Kids, Colds and Complement: Paroxysmal Cold Hemoglobinuria

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Manuscript: 1590 words

References: 40

Conflict of Interest: None

Paroxysmal cold hemoglolinuria (PCH) is a relatively rare autoimmune hemolytic anemia (AIHA).[1,2] It is characterized by a weak affinity, 'biphasic' autoantibody that only binds red cells 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/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 serum and red cells at 4C, followed by warming at 37C. 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 following 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

non-syphilitic and 3) acute, transient non-syphilitic.[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 RBC.[5] This specificity was refined by Worlledge and Rousso in 1965,

who identified an anti-P IgG after testing against P-positive and P^k red cells.[6] Following the identification

of the glycosphingolipid (GSL) globoside as the P-antigen, Marcus and colleagues used purified globoside

and Forssmann antigen to inhibit antibody-mediated hemolysis.[7] A more recent paper demonstrated

direct binding of PCH-serum to purified globoside and red cell 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 6-30% of all pediatric AIHAs.[3,16]

It is almost always a post-infectious complication following 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 1-3 weeks prior to presentation.[1-3] Hemolysis isoften sudden and severe,

with hemoglobin values often falling below 6 gm/dL.[1-3,17-19] Diagnostic findings are a C3+ 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/rouleaux in 50% and 17-25% of cases, respectively.[1,9,17] Red cell-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 et al describe a textbook-perfect case of PCH, and its workup, in a young male pediatric patient.[19] 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-positive red cells,[9,18] the authors performed the DL-test with a 5 cell panel that included I-negative and P-negative (p) red cells. In both episodes, they were able to demonstrate an autoanti-P. During both admissions, the patient developed significant anemia with hemoglobin levels below 5 gm/dL; however, his second episode of PCH was unusually severe with high output cardiac failure, resistance to early oral steroid treatment and circulating red cell-neutrophil rosettes and neutrophil erythrophagocytosis. He did recover but required aggressive RBC transfusion support (27 mL/kg in 24 hours), IV steroids, and IVIG, followed by oral steroids for many weeks. It is interesting to speculate whether his autoanti-P titer might have been higher upon re-stimulation.

It should also be noted that both PCH episodes were accompanied by reticulocytopenia during the first 2-4 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) under 6 years of age had low or normal reticulocyte counts despite a mean hemoglobin of 6.3 gm/dL (range, 4-9 gm/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 1-4 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 red cell 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 red cell membrane following 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 non-enveloped 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 co-expression 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 *H. influenzae* – a common cause of

upper respiratory tract infections 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 RBC despite widespread expression of globoside on other tissues and plasma.[29] In one respect, red cells may be uniquely susceptible to autoanti-P due to the sheer quantity of globoside expressed on their membrane. Overall, globoside constitutes 60-70% of the total red cell glycolipid and 6% of the total lipid.[26] This level is 2-3 fold 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 red cells in very young children due to immaturity of N-glycans on band 3 and other red cell glycoproteins.[24,37]

Treatment for PCH is supportive, including keeping the patient warm and blood transfusion as needed. RBC 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 RBC or provide rare P-negative RBC 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 red cell transfusion and removal of plasma and complement-coated red cells.[40]

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