlying pancytopenia is not well-understood, and it is not clear whether eculizumab can ameliorate pancytopenia, although this may be occurring in our patient. She no longer requires platelet transfusions, has an improved ANC, and has a decreased transfusion requirement beyond the initial effect of stopping intravascular hemolysis. Her eculizumab dose was escalated to try to obtain a further benefit and possibly RBC transfusion independence.

PNH additionally is associated with an increased risk of venous thromboembolic events (VTEs), in particular in unusual locations like inferior vena cava (IVC) and splenic or cerebral veins.³ One possible mechanism involves the free hemoglobin released in intravascular hemolysis causing decreased nitric oxide (NO), a potent natural vasodilator.³ Our patient

fortunately had no VTE with perhaps some protection with the suppression of hemolysis by eculizumab.

Allogeneic stem cell transplant (allo-SCT) is the only curative therapy for PNH. Allo-SCT is generally considered when patients have refractory pancytopenia or AA unresponsive to immunosuppressive therapy, like anti-thymocyte globulin (ATG) and cyclosporine (CSA) or high-risk MDS. Given improvements in supportive care, allo-SCT are being performed in patients into their 60s, particularly if they have a human leukocyte antigen (HLA)- matched sibling donor. Allo-SCT remains a potential future option.

REFERENCES

1. **Nicholson-Weller A, Spicer DB, Austen KF.** Deficiency of the complement regulatory protein, "decay-accelerating factor," on membranes of granulocytes, monocytes, and platelets in paroxysmal nocturnal hemoglobinuria. *N Engl J Med.* 1985 Apr 25;312(17):1091-7. PubMed PMID: 3845321.
2. **Parker CJ, Baker PJ, Rosse WF.** Increased enzymatic activity of the alternative pathway convertase when bound to the erythrocytes of paroxysmal nocturnal hemoglobinuria. *J Clin Invest.* 1982 Feb;69(2):337-46. PubMed PMID: 6915939; PubMed Central PMCID: PMC370983.
3. **Hillmen P, Young NS, Schubert J, Brodsky RA, Socié G, Muus P, Röth A, Szer J, Elebute MO, Nakamura R, Browne P, Risitano AM, Hill A, Schrezenmeier H, Fu CL, Maciejewski J, Rollins SA, Mojcik CF, Rother RP, Luzzatto L.** The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med.* 2006 Sep 21;355(12):1233-43. PubMed PMID: 16990386.
4. **Brodsky RA.** Paroxysmal nocturnal hemoglobinuria. *Blood.* 2014 Oct 30;124(18):2804-11. doi: 10.1182/blood-2014-02-522128. Epub 2014 Sep 18. Review. PubMed PMID: 25237200; PubMed Central PMCID: PMC4215311.
5. **List AF, Baer MR, Steensma DP, Raza A, Esposito J, Martinez-Lopez N, Paley C, Feigert J, Besa E.** Deferasirox reduces serum ferritin and labile plasma iron in RBC transfusion-dependent patients with myelodysplastic syndrome. *J Clin Oncol.* 2012 Jun 10;30(17):2134-9. doi: 10.1200/JCO.2010.34.1222. Epub 2012 Apr 30. PubMed PMID: 22547607.