Rituximab for primary chronic cold agglutinin disease: a prospective study of 37 courses of therapy in 27 patients

Running head: RITUXIMAB FOR CAD: A PROSPECTIVE STUDY

Sigbjørn Berentsen1, Elling Ulvestad2, Bjørn Tore Gjertsen3, Henrik Hjorth-Hansen4, Ruth Langholm5, Håvar Knutsen6, Waleed Ghanima7, Fuad Victor Shammas8, and Geir E Tjønnfjord9

1Dept. of Medicine, Haugesund Hospital, Haugesund, and University of Bergen, Bergen,
2Dept. of Microbiology and Immunology, The Gade Institute, Haukeland University Hospital, Bergen, 3Dept. of Medicine, Haukeland University Hospital, Bergen, 4Dept. of Medicine, St Olav University Hospital, Trondheim, 5Dept. of Pathology, The Norwegian Radium Hospital, Oslo, 6Dept. of Medicine, Akershus University Hospital, Lørenskog, 7Dept. of Medicine, Østfold Hospital Fredrikstad, Fredrikstad, 8Dept. of Hematology and Oncology, Rogaland Central Hospital, Stavanger, 9Dept. of Medicine, The National Hospital, Oslo, Norway

Declaration of commercial interest: This work was supported in part by Roche Norge AS by supplying rituximab at a reduced price.

Corresponding author: Sigbjørn Berentsen, Section of Hematology, Department of Medicine, Haugesund Hospital, P.O.Box 2170, N-5504 Haugesund, Norway. Phone: +47-52732000. Fax: +47-52770189. E-mail: s.beren@online.no

Word counts: Full text: 2766
Abstract: 194

Scientific heading: Clinical observations, interventions, and therapeutic trials
Conventional therapies for primary chronic cold agglutinin disease (CAD) are ineffective, but remissions after treatment with the anti-CD20 antibody rituximab have been described in a small, prospective trial and some case reports. In this study we report on 37 courses of rituximab administered prospectively to 27 patients. Fourteen of 27 patients responded to their first course of rituximab, and 6 of 10 responded to re-treatment. In both groups combined, responses were achieved after 20 of 37 courses, giving an overall response rate of 54%. We observed 1 complete and 19 partial responses. Two non-responders and 3 relapsed patients received second-line therapy with interferon-α combined with a new course of rituximab, and 1 non-responder and 2 relapsed patients achieved partial response. Responders achieved a median increase in hemoglobin levels of 4.0 g/dL. Median time to response was 1.5 months and median observed response duration 11 months. We conclude that rituximab is an effective and well-tolerated therapy for CAD. Histological and flowcytometric findings suggest that some of the effect may be mediated by other mechanisms than elimination of clonal lymphocytes. We were unable to predict response from the hematological, immunological, or histological parameters prior to therapy.
Introduction

Chronic cold agglutinin disease (CAD) is an uncommon autoimmune hemolytic anemia (AIHA) mediated by cold-reactive autoantibodies that bind to erythrocyte carbohydrate antigens, causing hemagglutination and complement-mediated hemolysis. CAD not associated with lymphoma or other diseases has traditionally been classified as “primary” or “idiopathic”. However, it has been shown that this condition represents a lymphoproliferative disorder of the bone marrow, characterized by clonal proliferation of CD20+,κ B-cells that produce monoclonal IgM cold agglutinin. Often, the histological features are those of lymphoplasmacytic lymphoma. Trisomy of the q arm of chromosome 3 has been shown in some cases.

The traditional classification of this disease as primary can therefore be questioned. In typical secondary CAD, however, the lymphoproliferative disorder is usually easily recognized as an aggressive lymphoma, and the monoclonal immunoglobulin tends to be of the IgMκ rather than κ type. In this work, we continued to apply the term primary CAD in patients not showing the typical criteria of the secondary type.

Many conventional treatment modalities used in other AIHA or indolent lymphomas, such as corticosteroids, alkylating agents, splenectomy, interferon-α (IFN) monotherapy, and purine analogues, have failed to demonstrate a convincing effect in primary CAD. However, favorable responses to the chimeric human-murine, monoclonal anti-CD20 antibody rituximab have been described in a small, prospective study and some case reports.

Complement dependant cytotoxicity (CDC) is probably one of the mechanisms of action of rituximab and has been proposed by some authors as the most important mechanism. Low C3 and very low C4 complement levels observed in most CAD patients
may therefore theoretically restrict the efficacy of rituximab therapy\textsuperscript{3,20,21}. However, C4 levels may be increased by the administration of IFN\textsuperscript{22}, and this cytokine may also upregulate CD20 expression\textsuperscript{23,24}. Furthermore, a synergistic antineoplastic effect of rituximab and IFN has been demonstrated in some lymphomas\textsuperscript{25}.

The purpose of this study was to further investigate the therapeutic efficacy of rituximab in CAD and evaluate the effect of adding interferon-\(\alpha\) in patients not responding to rituximab as a single agent.

Most reports on therapy for CAD do not state any well-defined response criteria\textsuperscript{26}, and results may be confounded by seasonal variations\textsuperscript{27} or acute phase reactions\textsuperscript{20,21}. In this work, therefore, we used previously published response definitions based on improvement of hemolytic anemia, serum paraprotein levels, and bone marrow disorder\textsuperscript{4,11}.

\section*{Patients and methods}

\subsection*{Study design}

Between 1 October 2001 and 1 May 2003, CAD patients from 10 Norwegian university and local hospitals were prospectively included in a phase 2 trial. The protocol was approved by the Regional Medical Research Ethics Committee of Southern Norway and the Norwegian Medicines Agency.

We have also included in this report 6 patients who had been treated with rituximab in our previously published, prospective trial\textsuperscript{4}, using the same inclusion and exclusion criteria, infusion schedule, and response definitions.
Before inclusion, all patients underwent clinical examination, chest radiograph, and abdominal ultrasonography. Hematological, immunological, and biochemical blood and serum parameters were recorded. Flowcytometric immunophenotyping of blood and bone marrow was performed as described previously\textsuperscript{1,3}. Bone marrow biopsies were examined by an experienced lymphoma pathologist (R.L.) and classified according to the World Health Organization (WHO) classification\textsuperscript{28}.

**Inclusion and exclusion criteria**

To be eligible for the study, patients were required to have CAD, as defined by the combination of chronic hemolysis with cold agglutinin titer $\geq 64$ and a typical pattern for the direct antiglobulin test (DAT). The typical pattern for DAT in CAD is a positive test when performed with polyspecific antiserum, negative or only weakly positive with anti-IgG, and strongly positive with anti-C3d\textsuperscript{1,27}. Serum and bone marrow examinations had to confirm the presence of a clonal lymphoproliferation of the CD20$^+$,\textkappa$^+$ phenotype, as defined by the combination of monoclonal IgM$^+$ band in serum with a clonal expansion of CD20$^+$,\textkappa$^+$ cells in the bone marrow, demonstrated by immune histochemistry and/or flowcytometric immunophenotyping\textsuperscript{1,29}. Additional requirements were clinical symptoms requiring treatment, e.g., anemia or Raynaud-like symptoms, and informed consent.

Patients with secondary CAD were not eligible. Patients were also excluded if they had blood lymphocyte count of more than 50 x10\textsuperscript{9}/L, non-lymphatic malignant disease, contra-indications to rituximab therapy, any severe disease other than CAD, or were unable to co-operate.
Therapy

Eligible patients received rituximab as described previously\textsuperscript{30,31} at a dose of 375 mg/m\textsuperscript{2} as an intravenous infusion day 1, 8, 15, and 22. Patients who did not respond within 3 months, or who relapsed during the study period, were offered second-line therapy with the combination of rituximab and IFN if there were no contra-indications to IFN. The combination consisted of a new, identical 4-week cycle of rituximab with the addition of IFN administered subcutaneously at a dose of 5 MU three times a week for 20 weeks. The IFN injections started 2 weeks before the first rituximab infusion.

The patients were assessed monthly for 6 months, and blood samples were collected at each visit. Cold-induced circulatory symptoms were assessed using a 0-2 scale (0 indicates no improvement; 2, complete resolution of clinical symptoms). Adverse effects related to rituximab or IFN were recorded. Assessments of bone marrow histology and flow cytometry were done after 3 and 6 months.

Response criteria

The criteria for complete response (CR) were absence of anemia, no signs of hemolysis, disappearance of clinical symptoms of CAD, undetectable monoclonal serum protein, and no signs of clonal lymphoproliferation as assessed by bone marrow histology, immunohistochemistry and flow cytometry. Partial response (PR) was defined as a stable increase in hemoglobin levels (Hgb) of at least 2.0 g/dL or to the normal range, combined with a reduction of serum IgM concentrations by at least 50\% of the initial level or to the normal range, improvement of clinical symptoms, and transfusion independence. Patients were classified as non-responders (NR) if they failed to achieve CR or PR.
Time to response was defined as the time from the first rituximab infusion to the achievement of any degree of response. Relapse was defined by Hgb below 10.0 g/dL or a decrease in Hgb by at least 2.0 g/dL from the highest level achieved after therapy, and/or need for re-treatment.

Statistics

Mean values were calculated for numeric variables showing a normal distribution. For the remaining numeric variables median values were used, with mean values stated in brackets where appropriate for comparison with other publications. The Chi squared test with Yates’ correction was used for significance testing of differences between frequencies in cross tables. For continuous variables, differences between groups were tested with Mann-Whitney test.

Results

Baseline characteristics

A total of 37 courses of rituximab were administered to 27 patients, 9 men and 18 women, with a mean age of 71 years (range 51-91). Prior to their first course of anti-CD20 therapy, 12 patients were previously untreated, 10 had received one, and 5 had received 2 or more other treatment modalities (corticosteroids, alkylating agents, purine analogues, and/or splenectomy). Additional baseline data are provided in Table 1 and 2.
Table 1. Baseline laboratory data

<table>
<thead>
<tr>
<th></th>
<th>Hemoglobin (g/dL)</th>
<th>IgM (g/L)</th>
<th>Cold agglutinin titer (4°C)</th>
<th>κ/λ ratio (bone marrow aspirate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>8.5</td>
<td>11.1</td>
<td></td>
<td>15.9</td>
</tr>
<tr>
<td>Median</td>
<td>8.2</td>
<td>5.0</td>
<td>6000</td>
<td>8.4</td>
</tr>
<tr>
<td>Range</td>
<td>6.2 – 12.3</td>
<td>0.6 – 51.7</td>
<td>64 - 256000</td>
<td>4.7 – 77.0</td>
</tr>
</tbody>
</table>

Table 2. Bone marrow histology at baseline

<table>
<thead>
<tr>
<th>WHO classification</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoplasmacytic lymphoma</td>
<td>15</td>
</tr>
<tr>
<td>Marginal zone lymphoma</td>
<td>2</td>
</tr>
<tr>
<td>Small B-cell lymphoma</td>
<td>2</td>
</tr>
<tr>
<td>Unclassified clonal lymphoproliferation</td>
<td>6</td>
</tr>
<tr>
<td>Reactive lymphocytic infiltration</td>
<td></td>
</tr>
<tr>
<td>no clonal lymphoproliferative disorder</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
</tr>
</tbody>
</table>

Response to rituximab first-line therapy and re-treatment

Fourteen of 27 patients responded to their first course of single agent rituximab (1 CR and 13 PR), and 13 were non-responders.

Two non-responders received re-treatment with the combination of rituximab and IFN, resulting in 1 PR and 1 NR. Eleven non-responders did not receive second-line therapy during the study period.

Eight relapses were treated with rituximab plus IFN (n=3) or rituximab monotherapy (n=5), resulting in 5 PR and 3 NR. In the relapsed patients receiving IFN, there were 2 PR and 1 NR. The reason for omitting IFN was patient’s refusal in 1 case and investigator’s decision...
in 4 cases. Two patients were re-treated with rituximab monotherapy for a second relapse, and both of them achieved a new PR.

Among the re-treated patients altogether, responses were observed after 6 of 10 courses.

**Overall response data**

The overall response data are shown in Table 3. Altogether, responses were achieved after 20 out of 37 courses of rituximab therapy. We observed 1 CR and 19 PR. Median time to response was 1.5 months (mean 1.7, range 0.5-4.0).

<table>
<thead>
<tr>
<th>Table 3. Overall response data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Response</strong> &amp; <strong>Frequencies</strong> &amp; <strong>Increase in Hgb. g/dL</strong> &amp; <strong>IgM reduction g/L</strong> &amp; <strong>IgM reduction %</strong></td>
</tr>
<tr>
<td>&amp; <strong>n</strong> &amp; <strong>%</strong> &amp; <strong>Median</strong> &amp; <strong>Range</strong> &amp; <strong>Median</strong> &amp; <strong>Range</strong> &amp; ** Median** &amp; ** Range**</td>
</tr>
<tr>
<td>CR &amp; 1 &amp; 3 &amp; 4.7 &amp; - &amp; 3.4 &amp; - &amp; 83 &amp; -</td>
</tr>
<tr>
<td>PR &amp; 19 &amp; 51 &amp; 3.9 &amp; 0.7 – 7.1 &amp; 5.1 &amp; 0 – 26.3 &amp; 54 &amp; 0 – 79 &amp; -</td>
</tr>
<tr>
<td>NR &amp; 17 &amp; 46 &amp; 1.3 &amp; -1.4 – +4.3 &amp; 1.2 &amp; 0 – 16.1 &amp; 32 &amp; 0 – 55 &amp; -</td>
</tr>
<tr>
<td>Total &amp; 37 &amp; 100 &amp; &amp; &amp; &amp; &amp;</td>
</tr>
</tbody>
</table>

Hemoglobin levels (Hgb) increased by a median of 4.0 g/dL (mean 4.1, range 0.7-7.1) among the responders. In addition, 4 patients classified as non-responders achieved an increase in Hgb of 2.0-4.3 g/dL. Peripheral circulatory symptoms improved by definition in all PR and CR, but also in 6 of 17 NR. One partial responder increased his Hgb level by only 0.7 g/dL. However, the initial Hgb level was within the normal range, and the clinical indication for therapy was circulatory symptoms. Another patient classified as PR showed no
decrease in IgM. This patient had a baseline IgM concentration within the normal range, but with a monoclonal band. Thus, both of these patients met the criteria for PR.

Response duration was calculated in 17 responders who have been observed until relapse or for more than 12 months after they achieved response. The median observed response duration was 11 months (mean 13, range 2-42). All responders observed for more than 12 months have relapsed except one, who is still in PR after 28 months. The only patient who achieved a CR retained the remission for 42 months.

**Clinical-hematological response compared to other parameters**

There was complete or partial histological regression of the bone marrow findings following 12 courses of rituximab, and no or uncertain histological response after 11. In the remaining 14 cases, such assessment was not relevant, not done, or unsuccessful. Table 4 shows the frequencies of histological regression in relation to clinical-hematological response.

### Table 4. Clinical-hematological response and histological response

<table>
<thead>
<tr>
<th>Regression of histological bone marrow findings</th>
<th>Clinical-hematological response Frequencies</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CR or PR</td>
<td>NR</td>
</tr>
<tr>
<td>Partial or complete</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>None or uncertain</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Not done, unsuccessful, or not relevant</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>17</td>
</tr>
</tbody>
</table>

Differences are not statistically significant (Chi-squared test with Yates’ correction)
Flowcytometric assessment of bone marrow aspirates for elimination of CD20\(^+\) cells was performed successfully after 26 courses of rituximab. A reduction of the percentage of CD20\(^+\) cells was achieved in all cases (responders and non-responders). This decrease was modest (about 50% from baseline) in two cases and near complete (reduction to 0-4% of the lymphocytes) after the remaining 24 courses. Following 5 courses of rituximab, a CD19/20 discrepancy was found 3 months after therapy. Following the remaining 21 courses, the reduction of CD20\(^+\) lymphocytes was paralleled by a reduction of CD19\(^+\) lymphocytes.

A comparison was made between responders and non-responders with regard to the following baseline parameters: age (mean values 72 and 71 years, respectively), Hgb concentration in blood (mean 8.3 and 8.8 g/dL), serum levels of IgM (median 5.2 and 4.4 g/L), complement protein C3 (median 0.78 and 0.72 g/L), C4 (median < 0.06 g/L in both groups), CD20\(^+\) cell percentage in bone marrow aspirates (median 24 and 25%), and \(\kappa/\lambda\)-ratio (median 9.3 and 7.8). These parameters did not show any significant difference between responders and non-responders (Mann-Whitney test).

**Tolerance**

There were no serious infusion-related adverse effects of rituximab, but 1 patient reported muscular pain during the first infusion. Hematological toxicity related to rituximab was observed in 1 patient (transient neutropenia WHO grade 4, fever grade 2, and infection grade 1). During IFN therapy we recorded flu-like symptoms in 4, and hematological toxicity grade 1 in 2 and grade 2 in 1 patient. Exacerbation of hemolysis after the administration of IFN did not occur. The dose of IFN had to be reduced or the administration discontinued before week 20 in 3 of 5 patients.
Two non-responders, 76 and 89 years old, who were treated with single agent rituximab, died from unrelated diseases after 12 and 4 months, respectively. The other patients are alive at 3-43 months after therapy.

**Discussion**

In this work we extended our experience with rituximab therapy for primary CAD to 37 courses in 27 patients. Six patients previously reported by us\(^4\) were included in this study in an appropriate way, since the inclusion and exclusion criteria and response definitions were identical in the two trials.

The results confirm our previous findings that rituximab is effective in primary CAD\(^4\). The response rates were 52% following rituximab single agent therapy in 27 patients who had not previously received rituximab, and 60% after 10 courses of re-treatment with or without the addition of IFN. In both groups combined, 20 responses were achieved following 37 courses, giving an overall response rate of 54%. The hemoglobin data may indicate a benefit even in some patients classified as non-responders.

The response rates are similar to those reported in follicular lymphoma and other indolent CD20\(^+\) B-cell lymphomas\(^25,30-32\). The observed response duration (median 11, mean 13 months) is also similar to that observed after rituximab therapy for follicular lymphoma\(^31\).

Re-treatment at relapse is feasible, and the response rate following re-treatment is in the same order of magnitude as the overall response rate. Furthermore, 2 PR were achieved following rituximab therapy for a second relapse.

Except for our previous study of 6 patients\(^4\), all original publications on rituximab therapy in CAD are case reports or retrospective observations of a few patients\(^12-16,26\).
According to review articles\textsuperscript{17,26}, a total of 23 cases have been published and responses observed in 21, both non-responders being reported in the only prospective study\textsuperscript{4}. A high proportion of the responses described in case reports have been classified by the authors as “complete”, without using well-defined response criteria\textsuperscript{26}. Our data are not in accordance with the combined results from the case reports. The discrepancy can easily be explained, since response rates estimated from case reports are very likely to be influenced by publication bias, lack of strict disease definitions, and heterogeneous response criteria.

Rituximab therapy was well tolerated, apparently better than in patients treated for other B-cell lymphomas\textsuperscript{31,32}. This finding is not unexpected, since the tumor burden is low in patients with primary CAD\textsuperscript{1}.

It was our intention to evaluate whether combining rituximab with IFN could improve efficacy, but patient and/or physician acceptability resulted in only 5 patients receiving the combination therapy. This small number makes us unable to put forward any firm statements on this issue. It may be interesting however, that one patient who had not responded to single agent rituximab, achieved a PR following second-line therapy with rituximab plus IFN. The 2 PR in 3 relapsed patients treated with rituximab plus IFN do not allow any conclusion to be drawn, since the response rate observed after rituximab single agent therapy for relapse was 63%.

In most cases in our series, a clinical response was accompanied by a histological response. However, we observed patients achieving PR without a significant histological regression of the bone marrow disorder, and in other patients we found a significant improvement of bone marrow histology without any clinical improvement. Furthermore, we observed a near complete elimination of CD20\textsuperscript{+} cells from bone marrow aspirates following most courses of rituximab in both responders and non-responders. In the majority of cases this effect was not caused by blocking of the receptor, since such blocking should result in
CD19/20 discrepancy. The histological and flowcytometric findings therefore suggest that some other mechanism than elimination of clonal CD20⁺ cells may be partly responsible for the therapeutic effect of rituximab in CAD.

Responses could not be predicted from the baseline levels of Hgb, IgM, C3, C4, or from the κ/λ-ratio or percentage of CD20⁺ cells in the bone marrow.

In conclusion, this prospective study documents the favorable effect of rituximab in primary CAD. Further studies should be undertaken in order to understand the characteristics of the non-responders and be able to propose therapies for this subgroup. The potential for increasing the response duration should also be investigated.

Acknowledgments

We want to thank Eva-Marie Jacobsen, Svein Arve Schjølseth, Oluf Herlofsen, and Yngve Sørum, who participated in therapy and follow-up of some patients, and Klaus Beiske who examined some of the bone marrow biopsies. We are also grateful to Kristin Nyberg for support and useful discussions.

References


Rituximab for primary chronic cold agglutinin disease: a prospective study of 37 courses of therapy in 27 patients