Comparison of efficacy and tolerability of azathioprine, mycophenolate mofetil, and cyclophosphamid among patients with neuromyelitis optica spectrum disorder: A prospective cohort study

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A B S T R A C T

Aims: To compare the efficacy and tolerability of azathioprine (AZA), mycophenolate mofetil (MMF), and cyclophosphamide (CTX) in patients with neuromyelitis optica spectrum disorder (NMOSD).

Methods: We performed a prospective cohort analysis of relapses, disability, and adverse events in NMOSD patients treated with AZA, MMF, or CTX (n = 119, 38, and 41, respectively). All the patients were co-treated with oral prednisone.

Results: A significant reduction in relapse rate was found in patients taking AZA (p < 0.001), MMF (p = 0.001) or CTX (p = 0.01). MMF was associated with a lower risk of relapse than AZA, but this difference was not statistically significant (p = 0.08). AZA and MMF decreased the mean Expanded Disability Status Scale (EDSS) scores significantly (AZA: p = 0.02; MMF: p = 0.01), whereas CTX did not. Compared with AZA, MMF had a significantly lower risk of treatment discontinuation due to drug-related adverse events (p = 0.02), whereas CTX had a comparable risk (p = 0.35).

Conclusions: MMF is a good first-line treatment option for NMOSD and AZA remains a valuable first-line drug if its side effects are tolerable while CTX can be a treatment option for patients who cannot tolerate AZA and MMF.

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1. Introduction

Neuromyelitis optica (NMO) is a severe inflammatory autoimmune disease of the central nervous system (CNS) that preferentially affects the optic nerves and spinal cord, and is associated with the production of autoantibodies against aquaporin-4 (AQP4-IgG) in approximately 70% of cases. NMO spectrum disorder (NMOSD) consists of diseases associated with NMO, which includes conditions associated with AQP4-IgG seropositivity with 1) limited or early forms of NMO such as longitudinally extensive transverse myelitis or recurrent or bilateral optic neuritis; 2) cerebral, diencephalic, or brainstem lesions; and 3) coexisting autoimmune disorders such as systemic lupus erythematosus (SLE) or Sjögren’s syndrome (SS) [1]. There are no differences in the clinical presentation, immunopathogenesis, biological characteristics, and immunotherapeutic strategies between NMO and NMOSD [2]. Furthermore, limited NMOSD syndromes often lead to attacks that are consistent with conventional NMO and, therefore, the International Panel for NMO Diagnosis proposed subsuming NMO into the single descriptive term NMOSD and established new diagnostic criteria in 2015 [2].

The disease course of NMOSD is often devastating, and only one or two acute attacks can lead to blindness or ambulatory disability [3,4]. Therefore, the effective prevention of relapses is critical in avoiding attack-related disability and, therefore, is the primary aim of treatment. Since the use of azathioprine (AZA) with prednisone was first reported in 1998 [5], various immunosuppressant drugs including corticosteroids [6], AZA [7–13], mycophenolate mofetil (MMF) [7,9,10,14,15], cyclophosphamide (CTX) [16,17], methotrexate [18–20], and mitoxantrone [21,22], have been used to prevent relapse in NMOSD patients, and most of them have shown some benefit. However, these observations have mostly been based on small case series or retrospective cohort studies, and there is no current consensus on optimum strategies for selecting an initial therapy. Considering the paucity of treatment data,
we conducted a prospective, observational cohort study to compare the efficacy (annualized relapse rate, ARR, first relapse, and disability) and tolerability (adverse events and treatment discontinuation due to side effects) of AZA, MMF, and CTX as initial immunosuppressive treatments for patients with NMOSD.

2. Material and methods

2.1. Patients

Institutional Review Board approval was obtained from the Peking Union Medical College Hospital while patient consent was not required because de-identified data were used in the study. The patient data were obtained from the MSNMOBase, which is an observational cohort study based on prospectively collected data. The MSNMOBase was conducted in the Department of Neurology at our hospital and commenced in 2011. At the time of data extraction, the MSNMOBase had enrolled 314 patients with NMOSD, who all met the 2015 NMOSD diagnostic criteria [2]. After excluding 80 patients who previously received immunosuppressive agents (including CTX, methotrexate, AZA, MMF, mitoxantrone and rituximab) and 30 patients who declined to receive treatments at enrollment, 204 patients with NMOSD were included in this prospective cohort study. Based on their individual economic and childbearing status and convenience of the administration route, the patients chose AZA, MMF, or CTX as their initial therapy. Of the 204 patients with NMOSD, 122, 38, and 44 were administered AZA, MMF, and CTX, respectively. After two and three patients administered AZA and CTX, respectively were lost to follow-up, and one patient administered AZA died in an accident, 198 patients (AZA, MMF, and CTX; n = 119, 38, and 41, respectively) were included in the final statistical analysis (Fig. 1).

The minimum baseline data requirements included demographics, date of onset, clinical presentation, Expanded Disability Status Scale (EDSS) score, serum AQP4-IgG, cerebral and spinal magnetic resonance imaging scans, and type and date of commencement of immunosuppressive agents. Other diagnostic test results were recorded, if performed, including the antinuclear antibody test, extractable nuclear antigen test, cerebrospinal fluid oligoclonal bands, and cerebral spinal fluid AQP4-IgG. Following the initial visit, a minimum annual follow-up was required, although all follow-up visits were recorded. The minimum data collected at follow-up were the date of visit, EDSS and AQP4-IgG scores before and after treatments while the time to first relapse and drug-related adverse events necessitating the discontinuation of treatment were also assessed.

2.2. Treatment protocol

One hundred and nineteen patients were prescribed AZA as their initial immunosuppressive agent, which was started at 50 mg daily and then increased to 100 mg daily 4 weeks later. Thirty-eight patients were prescribed MMF as their initial immunosuppressive agent with starting and maintenance doses of 1500 mg/day for adults and 600 mg/m² twice daily for children (no > 1500 mg/day). Forty-one patients were prescribed CTX as their initial immunosuppressive agent, and they received intravenous (iv) CTX at a dose of 400 mg weekly for 30 weeks, followed by and then AZA at 100 mg daily. All the patients were co-treated with oral prednisone, starting at 1 mg/kg daily (usually 60 mg/day) with a 5-mg dose reduction every week until a dose of 30 mg/day was attained, which was then maintained for 4 weeks. Then, a more gradual dose tapering process was initiated with a 5-mg reduction every 2 weeks, followed by a switch to 7.5 mg/day for long-term maintenance. In the acute relapsing stage, high-dose iv methylprednisolone was administered at a dose of 1000 mg/day for 5 consecutive days, which was then converted to oral prednisone. The initial therapy could be switched to an immunosuppressive agent alone (when patients were relapse-free for 2 years), another immunosuppressive agent (when patients had severe adverse reactions), or a more aggressive therapy such as rituximab (when patients relapsed during treatment). The switched treatments were not included in the present study analysis.

2.3. Clinical assessment

A relapse was defined as a neurological disturbance that increased the overall EDSS score by at least half a point, two functional system scores by 1 point, or one functional system score by 2 points for at least 24 h in the absence of other identifiable causes such as fever or infection. The annualized relapse rate (ARR) was calculated by dividing the number of relapses by the time in years. The ARR and EDSS scores of each group were compared before and after the initial treatment. The time to first relapse and drug-related adverse events necessitating the discontinuation of treatment were also assessed.

2.4. Statistical analysis

To compare the clinical characteristics of the groups, an analysis of variance (ANOVA) or Kruskal-Wallis test was used for continuous variables, and a Chi-squared or Fisher’s exact test was used for categorical variables. Wilcoxon signed-rank tests were used to compare the ARR and EDSS scores before and after treatments while the time to first relapse was compared using Kaplan-Meier survival analysis.

Fig. 1. Summary of patients analyzed in the present analysis. NMOSD = neuromyelitis optica spectrum disorder, AZA = azathioprine, MMF = mycophenolate mofetil, CTX = cyclophosphamide.
relapse and treatment discontinuation due to drug-related adverse events were described using the Kaplan-Meier (KM) method and then compared among the groups using Log-rank tests. The hazard ratios of relapse and treatment discontinuation due to drug-related adverse events were calculated by using the Cox proportional hazard model. The models were adjusted for age at onset and enrollment which was significantly different among the groups at baseline. A two-tailed p-value < 0.05 was considered statistically significant, and the statistical package for the social sciences (SPSS) version 22.0 was used for all the analyses.

3. Results

3.1. Baseline characteristics

Table 1 summarizes the demographic and baseline clinical characteristics of the three groups. No differences were found in the gender, AQP4-IgG-positive rates, disease duration before treatment, pretreatment ARR and, pretreatment EDSS, or treatment duration among the groups. Both the inclusion and onset ages were significantly lower for the MMF group than they were for the AZA and CTX groups (inclusion age: MMF vs. AZA, p = 0.00, MMF vs. CTX, p = 0.01; and onset age: MMF vs. AZA, p = 0.01, MMF vs. CTX, p = 0.02).

3.2. Effect on relapse

In the AZA group, the median pretreatment ARR was 0.8, but this value decreased to 0 during the median treatment duration of 15.2 months (range: 6.6–26.4 months, Table 1), with a significant reduction of 100% (p < 0.001, Table 2). In the MMF group, the median pretreatment ARR was 0.8, but this value decreased to 0 during the median treatment duration of 13.6 months (range: 0.6–64.0 months; Table 1), with a significant reduction of 100% (p = 0.01; Table 2).

The analysis of the KM curves (Fig. 2) showed no statistically significant difference among the three treatment groups in the occurrence of the first relapse (p = 0.22, Log-rank test). The multivariable analysis adjustment for the influence of baseline variables (inclusion and onset ages) on the risk of relapse revealed that the hazard ratios of the MMF and CTX groups compared to that of the AZA group were 0.44 (95% CI: 0.17–1.09; p = 0.08) and 1.30 (95% CI: 0.65–2.60; p = 0.45), respectively.

3.3. Effect on disability

After treatment, the mean EDSS score significantly decreased from 2.5 ± 1.8 to 2.3 ± 1.7 (p = 0.02) and 2.7 ± 2.0 to 2.0 ± 1.8 (p = 0.01) in the AZA and MMF groups, respectively while that of the CTX group increased from 2.6 ± 1.9 to 2.9 ± 1.7, but the increase was not statistically significant (p = 0.053). The EDSS scores improved or remained stable in 112 of 119 (94.1%), 37 of 38 (97.4%), and 33 of 41 (80.5%) patients administered AZA, MMF, and CTX, respectively. No significant difference in the proportion of patients with improved or stable EDSS scores was found between the AZA and MMF groups (p = 0.68). The proportion of patients with improved or stable EDSS scores was significantly lower in the CTX group than it was in the AZA and MMF groups (CTX vs AZA, p = 0.01; CTX vs MMF, p = 0.03, Table 2).

3.4. Tolerability

In the AZA group, treatment discontinuation due to drug-related adverse events occurred for 34 (28.6%) patients, 15 (12.6%), 14 (11.8%), 3 (2.5%), 1 (0.8%), 1 (0.8%), and 1 (0.8%) due to elevated liver enzymes, leukopenia, pancytopenia, gastrointestinal disturbances, alopecia, and anemia, respectively.

In the MMF group, treatment discontinuation due to drug-related adverse events occurred for two (5.3%) patients, 1 (2.6%) and 1 (2.6%) due to agranulocytosis and amenorrhea, respectively.

In the CTX group, treatment discontinuation due to drug-related adverse events occurred for 14 (34.1%) patients, seven (17.1%), three (7.3%), three (7.3%), one (2.4%), one (2.4%), and one (2.4%) due to leukopenia, elevated liver enzymes, amenorrhea, hemorrhagic cystitis, gastrointestinal disturbances, and thrombocytopenia, respectively.

The analysis of the KM curves (Fig. 3) showed statistically significant differences among the three treatment groups in the occurrence of treatment discontinuation due to side effects (p = 0.01, Log-rank test). After adjusting for the possible influence of baseline variables (inclusion and onset age), the MMF group had a significantly lower risk of treatment discontinuation due to drug-related adverse events than the AZA group did (hazard ratio: 0.19; 95% CI: 0.04–0.79; p = 0.02) while the CTX group had a higher risk than the AZA group did, but this result was not statistically significant (hazard ratio: 1.36; 95% CI: 0.72–2.55; p = 0.35).

4. Discussion

To the best of our knowledge, this is the first prospective cohort study comparing the efficacy and tolerability of AZA, MMF, and CTX in patients with NMOSD. Similar to previous studies [5,7–16], we found a

### Table 1
Demographic characteristics of study participants.

<table>
<thead>
<tr>
<th>Initial treatment</th>
<th>Azathioprine (n = 119)</th>
<th>Mycophenolate mofetil (n = 38)</th>
<th>Cyclophosphamide (n = 41)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean inclusion age (SD), years</td>
<td>39.7 (13.9)</td>
<td>31.6 (14.0)</td>
<td>40.2 (12.7)</td>
<td>.01‡</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>110 (92.4)</td>
<td>32 (84.2)</td>
<td>39 (95.1)</td>
<td>.18**</td>
</tr>
<tr>
<td>AQP-4-IgG positivity, n (%)</td>
<td>110 (92.4)</td>
<td>33 (86.8)</td>
<td>37 (90.2)</td>
<td>.57**</td>
</tr>
<tr>
<td>Mean onset age (SD), years</td>
<td>35.7 (14.0)</td>
<td>28.7 (13.0)</td>
<td>35.7 (11.6)</td>
<td>.02</td>
</tr>
<tr>
<td>Median disease duration before treatment (range), months</td>
<td>23.0 (6.6–220.3)</td>
<td>14.3 (1.9–276.7)</td>
<td>23.3 (6.3–208.0)</td>
<td>.44***</td>
</tr>
<tr>
<td>Median treatment duration (range), months</td>
<td>16.3 (0.2–53.2)</td>
<td>15.2 (6.6–26.4)</td>
<td>13.6 (6.6–64.0)</td>
<td>.97***</td>
</tr>
<tr>
<td>Median pre-treatment ARR (range)</td>
<td>0.8 (0.0–8.0)</td>
<td>0.8 (0.0–3.8)</td>
<td>0.7 (0.0–5.8)</td>
<td>.49***</td>
</tr>
<tr>
<td>MED (range)</td>
<td>2.0 (0.0–9.0)</td>
<td>2.0 (0.0–9.0)</td>
<td>3.0 (0.0–8.5)</td>
<td>.86***</td>
</tr>
</tbody>
</table>

AQP-4 = aquaporin-4, ARR = annualized relapse rate, EDSS = expanded disability status scale, SD = standard deviation.

* p-Values were determined by ANOVA testing with a threshold of p < 0.05.

** p-Values were determined by Fisher’s exact testing with a threshold of p < 0.05.

*** p-Values were determined by Kruskal-Wallis testing with a threshold of p < 0.05.
significant reduction in the relapse rate in patients with NMOSD with all three immunosuppressants. Compared with AZA, MMF therapy tended to carry a lower risk of relapse. However, efficacy with AZA, MMF therapy was not statistically different from AZA. The analysis of the disability-inducing effects of the agents revealed that AZA and MMF significantly decreased the mean EDSS scores, whereas CTX did not. Moreover, CTX treatment had the lowest proportion of patients with improved or stable EDSS scores among the three treatments. The tolerability analysis revealed that the MMF group exhibited a significantly lower risk of treatment discontinuation due to drug-related adverse events than the AZA group did, whereas this risk was not significantly different between the CTX and AZA groups.

AZA is the most commonly used NMOSD treatment at our institution due to its wide availability, familiarity, low cost, and convenient administration route. Previous studies, in which only a portion of the patients co-treated with AZA and prednisone, demonstrated that the reductions in relapse rate ranged from 39.1% to 72.1% [7–9,11]. In contrast, we found a 100% reduction in the relapse rate in the AZA group after a median treatment duration of 16.3 months. The enhanced prevention of relapse exhibited by AZA in our study might be associated with the long-term concomitant use of low-dose prednisone (7.5 mg/day). The synergistic effect of AZA and corticosteroids in patients with NMOSD patients has also been demonstrated by a recent study, which reported a 100% reduction in relapse rate by AZA and long-term concomitant use of low-dose corticosteroids is the potential risk of adverse events (e.g., ulceration, infection, or osteonecrosis of the femoral head). However, in our 198 patients (including patients taking MMF and CTX), we found no adverse events due to long-term use of low-dose prednisone. The median duration of treatment was 15.6 months (range: 0.2–64.0 months) in our cohort and, therefore, the synergistic effects and safety of immunosuppressants and low-dose corticosteroids should be further evaluated with a longer follow-up time.

Although AZA was more effective in combination with corticosteroids in patients with NMOSD in the present study, it had a higher risk of treatment discontinuation due to drug-related adverse events than MMF did, suggesting that AZA has additional safety risks and tolerability concerns. This result is consistent with those of Jeong et al. [7], who also found that MMF was tolerated better than AZA, which was the only agent associated with adverse events that necessitated the cessation of treatment. AZA is metabolized by the enzyme thiopurine methyltransferase (TPMT), and its activity is affected by genetic polymorphisms. Patients with a TPMT*3C heterozygous or homozygous genetic mutation are more likely to experience adverse events [23].
Therefore, screening for TPMT insufficiency before commencing treatment is recommended and might improve the tolerability of AZA.

NMO often coexists with systemic autoimmune diseases, especially SLE and SS [1]. CTX is an immunosuppressant that is widely used to treat patients with neuropsychiatric SLE [24] and CNS complications of SS [25]. The European Federation of Neurological Societies recommends the administration of CTX (iv, 7–25 mg/kg every month over a period of 6 months) as a second-line therapy for patients with NMO, based on a few case reports on NMO suggesting that CTX is partially effective [26]. However, the optimal treatment protocol and the efficacy of CTX have not been sufficiently investigated. Therefore, we prescribed the CTX to patients with NMO according to the treatment protocol of the neurological manifestations of SLE and SS, and hypothesized that CTX treatment might be more efficacious than direct AZA treatment would. However, we did not find a lower risk of relapse with CTX than with AZA. Moreover, in the disability analysis, CTX did not decrease the mean EDSS score and had the lowest proportion of patients with improved or stable EDSS scores among the three immunosuppressants. Considering its limited effect, the relatively higher risk of treatment discontinuation due to drug-related adverse events and inconvenient administration route observed with CTX than with the other agents, suggests it is better to prescribe CTX to patients with NMO who cannot tolerate AZA and MMF.

MMF is a good first-line treatment option for NMO, and it exhibited the best efficacy and tolerability of the three medications examined in this study. To date, three retrospective cohort studies have compared the efficacy and safety of AZA, MMF, and CTX [7,8–10]. Consistent with our results, all of these previous studies showed that MMF showed the greatest reduction in relapse rate and the lowest risk of side effects.

Our study was limited by its non-inclusion of a blinding design, relatively short follow-up duration, lack of an arm of rituximab and uneven assignment of patient to treatment groups. Rituximab is an effective and safe treatment option for NMOSD [7,9], however, we did not investigate this treatment option in this study.

In conclusion, MMF is a good first-line treatment option for NMO, and it exhibited the best efficacy and tolerability of the three medications examined in this study. To date, three retrospective cohort studies have compared the efficacy and safety of AZA, MMF, and CTX [7,8–10]. Consistent with our results, all of these previous studies showed that MMF showed the greatest reduction in relapse rate and the lowest risk of side effects.

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In conclusion, MMF is a good first-line treatment option for NMO and AZA remains a valuable first-line drug if its side effects are tolerated, while CTX can be a treatment option for patients who cannot tolerate AZA and MMF. Finally, the long-term concomitant use of low-dose prednisone (7.5 mg/day) is safe, and might increase the efficacy of immunosuppressants.

5. Conclusion

In conclusion, MMF is a good first-line treatment option for NMO and AZA remains a valuable first-line drug if its side effects are tolerated, while CTX can be a treatment option for patients who cannot tolerate AZA and MMF. Finally, the long-term concomitant use of low-dose prednisone (7.5 mg/day) is safe, and might increase the efficacy of immunosuppressants.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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