

Management of cold haemolytic syndrome

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Summary

Most haemolytic disease is mediated by immunoglobulin G (IgG) antibodies and leads to red blood cell destruction outside of the circulatory system. However, rare syndromes, such as paroxysmal cold haemoglobinuria, show IgG antibodies causing intravascular destruction. Haemolysis may also occur because of immunoglobulin M antibodies. Historically, these antibodies have been termed 'cold agglutinins' because they cause agglutination of red blood cells at 3°C. Cold agglutinin haemolytic anaemia has been associated with a number of autoimmune and lymphoproliferative disorders, and its management differs substantially from warm antibody-mediated haemolytic anaemia. This review of cold haemolytic syndromes describes new therapies and clinical strategies to determine a correct diagnosis.

Keywords: cold agglutinin, IgM monoclonal protein, MGUS, paroxysmal cold haemoglobinuria, Waldenström macroglobulinaemia.

Paroxysmal cold haemoglobinuria

Paroxysmal cold haemoglobinuria was first recognised as a distinct clinical entity in 1872 (Crosby, 1952), and the haemolytic antibody was described by Donath and Landsteiner (1904). Landsteiner later received the 1930 Nobel Prize in Medicine for his discovery of the ABO blood groups (Win *et al*, 2005). The antibody first was observed as a cross-reacting antibody to an antigen on *Treponema pallidum* and frequently was detected in patients with secondary or tertiary syphilis. Obviously, this is now rare. Today, it is identified in children with postviral illness, much like immune thrombocytopenic purpura. Albeit severe, paroxysmal cold haemoglobinuria is not persistent and generally is not a chronic disorder.

The test for paroxysmal cold haemoglobinuria [detection of the haemolytic (anti-P) antibody] is simple to perform. In its

original description, 2 aliquots of the patient's red blood cells are collected. One is incubated for 60 min at 37°C, and the other is incubated for 30 min at 3°C and then 30 min at 37°C. The blood is centrifuged, and the plasma is inspected for evidence of haemoglobin. Although it was not understood at the time the test was developed, complement-mediated haemolysis in the control tube (with never-chilled blood) does not occur because anti-P antibodies do not bind the red blood cell surface and complement is never fixed. In the second tube, incubation at 3°C allows binding of the polyclonal anti-P antibody and complement fixation. Subsequent warming to 37°C allows complement activation (C4 through C9), red blood cell lysis and intravascular haemoglobin release.

False-negative findings may occur with the Donath–Landsteiner test. If additional complement is not added to a blood specimen, lysis of cell membranes may not occur. Modern iterations of the Donath–Landsteiner test add complement to the specimens to guard against its depletion (a consequence of prior consumption by the antibody). The anti-P antibody also may be consumed by haemolysis. Further modifications of the assay include incubation of papain-treated group-O cells with the plasma. Papain digests the red blood cell membrane and exposes more P-antigen, thereby allowing the detection of low-titre antibodies. Finally, globoside in the serum may neutralise the anti-P antibody and cause a failure of reactivity. In a report of four patients (Sokol *et al*, 1998), two showed strong positive findings for the Donath–Landsteiner antibody. One was positive only when papain-treated red blood cells were used; for the other, a two-stage procedure was required to obtain a positive result. This report emphasised that initial tests may show negative results.

In a review of 52 patients with paroxysmal cold haemoglobinuria seen over 37 years (Sokol *et al*, 1999), patient age was as high as 82 years, but the median age was 5 years. The incidence rate was 0.4 cases per year per 100 000 people. Forty-four had acute transient paroxysmal cold haemoglobinuria, three had chronic disease, and one had syphilis-associated disease. For the remaining four, the positive Donath–Landsteiner test results were incidental findings without haemolysis. Acute paroxysmal cold haemoglobinuria typically occurred in young children with pallor, haemoglobinuria and mild jaundice. Patients generally had a history of recent viral infection, and no obvious association with

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exposure to cold was identified. Most patients completely recovered within 1 month. Sixty-eight per cent required blood transfusions, and many were treated successfully with prednisolone. Three patients with chronic paroxysmal cold haemoglobinuria did have true paroxysms that were induced by exposure to cold, and avoidance of cold was an effective therapy. Fifty of the 51 who were tested with the direct antiglobulin (Coombs) test had positive findings of C3d coating the red blood cells. The Donath–Landsteiner antibodies were in the immunoglobulin G (IgG) class, but this was shown only when assays were performed at 4°C. Twenty-seven of 30 patients had classic anti-P antibody specificity. Some haemolytic episodes occurred in patients with lymphoproliferative disorders, collagen vascular diseases and delayed haemolytic transfusion reactions. Many patients with unexplained haemolytic anaemia may have undiagnosed paroxysmal cold haemoglobinuria, particularly if they had a negative direct antiglobulin test.

Paroxysmal cold haemoglobinuria has been associated with varicella (Papalia & Schwarer, 2000) and with agnogenic myeloid metaplasia in a patient who also had an antiphospholipid antibody syndrome (Breccia *et al*, 2004). In a study of nearly 600 patients with immune haemolytic anaemia (Gottsche *et al*, 1990), the Donath–Landsteiner antibody was detected in 22 of 68 children (32.4%). All patients had an antecedent acute infection of viral origin. Haemolytic episodes were severe but resolved within a few weeks. All had a strongly positive direct antiglobulin (Coombs) test with anti-C3d antibodies but weak or negative results from IgG direct-acting Coombs' tests. The Donath–Landsteiner antibodies produced barely detectable haemoglobinaemia and were transient; however, they were detected with papain-treated red blood cells. When the red blood cells were untreated, the antibody was detected in only 12 of 22 patients. Paroxysmal cold haemoglobinuria may be recurrent (Taylor *et al*, 2003; Evans-Jones & Clough, 2004).

In children, the severity of the anaemia has resulted in sudden death. Therapy usually is expectant because the syndrome is self-limiting. Corticosteroid therapy may be successful, and, in adults, the syndrome has been treated with cyclophosphamide. Splenectomy has no role in treatment because the haemolysis is intravascular. One patient with paroxysmal cold haemoglobinuria after viral gastroenteritis underwent a 3-d course of total plasma exchange for haemolysis that was associated with a presenting haematocrit value of 12% (Roy-Burman & Glader, 2002). Transfusion therapy with 9 U of packed cells did not improve the anaemia, but after plasmapheresis was initiated, no further transfusions were required. Given the transient and relatively brief production of Donath–Landsteiner antibodies after a viral infection, clearance of the antibody may be possible with plasma exchange and may not cause a rebound in antibody production.

The degree of haemolysis may vary among patients because the characteristics of the haemolytic antibody are highly

variable. The amount of the antibody and the affinity of the antibody for the red blood cell surface are important determinants of the severity of haemolysis. Furthermore, some antibodies may fix complement less efficiently. Several explanations have been offered to explain why IgG antibodies result in intravascular haemolysis when the Ig antibodies of cold agglutinin disease do not. The Donath–Landsteiner antibodies are able to fix C4, but not C3, in the cold; however, the importance of this effect *in vivo* is unknown. Antigens of the Donath–Landsteiner antibodies may be closer to the membrane surface, thereby facilitating the fixation of complement, but, generally, the reasons for the differences in lytic action between paroxysmal cold haemoglobinuria and cold agglutinin haemolysis are still unknown.

Cold agglutinin haemolysis

Landsteiner also recognised that blood might autoagglutinate at 3°C (Landsteiner, 1903). The association between agglutination and respiratory disease was noted as early as 1918 (Clough & Richter, 1918). Horstmann and Tatlock (1943) reported that these agglutinins were detected in the serum of patients with primary atypical pneumonia. It was Marsh and Jenkins (1960) who first found two patients whose sera agglutinated adult I-negative cells and fetal i cells strongly but agglutinated normal (I) adult cells weakly. Marsh (1961) reported that erythrocytes from neonates had the i phenotype, whereas children aged 18 months and older had the I phenotype.

The literature has emphasised that patients with intravascular haemolysis may be distinguished from those with extravascular haemolysis by serum haptoglobin levels (Tabbara, 1992), but this idea recently was questioned. A study of 479 individuals, 16 with haemolytic disease, showed that all had markedly decreased plasma haptoglobin levels, and no differences were identified among patients with intravascular haemolysis and predominantly extravascular haemolysis (Kormoczi *et al*, 2006). Patients with a strongly positive direct Coombs' test or a high cold agglutinin titre but no further evidence for haemolysis had restoration of normal haptoglobin levels.

Increased amounts of red blood cell-bound C3b and C3d are caused by autoantibody activity. C3b is a marker of immunoglobulin M (IgM)-mediated complement binding. A sensitive, two-stage, enzyme-linked direct antiglobulin test has been described for the detection of C3b and C3d on the red blood cell surface (Bellamy *et al*, 1997). An enzyme-linked direct antiglobulin test is considerably more sensitive than the standard polyclonal agglutination studies for detection of red blood cell-bound C3b and C3d. In another study (Sokol *et al*, 2000), 221 patients with cold haemagglutinins with a thermal amplitude of 30°C or higher were classified into three groups. Group 1 included 116 patients with cold agglutinins but no haemolysins. Group 2 had 74 patients with only monophasic haemolysins. Group 3 had 31 patients with monophasic and

biphasic haemolysins. Patients in Groups 2 and 3 had lower haptoglobin levels. The Group 3 patients typically were elderly; most had chronic cold haemagglutinin disease, and several also had lymphoproliferative disorders. The condition remained active for long periods, but some required no treatment. Blood transfusion support was required when haemolysis was severe.

Between 1960 and 2005, physicians at Mayo Clinic have treated 34 633 patients with monoclonal gammopathies. Approximately 16% of these patients had an IgM monoclonal protein. Under the current classification scheme developed at the Second International Waldenström Workshop (Owen *et al*, 2003), patients with cold agglutinin-associated IgM are classified as having an IgM-related disorder that is distinct from IgM monoclonal gammopathy of undetermined significance and from symptomatic and smouldering Waldenström macroglobulinaemia. Patients with an IgM-related disorder may have haemolysis mediated by the IgM protein. Other IgM-related disorders that are separate from cold agglutinin disease include cryoglobulinaemia and amyloidosis. Cold agglutinin disease accounted for only 60 of the 34 000 monoclonal gammopathies (0.17%) treated at our institution, but it represents 1.1% of the 5405 patients with IgM monoclonal proteins. Cold agglutinin-mediated haemolysis by a monoclonal antibody was first reported by Dacie *et al* (1957); these were the first monoclonal proteins shown to have antibody activity (Christenson *et al*, 1957). Low-titre cold agglutinins may be found in the sera of healthy adults and may have no clinical consequences. Immune haemolytic anaemia with cold agglutinins occurs in 1 of 100 000 patients; the peak patient age is the seventh decade of life (Hadnagy, 1993; Mack & Freedman, 2000), which corresponds to the age of peak prevalence of monoclonal proteins. Adults with high-titre cold agglutinins have monoclonal proteins in the serum that may be removed by incubating the patient's serum with red blood cells bearing the I antigen (Christenson & Dacie, 1957).

Pathogenesis of cold agglutinin disease

The term 'cold agglutinin' is somewhat misleading because it implies a clear relationship between cell agglutination and cold exposure. The term is derived from the immunological properties of the disorder. Agglutination from warm antibody-mediated haemolytic anaemia requires incubation of red blood cells, antibody-containing serum and antihuman globulin antisera at 37°C (warm) for 2 h. Antiglobulin antisera (Coombs' antibody) are necessary to overcome electrostatic repulsive forces of red blood cells. The Coombs' antibody binds IgG on the red blood cell surface and leads to agglutination that is visible to the naked eye. In cold agglutinin disease, however, because the IgM molecule that mediates haemolysis has a molecular weight of 1 000 kD, the antibody can span the intercellular distance between red blood cells and cause agglutination at 4°C (cold), even when antiglobulin antisera are absent.

A transgenic strain of mice that expresses a pathogenic human cold agglutinin has been developed (Havouis *et al*, 2002). Experimental infections of these mice with mycoplasma resulted in a high antimycoplasma antibody response and induction of cold agglutinin production in the serum. This animal model mimics the induction of cold agglutinins in humans and is used to study the genesis of cold agglutinin anaemia. A second animal model consists of clones expressing genetically engineered pentameric IgM κ , which mimics the characteristics of cold agglutinins. Intraperitoneal injection of the antibody in BALB/c mice induced a typical haemolytic reaction (Dumas *et al*, 1997).

A study of 15 patients with chronic cold agglutinin disease (Ulvestad *et al*, 1999) showed that all had an IgM κ cold agglutinin encoded by *IGHV4-34* (the V_{H4-34} gene). Seven had reduced concentrations of T cells. CD56 and CD19 were increased in seven and three patients respectively and clonal κ -positive B cells were found in six of nine. Serum C3 levels were decreased in nine patients and C4 levels were decreased in 11, reflecting continuous low-grade complement consumption. Previously, *in vitro* experiments showed that the addition of active complement to a patient's serum increased haemolytic activity (Ulvestad, 1998); this suggested that haemolysis could be aggravated by increased complement production during an acute-phase reaction or by use of plasma-containing blood products. A follow-up study confirmed that the availability of complement determines the severity of the haemolysis and that increased complement synthesis after an acute-phase response may precipitate an increase in the severity of haemolysis (Ulvestad *et al*, 2001). Virtually, all anti-I cold agglutinins are IgM proteins, but cold agglutinins may also be anti-Pr antibodies that consist of IgG and IgA proteins. Haemolysis with IgM and IgG cold agglutinins is complement-mediated, but it is less severe in the IgG form of cold agglutinin disease (Roelcke *et al*, 1998).

In warm haemolytic anaemia, IgG molecules coat the red blood cell, and fragments of the cell membrane are removed during sequential passes through the spleen. The surface area of the red blood cell decreases gradually until it forms a spherocyte. Except in the severest forms of cold agglutinin disease, haemolysis tends to be extravascular. IgM proteins with high thermal amplitude bind the red blood cell surface in the cool peripheral circulation and fix complement proteins to the cell surface. Although IgM is no longer bound to the cell surface at 37°C, complement activation has caused the coating of the erythrocyte with C3b. Unlike the mechanism of paroxysmal cold haemoglobinuria, the complement fixed to the red blood cell in cold agglutinin disease does not induce complement-mediated intravascular lysis. Rather, C3b remains on the surface, binds to receptors in the mononuclear macrophage system in the liver, spleen, lungs and lymph nodes, and causes spherocyte formation and extravascular red blood cell destruction. Some cells escape destruction, and the surface complement is cleaved to C3d, which is resistant to further haemolytic action. Splenectomy is not considered an

effective therapy for most patients because the heavily complement-coated red blood cells are efficiently removed by hepatic Kupffer cells.

Routine screening for cold agglutinins in adults has shown that a high proportion of the population have agglutinins with low-thermal amplitude, reflecting their usually benign cause. Cold antibody-mediated haemolysis is estimated to occur in approximately 10% of patients with acquired immune haemolytic anaemia (Salama, 2004). When sera from 172 patients with IgM monoclonal proteins were assayed, 10 had cold agglutinin activity (5.8%) (Stone *et al*, 2003).

Studies of gene usage have shown that pathological cold agglutinins with anti-Pr sensitivity had a limited repertoire of Ig light-chain subgroups. Cold agglutinins with anti-I specificity exclusively used the *IGHV4-34* product in heavy chains. Five of 13 light chains were variable κ IV, five of 13 were variable κ I, and three of 13 were variable κ III. The single germline gene-derived subgroup variable κ IV was predominant (eight of 17) (Leo *et al*, 2004). Expression of *IGHV4-34* also was described in a patient with cold agglutinin disease who was subsequently diagnosed with chronic lymphatic leukaemia (Ruzickova *et al*, 2002). Unlike other blood group antigens, the I and i antigens are not produced by allelic pairs but are reciprocal (Reid, 2004). The I antigen is formed by an enzyme that adds branches to the i antigen; thus, I antigens are formed at the expense of the precursor i antigen. These antigens are present on all blood cells and are widely distributed in tissue.

Although most patients lack specific physical findings, livedo reticularis, acrocyanosis and Raynaud phenomenon have been suggested in the literature (Lauchli *et al*, 2001). Specific cytogenetic disorders, such as trisomy 3 (Michaux *et al*, 1995) and t(8;22) (Chng *et al*, 2004), are associated with cold agglutinin disease. Three patients with low-grade lymphoma associated with cold agglutinin syndrome were reported to have trisomy 3 q11-q29 (Michaux *et al*, 1998). In an overview of patients with IgM monoclonal protein disorders, cold agglutinins were identified in three of 105 (2.9%) (Owen *et al*, 2001). A population-based study of 86 patients (Berentsen *et al*, 2006) reported a prevalence of 16 per million and an incidence of one per million. The median age was 67 years, and the median survival was 12.5 years. Autoimmune diseases were identified in 8%, cold-induced symptoms in 91%, and increased evidence of haemolysis during febrile illnesses in 74%. Over half of the patients received red blood cell transfusions. The median monoclonal protein level was 40 g/l. Ninety per cent of patients had monoclonal IgM, whereas 3.5% of patients each had IgG or IgA; 94% had κ light chains. Lymphoplasmacytic lymphoma was seen in 50% of patients.

Immunoglobulin M antibodies resulting in haemolytic disease may be polyclonal (generally occurring in postinfectious children) or monoclonal (associated with adults with classic cold haemagglutinin disease). The polyclonal disorder is associated with viral infections, most commonly in children,

and is usually self-limiting but may require transfusional support (Finland & Barnes, 1958; Horwitz *et al*, 1977). Postinfectious cold agglutinins most notably are associated with mycoplasma pneumonia and infectious mononucleosis. Cold agglutinin disease, however, has been described and associated with systemic lupus erythematosus (Kotani *et al*, 2006), Epstein-Barr virus infection (Dourakis *et al*, 2006) and pregnancy (Batalias *et al*, 2006). Low-titre cold agglutinin disease has been associated with systemic sclerosis (Oshima *et al*, 2004) and after mycoplasma infection in a patient who already had chronic haemolysis from haemoglobin SC disease (Inaba *et al*, 2005).

A number of reports describe cold agglutinin disease developing after allogeneic bone marrow transplantation. One patient had an identified adenovirus infection (Mori *et al*, 2005). One paediatric patient had recurrent cold agglutinin disease after allogeneic transplantation (Azuma *et al*, 1996). Cold agglutinin disease has also been described in patients with solid tumours (uterine sarcoma) (Cao *et al*, 2000). Cold agglutinin disease has been associated with varicella (Terada *et al*, 1998) and legionella pneumonia (King & May, 1980). Cold agglutinin disease also was described in two of three patients with splenic marginal zone lymphoma (Murakami *et al*, 1997).

A report of a 22-year study described 18 patients with cold agglutinin disease (Go *et al*, 2005). The median patient age was 72 years. Although all had haemolysis, only two had physical signs. Eight patients had a concomitant lymphoproliferative disorder, and monoclonal proteins were identified in five. Therapy was required by 72%, which included corticosteroids for nine and cyclophosphamide for three. The steroid-related response rate was 78%, and the median response duration was 95 months. No patients had a response to cyclophosphamide. After a median follow-up of 56 months, eight were alive and five were in complete remission. Only one died of cold agglutinin disease. Lymphoproliferative disorder subsequently developed in two patients. This series was unique in the high rate of response to corticosteroid therapy.

Some patients will have acrocyanosis, but, for most, haemolytic anaemia is the only clinical manifestation. Recommendations to avoid cold exposure are primarily anecdotal, and the benefits of protection from cold are not as well established as they are for patients with cryoglobulinaemia. Haemolytic anaemia that is associated with monoclonal cold agglutinins is a more serious condition than that of polyclonal cold agglutinins because the former typically is chronic and sustained, whereas the latter is self-limiting. Cold agglutinin haemolysis tends to be more resistant to therapy than warm antibody-mediated haemolysis because the density of complement on the surface of the red blood cells is relatively high. Traditional therapies that inhibit IgG binding or reduce the number of available mononuclear phagocytic cells, such as splenectomy, therefore are less effective for cold agglutinin haemolysis.

The diagnosis of cold agglutinin disease may be suggested by occasional laboratory clues, such as extreme macrocytosis that results from agglutinated red blood cells. Cells may be passed through an automated particle counter, which can record a mean corpuscular volume as high as 140 fl (Kakkar, 2004). The typical laboratory features of haemolysis are hyperbilirubinaemia, elevated levels of lactate dehydrogenase and a positive direct antiglobulin test. A report from one institution described positive findings for the direct antiglobulin test for C3 in 74% of patients with cold agglutinin disease, C3 and IgG in 22%, and IgG alone in 3% (Chandesris *et al*, 2004). Seventy-eight per cent of patients with cold agglutinin disorders had an autoimmune disorder, an infection, or a lymphoproliferative disorder. Clinical haemolysis has been reported for patients with cold agglutinin titres as low as 1:64 (Schreiber *et al*, 1977), although most patients who have cold agglutinin disease have titers in excess of 1:1000.

Cold agglutinin disease can occur with antibodies that are not directed against the I/i system; they usually recognise the Pr antigen. Gene usage studies of anti-Pr cold agglutinins indicate a prevalent use of the light-chain variable domain κ IV (Leo *et al*, 2004). Bone marrow studies of 68 patients with cold agglutinin disease showed light-chain dominance in 90%, lymphoplasmacytic lymphoma in 50%, and any form of lymphoma in 76% (Berentsen *et al*, 2006). The histological findings included splenic marginal zone lymphoma, lymphoplasmacytic lymphoma and small lymphocytic lymphoma. The IgM monoclonal proteins were modest in size. Only half the patients were transfusion-dependent; these patients had a median cold agglutinin titre of 1:2048.

Haemolysis associated with a cold agglutinin disorder should not be confused with the normochromic normocytic anaemia of Waldenström macroglobulinaemia. If criteria for Waldenström macroglobulinaemia are not met (>10% lymphoplasmacytic cells in the bone marrow, IgM monoclonal protein of any size), cold agglutinin disease should be considered as the diagnosis. The Second International Workshop on Waldenström Macroglobulinaemia (Owen *et al*, 2003) defines cold agglutinin disease as an entity that is distinct from Waldenström macroglobulinaemia and categorises it as an IgM-related disorder, similar to cryoglobulinaemia, IgM-associated neuropathy and amyloidosis. Cold agglutinin haemolysis has been reported after treatment with ceftriaxone (Johnson *et al*, 2004). Transfusion may be required for patients with cold agglutinin disease. Cross-match testing is often difficult to perform because all red blood cells are reactive. Autoabsorption at cold temperatures is the method of choice to resolve the cases of suspected cold autoantibodies. In many instances, the use of type-specific red blood cells is required because of the reactivity of all panels (Nance & Arndt, 2004).

Eighteen patients with cold agglutinin disease were described in a report from a single institution (Go *et al*, 2005). Patients had a median age of 72 years. All had haemolysis, but only two had acral signs of disease. The median haemoglobin level was 84 g/l. A lymphoproliferative disorder was diagnosed

in eight, monoclonal IgM proteins were found in five and 13 of the 18 patients required therapy. The response rate to corticosteroid therapy was 78% and was durable. No patients responded to cyclophosphamide. After a median follow-up of 56 months, five patients had a complete response and three had partial responses. Ten patients had died by the time of report publication: one had ischaemic cardiac disease attributable to anaemia, and two had lymphoproliferative disorders. The clinical course was benign and was distinct from other reports because of the effectiveness of prednisone therapy.

Treatment of cold agglutinin haemolysis

The therapy for cold haemolysis has closely paralleled developments in the therapy of warm antibody-mediated haemolysis. As a consequence of splenectomy, patients may be treated with corticosteroids, Ig infusions, or cytotoxic or immunosuppressive medications. Generally, the durable response rate for therapy of cold haemolysis has been lower than that reported for the corresponding management of warm-antibody disease. The response to therapy may be partly determined by the density of antibody and complement on the red blood cell surface. In cold haemolysis, the number of C3 molecules on the red blood cell surface is much greater (Jaffe *et al*, 1976) than in warm-antibody disease (Schmitz *et al*, 1981). As a result, therapy is generally less effective, and responses have a shorter duration. The routine failure of corticosteroid therapy for cold-antibody disease is in striking contrast to its efficacy in warm-antibody disease (Frank, 1977). In a prospective study of 20 patients treated with rituximab, seven of whom had an associated malignant B-cell disorder, a response rate of 45% was observed, and one patient had a complete response (Schollkopf *et al*, 2006). Eight patients subsequently relapsed.

Surgical procedures that require hypothermia (e.g. coronary artery bypass) present unique problems for patients with cold haemolysis. Five patients were treated with cryofiltration apheresis. Two of five patients responded favourably and had a reduced cold agglutinin titre. The other three had chronic autoimmune haemolysis, but no complement activation was observed (Siami & Siami, 2004). Occasionally, multiple treatments with rituximab have been successful (Webster *et al*, 2004). After one course of rituximab treatment, a 71-year-old man had stabilised haemoglobin levels and was transfusion independent. A second course was given for 4 months, and he had a 9-month response. He further had a prompt response to a third course of rituximab. Another patient was treated with four doses of rituximab and oral cyclophosphamide (60 mg/m²/d) and achieved a haematological remission that lasted 8 months (Vassou *et al*, 2005).

Paradoxically, a patient with cold immune haemolysis had development of chronic lymphocytic leukaemia (Swords *et al*, 2006). The patient was treated with fludarabine, cyclophosphamide and rituximab, which resulted in a marked increase in haemolysis. Ultimately, the patient had a complete clinical

remission of leukaemia with persistent haemolysis; administration of additional rituximab resulted in remission of haemolysis. The authors postulated that fludarabine exacerbated the autoimmune haemolysis and that it subsequently was controlled with rituximab. A patient with haemolysis associated with cold agglutinins and a lymphoplasmacytic lymphoma was treated with combination chemotherapy and became refractory to treatment (Pulik *et al*, 2002). This patient was subsequently treated with rituximab and responded to therapy.

For patients with polyclonal cold agglutinin disease, therapy with corticosteroids may be effective. One patient with polyclonal cold agglutinin attributable to mycoplasma pneumonia showed marked improvement with the use of corticosteroids (Tsuruta *et al*, 2002). A French report of five patients treated with 4 weekly rituximab infusions indicated that four had partial recovery and one achieved a complete remission (Camou *et al*, 2003). In one of the largest patient cohorts to date (Berentsen *et al*, 2004), 37 courses of rituximab were administered to 27 patients with primary chronic cold agglutinin disease; 14 patients responded to the first course of treatment, and six of 10 responded to a second course. The overall response rate was 54%, and 19 of the 20 had a partial response. Second-line therapy with interferon- α and rituximab resulted in two of three partial responses. The median time to response was 1.5 months, and the median duration of response was 11 months. No predictors of response were identified. In a group of six patients treated with rituximab, the median haemoglobin level increased by 4.1 g/l (Berentsen *et al*, 2001). Patients with cold agglutinin haemolysis have responded to fludarabine therapy (Jacobs, 1996). Failure of cladribine therapy to control cold agglutinin disease was reported for five consecutive patients (Berentsen *et al*, 2000).

Conclusion

Paroxysmal cold haemoglobinuria is an intravascular, acute and life-threatening haemolytic process that primarily occurs in children after an infection. The syndrome is self-limiting, but corticosteroid therapy has aided recovery. Cold agglutinin haemolytic disease tends to occur in adults and may be a self-limiting, postinfectious, polyclonal syndrome with a good prognosis; or it may be a chronic, sustained, haemolytic syndrome associated with monoclonal IgM proteins, and a high proportion of these patients may have a lymphoproliferative disorder. Treatment is not curative, and in the past, chlorambucil and corticosteroids have shown some efficacy. Recently, the use of rituximab has resulted in partial responses in approximately half of the treated patients, but responses are not durable and relapses commonly are seen within 1 year.

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