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ORIGINAL ARTICLE

Rituximab is a safe and effective alternative treatment for patients with autoimmune hepatitis: Results from the ColHai registry

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Abstract

Background and Aims: Small series suggest that rituximab could be effective as treatment for autoimmune hepatitis (AIH), although data are scarce. We aimed to evaluate the efficacy and safety of rituximab in different cohorts of patients with AIH.

Methods: Multicentre retrospective analysis of the 35 patients with AIH and its variant forms treated with rituximab and included in the ColHai registry between 2015 and 2023.

Results: Most patients were female (83%), 10 (29%) had cirrhosis and four (11.4%) variant forms of AIH. Indication for rituximab were as follows: 14(40%) refractory AIH, 19(54%) concomitant autoimmune or haematological disorder, 2(6%) intolerance to prior treatments. In three (9%) subjects with a concomitant disorder, rituximab was the first therapy for AIH. Overall, 31 (89%) patients achieved or maintained complete biochemical response (CBR), including the three in first-line therapy. No difference in CBR was observed according to rituximab indication (refractory AIH 86% vs. concomitant disorders 90%, $p = .824$) or cirrhosis (80% vs. 92%, $p = .319$). Rituximab was associated with a significant reduction in corticosteroids (median dose: prior 20 vs. post 5 mg, $p < .001$) and the discontinuation of ≥1 immunosuppressant in 47% of patients. Flare-free rate at 1st, 2nd and 3rd year was 86%, 73% and 62% respectively. Flares were not associated with the development of liver failure and were successfully managed with repeated doses of rituximab and/or increased corticosteroids. Three

Abbreviations: AEEH, Association for the Study of the Liver; AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, anti-mitochondrial antibodies; ANA, antinuclear antibodies; ANCA, anti-neutrophil cytoplasmic antibody; AST, aspartate aminotransferase; CBR, complete biochemical response; CMX, trimethoprim-sulphamethoxazole; CS, corticosteroids; HCC, hepatocellular carcinoma; IAIHG, International AIH Group; IgG, immunoglobulin G; IQR, interquartile range; MMF, mycophenolate mofetil; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; SMA, anti-smooth muscle; ULN, upper limit of normality.

For affiliations refer to page 10.

(9%) patients experienced infusion-related adverse events (1 anaphylaxis and 2 flu-like symptoms) and five (14%) infections.

Conclusion: Rituximab is safe and effective in patients with refractory AIH and those treated due to concomitant autoimmune or haematological disorders.

KEYWORDS
anti-CD20 monoclonal antibodies, autoimmune hepatitis, corticosteroids, liver cirrhosis, rituximab

1 | BACKGROUND

Autoimmune hepatitis (AIH) is a chronic liver disease whose incidence has increased over recent years.[1,2] Although many patients debut as acute hepatitis with the development of jaundice among other symptoms, in many cases AIH has a completely asymptomatic course, leading to roughly one out every three adults presenting with liver cirrhosis at diagnosis.[2,3] Fortunately, AIH has a good prognosis with approximately 60% to 70% of patients achieving complete biochemical response (CBR) under first-line therapy with corticosteroids (CS) in monotherapy or in combination with azathioprine.[2,4] However, 30–40% of patients with AIH will need second-line therapy due to a lack of response or development of side effect or intolerance to immunosuppression.[2,5,6]

Second-line therapy includes mycophenolate mofetil (MMF),[7] though its efficacy among non-responder patients seems to be limited.[8] Third-line treatment encompasses tacrolimus and, to a lower extent, biological therapies.[2,9] Evidence for second and third-line therapy is based on real-life cohorts since data on clinical trials are lacking. Rituximab is a chimeric human monoclonal antibody against CD20, a glycoprotein specifically expressed at the surface of the B cells. Despite the fact of AIH being a T cell-mediated disorder, rituximab has been used as out-of-label therapy for AIH, though, to date, reports are very scanty and limited to some isolated case reports,[10–17] and two retrospective small cohorts.[18,19]

The association of AIH with other autoimmune disorders is very common.[2] For some of them, such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, idiopathic thrombocytopenic purpura or haemolytic anaemia, rituximab is a therapeutical option,[20–22] a fact that can lead patients with AIH to be treated with rituximab as the immunosuppressant for all their immunological disorders.

Our study aimed to evaluate the efficacy and safety of rituximab in a multicentre cohort of patients with AIH.

Key points

- Rituximab as therapy for autoimmune hepatitis has hardly been explored.
- We investigated the role of rituximab in patients with refractory autoimmune hepatitis, concomitant autoimmune or haematological disorders or intolerance to prior lines of therapy.
- In a cohort of 35 patients, rituximab led to the achievement of complete biochemical response in 89% patients, discontinuation of ≥1 immunosuppressive drugs in 47% and significant reduction in corticosteroid dose in most patients.
- Rituximab is a safe and effective therapy for autoimmune hepatitis, including patients with liver cirrhosis, refractory hepatitis and concomitant disorders.

2 | PATIENTS AND METHODS

2.1 | Study design and patients

This is a multicentre retrospective study that included all patients with AIH or its variant forms (AIH-primary biliary cholangitis [PBC] and AIH-primary sclerosing cholangitis [PSC]) treated with rituximab from January 2015 to June 2023 within the ColHai registry of the Spanish Association for the Study of the Liver (AEEH). The ColHai registry is an online database that collects data from adult patients (aged over 16 years) with either AIH or cholestatic diseases in Spain. Inclusion criteria for the present study were diagnosis of AIH based on the simplified criteria established by the International AIH Group (IAIHG) scoring system in 2008,[23] or AIH variant forms according to the Paris criteria,[24,25] and treatment with the anti-CD20 monoclonal antibody rituximab.

Indication for rituximab in this study included refractory AIH defined as failure to achieve CBR under first (azathioprine plus CS) and/or second/third line of therapy (MMF or tacrolimus), intolerance or adverse events to these treatments, or indication for rituximab due to a concomitant autoimmune or haematological disorder, either benign or malignant.

2.2 | Methods

Demographical and clinical variables included gender, age, race and history of autoimmune disorders. Data at diagnosis of AIH included: acute presentation (defined as transaminases increase

10-fold over upper limit of normality), presence of liver cirrhosis (based on liver biopsy, clinical evidence of decompensation or ultrasonographical findings such as surface nodularity, heterogeneous echostructure, segmental hypertrophy or atrophy), and laboratory parameters (platelet counts, prothrombin time, total and conjugated bilirubin, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), albumin and immunoglobulin G (IgG) levels, autoantibodies and treatment.

The severity of acute hepatitis was assessed according to the EASL criteria: *acute liver injury* was defined as INR>1.5 and *acute liver failure* as INR>1.5 plus signs of hepatic encephalopathy.[26] Antinuclear (ANA), anti-smooth muscle (SMA), anti-mitochondrial (AMA) and anti-neutrophil cytoplasmic (ANCA) antibodies were determined by indirect immunofluorescence on HEp-2 cells (ANA), on snap-frozen sections of rat liver, kidney and stomach (SMA, AMA) or indirect immunofluorescence using ethanol fixed neutrophils (ANCA).

In patients undergoing rituximab for a concomitant disorder, type of disease, either autoimmune or haematological, was recorded. Concerning liver-related outcomes, data on development of hepatic decompensation (hepatic encephalopathy, variceal bleeding and ascites), hepatocellular carcinoma (HCC), need of liver transplantation and liver cirrhosis among patients without significant liver fibrosis at diagnosis, were collected. Last access to data was September 2023 or at the death of patient.

Efficacy was assessed by improvement in transaminases and IgG levels. CBR was defined as normalization of aminotransferases and IgG according to the IAIHG criteria.[9] The upper limit of normality (ULN) for IgG was 1600 mg/dL, and transaminases 35 and 50 IU/mL, for female and male respectively. In patients achieving CBR, later development of flares was collected and defined as a two-fold increase in either AST or ALT above the ULN.[27]

Safety was assessed in terms of adverse reactions to rituximab infusion or development of flu-like symptoms, as well as infectious and tumoral complications developing until 1 year after the last dose of the anti-CD20 therapy.

This study was approved by the Vall d'Hebron Hospital Ethics Committee (EOM(AG)058/2021(5896), date 1st October 2021), and it was conducted in compliance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines and local regulatory requirements. Informed consent was waived due to the retrospective nature of the study. All data were anonymized.

2.3 | Statistical analysis

Quantitative variables were expressed as median and interquartile range (IQR) and analysed with the Mann–Whitney *U* test. Categorical variables were expressed as frequencies and percentages and compared using the chi-squared or Fisher's exact test, in the case frequencies were less than 5%. The results were considered statistically significant if *p*-value was lower than .05. All statistical analyses were performed using IBM SPSS, version 26.0 (SPSS Inc, Armonk, NY, USA).

3 | RESULTS

3.1 | Characteristics of patients at diagnosis of autoimmune hepatitis

In June 2023, 1317 patients with either AIH ($N=1041$) or AIH variant forms ($n=276$) were included in the ColHai registry. Thirty-five (3%) of these patients were treated with rituximab at 13 different Spanish hospitals. All patients had AIH except for four (11%) with variant forms of AIH, three AIH-PBC and one AIH-PSC. Briefly, most patients were women (29/35, 83%), with median age at diagnosis of 46 years (IQR, 34–58). Twenty-three (66%) subjects had at least one concomitant autoimmune disorder, with systemic lupus erythematosus ($n=6$), systemic scleroderma ($n=6$), multiple sclerosis ($n=5$) and benign haematological disorders ($n=5$) being the most frequently reported (Table 1). Presentation of AIH was as acute hepatitis in 25 (71%) cases, and nine (26%) of them met the criteria for acute liver injury. Liver biopsy was carried out in all patients but two, revealing typical findings of AIH. Despite the lack of histological confirmation, subjects without liver biopsy presented a pre-treatment Original Score for AIH of 16.[28]

At diagnosis of AIH, eight (23%) patients presented signs of cirrhosis, two of them decompensated. One patient (3%) had a history of two liver transplants (20 and 10 years prior to rituximab therapy) due to refractory AIH.

Initial therapy for AIH included CS in the majority of patients (33, 94%) with prednisone as the most common except for one patient who received budesonide. Concomitant immunosuppressant drugs were used in 30 (86%) subjects: azathioprine in 26 (74%), MMF in one (3%) and rituximab in three (9%). Only 10 (29%) individuals achieved CBR with the first line of therapy.

3.2 | Rituximab for treatment of autoimmune hepatitis

The median time from diagnosis of AIH to the beginning of therapy with rituximab was 25.3 (5.6–74.0) months. Prior to rituximab administration, two subjects with refractory AIH developed cirrhosis during the follow-up, leading to a total of 10 (29%) individuals with underlying cirrhosis receiving rituximab. Liver cirrhosis was most common among patients with refractory AIH compared to those with concomitant disorders (50.0% vs. 10.5%, $p=.036$).

Criteria for rituximab therapy were concomitant autoimmune ($n=17$) or haematological ($n=2$) disorders (19/35, 54%), refractory AIH (14, 40%) and adverse events to previous lines of therapy (2, 6%). Individual data at the time of rituximab beginning are summarized in Table 2.

First-line therapy with rituximab was used in three (9%) patients, all of them because of a concomitant autoimmune disease (2 individuals with multiple sclerosis) and one due to haematological disorder (1 autoimmune haemolytic anaemia). The remaining patients were receiving one (9, 26%), two (19, 54%) or three (4, 11%) immunosuppressant drugs for the management of AIH prior to anti-CD20 therapy.

TABLE 1 Characteristics of patients at diagnosis of autoimmune hepatitis and according to the criteria for therapy with rituximab.

	Overall ($n=35$)	Refractory AIH ($n=14$)	Concomitant disorder ($n=19$)	p value[a]
Female gender	29 (83%)	11 (79%)	16 (84%)	.510
Age, years	46 (34–58)	41 (24–56)	46 (34–62)	.534
Prior autoimmune disorder	23 (66%)	5 (36%)	17 (90%)	.002
Arterial hypertension	8 (23%)	5 (33%)	2 (11%)	.114
Diabetes	6 (17%)	3 (21%)	3 (16%)	.510
Dyslipidaemia	3 (9%)	0 (0%)	3 (16%)	.174
AIH variant forms	4 (11%)	1 (7%)	3 (16%)	.426
Acute hepatitis	25 (74%)	10 (71%)	13 (68%)	.581
Liver cirrhosis	8 (23%)	6 (43%)	2 (11%)	.042
Platelets, ×10E9/L	198 (171–253)	196 (145–226)	200 (184–290)	.071
Total bilirubin, mg/dL	1.7 (.7–6.5)	2.4 (1.2–8.7)	1.4 (.5–5.8)	.426
AST, IU/mL	635 (141–798)	659 (224–838)	456 (112–799)	.633
ALT, IU/mL	663 (246–795	677 (391–795)	478 (230–762)	.368
ALP, IU/mL	178 (118–283)	195 (161–383)	168 (110–283)	.242
GGT, IU/mL	195 (109–351)	178 (111–261)	234 (95–438)	.448
IgG, mg/dL	1894 (1353–2865)	2560 (1405–3290)	1748 (1250–2425)	.124
ANAs ≥1/80	30 (86%)	13 (93%)	15 (79%)	.278
SMA ≥1/40	12 (34%)	4 (29%)	7 (37%)	.453
Positive ANCAs	4 (13%)	4 (29%)	0 (0%)	.037
CBR at rituximab	10 (29%)	0 (0%)	10 (53%)	.001
Months to rituximab[b]	25.3 (5.6–74.0)	30.2 (9.7–117.8)	13.3 (4.4–65.8)	.231

Note: Factors are expressed as *n* (%) or median (IQR).

Abbreviations: AIH, autoimmune hepatitis; ANA, antinuclear antibodies; ANCA, anti-neutrophil cytoplasmic antibodies; CBR, complete biochemical response; SMA, anti-smooth muscle antibodies.

[a]Comparison between patients treated with rituximab for refractory AIH versus those with concomitant autoimmune disorders.

[b]From diagnosis of AIH.

Scheme of rituximab was only a 2-dose induction course with 1 gram every 2 weeks in 3 (9%) subjects, and periodical doses in 32 (91%), either fixed every 6 months in 16 (46%) and 16 (46%) in periods adjusted to immunological recovery (new dose of rituximab when CD19+ B-lymphocyte counts were once again detectable).

Only 14 (41%) received concomitant trimethoprim-sulphamethoxazole (CMX) prophylaxis, rate similar among those with or without CS-containing regimens (76% vs. 79%, $p=.602$).

At the last follow-up, 28 (80%) were still undergoing periodical therapy with rituximab. Follow-up was longer among those who discontinued rituximab (38 vs. 12 months, $p=.032$).

3.3 | Efficacy of rituximab in patients with AIH

The median time from the first dose of rituximab to the last follow-up was 20.9 (6.7–48.1) months. At the beginning of rituximab, 10 (29%) patients had already achieved a CBR of the AIH, all of them treated with rituximab because of a concomitant disorder. After rituximab, another 21 individuals achieved CBR after a median time of 8 (3–19) months after the first dose, leading to a total of 31 (89%) subjects with AIH who achieved or maintained CBR under therapy with rituximab (Figure 1). All three subjects receiving rituximab as first-line therapy for both AIH and their concomitant autoimmune or haematological disorder also achieved CBR. Among the group of 14 individuals with refractory AIH, 12 (86%) achieved CBR after rituximab therapy. CBR rate in individuals with concomitant autoimmune/haematological disorders was 90%.

No differences were observed in the rate of CBR according to the presence of underlying cirrhosis (80% vs. 92%, $p=.319$), the criteria for rituximab prescription (Refractory AIH: 86% vs. concomitant immune disorder: 90%, $p=.824$), gender (Male: 100% vs. female: 86%, $p=.454$), presence of ANAs (Positive: 87% vs. negative: 100%, $p=.523$) or diagnosis of AIH variant forms (AIH: 87% vs. variants: 100%, $p=.601$).

Overall, 11 (31%) patients experienced flares of the AIH. The rate free of flares at 1st, 2nd and 3rd year after the beginning of rituximab was 86%, 73% and 62% respectively (Figure 2).

There was a trend to a higher risk of flares in patients undergoing rituximab due to refractory AIH than those receiving the drug because of a concomitant disorder, but the difference was not statistically significant (43% vs. 28%, $p=.302$). None of these flares was associated with the development of acute-on-chronic liver failure, although one (3%) patient developed liver decompensation as a

TABLE 2 Individual data of included patients at the time of rituximab therapy beginning.

12	Sex	Age	AIH variant form	Concomitant AI disease	Cirrhosis	ANAs/SMA/ ANCA	Rituximab criteria	Rituximab scheme	CBR after rituximab	Adverse events	Flares	Follow-up (months)[a]
1	Male	43	No	Evans syndrome	Yes	1:320/1:80/neg	Refractory AIH	Periodical (CD-19 guided dose)	Yes	No	Yes	61
2	Male	36	No	Type 1 diabetes	No	1:160/neg/neg	Refractory AIH	Periodical (6-month dose)	Yes	No	Yes	74
3	Male	29	No	Eosinophilic pneumonia Multiple sclerosis	No	Neg/neg/neg	Concomitant AI disease	Periodical (CD-19 guided dose)	Yes	No	Yes	21
4	Female	73	No	SLE	Yes	1:160/neg/neg	Refractory AIH	Periodical (CD-19 guided dose)	Yes	No	Yes	22
5	Female	58	PBC-AIH	No	No	1:320/neg/neg	Refractory AIH	Periodical (CD-19 guided dose)	Yes	No	Yes	13
6	Female	55	No	No	Yes	1:640/neg/pos	Refractory AIH	Periodical (CD-19 guided dose)	Yes	No	No	6
7	Female	56	No	No	Yes	1:640/neg/neg	Refractory AIH	Periodical (CD-19 guided dose)	No	No	No	6
8	Female	19	No	SLE	No	1:640/neg/pos	Refractory AIH	Periodical (CD-19 guided dose)	Yes	No	No	7
9	Female	68	No	No	Yes	1:160/neg/pos	Refractory AIH	Periodical (CD-19 guided dose)	No	No	No	4
10	Female	19	No	Scleroderma	No	1:640/1:160/neg	Refractory AIH	Periodical (CD-19 guided dose)	Yes	No	No	48
11	Female	45	No	Scleroderma	No	1:320/1:160/neg	Refractory AIH	Periodical (CD-19 guided dose)	Yes	No	No	24
12	Female	50	No	Scleroderma	No	1:320/neg/neg	Refractory AIH	Periodical (CD-19 guided dose)	Yes	No	No	12
13	Female	50	No	Membranous glomerulonephritis Sjögren syndrome	No	1:320/neg/neg	Concomitant AI disease	Periodical (CD-19 guided dose)	Yes	No	No	8
14	Female	57	No	Scleroderma	No	1:160/1:40/neg	Intolerance to prior therapy	Periodical (CD-19 guided dose)	Yes	No	No	7
15	Female	44	No	Haemolytic anaemia Multiple sclerosis	No	1:80/neg/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	No	Yes	51
16	Female	48	No	Haemolytic anaemia Mixed connective tissue disease	No	1:160/neg/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	Yes (anaphylaxis)	No	98
17	Female	64	PSC-AIH	AI nephropathy	No	1:160/neg/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	No	No	6

(Continues)

TABLE 2 (Continued)

12	Sex	Age	AIH variant form	Concomitant AI disease	Cirrhosis	ANAs/SMA/ ANCA	Rituximab criteria	Rituximab scheme	CBR after rituximab	Adverse events	Flares	Follow-up (months)[a]
18	Female	53	No	Type 1 diabetes	No	1:160/1:160/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	No	No	39
19	Male	24	No	No	No	Neg/1:80/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	Yes (flu-like symptoms)	No	2
20	Female	79	No	No	Yes	1:320/1:160/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	Yes (flu-like symptoms)	No	2
21	Female	52	PBC-AIH	Scleroderma	No	1:320/neg/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	No	No	89
22	Female	41	PBC-AIH	Rheumatoid arthritis	No	1:320/1:160/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	No	Yes	51
23	Female	77	No	SLE	No	1:640/neg/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	No	No	24
24	Female	48	No	Rheumatoid arthritis	No	Neg/1:160/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	No	No	72
25	Female	53	No	SLE	No	1:640/neg/neg	Refractory AIH	Periodical (6-month dose)	Yes	No	No	12
26	Female	63	No	SLE Multiple sclerosis	No	1:640/neg/neg	Concomitant AI disease	Periodical (6-month dose)	No	No	No	9
27	Female	35	No	Scleroderma	Yes	1:80/1:40/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	No	No	4
28	Female	45	No	Type 1 diabetes Proliferative glomerulonephritis	No	1:80/neg/neg	Concomitant AI disease	Periodical (CD-19 guided dose)	Yes	No	No	68
29	Male	64	No	No	Yes	1:160/1:160/neg	Refractory AIH	Induction dose	Yes	No	Yes	23
30	Female	45	No	Multiple sclerosis	No	1:80/1:40/neg	Concomitant AI disease	Periodical (CD-19 guided dose)	Yes	No	No	22
31	Female	61	No	Multiple sclerosis	No	1:320/neg/neg	Concomitant AI disease	Periodical (6-month dose)	Yes	No	No	29
32	Female	31	No	SLE	Yes	1:640/neg/neg	Intolerance to prior therapy	Periodical (6-month dose)	Yes	No	Yes	12
33	Female	30	No	Thrombotic thrombocytopenic purpura	No	1:320/neg/neg	Concomitant AI disease	Induction dose	No	No	No	14
34	Male	65	No	Haemolytic anaemia	No	Neg/neg/neg	Concomitant AI disease	Induction dose	Yes	No	Yes	38
35	Female	44	No	No	Yes	Neg/neg/neg	Refractory AIH	Periodical (CD-19 guided dose)	Yes	No	Yes	55

Abbreviations: AI, autoimmune; ANA, antinuclear antibodies; ANCA, anti-neutrophil cytoplasmic antibody; CBR, complete biochemical response; *N*, number; neg, negative; Pos, positive; SLE, systemic lupus erythematosus; SMA, smooth muscle antibody.

[a]Months from the first dose of rituximab to the last follow-up.

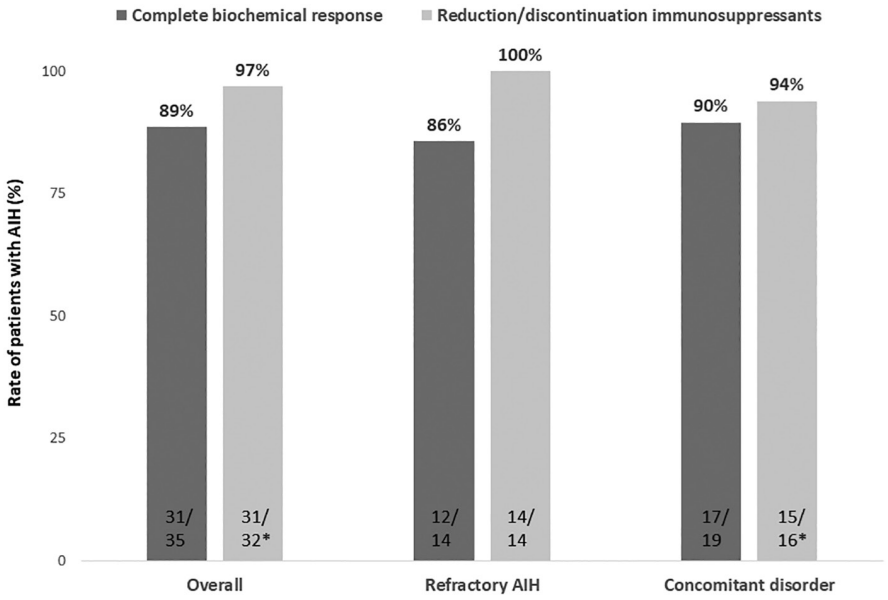

FIGURE 1 Efficacy of rituximab for patients with AIH in terms of complete biochemical response (CBR) and immunosuppressant drugs dose reduction or discontinuation after rituximab therapy. *Three patients received rituximab as first-line therapy for AIH, therefore they were excluded from the discontinuation/dose reduction data.

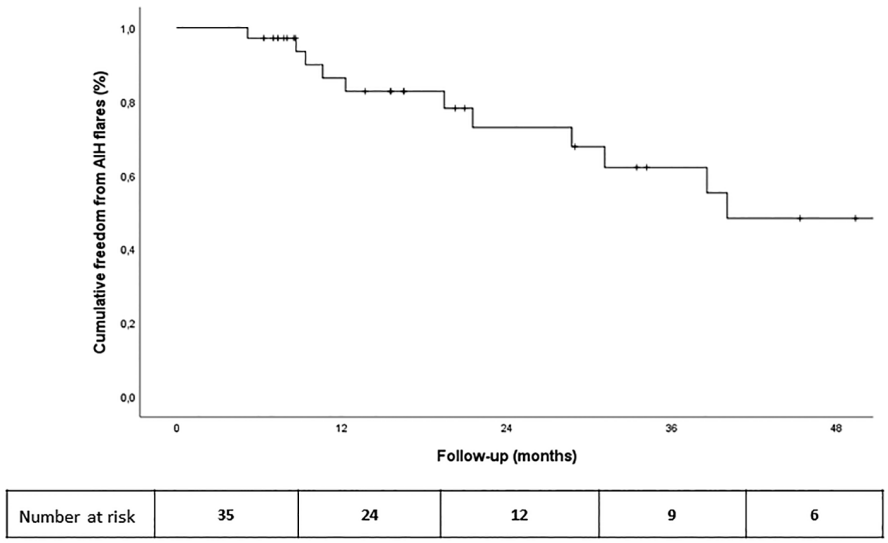

FIGURE 2 Kaplan–Meier curve representing the cumulative rate free of flare in the overall cohort of patients with autoimmune hepatitis treated with rituximab.

result in a delay in the rituximab therapy due to a respiratory infection. The vast majority of flares occurred at the time of CD-19 cell reconstitution when CD-19 cells reappeared.

All flares were successfully managed with repeated doses of rituximab and/or CS dose increase. Male gender was the only factor with a significant risk of flare development (83% vs. 24%, $p = .012$). The risk of flares among those receiving only an induction dose of rituximab (67% vs. 31%) or underlying liver cirrhosis (50% vs. 28%) was higher, but without statistical significance ($p = .266$ and $p = .198$ respectively).

Regarding the scheme of rituximab (fixed dose vs. CD-19 guided dosage), no differences were observed regarding the rate of CBR or flares (93.4% vs. 87.5%, $p = .500$; 25% vs. 31.4% $p = .500$ respectively).

Comparison of analytical data prior to rituximab to the last follow-up showed a significant drop in AST, ALT and IgG values (AST: 68 vs. 29 IU/mL, $p < .001$; ALT: 76 vs. 28, $p < .001$; IgG: 1400 vs. 1258, $p = .032$). Out of the 35 patients included in the study, the number of patients with follow-up data at 1, 3, 6, 12, 24, 36 and 48 months from rituximab were 35, 33, 30, 21, 14, 12 and 9 respectively. The evolution of transaminases and IgG values during rituximab are summarized in Figure 3 (3A overall cohort; 3B refractory AIH cohort). Improvement of both AST/ALT and IgG values was observed during all time points.

Among the cohort of 14 patients with refractory AIH, rituximab treatment led to a significant improvement in transaminases and IgG levels when contrasted with the last control (AST: 109 vs. 31 IU/mL, $p = .002$; ALT: 170 vs. 36, $p = .001$; IgG: 1721 vs. 1290, $p = .050$). Moreover, in this difficult-to-treat population, anti-CD20 therapy was also associated with an improvement in ALP and GGT values (ALP: 122 vs. 88, $p = .041$; GGT: 197 vs. and 45, $p = .002$). No changes were observed in bilirubin levels (.98 vs. .99, $p = .382$). As observed in the total cohort, the effect of rituximab in transaminases was maintained during the whole follow-up, except at year 3 (AST: 109

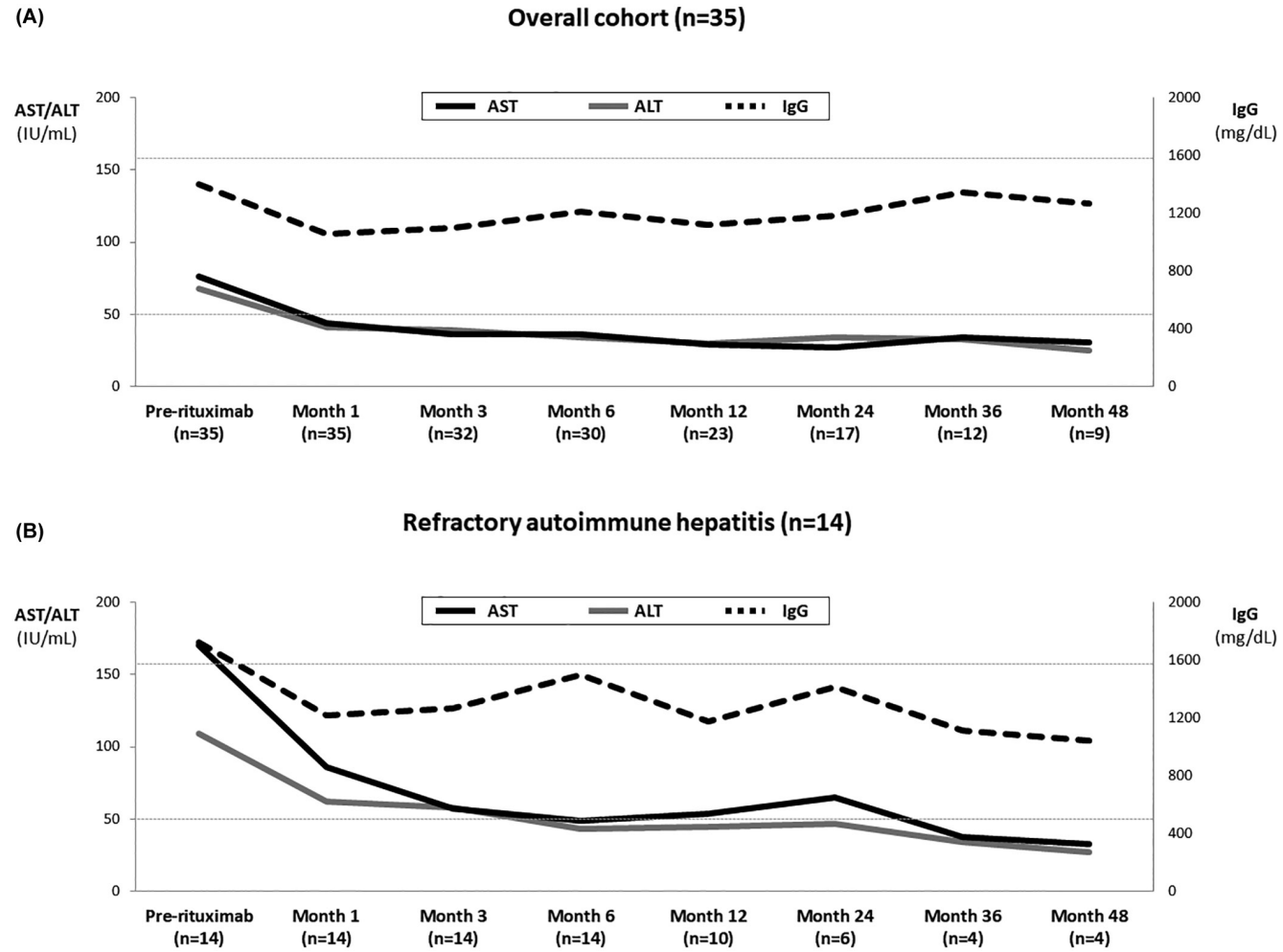

FIGURE 3 Evolution of transaminases and IgG values after therapy with rituximab. (A) Overall cohort ($n=35$). (B) Cohort of patients treated with rituximab due to refractory AIH. The dashed line marks the upper limit of normality of IgG (1600 mg/dL) and AST/ALT (50 IU/mL).

vs. 34 IU/mL, $p=.285$; ALT: 170 vs. 38, $p=.465$) and 4 (AST: 109 vs. 27 IU/mL, $p=.465$; ALT: 170 vs. 33, $p=.465$) of follow-up, probably due to the relatively low number of patients who achieved this time-point.

Three out of the seven patients who discontinued rituximab were initially treated due to refractory AIH. In all these cases, rituximab led to the achievement of CBR and the drug was successfully stopped after 2 years of therapy.

3.4 | Impact of rituximab in the immunosuppressive regimen of patients

Apart from the three patients whose first therapy for AIH was rituximab because of a concomitant disorder, among the remaining 32 subjects, therapy with rituximab was associated with immunosuppressant drug discontinuation or dose reduction in 31 (97%) subjects. Specifically, in 15 out of 32 (47%) subjects, therapy with rituximab led to the discontinuation of two ($n=10$, 31%) or one immunosuppressive drug ($n=5$, 16%). Figure 4 shows the evolution of the individual immunosuppressive treatment of each included patient. The proportion of patients undergoing CS prior to rituximab had dropped from 28 (80%) to 21 (60%) at the last follow-up ($p=.001$). Moreover, the median dose of CS was reduced from 20 (7.5–30) mg per day to 5 (2.5–10) mg per day ($p<.001$).

3.5 | Safety of rituximab in patients with AIH

Three (9%) patients experienced adverse events associated with the rituximab infusion: one anaphylactic shock and two flu-like symptoms. Five (14.3%) presented infections after rituximab: one pleuritis caused by nocardia (despite prophylaxis with CMX), one septic shock, one recurrent urinary tract infections, one recurrent folliculitis and one recurrent respiratory tract infections. Only the nocardia and the septic shock required admission to hospital. The presence of liver cirrhosis had no impact on the rate of infections (30% vs. 8%, $p=.128$). The incidence of infections was similar regardless of the scheme of rituximab among patients on periodical doses (fixed dose 18.8% vs. 12.5% CD-19 guided dosage, $p=.500$). Nevertheless,

FIGURE 4 Individual immunosuppressant drug regimen of each subject at the beginning for rituximab and therapeutical changes during follow-up.

only patients undergoing the fixed-dose scheme developed adverse events (18.8% vs. 0%), though this difference did not achieve statistical significance ($p = .113$).

Only one (2.9%) patient with an underlying liver cirrhosis and history of ascites presented a new decompensation (ascites) as a consequence of a flare due to a delay in rituximab dose caused by a respiratory infection. This flare was successfully managed with a temporary increase in the corticosteroid dose and a new cycle of rituximab. Despite being on complete biochemical response, this patient presented further decompensation and required liver transplantation. No cases of tumours, including hepatocellular carcinoma, were reported.

Only one (2.9%) patient died during follow-up because of the haematological disorder that motived the indication for rituximab therapy (MALT lymphoma).

4 | DISCUSSION

Herein, we report data on the efficacy and safety of treatment with rituximab in 35 patients with AIH, which is, to our knowledge, the largest cohort reported so far. Rituximab is an effective therapy for AIH as proved by the 89% (31/35) rate of CBR, including 12 out of 14 patients with refractory AIH. Moreover, this drug led to discontinuation or decrease in immunosuppressive drugs in almost all patients, with a good safety profile, and few cases of infusion reactions and infections.

The presence of cirrhosis has been shown to significantly reduce the chances of achieving CBR in a large retrospective study from the IAIHG.[6] However, the impact of cirrhosis in the response rate to second- and third-line therapies is largely unknown. This is due to the low number of patients with cirrhosis included in the series of cases or retrospective studies evaluating the efficacy of these therapies. In our cohort, with almost 30% of patients suffering from cirrhosis at the time of rituximab administration, CBR was similar in patients with and without cirrhosis (80% vs. 92% $p = .319$), and more importantly, the presence of cirrhosis did not significantly increase the rate of infectious complications.[18,19]

Despite the heterogeneity of the patients included in our cohort, the fact that only 29% of individuals achieved CBR with the first line of therapy for AIH highlights the difficult to treat population included in our study.

Notwithstanding the high rate of CBR observed in our cohort, the rate of flares was also relatively high, but not different from that reported in previous studies. Indeed, the ERN refractory AIH cohort reported a free-of-flare rate of 95% and 71% at years 1 and 2 after rituximab therapy,[19] which is very similar to the 86% and 73% rates detected in our cohort. Interestingly, we observed an increased risk of flares among males (83% vs. 24%, $p = .012$). While this is an interesting finding, the small number of male patients included in this cohort precluded us from performing further analysis and from drawing significant conclusions, but this is an important issue that should be explored in future studies. In addition, there was a trend to

a higher risk of flares in patients with cirrhosis though the difference did not reach statistical significance.

Moreover, rituximab had a significant impact on the patients' treatment regimen, allowing the discontinuation of at least one immunosuppressive drug in up to 47% of patients, and a reduction of the median CS dose from 20 mg per day to 5 mg per day.

Although the majority of the patients included in the study were women (83%) and one-quarter of our cohort were younger than 34 years old, no women became pregnant during therapy with rituximab. Despite the lack of studies from women with AIH, some cohorts from more prevalent disorders such as pANCA vasculitis and multiple sclerosis have provided promising data on rituximab safety and efficacy during pregnancy,[29,30] although the retrospective nature of these studies and the relatively low number of patients included resulted in the recommendation of rituximab discontinuation according to the international guidelines.[31]

The limited literature on rituximab for AIH has led to a lack of information about what is the best scheme of therapy. For instances, in the Alberta cohort from Burak et al. patients only received a 2-dose induction of 1 g every 2 weeks.[18] Nevertheless, at the ERN cohort, five out of the 22 included patients received additional courses of rituximab, mainly for management of later flares.[9] In our cohort, the vast majority of patients received repeated doses of rituximab (32, 91%), every 6 months (especially among those with concomitant disorders) or adapted to immunological recovery, a strategy in line with other autoimmune disorders such as ANCA-associated vasculitis.[32] Despite the retrospective nature of our study, the rate of flares observed not only in our cohort but also in the ERN study suggests the usefulness of the repeated-dose strategy, though further prospective studies are needed to support this management.

Interestingly, the strategy based on CD-19 guided dosage presented similar results in terms of efficacy though seemed to be associated with a tendency to lower incidence of adverse events (18.8% vs. 0%, $p = .113$). Another gap in knowledge is the duration of rituximab therapy. In our cohort, three out of the seven patients who discontinued rituximab were initially treated due to refractory AIH and in all these patients, rituximab led to the achievement of CBR and the drug was successfully stopped after 2 years of therapy, strategy in line with the scheme used for maintenance of response in other systemic disorders.[32]

Our study has some limitations. First, this was a retrospective study and therefore prior therapy of patients may have varied according to the available logistics of each hospital, as in the case of azathioprine metabolites. Second, the criterion for rituximab administration was not only refractory or intolerant AIH but also an indication for anti-CD20 therapy because of a concomitant autoimmune or haematological disorder. Whilst it is true that this fact limits the results on efficacy in difficult-to-treat AIH patients, it also provides an opportunity to assess rituximab as a first-line therapy for AIH, leading to a 100% CBR in the small group of three patients who were treated because of a concomitant disorder.

In summary, in our cohort rituximab was an effective and safe treatment option for patients with AIH, not only in difficult-to-treat cases but also it could be a useful option as a first-line therapy for subjects with concomitant disorders. However, prospective studies are needed to confirm these results.

AUTHOR CONTRIBUTIONS

Mar Riveiro-Barciela had full access to all the data in the study and took responsibility for the integrity and accuracy of the results. *Concept and design*: Mar Riveiro-Barciela, Ana Barreira-Díaz and María-Carlota Londoño. *Acquisition, analysis or interpretation of data*: Paula Esteban, Rosa Rota, Carmen Álvarez-Navascúes, Indhira Pérez-Medrano, Beatriz Mateos, Elena Gómez, Gema De-la-Cruz, Carlos Ferre-Aracil, Diana Horta, Álvaro Díaz-González, Javier Ampuero, Fernando Díaz-Fontenla, Magdalena Salcedo, Juan Carlos Ruiz-Cobo, María-Carlota Londoño. *Drafting of the manuscript*: Mar Riveiro-Barciela, Ana Barreira-Díaz and María-Carlota Londoño. *Critical revision of the manuscript for important intellectual content*: All authors.

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Mar Riveiro-Barciela: advisory board and/or speakers' bureau fees: Bristol Myers Squibb, Gilead, GlaxoSmithKline; Grants: Gilead. Álvaro Díaz-González: Advisory board and/or speakers' bureau fees: GlaxoSmithKline, IPSEN, AdvanzPharma. Magdalena Salcedo: Advisory board and/or speakers' bureau fees: Albireo. Maria-Carlota Londoño: advisory board and/or speaker's bureau fees: Intercept/Advanz Pharma, GlaxoSmithKline, Ipsen, Cymabay. The rest of authors have no conflicts to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, MRB, upon request.

ETHICS STATEMENT

This study was approved by the Vall d'Hebron Hospital Ethics Committee (EOM(AG)058/2021(5896), date 1/10/2021).

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