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Long-term follow-up of children and young adults with autoimmune hepatitis treated with cyclosporine

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ABSTRACT

Background: Cyclosporine (CSA) is an alternative treatment for autoimmune hepatitis (AIH), however, its unknown long-term safety and efficacy have limited its use.
Aims: Examine the long-term outcome of children and young adults with AIH treated with CSA for at least 4 years.
Methods: Twenty patients were included in this retrospective study: 15 with classical AIH and 5 with autoimmune hepatitis/autoimmune sclerosing cholangitis overlap syndrome (ASC). CSA was administered as first (12 patients) or second-line (8 patients) treatment, alone or in combination with azathioprine or mycophenolate mofetil and/or prednisone.
Results: CSA determined initial clinical and biochemical remission in all patients. At the end of follow-up (median 8.6; range 4–20.4 years), all patients are alive with their native liver; 15 in complete remission (75%), 2 with incomplete response to treatment and 3 listed for liver transplant. Side effects were mild and transitory after dose tapering or, in 1 case, after CSA withdrawal. Hypertrichosis and moderate gingival hyperplasia were the most frequent. Two patients presented mild transient glomerular filtration rate (GFR) reduction. Median GFR at the beginning and end of treatment was not statistically different for all patients.
Conclusions: CSA was effective and safe in the long-term treatment of our cohort of patients with AIH, tailoring the treatment remains key-points during CSA administration.

1. Introduction

Autoimmune hepatitis (AIH) is a progressive inflammatory disease of the liver, which if untreated may lead to cirrhosis and terminal liver failure [1,2]. AIH usually responds to immunosuppressive therapy, and the "so-called" conventional treatment consists of prednisone (PDN) and azathioprine (AZA). Although this treatment induces clinical and biochemical remission in most cases, it is frequently associated with incomplete response and relapses. Moreover, corticosteroids may cause moderate to severe side effects, which lead to poor treatment compliance, especially in adolescent girls. Azathioprine, on the other hand, may cause bone marrow suppression [3–5].

For these reasons the need for alternative treatments has been recognized by the medical community. The use of cyclosporine (CSA) was first reported in 1985 in an adult with type 1 AIH, and subsequently in 1987 in a 14-year-old boy with the same disorder [6,7]. Since then, several reports have been published where CSA has been shown to allow recovery from liver failure and shown to induce and safely maintain remission in children with AIH. However, since CSA has mainly been administered to induce remission as a bridge to a conventional treatment, its long-term safety and efficacy is unknown [8–11].

In 2004 we reported our experience with CSA in 12 patients with AIH who were followed-up for at least 18 months, and achieved complete remission without significant side effects [12]. The present study aims to report on the long-term outcome of

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patients who received a prolonged course of CSA. Disease remission, as well as the development of side effects, were analyzed in order to assess CSA efficacy and safety in the treatment of AIH in children. The second end point of the study was to investigate potential predictors of treatment outcome.

2. Materials and methods

This observational study was conducted retrospectively by chart review, from 1993 to 2004, and prospectively, from 2004 to 2015. The study was approved by the ethical committee of the Azienda Ospedaliera Universitaria Pisana. Twenty patients with AIH who were treated with CSA for at least 4 years were included. Fifteen patients were affected by "classical" autoimmune hepatitis and 5 patients by autoimmune hepatitis/autoimmune sclerosing cholangitis overlap syndrome (ASC).

Patients' characteristics at diagnosis are summarized in Table 1. In all patients, any known cause of liver damage was excluded, including viral infections by hepatotropic viruses (hepatitis A, B and C virus, Epstein–Barr virus, Cytomegalovirus, Herpes simplex virus), α1-antitrypsin deficiency and Wilson's disease, when appropriate. Serum autoantibodies were detected using indirect immunofluorescence techniques. Liver biopsy was performed at diagnosis in all except 3 patients, who presented with a prothrombin time (PT) <50%. Ishak scoring system was used for the assessment of necroinflammatory activity [13]. Biliary lesions were graded as absent, mild, moderate or severe. Diagnosis of AIH was made in 15 children according to the diagnostic criteria defined by the International Autoimmune Hepatitis Group [14], corresponding in all patients with a definite diagnosis. The diagnosis of ASC was made in 5 patients with anti-nuclear and/or anti-smooth muscle autoantibodies high titer reactivity, elevated immunoglobulin G (IgG) and histologic findings consistent with diffuse inflammatory bile duct damage as well as with interface hepatitis. Magnetic resonance cholangiography (MRC) and/or endoscopic retrograde cholangiography (ERPC) were performed in 5 patients. In two of them signs of sclerosing cholangitis were present 8.8 and 6 years after ASC diagnosis. Colonoscopy was performed in 4 of the 5 ASC patients and showed findings consistent with ulcerative colitis in one case.

2.1. Cyclosporine (CSA) treatment

CSA was administered either as first or second-line treatment. Indications for first-line treatment were the following: disease severity (4), patient steroid refusal (1), and presence of relative contraindication to steroid use (7) (age < 3 years, growth retardation, pubertal growth spurt in progress, overweight/obesity, candidiasis). CSA was used as a second-line treatment in patients who were non-responders (5) or intolerant to conventional treatment (3). Patients receiving second-line CSA treatment were all previously treated with PDN and AZA/mycophenolate mofetil (MMF).

Patients received CSA for a median time of 6.3 years (range 4–15.5), alone or in combination with AZA or MMF and/or prednisone. Ursodeoxycholic acid was added in ASC patients [Table 2]. In all cases a written consent to CSA treatment was obtained.

CSA was administered, in all cases, orally with a daily dosage ranging between 1.5 and 8 mg/kg with the aim to obtain and maintain initial trough blood levels of 150–200 ng/mL. Once remission was achieved, CSA doses were reduced to maintain trough levels between 100 and 150 ng/mL and, after 1 year of treatment, between 50 and 70 ng/mL.

Once CSA treatment had been established, blood levels of aminotransferases, albumin, IgG, PT and cyclosporine were periodically monitored (weekly during the first month, every other week during the second month, monthly for the following 3 months and every 3–6 months in the period after). Response to treatment was defined as complete remission (disappearance of symptoms, normalization of aminotransferases and gamma glutamyl transpeptidase activities, normalization of IgG levels, negative or < 1:40 autoantibody titre), incomplete response (partial improvement in clinical and laboratory features without normalization, with or without the need for second or third-line treatment) or treatment failure (worsening clinical, laboratory, and histological features despite compliance with therapy). Relapse was defined as an increase in serum aminotransferases activity of greater than twice the upper limit of normal, with or without reappearance of symptoms, after a complete remission as defined above.

Furthermore, in order to evaluate any adverse events related to CSA treatment, serum creatinine levels and blood pressure, as well as the presence of gingival hyperplasia, hypertrichosis and neurological signs, were routinely assessed in all patients. To assess kidney function, glomerular filtration rate (GFR) was estimated using the creatinine-based Schwartz equation in patients up to 18 years old and using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation in patients older than 18 years. GFR was defined as normal ($\geq$90 mL/min/1.73 m^2), mildly reduced (89–60 mL/min/1.73 m^2), moderately reduced (59–30 mL/min/1.73 m^2), severely decreased (29–15 mL/min/1.73 m^2), or consistent with kidney failure (<15 mL/min/1.73 m^2).

2.2. Assessment of predictive indicators of treatment outcome

To identify potential indicators of treatment outcome, patients were divided in two groups. The group of *responders* consisted of patients who at the end of follow-up were in complete remission. The group of *non-responders* comprised patients who, at the end of follow-up, showed incomplete response to treatment, or showed treatment failure. The prognostic value of 12 variables was analyzed [Table 3].

2.3. Statistical analysis

For descriptive analysis, categorical data are presented as numbers and percentages; continuous data are presented as medians and ranges. To assess predictive indicators of treatment outcome, a bivariate analysis was carried out in which each predictive variable was explored separately with respect to treatment outcome. Differences were evaluated with the Fisher exact test for categorical data and with the non-parametric Mann–Whitney test for continuous data (a non-normal distribution of data was shown both visually and with the Kolmogorov–Smirnov test). To assess the long-term safety of CSA, GFRs were compared using a non-parametric test for paired data (Wilcoxon test) at the beginning of CSA treatment and at the end of follow-up for each patient. A double-sided p value of <0.05 was considered statistically significant.

3. Results

3.1. Response to treatment and outcome in 12 patients receiving cyclosporine as first-line treatment

CSA treatment was associated with a 100% initial clinical and biochemical remission rate after a median period of 8.5 weeks (range: 4–36 weeks). No statistically significant difference was found in the time to achieve remission in patients receiving CSA as a first- and second-line treatment ($P = 0.10$).

Table 1
Patients' characteristics at diagnosis.

	AIH-1	AIH-2	ASC	All
Number of patients	5	10	5	20
Female gender, *n*	3	9	3	15
Age at diagnosis [median, in years (range)]	10.5 (2.4–12.9)	3.7 (1.4–14.2)	10.1 (8.2–13.5)	9.5 (1.4–14.2)
Mode of presentation, *n*				
• Acute hepatitis	1	3	0	4
• Insidious onset	0	0	2	2
• Incidental finding	4	7	3	14
Clinical findings, *n*				
• Hepatomegaly	3	6	3	12
• Splenomegaly	2	2	1	5
Biochemistry				
• ALT, xULN (median, range)	16 (7–25)	17 (5–73)	12 (10–20)	20 (5–73)
• GGT, xULN (median, range)	0 (0–2)	0 (0–2)	6 (2–10)	1.4 (0–10)
• PT <50%, *n*	2	2	2	6
• IgG, mg/dL (median, range)	3656 (1934–4430)	1860 (813–2200)	4000 (1730–4560)	2000 (813–4560)
Positive autoantibodies, *n*				
• ANA	2		3	5
• SMA	1			1
• ANA + SMA	2		1	3
• LKM1		8		8
• LKM1 + LC1		1		1
• LKM1 + LC1 + ANA		1		1
• ANA + pANCA			1	1
Liver histology*				
• Inflammatory activity Ishak Score (median, range)	12/18 (12–12)	12/18 (6–16)	6/18 (6–12)	12/18 (6–16)
• Fibrosis Ishak Score (median, range)	1/6 (0–2)	1/6 (0–5)	5/6 (4–6)	2/6 (0–6)
• Presence of biliary lesions, *n of patients*	2	0	5	7
Associated autoimmune disorders, *n* (%)	3 (60%)	5 (50%)	1 (20%)	9 (45%)
Family history of autoimmune disorders in first-degree relatives, *n* (%)	2 (40%)	5 (50%)	0 (0%)	7 (35%)

AIH-1: autoimmune hepatitis type 1, AIH-2: autoimmune hepatitis type 2, xULN: upper limit of normal, PT: prothrombin time, IgG: immunoglobulin G, ANA: antinuclear antibodies, SMA: anti-smooth muscle antibody, LKM1: anti-liver kidney microsomal type 1 antibody, LC1: anti-liver cytosol type 1 antibody, pANCA: perinuclear anti-neutrophil cytoplasmic antibodies.

* In 3 patients (2 AIH-1, 1 ASC) with PT <50% liver biopsy was not performed at diagnosis.

Table 2
Cyclosporine treatment features of 20 patients with autoimmune hepatitis.

Drug combinations in patients receiving first-line cyclosporine treatment (*n* = 12)	• Cyclosporine (10) • Cyclosporine + azathioprine (1) • Cyclosporine + prednisone (1) • Ursodeoxycholic acid (3)[a]
Drug combinations in patients receiving second-line cyclosporine treatment (*n* = 8)	• Cyclosporine (2) • Cyclosporine + prednisone (1) • Cyclosporine + azathioprine + prednisone (4) • Cyclosporine + mycophenolate + prednisone (1) • Ursodeoxycholic acid (2)[a]

Indications to cyclosporine first-line treatment (*n*)	• Relative controindication to steroid use (7)
	  • Age <3 years
	  • Growth retardation
	  • Pubertal growth spurt in progress
	  • Overweight/obesity
	  • Candidiasis
	• Patient steroid refusal (1)
	• Disease severity (4)

Indications to cyclosporine second-line treatment (*n*)	• Conventional treatment failure (5)
	• Steroid-related severe side effects (3)
	  • Obesity
	  • Growth failure
	  • Osteoporosis

Age at the beginning of cyclosporine treatment median (range)	10.3 years (2.2–23.1 years)
Cyclosporine treatment duration, median (range)	6.3 years (4–15.5 years)

[a] Ursodeoxycholic acid was added to patients with ASC.

At the end of a median follow-up of 8.6 years (range 4–20.4 years), all patients are alive with their native liver. One patient is listed for liver transplant.

3.1.1. Complete remission at the end of follow-up (n = *10)*

Ten of 12 patients are in complete remission at the end of follow-up (AIH-1, *n* = 2; AIH-2, *n* = 6; ASC *n* = 2): (1) seven patients were successfully shifted to azathioprine monotherapy after receiving cyclosporine treatment for a median time of 8.5 years (range 4–15.5 years); (2) one patient has been receiving cyclosporine and azathioprine for 4.6 years; (3) one patient received cyclosporine for 1.5 years before azathioprine was added to her treatment maintaining a complete remission for 6.5 years. She then experienced a relapse and shortly after was diagnosed with Systemic Lupus

Table 3
Analysis of prognostic indicators of treatment outcome in 20 children with autoimmune hepatitis.

Parameters	Responder n = 15	Non responder n = 5	Total N = 20	p
Sex, n (%)				1.00
M	4 (80.0%)	1 (20.0%)	5 (100.0%)	
F	11 (73.3%)	4 (26.7%)	15 (100.0%)	
Diagnosis, n (%)				0.56
AIH	12 (80.0%)	3 (20.0%)	15 (100.0%)	
OVLS	3 (60.0%)	2 (40.0%	5 (100.0%)	
Age at diagnosis, years	8.2 (1.4–14.2)	12.4 (8.7–13.2)	–	0.12
Signs of cirrhosis at diagnosis, n (%)				0.03
Yes	1 (25.0%)	3 (75.0%)	4 (100.0%)	
No	14 (87.5%)	2 (12.5%)	16 (100.0%)	
Jaundice at diagnosis, n (%)				1.00
Yes	5 (71.4%)	2 (28.6%)	7 (100.0%)	
No	10 (79.6%)	3 (20.4%)	13 (100.0%)	
ALT (xULN)	20.0 (7.0–73.0)	10.0 (5.0–42.0)	–	0.07
GGT (xULN)	1.0 (1.0–6.5)	2.5 (1.0–10.0)	–	0.07
PT (%)	75.0 (32.0–114.0)	54.0 (34.0–88.0)	–	0.25
Presence of autoimmune comorbidities, n (%)				0.13
Yes	5 (55.6%)	4 (44.4%)	9 (100.0%)	
No	10 (90.9%)	1 (9.1%)	11 (100.0%)	
Age at beginning CSA treatment, years	10.0 (2.2–23.1)	12.9 (9.9–18.8)	–	0.08
Previous treatment before CSA, n (%)				0.35
Yes	5 (62.5%)	3 (37.5%)	8 (100.0%)	
No	10 (83.3%)	2 (16.7%)	12 (100.0%)	
Relapses during CSA treatment, n (%)				0.04
Yes	6 (54.5%)	5 (45.5%)	11 (100.0%)	
No	9 (100%)	0	9 (100.0%)	

Row percentages.
Continuous data are expressed as median and range.

Erythematosus and continued treatment with prednisone alone; (4) one patient has been in stable remission for 6 months without any immunosuppressive maintenance drug, after 7.4 years of treatment with cyclosporine and prednisone followed by azathioprine monotherapy.

Four of the 10 patients had one or more episodes of relapse.

3.1.2. Incomplete response to treatment at the end of follow-up (n = 1)

One patient with AIH-1, liver cirrhosis, biochemical signs of liver failure (PT 43%) and severe hypergammaglobulinemia was initially started on cyclosporine achieving first remission after 36 weeks. Prednisone and azathioprine were added to her treatment after 2.6 and 7.9 years respectively due to failure to maintain a complete response.

3.1.3. Treatment failure (n = 1)

In one patient with ASC, cyclosporine allowed for remission lasting 2 years after which the patient relapsed. Subsequently he presented recurrent episodes of ascending bacterial cholangitis and 3.9 years after ASC diagnosis, ulcerative colitis was diagnosed. MRC performed 2 years later demonstrated extrahepatic bile duct changes characteristic of sclerosing cholangitis. Despite triple immunosuppressive therapy with cyclosporine, prednisone and azathioprine, liver disease progressed and enrollment in the liver transplant waiting list was necessary 6.3 years after diagnosis.

3.2. Response to treatment and outcome in 8 patients receiving cyclosporine as second-line treatment

CSA treatment was associated with initial clinical and biochemical remission in a median period of 6 weeks (range: 4–8 weeks) in all patients but two who were shifted to CSA despite being in remission with conventional treatment, because of severe obesity and severe growth impairment.

At the end of a median follow-up of 8.6 years (range 4–20.4 years) all patients are alive with their native liver. Two are listed for liver transplant.

3.2.1. Complete remission at the end of follow-up (n = 5)

Five of 8 patients are in complete remission at the end of a follow-up (AIH-1, $n = 2$; AIH-2, $n = 2$; ASC, $n = 1$): (1) two patients are on azathioprine monotherapy; (2) two patients are on cyclosporine and azathioprine; (3) one patient is on triple immunosuppressive therapy with cyclosporine, prednisone and MMF.

Two of the 5 patients had one or more episodes of relapse during treatment. One patient relapsed 6 months after withdrawal of immunosuppressive treatment; complete remission was achieved 4 weeks after cyclosporine administration was restarted.

3.2.2. Incomplete response to treatment at the end of follow-up (n = 1)

One patient with AIH-2 and celiac disease was initially started on prednisone and azathioprine; despite initial first remission, the patient relapsed and a repeat biopsy 2.3 years after diagnosis showed persistent inflammatory activity without worsening of fibrosis. Cyclosporine was added allowing achievement of remission and tapering of the steroid. After 7.6 years from diagnosis, however, and despite switching azathioprine to MMF, the patient shows incomplete response to triple immunosuppressive treatment.

3.2.3. Treatment failure (n = 2)

In two patients (AIH-2, $n = 1$; ASC, $n = 1$) with liver cirrhosis at diagnosis, cyclosporine was added to prednisone and azathioprine after 1.8 and 6.3 years, respectively, due to incomplete response and allowed for transient remission. In the patient with AIH-2, cyclosporine was replaced with tacrolimus after 5.7 years of therapy due to a reduction of the GFR. In the patient diagnosed with ASC, a MRC showed multifocal structuring and dilatation of intra- and extrahepatic bile ducts 8.8 after diagnosis. Liver disease pro-

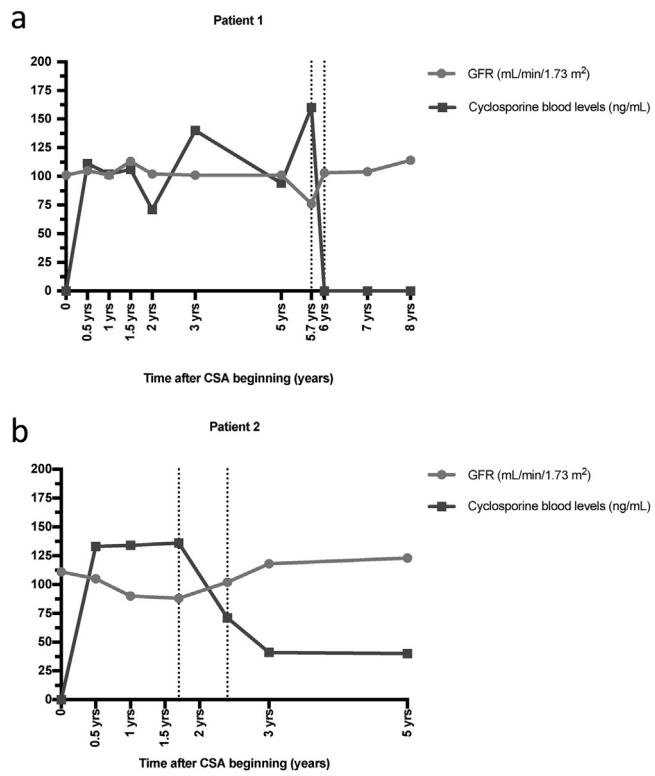

Fig. 1. Glomerular filtration rate and cyclosporine blood level relationship in two patients who presented mild transient GFR reduction.
(A) Patient 1, mild GFR reduction after 5.7 years of cyclosporine administration.
(B) Patient 2, mild GFR reduction after 1.7 years of cyclosporine administration.

gressed in both patients, and they were listed for liver transplant 11.2 and 14.4 years after diagnosis, respectively.

3.3. Prognostic indicators of treatment outcome

Univariate analysis showed that 2 of the investigated variables were significantly associated with treatment outcome. Specifically, an unfavorable outcome (*non-responder* group) was associated with signs of cirrhosis at diagnosis ($p = 0.03$) and presence of relapses during CSA treatment ($p = 0.04$) [Table 3]. The limited sample size and complete separation problems did not allow for development of a multivariate model.

3.4. Cyclosporine safety

Hypertrichosis was observed in 9 of 20 patients (45%), it resolved in all patients either spontaneously or after cyclosporine tapering.

Eleven patients (55%) presented gingival hyperplasia which disappeared in all once cyclosporine dosage was reduced.

A significant increase in serum creatinine levels up to 1.2 mg/dL and 0.96 mg/dL was observed in 2 patients (10%), respectively. In the first patient mild GFR reduction (76.5 mL/min/1.73 m^2) occurred after 5.7 years of cyclosporine administration. Cyclosporine, which at the time was administered at the dose of 1.9 mg/kg/day, was therefore switched to tacrolimus with a subsequent persistent normalization of serum creatinine (0.89 mg/dL) and GFR (103.2 mL/min/1.73 m^2) after 2 months. In the second patient, mild GFR reduction (88.8 mL/min/1.73 m^2) was observed after 1.7 years of cyclosporine administration. Cyclosporine dosage was accordingly reduced from 3.7 to 2.8 mg/kg/day; serum creatinine levels and GFR gradually returned in the normal range (0.85 mg/dL and 101.6 mL/min/1.73 m^2, respectively) within 8 months [Fig. 1].

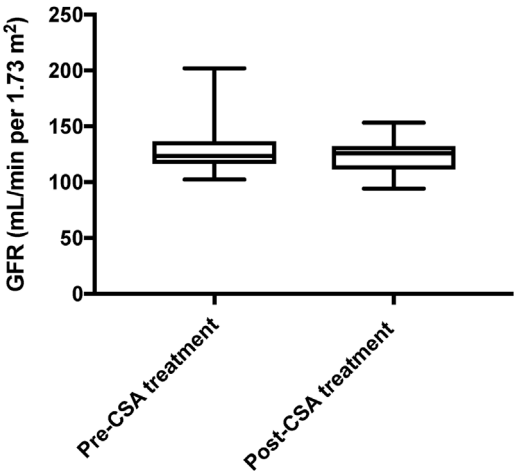

Fig. 2. Renal function of 20 patients with autoimmune hepatitis before and after cyclosporine treatment (median follow-up 8.6 years, range 4–20.4 years).

To assess the long-term safety of cyclosporine, GFR at the beginning of cyclosporine treatment and at the end of follow-up of each patient were compared. No statistically significant difference was found ($p = 0.41$) [Fig. 2].

Neither arterial hypertension nor any other known CSA-related side effects were registered throughout the entire follow-up period.

4. Discussion

This study is the first to report on the long-term efficacy and safety of cyclosporine treatment in children and young adults with AIH.

Conventional treatment, has been preferred over the last 30 years [1–3,15]. Its well-known benefits, however, need to be carefully weighed against the risks and flaws of such treatment. It is reported that 69% to 94% of pediatric patients relapse after drug withdrawal [16–18,28,29] and that 3% to 55% of children need to undergo liver transplant, despite treatment, with a recurrence rate of *de novo* AIH in transplanted patients ranging from 38% to 83% [2,16–18,28,29,31]. Furthermore, high dose steroids carry the risk of severe side effects. This risk increases with repetitive treatment of multiple relapses. Such side effects are often poorly tolerated, particularly by adolescent patients, and potentially cause scarce treatment compliance [1,5,18,19]. Azathioprine monotherapy has been demonstrated to maintain remission in most patients with AIH, although only in retrospective adult series, and its long-term use is potentially burdened by the risk of developing malignancies. All of these are compelling reasons to refine current treatment strategies and pursue new management options [20–23].

Cyclosporine was initially used to prevent allograft rejection after solid organ transplantation. Subsequently, it was also used for the treatment of numerous autoimmune conditions such as uveitis, rheumatoid arthritis, and psoriasis. The major CSA side effects are nephrotoxicity, arterial hypertension and gastrointestinal and neurological toxicity [24–27].

Recently Czaja reported that since 1985, CSA use has been documented in 10 reports including 133 adult patients with AIH. CSA was used as a salvage therapy with overall positive response in about 93% of patients and a negative response in 7% [20].

As for pediatric patients, there are only four major publications that include large series of patients with autoimmune liver disease. The reported remission rate achieved with CSA administration ranges between 83% and 100% with the most commonly reported side effects being hypertricosis and gingival hyperplasia. Transient

Table 4
Clinical features, outcome and side effects of cyclosporine treatment in selected studies featuring large series of patients with autoimmune liver disease.

Study	Number of patients	Diagnosis	*Median age at the beginning at CSA treatment (years)*	*Median duration of CSA treatment (years)*	% of remission	Side effects (% of patients)	Comments on side effects
Alvarez et al. [14]	32	AIH-1 AIH-2	10.1 (2–16.6)	0.5	83%	Mild hypertricosis Moderate gingival hypertrophy	Tolerance to CSA was very good. No patient presented with hypertension or renal complications.
EASL [15]	15	AIH-2	Not reported	Not reported	100%	Mild Hypertricosis (80%) Mild gingival hypertrophy (33%) GFR reduction > 20% (20%) Hypertension (6%) Tremor (6%) Headache (13%)	Renal function returned to normal after CSA dose reduction or discontinuation. Transient mild hypertension occurred in a child with associated autoimmune glomerulonephritis receiving also prednisone. All other side effects occurred within the first 6 months of treatment and then disappeared.
Maggiore et al. [18]	12	AIH-1 AIH-2 AC GCH	8 (3–12)	2.9 (0.7–7.4)	100%	Hypertricosis (17%) Gingival hypertrophy (25%) Creatinine elevation (8%)	Hypertricosis and gingival hypertrophy improved on changing to conventional treatment; renal function returned to normal after CSA dose reduction.
Saadah et al. [16]	84	AIH-1 AIH-2	9.9 (2.3–16)	0.5	94%	Mild hypertricosis (55%) Mild gingival hypertrophy (39%) Mild creatinine elevation (10%) Mild hypertension (4%)	Tolerance to CSA was satisfactory. All side effects were transient. Creatinine level increase and hypertension were detected in 1 occasion in 5 and 3 patients respectively (during the first month of treatment).

AIH-1: autoimmune hepatitis type-1, AIH-2: autoimmune hepatitis type-2, AC: autoimmune cholangitis, GCH: giant cell hepatitis, CSA: cyclosporine.

GFR reduction and creatinine elevation is reported in 8–20% of the patients in 3 of the studies [8–10,12] [Table 4].

In all of these studies, however, CSA has mostly been administered for short periods. It is likely that the lack of data concerning its long-term efficacy as well as its potential risks limited its use to short-term initial therapy in alternative to steroid-azathioprine or to salvage treatment.

This study was therefore undertaken to evaluate the long-term efficacy and safety of CSA in patients with AIH. Our patients were treated with CSA for a median period of 6.3 years (range: 4–15.5 years), the longest reported in literature to our knowledge.

Our results confirm the efficacy of CSA in treatment-naïve patients with AIH. Specifically, the efficacy of CSA in the induction and maintenance of clinical and biochemical remission in AIH was shown to be equal to that of conventional treatment reported in other studies [1,2,29].

Initial clinical and biochemical remission was indeed achieved in all of our patients; independent of the degree of hepatic impairment, CSA was equally effective in inducing remission both as first- and as second-line treatment.

CSA also determined long-term sustained clinical and biochemical remission in more than 80% of the treatment naïve patients, alone or in combination with azathioprine or prednisone. Shift to AZA monotherapy was achieved in 60% of patients and even after CSA withdrawal no relapse occurred, suggesting that CSA lead to a complete and persistent resolution of the liver inflammatory process. In 1 patient (8%), treatment was stopped and she has currently been out of therapy for the past 6 months.

In patients who received CSA as second-line treatment, CSA allowed complete prolonged remission in 62.5% of cases, alone or in combination with azathioprine and/or prednisone and/or MMF. Recently, a meta-analysis by Zizzo et al. compared the short-term response rate to second-line agents in pediatric refractory AIH. The highest response rate at 6 months was estimated to be with cyclosporine (86%) followed by MMF (38%) [30].

In our study, only 5 patients received CSA as a second-line treatment, however despite the small number of patients, the follow-up was prolonged and our results support the finding that CSA has a high-response rate as a second-line treatment, even well beyond the 6 month period.

In our cohort of patients, there were no deaths. Three patients are on transplant waiting list 6.3, 11.2 and 14.4 years after diagnosis, respectively. These three presented with more severe and aggressive features than the others. Two of them had ASC, which was associated with cirrhosis at diagnosis in one. Both of these presented with sclerosing cholangitis 8.8 and 6 years after diagnosis, respectively. The third patient presented initially with cirrhosis, PT <50% and portal hypertension, and was diagnosed with AIH-2 as well as with multiple extra-hepatic autoimmune disorders.

The association with inflammatory bowel disease is a well-known feature of autoimmune liver disorders and is more commonly reported in patients with ASC (45%) than with AIH-1 (20%). In our study population, only one of 5 patients with ASC was diagnosed with ulcerative colitis and he was one of the patients listed for liver transplant.

The analysis of prognostic indicators showed that cirrhosis at diagnosis and relapses during treatment were associated with an unfavorable outcome. The limited sample size and complete separation problems, however, did not allow for the development of a multivariate model, withholding the possibility to draw any definitive conclusion.

The frequency of relapses during CSA treatment in our study appeared to be equal to that which was reported in other studies with conventional treatment [1,2,29].

Cyclosporine treatment was very well tolerated, as observed in previous studies [8–12]; however both CSA treatment duration and follow-up were significantly longer in this study.

CSA side effects were mild and transitory in all patients either spontaneously, after dose tapering or, in 1 case, after CSA withdrawal. Transient hypertrichosis and moderate gingival hyperplasia were the most frequent side effects. Mild GFR reduction with return to normal renal function occurred in two patients. Median GFR was not statistically different at the beginning and at the end of treatment for all patients, and no patients presented with hypertension.

Severe and permanently disabling side effects such as the ones seen with prolonged steroid treatment are therefore not present even after prolonged CSA administration.

Satisfactory CSA tolerability in our patients seems to be related to the administration of a low CSA dose regimen and to its strict blood level monitoring.

This study presents several limitations, the main ones being the relatively small number of patients, the heterogeneity of diagnosis (AIH-1, AIH-2, ASC) and CSA treatment (first- or second-line) and the study design (partially retrospective and partially prospective). Moreover the presence of co-medication constitutes another potential confounder.

Despite these limitations, the major strength of this study is that it is the first one to describe the long-term outcome of CSA treatment in children and young adults with autoimmune hepatitis, reporting data on CSA efficacy and safety acquired over more than 20 years of experience.

5. Conclusions

In conclusion, CSA is an effective and safe long-term treatment for children and young adults with AIH constituting a viable alternative to conventional treatment. CSA administration should not be limited to a bridge to steroid-azathioprine treatment or to a salvage therapy, but could also be considered as a first-line and maintenance treatment.

Monitoring the occurrence of potential adverse effects, specifically the evaluation of renal function, and tailoring the treatment remain key points during CSA administration.

Conflict of interest

None declared.

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References

[1] Maggiore G, Nastasio S, Sciveres M. Juvenile autoimmune hepatitis: spectrum of the disease. World J Hepatol 2014;6:464–76.
[2] Gregorio GV, Portmann B, Reid F, Donaldson PT, Doherty DG, McCartney M, et al. Autoimmune hepatitis in childhood: a 20-year experience. Hepatology 1997;25:541–7.
[3] Maggiore G, Bernard O, Hadchouel M, Hadchouel P, Odievre M, Alagille D. Treatment of autoimmune chronic active hepatitis in childhood. J Pediatr 1984;104:839–44.
[4] Murray-Lyon IM, Stern RB, Williams R. Controlled trial of prednisone and azathioprine in active chronic hepatitis. Lancet 1973;1:735–7.
[5] Maggiore G, Bernard O, Hadchouel M, Alagille D. Life-saving immunosuppressive treatment in severe autoimmune chronic active hepatitis. J Pediatr Gastroenterol Nutr 1985;4:655–8.
[6] Mistilis SP, Vickers CR, Darroch MH, McCarthy SW. Cyclosporin, a new treatment for autoimmune chronic active hepatitis. Med J Aust 1985;143:463–5.
[7] Hyams JS, Ballow M, Leichtner AM. Cyclosporine treatment of autoimmune chronic active hepatitis. Gastroenterology 1987;93:890–3.
[8] Alvarez F, Ciocca M, Cañero-Velasco C, Ramonet M, de Davila MT, Cuarterolo M, et al. Short-term cyclosporine induces a remission of autoimmune hepatitis in children. Hepatology 1999;30:222–7.
[9] Debray D, Maggiore G, Girardet JP, Mallet E, Bernard O. Efficacy of cyclosporin A in children with type 2 autoimmune hepatitis. J Pediatr 1999;135:111–4.
[10] Cuarterolo M, Ciocca M, Velasco CC, Ramonet M, González T, López S, et al. Follow-up of children with autoimmune hepatitis treated with cyclosporine. J Pediatr Gastroenterol Nutr 2006;43:635–9.
[11] Cuarterolo ML, Ciocca ME, López SI, de Dávila MT, Alvarez F. Immunosuppressive therapy allows recovery from liver failure in children with autoimmune hepatitis. Clin Gastroenterol Hepatol 2011;9:145–9.
[12] Sciveres M, Caprai S, Palla G, Ughi C, Maggiore G. Effectiveness and safety of ciclosporin as therapy for autoimmune diseases of the liver in children and adolescents. Aliment Pharmacol Ther 2004;19:209–17.
[13] Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–9.
[14] Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31:929–38.
[15] European Association for the Study of the Liver. EASL clinical practice guidelines: autoimmune hepatitis. J Hepatol 2015;63:971–1004.
[16] Saadah OI, Smith AL, Hardikar W. Long-term outcome of autoimmune hepatitis in children. J Gastroenterol Hepatol 2001;16:1297–302.
[17] Radhakrishnan KR, Alkhouri N, Worley S, Arrigain S, Hupertz V, Kay M, et al. Autoimmune hepatitis in children-impact of cirrhosis at presentation on natural history and long-term outcome. Dig Liver Dis 2010;42:724–8.
[18] Maggiore G, Veber F, Bernard O, Hadchouel M, Homberg JC, Alvarez F, et al. Autoimmune hepatitis associated with anti-actin antibodies in children and adolescents. J Pediatr Gastroenterol Nutr 1993;17:376–81.
[19] Maggiore G, Bernard O, Homberg JC, Hadchouel M, Alvarez F, Hadchouel P, et al. Liver disease associated with anti-liver-kidney microsome antibody in children. J Pediatr 1986;108:399–404.
[20] Czaja AJ. Advances in the current treatment of autoimmune hepatitis. Dig Dis Sci 2012;57:1996–2010.
[21] Van Thiel DH, Wright H, Carroll P, Abu-Elmagd K, Rodriguez-Rilo H, McMichael J, et al. Tacrolimus: a potential new treatment for autoimmune chronic active hepatitis: results of an open-label preliminary trial. Am J Gastroenterol 1995;90:771–6.
[22] Burak KW, Urbanski SJ, Swain MG. Successful treatment of refractory type 1 AIH with methotrexate. J Hepatol 1998;29:990–3.
[23] Richardson PD, James PD, Ryder SD. Mycophenolate mofetil for maintenance of remission in autoimmune hepatitis in patients resistant to or intolerant of azathioprine. J Hepatol 2000;33:371–5.
[24] Minuk GY. Cyclosporin A in non-transplant-related liver diseases. Am J Gastroenterol 1989;84:1345–50.
[25] Treem W, Hyams JS. Cyclosporine therapy for gastrointestinal disease. J Pediatr Gastroenterol Nutr 1994;18:270–8.
[26] Mattos AM, Olyaei AJ, Bennett WM. Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. Am J Kidney Dis 2000;35:333–46.
[27] Choudhry S, Bagga A, Hari P, Sharma S, Kalaivani M, Dinda A. Efficacy and safety of tacrolimus versus cyclosporine in children with steroid-resistant nephrotic syndrome: a randomized controlled trial. Am J Kidney Dis 2009;53:760–9.
[28] Jiménez-Rivera C, Ling SC, Ahmed N, Yap J, Aglipay M, Barrowman N, et al. Incidence and characteristics of autoimmune hepatitis. Pediatrics 2015;136:e1237–48.
[29] Lee WS, Lum SH, Lim CB, Chong SY, Khoh KM, Ng RT, et al. Characteristics and outcome of autoimmune liver disease in Asian children. Hepatol Int 2015;9:292–302.
[30] Zizzo AN, Valentino PL, Shah PS, Kamath BM. Second-line agents in pediatric patients with autoimmune hepatitis: a systematic review and meta-analysis. J Pediatr Gastroenterol Nutr 2017;65:6–15.
[31] Mieli-Vergani G, Vergani D, Baumann U, Czubkowski P, Debray D, Dezsofi A, et al. Diagnosis and management of pediatric autoimmune liver disease: ESPGHAN hepatology committee position statement. J Pediatr Gastroenterol Nutr 2018;66:345–60.