Kids, colds, and complement: paroxysmal cold hemoglobinuria

Paroxysmal cold hemoglobinuria (PCH) is a relatively rare autoimmune hemolytic anemia (AIHA).[1,2] It is characterized by a weak-affinity, "biphasic" autoantibody that only binds red blood cells (RBCs) at low temperatures with recruitment and binding of early complement components. Although the antibody dissociates at higher temperatures, complement remains bound with complement activation and hemolysis. It has been proposed that the repetitive association and dissociation of antibody during normal circulation is a key element in the pathophysiology of the disease.[3]

Historically, PCH was a chronic condition associated with advanced syphilis. The first published case of PCH is generally attributed to Dr Dressler in 1854, who described intermittent hemoglobinuria in a 10-year-old child with congenital syphilis.[4] Over the next 50 years, several more cases of intermittent hemoglobinuria in syphilitic patients were documented, with episodes often precipitated by cold exposure. This observation led to two clinical tests—the Rosenbach test and the Ehrlich test.[4] In the Rosenbach test, an attack was precipitated by submerging the patient's feet in cold water for 10 minutes and close observation for the development of hemoglobinuria. In the Ehrlich test, a ligature was placed around one finger and the patient's hand was immersed in ice water. The presence of hemoglobinemia in the ligated finger was considered a positive test.

These two clinical tests informed the development of the Donath-Landsteiner (DL) test (1904), which remains the definitive test for PCH even today. In their assay, Donath and Landsteiner attempted to recapitulate the disease in vitro, by first incubating patient plasma and RBCs at 4°C, followed by warming at 37°C. Their findings clarified that PCH was caused by "a circulating hemolysin present in plasma, that the hemolysin was only able to bind red cells at low temperatures and that complement (alexin) was necessary for hemolysis or the 'second phase' of the disease."[4] Using the DL test, and the newly developed Wasserman assay for syphilis (1906), Landsteiner and others were able to confirm the strong correlation between PCH and syphilis during the early 20th century. It is interesting to note that multiple authors observed that children appeared to be particularly susceptible and often presented with more severe hemolysis than their adult counterparts.[4] Another clinical observation made by an early investigator was a paroxysmal enlargement of the spleen and sometimes the liver after a PCH attack, consistent with an element of extravascular hemolysis.[4]

As public health measures and antibiotics eliminated advanced syphilis, it became clear that PCH could occur in other conditions. By the 1950s, PCH was categorized as 1) chronic syphilitic; 2) chronic nonsyphilitic; and 3) acute, transient nonsyphilitic.[1] In 1963, Phillip Levine provided the first evidence that the antibody in PCH was directed against an antigen of the P-blood group system after testing six PCH samples against P_1, P_2, and p RBCs.[5] This specificity was refined by Worlledge and Rousso[6] in 1965, who identified an anti-P immunoglobulin (Ig)G after testing against P+ and P^k RBCs. After the identification of the glycosphingolipid (GSL) globoside as the P-antigen, Schwarting and colleagues[7] used purified globoside and Forssmann antigen to inhibit antibody-mediated hemolysis. A more recent article demonstrated direct binding of PCH serum to purified globoside and RBC neutral GSLs by thin-layer chromatography.[8] Although anti-P IgG is the most common antibody encountered in PCH, there are PCH cases with biphasic IgM and IgA antibodies,[9-12] and other carbohydrate specificities (I, i, HI, Pr).[1,11-15]

Today, PCH is predominantly a pediatric disease, accounting for 6% to 30% of all pediatric AIHAs.[3,16] It is almost always a postinfectious complication after an upper respiratory tract infection (URI; >70% cases) or gastrointestinal illness: other causes include vaccination, autoimmune disorders, and hematopoietic malignancies.[1-3] Pediatric PCH patients tend to be quite young (median age, 5 years) and male with a history of infection 1 to 3 weeks before presentation.[1-3] Hemolysis is often sudden and severe, with hemoglobin (Hb) values often decreasing to less than 6 g/dL.[1-3,17-19] Diagnostic findings are a C3+ direct antiglobulin test (DAT), the absence of warm autoantibodies, and a detectable biphasic DL hemolysin early in the disease.[2,3] On occasion, PCH can present with a negative DAT and positive DL test.[16,17] Despite recent infection, PCH is not associated with an increase in cold agglutinin titers.[3] Peripheral blood findings include spherocytosis and autoagglutination or rouleaux in 50% and 17% to 25% of cases, respectively.[1,9,17] RBC-neutrophil rosettes and erythrophagocytosis by neutrophils, which are usually cited as pathognomonic findings for PCH,[2] are encountered infrequently (9%).[1]

In this issue of **TRANSFUSION,** Prince and colleagues[19] describe a textbook-perfect case of PCH, and

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its workup, in a young male pediatric patient. Unlike most cases of pediatric PCH, this patient had two documented episodes of PCH almost 2 years apart. In both episodes, the patient had a prior URI, followed by hemolysis a few weeks later. While many laboratories (including my own) confine DL testing to P+ RBCs,[9,18] the authors performed the DL test with a five-cell panel that included I– and P– (p) RBCs. In both episodes, they were able to demonstrate an autoanti-P. During both admissions, the patient developed significant anemia with Hb levels of less than 5 g/dL; however, his second episode of PCH was unusually severe with high output cardiac failure, resistance to early oral steroid treatment, and circulating RBC-neutrophil rosettes and neutrophil erythrophagocytosis. He did recover but required aggressive RBC transfusion support (27 mL/kg in 24 hr), intravenous steroids, and intravenous immune globulin (IVIG), followed by oral steroids for many weeks. It is interesting to speculate whether his autoanti-P titer might have been higher upon restimulation.

It should also be noted that both PCH episodes were accompanied by reticulocytopenia during the first 2 to 4 days.[19] Severe anemia with a low or normal reticulocyte count has been documented in many PCH cases.[1,9,17-19] In one study, a third of children (8/25) less than 6 years of age had low or normal reticulocyte counts despite a mean Hb level of 6.3 g/dL (range, 4-9 g/dL).[1] This incidence of reticulocytopenia was twice the apparent rate in adult patients (1/7, 14%). In adult AIHA, a poor reticulocyte response is considered a bad prognostic sign, with mortality rates as high as 80% in older studies.[20,21] This level of clinical severity is not observed in pediatric patients with PCH, who typically experience a complete recovery within 1 to 4 weeks.[1,2] The relative reticulocytopenia observed in early PCH likely reflects the suddenness and severity of hemolysis, with depletion of the available reticulocyte pool in the marrow.[21] In addition, reticulocytes may be a target for autoantibodies in PCH:[19] globoside, Ii, and Pr antigens are present on reticulocytes.[22-24] Erythrophagocytosis is not a major contributor to anemia or reticulocytopenia, with only one known case of marrow hemophagocytosis attributed to PCH.[25]

One puzzle in PCH is the prevalence of anti-P in the disease. Proposed theories for the origin of autoanti-P have included viral modification of the RBC membrane with production of cross-reactive antibodies and/or altered immune regulation.[18] Many viruses and bacterial toxins bind glycoconjugates that are expressed on RBCs, including influenza, parainfluenza, HIV, parvovirus B19, and some strains of adenovirus.[26,27] At least one study has reported structural alterations of the RBC membrane after influenza binding to glycophorin and sialylated glycolipids.[28] Somewhat surprisingly, PCH is rarely, if ever, associated with parvovirus B19—a common febrile illness in children. A nonenveloped erythrovirus, parvovirus B19 recognizes RBC, erythroblasts, and other tissues via initial binding to globoside.[26]

A third hypothesis by which viral infection might induce an autoanti-P is stimulation of cross-reactive antibodies against the virus lipid envelope and/or viral glycoproteins.[7] Upon review, most of the viral infections associated with PCH are enveloped viruses belonging to the orthomyxovirus (influenza), paramyxovirus (mumps, measles, respiratory synctial virus, parainfluenza), and herpes families (chicken pox, Epstein-Barr, cytomegalovirus).[1,2,19] These viruses infect tissues of the respiratory tract, which are rich in globo-family GSLs including globoside.[29] Furthermore, many enveloped viruses require lipid rafts—membrane microdomains enriched in cholesterol and GSLs—for viral entry, assembly, and budding.[30-32] As a consequence, there is coexpression of viral antigens and host lipids on infected cells and secreted viral particles, which obtain their lipid envelope from the host cell membrane. In addition, viruses utilize host glycosyltransferases with expression of host-type glycosylation on viral glycoproteins.[33,34] Nor is the possibility of cross-reactive antibodies limited to viruses. Blood group–like epitopes have been described on many bacterial species,[26] including *Haemophilus influenzae*—a common cause of URIs that has also been linked with PCH.[1,35] Cross-reactive antibodies to bacterial polysaccharides may account for PCH following pneumococcal vaccination.[10]

Another puzzle is why complement-mediated cell damage is limited to RBCs despite widespread expression of globoside on other tissues and plasma.[29] In one respect, RBCs may be uniquely susceptible to autoanti-P due to the sheer quantity of globoside expressed on their membrane. Overall, globoside constitutes 60% to 70% of the total RBC glycolipid and 6% of the total lipid.[26] This level is two- to threefold higher than most other human tissues.[26,29] Moreover, globoside is irregularly distributed in large, confluent patches,[36] resulting in a high-density, multivalent epitope for antibody binding and complement activation. Finally, globoside expression is enhanced on RBCs in very young children due to immaturity of N-glycans on Band 3 and other RBC glycoproteins.[24,37]

Treatment for PCH is supportive, including keeping the patient warm and blood transfusion as needed. RBCs selected for transfusion should be leukoreduced, ABO, and crossmatch-compatible as appropriate. Because the PCH hemolysin only reacts at low temperatures, it does not interfere with compatibility testing. It is not necessary to wash RBCs or provide rare P– RBCs in these patients.[3] Given the binding characteristics of the antibody and the need to avoid chilling, it is common practice to use a blood warmer.

Many patients also receive steroid immunosuppression, although the efficacy and appropriateness of steroids in PCH is not clear.[1,3] Unusually severe hemolysis and/or recurrent chronic PCH has been treated with IVIG, azathioprine, or rituximab.[11,12,19,38] Surprisingly, eculizumab, which blocks complement at the C5 stage, may not be effective based on one case report.[39] Patients with severe, ongoing hemolysis requiring massive transfusion support and steroids are at risk for hypertension and volume overload. In these rare patients, it may be worthwhile to consider manual whole blood exchange, which would permit isovolemic RBC transfusion and removal of plasma and complement-coated RBCs.[40]

CONFLICT OF INTEREST

The author has disclosed no conflicts of interest.

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