# Efficacy of rituximab in difficult-to-manage autoimmune hepatitis: Results from the International Autoimmune Hepatitis Group

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## Graphical abstract
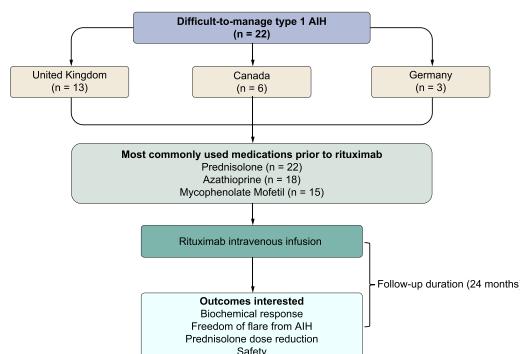


## Highlights

- Study of rituximab therapy in 22 patients with autoimmune hepatitis over a follow-up period of 24 months.

- No serious adverse events were noted during follow-up in patients treated with rituximab.

- Rituximab therapy improved liver enzymes significantly during the 2 years of follow-up.

- Prednisolone dose reductions were seen in 62% of patients at 12-month follow-up.

- A total of 71% of patients were free of AIH flares during the 24 months of follow-up.

## Lay summary
Autoimmune hepatitis is an autoimmune condition of the liver, usually treated with medications that suppress the immune system, such as steroids. However, some patients do not respond to this treatment. We analysed the safety and efficacy of rituximab in patients who were not responding to first- or second-line therapies. Rituximab was safe and improved liver blood tests in 70% of patients over a 2-year follow-up period, while enabling steroid doses to be reduced in two-thirds of patients, which is a very positive clinical outcome.

# Efficacy of rituximab in difficult-to-manage autoimmune hepatitis: Results from the International Autoimmune Hepatitis Group

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**Background & Aims:** Treatment options remain limited for patients with autoimmune hepatitis (AIH), while there are still concerns over the consequences of long-term corticosteroid use. A few studies have suggested a role for B cell-driven autoimmune liver injury in AIH. This multicentre, international retrospective cohort study from the International Autoimmune Hepatitis Group aims to evaluate the clinical efficacy and safety of rituximab in difficult-to-manage AIH.

**Methods:** Clinical data from 22 patients who received rituximab between 2007 and 2017 were collected from centres in the United Kingdom, Germany and Canada. Clinical response was assessed using changes in biochemical and immunological parameters up to 24 months post-rituximab infusion. In addition, we compared the doses of prednisolone used 3 months before and 12 months after treatment, and assessed freedom from AIH flares over the post-treatment period.

**Results:** Twenty-two patients with type-1 AIH were included, with a median age of 40 years at diagnosis (range 19–79); 15/22 (68%) were female and 18/22 (82%) were Caucasian. The median period from diagnosis to the end of follow-up in these patients was 11 years (range 3–28). Values of alanine aminotransferase, aspartate aminotransferase and albumin improved significantly following rituximab therapy, and were sustained for up to 2 years (all $p$ <0.001). Prednisolone doses were significantly reduced by 12 months post-treatment ($p$ = 0.003), with 13/21 (62%) patients having a dose reduction. Over a median post-treatment follow-up period of 6 years (range 1–10), 5 patients developed AIH flares at a median of 22 months post-treatment, giving an estimated 71% freedom from AIH flare at 2 years. Four of these patients received a second course of treatment, of whom 2 had subsequent further flares. No serious adverse events attributable to rituximab were recorded.

**Conclusion:** In patients with difficult-to-manage AIH, rituximab appears to be clinically effective and well tolerated. Rituximab was associated with sustained improvements in serum liver tests, an absence of clinical disease flares, and a reduction in prednisolone dose. Controlled trials are warranted to further evaluate B cell-targeting therapies in patients with AIH.

## Introduction

Autoimmune hepatitis (AIH) is a rare, chronic immune-mediated liver disease, with a reported prevalence of 10–17 per 100,000 in Europe.[1,2] Due to the lack of a precise understanding of disease pathogenesis, AIH remains a therapeutic challenge. Current treatments are based on broad immunosuppression and associated with significant side effects.[3,4] Although AIH is a T cell driven disease,[4–6] immunoglobulin and antibody producing B cells also play an important role in its pathogenesis.[7] The diagnostic criteria proposed by the International Autoimmune Hepatitis Group (IAIHG) include elevated IgG concentrations, the presence of autoantibodies,[8,9] and the histological findings of a plasma cell rich interface hepatitis.[4,10]

Historical data demonstrates that patients with uncontrolled AIH progress to end-stage liver disease, with 5-year mortality of around 50%.[11] Progression of liver disease is more common in

patients with incomplete treatment response or treatment failure.[12,13] A combination of prednisolone and azathioprine is the accepted first-line treatment used for induction and maintenance of remission in AIH.[14] Approximately 15% of patients experience insufficient response or intolerance to standard therapy and require alternative agents.[15] Mycophenolate mofetil (MMF)[11] and calcineurin inhibitors, such as tacrolimus[15] are second- and third-line rescue therapies for those who are unable to tolerate azathioprine or mercaptopurine.[16] Difficult-to-manage AIH includes patients for whom these therapies are ineffective, not tolerated, and/or associated with an unacceptable long-term side effect burden. Successful treatment with biologics, such as infliximab, an anti-tumour necrosis factor antibody,[17] has been reported for such patients. In addition to infliximab, reports have suggested some patients may respond to anti-B cell therapy with rituximab.

Rituximab is a chimeric human monoclonal anti-CD20 antibody that targets CD20, a cell surface glycoprotein restricted to B cell lineages.[4,12] The pathogenesis of AIH is not fully understood, but the current hypothesis is that hepatic autoantigens present to B cells, which triggers a dysregulated T cell response against autoantigens, leading to the release of a cascade of cytokines. Interferon and tumour necrosis factor are produced upon this trigger, contributing to hepatocyte injury, cell death and release of liver enzymes in individuals who are genetically susceptible to AIH.[18] The binding of rituximab to CD20 results in apoptotic lysis and depletion of B cells via complement and antibody-mediated pathways.[19] The proposed mechanism of action of rituximab is illustrated in Fig. 1.

Rituximab has been licenced for clinical use in adults with CD20-positive B cell lymphoma, rheumatoid arthritis, or vasculitis associated with anti-neutrophil cytoplasmic antibodies.[20] However, rituximab is not currently a licenced therapy in AIH and representative, multicentre, long-term data on the efficacy of rituximab therapy in AIH is lacking. Herein we report on the efficacy and safety of rituximab in patients with difficult-to-manage AIH.

## Patients and methods

### Study population
Twenty-two patients with difficult-to-manage type 1 AIH were identified for inclusion in the study. We defined patients with
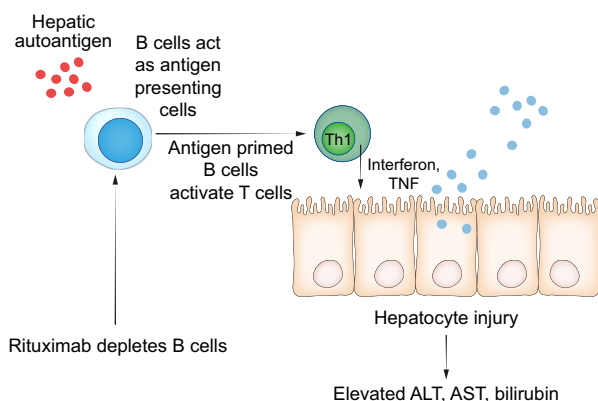


**Fig. 1. Proposed mechanism of rituximab therapy in AIH.** The proposed pathogenesis of AIH was that B cells act as antigen presenting cells by presenting autoantigens on hepatocytes to CD4 T- cells (Th1), which leads to the release of a cascade of cytokines (interferon and TNF). TNF and interferon subsequently cause hepatocyte injury, cell death and release of liver enzymes and bilirubin. Rituximab depletes B cells and as a result Th1 activation, which subsequently prevents hepatocyte injury. Th1, T helper 1 cell; TNF, tumour necrosis factor, ALT, alanine aminotransferase, AST, aspartate aminotransferase.

"difficult-to-manage" AIH as those who i) had not achieved normalisation of aminotransferase despite being on first-line therapy (corticosteroid), second-line therapies (either azathioprine or mycophenolate mofetil) or even third-line therapies (tacrolimus or cyclosporine), ii) were compliant with medications and iii) after exclusion of primary sclerosing cholangitis. This is the real-world definition used by hepatologists who are managing difficult AIH cases based on current guidelines.

The patients in this study fulfilled the criteria for a definite diagnosis of AIH, based on international guidelines[11] after excluding other causes of hepatitis, and despite being compliant with treatments, these patients continued to have elevated liver enzymes, with median alanine aminotransferase (ALT) of 214 IU/L (interquartile range 115–323 IU/L) and median IgG levels of 18.6 g/dl (interquartile range 12.2–21.9 g/dl) prior to rituximab treatment. The decision to use rituximab in these patients was made by the clinician at each centre.

For each course of treatment, patients received 2 doses of rituximab (1,000 mg in each dose) via intravenous infusion, administered 2 weeks apart, with the exception of a patient from Germany, who received 375 mg/m$^2$ rituximab. Patients received their first dose of rituximab between the years 2007 and 2017, a median of 5 years after AIH diagnosis (range 0–18 years). The data for biochemical parameters and IgG values were collected 1 month before, and at 1, 3, 6, 12 and 24 months after rituximab therapy. Five patients received additional doses of rituximab during the follow-up period, and these patients were censored from the analysis of biochemical parameters and IgG at the time the second dose was administered, with data from later time points excluded from the analysis.

Biochemical assessments included ALT, aspartate aminotransferase (AST), bilirubin and albumin. Data were also collected for haematological measures, platelet count, prothrombin time or international normalised ratio (INR) and immunology parameters (autoantibodies and serial IgG concentrations). In addition, the model for end-stage liver disease (MELD) and Child-Pugh scores, as well as data on prednisolone doses, were retrieved at 3 months before and 12 months after rituximab treatment. A flare of AIH was defined according to IAIHG criteria, as an elevation of ALT (3x upper limit of normal [ULN]).

The study was discussed and approved by IAIHG at a meeting where investigators were present. Each centre obtained its own ethical approval to collect patient data.

### Statistical analysis
Initially, the changes in a variety of measures (bilirubin, ALT, AST, IgG, albumin, INR) over the period of follow-up were assessed. As data were not complete for all patients, particularly at later time points, a generalised estimating equation approach was used. This accounted for the non-independence of repeated measures on the same patient, whist allowing for the inclusion of patients who did not have complete data for all of the time points, hence maximising the included sample size. The time point of the measurement was included as a nominal factor in the model, and a first order autoregressive correlation structure was used to account for within-patient correlations. As the biochemical and immunological values analysed all follow skewed distributions, these values were log$_{10}$-transformed, prior to analysis, to normalise the distributions. As a result, the data were summarised using geometric means and 95% CIs. Any patients that had received a second rituximab treatment were excluded from the analysis of subsequent time points. As a sensitivity analysis, a complete cases analysis was performed for those patients with measurements of each marker pre- and 1-, 3- and 6-months

post-treatment. A non-parametric approach was used, with data summarised using medians and IQRs, and comparisons performed using Freidman's tests, followed by pairwise *post hoc* tests where the overall effect was significant.

Prednisolone doses and MELD scores were then compared between the pre- and post-treatment assessments using Wilcoxon's tests. The period of freedom from a flare was estimated using Kaplan-Meier curves. It was assumed that patients would be given rituximab in the presence of a flare. Hence, for each patient, the time from the first treatment of rituximab to any subsequent treatment was calculated, with patients that only received a single rituximab treatment censored at the end of follow-up. A similar analysis was then performed starting with the second dose of rituximab. All analyses were performed using IBM SPSS 22 (IBM Corp. Armonk, NY) and GraphPad Prism version 7.0, with $p < 0.05$ deemed to be indicative of statistical significance.

## Results

### Demographics

Data were collected retrospectively from 22 patients with difficult-to-manage type 1 AIH, who had initial diagnoses of AIH between 1989 and 2012. Of those patients, the majority were recruited from the UK (Birmingham, Leeds, Cardiff; n = 13), with the remainder from Canada (n = 6) and Germany (n = 3). We included previously published data of 6 patients from Canadian Liver Unit [55], as additional long-term follow-up data were now available for these patients beyond 72 weeks follow-up. Most patients were female (n = 15, 68%) and of Caucasian ethnicity (n = 18, 82%), with a median age at diagnosis of 40 years (range 19–79). The median period from diagnosis to the end of follow-up in these patients was 11 years (range 3–28). At initial diagnosis, 10/17 (59%) were positive for antinuclear antibodies and 13/16 (81%) tested positive for anti-smooth muscle antibodies. A total of 18 patients had liver biopsies at the time of initial diagnosis, of whom 3 (17%) were Metavir liver fibrosis stage 4. Of these 3 patients, 2 were Child-Pugh A and 1 was Child-Pugh C. The clinical presentations that led to the diagnosis of AIH were abnormal liver blood tests without any symptoms (n = 9), arthralgia (n = 5), jaundice (n = 4), lethargy (n = 3) and abdominal pain (n = 1). Further demographic data are reported in Table 1.

The details of all the immunosuppressive medications that each patient had received at any time point prior to rituximab therapy since diagnosis are shown in Fig. 2. The most commonly used immunosuppressant medications used were prednisolone (n = 22, 100%), azathioprine (n = 18, 82%) and MMF (n = 15, 68%). The immunosuppressive medications that were used within 3 months prior to initiating rituximab and 12 months post therapy are reported in Fig. 3 and Table S4.

One patient had a previous HBV infection (they were positive for HBV core antibody) and received prophylactic anti-HBV treatment with lamivudine 100 mg per day prior to starting rituximab. This patient did not develop a hepatitis B flare during treatment. Two patients (9%) from the UK (Birmingham centre) underwent liver transplantation at 2 and 8 years, respectively, after their first dose of rituximab. Both patients received a second course of rituximab treatment prior to ultimate liver transplantation, due to AIH flares that occurred at 4 and 23 months after their first rituximab treatments, and both are still alive after liver transplantation at the time of writing. Only 1 patient from the cohort died, due to *Staphylococcus aureus* bacteraemia, with this occurring 12 years after their AIH diagnosis, and 6 years from the last dose of rituximab infusion. We concluded that the death was unrelated to the rituximab therapy.

### Coexisting autoimmune diseases

Thirteen patients (59%) had associated autoimmune conditions, with the most common being systemic connective tissue disorders, followed by endocrine-related conditions. Further details of the associated coexisting autoimmune conditions in the cohort are described in Table 2.

### Rituximab treatment leads to a significant improvement in AIH disease activity

Analyses of biochemical and haematological markers in the period after rituximab therapy are reported in Fig. 4 and Table S2, with the normal ranges of the blood tests shown in Table S1. The primary analysis found the average ALT and AST levels were significantly improved 1 month after the first dose of rituximab, with this improvement sustained up until the end of follow-up at 24 months ($p < 0.001$). Albumin levels were significantly improved 3 months after treatment ($p = 0.024$), with this sustained until 24 months post-treatment ($p < 0.001$). No significant changes were seen in either platelet count ($p = 0.517$), INR ($p = 0.093$) or bilirubin ($p = 0.097$) during the period of follow-up. IgG concentrations declined significantly 1-month after

**Table 1. Demographic and diagnostic factors for patients at diagnosis.**

|  | Median (range)/n (%) |
|---|---|
| **Patient demographics** |  |
| Age (years) | 40 (19–79) |
| Gender (% female) | 15 (68%) |
| Ethnicity (% caucasian) | 18 (82%) |
| **Biochemical parameters at diagnosis** |  |
| Platelets (x10⁹/L) | 323 (138–582) |
| Alanine aminotransferase (U/L) | 137 (47–518) |
| Aspartate aminotransferase (U/L) | 175 (19–1,208) |
| Bilirubin (µmol/L) | 15 (6–476) |
| Albumin (g/L) | 40 (23–46) |
| International normalised ratio | 1.0 (0.9–1.7) |
| **Immunological parameters at diagnosis** |  |
| IgG (g/L) | 23 (10–45) |
| Antinuclear antibody positivity | 10/17 (59%) |
| Anti-smooth muscle antibody positivity | 13/16 (81%) |
| Prognostic scoring at diagnosis |  |
| Simplified autoimmune hepatitis score | 6 (2–8) |
| Model for end-stage liver disease score | 8 (5–25) |
| Metavir fibrosis score | n = 18 |
| Stage 0 | 4 (22%) |
| Stage 1 | 2 (11%) |
| Stage 2 | 3 (17%) |
| Stage 3 | 6 (33%) |
| Stage 4 | 3 (17%) |
| Child-Pugh score for stage 4 fibrosis | n = 3 |
| A | 2 (67%) |
| B | 0 (0%) |
| C | 1 (33%) |

| Patient | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | N (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Prednisolone | | | | | | | | | | | | | | | | | | | | | | | 22 (100%) |
| Azathioprine | | | | | | | | | | | | | | | | | | | | | | | 18 (82%) |
| MMF | | | | | | | | | | | | | | | | | | | | | | | 15 (68%) |
| Tacrolimus | | | | | | | | | | | | | | | | | | | | | | | 6 (27%) |
| Cyclosporine | | | | | | | | | | | | | | | | | | | | | | | 5 (23%) |
| Budesonide | | | | | | | | | | | | | | | | | | | | | | | 3 (14%) |
| Sirolimus | | | | | | | | | | | | | | | | | | | | | | | 2 (9%) |
| 6-MP | | | | | | | | | | | | | | | | | | | | | | | 1 (5%) |

Fig. 2. Medications used by individual patients before rituximab treatment (at any time point). MMF, mycophenolate mofetil.

treatment (p = 0.006) and were sustained until the end of follow-up at 24 months (p <0.001).

In addition, sensitivity analyses were performed for each of the markers using a complete cases approach over the first 6 months post-treatment (Table S3). This returned consistent results to those above, with significant improvements in ALT, AST, albumin and IgG observed post-treatment. This analysis also identified a significant change in bilirubin, with median levels declining from 17 to 11 umol/L by 6 months post-treatment (p = 0.007).

MELD scores were recorded at both pre-treatment and 12 months post-treatment in 18 patients, and were not found to change significantly, with medians of 8 (IQR 6–9) and 7 (6–12), respectively (p = 0.821).

**Rituximab therapy leads to a lower burden of corticosteroid use**
Changes in prednisolone doses between 3 months pre- and 12 months post-rituximab therapy are shown in Fig. 5. One patient was excluded from this analysis, as they were treated with budesonide (at a dose of 3 mg at both time points). For the remaining 21 patients, a significant reduction in prednisolone doses was detected, from a median of 15 mg to 10 mg (p = 0.003), with a total of 13 (62%) patients having a dose reduction.

**Rituximab treatment leads to freedom from AIH flares**
A total of 5 patients (23%) developed flares of AIH during the follow-up period. The earliest flare occurred 135 days (4 months) after the first dose of rituximab, with the other 4 cases occurring
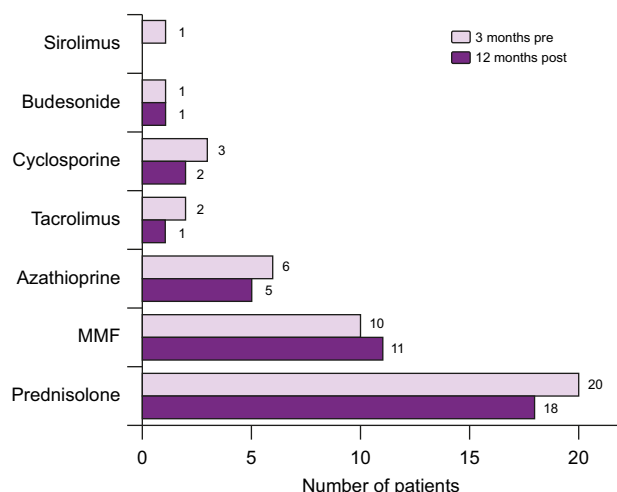
at 21, 22, 23 and 24 months. One additional patient had a second course of rituximab 9 months after their first dose for the treatment of rheumatoid arthritis rather than AIH, and hence was censored from analysis at this point.

Kaplan-Meier analysis estimated the rates of freedom from flare to be 95% and 71% at 1 and 2 years, respectively, after the first dose of rituximab (Fig. 6). Of the 5 patients who developed AIH flares, 4 (80%) were treated with a second dose of rituximab. Two of these 4 patients subsequently developed recurrence of AIH activity at 236 days (8 months) and 471 days (15 months) after their second rituximab infusions.

**Safety profile**
Rituximab was well tolerated in this cohort of patients. One patient suffered with recurrent urinary tract infection, and a second patient developed human papilloma virus-related tongue cancer. Although unlikely, a causative relationship with rituximab use could not be excluded in these cases. One patient died 6 years post-rituximab infusion from *Staphylococcus* infection, which was

Table 2. Associated autoimmune conditions.

| Patient ID | Associated autoimmune conditions |
|---|---|
| 1 | Colitis |
| 2 | Multiple sclerosis |
| 3 | Hypothyroidism |
| 4 | Diabetes mellitus, diabetes insipidus Addison's disease, hypopituitarism, hypoadrenalism Arthritis Alopecia |
| 5 | Lupus nephritis Systemic lupus erythematosus |
| 6 | Rheumatoid arthritis |
| 7 | Diabetes mellitus Systemic lupus erythematosus |
| 8 | Wegner's vasculitis |
| 9 | Coeliac Type 1 diabetes |
| 10 | Psoriasis |
| 11 | Primary biliary cholangitis |
| 12 | Colitis Systemic lupus erythematosus, Vasculitis |
| 13 | Membranous glomerulonephritis Systemic lupus erythematosus |



Fig. 3. Medications used 3 months before and 12 months after rituximab therapy in patients with autoimmune hepatitis.

Only those patients with associated autoimmune conditions are included in the table. The remainder of the cohort (n = 9) had no associated autoimmune conditions.
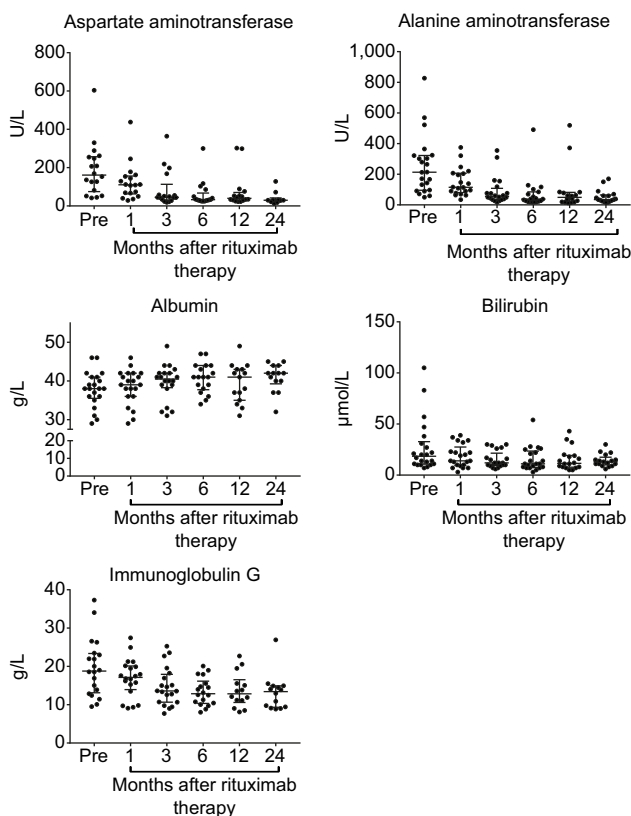
**Fig. 4. Changes in aspartate aminotransferase, alanine aminotransferase, bilirubin, albumin and IgG after rituximab treatment.**

thought to be unrelated to the infusion. No other significant side effects with treatment were reported by the investigators.

## Discussion

AIH is an immune-mediated liver disease with many unanswered questions relating to its pathogenesis. In most patients, AIH can be controlled with inexpensive, non-specific immunosuppression using prednisolone and azathioprine, which have limited the
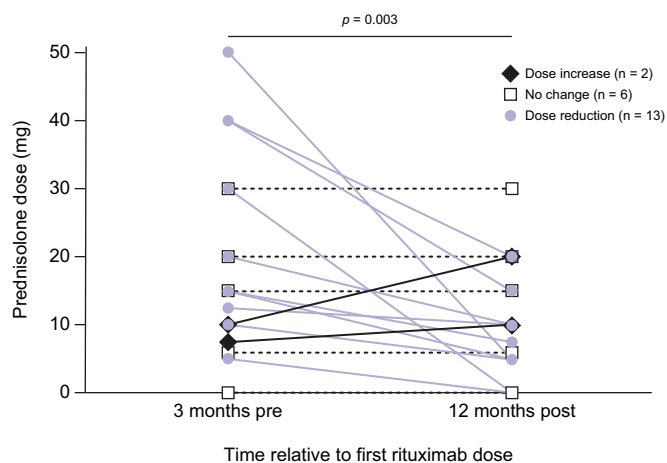


**Fig. 5. Changes in prednisolone dose following rituximab therapy.**
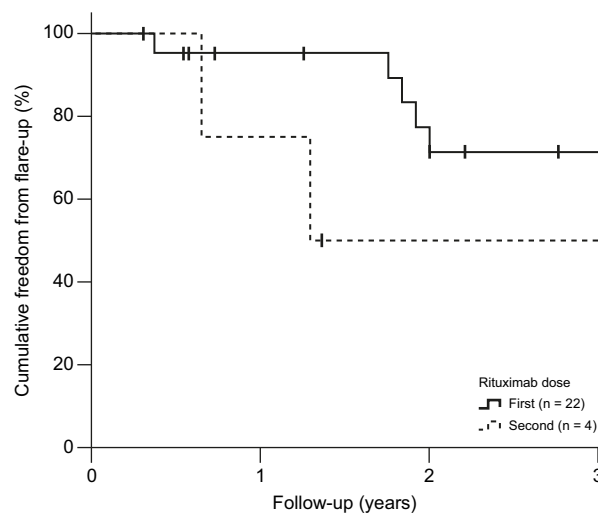


**Fig. 6. Kaplan-Meier curve of freedom from autoimmune hepatitis flares following rituximab therapy.**

need to test more targeted immunotherapies for this disease.[21] However, a significant minority of patients have AIH that is difficult to control with conventional therapies, and there is no clear evidence relating to which second- or third-line treatments should be used in such patients. We report the results of an international multicentre study of anti-B cell therapy with rituximab for difficult-to-manage AIH. Our demonstration of efficacy and acceptable safety highlights the potential of B cell-targeting therapies for patients with AIH.

The aim of treatment in AIH is to achieve biochemical, immunological and histological remission to prevent the development of cirrhosis and liver-related death.[22,23] Corticosteroid therapy followed by the addition of azathioprine has been used as the first-line treatment to induce and then maintain remission on the lowest possible dose of corticosteroid.[24–30] Mercaptopurine can be used in patients who are intolerant to azathioprine.[16] For those who are intolerant to azathioprine and mercaptopurine, MMF[31–33] is recommended as second-line treatment. However, in some patients, neither the first- or second-line therapies can be tolerated, due to side effects or contraindications associated with the therapies. For example, azathioprine is contraindicated in those with a previous history of squamous cell carcinoma of the skin[34] and MMF should be avoided in young women who intend to have a family, because of the reported risk of teratogenicity.[35] In these circumstances, calcineurin inhibitors such as tacrolimus[15,31,36] or cyclosporine[37,38] can be used as third-line treatment. However, the current approach to management of AIH is only effective in 80% of patients,[39] and a subgroup of patients with AIH continue to have elevated liver enzyme activities and IgG, despite being adherent to current treatments. This group of patients are known to clinicians as difficult-to-manage AIH cases. However, it is necessary to exclude other causes of hepatitis and evaluate treatment compliance before definitively diagnosing these patients with "difficult-to-manage" AIH.[40–45]

AIH is an immune-mediated liver disease, thus biological agents, such as infliximab (anti-tumour necrosis factor [TNF] therapy), rituximab (anti-B cell therapy) or even tocilizumab (anti-IL-6

therapy) may have a potential role as disease-modifying agents and salvage therapies.[17,46,47] Anti-TNF treatment (infliximab) has been applied successfully as a rescue therapy in AIH.[17] Adult patients with AIH who have not responded sufficiently or are intolerant to standard of care are currently being recruited for the first randomised placebo-controlled trial of the B cell-depleting anti-BAFF receptor antibody (VAY736) (NCT03217422).

However, there is currently no consensus or guideline on the optimal timing to introduce biologics. In addition, the paucity of data on treatment response and safety of biologics in AIH does not help clinicians justify what are comparatively expensive therapies. Rituximab binds to the CD20 glycoprotein expressed on the surface of most B cell lineages (although not plasma cells), with little expression found on other cell types. When administered, it leads to a depletion of B cells, thereby reducing the ability of B cells to present antigen to T cells.[48] Rituximab has been used for several years in the treatment of various autoimmune conditions, including systemic lupus erythematosus, rheumatoid arthritis and autoimmune haemolytic anaemia.[47,49,50] Despite its potential efficacy, it is not currently approved for the treatment of AIH. Although there are case reports of rituximab treatment of AIH,[19,51–55] cumulative multicentre data are lacking. Published data from other autoimmune diseases suggest that rituximab therapy is safe,[19,49,50,55] with the most common side effect being an allergic reaction related to the infusion.[19] All the patients in the current study tolerated rituximab well.

We report clinically meaningful reductions in liver enzyme values following rituximab treatment which, in most patients, were maintained over 24 months. This translated into freedom from further AIH flares in 95% and 71% of patients receiving rituximab at 1 and 2 years, respectively. This is an important clinical outcome, suggesting that rituximab may have the potential to settle biochemical response in patients with difficult-to-manage AIH for up to 18 months, allowing the physician to reformulate the next treatment strategy to maintain remission.

Only 2 of the 4 patients who received a second course of infusions for a subsequent AIH relapse were flare-free 2 years following therapy. This could potentially be related to the emergence of anti-rituximab antibodies,[56,57] although this was not tested in our study. A significant improvement in IgG was also observed following rituximab therapy. However, longitudinal IgG level assessment may not be a reliable clinical indicator of treatment response in this cohort, since rituximab depletes B cells and, as a consequence, can lead to a reduction in immunoglobulins.

There was no evidence of significant changes in INR with rituximab treatment, reflecting the fact that the majority of patients in the study were non-cirrhotic.

The reduction in prednisolone dose following rituximab is a further clinically relevant observation. Prednisolone doses declined significantly by 12 months after treatment, from a median of 15 mg to 10 mg, with almost two-thirds of patients having a dose reduction following rituximab infusion. As most patients with difficult-to-manage AIH have been exposed to a protracted course of corticosteroid treatment, reducing prednisolone dosage is important to prevent patients from developing the serious side effects of corticosteroids. Furthermore, corticosteroid-free therapy in AIH has the potential to substantially enhance patients' quality of life.[44,58]

Our study is a multicentre collaboration within the IAIHG arena, which sought to use retrospective data to determine whether rituximab could be used as a treatment in patients with difficult-to-manage AIH. AIH is a rare disease, and access to rituximab for non-approved indications is difficult, which explains the relatively small sample size of the study. Our study also suffers from all the limitations associated with a retrospective study design, especially relating to the nature of data collection, the potential for ascertainment bias, cohort heterogeneity and the lack of formal safety monitoring. Furthermore, reimbursement constraints bring in potential bias from non-medical factors that may impact on rituximab use. One of the patients developed human papillomavirus-related tongue cancer and it was difficult to know for definite whether this particular complication was related to rituximab therapy. We acknowledge that some side effects may have been underestimated due to the retrospective study design, which is also regarded as an important limitation of the study.

However, this study provides cumulative data from several centres, providing useful information for the design of future clinical trials in this area. We previously reported the immunological profile of treatment-naïve patients with AIH from the UK, which demonstrated that there is a subgroup of patients with elevated B cells[59] before any exposure to immunosuppression. This suggests that rituximab could be useful, even as first-line therapy for selected patients with AIH.

In conclusion, our data clearly demonstrate a temporal association between rituximab therapy in patients with AIH and significant clinically meaningful impacts on serum liver tests, immunoglobulin concentrations, corticosteroid burden, and the subsequent rate of AIH flares. These findings, together with a satisfactory safety profile, provide compelling evidence that further controlled, licencing studies of rituximab and/or other B cell-targeting therapies should be considered for patients with AIH.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhepr.2019.10.005.

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