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A real-world study focused on the long-term efficacy of mycophenolate mofetil as first-line treatment of autoimmune hepatitis

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SUMMARY

Background
Front-line therapy with mycophenolate mofetil (MMF) in autoimmune hepatitis (AIH) has shown high on-treatment remission rates.

Aim
To study prospectively in a real-world fashion the long-term outcome of a large group of consecutive treatment-naïve AIH patients.

Methods
Between 2000 and 2014, 158 patients were recruited but only 131 were eligible for treatment (109 MMF/prednisolone; 22 prednisolone ± azathioprine). Long-term data on outcome after drug withdrawal were evaluated. Patients stopped treatment after having achieved complete response (normal transaminases and IgG) for at least the last 2 years.

Results
At diagnosis, 31.6% of patients had cirrhosis and 72.8% insidious presentation. A total of 102 of 109 (93.6%) responded initially to MMF within 2 (1–18) months. A total of 78 of 109 (71.6%) had complete response on treatment and 61 of 78 (78.2%) maintained remission off prednisolone. MMF-treated patients had increased probability of complete response compared to those receiving azathioprine ($P = 0.03$). Independent predictors of complete response were lower ALT at 6 months ($P = 0.001$) and acute presentation ($P = 0.03$). So far, treatment withdrawal was feasible in 40/109 patients and 30 (75%) are still in remission after 24 (2–129) months. Remission maintenance was associated with longer MMF treatment ($P = 0.005$), higher baseline ALT ($P < 0.02$), lower IgG on 6 months ($P = 0.004$) and histological improvement.

Conclusions
Mycophenolate mofetil proved to be an efficient first-line treatment for AIH, achieving so far the highest rates of remission maintenance off treatment (75%) ever published for at least a median of 2 years, although the remission criteria used were strict. However, the risk of potential bias and overestimation of intervention benefits from MMF cannot be completely excluded as this is a real world and not a randomised controlled trial.

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INTRODUCTION

Autoimmune hepatitis (AIH) is a progressive liver disease of unknown aetiology with female predominance occurring in all ages and races that is characterised by hypergammaglobulinemia, circulating autoantibodies and interface hepatitis.[1–3] The disease is divided into two major types AIH type 1 (AIH-1) and AIH type 2 (AIH-2) according to the autoantibody profile: antinuclear antibodies (ANA), smooth muscle antibodies (SMA) and/or soluble liver antigen/liver pancreas antibodies (anti-SLA/LP) in AIH-1, and liver/kidney microsomal antibody type 1 (anti-LKM1), liver/kidney microsomal antibody type 3 (anti-LKM3) and/or liver cytosol antibody type 1 (anti-LC1) in AIH-2.[1, 3–5]

The natural history of AIH depends vastly on treatment response, as the prognosis of untreated patients or those who do not respond is very poor leading to cirrhosis and need for orthotopic liver transplantation or death.[1–3] Indeed, 10-year survival of untreated patients has been reported as low as 10%.[1, 2, 4, 6] Treatment with corticosteroids with or without azathioprine (AZA) is the standard of care inducing clinical, laboratory and histological on treatment improvement in up to 65–80% of patients.[1, 2, 4, 6, 7]

However, approximately 15–20% of patients do not respond or are intolerant to conventional therapy with predniso(lo)ne alone or in combination with AZA.[2, 4] In addition, Lamers *et al.*[8] after reviewing all 11 published randomised controlled trials from 1950 to 2009 including 578 patients (363 treatment naïve) found a much lower proportion of responders (approximately 43%) compared with the current literature remission rates of 65–80% with conventional therapy.[9] Therefore, they concluded that AIH treatment with prednisolone in combination or not with AZA is far from ideal, and the search for drugs with a favourable risk–benefit ratio seems mandatory.[8] Furthermore, it has been shown recently that the application of the 2010 response criteria of the AASLD practice guidelines[10] (similar to the recent EASL clinical practice guidelines[4]) compared to the previous 2002 criteria[11] flips the previously codified remission rate with conventional therapy from 73% to 26%.[12]

In parallel with the abovementioned data, a very recent large multicentre study showed that relapse is almost universal when immunosuppression with AZA is discontinued in AIH patients in long-term remission further enhancing the concerns for a lack of long-term efficacy of conventional treatment.[13] On the other hand, although it is over 40 years that treatment with corticosteroids with or without AZA has been established, there are still open issues regarding the impact of gender and age at onset on disease expression and prognosis, the significance of disease presentation and serological markers like immunoglobulin G (IgG) titres on disease outcome and/or response as well as the ultimate goal of immunosuppression.[1, 4, 10, 14–18] Regarding the latter, the optimal treatment duration, the impact of treatment duration on outcome and the percentage of remission off treatment remain subjects of investigation.[1, 4, 19–22] In addition, it is well known that 80% of patients on corticosteroid therapy develop cosmetic changes and truncal obesity after 2 years.[1, 2, 4, 9, 10] Severe, debilitating complications, such as osteoporosis, vertebral compression, diabetes, hypertension and psychosis, usually develop after 18 months of continuous therapy and at doses of prednisolone that exceeds 10 mg daily.[1, 2, 4, 9, 10]

Therefore, alternative immunosuppressive agents to AZA, such as ciclosporin, tacrolimus and mycophenolate mofetil (MMF), have been used constituting a core repertoire of regimens with selective actions that target critical pathogenic pathways in AIH.[1, 2, 4, 9, 10, 22] Ciclosporin and tacrolimus have been used as salvage therapy in AIH, but they proved to have significant side effects hampering their use as maintenance therapy.[1, 2, 4, 10]Under this context, we have recently reported in the largest prospective series of treatment-naïve AIH patients (n = 59) ever published that MMF as an alternative first-line treatment resulted in high rates of remission (88% initial complete response) within only 3 months even though the definition of complete response used in that study was very strict, fewer side effects, quick corticosteroid withdrawal and a zero rate of primary no response.[23]

Accordingly, the aim of this study was to explore further the MMF role on the natural history of AIH, by studying prospectively in a noncontrolled setting but in a real-world observational setting the long-term outcome of a large group of consecutive treatment-naïve patients receiving MMF as front-line treatment. In addition, a particular emphasis was given on the outcome of those patients who stopped treatment as similar studies are missing.

MATERIALS AND METHODS

Patients

All eligible patients between 2000 and 2014, who fulfilled the diagnostic criteria for AIH established by the

International AIH Group (IAIHG),[24] were included in the study. Accordingly, in this specific period 158 consecutive patients were diagnosed in our Department with AIH (Table S1). Among them 55 patients have already been described in our previous preliminary report[23] where the efficacy and safety of treatment induction using prednisolone plus MMF were assessed. At the same period, additional 26 consecutive patients with AIH/primary biliary cholangitis (PBC) variant or AIH/primary sclerosing cholangitis (PSC) variant were diagnosed (Figure 1). Genetic, toxic, metabolic causes and significant alcohol intake were appropriately ruled out.

Clinical presentation was considered 'acute' when features of acute icteric hepatitis [transaminases above 10× upper limit of normal (ULN) plus clinically evident icterus] were present and 'insidious' when either symptoms were vague and nonspecific (e.g. fatigue, arthralgia, malaise, anorexia, etc.) or when altered liver function tests were occasionally found in the absence of symptoms. During follow-up (median: 67; range: 3–168 months), clinical and laboratory evaluation every 3–6 months was routinely performed in all patients irrespective of the kind of treatment by determining signs and symptoms as well as standard liver biochemistry tests and IgG to monitor treatment response and to guide the fine tuning of immunosuppression.

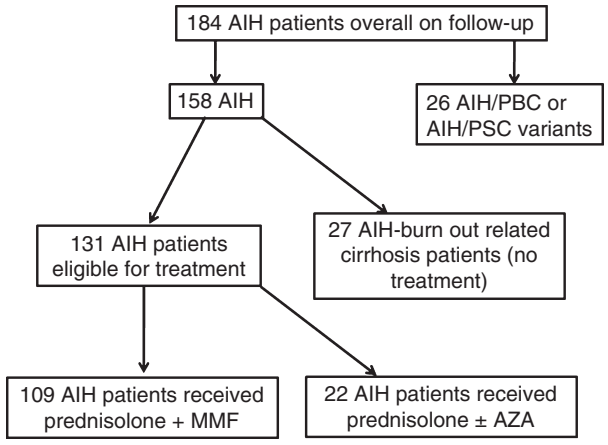

Figure 1 | Flow chart of the AIH patients being followed at the Department of Medicine and Research Laboratory of Internal Medicine, Medical School, University of Thessaly, Larissa, Greece. One hundred and thirty-one consecutive patients were eligible for the treatment protocol (after excluding 26 patients with AIH/variant syndromes and 27 patients with AIH-related burn-out cirrhosis); 109/131 patients consented to participate in the protocol using MMF as first-line treatment, whereas the remaining 22 who refused management with MMF received conventional immunosuppression.

Autoantibody testing

ANA, SMA, anti-LKM1, anti-LC1 antibodies were initially detected by indirect immunofluorescence on 5-μm fresh-frozen sections of in-house rodent multiorgan (kidney, liver and stomach) tissue substrates as we described previously.[2, 3, 23, 25, 26] Anti-LKM1, anti-LC1 and anti-SLA/LP reactivity were also evaluated by Western immunoblot using rat liver microsomal or cytosolic extracts.[2, 3, 25, 26] Commercially available ELISA (INOVA, Diagnostics Inc., San Diego, CA, USA) kits using recombinant SLA/LP/tRNP(Ser)Sec were also used for anti-SLA/LP determination according to the manufacturer's instructions.

Determination of human leucocyte antigens (HLA)

At the time of interview, 102 patients (64.5%; 95% CI: 57–72%) consented for determination of HLA pattern by polymerase chain-reaction sequence–specific oligonucleotides (Table S1).

Liver histology

Liver biopsy either typical or compatible with AIH[22] was performed at baseline in 131 patients (83%; 95% CI: 77–89%) (Table S1). In the remaining patients, liver biopsy could not be performed either because of the acute/severe mode of presentation with significant coagulation impairment or because patients refused the procedure, but all these patients fulfilled the remaining criteria for a definite diagnosis of AIH-like positive liver autoimmune serology, increased IgG, exclusion of viral and other liver disorders and a favourable response to immunosuppression. Histological evaluation was assessed by one experienced liver immunopathologist (G.K.) who was unaware of the clinical diagnosis of patients using the Knodell histologic/activity index score.[27]

According to previous publications of our group[23, 26, 28] and for statistical reasons, patients were divided into two groups according to inflammation: minimal–mild (score: 0–8) and moderate–severe (score: 9–18); and according to fibrosis: minimal–mild–moderate (score: 0–2) and severe fibrosis–cirrhosis (score: 3–4).

Treatment

In this open, real-world observational study, 131 consecutive AIH patients were eligible for immunosuppressive treatment (131/158; 83%; 95% CI: 77–89), as the remaining 27 patients had already established burn-out cirrhosis

while the 26 patients with AIH/PBC or AIH/PSC variants received combination treatment with conventional immunosuppression plus ursodeoxycholic acid and, therefore, they were not included in the analysis (Figure 1). According to the protocol published by our group recently,[23] 109 patients were treated with combination of prednisolone plus MMF (Myfetil 500 mg FC; Specifar SA, Athens, Greece), whereas 22 patients received conventional immunosuppression as these patients did not give written consent to participate to the MMF protocol. Prednisolone in the AZA group was given exactly at the same dose and tapering schedule as in the MMF group (see below) either alone or in combination with AZA at a dose of 1.5–2 mg/kg/day. In more detail, the final dose of MMF was 1.5–2 g daily. MMF was started with 1 g/day and after 3 weeks the dose was gradually increased to 1.5–2 g/day which was maintained for at least 2 years after CR. This fixed final dose was decided according to its proven efficacy in suppressing the immune response in our previous report[23] as well as in reports on other autoimmune diseases[29] and also in AIH patients intolerant and/or nonresponsive to standard therapy.[30, 31] In icteric patients, MMF was started if only bilirubin had decreased to less than 4 mg/dl. Prednisolone was instituted concurrently with MMF at one daily dose in the morning (0.5–1 mg/kg/day according to the recent EASL clinical practice guidelines[4]), followed by a gradual tapering (5 mg per week till the dose of 15 mg and then the tapering rate was 2.5 mg per week according to the biochemical and clinical response until complete withdrawal). After corticosteroid withdrawal and when normalisation of biochemical parameters had been achieved for at least 6 months, in addition to the absence of any sign of clinical exacerbation, the dose of MMF was gradually reduced to 1–1.5 g daily in an attempt to achieve maintenance of immunosuppression at a minimal effective dose while minimising the likelihood of its long-term side effects.

Treatment end points were defined according to the AASLD and EASL guidelines as well as our recent report.[4, 10, 23] In brief, a response was considered complete when serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and IgG had normalised, symptoms had improved or disappeared and liver histology, if performed, showed minimal or no inflammation. A partial response was defined as partial decrease in ALT or AST below 2× ULN without achieving complete normalisation and inability to withdraw or taper prednisolone. No response was defined as persistently elevated transaminases more than 3× ULN and/or increased IgG despite intensive immunosuppression and reassurance of compliance to therapy. Relapse was defined as a rise of AST and ALT above 3× ULN and/or increase in IgG above 2000 mg/dL accompanied or not by reappearance of symptoms at any time point during therapy following an initial complete response. Relapses were treated using the same treatment schedule as at baseline.

According to the EASL guidelines,[4] treatment could be withdrawn when immunosuppression had been administered for at least 3 years and if only patients had achieved continuous complete response at least for the last 2 years of treatment. However, as it has been suggested that the probability of sustained remission without therapy is at least 3-fold higher, when patients with AIH receive continuous immunosuppressive treatment for more than 4 years,[32] while repeated relapse and retreatment are associated with progression to cirrhosis,[33] our aim and intention was to maintain immunosuppression for at least 4 years (maximum 5 years) in every treated patient irrespective of the treatment schedule has received.

Liver biopsy was recommended to all patients fulfilling the above criteria before treatment withdrawal to assess the efficacy of treatment at the histological level. Relapse after treatment withdrawal was defined according to the above definition of relapse after an initial complete response while on treatment.[4, 10] In this case, treatment was reconstituted as it was given at baseline.

All patients gave written informed consent. Female patients at child-bearing age were informed about the possible teratogenicity effect in particular of MMF and to a lesser extent of AZA (both in D category by FDA) and were counselled for adequate contraceptive measures throughout the study. In brief, all women of child-bearing potential must have a negative serum pregnancy test at screening. They should be using or willing to use two highly effective method of birth control among the following: diaphragm, condom (by the partner), copper intrauterine device (or hormonal), sponge (or spermicide) and hormonal contraceptives. Reliable contraception should be maintained from the screening, throughout the whole period of MMF or AZA administration and at least 6 months apart the potential drug withdrawal. The study was approved by the Ethics Committee of Thessaly University, Medical School.

Assessment of safety

Safety was monitored by assessment of vital signs and physical examination in every visit on month 1, 3 and 6

and every 4 months thereafter depending on response, along with follow-up assessment of blood count and biochemical parameters. All adverse events were encountered and characterised as serious or not as well as drug related or not.

Statistical analysis

Results are expressed as median (range) and mean ± standard deviation (s.d.) where appropriate. Data were analysed by student's *t*-test, Mann–Whitney *U*-test, χ^2 with Yate's correction, Fisher's exact test, RR, likelihood ratio, χ^2 test, Kruskal–Wallis, ANOVA and binary and multinomial logistic regression analysis with Wald statistic, where applicable. Two-sided $P < 0.05$ was considered as statistically significant. 95% confidence intervals (CI) were calculated by Wilson procedure with a correction for continuity.

RESULTS

Baseline characteristics of AIH patients

The main clinical, biochemical, serological and histological characteristics of patients at presentation according to gender are shown in Table S1. Age distribution at disease onset was similar between females and males, with peaking incidence at the fifth and sixth decade, respectively (Figure 2). A total of 50 of 158 (31.6%; 95% CI: 24–39%) patients had already cirrhosis at diagnosis (clinically and/or by liver histology), which was strongly associated with the time to diagnosis defined as the time spent between the appearance of first symptoms or the presence for the first time of abnormal aminotransferases to the establishment of a firmly AIH diagnosis [time to diagnosis (months): noncirrhotics 6.5 (0–192) vs. cirrhotics 34.5 (0–240); $P < 0.001$]. Overall, complete corticosteroid withdrawal was feasible in 56/131 patients (47.2%; 95% CI: 34–51%), 26/158 (16.5%; 95% CI: 11–22%) had disease progression [(decompensation, development of hepatocellular carcinoma (HCC) and/or liver-related death or liver transplantation)], whereas 15/158 (9.5%; 95% CI: 5–14%) died due to liver-related causes.

One third of patients (31.6%; 95% CI: 24–39%) had concurrent extrahepatic autoimmune diseases including 14 with Hashimoto's thyroiditis, 10 multiple sclerosis, 8 Biermer's anaemia, 4 psoriasis, 3 rheumatoid arthritis, 1 Grave's disease, 1 coeliac disease, 3 Sjögren's syndrome, 3 inflammatory bowel disease, 2 scleroderma, 1 retroperitoneal fibrosis and 1 idiopathic thrombocytopenic purpura. One patient suffered from nephrotic syndrome along with Sjögren's syndrome, myositis and Hashimoto thyroiditis.[34] Patients with extrahepatic autoimmune diseases were more frequently ANA positive (OR 3.2; 95% CI: 1.3–7.8), had a delayed AIH diagnosis [time to diagnosis (months): patients with extrahepatic autoimmune diseases 44 ± 51 vs. those without extrahepatic autoimmune diseases 28 ± 46; $P < 0.05$], while they had longer disease duration (months) compared to patients without extrahepatic diseases (117 ± 68 vs. 94 ± 68; $P < 0.05$).

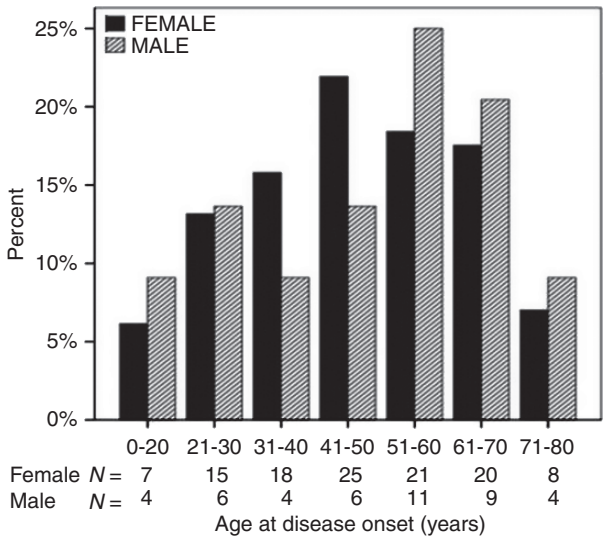

Figure 2 | Similar age distribution at disease onset between females and males in 158 AIH patients. The peaking incidence was at fifth and sixth decade, respectively.

AIH-2 patients did not differ from AH-1 in respect to clinical and laboratory parameters (data not shown), apart from less frequent HLA-DR3 detection [0/10 in AIH-2 vs. 36/92 (39%) in AIH-1; $P = 0.01$; RR 1.6; 95% CI: 1.4–1.9].

HLA-DR3 was the most prevalent HLA (36/102; 35.3%; 95% CI: 26–44%), whereas HLA-A1B8DR3 was present in 9.6% of patients (95% CI: 4–15%) and associated with necroinflammatory activity [9/58 (15.5%) in patients with severe inflammation vs. 0/33 in those with mild activity; $P = 0.02$; RR 1.6; 95% CI: 1.4–2%]. None of HLA haplotype was associated with gender, age at onset, disease severity or disease outcome (data not shown) apart from HLAB8 which was associated more frequently with acute presentation [9/25 (36%) in acute disease vs. 12/77 (15.6%) in insidious presentation, $P = 0.05$; RR 2.17; 95% CI: 1.12–4.2] and symptomatic disease [17/60 (28%) in symptomatic vs. 4/42 (9.5%) in

asymptomatic patients; $P < 0.05$; RR 1.5; 95% CI: 1.14–2%).

Moderate or severe inflammation had 86/131 of patients (65.6%; 95% CI: 57.5–74%), whereas 50/131 (38.2%; 95% CI: 30–46.5%) had severe fibrosis ($n = 14$) or cirrhosis ($n = 36$) (Tables S1 and S2).

Baseline characteristics according to age (Table S2)

No significant differences were found between patients >60 years (group A; $n = 45$) and those of <60 years (group B; $n = 113$) with respect to mode of presentation, extrahepatic autoimmune diseases, HLA, autoantibody profile, time to diagnosis, treatment response and rate of corticosteroid withdrawal. However, younger patients had longer disease duration and more frequently ALT levels above 2×ULN at presentation ($P < 0.05$; RR: 1.92; 95% CI: 1.1–3.3%) compared to the elderly. The presence of cirrhosis at diagnosis was more frequent in group A patients ($P = 0.006$; RR: 1.97; 95% CI: 1.3–3%) who received significantly less commonly immunosuppression, had more frequently progression of liver disease (RR 4.02; 95% CI: 1.97–8.2%) and higher prevalence of liver-related death (RR 9.2; 95% CI: 2.7–31.4%) compared to group B.

Characteristics of patients according to acute or insidious presentation

The insidious disease onset was the most prevalent (115/158; 72.8%; 95% CI: 66–80%). Disease presentation was not associated with gender, age, extrahepatic autoimmune diseases, cirrhosis at diagnosis, HLA and autoantibody profile (data not shown). None of the patients with acute presentation progressed to fulminant hepatitis. However, patients with acute AIH had more frequently moderate/severe inflammation in biopsy (34/38; 89.5% vs. 52/93; 55.9%; $P = 0.001$; RR: 1.6; 95% CI: 1.3–1.9%), higher baseline IgG [2013 (1020–6410) vs. 1905 (942–4520) mg/dL; $P = 0.02$] and γ-GT [130 (23–566) vs. 52 (10–618) IU/mL; $P < 0.001$], and lower ALT at month 6 [24 (11–74) vs. 31 (7–339) IU/ml; $P = 0.01$] compared to patients with insidious disease onset.

Patients with acute presentation carried higher probability of complete response (37/43 vs. 51/88; $P = 0.003$; RR 3; 95% CI: 1.4–6.6), and complete corticosteroid withdrawal [35/43 (81.4%) vs. 55/88 (62.5%); $P < 0.05$; RR: 1.3; 95% CI: 1.05–1.6%], lower disease progression rates [2/43 (4.7%) vs. 24/115 (20.9%); $P < 0.03$; RR: 1.2; 95% CI: 1.07–1.3%) and higher 10-year survival (100% vs. 87%; $P < 0.03$; Figure 3) compared with those presenting with an insidious onset.

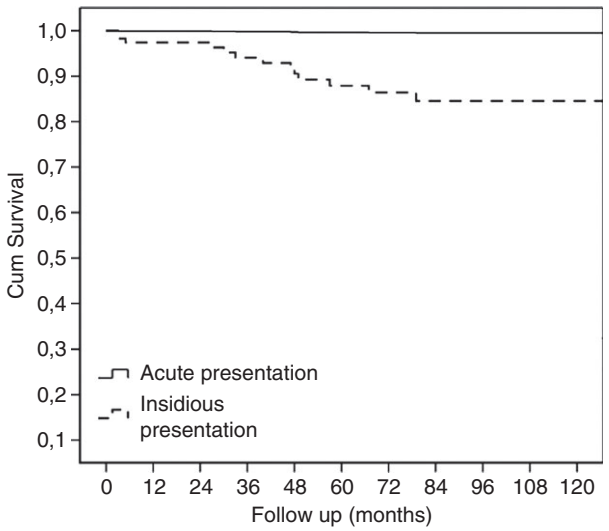

Figure 3 | Higher 10-year survival was found after Cox regression survival analysis in patients with acute presentation compared to those with insidious onset of AIH (100% vs. 87%; $P < 0.03$).

Follow-up data of 109 patients receiving MMF as first-line treatment

Patients were followed for 72 (3–168) months. Initial complete response was achieved in 102/109 (93.6%; 95% CI: 89–98%) of patients (16/22; 72.7%; 95% CI: 54–91.3% in those treated with conventional therapy; $P = 0.01$; RR: 1.28; 95% CI: 0.99–1.67%). This CR was achieved within 2 months (range: 1–18) with 83/102 patients (81.3%; 95% CI: 73.7–88.8%) accomplishing this end point in 3 months maximum (11/16; 68.7%; 95% CI: 46–91% in the conventional group; P = N.S.). Complete response can be documented by the statistically significant decrease in AST, ALT and IgG from the first month of MMF treatment ($P < 0.001$, for each; Figure 4). In more detail, 78/109 patients (71.6%; 95% CI: 63–80%) had complete response (10/22; 45.5%; 95% CI: 24.7–66.3% in the conventional group; $P = 0.03$; RR: 1.57; 95% CI: 0.98–2.5%) including 61 (61/78; 78.2%; 95% CI: 69–87%) who maintained complete response after prednisolone withdrawal (CR off prednisolone), while at the time of this writing, the remaining 17 patients were in the process of prednisolone tapering. The remaining 24/102 patients (23.5%; 95% CI: 15.3–32%) with initial complete response relapsed during prednisolone tapering or withdrawal and required either retreatment with prednisolone or increase in prednisolone dose (complete response but prednisolone dependent) with a subsequent new complete response (6/16 patients; 37.5% with initial complete response

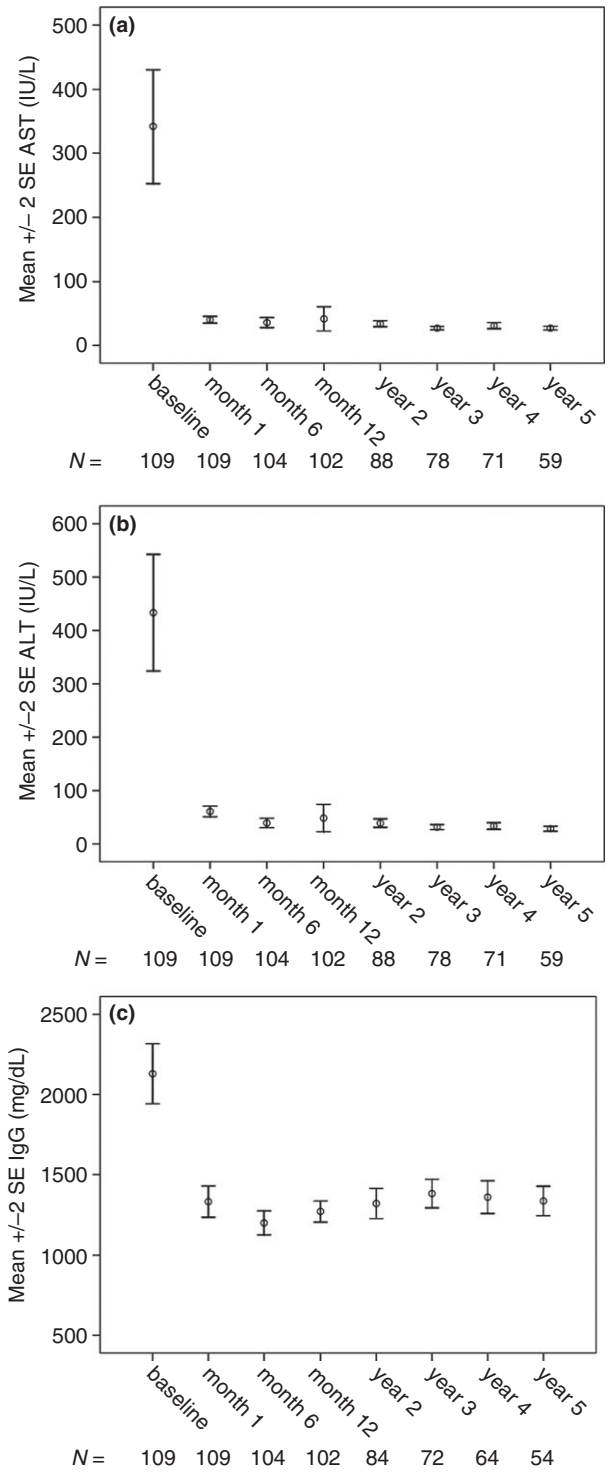

Figure 4 | Significant decrease (mean ± 2S.E.M.) in AST (a), ALT (b) and IgG (c) levels from the first month of treatment ($P < 0.001$, for each).

relapsed during prednisolone withdrawal in the conventional group; P = N.S.). The remaining 7/109 of patients (6.4%; 95% CI: 1.8–11%) had partial response (5/22; 22.7%; 95% CI: 5.2–40.2% in the conventional group; $P = 0.04$; RR: 1.2; 95% CI: 0.96–1.5). So far, none of patients is no responder (one in the conventional group).

The characteristics of patients who received MMF as front-line treatment according to their response are shown in Table 1. After multinomial logistic regression model (stepwise, forward entry), lower ALT at month 6 ($P = 0.001$) and acute presentation ($P = 0.03$) were identified as independent predictors of on-treatment complete response.

In respect to outcome, none of the 83 patients who did not have cirrhosis at the time of diagnosis [follow-up: 72 (3–168) months] developed cirrhosis. In contrast, 6/24 patients with cirrhosis at diagnosis decompensated (29%; 95% CI: 11–47%), 4 died due to liver-related causes, 1 was transplanted and 4 developed HCC. Overall, in 7/109 (6.4%) of patients the disease progressed. Baseline factors associated with disease progression were as follows: presence of symptoms ($P < 0.05$), cirrhosis ($P < 0.001$) and lower platelets ($P < 0.02$) (data not shown).

Characteristics of MMF-treated patients in whom treatment was withdrawn

At the time of this writing, MMF was withdrawn in 40/109 (36.7%) patients who had received therapy for 60 (24–132) months. In additional four patients, although the criteria for treatment withdrawal were fulfilled, treatment was not stopped because of concurrent extrahepatic autoimmune diseases (one with ulcerative colitis, one with nephrotic syndrome and two with multiple sclerosis). Discontinuation of treatment before completing 4 years was done in 12/40 (6 on month 40 and 6 when completing 2 years because of their personal reasons and decisions). According to the relapse criteria published by the AASLD[10] and EASL[4] clinical practice guidelines (relapse is defined as a rise of AST and ALT above 3×ULN and/or increase in IgG above 2000 mg/dL), at the end of follow-up 33 patients (33/40; 82.5%; 95% CI: 70.7–94.3%) did not relapse. However, to avoid overestimation of complete response rates after drug withdrawal, complete response at the end of follow-up was also calculated by taking into account the definition of complete response (normal serum AST, ALT and IgG levels, improvement or disappearance of symptoms and stable or improvement of liver histology). After taking into consideration these strict remission criteria, 30 patients (75%; 95% CI: 61.6–88.4%) still remained in complete response for 24 (2–129) months (remission group). The remaining 10 patients relapsed in 5 (2–24) months

Table 1 | Demographic, clinical, laboratory and histological characteristics of MMF-treated patients according to response to treatment

	AIH patients with complete response (n = 78)	AIH patients with initial complete response followed by relapses (RG) (n = 24)	AIH patients with partial response (n = 7)	P
Age at disease onset (years)	48 (16–75)*	44 (12–70)	24 (14–53)*	<0.05
Time to diagnosis (months)	24.5 ± 44.4	36.6 ± 50	24 ± 28.6	N.S.
Female	59 (75.6%)	17 (70.8%)	4 (57.1%)	N.S.
Presentation				
Acute	33 (42.3%)**	5 (20.8%)	0**	0.021
Insidious	45 (57.7%)	19 (79.2%)	7 (100%)	
Total follow-up (months)	70 ± 45.6***	91.5 ± 48***	101 ± 28.5	<0.05
Disease duration till last follow-up (months)	98.7 ± 67	116 ± 73.6	136 ± 53	N.S.
Treatment duration (months)	56 (3–156)†,‡	84 (64–171)‡	60 (15–156)†	0.003
Concurrent autoimmune diseases	22 (28.2%)	9 (37.5%)	3 (42.9%)	N.S.
AIH score				
Revised	14.6 ± 3.6	14 ± 3.4	13.5 ± 4	N.S.
Simplified	6.5 ± 1	6.2 ± 1.3	6.4 ± 1.3	N.S.
AST (IU/L, ULN: 40)	410 ± 548	292 ± 377	178 ± 127	N.S.
AST (IU/L) month 6 of treatment	27 ± 9.2§,¶	66 ± 100§	79 ± 102¶	0.006
ALT (IU/L, ULN: 40)	519 ± 667	354 ± 795	287 ± 199	N.S.
ALT (IU/L) month 6 of treatment	28.6 ± 11§,¶	75 ± 102§	87 ± 103¶	0.001
IgG (mg/dL, ULN: 1500)	2068 ± 912	2075 ± 819	2405 ± 538	N.S.
γ-GT (IU/L, ULN: 40)	118 ± 121	147 ± 182	197 ± 184	N.S.
Billirubin (mg/dL, ULN: 1.1)	2.8 ± 3.9	3.7 ± 6.3	1.1 ± 0.3	N.S.
Anti-SLA/LP	13 (16.7%)	4 (16.7%)	1 (14.3%)	N.S.
Anti-LKM	4 (5.1%)	4 (16.7%)	0	N.S.
HLA typing	N = 49	N = 16	N = 7	
HLA-DRB1*0301	16 (32.7%)	7 (43.8%)	5 (71.4%)	N.S.
HLA-DRB1*0401	6 (12.2%)	4 (25%)	1 (14.3%)	N.S.
HLA-DRB1*0701	4 (8.2%)	3 (18.8%)	0	N.S.
HLA-DRB1*13	13 (26.5%)	2 (12.5%)	2 (28.6%)	N.S.
HLA-B8	9 (18.4%)	6 (37.5%)	2 (28.6%)	N.S.
HLA-A1,B8,DRB1*0301	5 (10.2%)	3 (18.8%)	1 (14.3%)	N.S.
Cirrhosis at presentation	15 (19.2%)	9 (37.5%)	2 (28.6%)	N.S.
Liver histology at baseline	n = 68	n = 23	n = 7	
Moderate/severe inflammation	48 (70.6%)	20 (87%)	5 (71.4%)	N.S.
Severe fibrosis/cirrhosis	22 (32.4%)	10 (43.5%)	3 (42.9%)	N.S.

N.S., not statistically significant.

Data are expressed as mean ± s.d. or median (range) where appropriate. Abbreviations are same as in the text.

*, **, ***, †, ‡, §, ¶, indicates which group have been compared and p significance of these comparisons are provided on the last column.

(relapse group). Patients' characteristics according to maintenance or not of complete remission after stopping therapy are shown in Table 2. Liver biopsy at the end of treatment was performed in 35/40 (87.5%) patients as 5 denied the procedure. Factors associated with maintenance of remission were longer treatment duration with MMF (Table 2; Figure 5a), higher ALT levels at baseline (Table 2; Figure 5b) and lower IgG on month 6 of treatment (Table 2; Figure 5c). In addition, all candidates for drug withdrawal had significant improvement of necroinflammatory activity at second liver biopsy while fibrosis score was also stable and/or improved (Table 2). Interestingly, complete response vs. relapse during treatment did not play any role in remission maintenance after stopping treatment (Table 2). When the significant variables in the univariate analysis entered the binary logistic regression model, the only variable which independently predicts maintenance of remission after stopping treatment was the longer duration of treatment with MMF ($P < 0.05$).

Table 2 | Demographic, clinical, laboratory and histological characteristics of AIH patients according to maintenance of remission or not after complete treatment withdrawal

	Remission group (n = 30)	Relapse group (n = 10)	P
Age at disease onset (years)	47 ± 16	40 ± 14	N.S.
Time to diagnosis (months)	33 ± 49	45 ± 45	N.S.
Female/male	21/9	9/1	N.S.
Presentation			
Acute	11 (36.7%)	2 (20%)	N.S.
Insidious	19 (63.3%)	8 (80%)	
Disease duration till last follow-up (months)	126 ± 63	164 ± 63.5	N.S.
Treatment duration (months)	62 ± 24	36.6 ± 21	0.005
Concurrent autoimmune diseases (yes/no)	12/18	1/9	N.S.
AIH score			
Revised	14.5 ± 4	14 ± 4	N.S.
Simplified	6.4 ± 1.4	6.1 ± 1.4	N.S.
AST at baseline (IU/L, ULN: 40)	106 (21–3050)	66 (35–271)	N.S.
ALT at baseline (IU/L, ULN: 40)	176 (11–3320)	79 (40–264)	0.01
IgG at baseline (mg/dL, ULN: 1500)	1871 ± 582	2118 ± 738	N.S.
IgG month 6 (mg/dL)	1121.7 ± 245	1515 ± 382	0.004
γ-GT at baseline (IU/L, ULN: 55)	95.4 ± 97.6	71 ± 81	N.S.
Bilirubin at baseline (mg/dL, ULN: 1.1)	1.15 (0.26–21.6)	0.85 (0.5–2.5)	N.S.
Anti-SLA/LP	3 (10%)	2 (20%)	N.S.
Anti-LKM	5 (16.7%)	0	N.S.
HLA typing	N = 25	N = 8	
HLA-DRB1*0301	10 (40%)	2 (25%)	N.S.
HLA-DRB1*0401	3 (12%)	3 (37.5%)	N.S.
HLA-DRB1*0701	4 (16%)	1 (12.5%)	N.S.
HLA-DRB1*13	7 (28%)	2 (25%)	N.S.
HLA-B8	7 (28%)	1 (12.5%)	N.S.
HLA-A1B8DRB1*0301	5 (20%)	0	N.S.
Cirrhosis at presentation	4 (13.3%)	4 (40%)	N.S.
Necroinflammatory activity			
At baseline: minimal/mild–moderate–severe	9-10-10 (n = 29)*	2-5-2 (n = 9)	
At drug withdrawal: minimal/mild–moderate–severe	27-2-0 (n = 29)*	4-2-0 (n = 6)	
Fibrosis			
At baseline: minimal/mild–moderate–severe–cirrhosis	14-6-6-3 (n = 29)**	4-1-1-3 (n = 9)***	
At drug withdrawal: minimal/mild–moderate–severe–cirrhosis	16-7-4-2 (n = 29)**	3-0-1-2 (n = 6)***	
Complete response vs. relapse during treatment	23/7	5/5	N.S.

N.S., not statistically significant.

Data are expressed as mean ± s.d. or median and range where appropriate. Abbreviations are same as in the text.

*P < 0.0005; **P < 0.000005; ***P < 0.02.

Safety issues

In general, MMF was well tolerated (in only two cirrhotic patients, MMF discontinued because of septicaemia). Four patients reported mild gastrointestinal symptoms, which were temporary and did not need dose reduction. Five patients developed leukopenia during treatment, three of whom had also mild thrombocytopenia. Of note, three of these five patients were already cirrhotic at diagnosis, whereas the remaining had severe fibrosis. MMF was reduced in all of them (five patients) without on-going problems. Six patients developed respiratory tract infections and two herpes zoster. Although none of these eight patients needed hospitalisation, MMF was stopped temporary and started gradually to the standard dose, 15 days after recovery without new side effects. No pregnancy among females at child-bearing age was recorded during the whole study period. Overall, discontinuation or permanent reduction in MMF was observed in 2/109 (1.8%) and 5/109 (4.6%), respectively.

DISCUSSION

The current open real-world observational prospective study assessed the natural history of AIH in a large

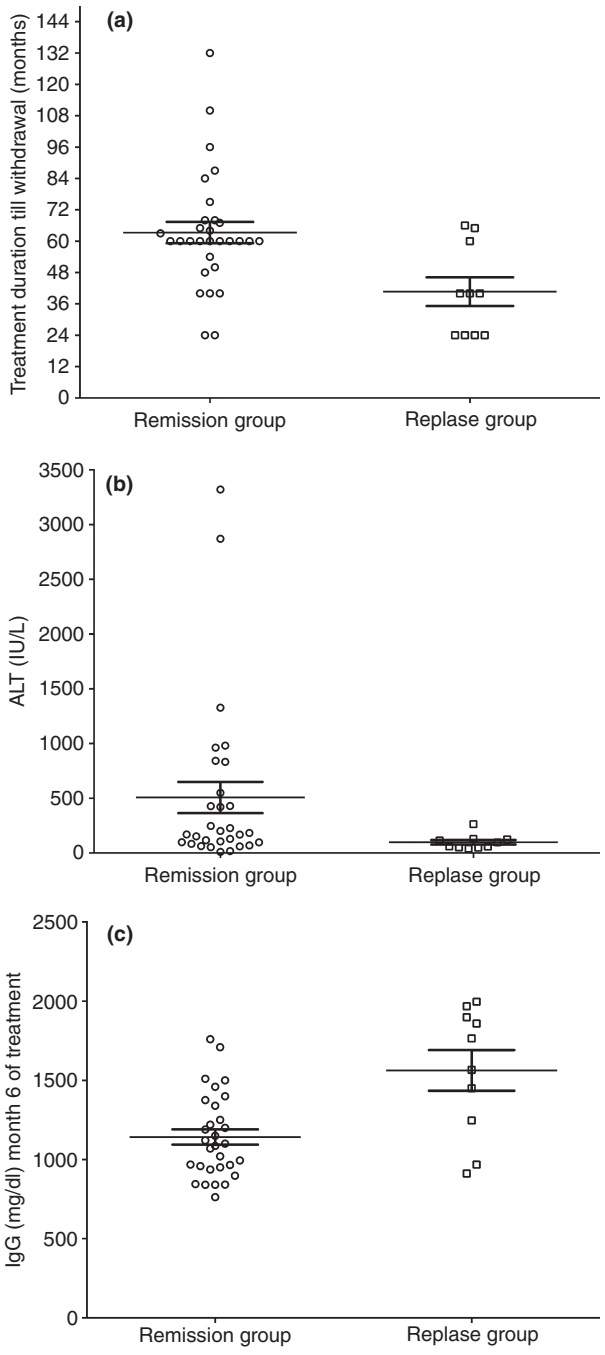

Figure 5 | Longer treatment duration with MMF (a; $P = 0.005$), higher ALT levels at baseline (b; $P = 0.01$) and lower IgG levels on month 6 of treatment (c; $P = 0.004$) were associated with maintenance of complete remission after MMF withdrawal.

cohort of consecutive treatment-naïve patients followed in Greece for a long period. Special emphasis was given in patients receiving MMF as first-line treatment and more specifically on the outcome of patients in whom treatment was withdrawn. The following major points have been raised: (i) disease expression and response to treatment was independent of the gender and age, apart from a more frequent presence of cirrhosis at diagnosis in the older patients; (ii) extrahepatic autoimmune diseases seems to affect negatively a timely and prompt diagnosis; (iii) disease presentation was associated with the outcome (acute onset was associated with better on-treatment response, whereas insidious with disease progression) and, most importantly, (iv) MMF as front-line treatment not only accomplishes high rates of on-treatment response, but so far results also in maintaining complete remission off treatment in 75% of patients for a median of 2 years.

This study confirms that AIH can affect patients at any age and also males[15, 16, 23, 35, 36] with a peak incidence in fifth and sixth decade in men and women, respectively. Interestingly, one third of patients were >60 years, indicating that considerably increasing number of AIH patients are older.[36, 37] Disease presentation did not differ between the elderly and younger patients, although older patients had more advanced disease at diagnosis. Furthermore, although the response rates to treatment were similar between younger and older patients, substantially lower number of older patients received treatment. The latter may explain why contrary to previous studies,[36, 37] we found a more frequent disease progression in the elderly group.

Extrahepatic autoimmune diseases were frequent (30%) in AIH patients.[34, 38, 39] Neither disease presentation nor response to treatment or outcome was affected by the presence of extrahepatic autoimmune diseases. However, a delay in diagnosis was apparent in patients with extrahepatic diseases which points out to the need of a more thorough evaluation for the probable concurrence of AIH in patients with diverse autoimmune diseases and abnormal liver biochemistry, as a hidden or even misdiagnosed AIH may lay behind.

Acute AIH was observed in 27% of patients.[17, 23, 35, 40] Apparently, the acute onset, although higher in children, is also observed in nearly one third of adults, without any significant difference in age at onset between acute and insidious disease. Of interest, one fifth of patients with acute AIH had already cirrhosis at diagnosis, suggesting that in this subgroup actually an exacerbation of well-established but previously underdiagnosed or misdiagnosed AIH was the case.[2, 40]

In addition, patients with acute onset had lower probability of disease progression and a better 10 years survival compared to those with an insidious onset as acute

disease proved to be an independent predictor of on-treatment complete response in MMF-treated patients carrying also higher probability of corticosteroid withdrawal. In a recent study, Ngu *et al.*[41] found as indicators of poorer outcome incomplete normalisation of ALT at 6 months, low serum albumin and age at presentation of <20 or >60 years. Lower ALT levels on month 6 were found the strongest predictor of complete response in our study, as well. However, it is not clear if the kind of disease presentation was taken into account in the analysis from New Zealand.[41] On the other hand, early studies[42] have linked acute onset AIH with worse prognosis, although the outcome thought to be related mainly to prompt diagnosis, severity of clinical findings and early response to treatment.[43] This discrepancy could be attributed to the fact that, in our study under the term 'acute', we grouped patients not only with acute/severe disease (>×10 ULN of aminotransferases, increased bilirubin and coagulopathy) but also with normal liver biosynthetic capacity. Further analysis, which was beyond the aims of this study, is needed to assess if there are subgroups of patients with acute onset of AIH and diverse prognosis or the determinant factors of these patients' outcome.

Recently, we have shown[23] that treatment with prednisolone and MMF not only is effective in inducing and maintaining on-treatment remission in AIH patients but also permits a rapid steroid withdrawal, which implies the avoidance of the multiple corticosteroid side effects. In parallel with our previous study, this long-term real-world observational study not only showed a high rate of initial response (93.6%) but also a high on-treatment complete response rate (71.6%) carrying a good safety profile and a rapid achievement of complete response in a median of only 2 months, which in turn may have implications on outcome as it has been linked with retardation of disease progression to cirrhosis.[44] In addition, although not included in the aims of this study, the treatment schedule used (MMF vs. AZA) was independently associated with higher initial complete response ($P = 0.01$) and increased probability of on-treatment complete response ($P = 0.03$) in MMF-treated patients (Table S3), although the two groups did not differ in respect to baseline demographic, clinical, genetic, laboratory and histological characteristics (data not shown). A potential bias cannot be completely excluded when a decision for MMF or AZA was made, but this decision was exclusively taken by the index patient after a detailed explanation of the treatment protocol along with the potential risks from MMF use and, therefore, we believe that as controlled trials in AIH are somehow difficult to perform due to the rarity of the disease, our case series from the every-day clinical practice seem important.

MMF is prodrug of mycophenolic acid, which blocks purine synthesis, inhibits DNA synthesis and exerts a selective anti-proliferative effect on B- and T-lymphocytes.[45] MMF has a 5-fold potent inhibitory effect on type-II isoform of inosine-5′-monophosphate dehydrogenase, an enzyme of the purine synthesis pathway, that depletes guanosine nucleotide specifically in activated B- and T-lymphocytes, without affecting type-I isoform expressed in other cell types. As a result, MMF tends to be more powerful and better tolerated agent, providing, additionally, selective immunosuppression with minimal side effects, which is the requested standard of therapy in transplantation and autoimmune diseases.

Concerning the risk of AIH relapse after withdrawal of conventional treatment, the recent EASL clinical practice guidelines for AIH have reported that unfortunately according to the present published data, only a small minority of patients stay in remission without AZA maintenance therapy.[4] Indeed, although recently a systematic review of studies published from 1972 to 2014 has shown that permanent drug withdrawal could be achievable in 19–40% of patients,[20] the experience from Bologna, Italy,[17] was different with 100% relapse rate after stopping conventional immunosuppression, whereas similar high relapse rates were recorded by a large recent multicentre study from the Netherlands.[13] Actually in that study, it was shown that 1 year after AZA withdrawal, 59% of patients required retreatment, after 2 years the percentage raised to 73% and after 3 years to 81%.[13] The authors therefore concluded that 'loss of remission or relapse occurs in virtually all AIH patients in long-term remission when immunosuppression is discontinued'.[13] Interestingly, this was not the case in our study as, so far, 75% of patients in whom MMF treatment was withdrawn remained in complete remission for a median of 2 years. Therefore, we believe that we give convincing evidence for the high efficacy of MMF in AIH treatment as, herein, we report the highest remission rates off treatment ever published under real life conditions. As a result, the clinicians seem to have the opportunity to consider discontinuation of MMF treatment in AIH patients without the fear of an almost universal relapse. In this context, longer duration of MMF treatment, higher ALT levels at baseline, normalisation of IgG on month 6 of treatment and significant improvement of inflammatory activity with at least stable fibrosis in liver biopsy before drug withdrawal could be helpful

for the clinicians to take difficult decisions like MMF withdrawal. Of note, the longer duration of MMF therapy proved to be an independent predictor of remission maintenance off treatment, whereas complete response vs. relapse with subsequent new complete response during treatment does not seem to play any role in remission maintenance after stopping treatment.

In conclusion, we showed that AIH can affect patients at any age and gender; concurrent extrahepatic autoimmune diseases are frequent and can result in significant delay of diagnosis. Acute presentation seems to associate with lower probability of disease progression and higher 10-year survival. Most importantly, the present large cohort study not only confirmed our previous findings concerning the high efficacy and safety of MMF use as first-line treatment for AIH[23] but also showed for the first time the highest rates of maintenance of complete remission off treatment (75%) ever published, although the remission criteria were strict. As relapse after drug withdrawal is almost universal with conventional therapy (73–100% in 2 years off treatment follow-up,[13, 17] with almost 50–90% of the relapses occurring typically in the first 12 months after stopping treatment[4]), MMF seems a reasonable, safe and important first-line treatment of AIH which should seriously and urgently be considered in the future, although the risk of potential bias and overestimation of intervention benefits due to the presently relative small time of follow-up off treatment (median of 24 months, so far) cannot be excluded in this real-world study. In every-day clinical practice, however, some easy markers as the high ALT levels at baseline, normal IgG on month 6 of treatment and improvement of liver histology could help physicians to take difficult decisions like the cessation of immunosuppression and minimise the possibility of relapse after drug withdrawal.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Baseline demographic, clinical, laboratory and histological characteristics of the 158 AIH patients according to gender.

Table S2. Baseline demographic, clinical, laboratory and histological characteristics of the 158 AIH patients according to the age.

Table S3. Binary logistic regression analysis of baseline factors associated with response to treatment in the 131 patients who received treatment.

AUTHORSHIP

Guarantor of the article: GND.

Author contributions: GND, KZ and NKG had the original idea, designed the study and wrote the first draft of the paper; KZ, NKG, PA and SG performed the laboratory analysis, collected the data and did the statistical analysis; GND, KZ and EIR treated and followed the patients; GKK did the interpretation of the histological data of the patients and along with GND and KZ made the final critical revision of the manuscript for important intellectual content; all authors have seen and approved the final draft of the paper.

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