Review: acute Donath-Landsteiner hemolytic anemia

A.F. EDER

The term paroxysmal cold hemoglobinuria (PCH) was first used in the early 1900s to describe immune hemolysis caused by the Donath-Landsteiner (DL) antibody, an IgG antibody with anti-P specificity that mediates biphasic hemolysis. The disease was aptly named for a recurrent complication in late-stage or congenital syphilis, observed as sudden attacks (paroxysms) of constitutional symptoms and hemoglobinuria precipitated by exposure to cold temperatures.[1(pp12–3)] Today, the diagnosis of chronic PCH in adults is extremely rare, its decline prompted by the advent of effective treatment for syphilis (Table 1). In contrast, the presence of DL antibodies should be immediately suspected in children with the abrupt onset of intravascular hemolysis that follows a recent viral or bacterial illness.[2–4] In children, the symptoms are not induced by cold exposure, resolve completely within a few weeks, and do not recur. Because the clinical presentation of acute disease in children differs from that of chronic PCH in adults, the condition is alternately referred to as Donath-Landsteiner hemolytic anemia (DL-HA).[3] Overall, immune hemolysis is an uncommon diagnosis in pediatrics, but DL antibodies are disproportionately implicated in 30 to 40 percent of cases of autoimmune hemolytic anemia in young children.[2–4] This review summarizes the clinical and serologic aspects of the acute transient form of hemolysis caused by DL antibodies in children.

Table 1. Classification of DL antibodies in a single institution[2]

Clinical classification	Age	Number (percent)
Acute (Acute transient nonsyphilitic PCH)	Children (< 13 years old)	30 (58%)
	Adult (> 27 years old)	14 (27%)
Chronic, nonsyphilitic PCH	Adult	3 (6%)
Chronic, syphilitic PCH	77 years old	1 (2%)
Unknown; incidental	67–82 years old	4 (7%)
		Total = 52

Pathogenesis

Julius Donath and Karl Landsteiner in 1904 were the first to attribute the temperature-dependent hemolysis in PCH to a cold-reacting autohemolysin and warm-reacting lytic factor.[1(pp12–3)] Their test for biphasic hemolysis after a cold-to-warm transition was the first immunohematologic test ever described and still serves as the definitive test for the Donath-Landsteiner autoantibody.[1(pp12–3)] The DL autoantibody is an IgG antibody that sensitizes RBCs at cold temperatures (< 20°C) by fixing the early components of complement, then dissociates from the RBCs at warmer temperatures. Complement activation is maximal at 37°C and proceeds to completion to produce hemolysis only after the warm incubation. Although initial incubation at low temperatures (0°C–20°C) is required for hemolysis in vitro, DL antibodies are also capable of sensitizing RBCs under physiologic conditions, even though peripheral body temperature rarely falls below 30°C. The mechanism by which DL antibodies produce such severe intravascular hemolysis is incompletely understood. Possible explanations include a heterogeneous population of DL antibodies with differential affinity in vivo and the ability of DL antibodies to initiate repeated cycles of complement activation by binding, dissociating, then reattaching to unaffected RBCs as blood circulates from cooler peripheral temperatures to warmer core temperatures.[2,3] In addition to the intrinsic characteristics of the DL antibody, patient-related factors, such as concurrent infection, may also contribute to an increased susceptibility to complement-mediated hemolysis in DL-HA.

The inciting stimulus for autoantibody formation is unknown, but infection or immune dysregulation may alter pathways that otherwise would suppress formation of antibodies against self-antigens.[5] Alternatively, pathogens may alter the RBC membrane to stimulate autoantibody formation or may possess antigens similar to those on RBC membranes so that

the resultant antibodies cross-react with RBCs (e.g., molecular mimicry).[6] DL-HA often occurs 1 to 3 weeks after a viral or bacterial infection and a wide variety of pathogens have been implicated in case reports (Table 2).[1(pp73–9)–4] More commonly, a specific etiologic agent is not identified, but an upper respiratory tract infection precedes the hemolysis in a vast majority of cases.[2,3] DL antibodies have specificity for the P antigen, a glycosphingolipid globoside that is also commonly expressed on microorganisms. The erythrocyte P antigen is the cellular receptor for parvovirus B19, and individuals who lack the P antigen (p or P_k phenotypes) are resistant to infection with the virus.[7,8] Acute infection with parvovirus B19 has been described in a child with DL-HA, but it does not appear to be a primary or underrecognized cause of DL-HA.[9,10] In a small case series of patients with DL antibodies, none had IgM antibodies against parvovirus B19, and IgG antibodies were not more common in the patients (7 of 13) compared to the control group (11 of 18).[10] Finally, several temporal clusters of acute DL-HA have been reported, but a common etiologic agent was not implicated in the cases.[2,4] Apparently, a wide variety of infections can trigger production of DL antibodies; moreover, increased awareness of the condition likely results in improved detection.[4] Rare examples of DL antibodies have been described in patients, typically adults, with other immunologic disorders, such as lymphoproliferative malignancies, collagen disease, myelodysplastic syndrome, delayed hemolytic transfusion reaction, and other types of autoimmune hemolytic anemia.[2,11–13]

Table 2. Pathogens implicated in case reports of acute DL-HA

Upper respiratory tract infection
Gastroenteritis, enteritis
Measles
Mumps
Chicken pox
Cytomegalovirus (CMV)
Epstein-Barr virus
Influenza virus
Adenovirus
Parvovirus B19
Coxsackie virus A9
Haemophilus influenza
Mycoplasma pneumoniae
Klebsiella pneumoniae
Measles vaccine

Clinical Presentation and Management

The typical presentation of DL-HA is a young child with a recent history of an upper respiratory tract infection or other acute illness associated with recurrent fever, and the passage of red-brown urine. Common characteristics in childhood cases from the two largest observational series describing DL antibodies are summarized in Table 3. DL-HA most often occurs in children under the age of 5 years (range, 8 months to 13 years).[2,3] In some reports, there is a slight male preponderance, with a male to female ratio of about 2:1.[2–4] Overall, DL-HA is an uncommon condition, but it may account for 30 to 40 percent of cases of autoimmune hemolytic anemia in young children.[2–4] The precise incidence and prevalence of DL-HA is unknown, and estimates based on small numbers of cases reported to blood services or reference laboratories, while informative, should be interpreted cautiously. Sokol et al. estimated the annual incidence of DL-HA as 0.4 per 100,000 for children under the age of 5 years.[2] The transient nature of the DL antibody, the level of awareness among primary care physicians, and the availability of the DL antibody are factors that potentially affect recognition and correct diagnosis of DL-HA.

The sudden onset of hemoglobinuria is reported in almost all cases of acute PCH, often accompanied by pallor, jaundice, and fever. The intravascular hemolysis and a rapidly progressing anemia may have a dramatic clinical presentation as high fever, shaking chills, and abdominal pain. Headache, nausea, vomiting, anorexia, and diarrhea may also occur. Physical findings included an enlarged liver and spleen in 25 percent of cases.[4] Typically, symptoms in children with acute DL-HA are not precipitated by cold exposure, in contrast to the presentation of chronic, syphilitic PCH in adults.

The degree of anemia is variable but may be severe in children with DL-HA. About one third of patients have hemoglobin concentrations of 5 g/dL or less (range: 2.5–12.5 g/dL) at presentation, which may decrease rapidly in the first 12 to 24 hours.[2–4] Differences in the severity of anemia among patients may reflect the characteristics of the DL antibody, such as serum titer or thermal range, or the interval of time between the onset of hemolysis and diagnosis. In addition, reticulocytopenia is often observed early in the course of the disease and the delayed hematopoietic response aggravates the initial anemia. Reticulocytopenia may result from viral suppression of the bone marrow or preferential destruction of reticulocytes by the DL autoantibody. However, the period of reticulocytopenia usually is brief and reticulocytosis commensurate with the degree of hemolysis promptly ensues. The peripheral blood

Table 3. Common findings in DL-HA in children

	Gottsche et al., 1990	Sokol et al., 1999
Number of cases (duration of study)	22 cases (4 years)	30 cases (37 years)
Patient age mean (range)	2.6 years (8 months–5 years)	3.5 (1–13 years)
Clinical presentation		
Percent (number) with precedent (< 3 weeks) infection	URI 77% (17/22) Other 23% (5/22)	URI 87% (26/30) Other 7% (2/30) None 7% (2/30)
Percent (number) with severe anemia (Hb < 5 g/dL)	27% (6/22)	40% (12/30)
Hemoglobin (g/dL)(range)	6.1 (4.4–8.8)	6.0 (3.4–12.9)
Percent (number) with hemoglobinuria	77% (17/22)	100% (30/30)
Serologic characteristics		
DAT		
Complement only	100% (22/22, at 37°C) 73% (16/22, at 20°C)	90% (27/30)
Complement + IgG	27% (6/22, at 20°C)	7% (2/30)
Not tested	–	3% (1/30)
Positive DL test		
Untreated RBC	59% (13/22)	93% (28/30)
Enzyme-RBC, only	41% (9/22)	3% (1/30)
Two-stage, only	–	3% (1/30)
Anti-P, if evaluated	NR	13/13 tested

URI = upper respiratory tract infection
NR = not reported

smear demonstrates RBC agglutination, polychromasia, nucleated RBCs, anisopoikilocytosis, occasional spherocytes, and erythrophagocytosis (Fig. 1). Erythrophagocytosis by neutrophils, rather than monocytes, is a relatively frequent phenomenon in PCH, but is rarely observed in other types of autoimmune hemolytic anemia.[14] The significance of this phenomenon to the RBC destruction that occurs is unclear, but extravascular hemolysis may contribute to the anemia and is further evidenced by occasional spherocytes on the peripheral blood smear. In addition, the monocyte monolayer assay (MMA) performed with mononuclear cells from patients with PCH demonstrated significantly more phagocytic activity than with those from normal individuals, suggesting enhanced reticuloen-dothelial cell function in the disease.[4] The practical significance of erythrophagocytosis, especially by neutrophils, in a young child is that it should trigger further investigation for DL-HA.[4] Additional serum chemistry findings in patients with DL-HA include increased LDH and unconjugated (indirect) bilirubin, decreased or absent haptoglobin, increased blood urea nitrogen (BUN), and increased serum creatinine.

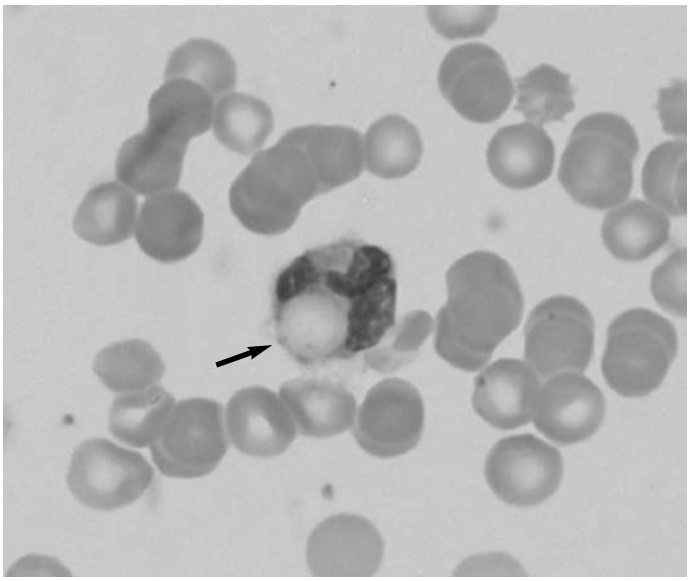

Fig. 1. Erythrophagocytosis in DL-HA. Peripheral blood from a 2-year-old girl with DL-HA and sudden onset of severe anemia (Hb 4.2 g/dL), showing erythrophagocytosis and RBC agglutination (100×; Wright stain).

Acute DL-HA usually resolves spontaneously and completely within several weeks and does not recur. There is a single case report of acute PCH occurring on two separate occasions in a child, each time after an upper respiratory tract infection, although the specificity of the antibody was not anti-P.[15] Treatment is supportive; glucocorticoids do not shorten the course of the disease. If glucocorticoids were started empirically for treatment of AIHA, they can be discontinued once the diagnosis of DL-HA is confirmed. Transfusion may be needed if anemia is severe.

DL antibodies do not interfere in routine pretransfusion and compatibility tests, because the causative autoantibody rarely causes RBC agglutination above 20°C. Patients with DL-HA typically demonstrate a DAT that is positive for complement, but negative for IgG, with a corresponding eluate that is negative. The antibody screen is usually negative, because DL antibodies do not react with RBCs under routine reaction conditions. DL antibodies may cause direct agglutination at 0°C or may be detected if the IAT is performed under strict conditions in the cold.

Although the specificity of the DL antibody is almost always anti-P, P– RBCs (i.e. p, P_1^k, or P_2^k) are extremely rare and are not available in routine practice for patients who require urgent transfusion. Fortunately, most patients with DL-HA who require

transfusion achieve a favorable clinical response and an adequate posttransfusion increment with P+ RBCs despite the presumed, or demonstrated, incompatibility. P– RBCs from rare donor registries have been used in case reports of PCH when hemolysis was severe and prolonged, although it is not clear whether transfusion of P– RBCs was beneficial or spontaneous recovery was coincident with transfusion.[16] Although cold-induced hemoglobinuria is rarely a feature of DL-HA, the patient should be kept warm during the transfusion and the use of a blood warmer is prudent despite the paucity of data that support this practice. Washing RBCs to remove residual complement may have theoretical benefit, but likely does not improve transfusion safety and often is not performed.[1(p396)] In rare cases of life-threatening anemia, plasmapheresis has been used to acutely remove IgG autoantibodies and alleviate symptoms.[17]

Donath-Landsteiner Antibodies

Donath-Landsteiner antibodies are typically IgG with anti-P specificity that demonstrate a low titer (< 32), a low thermal amplitude (< 20°C), and biphasic hemolysis. The vast majority of DL antibodies have these characteristics; however, the following exceptions have been described in case reports:

- *Specificity:* Biphasic IgG antibodies may demonstrate specificity for antigens other than P, such as anti-I, anti-p (anti-Gd), anti-i, and anti-"Pr-like."[1(p254)]
- *Thermal activity:* Biphasic IgG antibodies with anti-P specificity may demonstrate thermal activity above 20°C, agglutinate RBCs, or react by IAT at 37°C.[1(pp223–7),18–21]
- *Immunoglobulin subclass:* IgM antibodies may give a positive DL test, but these usually represent falsely positive results caused by monophasic hemolysis. Regardless, patients demonstrating IgM antibodies with biphasic hemolytic properties have been diagnosed as having PCH. The antibodies had anti-I, anti I^TP, or anti-P specificity.[1(pp195-6)]

Although there is no established consensus, diagnosis of DL-HA should require, at a minimum, a biphasic IgG antibody even if the specificity is not anti-P and evidence of intravascular hemolysis.[1(pp195–6)] The diagnosis of DL-HA should be questioned when the clinical history is atypical or the antibody is IgM or has other characteristics unusual for DL antibodies.

The IgG class of DL antibodies can be confirmed by performing the DAT under cold conditions, including cold washes to avoid eluting antibody. In addition, IgG may be demonstrated if IAT is performed in the cold with monospecific antihuman globulin reagents, although this test is susceptible to interference from normal cold agglutinins. In one report, a DL antibody was identified as IgG3, although it is not known whether this is a consistent finding in cases of DL-HA.[22] Finally, the MMA may be strongly positive in patients with PCH, suggesting the presence of IgG on the surface of their RBCs, and may be more sensitive than the IAT.[4,23]

Donath-Landsteiner Assay for Biphasic Hemolysis

The essential test for DL-HA is the Donath-Landsteiner assay for biphasic hemolysis, which can be performed either by the direct method using a sample of whole blood or by the indirect method using separated serum.[1(pp223–7),2] The patient's blood specimens must be maintained at 37°C after collection. Both assays first require an incubation of sample tubes in a melting ice bath (approximately 0°C) followed by an incubation at 37°C. The endpoint is the presence or absence of visible hemolysis in the plasma compared to control tubes kept at a constant temperature (0°C or 37°C). The direct test is an easy screening test but suffers from several limitations, as it requires more whole blood and is less sensitive than the indirect test.[4] Autologous RBC lysis may not be observed because of the protective effect of C3dg deposited on the surface of circulating RBCs during the hemolytic episode. Moreover, recent or ongoing hemolysis may deplete serum complement so that RBC lysis is not detected in the direct assay (Table 4).

The indirect test is performed with the patient's serum which has been maintained and promptly

Table 4. Limitations of DL tests

Falsely-negative results
Direct DL test (lysis of autologous RBCs)
Low antibody titer
Low serum complement
Resistance to lysis due to C3dg
Indirect DL test (lysis of reagent RBCs)
Low antibody titer
Inhibition of DL antibody by globoside in normal serum
Autoadsorption of antibody during serum separation
Falsely-positive results
Direct and indirect DL test
Monophasic lysis by an IgM autoantibody

separated at 37°C. A common method used for the DL indirect assay requires three sets of three tubes (Fig. 2).[24] The first set contains the patient's serum (row 1), the second set contains the patient's serum and fresh normal serum as an added source of complement (row 2), and the third set contains fresh normal serum as a control (row 3). Reagent ABO-compatible RBCs are added to all tubes. One tube from each set is incubated in a melting ice bath (approximately 0°C), then transferred to 37°C. The control tubes in each set are kept at a constant temperature, either 37°C or 0°C. Visible hemolysis in the patient's samples, with or without the additional complement, and the absence of hemolysis in all control tubes is a positive test result. Although maximal hemolysis is observed when the initial incubation is 0°C, DL antibodies in acute PCH may have thermal activity up to 24°C, but rarely cause lysis with higher temperatures in the initial incubation.

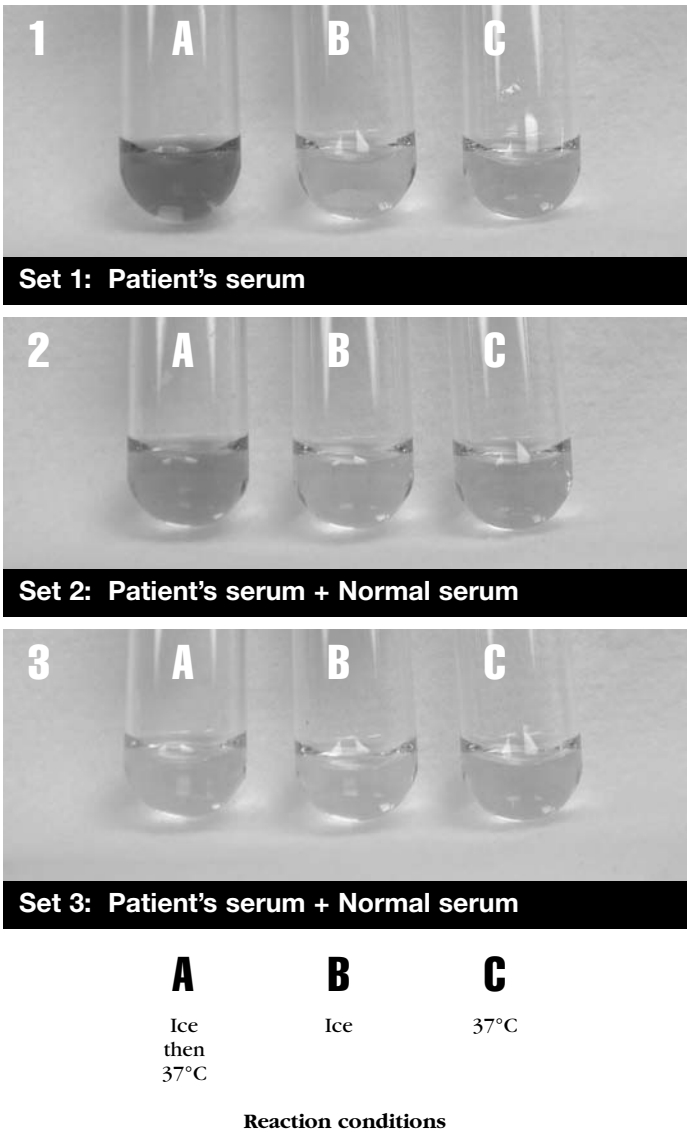

Fig. 2. Indirect Donath-Landsteiner test: sample conditions.

If RBCs with the p or P^k phenotype are available, they can be used as a negative control and should remain intact in the assay, confirming the expected anti-P specificity of the DL antibody. If an antiglobulin test is performed on the unlysed cells, antibody binding to P+ but not to p or P^k RBCs will be demonstrated. Because these RBCs are often not available, this additional testing may not be possible.

The most common cause of negative DL tests in suspected cases of DL-HA, with a classic clinical presentation and otherwise consistent laboratory findings, is the failure to detect the transient autoantibody that disappears quickly from the plasma during recovery from the acute illness. DL antibody titers quickly wane soon after recovery from the initial hemolytic episode. False-negative results may occur in both the direct and indirect test owing to low antibody titers. Because the indirect test evaluates the patient's serum only, false negatives may result from autoabsorption of antibody during serum separation or inhibition of antibody by neutralizing carbohydrate antigens (e.g., globoside) present in the added fresh serum (Table 4).

If the diagnosis of DL-HA is strongly suspected, but the indirect DL test is negative, the biphasic hemolysis test can be modified to increase its sensitivity by treating the reagent RBCs with enzymes, by performing a two-stage assay, or by testing for the DL antibody by the IAT. In addition, interpretation of the DL test may be difficult if the initial sample drawn for analysis is hemolyzed. In these cases, the extent of additional hemolysis during the assay may be gauged by the size of the red cell button after centrifugation. Alternatively, the two-stage test can be performed. In rare patients with classic DL-HA, the DL test was only positive with one of the following modified tests (Table 3)[2,3]:

- *Enzyme-treated RBCs:* The reagent RBCs may be treated with enzymes, such as 1% papain, which exposes more P antigen on the RBC membrane. Enzyme treatment, however, makes the RBCs more susceptible to lysis by cold agglutinins, and careful interpretation of appropriate controls is necessary.
- *Two-stage test:* For the two-stage test, the patient's serum is replaced with normal ABO-compatible serum after the first incubation at 0°C. Because antibody binding to the RBC occurs

only in the first phase, the patient's serum can be removed after this incubation occurs. Serum replacement not only removes the patient's serum that may have been hemolyzed at baseline, but also provides additional complement for the second phase of the assay at 37°C without diluting the sample.

- *IAT:* DL antibodies may be detectable by the IAT with antihuman globulin after the cold incubation with monospecific reagents.[1(p224)] However, interpretation of the test is problematic because the DL antibody or coincidental IgM antibody may cause direct agglutination at 0°C, and normal incomplete cold antibody may give false-positive results. A negative control with p or P_k phenotype RBCs should be used because of the propensity for false-positive results.

Conclusion

The diagnosis of DL-HA is readily made in most cases, but requires an index of clinical suspicion to initiate investigation, because the DL test is not performed routinely by most transfusion services. Despite the excellent reviews that have appeared in recent journals, the diagnosis is often still delayed, which confounds demonstration of the pathognomonic DL antibodies. A young child with a precedent viral illness and the sudden onset of intravascular hemolysis strongly suggests a diagnosis of DL-HA, even in light of a negative DL test. The most common cause of negative DL tests in acute cases is the failure to detect the transient autoantibody that disappears quickly from the plasma soon after the initial hemolytic episode. Drug-induced hemolysis (e.g., ceftriaxone), or possibly transfusion-associated hemolysis, may be associated with acute intravascular red cell destruction like acute DL-HA, but can be distinguished by the clinical history and immunohematologic findings.[2,25] On occasion, a cold-reactive IgM autoantibody may demonstrate apparent biphasic behavior; conversely, a DL antibody may appear to be monophasic because its thermal amplitude is close to physiologic temperatures. DL-HA is differentiated from cold agglutinin syndrome on clinical grounds and laboratory findings.[1(p196)] Although a rare disease, and rarely a diagnostic dilemma, DL-HA nonetheless requires prompt recognition for laboratory diagnosis and appropriate clinical management.

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Anne F. Eder MD, PhD, Executive Medical Officer, Biomedical Services, National Headquarters, American Red Cross, 2025 E Street NW, Washington, DC 20006.