Effects of temporal withholding of mycophenolate in patients with systemic erythematosus lupus erythematosus

Arthritis Australia Project Grant

Final Report

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Grants and Other Financial Support for the Study:

We thank Arthritis Australia for its funding support as a Project Grant awarded in 2023, with extension.

Background

Mycophenolate mofetil (MMF) is a commonly used immunosuppressant in the management of moderate to severe systemic lupus erythematosus (SLE). Its primary action involves inhibiting the *de novo* synthesis of guanosine nucleotides, thereby reducing immune cell function and, consequently, inflammation and tissue damage associated with SLE. However, MMF's immunosuppressive effects can also diminish patients' responses to vaccinations, raising concerns about the efficacy of vaccines like those for influenza and SARS-CoV-2 in this population (1-3). At the Monash Lupus Clinic, approximately 42% of patients are treated with mycophenolate, with an even higher proportion seen among those with higher disease activity (4).

To address this issue, one strategy to optimise vaccine response is to temporarily withhold the immunosuppressive treatment around the time of vaccination, allowing the immune system to respond more robustly. This strategy has been successful in rheumatoid arthritis patients on methotrexate, where withholding methotrexate temporarily increased antibody responses to influenza vaccination (5, 6). While there are limited data on withholding mycophenolate specifically, a small cohort study involving patients with mixed rheumatic diseases demonstrated improved antibody responses to SARS-CoV-2 mRNA vaccination after a temporary interruption of the drug (7). These findings provide a rationale for further exploring the safety and efficacy of this approach in SLE patients.

Currently, there is a lack of data on the optimal timing and duration of MMF withdrawal in the context of vaccination. Our study will help fill the knowledge gap by investigating the effects of suspending mycophenolate treatment in SLE patients with stable disease for their seasonal influenza vaccination. Building on lessons learned from methotrexate studies in rheumatoid arthritis, we proposed a two-week post-vaccination interruption of mycophenolate in stable SLE patients and evaluated the immune responses to influenza vaccination and disease control in those who continued versus those who withheld mycophenolate.

Methods

Participants and setting: Adult SLE patients were recruited from the Monash Lupus Clinic if they were also participants of the Australian Lupus Registry & Biobank. Participants were invited to take part in the study if they were between 18-65 years of age, have been on stable doses of mycophenolate for treatment of their SLE, without a disease flare in the last three months. Eligible patients on the ALRB were those who had been on mycophenolate were sent two email invitations. Information regarding the study was provided to eligible patients at the time of the clinic review. Combination therapy with hydroxychloroquine is permitted and prednisolone dose must be equal to or less than 7.5mg/day. Patients are excluded if they have been exposed to rituximab or belimumab in the last six months. Patients who are also taking concomitant biologic therapy or targeted synthetic anti-rheumatic drugs were excluded. All the patients participating in the study have provided written consent (Monash HREC RES-22-649A)

Study Design

In this prospective randomised controlled study, we assessed immune responses in those who temporarily withhold their mycophenolate versus those who continued their usual dosage, following seasonal influenza

vaccination. The study is part of the broader project called “Temporary Withholding of Immunosuppressants in Rheumatic Diseases”, or TWIRL, which intends to evaluate effects of temporary withholding of medications and effects on immune function in SLE patients. The study (TWIRL-MMF) evaluated the effects of mycophenolate withdrawal on immune response in stable SLE patients, receiving Afluria Quad®, which is a quadrivalent inactivated influenza vaccine indicated for children >5 years of age and adults. The National Immunisation Program recommends that for patients over 65 years of age, the preferred influenza vaccine is the adjuvant version (eg Fluad® Quad). We have therefore excluded also those patients who are over 65 years of age. Participants were randomly assigned to the continuing group versus the withholding group in a 1:1 ratio. This study has been registered on ANZCTR number 12623000397617.

Seasonal Influenza Vaccines

All participants received a cell-based quadrivalent seasonal influenza vaccines (Afluria Quad®), which was donated by CSL Seqirus.. The influenza strains in the southern hemisphere vaccines contained the following influenza A strains in 2023 are: H3N2 A/Darwin/6/2021 and H1N1 A/Sydney/5/2021 and in 2024 are: H3N2 A/Massachusetts/18/2022 and H1N1 A/Wisconsin/67/2022. In both seasons vaccines contained the same influenza B stains B/Austria/1359417/2021 (B Victoria Lineage) and B/Phuket/3073/2013_HA (B Yamagata Lineage).

Intervention

The influenza vaccine was delivered in a 0.5ml prefilled syringe, given as an intramuscular injection in the deltoid muscle by immunisation nurse. After vaccination, subjects were randomised to either the continuing group or the withholding group. The continuing group continued their usual mycophenolate dose, whereas the withholding group stopped the mycophenolate for 2 weeks, then they received a reminder via text message to resume the mycophenolate at their previous dose.

Immunophenotyping and immunoglobulins

Blood was collected from participants at baseline (before vaccination, week 0), and at week 4 after vaccination for anti-influenza antibodies, immunophenotyping and immunoglobulin levels. CD4+ and CD8+ T-cells and CD19+ B-cells were performed by Monash Pathology.

Detection of anti-influenza antibodies

Chemiluminescent ELISAs were developed to detect the generation of antibodies against influenza antigens haemagglutinin and neuraminidase. For each participant, plasma from visit 1 (baseline) and visit 2 (4 weeks post-immunisation) was serially diluted to allow for the calculation of titres. Depending on the antigen, using either a cut off of 20 or 10 times the assay background titres for each sample were calculated using non-linear regression curve fit analysis. Satisfactory seropositivity to the influenza strain and specific antigens were defined by greater than or equal to four-fold increase in antibody titre compared to baseline prior immunization in ≥2 of four influenza vaccine antigens.

SLE disease activity assessment

For each patient enrolled into the study, clinical parameters regarding their SLE disease activity were collected from the Australian Lupus Registry & Biobank Lupus Connect database. Their overall time adjusted mean SLE Disease Activity Index (SLEDAI-2K), most recent SLEDAI-2K score before their vaccination date, rate of flares based on SLE Flare Index (SFI) in the preceding 12 months, and the most recent physician global assessment (PGA) were collected. Disease severity will also be classified according to High Disease Activity Status (HDAS) (8). Additional patient-reported outcome measures in the form of RAPID3 (score 0-30), was recorded at five timepoints, at baseline/week 0, week 2, 4, 8, and 12. Flares were captured by either physician-reported SFI or PGA ≥1 at week 4 or patient-reported RAPID3 score of >12 (9).

Monitoring for influenza infection

Patients will be asked on week 4, 8 and 12 on their questionnaire, whether they have had symptoms that are suggestive of influenza infection, and whether they have had microbiological confirmation. The effects of influenza infection on work commitment and any hospitalisation will be recorded.

At enrolment, patients will be provided with a pathology request slip for a nasopharyngeal swab and symptom diary (paper and electronic form). Patients are encouraged to seek a nasopharyngeal swab to confirm the type of respiratory illness they may have during the study period. They are instructed to seek care from their usual doctor but let the study coordinator know if they have done a swab. It is standard of care to clarify the nature of the pathogen, where possible. Patients will be asked to fill out the symptom diary on a daily basis from the start of their symptoms until their resolution.

Statistical analysis

The results were described as numbers (percentages), mean plus standard deviation (SD) or standard error (SEM), or median and interquartile range (IQR). [6]. Comparison of those who achieve a satisfactory vaccine response from each group will be reported and compared using $\chi 2$ tests or Fisher's exact test, as appropriate. Other continuous variables are analysed by using a t-test or Mann Whitney U test as appropriate. Binary secondary efficacy variables (such as frequency of disease flare and incidence of infection) will be compared by using $\chi 2$ tests or Fisher's exact test, as appropriate. Logistic regression will be used to assess association of clinical variables such as absolute B-cell and/or T-cells numbers at baseline, study group assignment (temporary interruption vs continuation), and other disease related parameters with adequate vaccine response. $P<0.05$ is considered to indicate statistical significance.

Results

Study subjects

Study participants were recruited from Monash Lupus Clinic. Out of 120 eligible patients in the database, 14 patients had discontinued mycophenolate, and another 14 were excluded due to concomitant medications. A further 9 were excluded based on their age (>65 years), as they ideally should receive the adjuvant version of the influenza vaccine. An additional 14 were excluded for other medical reasons. We observed that 18% (n=22) declined participation and another 13% (n=16) had already their influenza vaccines with another provider. The study began in mid 2023, during which we managed to recruit only 10 patients. A second round of recruitment

took place in 2024, but similar proportions of patients were either excluded, declined, or were not contactable. We successfully recruited an additional 11 patients for the 2024 season (See Figures 1 and 2).

Lupus disease activity of participants

The participants of the TWIRL-MMF study were representative of the overall Australian lupus registry participants. The median age at disease onset was 30 years (IQR 20.5, 33), with a female predominance (88.2%) (see Table 1). A significant proportion of participants were of the Southeast or Northeast Asian descent (64.7%) and had a higher rate of tertiary education (70.6%) compared to general cohort of the Australian Lupus Registry participants. The overall disease activity, as measured by time adjusted SLEDAI throughout the observation period in the registry, showed an AMS of 3.96 (IQR 3.19, 6.56). The proportion of HDAS patients in the TWIRL-MMF study was 58.8% which was higher than in the overall ALRB cohort. This was expected, as the recruitment criteria, which required patients to be on mycophenolate, likely selected for those with more severe disease. Consequently, participants demonstrated greater organ involvement as indicated by the number of classification criteria met (Table 2). They also showed higher levels of previous renal (70.6%) and haematological (47.1%) disease activity, using SLEDAI-2K measures. All our participants were taking immunosuppressants, as mandated by the recruitment criteria, and 88.2% was exposure to prednisolone.

Baseline blood counts and immunoglobulin levels

Cytopenia, particularly lymphopenia and neutropenia, is commonly observed in SLE patients. We compared baseline levels between the continuing group and the withholding group, to ensure that our randomisation process was adequate. The mean lymphocyte counts were close to the lower limit of normal in both groups with no significant difference between them (Table 3). The mean neutrophil counts were also within the normal range. The different types of lymphocytes, including B cells (CD19), CD4+ and CD8 + cells were within the normal range, and showed no difference between the groups. Baseline immunoglobulin levels were measured and did not differ between the two groups.

Effects of withholding mycophenolate

Anti-influenza haemagglutinin inhibition

Based on assays of the 2023 season vaccine strain responses, haemagglutinin inhibition was significantly higher in the withholding group, compared to the continuing group. There was a significant increase in the fold rise of haemagglutinin antibody, and this fold rise in influenza antibody titre differed according to the influenza vaccine strain. (Figure 4) H1N1A/Syd/5/2021 had the lowest fold rise in titre, whereby no patients in either group achieved a fourfold rise in titre post vaccination. There was a significant difference in haemagglutinin response in the 2023 group, where the fold change was significantly higher in the withholding group (Figure 5a).

Immunoglobulin levels and other cell counts

We also observed a small but statistically significant increase in IgG and IgA and IgM in the withholding group (Figure 6). The magnitude of change was small with the most notable increase observed in the IgM levels. There

was no significant difference between lymphocyte or neutrophil counts between the withholding group and the continuing group (Figure 7), nor in the individual lymphocyte subsets (Figure 8).

No difference in disease or infection control

We examined the lupus disease control among the participants and found that temporary withholding of mycophenolate for two weeks was well tolerated. Disease activity was measured using RAPID3, a self-reported symptom score (Figure 9) which showed no significant difference between the withholding or continuing group. We also measured the SLEDAI-2K activity and physician global assessment at baseline and five weeks post vaccination (Table 4) and similarly, there was no significant difference between the two groups. None of the participants experienced flu-like symptoms, or needed to undergo nasal swabbing, to proceed to do the symptom diary.

Discussion

The findings from this study suggest that a temporary cessation of mycophenolate mofetil (MMF) around the time of vaccination can enhance antibody responses to influenza vaccine as shown by the haemagglutinin inhibition response in patients with systemic lupus erythematosus (SLE) who withheld MMF, without compromising disease control. These results contribute valuable insights into the potential strategies for improving vaccine efficacy in immunosuppressed populations. However, this study encountered several limitations that should be taken into consideration when interpreting the outcomes.

Project delays and challenges: One significant limitation was the delay in recruitment, which impacted the study's timeline and sample size. Recruitment delays were influenced by various factors, including patient hesitancy and scheduling conflicts with clinic visits. This was higher than what we anticipated, and did not improve despite starting much earlier with our recruitment in the 2024 season, reflecting a general vaccine hesitancy in our population. In our planning we also did not envisage the barrier of the National Immunisation Program recommendation for adults over 65 years and older to receive a different influenza vaccine making the study design and recruitment more difficult. These factors collectively limited the number of participants and prolonged the duration of enrolment. Despite multiple rounds of recruitment, the study only managed to enrol a relatively small cohort, which may affect the generalisability of the findings.

Running immunological assays at the Burnet Institute, a key partner in this research, posed logistical challenges. Collaboration difficulties included delays in processing and analysing samples due to competing priorities and resource allocation at the Burnet Institute. This led to extended waiting periods for communication, results and potential variability in assay timing. To date, we are still waiting for the 2024 sample assays and additional analyses. These logistical barriers highlighted the complexities of conducting multi-institutional studies where external collaborators manage essential components of data analysis.

Small Sample Size and Statistical Power The study's relatively small sample size also limits the statistical power to generalise results to a broader SLE population. While the observed trends suggest a beneficial effect of temporarily withholding MMF, the small cohort may not fully capture the variability in patient responses. We

have tried to demonstrate that our cohort is a good representation of the patients with the more severe disease spectrum, and among them have more uniformity and consistency to provide a valid observation.

Implications for Practice Despite these limitations, this study provides an important starting point for understanding how temporary MMF withdrawal can influence vaccine efficacy in SLE patients. Clinicians should weigh the potential benefits of improved vaccine response against the risks of a short-term pause in immunosuppressive therapy. Further research with larger sample sizes, streamlined collaborative efforts, and clearer logistical pathways will be essential to corroborate these findings and refine practical recommendations.

In conclusion, while the study demonstrated promising results, the limitations underline the need for continued investigation. Addressing recruitment delays, enhancing collaboration efficiency, and expanding sample size will be crucial for future studies to build on these findings and provide robust clinical guidance.

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