Long-term results of mycophenolate mofetil vs. azathioprine use in individuals with autoimmune hepatitis

Authors
George N. Dalekos, Pinelopi Arvaniti, Nikolaos K. Gatselis, Stella Gabella, Anna Samakidou, George Giannoulis, Eirini Rigopoulou, George K. Koukoulis, Kalliopi Zachou

Correspondence
georgedalekos@gmail.com (G.N. Dalekos).

Graphical abstract

Long-term outcome and treatment response in AIH

AZA-based treatment (prednisolone + AZA)

- Lower primary non-response rates
- Higher rates of complete biochemical response at 12-months and at the end of follow-up

Alternative treatment (prednisolone + MMF)

- Lower rates of intolerance and serious adverse events
- More frequently eligible to stop immunosuppression according to the guidelines

MMF vs. standard

- MMF vs. standard

Highlights
- Corticosteroids ± AZA have been the standard treatment in AIH for decades.
- MMF seems to induce significantly lower non-response and higher CBR rates than AZA.
- Intolerance was significantly lower in MMF- than in AZA-treated individuals.
- Probability to stop therapy was significantly higher in MMF- than in AZA-treated individuals.
- MMF proved a safe and efficient alternative first-line treatment option in AIH.

Impact and implications
For more than 40 years, azathioprine (AZA) has been considered the standard treatment for induction and maintenance of response in autoimmune hepatitis (AIH). However, treatment usually needs to be maintained for life, as relapses are common after AZA cessation. Therefore, alternative treatment options are needed. Herein, we showed that the use of mycophenolate mofetil (MMF) as an alternative first-line immunosuppressant was much more efficient in the long-term than AZA as attested by the lower non-response rates at 4 weeks and higher response rates at 12 months and the end of follow-up. Moreover, AZA-treated patients were more prone to change treatment because of intolerance, whereas MMF-treated patients were more often eligible to achieve treatment withdrawal.

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Long-term results of mycophenolate mofetil vs. azathioprine use in individuals with autoimmune hepatitis

George N. Dalekos,1,2,* Pinelopi Arvaniti,1,2 Nikolaos K. Gatselis,1,2 Stella Gabeta,1,2 Anna Samakidou,1 George Giannoulis,1 Eirini Rigopoulou,1,2 George K. Koukoulis,3 Kalliopi Zachou1,2

1Department of Medicine and Research Laboratory of Internal Medicine, National Expertise Center of Greece in Autoimmune Liver Diseases, General University Hospital of Larissa, Larissa, Greece; 2 European Reference Network on Hepatological Diseases (ERN RARE-LIVER), General University Hospital of Larissa, Larissa, Greece; 3 Department of Pathology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

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Background & Aims: We have shown previously that mycophenolate mofetil (MMF) might be used as first-line treatment instead of azathioprine (AZA) in individuals with autoimmune hepatitis (AIH). Herein, we present our long-term prospective data on response and outcome after first-line therapy with MMF in treatment-naïve individuals with AIH, as similar data are missing.

Methods: During the 21 years of the study, 292 individuals with AIH were included (females: 213; median age: 59 [17–85] years). Patients received either prednisolone 0.5–1 mg/kg/day alone (n = 19) or in combination with AZA 1–2 mg/kg/day (n = 64) or MMF (n = 183). The tapering schedule of prednisolone was identical between groups. We assessed the rates of complete biochemical response (CBR) at 6 months, 12 months, and the end of follow-up; non-response (4 weeks of treatment); CBR off prednisolone; adverse effects; CBR off treatment; histological remission; and overall and liver-related mortality between the AZA and MMF groups.

Results: The MMF group had lower non-response (p = 0.02) and higher CBR rates at 12 months (86 vs. 71.8%; p <0.05) and the end of follow-up (96 vs. 87.2%; p = 0.03) than the AZA group. Treatment change was more frequent in the AZA group (43.7 vs. 11%; p <0.001), mostly because of intolerance, whereas MMF was proven safe (serious complications 3.8 vs. 18.8%; p = 0.0003). MMF-treated patients were more frequently eligible to stop immunosuppression according to the guidelines (p <0.05). Cirrhosis at diagnosis, age at diagnosis >60 years, and longer disease duration were independent predictors of liver-related mortality.

Conclusions: MMF seems an efficient alternative first-line treatment option for AIH, bearing lower non-response at 4 weeks and higher CBR rates at 12 months and the end of follow-up than AZA. In addition, MMF was proven to be safe, leading more frequently to the eligibility for stopping immunosuppression according to the guidelines.

Impact and implications: For more than 40 years, azathioprine (AZA) has been considered the standard treatment for induction and maintenance of response in autoimmune hepatitis (AIH). However, treatment usually needs to be maintained for life, as relapses are common after AZA cessation. Therefore, alternative treatment options are needed. Herein, we showed that the use of mycophenolate mofetil (MMF) as an alternative first-line immunosuppressant was much more efficient in the long-term than AZA as attested by the lower non-response rates at 4 weeks and higher response rates at 12 months and the end of follow-up. Moreover, AZA-treated patients were more prone to change treatment because of intolerance, whereas MMF-treated patients were more often eligible to achieve treatment withdrawal.

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that standard treatment failed to result in histological resolution of the disease or prevention of fibrosis progression in many patients. Concerns about the long-term efficacy of the conventional treatment with AZA were also shown by a recent multicentre study from the Netherlands. In that study, almost all patients who were in CBR for more than 2 years relapsed after treatment withdrawal. By contrast, it is generally accepted that approximately 15–25% of cases develop intolerance or non-response to AZA, whereas a remarkable proportion of patients suffer also from corticosteroids-related adverse effects when prednisolone is given for more than 18 months at doses higher than 7.5–10 mg/day.

In this context, we and others have shown in real-world studies, propensity matching trials and/or meta-analysis, that mycophenolate mofetil (MMF) could be a reliable and effective first-line treatment option apart from AZA, for induction and maintenance of response. As a result, since early 2015, the Hellenic Association for the Study of the Liver (HASL) has included, apart from AZA, MMF as a potential first-line treatment for induction and maintenance of response in individuals with AIH. Accordingly, we present herein our experience from our large cohort of individuals with AIH who were followed up for 10 years.

Patients and methods

Patients
From 1 January 2000 to 31 December 2020, all consecutive treatment-naive adult individuals with AIH (≥16 years; n = 292), who were diagnosed and followed up prospectively according to the criteria of the International AIH Group (IAIHG) were included (Table S1). Other causes of liver diseases such as viral, metabolic, genetic, and toxic (including alcohol misuse) were excluded in all patients by appropriate investigation. During the same period, 25 consecutive individuals with primary biliary cholangitis/AIH or primary sclerosing cholangitis/AIH variants were excluded from the analysis (Fig. S1).

The presentation of AIH was considered ‘acute’ when patients had, at onset, aminotransferases >10 × the upper limit of normal (ULN) with or without clinically evident jaundice and ‘insidious’ when deranged liver biochemistry was found in conjunction with or without non-specific general symptoms such as fatigue, arthralgias, malaise, and anorexia. The ‘acute-severe’ variant was defined as an acute presentation of newly (<24 weeks) diagnosed acute episode of hepatitis with or without hepatic encephalopathy characterised by elevated international normalised ratio (INR) ≥1.5 at any time during the acute course and without lesions of chronic disease on histology. All patients were systematically evaluated during follow-up (75 ± 62 months) every 3–6 months by physical and laboratory examination. All signs and symptoms, routine liver function tests, and IgG were recorded to monitor response and guide the fine-tuning of immunosuppression.

All patients agreed to the use of their data after anonymous analysis by written consent at the time of initial evaluation. The ethical committee of the General University Hospital of Larissa approved the study protocol, which conforms to the ethical guidelines of the 1975 Declaration of Helsinki as revised in Brazil in 2013, as reflected in a priori approval by the institution’s human research committee (2258/21-3-2016).

Autoantibodies testing
According to the guidelines and our previous works, antinuclear antibodies (ANA), smooth muscle antibodies (SMA), anti-liver cytosol type-1 (anti-LC1), and anti-liver/kidney microsome type-1 antibodies (anti-LKM1) were initially investigated by indirect immunofluorescence on 5-μm fresh frozen sections of in-house rodent multi-organ (kitchen, liver, and stomach) substrates. Anti-LC1, anti-LKM1, and antibodies against soluble liver antigen/liver pancreas (anti-SLA/LP) were also assessed by Western blotting using rat liver microsomal or cytosolic extracts. Commercially available ELISA kits using recombinant forminotransferase cyclodeaminase (Euromimmune, Medizinische Labor Diagnostika AG. D23560 Lubeck, Deutschland), cytochrome P450 2D6 (INOVA, Diagnostics Inc., San Diego, CA, USA), and SLA/LP/trRNPSerSec (INOVA) antigens were also used for the detection of anti-LC1, anti-LKM1, and anti-SLA/LP, respectively, according to the manufacturer’s instructions.

Liver histology
Liver biopsy was performed in 251 patients. In the rest of the patients, biopsy was not done either because they refused the procedure or because they suffered from an acute-severe onset of the disease with significant impairment of coagulation markers. However, all these 41 patients fulfilled the other criteria of AIH diagnosis such as seropositivity for autoantibodies, high IgG levels, absence of other liver diseases, and favourable response to immunosuppression. All biopsies were evaluated blindly by 1 experienced liver immunopathologist (GK) using the mHAI. In individuals with cirrhosis who had not undergone liver biopsy, the diagnosis was based on clinical findings of decompensated disease, and/or supportive ultrasonography (coarse echo pattern of the parenchyma in association with irregular hepatic margins, spleen >12 cm, portal vein >16 mm), and/or liver stiffness measurements (LSM) using FibroScan® 502 (Echosens, Paris, France) >9 kPa, and/or endoscopic findings of portal hypertension, as we have reported recently.

Treatment
From the 292 patients, 266 (females: 195; 57 ± 16 years) were eligible for treatment according to the EASL and HASL guidelines. The remaining 26 patients did not receive any treatment because they had already well-established burn-out compensated or compensated cirrhosis with minimal or no necroinflammatory activity on liver histology. Patients eligible for therapy received for at least 3 years (not more than 5–6 years) either prednisolone 0.5–1 mg/kg/day alone (n = 19) or prednisolone at the same dose in combination with AZA 1–2 mg/kg/day (n = 64) or MMF 1.5–2 g/day (n = 183; Table 1). The tapering schedule of prednisolone was identical between the MMF and AZA groups (Table 1). The reasons for prednisolone monotherapy in the small subgroup of individuals with AIH were as follows: recent (<5 years) or current history of malignancy (n = 3), mild disease (n = 9), or denial of patients to receive prednisolone in combination with AZA or MMF (n = 7). Because of the low number, patients who were treated with prednisolone monotherapy were excluded from further analysis. These 19 patients had never received combination treatment with AZA or MMF.

The decision for MMF or AZA administration was made exclusively by the index patient after a detailed explanation of the treatment protocol and our published experience along with the provided information regarding the potential risks
If bilirubin was <6 mg/dl.

12 months, and the end of follow-up and was discontinued in every index patient who had received at least 3 consecutive years of immunosuppression.

Treatment of CBR besides compliance to treatment and intensive tapering of MMF (500 mg/year) up to 1 g/day.

Tapering AZA (25 mg/6 months) up to 1 mg/kg/day.

The primary endpoints were as follows:

- Rapidity of achieving CBR at 6 months, 12 months, and the end of follow-up
- Duration of CBR off prednisolone
- Relapse rates during tapering or withdrawal of corticosteroids
- Stable or improved liver disease at the histological level in the second liver biopsy
- Changes after serial LSM determinations by transient elastography

Safety assessment

Safety was assessed by vital signs and physical examination in every visit on the first, third, and sixth months and every 3–6 months thereafter depending on the response, along with follow-up investigations of the whole blood count and biochemical markers. All adverse events were encountered and characterised as serious or not and regimen-related or not.

Statistical analysis

Analysis was made using the SPSS 20 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7.0 software (GraphPad Software, San Diego, CA, USA). Results were expressed as median (range) and mean ± SD. Data were compared using Student’s t test or the Mann–Whitney U test. The Chi-square test and Fisher’s exact test were used for comparison between categorical variables. Overall survival and liver-related deaths were presented by Kaplan–Meier analysis. In addition, univariate and backward stepwise multivariate Cox regression analyses were used for estimating survival differences according to diverse factors included in the dataset. Two-sided p values <0.05 were considered as statistically significant in 95% CI.

Results

Baseline characteristics

The baseline demographics, clinical, laboratory, serological, and histological characteristics of patients did not significantly differ between the groups apart from the significantly higher presence of anti-SLA/LP antibodies in the MMF group than in the AZA group (Table 2). At the time of this writing, the total follow-up of patients was 57 (2–250) months and the duration of immunosuppression 52 (2–328) months. The median initial prednisolone dose was 40 (5–125) mg/day in the MMF group and 40 (10–100) mg/day in the AZA group (p >0.05). In addition, the median prednisolone dose at the end of follow-up was 0 (0–35) mg/day and 0 (0–20) mg/day in the MMF and AZA groups, respectively (p >0.05).

The treatment protocol in the two groups of the study.

| Week | Prednisolone (0.5–1 mg/kg/day) | AZA (mg/day) | MMF (g/day) |
|------|-------------------------------|--------------|-------------|
| 1    | Initial dose                  | 1 g          |             |
| 2    | Initial dose                  | 1 g          |             |
| 3    | Tapering 5 mg                 | 50 mg        | 1.5 g       |
| 4    | Same dose as in Week 3        | 50 mg        | 2 g         |
| 5    | Tapering 5 mg                 | 75 mg        | 2 g         |
| 6    | Same dose as in Week 5        | 75 mg        | 2 g         |
| 7    | Tapering 5 mg                 | 100 mg       | 2 g         |
| 8    | Same dose as in Week 7        | 100 mg       | 2 g         |
| 9    | Tapering 5 mg                 | 150 mg       | 2 g         |
| 10   | Same dose as in Week 9        | 150 mg       | 2 g         |
| 11   | Tapering 5 mg                 | 150 mg       | 2 g         |
| 12   | Tapering 5 mg                 | 150 mg       | 2 g         |
| 13   | Tapering 5 mg                 | 150 mg       | 2 g         |
| 14   | Tapering 5 mg                 | 150 mg       | 2 g         |
| 15 and thereafter | Tapering 2.5 mg/week up to complete withdrawal | 150 mg<sup>2</sup> | 2 g<sup>1</sup> |

AZA, azathioprine; MMF, mycophenolate mofetil.<sup>1</sup>

† If bilirubin was <6 mg/dl.

‡ In sustained (>6 months) complete biochemical response off prednisolone, tapering of AZA (25 mg/6 months) up to 1 mg/kg/day.

A second liver biopsy before treatment cessation was desirable and was not accepted by all patients. However, in these cases, serial LSM determinations were done to follow-up changes in fibrosis.

Primary and secondary treatment endpoints

Primary endpoints

The primary endpoints were as follows:

- CBR rates at 6 months, 12 months, and the end of follow-up
- Rates of non-response (defined as <50% decrease of aminotransferases within 4 weeks after treatment initiation)
**Table 2. Baseline demographics, clinical, laboratory, serological and histological characteristics in the two groups of the study.**

| Characteristics                              | MMF (n = 183) | AZA (n = 64) | p value |
|----------------------------------------------|---------------|--------------|---------|
| Sex (female/male)                            | 134/49        | 47/17        | 1       |
| Age at diagnosis (years)                     | 49 (10–81)    | 48 (8–77)    | 0.792   |
| Time to diagnosis (months)                   | 34 (1–402)    | 29 (1–194)   | 0.211   |
| Acute presentation (%)                       | 87 (47.5)     | 32 (50.0)    | 0.847   |
| Insidious/asymptomatic presentation          | 43/53         | 18/14        | 0.358   |
| Follow-up (months)                           | 66 (2–250)    | 63 (1–238)   | 0.319   |
| Treatment duration (months)                  | 51 (1–220)    | 47 (3–241)   | 0.565   |
| Concurrent autoimmune diseases (%)           | 81 (44.3)     | 20 (31.3)    | 0.06    |
| AIH revised diagnostic score                 | 18 (8–24)     | 17 (9–22)    | 0.305   |
| AIH simplified diagnostic score              | 6 (5–8)       | 6 (6–8)      | 0.99    |
| ALT (IU/ml, ULN: 40)                        | 314 (27–4,925) | 340 (17–2,056) | 0.769   |
| Bilirubin (mg/dl, ULN: 1.1)                  | 1.07 (0.18–279) | 1.08 (0.25–17) | 0.516   |
| γ-GT (IU/L, ULN: 40)                        | 76 (8–747)    | 84 (7–1,360) | 0.6     |
| IgG (mg/dl, ULN: 1,500)                     | 1,810 (701–6,410) | 1,750 (782–4,594) | 0.314   |
| ANA positive (%)                             | 82 (44.8)     | 35 (54.7)    | 0.224   |
| SMA positive (%)                             | 172 (94.0)    | 57 (91.9)    | 0.305   |
| Anti-SLA/LP positive (%)                     | 27 (14.8)     | 4 (6.0)      | 0.02    |
| Anti-LKM positive (%)                        | 12 (6.6)      | 1 (1.6)      | 0.224   |
| Anti-Ro52 positive (%)                       | 46 (25.1)     | 17 (26.6)    | 0.953   |
| Cirrhosis at diagnosis (%)                   | 38 (20.7)     | 12 (18.8)    | 0.869   |
| mHAI activity score                         | 6 (2–17)      | 7 (3–14)     | 0.137   |
| mHAI fibrosis score                         | 1 (1–6)       | 1 (1–6)      | 0.622   |

Data are expressed as median (range). The Chi-square test with Yate’s correction was used for comparison between categorical variables and the Mann–Whitney U test for numerical variables.

γ-GT, gamma-glutamyl transpeptidase; AIH, autoimmune hepatitis; ALT, alanine aminotransferase; ANA, antinuclear antibodies; anti-LKM, anti-liver/kidney microsomal type 1 antibodies; anti-SLA/LP, antibodies against soluble liver antigen/liver pancreas; AZA, azathioprine; mHAI, modified hepatitis activity index; MMF, mycophenolate mofetil; SMA, smooth muscle antibodies; ULN, upper limit of normal.

**CBR, non-response and, prednisolone withdrawal**

CBR at 6 months was not significantly different between the groups (Table 3). However, CBR at 12 months and the end of follow-up was significantly higher in the MMF group ($p <0.05$ and $p = 0.03$, respectively; Table 3) than in the AZA group. The non-response rate was significantly lower in the MMF-treated patients than in the AZA-treated patients ($p = 0.02$; Table 3). As AZA-treated patients initiated the drug after being 2 weeks on corticosteroids monotherapy (Table 1), we also compared the non-response rate on Week 6 of treatment. Again, MMF-treated patients showed significantly lower non-response rates than the AZA-treated patients ($p <0.05$, data not shown). The rate of corticosteroids withdrawal at 6 months, 12 months, and the end of follow-up did not differ between the groups ($p >0.05$; Table 3).

The rapidity of CBR was not associated with the treatment schedule (Table 3). Similarly, the rapidity of prednisolone withdrawal was not associated with the treatment schedule, neither was the duration of CBR off prednisolone (Table 3). Relapses during tapering or withdrawal of corticosteroids were observed in 73/193 (37.8%) of the MMF-treated patients and 17/47 (36%) of the AZA-treated patients, respectively.

**Table 3. Treatment response and endpoints in the two groups of the study.**

| Characteristics                              | MMF* | AZA* | p value |
|----------------------------------------------|------|------|---------|
| CBR at 6 months (%)                          | 158/188 (84.0) | 39/47 (83.0) | 1 |
| CBR at 12 months (%)                         | 157/182 (86.3) | 28/39 (71.8) | 0.04 |
| CBR at the end of follow-up (%)             | 186/193 (96.4) | 41/47 (87.2) | 0.03 |
| Non-response (%)                             | 14/182 (7.7) | 11/57 (19.3) | 0.02 |
| Corti stop at the end of follow-up (%)      | 144/193 (74.6) | 36/47 (76.6) | 1 |
| Time to CBR at the 6-month period (months)  | n = 158 | n = 39 | 0.591 |
| –                                             | 3.4 ± 3 | 2.69 ± 1.9 | |
| Time to CBR at the 12-month period (months) | n = 157 | n = 28 | 0.102 |
| –                                             | 3.5 ± 3.2 | 2.7 ± 1.9 | |
| Time to CBR at the end of follow-up (months)| n = 186 | n = 41 | 0.208 |
| –                                             | 3.1 ± 2.7 | 2.6 ± 1.7 | |
| Time to stop corti at the end of follow-up (months) | 13 (1–249) | 12 (3–244) | 0.312 |
| CBR duration off prednisolone                | 27 (1–124) | 23 (2–204) | 0.09 |
| Treatment change (%)                         | 20/183 (10.9) | 28/64 (43.7) | <0.001 |
| CBR duration off treatment (months)          | 50 (1–192) | 19 (1–60) | 0.255 |
| Overall survival (%)                         | 142/156 (91.0) | 39/41 (95.1) | 0.594 |
| Liver-related mortality (%)                  | 7/156 (4.5) | 1/41 (2.4) | 0.883 |
| mHAI fibrosis score                          | n = 42 | n = 6 | 0.962 |
| –                                             | 2.27 ± 1.7 | 2.6 ± 20.1 | |
| Progression of the disease (%)               | 15/193 (7.8) | 6/47 (12.7) | 0.277 |

The Chi-square test with Yate’s correction was used for comparison between categorical variables and Student’s t test, expressed as mean ± SD, or the Mann–Whitney U test, expressed as median (range), for numerical variables.

AZA, azathioprine; CBR, complete biochemical response; corti, corticosteroids; mHAI, modified hepatitis activity index; MMF, mycophenolate mofetil.

* For the number of patients included in each subgroup, refer to Fig. 52.
the AZA group (p > 0.05). Overall, at the end of follow-up, 144/193 MMF-treated patients and 36/47 from the AZA group stopped corticosteroids (Table 3). The need for reintroduction of prednisolone was identical between the groups (60/144 [42%] in the MMF group vs. 14/36 [39%] in the AZA group; p > 0.05).

Treatment changes because of insufficient response and/or intolerance to first treatment

Individuals with AIH who were receiving AZA-based therapy were more prone to switching treatment than the MMF-treated patients (Table 3; p < 0.001). The reasons of the 28/64 (43.7%) patients in the AZA group who switched treatment to MMF were as follows: insufficient response (n = 16), intolerance (n = 9), and serious infections (n = 3). In addition, 20/183 (11%) patients in the MMF-group changed treatment to AZA (n = 11; insufficient response = 3, unavailability of the drug = 4, willingness of pregnancy = 3, and intolerance = 1) or low-dose prednisolone (2.5–5 mg/day; n = 9; serious infection = 4, malignancy = 2, and maintenance of CBR under low-dose corticosteroids = 3). At the time of this writing, the overall CBR rate after treatment changes was significantly higher in the MMF-group (186/193, 96%) than the overall response of the AZA group (41/47; 87%; p = 0.03). The duration of CBR was 51 (2–222) in the MMF group and 42 (3–328) months in the AZA group (p > 0.05).

Histological remission at second liver biopsy and CBR off treatment

In 44 patients who had either positive anti-SLA/LP, anti-LKM1, and anti-LC1 or established cirrhosis, treatment was not withdrawn, as it is well known from published data and our experience that these patients may never be able to stop treatment even if they had long-term CBR (>2 years). In 6 additional patients, although they fulfilled the criteria for treatment cessation, immunosuppression was not withdrawn owing to concurrent extrahepatic autoimmune diseases (2 rheumatoid arthritis, 2 ulcerative colitis, 1 nephrotic syndrome, and 1 multiple sclerosis). Consequently, at the time of this writing, 72 patients, who were eligible for stopping treatment according to the EASL and HASL guidelines, withdrew therapy. Of note, the MMF-treated patients were significantly more frequently eligible for complete treatment cessation according to the guidelines than were the AZA group (64/193 [33%] in the MMF group and 8/47 [17%] in the AZA group; p < 0.05).

Among them, second liver biopsy was performed at the end of treatment in 48/72 (66.6%; 6/8 under AZA and 42/64 under MMF) as 24 patients denied the procedure. Inflammation improved in 44/48 patients (91.6%; 38/42 MMF vs. 6/6 AZA; p > 0.05). Fibrosis also improved or remained stable in 40/48 patients (83%; 35/42 MMF vs. 5/6 AZA; p > 0.05).

Regarding CBR off treatment, 38/64 (59%) patients from the MMF group remained in CBR off treatment for 19 (1–80) months (Table 3). Maintenance of CBR off treatment in the MMF group was associated with absence of relapses (7/38 vs. 18/26; p < 0.001) and shorter treatment duration (67 ± 36 vs. 89 ± 43 months; p = 0.03).

Outcome

Of 292 patients, 56 were lost to follow-up. From the remaining 236 patients, 40 (17%) died during follow-up (30 of them because of liver-related death; 30/236; 12.7%). No patient underwent liver transplantation. However, 8/236 (3.4%) patients were eligible for liver transplantation (4 individuals with AIH with acute-on-chronic liver failure died waiting on the transplant list, whereas 4 were excluded from the list according to our legislation because of age [all above 70-years]). Disease progression according to histology and/or liver elastography was observed in 22/292 (7.5%) patients, 15/193 (8%) from the MMF group, 6/47 (12.7%) from the AZA group, and 1 patient under corticosteroid monotherapy. Among them, 3 patients developed cirrhosis (1 from the MMF group, 1 from the AZA group, and 1 under corticosteroids monotherapy), whereas 7 individuals with cirrhosis developed hepatocellular carcinoma (5 from the MMF group, 1 from the AZA group, and 1 under corticosteroids monotherapy).

Overall survival at the end of follow-up (n = 236; 75 ± 62 months) was 83%, whereas liver-related survival was 87.3% (Fig. 1). When treatment schedule (AZA vs. MMF) was considered, neither overall nor liver-related survival was different between the groups.

In the univariate Cox regression survival analysis, overall survival and liver-related death did not differ between the groups. Factors associated with liver-related death were as follows: increased time to diagnosis (hazard ratio [HR] 1.004; 95% CI 1.001–1.008; p = 0.041), age >60 years at diagnosis (HR 0.125; 95% CI 0.055–0.285; p < 0.001), increased disease duration (HR 0.991; 95% CI 0.985–0.998; p = 0.007), insidious presentation (HR 0.130; 95% CI 0.039–0.429; p = 0.001), higher histological staging at diagnosis (HR 3.147; 95% CI 1.89–5.241; p < 0.001), cirrhosis at diagnosis (HR 75.175; 95% CI 10.23–552.4; p < 0.001), increased duration of corticosteroids administration (HR 0.995; 95% CI 0.977–1.006; p < 0.001), higher total duration of treatment (HR 0.962; 95% CI 0.939–0.985; p = 0.002), no treatment (HR 0.057; 95% CI 0.027–0.119; p < 0.001), lower alanine aminotransferase (ALT; HR 0.997; 95% CI 0.995–0.999; p = 0.003) and albumin levels (HR 0.5; 95% CI 0.304–0.821; p = 0.006) at diagnosis, and increased IgG at the end of follow-up (time-dependent analysis; HR 1.002; 95% CI 1.001–1.028; p = 0.003).
Fig. 2. Treated individuals with cirrhosis had better survival than untreated individuals with cirrhosis (Cox regression survival analysis; HR 0.269; 95% CI 0.128–0.566; p = 0.001).

When these parameters entered the multivariate Cox regression model, independent predictors of liver-related death were the increased disease duration (p = 0.04), presence of cirrhosis at diagnosis (p = 0.004), low albumin level at diagnosis (p < 0.03), delayed diagnosis (p = 0.05), and age at diagnosis >60 years (p = 0.05). Individuals with cirrhosis who received immunosuppression had better survival than untreated individuals with cirrhosis (HR 0.269; 95% CI 0.128–0.566; p = 0.001; Fig. 2).

Safety issues
MMF was well tolerated, and only 1 patient (1/20; 5%) changed treatment to AZA owing to MMF intolerance (development of rash). In contrast, 9/30 (30%) patients in the AZA group changed to MMF owing to AZA intolerance (5 myelotoxicity and 4 hepatoxicity). At the beginning of therapy, mild gastrointestinal symptoms were reported in 26/183 (14.2%) MMF-treated and 18/64 (28.1%; p = 0.02) AZA-treated patients. These adverse effects were temporary and did not need dose reduction in both arms. Infections were managed in the outpatient clinic with antibiotics. Temporary treatment discontinuation was observed in 16 MMF-treated and 8 AZA-treated patients. More severe infections requiring permanent MMF or AZA discontinuation were observed in 4/183 and 3/64 patients, respectively (p >0.05). Malignancy was developed in only 2 patients receiving MMF (1 lymphoma and 1 melanoma). Up to the present, none of females at childbearing age became pregnant. In overall, discontinuation of immunosuppression as a result of serious adverse events was required in 7/183 (3.8%) MMF-treated and 12/64 (18.8%) AZA-treated patients (p = 0.0003), whereas permanent dose reduction was needed in 18/183 (9%) and 8/64 (12.5%) patients, respectively.

Discussion
This study presents the long-term prospective follow-up data from a large cohort of Greek individuals with AIH followed up for 21 years. To the best of our knowledge, this is the biggest study ever published with 183 naïve individuals with AIH treated with MMF as first-line treatment and followed up for a mean of 87 ± 64 months; thus, general conclusions can safely be drawn. Additionally, in this study, the new definitions of response criteria and endpoints proposed recently by the IAIHG were assessed. The following major points arise from the present study: (a) the non-response rate was significantly lower in the MMF group than in the AZA group; (b) CBR rates at 12 months, at the end of follow-up, and in overall were significantly higher in the MMF-treated patients than in the AZA-treated patients; (c) patients receiving AZA were more prone to switching to MMF than those receiving MMF; (d) MMF-treated patients were more frequently eligible for stopping immunosuppression according to the guidelines than AZA-treated patients; and (e). Lower disease duration, absence of cirrhosis or higher albumin levels at diagnosis, early diagnosis, and age at diagnosis <60 years were independent predictors of better liver-related survival. Importantly, the present study confirmed and explored further the results of our previous real-world prospective and propensity matching studies. Our results are in accordance with Hlviko et al., who found 84% response in 29 individuals with AIH (17 naïve patients), as well as with a recent meta-analysis that showed that MMF combination with prednisone led to significantly higher CBR rates and lower non-response rate compared with standard therapy.

Many retrospective studies have assessed MMF safety and efficacy in AIH but, in most cases, as second-line therapy. In this context, an overall high efficacy of MMF was reported recently in a review and meta-analysis. In another study from the Australian Liver Association Clinical Research Network, MMF also proved an excellent treatment option for individuals with AIH either intolerant or refractory to standard therapy with those most likely to respond having lower IgG or INR levels at baseline or being older at the time of MMF initiation.

Mycophenolic acid – the active form of MMF – is the first selective, potent, reversible, and non-competitive inhibitor of type II isomerase of inosine-5’-monophosphate dehydrogenase discovered almost 100 years ago. As a result, MMF leads to selective immunosuppressant effect with few adverse events, which is the requested target in transplant patients or individuals with autoimmune diseases. Similar to that in previous studies, tolerability to MMF in the current prospective long-term trial was excellent, as it was also reported in transplant recipients and systemic lupus erythematosus.

At the time of this writing, only 11% of MMF-treated patients discontinued treatment. However, in most of them, this was not done because of adverse effects or intolerance to the drug but for other reasons (willingsness of pregnancy, unavailability of the drug, etc.). This is in parallel with previous published cohort studies. In contrast, patients receiving AZA were significantly more prone to discontinuing treatment because of intolerance or serious adverse events. Interestingly, CBR rates remained significantly higher in the MMF group than in the AZA group even after including in the former patients who had insufficient response or were intolerant to AZA. Of note, CBR rates in the MMF group were not affected by the presence of significantly higher proportion of SLA/LP-positive patients in the MMF-group compared to the AZA-group. However, it should be emphasised that MMF administration should be cautious under strict contraceptive measures in women of childbearing age because contrary to AZA, it is an absolute contraindication during pregnancy. Another probable disadvantage of MMF is the financial issues. MMF is more expensive than AZA; however, the use of generics, as it happens in our case, minimises the cost.
Abbreviations

AIH, autoimmune hepatitis; ALT, alanine aminotransferase; ANA, anti-nuclear antibodies; anti-LC1, anti-liver cytosol type 1 antibodies; anti-LKM1, anti-liver/kidney microsomal type 1 antibodies; anti-SLA/LP, antibodies against soluble liver antigen/liver pancreas; AZA, azathioprine; CBR, complete biochemical response; HASL, Hellenic Association for the Study of the Liver; HR, hazard ratio; IAIHG, International AIH Group; LSM, liver stiffness measurement; mHAI, modified hepatitis activity index; MMF, mycophenolate mofetil; SMA, smooth muscle antibodies; ULN, upper limit of normal.

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Conflicts of interest

The authors have nothing to declare.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors’ contributions

Study concept and design: GND, KZ. Acquisition of research data: PA, NKG, SG, AS, GG. Analysis and interpretation of data: PA, KZ, GND, NKG, ER, GKK. Drafting of the manuscript: GND, PA, KZ. Critical revision and editing of the manuscript: all authors.

Data availability statement

All data used to support the findings of this study are included within the article and supplementary files.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2022.100601.

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