How I manage cold agglutinin disease

Sigbjørn Berentsen

Department of Medicine, Haugesund Hospital, Haugesund, Norway

Summary

Primary chronic cold agglutinin disease (CAD) is a clonal lymphoproliferative disorder accounting for 13–15% of autoimmune haemolytic anaemias. Significant advances have been made in treatment, which was largely unsuccessful until recently. The essential clinical, immunological and pathological features are reviewed, focusing on their relevance for therapy. Non-pharmacological management still seems sufficient in some patients. With the recent improvements, however, drug therapy seems indicated more often than previously thought. Corticosteroids should not be used to treat CAD. Half of the patients respond to rituximab monotherapy; median response duration is 11 months. Fludarabine-rituximab combination therapy is very effective, resulting in 75% response rate, complete remissions in about 20%, and more than 66 months estimated response duration. Toxicity is a concern, and benefits should be carefully weighed against risks. An individualized approach is discussed regarding the choice of fludarabine-rituximab combination *versus* rituximab monotherapy. Patients requiring treatment should be considered for prospective trials.

Keywords: cold agglutinin disease, autoimmune haemolytic anaemia, lymphoproliferative, rituximab, fludarabine.

Autoimmune haemolytic anaemias (AIHA) are classified into warm and cold reactive antibody types. Primary chronic cold agglutinin disease (CAD) accounts for 13–15% of patients with AIHA (Sokol *et al*, 1981; Dacie, 1992a; Genty *et al*, 2002), with a reported prevalence in Scandinavia of about 16 per million inhabitants and an incidence rate of one per million inhabitants per year (Berentsen *et al*, 2006). In rare cases, secondary CAD occurs as a complication to aggressive or overt extramedullary lymphoma or other cancers. Cold-antibody AIHA may also occasionally complicate *Mycoplasma pneumoniae* or viral infections. Only primary CAD will be further addressed in this review.

Correspondence: Sigbjørn Berentsen, Department of Medicine, Haugesund Hospital, PO Box 2170, N-5504 Haugesund, Norway.
E-mail: sigbjorn.berentsen@haugnett.no
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Cold agglutinins (CA) are antibodies that agglutinate erythrocytes at an optimum temperature of 0–4°C (Landsteiner, 1903; Ulvestad *et al*, 1999). CA are often found in the sera of healthy individuals. As compared to normally occurring, polyclonal CA, the CA in CAD are monoclonal, are present at much higher titres and have a high thermal amplitude, which contributes to their pathogenicity at temperatures approaching 37°C (Harboe & Deverill, 1964; Harboe *et al*, 1965; Rosse & Adams, 1980; Ulvestad *et al*, 1999).

Cold agglutinin disease is diagnosed when the following criteria are met: chronic haemolysis, CA titre ≥ 64 at 4°C and typical findings by the direct antiglobulin test (DAT). The typical DAT pattern is defined as a positive polyspecific test with monospecific test positive for complement protein C3d and negative (or occasionally weakly positive) for IgG (Berentsen *et al*, 2006, 2007a). CAD is termed 'primary' if no malignant disease can be found by clinical and radiological assessment (Dacie, 1992a; Berentsen *et al*, 2007a). For reasons discussed below, measurements of serum immunoglobulin classes, electrophoresis and immunofixation should always be done, as well as flow cytometry of bone marrow aspirate and examination of a bone marrow biopsy sample by an experienced haematopathologist. Importantly, in order to achieve sufficient sensitivity, serum for CA titration and immunoglobulin assessments must be obtained from blood specimens kept at 37–38°C from sampling until the serum has been removed from the clot (Berentsen *et al*, 2007a). Immunofixation should be performed even if no monoclonal band is visible on electrophoresis (Berentsen *et al*, 2006).

Treatment was largely unsuccessful until the last decade (Dacie, 1992b; Berentsen *et al*, 2006). More recently, considerable progress has been made in the knowledge of clinical features, pathogenesis and possible targets for therapy, and new treatment options have become available. This review initially focusses on the clinical, immunological and pathological findings relevant for the development of more efficient therapy. Based on these data and more recently published prospective therapeutic trials, I will discuss the optimal current management.

The clinical background for therapy

Most patients with CAD are elderly. In a population-based study from Norway, the median age was found to be 76 years

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(range, 51–96), while the median age at onset of symptoms was 67 years (range, 30–92). Median survival was 12·5 years, which is similar to the expected survival in an age-matched Scandinavian general population. Although the clinical course is variable and unpredictable, CAD does not generally worsen with time (Berentsen *et al*, 2006).

According to some previous reviews, the anaemia is usually not severe (Dacie, 1992c; Nydegger *et al*, 1991). However, this is definitely not always the case. Five of 16 patients described in an early report had haemoglobin (Hb) levels below 70 g/l and one had levels below 50 g/l (Schubothe, 1966). Our descriptive study of 86 unselected CAD patients found a median Hb level of 89 g/l, while the lower tertile was 80 g/l and the lower range was 45 g/l. Approximately 50% of the patients had received transfusions at some time during the course of the disease, and drug therapy had been attempted in 70% (Berentsen *et al*, 2006).

Cold-induced circulatory symptoms are considered typical for CAD (Schubothe, 1966; Nydegger *et al*, 1991) but are not always appreciated by physicians. We recorded such symptoms in more than 90% of unselected patients, ranging from moderate acrocyanosis to disabling Raynaud phenomena triggered by slight cold exposure (Berentsen *et al*, 2006). Although patients may have considerable haemolysis and clinical symptoms even in warm climates, characteristic seasonal variations have been well documented (Lyckholm & Edmond, 1996). About two-thirds of the patients experience 'paradoxical' exacerbations of haemolytic anaemia precipitated by febrile illnesses or major trauma (Ulvestad, 1998; Ulvestad *et al*, 2001; Berentsen *et al*, 2006).

These observations show that in many patients, CAD is not an 'indolent' disease in terms of major clinical symptoms and quality of life.

The immunological background for therapy

Cold agglutinins in CAD are usually specific for the I antigen, a red-cell surface carbohydrate macromolecule (Wiener *et al*, 1956; Dacie, 1992d). During passage through the peripheral circulation, cooling allows high-thermal amplitude CA to bind to the antigen, leading to agglutination of erythrocytes and, thereby, impaired microcirculation. The antigen-antibody complex activates the classical complement reaction pathway (Jonsen *et al*, 1961; Rosse & Adams, 1980; Ulvestad *et al*, 1999), resulting in a predominantly extravascular haemolysis mediated by the reticulo-endothelial system and occurring mainly in the liver (Jaffe *et al*, 1976; Kirschfink *et al*, 1994; Zilow *et al*, 1994). Activation of the terminal complement components with intravascular hemolysis, as evidenced by e.g. haemoglobinuria, may occasionally occur in severe exacerbations (Schubothe, 1966; Nydegger *et al*, 1991). Figure 1 illustrates the complement-mediated red cell destruction.

Because of constant consumption, serum levels of complement proteins C3 and, in particular, C4 are low in most CAD patients (Ulvestad, 1998; Ulvestad *et al*, 1999). The complement depletion is assumed to be rate-limiting for the extravascular haemolysis and probably prevents full-blown activation of the terminal complement pathway which would result in intravascular haemolysis. During the acute phase reaction, complement production is enhanced and C4 again becomes available, which explains the 'paradoxical' exacerbations complicating febrile diseases (Ulvestad *et al*, 2001; Berentsen *et al*, 2006).

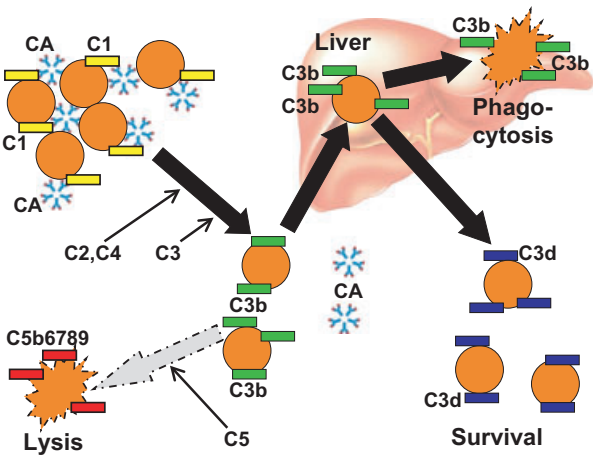

Fig 1. Predominant pathways of complement-mediated haemolysis in cold agglutinin disease. Cooling during passage through the peripheral circulation allows cold agglutinin (CA) to bind to erythrocytes, causing agglutination and fixation of complement C1 complex. C1 esterase activates complement proteins C4 and C2, generating C3 convertase, which binds and splits C3, resulting in deposition of C3b on the cell surface. Upon subsequent warming, CA is dissolved and the agglutinated cells detach from each other, while C3b remains bound. C3b may in turn activate C5, leading to the formation of the membrane attack complex and intravascular haemolysis. In steady-state disease, however, destruction of C3b-coated erythrocytes is mediated by reticulo-endothelial cells, mainly in the liver. C3b is converted to C3d on the surviving erythrocytes, which are then released into the systemic circulation.

The essential role and specific features of the complement system involvement may have therapeutic implications. First, the administration of complement containing plasma products should probably be avoided. Second, the non-functional classical complement pathway may, hypothetically, affect the therapeutic potential of some monoclonal antibodies, e.g. rituximab, depending on the importance of complement-dependent cytotoxicity (Harjunpaa *et al*, 2000). Third, a future therapeutic roll of complement blocking agents cannot be excluded in some specific situations.

Clonality as background for therapy

The first monoclonal immunoglobulin ever described was a CA from a patient with CAD (Christenson *et al*, 1957), and monoclonal immunoglobulin (Ig) Mκ was a recurrent finding in subsequent studies (Harboe *et al*, 1965). In a larger cohort of 86 patients, we detected monoclonal IgMκ in more than

90%, whereas monoclonal IgG, IgA or λ light chain restriction were rare findings (Berentsen *et al*, 2006). Based on the autoantibody characteristics, a relationship between CAD and Waldenström macroglobulinemia (WM) had been suggested in early works (Schubothe, 1966; Oluboyede *et al*, 1976). Anti-I CA in patients with primary CAD are preferentially encoded by the *IGHV4-34* gene segment (Pascual *et al*, 1992; Thorpe *et al*, 1997).

We reported the findings of immunocytoma [currently termed lymphoplasmacytic lymphoma (LPL)] in bone marrow biopsy samples from three consecutive patients diagnosed with primary CAD (Berentsen, 1995). In a subsequent flow cytometric and histopathological study of bone marrow specimens from CAD patients without any clinical or radiological evidence of lymphoma, we detected a $CD19^+$, $CD20^+$, κ^+ clonal lymphocyte population in 10 of 11 patients (Berentsen *et al*, 1997). More recently we re-examined the medical records of 86 patients otherwise classified as having primary CAD with regard to findings indicating a clonal bone marrow lymphoproliferation (Berentsen *et al*, 2006). Monoclonal $CD20^+$, κ^+ lymphocytes were detected in aspirates from 90% of patients in whom flow cytometric immunophenotyping had been performed. Morphological and immunohistochemical signs of a clonal lymphoproliferative B-cell disorder were described in trephine biopsies in 50 (76%) of 66 patients with relevant data. These findings, classified according to the 2001 version of the World Health Organization (WHO) classification (Jaffe *et al*, 2001), are shown in Table I. The most frequently described well-defined histological disorders were LPL and marginal zone lymphoma (MZL).

Given that bone marrow LPL was reported in 50% of patients (Table I) and that monoclonal IgM could be detected in almost all cases, it has been concluded that nearly 50% of patients with primary CAD also fulfil the diagnostic criteria for WM (Owen *et al*, 2003; Berentsen, 2009). IgM-related disorders (IgM-RD) are gammopathies clinically characterized by specific properties of monoclonal IgM proteins and without evidence of lymphoma (Cesana *et al*, 2005). In most patients not having LPL/WM or MZL, primary CAD may be classified as an IgM-RD. Clinically, however, CAD with marked or merely detectable clonal B-cell proliferation should be regarded a continuous spectrum, not different entities. Furthermore, preliminary findings in a recent systematic re-examination of bone marrow biopsy samples from more than 40 patients with primary CAD indicate a more uniform histological picture than previously reported, distinct from both LPL and MZL (Randen, U., Tierens, A., Tjonnfjord, G., Berentsen, S., Beiske, K., & Delabie, J, unpublished data). Until the final results of this study have been published, it remains to be decided whether CAD-associated lymphoproliferation should be proposed as a distinct histopathological entity.

Table I. Bone marrow histology in 66 patients with primary chronic cold agglutinin disease.

Histological findings	*n*	%
Normal findings or reactive lymphocytosis	7	11
Irregular lymphoid hyperplasia	9	13
Non-Hodgkin B-cell lymphoma	50	76
Lymphoplasmacytic lymphoma	33	50
Marginal zone lymphoma	5	8
Small lymphocytic B-cell lymphoma	4	6
Clonal lymphocytosis/Other small B-cell lymphoma	8	12
Total	66	100

The presence of a clonal, B-cell lymphoproliferative bone marrow disorder in most if not all patients with primary CAD, as well as the relationship to WM, provide an important basis for therapeutic trials.

The problem of evidence-based therapy in an uncommon disease

As in most autoimmune cytopenias, the low prevalence of CAD makes it difficult to design and conduct randomized trials. Even non-randomized prospective studies or larger, well-designed retrospective series are few, and conclusions found in the literature have quite often been based on pooled data from case reports or very small retrospective series. Reports published in 1998–2003 may serve to illustrate the problem. During this period, several case reports on rituximab monotherapy for CAD appeared in the literature (Lee & Kueck, 1998; Cohen *et al*, 2001; Layios *et al*, 2001). A small, prospective trial was reported in 2001 (Berentsen *et al*, 2001); two larger phase 2 trials were published within the next 5 years (Berentsen *et al*, 2004; Schollkopf *et al*, 2006); and the results from a relatively large, retrospective series of consecutive patients appeared in 2006 (Berentsen *et al*). By 2003, 23 cases had been published altogether and responses had been reported in 21 (91%) (Camou *et al*, 2003; Finazzi, 2002); the two non-responders were observed in the only prospective series (Berentsen *et al*, 2001). In the two more recent, larger prospective trials and the large retrospective series, however, overall response rates were between 45% and 58% (Berentsen *et al*, 2004, 2006; Schollkopf *et al*, 2006). In many case reports, remissions were classified as complete, whereas the systematic studies showed that complete responses (CR) to rituximab monotherapy are rare. These major discrepancies highlight the well-known fact that response rates calculated from pooled case reports are highly likely to be influenced by publication bias and heterogeneous or poorly defined response criteria.

In CAD, it would be unrealistic to require more than two well-performed phase 2 trials for a given therapy modality to be considered evidence-based. If safety and efficacy have been documented in two or more treatment modalities, it may be impossible to put forward evidence-based guidelines on which regimen should be preferred. Response rates derived from pooled case reports should not be accepted as a basis for recommendations.

Management

Non-pharmacological management

Given that medical therapy has been largely ineffective until recently, counselling has been considered the mainstay of management (Schubothe, 1966; Dacie, 1992b). It should be realized, however, that the term 'cold' refers to the biological properties of the CA rather than the clinical effect of low ambient temperatures (Gertz, 2006). Because of the high thermal amplitude of the CA in many patients, the physiological cooling of the blood in the peripheral vessels is usually sufficient to cause haemolysis and circulatory symptoms. Furthermore, only anecdotal documentation exists for the therapeutic effect of particularly warm clothing (Bartholomew *et al*, 1987; Dacie, 1992b; Ness *et al*, 2003). Nevertheless, in our clinical experience, such measures seem to alleviate the symptoms to a varying extent and can probably prevent severe exacerbations of haemolysis.

Most authors agree that patients should avoid cold exposure, particularly of the head, face and extremities (Dacie, 1992b; Nydegger *et al*, 1991; Schubothe, 1966). Those living in cold climates will often, even before the diagnosis has been established, tell the doctor that they use warm clothing and, in many cases, stay indoors during winter. Some patients report on improvement of Hb levels and ischaemic symptoms after temporarily moving to warmer regions during the cold season, but severely symptomatic CAD does exist even in the subtropics. Any intravenously infused liquids should be prewarmed; and surgery under hypothermia should be avoided or specific precautions undertaken.

Transfusions can be given provided appropriate precautions are observed (Berentsen *et al*, 2007a; Dacie, 1992b). In contrast to the transfusion problems encountered in warm-antibody AIHA, it is usually easy to find compatible donor erythrocytes, and screening tests for irregular blood group antibodies are most often negative. Antibody screening and, if required, compatibility tests should be performed at 37°C. The patient and, in particular, the extremity chosen for infusion should be kept warm, and the use of an in-line blood warmer is recommended.

According to clinical experience, plasmapheresis is efficient as a 'first-aid' in acute situations or before surgery requiring hypothermia (Nydegger *et al*, 1991; Zoppi *et al*, 1993); however, the remissions achieved are very short-lived and some conflicting data have been reported (Rosenfield & Jagathambal, 1976; Dacie, 1992b). Since the extravascular haemolysis does not take place in the spleen selectively, splenectomy should not be used in the treatment of CAD. Three splenectomized patients were described in our population-based retrospective study, none of whom responded (Berentsen *et al*, 2006). Response to splenectomy has occasionally been reported among the rare patients with CAD mediated by an IgG CA instead of IgM (Silberstein *et al*, 1987).

Indications for drug therapy

The above-mentioned data may indicate a discrepancy between the restrictive attitude to medications for CAD often found in the literature and the real requirement for therapy (Berentsen *et al*, 2007a). Recommendations to avoid drug treatment may simply reflect the fact that in the past, therapy was ineffective. In addition, underestimation of the severity of anaemia and clinical symptoms in the patient population may have influenced the recommendations. Actually, the ischaemic symptoms may sometimes be sufficiently disabling to justify therapy even if the haemolysis is fully compensated (Berentsen *et al*, 2010). A considerable number of patients, however, do have a mild disease in which the anaemia is slight and the circulatory symptoms are tolerable or absent. Therefore, CAD should still not be regarded an indication for therapy in every case, and the decision to treat should be based on an individualized assessment. Reasonable criteria for initiating drug therapy are symptom-producing anaemia, transfusion dependence, or disabling circulatory symptoms (Berentsen *et al*, 2007a, 2010).

Conventional immunosuppressive, cytotoxic or supportive therapies

Cold agglutinin disease has often been treated with corticosteroids, although this practise has never been supported by systematic studies. In a historical description of 38 patients seen at the Hammersmith Hospital in London up to 1990, only occasional patients were reported to respond to steroids (Dacie, 1992b). This observation is in perfect accordance with comprehensive clinical experience obtained elsewhere (Nydegger *et al*, 1991; Schubothe, 1966; Worlledge *et al*, 1968). Studied retrospectively, 43% of unselected Norwegian patients with CAD had received corticosteroids for one or more periods (Berentsen *et al*, 2006). Responses had been observed in only 14% of those treated, which is an unacceptably low response rate. Furthermore, some of the few patients who did respond required unacceptably high doses in order to maintain the remission. The requirement for high maintenance doses in the occasional responder has also been described by others (Schreiber *et al*, 1977).

Monotherapy with alkylating agents has shown some beneficial effect on laboratory parameters, and clinical improvement has been observed (Worlledge *et al*, 1968; Hippe *et al*, 1970). The clinical response rates, however, are probably in the same low order of magnitude as for corticosteroids (Berentsen *et al*, 2006). In two small series of patients treated with interferon-α or low-dose cladribine respectively, these drugs were not shown to be useful (Hillen & Bakker, 1994; Berentsen *et al*, 2000), although some conflicting data exist for interferon-α (O'Connor *et al*, 1989; Fest *et al*, 1994; Rordorf *et al*, 1994). Only a few patients treated with azathioprine have been reported in the literature, none of whom responded (Berentsen *et al*, 2006).

Exacerbations precipitated by febrile illnesses should warrant immediate treatment of any bacterial infection (Ulvestad *et al*, 2001; Berentsen *et al*, 2007a). In my experience, supportive therapy for CAD with erythropoietin or its analogues seems quite widely used in North America but not so often in Scandinavia and Western Europe. No studies have been published to support or discourage its use.

Rituximab monotherapy

Several case reports on remission following rituximab monotherapy have been published since 1998 (Lee & Kueck, 1998; Cohen *et al*, 2001; Layios *et al*, 2001; Engelhardt *et al*, 2002); and a small, prospective series was reported in 2001 (Berentsen *et al*, 2001). Two larger, prospective uncontrolled trials of 37 and 20 courses of therapy respectively, have also been published (Berentsen *et al*, 2004; Schollkopf *et al*, 2006). In both studies, the dosage was 375 mg/m^2 weekly for 4 weeks. The response criteria used in our study (Berentsen *et al*, 2004) are shown in Table II, and similar strict definitions were used in the Danish study (Schollkopf *et al*, 2006). The overall response rate was found to be 54% and 45% respectively, in the two trials. With the exception of one CR observed in our trial, all remissions were partial responses (PR). Ten patients were treated for relapse after previously having received rituximab therapy, and six of them responded to a second course. In our study, the responders achieved a median increase in Hb levels of 40 g/l, with a median time to response of 1·5 months (range, 0·5–4 months), and the median observed response duration was 11 months (range, 2–42 months).

In our population-based study of 86 Norwegian patients with primary CAD, 40 patients were reported to have received rituximab monotherapy (Berentsen *et al*, 2006). As far as permitted by available data, the same response criteria as previously published (Table II) were used for the retrospective analysis. Twenty-three patients (58%) responded; 2 (5%) achieved CR and 21 (53%) achieved PR. Responses had been observed following a second and even a third course of rituximab in patients who had relapsed after previous therapy. These findings confirm the essential results of the prospective studies; rituximab monotherapy is an efficient treatment for primary CAD. However, CR is uncommon, the median response duration is relatively short and the number of non-responders is considerable.

Table II. Response definitions in cold agglutinin disease.

Response level	Criteria
Complete response (CR)	Absence of anaemia No signs of haemolysis Disappearance of clinical symptoms of CAD Undetectable monoclonal serum protein No signs of clonal lymphoproliferation as assessed by bone marrow histology, immunohistochemistry and flow cytometry
Partial response (PR)	A stable increase in haemoglobin levels by at least 20 g/l or to the normal range A reduction of serum IgM concentrations by at least 50% of the initial level or to the normal range Improvement of clinical symptoms Transfusion independence
No response (NR)	Failure to achieve complete or partial response

In order to qualify for any given response level, all criteria have to be fulfilled.

Adverse effects were few and tolerable in all three series (Berentsen *et al*, 2004, 2007a; Schollkopf *et al*, 2006). Data from rituximab maintenance in patients with follicular lymphoma indicate that even prolonged or repeated administration of this monoclonal antibody is safe with regard to infections (Ghielmini *et al*, 2004), though rare cases of progressive multifocal leucoencephalopathy and hepatitis B virus reactivation have been reported in patients receiving rituximab for polyclonal autoimmune disorders (Cooper & Arnold, 2010). Causal associations are somewhat unclear because of concomitant immunosuppressive therapies and immune dysregulation as part of the autoimmune disease.

Fludarabine and rituximab combination therapy

The purine analogues, e.g. fludarabine, are powerful therapeutic agents in several lymphoproliferative diseases. Remission of CAD following fludarabine monotherapy has been reported in two patients (Jacobs, 1996; Berentsen *et al*, 2006), and the fludarabine-rituximab combination has yielded high response rates in WM (Treon *et al*, 2009) and other low-grade non-Hodgkin lymphoma (Czuczman *et al*, 2005).

In an attempt to improve on the results achieved by rituximab monotherapy, we performed a prospective, uncontrolled trial of combination therapy with fludarabine and rituximab in patients with primary CAD requiring treatment (Berentsen *et al*, 2010). Twenty-nine patients aged 39–87 years (median, 73 years) received rituximab 375 mg/m^2 on days 1, 29, 57 and 85; and fludarabine orally, 40 mg/m^2 on days 1–5, 29–34, 57–61 and 85–89. We used the same response criteria as previously published (Table II). Twenty-two patients (76%) responded, with 6 (21%) achieving CR and 16 (55%) achieving PR. Among 10 patients non-responsive to rituximab monotherapy, CR was observed in one patient and PR in six. Median increase in Hb level was 31 g/l in the responders and 40 g/l among those who achieved CR. Median time to response was 4 months. Lower quartile of response duration was not reached after 33 months, and estimated median response duration was more than 66 months (Fig 2).

Grade 3–4 haematological toxicities occurred in 12 patients (41%); neutropenia accounted for all cases of grade 4 toxicity. Seventeen patients (59%) had grade 1–3 infection, which was successfully treated in all except for one elderly, frail non-responder who died of pneumonia 9 months after treatment. Infection grade 4 or *Pneumocystis jirovecii* pneumonia did not

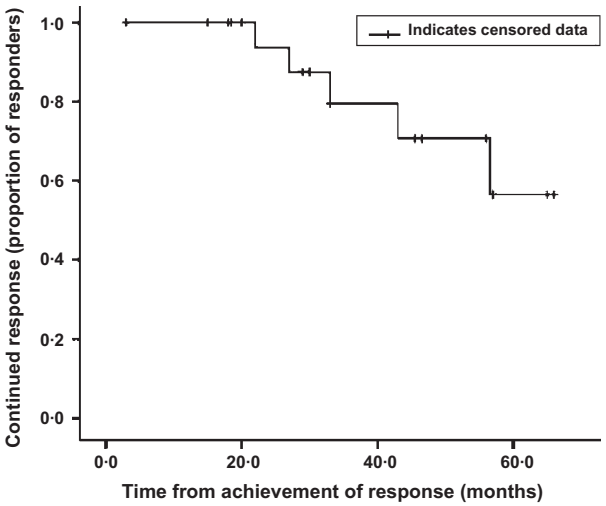

Fig 2. Response duration following fludarabine-rituximab combination therapy. Kaplan–Meier graph relating response status to duration (time from achievement of response to relapse or censoring of data) in 22 complete or partial responders. This figure was originally published in *Blood* (Berentsen *et al*, 2010). © the American Society of Hematology.

occur. Antiviral prophylaxis was not given routinely, and three patients (10%) experienced herpes zoster reactivation. Transient exacerbation of haemolytic anaemia was seen in three patients (10%). All three were found to have exacerbation of CAD precipitated by acute phase reaction (Ulvestad, 1998; Ulvestad *et al*, 2001), whereas fludarabine-induced warm-antibody AIHA was not observed. Nearly half of the patients had their doses of fludarabine reduced because of haematological toxicity. We found no significant association, however, between response level and adherence to the scheduled dose (Berentsen *et al*, 2010).

Comparison of non-randomized trials should be undertaken with care. Nevertheless, the baseline data reported in the fludarabine-rituximab trial (Berentsen *et al*, 2010) matched well with those described in our trial of rituximab single agent therapy (Berentsen *et al*, 2004). The response criteria (Table II) were identical in the two studies and very similar to those used in the Danish monotherapy trial (Schollkopf *et al*, 2006). The 76% response rate and more than 66 months estimated response duration achieved by using fludarabine and rituximab in combination, therefore, compared favourably with the 45–58% response rate and 11 months response duration observed after monotherapy with rituximab. In addition, CR was achieved in 21% of the patients following the combination therapy, whereas remissions are rarely complete after rituximab monotherapy. Furthermore, 10 patients acted as their own controls by receiving the combination after rituximab single agent therapy had failed (Berentsen *et al*, 2010). With the achievement of one CR and six PR in this subgroup, the combination has been shown to be effective even in patients non-responsive to monotherapy with rituximab. Provided the results can be reproduced, fludarabine and rituximab in combination represents a major step forward in therapy for primary CAD (Berentsen *et al*, 2010; Stone, 2010).

Because of the toxicity, the therapeutic potential should be carefully weighed against the short-time risks, particularly in very old and comorbid patients. We found, however, no significant association between adverse events and age itself (Berentsen *et al*, 2010). The study was not designed to investigate the risk of secondary haematological malignancies. Although not specific to purine nucleoside analogues, late-occurring acute myeloid leukaemia and myelodysplastic syndromes have been observed after fludarabine-based therapy for WM (Leleu *et al*, 2009; Treon *et al*, 2009). This concern should not be prohibitive to the use of rituximab and fludarabine in combination, but lead to a balanced, individualized consideration of benefit *versus* long-time risk, particularly in younger patients.

Unspecific immunosuppression or targeting clonal B-cells?

The exact mechanism of action of therapy for primary CAD has not been established. Since corticosteroids and conventional immunosuppressive agents are largely ineffective, it may be assumed that unspecific suppression of immune effector mechanisms is not important. The rituximab trials as well as the study of fludarabine and rituximab in combination were based on the idea that targeting the pathogenic, monoclonal B-lymphocytes might provide an efficient therapeutic measure (Berentsen *et al*, 2007b). In studying rituximab monotherapy, however, we observed that PR was often achieved even though the decline in IgM level was modest (median 54%) (Berentsen *et al*, 2004). Moreover, the administration of either rituximab or purine nucleoside analogues has been found beneficial in polyclonal autoimmune disorders (Beutler *et al*, 1996; Berentsen, 2007; Silverman, 2007). Several alternative mechanisms of action have been proposed for CD20-directed therapy in autoimmune diseases, such as modulation of more global B-lymphocyte functions, receptor blocking, or interfering with antigen-presenting cells (Mease, 2008; Taylor & Lindorfer, 2007, 2008).

In our study of fludarabine and rituximab combination therapy, clinical and haematological remissions were accompanied by resolution of the lymphoproliferative bone marrow disorder (Berentsen *et al*, 2010). As compared to the rituximab monotherapy trials, the higher response rate observed after combination therapy was associated with a more profound decrease in IgM levels. Moreover, no relapses were seen during the study period in patients who achieved CR, which by definition included disappearance of monoclonal IgM and complete histological resolution. These data support the hypothesis that targeting the pathogenic B-cell clone efficiently is essential for the clinical effect of treatment.

Perspective for future studies

Future achievements in treating primary CAD might include, hopefully, still higher response rates and numbers of CR, less

toxic regimens, even more prolonged response duration and efficient second-line therapies. It has already been proposed (Stone, 2010) to investigate the safety and efficacy of fludarabine and rituximab in combination using reduced doses of fludarabine. Furthermore, some data have indicated that the doses of rituximab may be reduced without losing therapeutic efficacy, although this conclusion has been based on studies of patients with mainly polyclonal autoimmune cytopenias (Provan *et al*, 2007).

The relationship between primary CAD and WM provides a basis for exploring the potential of several, more or less targeted therapies shown to be feasible and efficient in WM (Treon, 2009). Of interest, high response rates have been achieved in WM following treatment with a bortezomib-based combination regimen (Treon *et al*, 2008). Probable improvement of CAD has been reported in two patients who received bortezomib monotherapy (Carson *et al*, 2010). The monoclonal anti-C5 antibody, eculizumab, has been established as a powerful therapeutic agent in paroxysmal nocturnal haemoglobinuria (Hillmen *et al*, 2006). As discussed above, most of the haemolysis is not C5-mediated in steady-state CAD (Fig 1). Infusions of eculizumab have been reported, however, to result in stable improvement in one single patient (Roth *et al*, 2009). Hypothetically, it may prevent exacerbations with intravascular haemolysis due to acute phase complement production (Ulvestad *et al*, 2001) and prove useful in subgroups, e.g. in those who have a substantial component of intravascular haemolysis or in exacerbations associated with intravascular haemolysis.

None of these possibilities have been systematically explored and, therefore, new studies are warranted in order to further improve on current therapy in this challenging disease.

Conclusions

Significant advances have been made during the last decade in the management of primary CAD. Non-pharmacological management is probably still sufficient for some patients. Those requiring drug therapy should be considered for prospective trials whenever such studies are available. Several conventional therapies for autoimmune disorders should not be used to treat primary CAD, including corticosteroids, monotherapy with alkylating agents, azathioprine, interferon-α and splenectomy. Fludarabine and rituximab in combination has yielded very high response rates, including a significant number of complete remissions, as well as prolonged response duration. Short-term and long-term toxicity, however, remains a concern. Rituximab monotherapy has resulted in somewhat lower response rates, only occasional complete remissions and much shorter response duration, but should still be regarded an efficient treatment with low toxicity.

Outside clinical trials, elderly patients severely affected by primary CAD should be considered for fludarabine and rituximab combination therapy if they are otherwise reasonably fit and have no relevant co-morbidity. The combination has also proved useful in patients non-responsive to monotherapy with rituximab. An individualized, balanced consideration of risk *versus* benefit should always be undertaken. In the occasional young patients as well as the very old and co-morbid ones, rituximab as a single agent should often be preferred in the first-line situation. Patients who relapse after having responded to rituximab may receive another course of rituximab or proceed to the combination therapy, depending on an individualized assessment.

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Conflicts of interest

None.

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