Profiling the patient with autoimmune hepatitis on calcineurin inhibitors: a real-world-experience

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Objective Therapy for autoimmune hepatitis (AIH) consists of steroid induction therapy, followed by maintenance therapy with azathioprine. However, up to 20% of patients experience either insufficient response or intolerance on first-line therapy. Calcineurin inhibitors (CNIs) are frequently used when first-line therapy fails. Although a number of studies report on efficacy, less is known on the patient trajectory before switch to CNIs. Our aim was to describe the road toward CNI therapy in AIH patients.

Methods Patients with an AIH diagnosis who used CNIs as either second- or third-line treatment were included in the study. Reason for switch to CNI was assessed as either an insufficient response or intolerance to prior therapy. Efficacy was assessed by normalization of transaminases at last moment of follow-up.

Results Final analysis included 20 patients who were treated with CNIs. Ten patients were treated with tacrolimus and ten patients received cyclosporine. In patients who used CNI treatment as third-line therapy (n = 13), duration of first-line therapy was almost twice as long as duration of second-line therapy (2.58 years vs. 1.33 years; $P = 0.67$). Patients treated with tacrolimus had relatively high trough levels (7.6 ng/mL) and more (minor) adverse events. Fifty-five percent of patients had normalization of transaminases at last moment of follow-up.

Conclusion CNI treatment in AIH as second- or third-line therapy is effective in ~50% of patients. The trajectory before switch varies considerably between patients. Eur J Gastroenterol Hepatol 32: 727–732

Introduction

Autoimmune hepatitis (AIH) is a rare, chronic liver disease characterized by circulating autoantibodies, elevated levels of serum immunoglobulin G (IgG) and inflammatory liver histology. When left untreated, AIH can lead to development of cirrhosis and end-stage liver disease [1,2].

Standard treatment of AIH consists of corticosteroids alone or in combination with azathioprine (AZA) and is effective in the vast majority of patients [3]. However, up to 20% of patients show insufficient response or experience adverse events that warrant cessation of the drug [4]. Among factors that predict a poor response to treatment are hyperferritinemia, younger age, increased mean platelet volume, and cirrhosis at diagnosis [5–7]. Various immunosuppressive agents have been proposed as alternative options for second-line therapy in AIH, including mycophenolate mofetil (MMF), 6-mercaptopurine and 6-tioguanine. Exact numbers are lacking, but reports suggest that 75–90% of patients will achieve a satisfactory response on second-line therapy [8–11].

There is scarce evidence on the calcineurin inhibitors (CNIs) cyclosporine (CsA) and tacrolimus (TAC) for treatment in AIH. Both drugs act through suppression of activated T-cells via inhibition of the intracytoplasmic enzyme calcineurin, blocking nuclear transcription of proinflammatory cytokines such as interleukin-2. To date, CNIs are the mainstay of treatment for the prevention of allograft rejection. Both drugs require therapeutic drug monitoring, because of their narrow therapeutic index and significant interindividual variability in blood concentrations [12]. Data on both CsA and TAC in AIH are limited and mainly focused on response rates rather than characterization of patients in their trajectory before switch to CNI therapy. Notably, most of these studies were done in patients who received CNIs as second-line therapy.

This study aims to describe a cohort of AIH patients who are treated with CNIs in two expert centers in The Netherlands and Belgium. We specifically aimed to describe the road toward CNI therapy in AIH patients with emphasis on duration of prior treatment and reasons for therapy switch. Additionally, we aim to investigate to efficacy of CNIs as second- or third-line treatment.

Methods

Patients

Patients with an established AIH diagnosis were identified by local databases from the University Hospital KU Leuven in Belgium and the Radboud University Medical Center,

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Nijmegen, The Netherlands. AIH diagnosis was based on the simplified IAIHG diagnostic criteria [13,14]. Inclusion criteria for this study were as follows: all patients with a probable or definite AIH diagnosis on current or previous treatment with either CsA or tacrolimus as second- or third-line therapy for AIH. We defined second- and third-line therapy as a second or third drug used for maintenance therapy regardless of reason of switch from prior therapy. Patients with variant syndromes with primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC) were included in this study. Variant syndrome with PBC was defined according to the Paris criteria with an antimitochondrial antibody (AMA) titer >1:80 in combination with compatible histology [15,16]. Variant syndrome with PSC was defined as having typical radiological findings on imaging (magnetic resonance cholangiopancreaticography/endoscopic retrograde cholangiopancreaticography) [17]. Liver transplantation recipients were excluded. Data on initiation and cessation of therapy, laboratory values and other variables of interest were retrospectively collected from (electronic) patient records and databases. Ethics approval was waived by institutional review board after local review.

Analysis

We analyzed baseline and treatment characteristics of patients who used CNI treatment as third-line therapy compared to patients who used CNI treatment as second-line therapy. The call to switch to CNI was made by the treating physician in case there was an insufficient response or intolerance to prior therapy. Efficacy was assessed by normalization of transaminases at last moment of follow-up. Biochemical remission was defined according to international guidelines as normal serum transaminases and IgG [18]. Drug-related adverse events were ascribed at the discretion of the treating physician. Univariate comparisons were made using Fisher's exact test, Mann–Whitney U test or *t*-test as appropriate. Patients with ongoing treatment were censored at last moment of follow-up. *P*-values <0.05 were considered statistically significant. Analysis was done with SPSS version 24 (IBM Corporation, Armonk, New York, USA).

Results

Population

The total cohort of AIH patients in Leuven and Nijmegen consisted of 393 patients. We identified 26 patients who had been, or were actively treated with CNIs. We excluded three patients who used CNIs for prevention of allograft rejection after liver transplantation. Furthermore, we excluded three patients who started CNIs as first-line therapy for AIH. Final analysis included 20 patients: 13 patients used CNIs as third-line treatment and seven patients used CNIs as second-line treatment. Most patients were female (70%) and mean age at diagnosis was 34 years (range 13–66). Mean duration of follow-up since diagnosis was 12.5 years (SD 8.95) and mean duration of follow-up on CNI treatment was 26.6 months (SD 40.3). Fourteen patients (70%) were diagnosed as probable AIH and six patients (30%) as definite AIH. Nineteen patients were diagnosed with AIH type 1 and one patient with AIH type 2.

There were no significant baseline differences between patients who used CNIs as third-line treatment when compared to patients who used CNIs as second-line treatment (Table 1).

Patients who used calcineurin inhibitors as second-line treatment

Seven patients received CNI treatment as second-line therapy: four patients were treated with CsA and three patients received TAC. Most patients (6/7) were treated with AZA before switching to CNIs. Median AZA dose before switch was 87.5 mg (range 25–100 mg) (P = 1.00 compared to third-line treated patients). The other patient was treated with MMF 1000 mg as first-line therapy. Patients were on first-line therapy for a median duration of 6.83 years (range: from 3 months to 24 years). Three patients switched to CNIs because of intolerance to first-line treatment and four patients switched because of insufficient response. Most patients still had evidence of biochemical disease activity at the time of switch to CNI treatment: median alanine aminotransferase (ALT) at AIH diagnosis was 171 U/l (94–1692) and had barely dropped at the moment of switch to CNI therapy: 134 U/l (21–295).

Patients who used calcineurin inhibitors as third-line treatment

Thirteen patients received CNI treatment as third-line therapy: six patients were treated with CsA and seven patients received TAC. Most patients (76.9%) received prior therapy consisting of AZA followed by MMF. For this combination, the last used median AZA and MMF dosages before switch to CNIs were 50 mg (range: 25–200 mg) and 1000 mg (range: 1000–2000 mg), respectively. Other treatment combinations are presented in Table 1. Patients were on first-line therapy for a median duration of 2.58 years (range: from 1 month to 17.17 years). Interestingly, duration of second-line therapy was shorter with a median therapy duration of 1.33 years (range: from 1 month to 16.75 years) (Fig. 1), this difference was not statistically significant (P = 0.67). Most patients (n = 9) switched to CNI therapy due to an insufficient response on second-line therapy and three patients switched because of intolerance to second-line treatment. One patient switched from MMF to CsA because of pregnancy wish. Most patients had evidence of biochemical disease activity at the time of switch from second-line therapy to third-line CNI treatment: median ALT at diagnosis was 278 U/l (range 92–1355) and decreased to 84 (13–703) U/l at moment of switch to second-line treatment. However, at the moment of switch from second-line therapy to CNI, ALT had increased to 96 U/l (16–794).

Differences between third- and second-line calcineurin inhibitor treatment

Patients on CsA treatment were started on a median dose of 1.83 mg/kg (1.36–3.75) when on third-line therapy compared to 2.11 mg/kg (1.23–2.99) and when on second-line therapy (P = 0.48). CsA dosage at last moment of follow-up was equal in both second- and third-line treated patients [2.11 mg/kg (1.23–2.99) vs. 2.11 mg/kg (1.36–3.75); P = 0.64]. Initial median doses of TAC treatment did

Table 1. Vignette of patients who used calcineurin inhibitors as third-line therapy vs. patients who used them as second-line therapy

	Third-line therapy; N = 13	Second-line therapy; N = 7	*P* value
Female gender, n (%)	9 (69.2%)	5 (71.4%)	1.00
Weight (kg), mean (SD)	68.4 (4.5)	71.7 (10.9)	0.64
Age at diagnosis, mean (SD)	35.5 (15.9)	38.3 (13.6)	0.63
Fibrosis stage (biopsy)			
F0–F2	10 (76.9%)	4 (57.1%)	0.61
F3–F4	3 (23.1%)	3 (42.9%)	0.61
Overlap PBC/PSC, n (%)	3 (23.1%)	1 (14.3%)	0.52
Therapies before switch			
AZA	ND	6 (85.7%)	
MMF	ND	1 (14.3%)	
AZA + MMF	10 (76.9%)	ND	
AZA + 6-TG	1 (7.7%)	ND	
AZA + 6-MP	1 (7.7%)	ND	
6-TG + MMF	1 (7.7%)	ND	
Therapy duration			
First-line therapy (years), median (range)	2.58 (0.08–17.17)	6.83 (0.25–24.42)	0.28
Second-line therapy (years), median (range)	1.33 (0.08–16.75)	ND	ND
ALT start CNI (U/L), median (range)	96 (16–794)	134 (21–295)	0.84
IgG start CNI (g/L), median (range)	10.90 (8.27–25.44)	14.10 (6.15–27.80)	0.48
Reason for switch to CNI			
Intolerance, n (%)	3 (23.1%)	3 (42.9%)	0.54
Insufficient response, n (%)	9 (69.2%)	4 (57.1%)	
Other, n (%)	1 (7.7%)	0	
Use of CsA, n (%)	6 (46.2%)	4 (57.1%)	
Use of TAC, n (%)	7 (53.8%)	3 (42.9%)	1.00
Initial dose CNI			
CsA (mg/kg)	1.83 (1.36–3.75)	2.11 (1.23–2.99)	0.48
TAC (mg/kg)	0.08 (0.05–0.08)	0.06 (0.04–0.10)	0.86
Predniso(lo)ne dose at switch (mg), median (range)	10 (5–40)	20 (10–30)	0.38
Dose CNI at last FU, median (range)			
CsA (mg/kg)	2.11 (1.36–3.75)	2.11 (1.23–2.99)	0.64
TAC (mg/kg)	0.07 (0.04–0.10)	0.04 (0.01–0.07)	0.20
Trough levels CNI at last FU			
CsA (ng/mL), median (range)	107 (18–125)	82 (74–89)	0.50
TAC (ng/mL), median (range)	7.6 (5.2–8.3)	12.3 (7.6–14.0)	0.14
Predniso(lo)ne dose at last FU (mg), median (range)	9.0 (5.0–12.0)	15.0 (2.5–30)	0.19

6-MP, 6-mercaptopurine; 6-TG, 6-tioguanine; ALT, alanine aminotransferase; AZA, azathioprine; CNI, calcineurin inhibitor, CsA, cyclosporine; FU, follow-up, IgG, immunoglobulin G; ND, no data; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis G; TAC, tacrolimus.

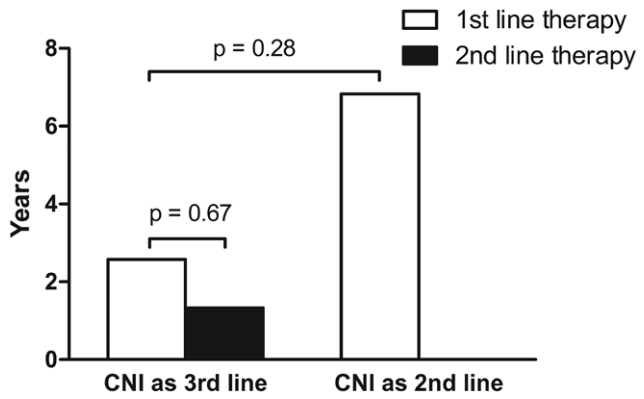

Fig. 1. Duration of treatment before CNI initiation. Patients who used CNIs as third-line treatment used first-line therapy shorter than patients who used CNIs as second-line treatment, however NS. CNI, calcineurin inhibitor.

not differ between third- and second-line treated patients [0.08 mg/kg (0.05–0.08) vs. 0.06 mg/kg (0.04–0.10); *P* = 0.86]. TAC dose at last moment of follow-up was non-significantly higher in third-line treated patients: 0.07 mg/kg (0.04–0.10) vs. 0.04 mg/kg (0.01–0.07) for second-line treated patients (*P* = 0.20).

All patients used concomitant steroids at the time of therapy switch to CNI. Median daily prednisolone dose was 10 mg (range 5–40) for patients on third-line CNI therapy vs. 20 mg (range 10–30) for patients on second-line CNI therapy (*P* = 0.38). At last moment of follow-up, six patients were successfully withdrawn from steroids. In patients who were still steroids, median prednisolone dosages had dropped to 9 mg (5.0–12 mg) in third-line patients compared to 15 mg (2.5–30 mg) in second line patients (*P* = 0.19).

Two patients (Table 3: patients 13 and 18) used additional immunosuppression next to CNI treatment: one patient used MMF 1000 mg in addition to CsA 200 mg and one patient was on AZA 100 mg in addition to CsA 150 mg. Median trough level of CsA at last follow-up was 107 ng/mL for patients on third-line treatment vs. 82 ng/ml in patients on second-line treatment (*P* = 0.50). For TAC, the median trough level was lower in patients on third-line treatment that in patients on second-line treatment: 7.6 ng/mL (5.2–8.3) vs. 12.3 ng/mL (7.6–14.0); (*P* = 0.14).

Efficacy of calcineurin inhibitor therapy

At last moment of follow-up (median follow-up on CNI treatment: 26.6 months), 7/13 (53.8%) patients who used CNIs as third-line therapy had normalization of serum transaminases compared to 4/7 (57.1%) patients who used CNIs as second-line therapy (*P* = 1.00) (Table 2). ALT kinetics per group are presented in Fig. 2. From the 13 patients who had available IgG at last moment of follow-up, 4/9 (44.4%) patients who were on third-line therapy reached biochemical remission compared to 3/4 (75.0%) patients who were on second-line therapy (*P* = 0.31). There were no differences in rates of normalization

Table 2. Treatment outcomes of patients who used calcineurin inhibitors as third-line therapy vs. patients who used them as second-line therapy

	Third-line therapy; N = 13	Second-line therapy; N = 7	*P* value
Years of follow-up since diagnosis, median (range)	15 (2–28)	8 (1–29)	0.24
Months of follow-up on CNI therapy, median (range)	12 (1–154)	16 (3–23)	0.78
Normal transaminases at last moment of FU, n (%)	7/13 (53.8%)	4/7 (57.1%)	1.00
Biochemical remission at last moment of FU, n (%)[a]	4/9 (44.4%)	3/4 (75.0%)	0.31

CNI, calcineurin inhibitors; FU, follow-up.
[a]IgG was available in 13 patients.

Table 3. Individual patient data of patients who are treated with calcineurin inhibitors

Patient	Age/ gender	Fibrosis	Medication before CNI switch	Reason CNI switch	ALT at start CNI	CNI + last dose	Medication while on CNI	Normal TA at last FU
1	33/F	F4	AZA 100 mg	IR	134	TAC 5 mg	Pred 2.5 mg	Yes
2	46/F	F0	AZA 75 mg	INT	43	TAC 3 mg	None	Yes
3	21/F	F2	6-TG 50 mg → MMF 1000 mg	IR	118	TAC 3 mg	Pred 10 mg	Yes
4	57/F	F1	AZA 50 mg → MMF 2000	IR	83	TAC 1 mg	Pred 5 mg	Yes
5	42/F	F0	AZA 25 mg → 6TG 20 mg	IR	34	TAC 1 mg	Pred 10 mg	No
6	49/F	F1	AZA 50 mg → MMF 2000 mg	IR	51	TAC 5 mg	Pred 7.5 mg	Yes
7[a]	13/M	F3	AZA 175 mg →MMF 2000 mg	IR	108	TAC 3 mg	Pred 10 mg	No
8[b]	50/F	F2	AZA 50 mg → MMF 2000 mg	IR	159	TAC 3 mg	Pred 10 mg	No
9[a]	37/F	F4	MMF 1000 mg	INT	148	TAC 5 mg	Pred 30 mg	No
10[b]	32/M	F3	AZA 75 mg	IR	164	TAC 6 mg	Pred 15 mg	No
11	20/F	F0	AZA 50 mg → MMF 1000 mg	IR	623	CsA 125 mg	none	No
12	33/M	UNK	AZA 50 mg → MMF 2000 mg	INT	96	CsA 100 mg	Pred 8 mg	No
13	35/M	F2	AZA 100 mg	IR	37	CsA 150 mg	AZA 100 mg	Yes
14	19/M	F3	AZA 200 mg → MMF 1000 mg	IR	429	CsA 125 mg	Pred 12 mg	Yes
15	28/M	F1	AZA 100 mg → MMF 1000 mg	Pregnancy	54	CsA 120 mg *stopped therapy*	None	Yes
16	24/F	UNK	AZA 100 mg	IR	295	CsA 175 mg	Pred 15 mg	No
17	50/F	F0	AZA 75 mg → 6MP 50 mg	IR	794	CsA 200 mg	Pred 5 mg	Yes
18[a]	57/F	F3	AZA 100 mg → MMF 1000 mg	INT	16	CsA 200 mg	MMF 1000 mg	Yes
19	22/F	UNK	AZA 150 mg → MMF 1000 mg	INT	71	CsA 200 mg	none	No
20	66/F	UNK	AZA 25 mg	INT	21	CsA 100 mg	Bud 3 mg	Yes

ALT, alanine aminotransferase; AZA, azathioprine; Bud, budesonide; CNI, calcineurin inhibitors; F, female; INT, intolerant; IR, insufficient response; M, male; MMF, mycophenolate mofetil; FU, follow-up; CsA, cyclosporin; Pred, prednisone; TA, transaminases; TAC, tacrolimus; UNK, unknown.
[a]Indicates patients with a variant syndrome with primary biliary cholangitis.
[b]Indicates patients with a variant syndrome with primary sclerosing cholangitis.

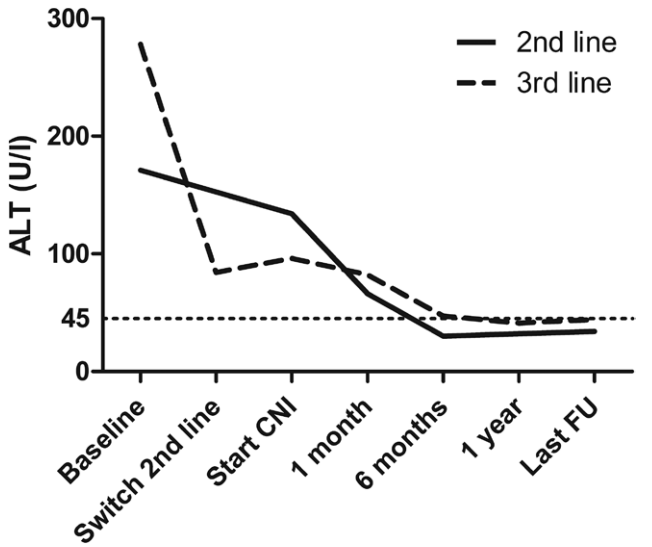

Fig. 2. Median ALT during CNI treatment. Comparison of patients who use CNIs as third-line therapy vs. patients who use CNIs as second-line therapy. ALT, alanine aminotransferase; CNI, calcineurin inhibitor; FU, follow-up.

of transaminases between patients who switched because of intolerance on prior therapy (n = 6) when compared to patients who switched due to insufficient response (n = 13): 50% vs. 53.8% (*P* = 0.64). There were no patients with a second (follow-up) biopsy after initiation of CNI treatment to assess histological response.

Adverse events on calcineurin inhibitor treatment

Overall, CNI treatment was well tolerated. Renal function remained stable in the majority of patients: median serum creatinine before start of CNI treatment was 62 µmol/L (range 45–86) compared to 65.5 µmol/L (44–132) at last moment of follow-up (*P* = 0.36). Patients on third-line CNI treatment were more frequently subject to adverse events than patients on second-line treatment: 46.2% vs. 28.6% (*P* = 0.44). Most commonly reported adverse events for TAC treatment were tremor (n = 3) and nausea (n = 2), followed by diarrhea and vertigo. Adverse events with CsA treatment were less common and were limited to headache, flu-like symptoms (leading to discontinuation of therapy) and gingival hypertrophy. One patient developed hepatocellular carcinoma shortly after CsA initiation and underwent a curative resection. There was no occurrence of other liver-related events (liver transplantation or liver related death). One patient on second-line TAC treatment, who had presented with Child-Pugh B cirrhosis at diagnosis, died of complications after an unexpected and unrelated event.

Discussion

We identified 20 AIH patients from two university hospitals who switched to CNI treatment when standard therapy failed. Thirteen patients received CNIs as third-line treatment for AIH. Most common reason for switch to CNI was insufficient response to the previous therapeutic

regimen. Interestingly, we found that duration of first-line therapy was almost twice as long as duration of second-line therapy. Disease characteristics of patients on third-line CNI treatment (n = 13) were comparable to those who used CNI as second-line therapy.

Both second- and third-line treated patients achieved rates of ~50% for normalization of transaminases. Additionally, 7/13 patients who were on CNI treatment and had available IgG at last moment of follow-up achieved complete biochemical remission. Previous studies on both TAC and CsA in AIH are of a limited sample size, mostly retrospective of nature and focus on a heterogeneous study population. Evidence from these studies show response rates varying from 27 to 94% [19–26]. The use of different definitions for response may be the root cause for the wide range of response rates reported in these studies. The largest study to date that investigated CNI therapy in AIH, which uses the same definitions of response as our study, found that the overall response rate in 80 TAC treated patients with a prior insufficient response to standard therapy was 56.5% [27], which is in line with our results and probably more accurate to clinical practice than the higher response rates reported in other studies.

Treatment with CNI is complex and should be tailored to the individual patient with frequent measuring of trough levels to avoid incorrect dosing. The exact target trough level TAC in AIH is unknown, although a level of 6 ng/mL is suggested in the EASL guideline [18]. In our cohort, we found that patients treated with TAC had relatively high trough levels associated with minor adverse events.

Although most studies report only on separate use of CNI treatment as second-line therapy option in AIH, there are reports on using either TAC or CsA in combination with other immunosuppressive agents to control the disease [25,28]. A recent survey among AIH experts showed that CNI treatment was mainly initiated by physicians working in transplant-centers. The survey does not report on the combination of two (or more) immunosuppressive agents in the management of AIH [29,30]. It is therefore unclear how many physicians use CNI treatment as separate therapy or in combination with other immunosuppressive drugs. In our cohort, two patients were using additional immunosuppression (AZA and MMF) in addition to treatment with CNIs.

The fact that patients on third-line CNI treatment received relatively shorter second-line treatment regimens when compared to duration of first-line therapy questions the optimal timing for switch to other therapies. A possible explanation for our finding could be that physicians and patients grow impatient when, after therapeutic failure on a first agent, a second drug also fails to be effective. The low occurrence of third-line therapy in AIH creates paucity of data in this field, leading to low-evidence recommendations in difficult-to-treat patients [18]. Ideally, international adapted timelines and criteria for initiation of third-line AIH treatment would exist, which would minimize practice variation.

Our study naturally comes with its limitations. First, because of the retrospective design, this study has its inherent selection bias. Second, we were not able to report on biochemical remission (normalization of transaminases and IgG) in every patient because of missing IgG values at last moment of follow-up. Third, in some patients standard therapy might have been dosed in suboptimal regimens, which could have been improved. Current guidelines advise to increase AZA dose up to 2 mg/kg/day in case of insufficient response, but this was not done in every patient. Last, our sample size limited us to conduct a multivariable analysis, which would be preferred for this type of study.

The relatively low response rates in our study and those that are reported in other studies raise the question whether CNI therapy is the optimal treatment for difficult-to-treat AIH. Currently, few alternatives exist such as MMF and monoclonal biologicals, MMF is widely accepted in patients who are intolerant to or have an insufficient response on first-line therapy. Recent reports showed that MMF is mainly successful in AZA intolerant patients in contrast to patients with an insufficient response [31]. Remission rates from recent studies vary between 34 and 57% in nonresponders, while remission rates varied from 62 to 91.9% in AZA intolerant patients [27,31]. However, the same studies report on high discontinuation rates due to infectious complications. Furthermore, the teratogenic properties of MMF raise questions about its applicability in a disease that mainly targets females in childbearing age. There is some familiarity in expert centers with biological treatment. Infliximab is able to act successfully as salvage therapy for difficult-to-treat AIH, although the large majority of patients developed infectious complications [32]. Alternatively, there are anecdotal reports on use of rituximab, which was deemed as a safe and effective treatment in AIH patients who failed on AZA therapy [33]. Other experimental therapies currently under investigation for AIH treatment are low-dose interleukin-2 and anti-B-cell activating factor antibody therapy (NCT03217422) [34].

In conclusion, we demonstrate that difficult-to-treat AIH patients on CNI treatment have a heterogeneous trajectory before switch to CNI. Treatment with CNIs was effective in ~50% of patients to achieve remission of the disease. Patients who are treated with third-line CNIs might have a longer duration of first-line therapy than second line therapy.

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Conflicts of interest

There are no conflicts of interest.

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