Personalised therapy during preconception and gestation in SLE: usefulness of 6-mercaptopurine metabolite levels with azathioprine

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Although azathioprine (AZA) is the immunosuppressive of choice in SLE pregnancies, no one has evaluated 6-mercaptopurine (6-MP) metabolite levels in this population. Even outside pregnancy, the use of metabolite testing has not been widely applied in SLE.1 AZA is a prodrug that is cleaved to 6-MP, which is converted to the active nucleotides 6-thioguanine (6-TG) and via the enzyme thiopurine methyltransferase (TPMT) to 6-methylmercaptopurine (6-MMP) (figure 1).1 Studies in inflammatory bowel diseases (IBD) established the therapeutic range for 6-TG concentrations between 235 and 450 pmol/8×10⁸ red blood cells (RBC), as higher concentrations are associated with higher risk of myelotoxicity without increased efficacy.1 6-MMP levels >5700 pmol/8×10⁸ RBC are associated with a higher risk of hepatotoxicity.1 Additionally, a subgroup of patients resistant to AZA shunts 6-MP towards the overproduction of 6-MMP, which is reflected in an inability to achieve therapeutic 6-TG levels despite dose escalation.1

In one study, 31% of patients on AZA were identified as ‘shunters’.1 Identifying patients as non-adherent, treatment-refractory or undertreated, as well as identifying drug toxicity, could improve the efficacy and safety of clinical decision-making. As pregnancy is a particularly critical period to optimise disease control and minimise drug toxicity, we evaluated 6-MP metabolite levels in women with SLE taking AZA during the preconception and/or gestational periods.

We performed a retrospective assessment of women with SLE aged 18–40 years with at least one McGill Lupus Cohort annual study visit between January 2017 and July 2019. Among all females on AZA who were pregnant or trying to conceive, we identified those with 6-MP metabolite levels during this interval. All patients in this cohort are tested for TPMT enzymatic levels and only those with normal levels are started on AZA. We characterised patients with undetectable, low or normal 6-TG levels, as well as ‘shunters’ (ie, 6-MMP to 6-TG ratio ≥20) with high 6-MMP) using therapeutic reference ranges for IBD, since none exist for SLE.2 We suggested a possible metabolite interpretation as ‘shunter’ (ie, 6-MMP to 6-TG ratio ≥20 with high 6-MMP), non-adherent (undetectable or barely detectable metabolite levels despite appropriate dosing) or subtherapeutic (low metabolite levels with AZA dose ≤2.0 mg/kg/day). We summarised key clinical characteristics (ie, prior lupus nephritis, lupus low disease activity state attainment, pregnancy outcomes) of these patients.

Among 29 women of reproductive age with SLE over the study period, eight were pregnant or trying to conceive. Of these, six had 6-MP metabolite levels performed at least once (see table 1 for patients’ characteristics). All except one had prior lupus nephritis.
### Table 1  Characteristics of patients with SLE on AZA who were pregnant or trying to conceive at the time of 6-mercaptopurine metabolite level monitoring

| Case | Conception status | Prior lupus nephritis | AZA dose (mg/kg/day) | Metabolite levels (pmol/8×10^8 RBC) | Disease activity at time of metabolite measurement | Pregnancy outcome | Metabolite levels interpretation | Action based on therapeutic drug monitoring |
|------|-------------------|-----------------------|----------------------|-----------------------------------|-----------------------------------------------|------------------|--------------------------------|----------------------------------------|
| 1    | Preconception     | Yes                   | 2.6                  | 141 (low) 5899 (elevated)         | LLDAS                                         | Not applicable   | ‘Shunter’*                    | Switched to tacrolimus                |
|      |                   |                       | 2.6                  | 129 (low) 5888 (elevated)         | LLDAS                                         |                  |                              |                                        |
| 2    | Preconception     | Yes                   | 1.9                  | Not detectable Not detectable     | LLDAS                                         | Not applicable   | Non-adherence†                | Adherence discussion                  |
| 3    | Pregnant          | No                    | 1.2                  | 109 (low) 384                    | No LLDAS                                      | Pre-eclampsia and preterm birth               | Subtherapeutic dosing‡                | Dose increased                        |
|      |                   |                       | 1.8                  | 207 (low) 3361                   | LLDAS                                         |                  |                              |                                        |
|      |                   |                       | 2.1                  | Not detectable Not detectable    | LLDAS                                         |                  |                              |                                        |
| 4    | Preconception     | Yes                   | 2.3                  | 330 (normal) 3019                | LLDAS                                         | Not applicable   | Therapeutic dosing            | Continued same dose                   |
| 5    | Pregnant          | Yes                   | 2.5                  | 44 (low) 269                     | LLDAS                                         | Uncomplicated term pregnancy               | Non-adherence versus subtherapeutic dosing | Adherence discussion                  |
| 6    | Pregnant          | Yes                   | 1.5                  | 155 (low) 3392                   | LLDAS                                         | Uncomplicated term pregnancy               | Subtherapeutic dosing                | Discussion about dose increase (patient refused) |

*Shunter: 6-MMP to 6-TG ratio ≥20 with high 6-MMP.
†Non-adherence: not detectable or barely detectable metabolite levels (ie, up to twice the minimal detectable 6-TG levels which is 30 pmol/8×10^8 RBC) despite adequate AZA dosing.
‡Subtherapeutic dosing: 6-TG levels <235 pmol/8×10^8 RBC while receiving a dose of AZA <2.0 mg/kg.
§Reference range for metabolite levels: 6-TG concentrations between 235 and 450 pmol/8×10^8 RBCs and 6-MMP levels <5700 pmol/8×10^8 RBC; LLDAS: as per previously validated definition: (1) SLEDAI-2K ≤4, with no activity in major organ systems (renal, central nervous system, cardiopulmonary, vasculitis, fever) and no haemolytic anaemia or gastrointestinal activity; (2) no new lupus disease activity compared with the previous assessment; (3) a Safety of Estrogens in Lupus Erythematosus National Assessment-SLEDAI physician global assessment (scale 0–3) ≤1; (4) a current prednisolone (or equivalent) dose ≤7.5 mg/day and (5) well-tolerated standard maintenance doses of immunosuppressive drugs and approved biological agents.
AZA, azathioprine; LLDAS, lupus low disease activity state; 6-MMP, 6-methylmercaptopurine; RBC, red blood cells; SLEDAI, SLE Disease Activity Index; 6-TG, 6-thioguanine.
and all except one had quiescent disease over the study interval. Half (3/6) of the women were planning to conceive, while the other half (3/6) became pregnant. In most (5/6), 6-TG levels were below the normal range. Among these, three patients had non-detectable or barely detectable levels, despite appropriate drug dosing, suggesting non-adherence; two of these were pregnant at the time of measurement. One patient was determined a ‘shunter’ (i.e., case #1 in table 1) and was thus switched to tacrolimus. Of the three pregnancies, two had no adverse outcome (despite low maternal 6-TG) and one was complicated by pre-eclampsia and preterm birth (with non-detectable maternal 6-TG). Given that approximately 20% of SLE pregnancies experience placenta-mediated complications, it is unclear if the non-detectable 6-TG levels contributed to pre-eclampsia in this patient.

Our study is the first to assess 6-MP monitoring in women with SLE prior to conception and during pregnancy. Despite small numbers, our findings highlight key opportunities to personalise therapy during this critical period. In particular, identification of ‘shunting’ helps avoid unnecessary and potentially harmful dose escalation, with the option to switch patients to an alternative pregnancy-compatible drug, such as tacrolimus. We also demonstrate how dose escalation can be done safely, as we can avoid 6-TG and 6-MMP levels that are known to be toxic in the IBD population. Lastly, we observed a substantial number of non-adherent patients, which allows focus to be placed on discussing adherence during clinic visits and may guide future treatment approaches. The importance of appropriate control of disease is of heightened importance during pregnancy, a vulnerable time for both mother and baby.

We need to acknowledge potential limitations. We used therapeutic reference ranges for IBD, as none exist for SLE. It has been suggested that target ranges in SLE may be lower than in IBD.\(^1\) Furthermore, in a small study (n=30), pregnancy was found to potentially affect AZA metabolism resulting in a mild decrease in 6-TG levels during gestation with a return to preconception baseline levels after delivery.\(^3\) However, adherence to AZA was not assessed in this study and might have contributed at least in part to the findings.

In conclusion, the use of personalised metabolite monitoring is a promising strategy as it might improve efficacy and safety in our vulnerable patient population prior to or during pregnancy. A metabolite-guided approach could likely be applied to a broader SLE population. In patients with active SLE despite AZA, it may allow identification of the cause of treatment failure as non-adherence, undertreatment or treatment-refractoriness. There is a great need for larger prospective studies to establish target 6-MP levels in pregnant and non-pregnant patients with SLE.

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**REFERENCES**
1 Askanas AD, Wallace DJ, Weisman MH, et al. Use of pharmacogenetics, enzymatic phenotyping, and metabolite monitoring to guide treatment with azathioprine in patients with systemic lupus erythematosus. J Rheumatol 2009;36:89–95.
2 Chapdelaine A, Mansour A-M, Troyanov Y, et al. Metabolite monitoring to guide thiopurine therapy in systemic autoimmune diseases. Clin Rheumatol 2017;36:1341–8.
3 Jharap B, de Boer NKH, Stokkers P, et al. Intrauterine exposure and pharmacology of conventional thiopurine therapy in pregnant patients with inflammatory bowel disease. Gut 2014;63:451–7.