

Characteristics of azathioprine use and cessation in a longitudinal lupus cohort

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ABSTRACT

Objective: Guidelines for azathioprine (AZA) use in systemic lupus erythematosus (SLE), including indications for initiation and cessation, are lacking. Clinical decision-making could be improved if reasons for cessation of AZA treatment were standardised.

Methods: We determined the characteristics of AZA use in a cohort of patients with SLE and evaluated reasons for AZA cessation. Patients with SLE in a single centre had longitudinal recording of disease activity (Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI)-2k), laboratory investigations and treatment from 2007 to 2012.

Results: Of 183 patients studied, 67 used AZA on at least one occasion. There was no significant difference between AZA users and non-users in age or American College of Rheumatology criteria. Compared with those not treated with AZA, patients treated with AZA had higher disease activity (time-adjusted mean SLEDAI 5.2 ± 0.3 vs 3.8 ± 0.3 , $p=0.0028$) and damage (Systemic Lupus International Collaborating Clinics (SLICC)-SDI 1.6 ± 0.3 vs 1.2 ± 0.1 , $p=0.0445$), and were more likely to have a positive dsDNA ($p=0.0130$) and receive glucocorticoids ($p<0.0001$). AZA therapy was ceased in 30/67 (45%) patients. The predominant reasons for cessation were treatment de-escalation 14 (47%), treatment failure 12 (40%) and toxicity 3 (10%). AZA was switched to mycophenolate mofetil (MMF) in 9/12 (75%) of treatment failures, and this choice was strongly associated with active lupus nephritis.

Conclusions: AZA toxicity was uncommon, and many patients ceased therapy in the context of treatment de-escalation. However, the frequent development of active lupus nephritis requiring MMF suggests the need to distinguish refractoriness, under-treatment and non-adherence to AZA in patients with SLE. These findings suggest that future studies of AZA metabolite measurement could prove valuable in the management of SLE.

KEY MESSAGES

- ▶ Azathioprine is a commonly used immunosuppressant for all manifestations of SLE, however indications for its use and cessation are not standardized.
- ▶ We describe the use and cessation of AZA in our SLE cohort, with 50% of cessation due to treatment failure or toxicity and the remainder undergoing de-escalation.
- ▶ Distinguishing true treatment refractoriness from undertreatment or non-compliance, and toxicity from disease activity may be enhanced with use of AZA metabolite measures.

both inducing remission and as maintenance therapy. However, in a patient population consisting predominantly of women in their child-bearing years, AZA is one of only a few medications deemed safe for use during pregnancy,^{9–11} and it remains widely used. Pre-therapy measurement of thiopurine methyltransferase (TPMT) enzyme levels is not universally implemented in rheumatology practice, and there is a lack of consensus about indications for initiation and cessation of AZA therapy in SLE, with no widely accepted treatment guidelines for its use.⁵ In addition, current weight-based dosing regimens for AZA fail to reflect inter-patient variability of AZA metabolism.^{12–13}

Evidence in autoimmune hepatitis¹⁴ and inflammatory bowel disease (IBD)¹⁵ designates a target range for AZA metabolites that correlates with avoidance of serious toxicity, attaining and sustaining remission, as well as providing an indication of treatment adherence.¹⁶ This has not been applied in SLE. For patients with active SLE despite AZA, appropriate designation as non-compliant, treatment-refractory or undertreated could improve clinical decision-making as has been shown most clearly in IBD.¹⁵

Given the evolving prospects for improving clinical decision-making via therapeutic drug monitoring of AZA, we sought to evaluate patterns of AZA cessation in an SLE cohort, to determine whether reasons for cessation



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INTRODUCTION

Azathioprine (AZA) is a valuable immunosuppressant for the treatment of multiple manifestations of systemic lupus erythematosus (SLE).^{1–5} Following the MAINTAIN trial^{6–7} and ALMS study,⁸ mycophenolate mofetil (MMF) has largely usurped AZA as the therapy of choice in lupus nephritis, for

of AZA in routine practice supported the potential utility of metabolite monitoring. We found that a significant proportion of patients ceased AZA therapy due to poor control of disease, implying that designation of the cause of therapeutic failure through metabolite monitoring could frequently be of benefit.

METHODS

We performed a retrospective study of longitudinal data, acquired prospectively in a single centre. As previously described,^{17–19} all patients with SLE (≥ 4 American College of Rheumatology (ACR) criteria²⁰) attending the Lupus Clinic at Monash Medical Centre, Melbourne, Australia, were invited to be included in a database, approved by the Human Research Ethics committee of Monash Health. After written informed consent, baseline demographic and clinical data were collected on all patients during routine visits to the SLE clinic by the attending physician using standard proformas. Recording of disease activity (hybrid SLENA-Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2k)²¹), laboratory investigations (serum complement, anti-dsDNA antibodies, full blood count, electrolytes, liver function tests, proteinuria (urine protein: creatinine ratio) and urine microscopy) and treatment details are completed at each visit as previously described.^{17–19} Disease-related damage is recorded annually using the Systemic Lupus International Collaborating Clinics (SLICC)-SDI²² and time-adjusted mean SLEDAI (AMS), a measure of mean disease activity adjusted for the period of observation, calculated.

All patients included in the database between January 2007 and November 2012 were assessed for inclusion; data on medication use prior to enrolment in the database were not available. Inclusion criteria for this study were at least one complete set of visit data during the study period. Patients using AZA at any time during the period of observation were identified and details of disease activity and medication use extracted. AZA cessation was defined when documented use of AZA was terminated during the period of observation. Medications used prior to, during and following AZA use were documented, as were prednisolone dose changes at time of AZA cessation. Reasons for AZA cessation were designated as toxicity, treatment failure or de-escalation. Toxicity was defined when abnormal liver function or haematological parameters were present at the time of cessation. Treatment failure was defined where patients ceasing AZA underwent escalation of corticosteroid dose or institution of replacement immunosuppressive therapy. De-escalation was defined when AZA was ceased but no escalation in glucocorticoids or treatment replacement for AZA was instituted, assuming the definitions of toxicity or treatment failure were not met. Disease activity at the time of AZA cessation was evaluated to verify the presence of treatment failure.

Attainment of the recently described lupus low-disease activity state (LLDAS)²³ at AZA cessation was assessed.

For comparison of means, continuous data were analysed using Student's *t* tests, while discontinuous data were analysed using Mann–Whitney rank tests. For comparisons of prevalence and calculation of ORs, Fisher's exact test was used. All statistical analyses were completed using GraphPad Prism Software V.6.0.

RESULTS

A total of 183 patients were studied. Characteristics of the study population are shown in table 1. Of 183 patients studied, 67 (37%) used AZA on at least one occasion during the study period. Among patients who had used AZA, the median (range) dose of AZA used across the period of observation was 95 mg/day (50–150 mg/day).

We next compared clinical characteristics between patients who used AZA and those who did not. As shown in table 2, there was no significant difference in age or number of ACR criteria fulfilled between patients using AZA and those not.

Patients using AZA had a significantly longer total period of observation, and a greater number of visits recorded than patients not using AZA (table 2). Patients who were treated with AZA at any time during the period of observation had significantly higher AMS, indicating higher disease activity across the period of observation ($p=0.0028$, table 2). Ethnicity differed significantly, in that patients of Caucasian ethnicity were significantly over-represented among patients using AZA (table 2). No

Table 1 Characteristics of study population

N	183
Age (years, mean \pm SEM)	44.4 \pm 15.0
Ethnicity (Asian:Caucasian:other*)	90:54:39 (49%:30%:21%)
Gender (female:male, n (%))	154:29 (84%:16%)
ACR criteria (median (range))	5 (4–9)
Duration SLE (years, mean \pm SEM)	9.9 \pm 7.7
Visits observed (n, median (IQR))	12 (6–21)
Period observed (days, median (IQR))	1113 (469–1603)
ANA positive (n (%))	180 (100%)
ANA titre (median (range))	1280 (80–2560)
Anti-dsDNA positive (n (%))	133 (72.7%)
Hypocomplementemia (n (%))	139 (75.9%)
Use of prednisolone (n (%))	138 (75.4%)
Adjusted mean prednisone dose (mg/day, mean \pm SEM)	7.6 \pm 0.66
AMS (mean \pm SEM)	4.34 \pm 0.23
SDI (median (range))	1 (0–12)

*Other—those identifying as 'other' or declining to be identified ethnically.

ACR, American College of Rheumatology; AMS, (time) adjusted mean SLEDAI; ANA, antinuclear antibody; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

Table 2 Characteristics of azathioprine (AZA) users versus non-AZA users

	AZA users	Non-AZA users	p Value
N	67	116	
Age (year, mean±SEM)	43±2	45±1	0.4553
Ethnicity (n; %) (Asian:Caucasian:other*)	29:32:6	61:22:33	<0.0001
	43%:47%:10%	53%:19%:28%	
ACR criteria (median (range))	5 (4–9)	5 (4–9)	0.5796
Duration SLE	10.09±0.8840	9.776±0.7368	0.7906
Visits observed (n, median (IQR))	18 (9–26)	9 (4–16)	<0.0001
Period observed (days, median (IQR))	1316 (798–1701)	889 (369–1545)	0.0094
Anti-dsDNA-positive	54 (80.6%)	77 (67.5%)	0.0399
Hypocomplementemia (n (%))	60 (89.5%)	79 (68.1%)	0.0011
AMS (mean±SEM)	5.23±0.29	3.82±0.31	0.0028
SDI (median (range))	1 (0–12)	1 (0–7)	0.1422
Corticosteroid use	65 (97%)	73 (63%)	<0.0001
Adjusted mean prednisone dose (mg/day, mean±SEM)	7.6±0.66	5.6±0.62	0.0386
Hydroxychloroquine use	65/67 (97%)	104/116 (89.7%)	NS

Data refer to period of observation recorded.

*Other—those identifying as 'other' or declining to be identified ethnically.

ACR, American College of Rheumatology; AMS, (time) adjusted mean SLEDAI; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

significant difference in cumulative damage, as measured using SLICC-SDI at the last assessment, was observed (table 2). Patients using AZA were more likely to have anti-dsDNA antibodies ($p=0.0130$) and to receive glucocorticoids ($p=0.0001$), and were exposed to a significantly higher adjusted mean glucocorticoid dose ($p=0.0386$, table 2), than patients not exposed to AZA. Concomitant treatment with hydroxychloroquine (HCQ) occurred in 65/67 (97%) of the AZA cohort, and 104/116 (89.7%) of non-AZA cohort (NS).

Of the 67 AZA users, 29 commenced AZA during the period of observation, allowing us a view of preceding medications and disease trajectory. Of those starting AZA during the period of observation, 24/29 had used preceding lupus medications including MMF (6 patients) methotrexate (MTX) (4), cyclophosphamide (2), rituximab (1), HCQ (22) and prednisolone (23). Thus, five patients commenced AZA de novo with no preceding medications during database capture.

We next assessed reasons for AZA cessation. Also, 30/67 (45%) patients ceased AZA during the period observed, affording the opportunity to examine reasons for treatment cessation. Using the definitions outlined above, 14 patients (21%) ceased AZA due to de-escalation, 12 (18%) due to treatment failure and 3 (4%) due to toxicity, with one patient unable to be classified. Among patients assessed as ceasing AZA due to de-escalation, 6/14 (43%) met criteria for LLDAS, while 8/14 (57%) did not. There was no difference in ethnicity among patients who did, or did not, cease AZA (not shown). As details of AZA treatment prior to enrolment in the database were not available, no analysis of treatment duration was undertaken.

Among 30 patients ceasing AZA, treatment failure was identified as the reason for cessation in 40%. Treatment

was changed to MMF in 9/12 (75%) patients who ceased AZA due to treatment failure. Overall disease activity as measured by SLEDAI was numerically but not significantly higher in those in whom AZA was switched to MMF (AZA-MMF) compared with those who switched to other treatments (AZA-other) (table 3). None of the patients who ceased AZA due to treatment failure met criteria for LLDAS.

On the basis that MMF is most frequently used for the treatment of lupus nephritis, we analysed renal disease activity using the renal domains of SLEDAI (urinary casts, proteinuria, haematuria, pyuria).^{24 25} AZA-MMF patients had significantly higher renal SLEDAI than AZA-other patients ($p=0.0214$, table 3). Data on histological class of lupus nephritis are stored in our database; however, only patients with Class III/IV/V lupus nephritis qualify for MMF treatment in our centre. Contingency analysis revealed that the relative risk of active renal disease in AZA-MMF patients compared with AZA-other patients was 4.6 (95% CI 1.1 to 18.4, $p=0.019$). This suggests that active lupus nephritis developing during AZA therapy was driving treatment escalation to MMF. Among the patients who switched from AZA to MMF, the median dose was 100 mg/day (range 53–143); a median (range) dose of 1.521 mg/kg/day (0.839–2.123). This was not different from the median (range) 1.527 (0.547–2.50) mg/kg/day in the overall population on AZA. In contrast, lupus nephritis was not the major lesion in the 3/12 treatment failure patients whose therapy was changed to immunosuppressive treatment other than MMF.

Toxicity, in the form of leukocytopenia, was the reason for cessation in three patients (10% of patients ceasing AZA). Of the three neutropenic patients, a nadir of 1.3×10^9 leucocytes/L was observed in a patient with

Table 3 Reasons for AZA cessation

	Total 67	Cessation 30	No cessation 37
De-escalation		14	
Toxicity		3	
Treatment failure		12	
	AZA-MMF*	AZA-other†	p Value
(n, (%))	9 (75%)	3 (25%)	
SLEDAI (mean±SEM)	10.14±2.45	5.59±1.2	NS
Renal SLEDAI (mean±SEM)	4.44±1.0	1.52±0.65	0.0214

*AZA-MMF: switched from AZA to MMF.

†AZA-other: switched from AZA to treatment other than MMF.

AZA, azathioprine; MMF, mycophenolate mofetil; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

likely lupus-induced neutropenia, evidenced by further reduction to 0.48×10^9 leucocytes/L 14 months post cessation of AZA, with no other cytopenia-inducing medications. The other two patients had neutrophil nadirs of 0.97 and 1.78×10^9 leucocytes/L, both of which improved over 1–4 months with cessation of AZA and use of prednisolone, consistent with AZA-mediated bone marrow suppression.

DISCUSSION

In SLE, AZA is generally prescribed for active non-renal disease, using weight-based dosing with varying suggested adjustments for renal impairment.^{26–27} As control of disease activity is a key goal in SLE,^{28–29} the presence of active disease despite AZA therapy may result in the presumption of inefficacy and switching of AZA to another therapy. Dose escalation is an alternative to switching, but the frequency of dose-limiting AZA toxicity in patients with SLE is not well described. An individualised metabolite monitoring-based approach to AZA therapy in SLE has been suggested as a way to distinguish disease activity due to underexposure or non-adherence as opposed to medication refractoriness, and potentially distinguish AZA toxicity from active SLE as a cause of leucopenia.³⁰ However, the frequency of these differing outcomes in AZA-treated patients with SLE is poorly understood, and hence the potential benefit of the application of AZA metabolite monitoring as a standard of care in SLE is unclear. We therefore undertook a study of AZA use in a longitudinally followed SLE cohort, focusing on reasons for treatment cessation, to determine whether reasons for AZA termination in routine practice supported the potential use of monitoring.

First, we observed that AZA use was common. In this cohort, AZA was prescribed in patients with more severe disease, as suggested by higher disease activity, anti-dsDNA positivity and use of corticosteroids. An average AMS of >5 indicates inadequate overall disease control in AZA-treated patients. This finding alone

suggests the need for improved use of AZA in SLE, potentially using AZA metabolite monitoring to optimise dosing.

Second, a significant proportion of patients ceased AZA therapy during the period of observation. The most common reason for AZA cessation was treatment de-escalation, followed by treatment failure. In contrast, cessation of AZA due to toxicity was uncommon. Nearly a quarter of all patients with SLE treated with AZA ceased therapy in a setting of treatment de-escalation. Long-term continuation of HCQ is now commonplace,³¹ but there is a lack of data regarding the optimal duration of other therapies, especially in the setting of low-disease activity.³ Interestingly, not all patients who ceased AZA for treatment de-escalation had ‘low-disease activity’ according to a definition proposed recently.²³ The current study was not designed to assess outcomes after treatment de-escalation, but it would be useful in future studies to investigate outcomes subsequent to de-escalation of AZA therapy in patients with stable disease.

Outside the setting of clinical trials, treatment cessation decisions are based on a myriad of factors, and while many are captured in longitudinal cohort databases such as that studied here, others, including the impact of individual patient preference, and subjective measures of intolerability (nausea) are not.³² This highlights a limitation of the current study, in that the data were originally collected for the purpose of analysing longitudinal disease activity^{17–19} and were retrospectively analysed. Further limitations include the potential bias arising from studying a single-centre cohort. Finally, compliance and appropriate documentation cannot be assessed in this format.

The second most frequent reason for AZA cessation was treatment failure, defined as an escalation of therapy in the setting of active disease. There is increasing acceptance of the use of MMF for the treatment of lupus nephritis,⁸ and active nephritis was the dominant clinical feature in patients changing from AZA to MMF in the current study. The observation that 9 of 67

AZA-treated patients developed active lupus nephritis requiring escalation to MMF suggests these cases were regarded by the treating physician as AZA-refractory. While AZA dosing recommendations vary according to disease manifestations of SLE, a maximum dose of 2 mg/kg is advised as maintenance therapy for lupus nephritis.^{3 6} The median dose in AZA-MMF patients was 1.5 mg/kg/day, and only two patients who transitioned to MMF in the setting of active renal disease were receiving a dose ≥ 2 mg/kg, suggesting these patients may have been undertreated.

We propose that distinguishing between true treatment refractoriness, as opposed to under-dosing and non-adherence, and between toxicity and lupus-related leucopenia, would be possible through the measurement of AZA metabolites. Half of all patients on AZA in this study ceased treatment due to presumed inefficacy or toxicity, suggesting that as many as half of all patients with SLE treated with AZA could benefit from metabolite monitoring. One study in IBD using an AZA metabolite-based dosing algorithm found 24% of patients avoided inappropriate dose escalation, and 87% of patients achieved improved clinical outcomes.¹⁵ The application of such an approach in SLE has been suggested,³⁰ but application of such an approach is limited by the fact that an empirically defined therapeutic range for AZA metabolites in SLE is lacking. Two small studies in mixed cohorts of connective tissue disease patients confirmed high inter-individual variation of metabolite levels, and poor correlation of metabolite levels with weight-adjusted doses.^{12 33} A 6-month dose escalation study of 50 patients with SLE identified a target level of AZA metabolites that correlated with efficacy.³⁴ Doses required to achieve this were approximately 3 mg/kg/day, higher than current dosing guidelines for lupus nephritis maintenance³ and similar to maximum dosing recommendations for other autoimmune manifestations.^{35 36} This suggests that inappropriate designation of patients as AZA-refractory may be common in SLE.

Pre-drug TPMT testing was not routinely undertaken in our cohort. It remains a matter of debate whether genotype or phenotype (TPMT enzyme testing) is superior in SLE.^{37 38} The observation that TPMT levels may not always determine potential marrow suppression³⁹ nor individual dose requirements⁴⁰ promotes ongoing conjecture regarding its utility. TPMT measurements potentially provide pretreatment information regarding potential risk of bone marrow suppression, whereas measurement of AZA metabolites can provide a real-time representation of an individual's handling of AZA.⁴¹

In this study, cessation of AZA treatment for toxicity was distinctly uncommon. Insufficient data preclude definitive attribution of the three cases of cytopenia to drug toxicity or disease activity. The ability to distinguish between drug toxicity and disease activity in patients with cytopenia during AZA therapy has potential to inform treatment choices and would also be assisted by metabolite measurement. The fourfold higher prevalence of

treatment cessation for treatment failure than for toxicity implies that current focus of laboratory testing on adverse effects of AZA may be less valuable to clinicians than metabolite measurement enabling dose optimisation and hence disease control.

In conclusion, AZA was a frequently used immunosuppressant in this SLE cohort, but was withdrawn in a high proportion of cases. AZA cessation for toxicity was infrequent, while development of active lupus, including nephritis, requiring treatment escalation to mycophenolate was much more frequent. This suggests that breakthrough disease activity, implying AZA-refractoriness, undertreatment or non-adherence, greatly exceeds AZA toxicity as a management issue in SLE. In addition, when combined, the potential for AZA metabolite monitoring to influence physician decisions was present in half of all patients with SLE treated with AZA. We conclude that knowledge of AZA metabolite concentrations could potentially aid decision-making as part of the standard of care in SLE, for which prospective studies of AZA metabolite levels in SLE and strategy trials of recently proposed metabolite-based dosing algorithms³⁰ are required. The outcomes of such research could include more stringent guidelines for the use of AZA in SLE, potentially resulting in highly cost-effective reductions in disease activity and prevention of treatment escalation.

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