

A phase III randomized trial comparing glucocorticoid monotherapy *versus* glucocorticoid and rituximab in patients with autoimmune haemolytic anaemia

Henrik Birgens,¹ Henrik Frederiksen,² Hans C. Hasselbalch,³ Inge H. Rasmussen,⁴ Ove J. Nielsen,⁵ Lars Kjeldsen,⁵ Herdis Larsen,⁶ Torben Mourits-Andersen,⁷ Torben Plesner,⁸ Dorte Rønnev-Jessen,⁶ Hanne Vestergaard,⁸ Tobias W. Klausen¹ and Claudia Schöllkopf³

¹Department of Haematology, Copenhagen University Hospital, Herlev, ²Department of Haematology, Odense University Hospital, Odense, ³Department of Haematology, Roskilde University Hospital, Roskilde, ⁴Department of Haematology, Aalborg University Hospital, Århus University, Aalborg, ⁵Department of Haematology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, ⁶Department of Internal Medicine, Haematology section, Viborg Hospital, Viborg, ⁷Department of Haematology and Infectious Diseases, Esbjerg Hospital, Esbjerg, and ⁸Department of Haematology, Vejle Hospital, Vejle, Denmark

Received 9 April 2013; accepted for publication 27 June 2013

Correspondence: Henrik Birgens, Department of Haematology, Copenhagen University Hospital Herlev, Herlev Ringvej 75, DK-2730 Herlev, Denmark.
E-mail: henrik.birgens@regionh.dk

Warm-antibody reactive autoimmune haemolytic anaemia (WAIHA) is one of the most common forms of acquired haemolytic anaemia, and is caused by the formation of autoantibodies with a temperature maximum of about 37°C against antigens bound to the surface of the red cell membrane (Petz, 2001). WAIHA can be primary or secondary to another disease, mainly occurring secondary to chronic lymphocytic leukaemia (CLL) or an inflammatory condition, such as systemic lupus erythematosus (SLE) or ulcerative colitis (Petz & Garratty, 2004).

Conventional treatment of WAIHA includes corticosteroids as first-line therapy, which is associated with long-term remission in approximately 30% of patients (Petz, 2001). In

Summary

The impact of first-line treatment with the anti-CD 20 chimeric monoclonal antibody rituximab in patients with warm-antibody reactive autoimmune haemolytic anaemia (WAIHA) is unknown. We report the first randomized study of 64 patients with newly diagnosed WAIHA who received prednisolone and rituximab combined ($N = 32$) or prednisolone monotherapy ($N = 32$). After 12 months, a satisfactory response was observed in 75% of the patients treated with rituximab and prednisolone but in a significantly smaller proportion (36%) of those given prednisolone alone ($P = 0.003$). Furthermore, relapse-free survival was significantly better after the combined therapy than after prednisolone monotherapy ($P = 0.02$). After 36 months, about 70% of the patients were still in remission in the rituximab-prednisolone group, whereas only about 45% were still in complete or partial remission in the prednisolone group. There was no significant difference between the two groups regarding adverse reactions to the studied medications. Likewise, serious adverse events were equally distributed, and no allergic reactions to rituximab were recorded. In conclusion, our data show that using rituximab and prednisolone combined rather than prednisolone alone as first-line treatment in WAIHA increases both the rate and the duration of the response.

Keywords: immune haemolytic anaemia, clinical trials, antibody therapy.

cases involving relapse or refractory disease, splenectomy may be the initial second-line therapy, or, if that is not feasible, other immunosuppressive drugs, such as azathioprine, cyclophosphamide, ciclosporin A, danazol, and rituximab (Petz, 2001). However, splenectomy and prolonged treatment with these drugs entails a considerable risk of serious infections as the result of profound long-term immunosuppression. Indeed, a recent review concluded that no controlled clinical trials have yet been performed that can guide the choice of treatment in WAIHA (Crowther *et al*, 2011).

Rituximab is a chimeric human IgG1- κ monoclonal antibody directed specifically against the CD20 antigen expressed

on the surface of B-lymphocytes, and it is known that this agent depletes the blood of circulating B-lymphocytes for several months (Roll *et al*, 2006). Rituximab is licensed for the treatment of malignant B-cell lymphomas and rheumatoid arthritis, but has also been reported to be effective against other autoimmune diseases, such as SLE, multiple sclerosis, thrombotic thrombocytopenic purpura and acquired haemophilia (Garvey, 2008). Its influence on B-lymphocytes is mediated by several mechanisms, including antibody-dependent cellular cytotoxicity and complement-mediated cytotoxicity (Reff *et al*, 1994). However, the efficacy of rituximab in autoimmune haematological disorders is probably mediated not only through elimination of pathogenic auto-antibodies, but also by disturbing the potency of B-lymphocytes as antigen-presenting and cytokine-producing cells (Reff *et al*, 1994; Stasi, 2010).

Rituximab has been used successfully to treat immune-mediated cytopenias, such as immune thrombocytopenic purpura (Zaja *et al*, 2010; Arnold *et al*, 2012), WAIHA (Shanafelt *et al*, 2003; Narat *et al*, 2005; Ramanathan *et al*, 2005; D'Arena *et al*, 2007; Bussone *et al*, 2009; Dierickx *et al*, 2009; Barcellini *et al*, 2012), and cold-antibody reactive autoimmune haemolytic anaemia (Berentsen *et al*, 2004; Schöllkopf *et al*, 2006). These studies have included adult patients with idiopathic (primary) immune cytopenias as well as adult patients with secondary forms in relation to CLL (Chemnitz *et al*, 2002; Gupta *et al*, 2002) and SLE (Perrotta *et al*, 2002). Similarly, rituximab has also been reported to be effective in children with immune cytopenias (Quartier *et al*, 2001; Zecca *et al*, 2003; Rao *et al*, 2008). Nevertheless, the majority of published studies of rituximab in immune cytopenias have included only a few or small series of patients, and many of them have been retrospective in design and have focused chiefly on patients who had received other treatment modalities before being given rituximab. In addition, none of the investigations have been performed in a randomized, prospective fashion.

In the present phase III trial, patients with newly diagnosed primary and secondary WAIHA were randomized to either prednisolone alone or prednisolone and rituximab combined in order to determine whether use of this combination as first-line therapy could increase the response rate and prolong event-free survival (EFS).

Materials and methods

Study design

This multicentre, randomized, open-label phase III trial was initiated in March 2005 and concluded in June 2012. The treatment and the response evaluation phase lasted from the time of enrollment up to 12 months (Fig 1). The follow-up phase lasted from 1 to 4 years, thus all patients were followed for at least 12 months.

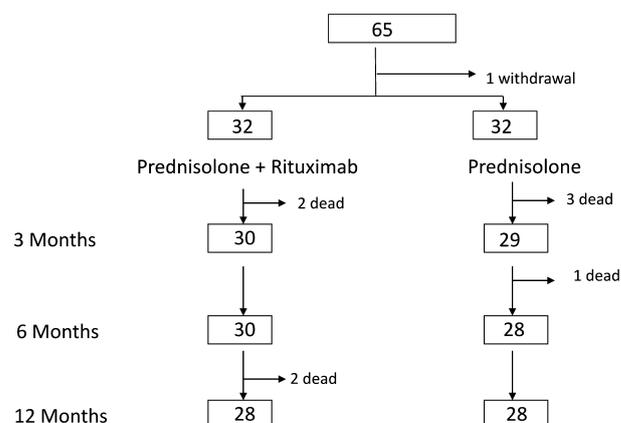


Fig 1. Flow chart illustrating the number of patients available for response assessment 3, 6 and 12 months after initiation of treatment with either rituximab and prednisolone combined or prednisolone alone.

Patients

Patients treated at eight haematological centres in Denmark participated in the study, which was approved by the Danish National Committee of Health Research Ethics (Reg. no. KA 0462gms) and the Danish Data Protection Agency (j.nr. 2004-41-40679). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Clinicaltrials.gov identifier NTC001134432.

Eligible patients had newly diagnosed and previously untreated WAIHA, defined as symptomatic haemolytic anaemia associated with a positive direct antiglobulin test with either anti-IgG alone or a combination of anti-IgG and anti-C3d tested by a gel agglutinating system (ID-system, Diamed GmbH, Cressier, Switzerland). Patients with primary AIHA, as well as those with a concomitant autoimmune disease or a low-grade B-cell lymphoproliferative neoplasia, were also included. Patients with overt drug-induced immune haemolytic anaemia were not included. Patients were allowed to have received oral prednisolone for up to 1 week before enrollment.

The exclusion criteria were as follows: age under 18 years; Eastern Cooperative Oncology Group performance status above 2; previous rituximab treatment; immunosuppressive or anti-neoplastic drugs within the last 3 months; haemolytic anaemia secondary to autoimmune disease within the last 6 months; other serious diseases, including malignancy; pregnancy or breast-feeding; hypersensitivity to rituximab, and active infection requiring systemic treatment. In addition to careful clinical assessment, a bone marrow aspirate and biopsy and an abdominal ultrasound scan or a computerized tomography scan were performed on all patients to determine whether the AIHA was primary or secondary to a B-cell lymphoproliferative disease. Anti-nuclear antibodies, DNA antibodies, and rheumatoid factors were assessed in all patients. All blood analyses were performed in local laboratories

according to good laboratory practice. All Danish laboratories for clinical chemistry are subject to a common quality assurance system to ensure consistent quality throughout the country.

Treatment

Eligible patients were randomized 1:1 by use of pre-coded envelopes. All patients received prednisolone 1.5 mg/kg/d for 2 weeks followed by tapering according to this schedule: 0.75 mg/kg/d for 1 week (week 3), thereafter 0.5 mg/kg/d for 1 week (week 4), followed by a gradual reduction over the next 4–8 weeks to the lowest dose that was effective in maintaining a normal haemoglobin level.

In the group allocated to a combination of prednisolone and rituximab, the patients received prednisolone at the same dose and schedule as in the monotherapy group and were given rituximab at a dosage of 375 mg/m² as an intravenous infusion once a week for 4 weeks. All patients received oral folic acid 5 mg/day, and those given a rituximab infusion also received premedication with acetaminophen 1 g and clemastine 2 mg intravenously 30–60 min before the infusion.

Assessments and outcome measures

The patients underwent a full clinical examination and complete blood counts including haemolytic parameters (e.g., haemoglobin, reticulocyte count, total bilirubin, lactate dehydrogenase, and haptoglobin) at enrollment, on days +7, +14, +21, +28, +42, +56, +70 and +84, then monthly until month 6, and finally, every third month until the end of follow-up. This applied to all patients until they showed a lack of response necessitating either a switch to some other immunosuppressive treatment or splenectomy, or they relapsed after an initial positive response. The minimum and maximum follow-up times after initiating treatment were 12 and 48 months, respectively.

The primary objective of the study was to analyse differences in treatment responses between the two groups. Responses were evaluated at 3, 6 and 12 months after treatment was initiated. Complete response (CR) was defined as normalization in haemoglobin concentration without any ongoing immunosuppressive treatment and without any biochemical signs of haemolytic activity. Partial response (PR) was defined as being similar to CR but requiring continued low-dose prednisolone (<10 mg/day), or appearing as compensated haemolytic anaemia entailing a stable, acceptable haemoglobin level without any need of treatment except < 10 mg/day prednisone. Neither CR nor PR was attained by any of the non-responders (i.e., those patients who required more than 10 mg prednisone per day or other immunosuppressive treatment or splenectomy).

Secondary objectives of our investigation were to evaluate differences in relapse-free survival (RFS), red blood cell transfusion requirement after treatment, the need for splenectomy,

and safety profiles associated with the treatments up to 12 months after enrollment.

Statistical analysis

The chi-square test was used to analyse the two groups regarding differences in the proportion of cases with CR or PR and the Fisher's exact test was used for analysis of adverse events due to low cell counts. Time to relapse was compared using the Kaplan-Meier method and log rank test. For descriptive statistics, we applied the Mann-Whitney test. All *P*-values were two-sided, and those below 0.05 were considered statistically significant. All calculations were performed using IBM SPSS Statistics version 19.0.0 (2010, IBM corp., Armonk, NY) or R Statistical Software version 2.14.1 (2011, R Foundation for Statistical Computing, Vienna, Austria).

Results

Patients

A total of 65 patients were initially allocated to either of the two treatment groups, but one patient withdrew informed consent and was removed from the study. Thus 32 patients were randomized to prednisolone and 32 to a combination of prednisolone and rituximab (Fig 1). Patient characteristics are given in Table I. There was no statistically significant

Table I. Baseline characteristics of the patients.

	Prednisolone	Prednisolone and Rituximab	<i>P</i> -value
Number	32	32	
Age (years)	67 (35–90)	65 (41–89)	0.62
Median (range)			
Sex ratio	17/15	20/12	0.61
(men/women)			
Lymphoproliferative disease	2	4	0.35
Autoimmune disease	1	0	1.0
Haemoglobin (g/l)	74 (48–128)	80 (48–112)	0.18
Median (range)			
Reticulocytes (x10 ⁹ /l)	197 (2–715)	205 (45–746)	0.58
Median (range)			
Platelets (x10 ⁹ /l)	317 (20†–750)	327 (127–540)	0.96
Bilirubin (µmol/l)	35 (8–114)	51 (4–207)	0.44
Median (range)			
LDH (U/l)	511 (25–918)	431 (88–1566)	0.85
Median (range)			
ALAT (U/l)*	30 (6–300)	26 (8–88)	0.54
Median (range)			
Creatinine (µmol/l)	79 (47–122)	85 (45–142)	0.21
Median (range)			

*Alanine aminotransferase.

†This patient had Evans syndrome.

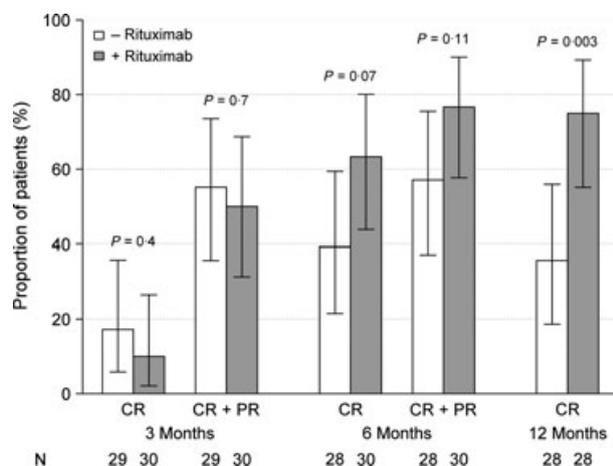


Fig 2. Proportions of patients with newly diagnosed warm-antibody reactive autoimmune haemolytic anaemia that showed any response (CR + PR) or a complete response (CR) at 3, 6 and 12 months after initiating treatment with rituximab and prednisolone combined ($N = 32$) or prednisolone alone ($N = 32$). CR, complete response; PR, partial response.

differences between the two groups with respect to age, sex ratio, haemolytic activity, liver and renal function, or the proportion with an underlying lymphoproliferative or autoimmune disease. Fig 1 shows the number of patients available for response evaluation 3, 6 and 12 months after initiating treatment.

Response to treatment

Three months after initiating treatment, CR or PR was attained in about 50% of the patients in each of the two groups (Fig 2). After 6 months, the majority of responding patients developed CR and those co-treated with combination of prednisolone and rituximab showed a tendency towards a higher response rate, considering both CR and a combination of CR and PR. After 12 months, all patients demonstrated only CR or persistent disease, and the difference between the two treatment groups was highly significant ($P < 0.003$). In the prednisolone-rituximab group, the CR rates after 6 and 12 months were 63% [95% confidence interval (CI): 44–80] and 75% (95% CI: 55–89), respectively. This compared to CR rates of 39% (95% CI: 22–59) at 6 months and 36% (95% CI: 19–56) at 12 months in the group treated with prednisolone monotherapy.

Cumulative RFS was evaluated in all patients who exhibited any response to treatment (CR or PR) and the results for the patients on prednisolone monotherapy were compared with those obtained for the subjects given rituximab plus prednisolone. The RFS in all responders was significantly higher in the patients receiving rituximab and prednisolone combined ($P = 0.02$; Fig 3) corresponding to a hazard ratio of 0.33 (95% CI: 0.12–0.88). Thirty-six months after start of treatment, about 70% of the patients who showed

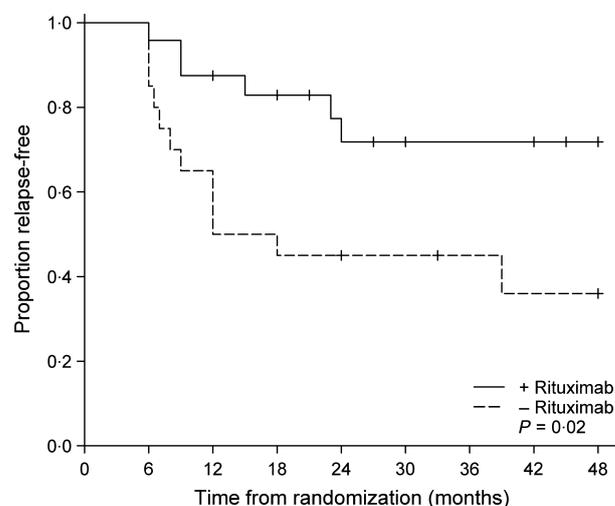


Fig 3. Relapse-free survival in patients with newly diagnosed warm-antibody reactive autoimmune haemolytic anaemia randomized to receive rituximab and prednisolone combined or prednisolone alone as first-line therapy.

either CR or PR were still relapse-free in the combination treatment group, whereas the corresponding proportion in the group receiving prednisolone monotherapy was only about 45%. The study was not powered to stratify analyses of remission in subgroups of the patients.

We also examined the number of red blood cell transfusions that was given to the patients after treatment. There was no difference between the prednisolone/rituximab group and the prednisolone monotherapy group when comparing responders (total and partial) from enrollment to end of response or to the end of study follow-up (34 units *versus* 30 units, median (range): 0 (1–6) vs. 0 (1–5), $P = 0.81$).

The number of patients that underwent splenectomy due to relapse or lack of response was essentially equivalent in the two treatment groups (4 in the rituximab group and 3 in the non-rituximab group).

Safety

The side effects and the number of Serious Adverse Events (SAEs) with a non-fatal or a fatal outcome did not differ between the two treatment groups (Tables II and III).

The most common complaints were dyspnea, dyspepsia, insomnia, headache and fatigue, several of which were probably related to prednisolone.

Most of the non-fatal SAEs were not considered to be related to treatment. However, the responsible investigator could not exclude the possibility of an association with the immunosuppression caused by the therapy in 3 patients with infections.

One of the seven cases with a fatal SAE was a patient who contracted pneumonia and was transferred to the intensive care unit; in this case, the investigator could not exclude the

Table II. Warm-antibody reactive autoimmune haemolytic anaemia patients randomized to prednisolone and rituximab in combination or prednisolone monotherapy: proportions exhibiting adverse events of grade 1 or higher according to the National Cancer Institutes Common Terminology Criteria for Adverse Events (NCI CTCAE) version 3.0.

Side effect	Rituximab + Prednisolone (%)	Prednisolone (%)	P-value
Fever	3.3	6.7	0.6
Nausea	0	10	0.1
Dyspnea	13.3	16.7	0.7
Dyspepsia	3.3	13.3	0.2
Restless legs	6.7	0	0.5
Vertigo	0	10	0.1
Increased appetite	0	10	0.1
Insomnia	10	10	1.0
Arthralgia	6.7	3.3	1.0
Headache	6.7	13.3	0.4
Fatigue	13.3	13.3	1.0
Hypotension	6.7	3.3	1.0
Palpitations	0	6.7	0.7
Hyperglycaemia	0	6.7	0.7

(http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae3.pdf).

Table III. Warm-antibody reactive autoimmune haemolytic anaemia patients randomized to either prednisolone and rituximab in combination or prednisolone as monotherapy: number of serious adverse events (SAEs) with a non-fatal or a fatal outcome.

	Prednisolone + Rituximab (n)	Prednisolone (n)
Non-fatal SAEs	8 (5 patients)	4 (4 patients)
Pneumonia	3	2
Fever	2	
Urinary tract infection	2	1
<i>Chlostridium difficile</i> enteritis	1	
Pulmonary embolism		1
SAE with fatal outcome	4 (4 patients)	3 (3 patients)
Pneumonia	1	
Ischaemic stroke	2	1
Heart failure	1	1
Homicide victim		1

possibility that this complication was due to the immunosuppressive treatment with rituximab and prednisolone. The other 6 fatal SAEs were not considered as related to the immunosuppressive therapy.

No cases of allergic reaction were observed in the patients treated with rituximab.

Discussion

For several years, the conventional first-line treatment for WAIHA has been high-dose corticosteroids. The majority of patients show an initial response to this treatment, but only

about 30% exhibit a sustained response with long-term remission. Furthermore, most patients ultimately require additional treatment modalities, i.e., either splenectomy or some other type of immunosuppressive therapy (i.e. azathioprine, cyclophosphamide, danazol, or rituximab) (Petz, 2001).

To the best of our knowledge, the present study is the first randomized trial to investigate the treatment of WAIHA.

Overall, even though only 32 patients were enrolled in each of the two treatment arms, our results convincingly demonstrate that using a combination of prednisolone and rituximab as first-line therapy in patients with newly diagnosed WAIHA leads to significantly higher response rates and longer RFS than can be achieved by prednisolone monotherapy.

Remarkably, the response rate observed in patients who received the combination of prednisolone and rituximab gradually increased over a period of 6 months after discontinuation of treatment. Patients treated with prednisolone alone seemed to show a maximum total response after 3 months, suggesting a more late effect of Rituximab. The difference between the patients with the combination therapy and those on prednisolone monotherapy was detectable after 6 months but was not statistically significant until after 12 months.

Our patients receiving the combined treatment had a response rate of 75%, which is somewhat lower than the rate of 90% recently observed by Barcellini *et al* (2012) in 14 WAIHA patients 12 months after initiating treatment with a combination of oral prednisone and low-dose rituximab (fixed dose of 100 mg in 4 weekly infusions). One explanation for this discrepancy might be a difference in the response criteria applied in the two investigations.

In our study, the difference in RFS in favour of the combination therapy was obvious after 6 months, and relapse occurred in a much larger proportion of the patients in the prednisolone monotherapy group over the 6–12 months after initiation of treatment. The RFS rate in our patients receiving the combination therapy was 70–80%, which is similar to the long-term RFS of slightly more than 75% reported by Barcellini *et al* (2012) in their 14 WAIHA patients 12 months after initiation of treatment. It can also be noted that the long-term RFS rate of approximately 40% observed in our prednisolone monotherapy group agrees with the relatively poor results of such treatment reported in the literature (Petz, 2001).

The blood transfusion requirement from the start of treatment to relapse or discontinuation of the study was not significantly different between the 2 treatment groups. One explanation could be that prednisolone, where the dose was similar in the 2 groups, is responsible for the rapid control of haemolysis, but rituximab primarily reduces the risk of relapse.

The observed findings were not biased by differences in the treatment groups. The 2 groups were comparable with regard to demographic data and haemolytic activity.

One of our objectives was to ascertain whether the combination of rituximab and prednisolone as first-line treatment in WAIHA could reduce the need for splenectomy compared to prednisolone monotherapy. However, the number of patients that were splenectomized in our trial was too small to demonstrate such a difference. Moreover, in general, the indication for splenectomy is not clear and may vary between different treatment centres.

The limitation of this study is obviously the relatively small number of patients in the two treatment groups. However, in a power analysis of our results in terms of proportions of CR at 12 months we found a power of 0.86, indicating that 86% of equivalent trials will show a significant result. In addition, in terms of EFS, we found that a hazard ratio of 0.267 (in contrast to 0.33) would be detectable with 80% certainty given the applied population size.

Our patients received 375 mg/m² rituximab (as 4 weekly infusions), which is equivalent to the dose that is conventionally applied in the majority of rituximab studies, including those evaluating malignant lymphoma. Importantly, the same results might have been achieved using the low-dose principle of a fixed level of 100 mg, as in the above-mentioned study by Barcellini *et al* (2012) and in a previous study that assessed patients with immune thrombocytopenia (Zaja *et al*, 2008). Obviously, such a strategy might reduce the costs and probably also decrease the side effects of rituximab, and hence deserves evaluation in another randomized study.

Notably, the side effects that occurred did not differ between the two treatment groups in our trial and, perhaps most importantly, the SAEs were equally distributed and there were no serious allergic reactions to rituximab.

In conclusion, this is the first randomized study of the combination therapy of rituximab and prednisolone *versus* prednisolone monotherapy as first-line treatment in patients with WAIHA. The results show a significantly higher response rate and longer RFS in favour of the combination therapy. Furthermore, the rituximab-prednisolone therapy has an acceptable safety profile and is not associated with more serious side effects than the conventional steroid treatment.

Acknowledgements

The trial was investigator-initiated but was supported in part by research funding from Roche A/S, Denmark. The design of the study, protocol writing, interpretation of the data, and preparation of the manuscript were conducted without any interference from Roche. We thank research nurses Christina Rønsholt, Lars Harpelund, Lidia Wojnicka Ginsbo, and Pia Høigaard-Sørensen for careful collection and storage of data during the trial.

Author contributions

HB designed the research study, was the principal investigator, analysed the data and wrote and edited the manuscript. HCH and CS designed the research study, collected participants, analysed the data and edited the manuscript. HF, IHR, OJN, LK, HL, HTM-A, TP, DR-J and HV collected participants, analysed the data and edited the manuscript. TWK performed the statistical data-analyses and wrote and edited the manuscript.

References

- Arnold, D.M., Heddle, N.M., Carruthers, J., Cook, D.J., Crowther, M.A., Meyer, R.M., Liu, Y., Cook, R.J., McLeod, A., MacEachern, J.A., Mangel, J., Andersen, D., Vickers, L., Timmouth, A., Schuh, A.C. & Kelton, J.G. (2012) A pilot randomized trial of adjuvant rituximab or placebo for nonsplenectomized patients with immune thrombocytopenia. *Blood*, **119**, 1356–1362.
- Barcellini, W., Zaja, F., Zaninoni, A., Imperiali, F.G., Battista, M.L., Di Bona, E., Fattizzo, B., Consonni, D., Cortelezzi, A., Fanin, R. & Zanella, A. (2012) Low-dose rituximab in adult patients with idiopathic autoimmune haemolytic anaemia: clinical efficacy and biological studies. *Blood*, **119**, 3691–3697.
- Berentsen, S., Ulvestad, E., Gjertsen, B.T., Hjorth-Hansen, H., Langholm, R., Knutsen, R., Ghanima, W., Shammass, F.V. & Tjønfjord, G.E. (2004) Rituximab for primary cold agglutinin disease: a prospective study of 37 courses of therapy in 27 patients. *Blood*, **103**, 2925–2928.
- Bussone, G., Ribeiro, E., Dechartres, A., Viillard, J.-F., Bonnotte, B., Fain, O., Godeau, B. & Michel, M. (2009) Efficacy and safety of rituximab in adults' warm antibody autoimmune haemolytic anaemia: retrospective analysis of 27 cases. *American Journal of Hematology*, **84**, 153–157.
- Chemnitz, J., Draube, A., Diehl, V. & Wolf, J. (2002) Successful treatment of steroid and cyclophosphamide-resistant hemolysis in chronic lymphocytic leukemia with rituximab. *American Journal of Hematology*, **69**, 232–235.
- Crowther, M., Chan, Y.L.T., Garbett, I.K., Lim, W., Vickers, M.A. & Crowther, M.A. (2011) Evidence-based focused review of the treatment of idiopathic warm immune haemolytic anaemia in adults. *Blood*, **118**, 4036–4040.
- D'Arena, G., Califano, C., Annunziata, M., Tartarone, A., Capalbo, S., Villani, O., Amendola, G., Pietrantonio, G., Ferrara, F., Pinto, A., Musto, P., D'Arco, A.M. & Cascavilla, N. (2007) Rituximab for warm-type idiopathic autoimmune haemolytic anaemia: a retrospective study of 11 adult patients. *European Journal of Haematology*, **79**, 53–58.
- Dierickx, D., Verhoef, G., Van Hoof, A., Mineur, P., Roest, A., Triffet, A., Kentos, A., Pierre, P., Boulet, D., Bries, G., Le, P.-Q., Janssens, A. & Delannoy, A. (2009) Rituximab in auto-immune haemolytic and immune thrombocytopenic purpura: a Belgian retrospective multicentric study. *Journal of Internal Medicine*, **266**, 484–491.
- Garvey, B. (2008) Rituximab in the treatment of autoimmune haematological disorders. *British Journal of Haematology*, **141**, 149–169.
- Gupta, N., Kavuru, S., Patel, D., Janson, D., Driscoll, N., Ahmed, S. & Rai, K.R. (2002) Rituximab-based chemotherapy for steroid-refractory autoimmune haemolytic anaemia of chronic lymphocytic leukemia. *Leukemia*, **16**, 2092–2095.
- Narat, S., Gandla, J., Hoffbrand, A.V., Hughes, R.G. & Mehta, A.B. (2005) Rituximab in the treatment of refractory autoimmune cytopenias in adults. *Haematologica*, **90**, 1273–1274.
- Perrotta, S., Locatelli, F., La Manna, A., Cennamo, L., De Stefano, P. & Nobili, B. (2002) Anti-CD20 monoclonal antibody (Rituximab) for life-threatening autoimmune haemolytic anaemia in a patient with systemic lupus erythematosus. *British Journal of Haematology*, **116**, 465–467.

- Petz, L.D. (2001) Treatment of autoimmune haemolytic anaemias. *Current Opinion in Hematology*, **8**, 411–416.
- Petz, L.D. & Garratty, G. (2004) Immune Haemolytic Anaemias, 2nd edn. Churchill Livingstone, Philadelphia.
- Quartier, P., Brethon, B., Philippet, P., Landman-Parker, J., Le Deist, F. & Fischer, A. (2001) Treatment of childhood autoimmune haemolytic with rituximab. *The Lancet*, **358**, 1511–1513.
- Ramanathan, S., Koutts, J. & Hertzberg, M.S. (2005) Two cases of refractory warm autoimmune haemolytic anaemia treated with rituximab. *American Journal of Hematology*, **78**, 123–126.
- Rao, A., Kelly, M., Musselman, M., Ramadas, J., Wilson, D., Grossman, W. & Shenoy, S. (2008) Safety, efficacy, and immune reconstitution after rituximab therapy in pediatric patients with chronic and refractory hematologic autoimmune cytopenias. *Pediatric Blood & Cancer*, **50**, 822–825.
- Reff, M.E., Carner, K., Chambers, K.S., Chinn, P.C., Leonard, J.E., Raab, R., Newman, R.A., Hanna, N. & Anderson, D.R. (1994) Depletion of B-cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood*, **83**, 435–445.
- Roll, P., Palanichamy, A., Kneitz, C., Dorner, T. & Tony, H.-P. (2006) Regeneration of B cell subsets after transient B cell depletion using anti-CD20 antibodies in rheumatoid arthritis. *Arthritis and Rheumatism*, **54**, 2377–2386.
- Schöllkopf, C., Kjeldsen, L., Bjerrum, O.W., Mourits-Andersen, H.T., Nielsen, J.L., Christensen, B.E., Jensen, B.A., Pedersen, B.B., Taaning, E.B., Klausen, T.W. & Birgens, H. (2006) Rituximab in chronic cold agglutinin disease: a prospective study of 20 patients. *Leukemia & Lymphoma*, **47**, 253–260.
- Shanafelt, T.D., Madueme, H.L., Wolf, R.C. & Tefferi, A. (2003) Rituximab for Immune Cytopenia in Adults: idiopathic Thrombocytopenic Purpura, Autoimmune Haemolytic Anaemia, and Evans Syndrome. *Mayo Clinic Proceedings*, **78**, 1340–1346.
- Stasi, R. (2010) Rituximab in Autoimmune Hematologic Diseases: not Just a Matter of B Cells. *Seminars in Hematology*, **47**, 170–179.
- Zaja, F., Battista, M.L., Pirota, M.T., Palmieri, S., Montagna, M., Vianelli, N., Marin, L., Cavallin, M., Bocchia, M., Defina, M., Ippoliti, M., Ferrara, K., Patriarca, F., Avanzini, M.A., Regazzi, M., Baccarani, M., Isola, M., Soldano, F. & Fanin, R. (2008) Lower dose rituximab is active in adults patients with idiopathic thrombocytopenic purpura. *Haematologica*, **93**, 930–933.
- Zaja, F., Baccarani, M., Mazza, P., Bocchia, M., Gugliotta, L., Zaccaria, A., Vianelli, N., Defina, M., Tieghi, A., Amadori, S., Campagna, S., Ferrara, F., Angelucci, E., Usula, E., Cantoni, S., Visani, G., Fornaro, A., Rizzi, R., De Stefano, V., Casulli, F., Battista, M.L., Isola, M., Soldano, F., Gamba, E. & Fanin, R. (2010) Dexamethasone plus rituximab yields higher sustained response rates than dexamethasone monotherapy in adults with primary immune thrombocytopenia. *Blood*, **115**, 2755–2762.
- Zecca, M., Nobili, B., Ramenghi, U., Perrotta, S., Amendola, G., Rosito, P., Jamkovic, M., Pierani, P., De Stefano, P., Borana, M.R. & Locatelli, F. (2003) Rituximab for the treatment of refractory autoimmune haemolytic anaemia in children. *Blood*, **101**, 3857–3861.