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Azathioprine metabolite levels and outcomes during pregnancies with rheumatic disease

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To cite: Balevic S, Sims CA, Eudy A, *et al*. Azathioprine metabolite levels and outcomes during pregnancies with rheumatic disease. *Lupus Science & Medicine* 2024;**11**:e001036. doi:10.1136/lupus-2023-001036

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/lupus-2023-001036).

Received 29 August 2023
Accepted 14 December 2023

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ABSTRACT

Objective Despite widespread use of azathioprine (AZA) during pregnancy, no studies evaluated the impact of pregnancy on AZA metabolites 6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine nucleotide (6-MMPN) disposition in rheumatic diseases. This study characterises changes in AZA metabolite concentrations throughout pregnancy in women with rheumatic disease and explores relationships between metabolite concentrations, maternal disease activity, and neonatal outcomes.

Methods Patients with rheumatic disease from a single centre prescribed AZA prior to pregnancy and ≥1 blood sample during pregnancy (5/2016 to 4/2022) were included. Commercial laboratories quantified AZA metabolite concentrations. The upper safety limit for 6-MMPN was >5700 pmol/8×10^8 RBC. The therapeutic target for 6-TGN was ≥159 pmol/8×10^8 RBC. Repeated correlation measures were used to evaluate the relationship between metabolite concentrations and pregnancy duration, and the relationship between 6-TGN concentration and SLE Physician Global Assessment (PGA). The relationship between pregnancy average 6-TGN and neonatal gestational age at birth was analysed using linear regression.

Results Thirty-seven pregnancies in 35 women with 108 serum samples were included. There was no significant difference in dose-adjusted 6-TGN concentrations across pregnancy and peripartum, whereas 6-MMPN concentrations appeared higher during pregnancy. No elevated transaminases or cholestasis were observed concurrently with 6-MMPN above 5700 pmol/8×10^8 RBC. Metabolite concentrations were related to total AZA dosage, weight-based dosage and TPMT phenotype. In pregnant women with SLE achieving average 6-TGN in the therapeutic range, we observed a non-significant reduction in PGA and increase in neonatal gestational age at birth.

Conclusions In this exploratory study, we did not observe systematic changes in 6-TGN concentrations throughout pregnancy and peripartum, whereas 6-MMPN concentrations were higher during pregnancy. Monitoring AZA metabolite concentrations in pregnancy is a potential tool to identify medication non-adherence as well as patients with high 6-MMPN in whom dosage adjustment or close laboratory monitoring may optimise safety.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Azathioprine is commonly prescribed to pregnant women with rheumatic disease to control disease activity. The azathioprine metabolite 6-thioguanine nucleotide is associated with efficacy, whereas the metabolite 6-methylmercaptopurine nucleotide (6-MMPN) is associated with hepatotoxicity. However, the physiological changes of pregnancy can impact drug pharmacokinetics, potentially resulting in therapeutic failure or medication toxicity.

WHAT THIS STUDY ADDS

⇒ We found that concentrations of the hepatotoxic metabolite 6-MMPN is higher during pregnancy and varies widely between patients.

⇒ As in non-pregnant patients, azathioprine metabolite concentrations in pregnancy were related to total daily dosage, weight-based dosage and thiopurine methyltransferase phenotype.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our study highlights the importance of monitoring azathioprine metabolite concentrations and liver function tests during pregnancy.

INTRODUCTION

Active rheumatic disease during pregnancy, particularly lupus nephritis, can result in devastating outcomes, with up to two-thirds of mothers with active SLE delivering preterm, and many others having preeclampsia or other poor pregnancy outcomes.[1] Azathioprine (AZA) is considered safe during pregnancy and breastfeeding, with no evidence of increased rates of miscarriage or congenital malformation in over 1300 studied pregnancies[2]; therefore, the American College of Rheumatology recommends using AZA to control disease activity in pregnant women.[3] Moreover, the use of AZA to control disease activity in pregnancy can reduce the need for concomitant glucocorticoids, which independently increases the risk for preterm birth.[4]

AZA is a pro-drug that is eliminated through the kidneys. AZA is metabolised by multiple enzymes to two relevant metabolites: 6-thioguanine nucleotide (6-TGN), which correlates with efficacy in rheumatic diseases, and 6-methylmercaptopurine nucleotide (6-MMPN), which correlates with hepatotoxicity.[5 6] The thiopurine methyltransferase (TPMT) enzyme catalyses formation of 6-MMPN; although changes in TPMT activity during pregnancy are unknown, TPMT polymorphisms with reduced function result in low 6-MMPN and high 6-TGN.[7] Characterising 6-TGN levels during pregnancy is clinically important, as 6-TGN is known to cross the placenta and may cause neonatal bone marrow suppression,[8] whereas 6-MMPN poorly crosses the placenta. However, 6-MMPN may still be associated with maternal hepatotoxicity[9]; although no guidelines currently exist for monitoring metabolites in pregnancies with rheumatic disease.[9]

Changes in drug pharmacokinetics (PK) during pregnancy may include alterations in the volume of distribution secondary to increasing adipose tissue and total body water and changes in clearance secondary to increased glomerular filtration and alterations in drug-metabolising enzymes.[10 11] Because AZA undergoes extensive metabolism and is eliminated largely through the kidneys,[12 13] the changes in physiology that occur during pregnancy may alter AZA and metabolite blood levels, thereby potentially resulting in therapeutic failure or increased risk of toxicity.[10] Limited evidence in inflammatory bowel disease suggests that 6-TGN decreases during pregnancy while 6-MMPN increases.[14]

Despite AZA being used extensively in pregnant patients with rheumatic disease, and despite the significant impact of potential treatment failure, the effect of pregnancy on 6-TGN and 6-MMPN disposition in women with rheumatic disease remains virtually unknown. Therefore, the aim of this study was to characterise changes in AZA metabolite concentrations throughout pregnancy in women with rheumatic disease and explore the relationship between metabolite concentrations, maternal disease activity, and neonatal outcomes.

METHODS

Study design

We analysed data from a single centre collected from the Duke Autoimmunity in Pregnancy (DAP) and Maternal Autoimmune Disease Research (MADRA) registries, which prospectively collected clinical data on maternal disease activity, demographics, medication use and standard of care laboratory studies during pregnancy and postpartum. Whole blood samples were tested for AZA metabolites (6-TGN and 6-MMPN) for routine clinical care and extracted from the medical record. 6-TGN and 6-MMPN concentrations were quantified by commercial laboratories using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) assays. AZA doses were adjusted at the discretion of the treating provider per routine clinical care.

Participants

From 2016 to 2022, patients with rheumatic disease taking AZA were identified from the DAP and MADRA registries. From 2016 to 2017, patients with any rheumatic disease were included as AZA PK are not known to be different based on disease; from 2018 to 2022, only patients with SLE were included due to preliminary data suggesting a relationship between metabolite concentrations and outcomes. All patients with SLE met either ACR or SLICC classification criteria, and patients with mixed connective tissue disease (MCTD) were categorised as SLE if they had a phenotype with predominant SLE features (eg, lupus nephritis) per the treating physician. Eligible patients had to be maintained on AZA longitudinally during pregnancy with at least two blood samples available for analysis: one blood sample had to be from pregnancy, while the other sample could be from pregnancy, pre-pregnancy or postpartum. Patients were also eligible if they started on AZA during pregnancy. Patients with multiple gestations (eg, twins) were excluded due to potential confounding with metabolite concentrations and outcomes.

Data collection

Registry study visits occurred on average two to three times during pregnancy and once after delivery. TPMT activity or genotype was measured per standard of care and recorded in the electronic health records. Patients were then classified as normal, intermediate or low TPMT phenotypes according to the laboratory's report. TPMT measurement could have occurred at any time in the patient's history. For subjects with SLE, disease activity was measured by one rheumatologist using the Physician Global Assessment (PGA) and the SLE Pregnancy Disease Activity Index (SLEPDAI).[15] The PGA for SLE is a Visual Analogue Scale ranging from 0 (no disease activity) to 3 (severe disease activity); and the SLEPDAI uses the same definitions as the SELENA-SLEDAI.[16] Recent lupus nephritis was defined as nephritis occurring ≤3 years from study inclusion, and active lupus nephritis was defined as proteinuria (≥500 mg/d) occurring before 20 weeks gestation.

AZA dosing was analysed as total daily dose as reported by the patient. When dosing was not reported by the patient, or if dosing was unclear, the total daily dose was determined based on review of the patient's medical record. Preference for determining dose discrepancy was (1) physician-reported dosing in clinic visit note, and (2) prescribed dosing in the pharmacy record. A 'visit' was defined as any encounter with an AZA metabolite level, which could have occurred in the context of an outpatient clinical encounter, a hospitalisation or a laboratory-only visit. Accordingly, some covariates such as disease activity measures may not have been collected for certain visits (eg, laboratory-only encounters or hospitalisations). If covariates were not available on the same day as metabolite testing, the closest values within 30 days of the metabolite level were recorded.

Pregnancy duration in weeks was determined using the date of the last menstrual period and rounded down to represent the most recent 'completed' week. When the last menstrual period was unknown, it was calculated to be 40 weeks prior to the estimated date of delivery. Using American College of Obstetrics and Gynecology guidelines, trimesters were defined as <14 weeks (first trimester), 14–27 weeks (second trimester) and ≥28 weeks (third trimester).[17]

In 2018, we began collecting patient-reported adherence using the Medication Adherence Self-Reported Inventory (MASRI). For patients with MASRI scores, each visit was dichotomised as adherent (MASRI score ≥80) or non-adherent (MASRI score<80).[18 19]

Statistical analysis

AZA metabolite concentrations during pregnancy

We adjusted metabolite levels according to dose by dividing the concentration by the total daily dose in milligrams (mg). We compared metabolite concentrations descriptively as a function of dose and trimester and used repeated correlation measures to generate the correlation coefficient for the relationship between metabolite concentrations and pregnancy duration.[20] In addition, we analysed the correlation between 6-TGN and 6-MMPN concentrations by total daily dose and weight-based dose using repeated correlation measures. Scatterplots were used to visualise adjusted and unadjusted 6-TGN and 6-MMPN levels by weeks of pregnancy gestation for all subjects, excluding those with a dose change as well as those grouped by TPMT phenotype. We summarised 6-TGN and 6-MMPN concentrations descriptively by MASRI adherent category. We defined the upper safety limit for 6-MMPN as >5700 pmol/8×10^8 RBC and a minimum therapeutic target for 6-TGN as≥159 pmol/8×10^8 RBC based on prior literature.[5 6] However, we also explored a higher concentration target of>234 pmol/8×10^8 RBC.[7]

We included all subjects with available data in the primary analyses; however, we performed several sensitivity analyses that excluded (1) visits with metabolite concentrations below the quantifiable limit (BQL); (2) patients with dose changes; (3) patients with a recent history of lupus nephritis and (4) patients who had not achieved steady state, defined as being on a stable dose of AZA for at least 1 month.[21]

AZA metabolite concentrations and maternal disease activity

We stratified patients by disease (SLE and non-SLE) given differences in disease activity measures between the two groups; we did not analyse disease activity in non-SLE subjects due to small sample sizes. We analysed the correlation between SLE PGA and metabolite concentrations using repeated correlation measures. We descriptively analysed the relationship between SLE PGA and two different categorical cutoffs of 6-TGN therapeutic exposure (≥159 and >234 pmol/8×10^8 RBC, respectively).

Neonatal outcomes

We restricted the neonatal outcome analysis to patients with SLE due to differences in risks of preterm birth and low sample size in patients without SLE. We analysed the relationship between pregnancy average 6-TGN and neonatal gestational age at birth using linear regression, unadjusted and adjusted for active lupus nephritis and concomitant prednisone use. We also explored a model including SLE PGA in place of active lupus nephritis. We also analysed the relationship between therapeutic 6-TGN category (<159 vs ≥159 pmol/8×10^8 RBC) and neonatal gestational age at birth using analysis of variance.

All analyses were performed using R and RStudio V.1.4.1717 (2021, RStudio, Boston, Massachusetts, USA). Repeated correlations were conducted using the 'rmcorr' package in R.[20] Samples with metabolite levels BQL were analysed based on the raw value reported by the laboratory.

RESULTS

Clinical characteristics and samples

We enrolled 37 pregnancies from 35 women into the study (table 1). From these 37 pregnancies, we obtained a total of 108 samples for 6-TGN and 6-MMPN across pregnancy and prepartum/postpartum (first trimester=26, second trimester=40, third trimester=22, prepartum=6, postpartum=14). One patient visit at 11 days post-last menstrual period was considered prepartum. Each patient contributed between 2 and 6 samples. The median (IQR) age averaged across each visit was 29 (27–30.5) years. The median (IQR) total daily dosage of AZA was 112.5 (100–150) mg and the median (IQR) weight-based dosage across 104 visits with available weights was 1.56 (1.17–1.96) mg/kg.

Overall, 33 patients (35 pregnancies) had an SLE diagnosis or a predominantly SLE phenotype; disease activity was mild to moderate in these patients as measured by their PGA with a median (IQR) PGA of 0.5 (0–1) across all visits. For patients with SLEPDAI scores available (90/101 visits), the most common disease manifestations at each visit were elevated dsDNA antibody (40%), low complement (32.2%) and rash (17.8%). Eight visits had active arthritis and one visit was complicated by vasculitis. About one-third of pregnancies had moderate to high disease activity (PGA>1) at some point during pregnancy. Twelve patients had a history of lupus nephritis, of which eight had active nephritis ≤3 years prior to study inclusion and six had active nephritis by 20 weeks in at least one pregnancy during the study.

Medication adherence

Excluding non-pregnant visits, there were a total of 22 pregnancies in 20 patients (38 visits) who had MASRI scores. Of those, 35 visits (21 pregnancies) were classified as adherent and 3 visits (3 pregnancies) were classified as non-adherent. Metabolite concentrations in adherent patients are reported in table 2. The concentrations for

Table 1 Demographics and clinical characteristics

N=35 patients, 37 pregnancies, 108 visits	
	N (%) or median (IQR)
Age (years)	29 (27–30.5)
Race	
Black/African American	16/35 (45.7%)
White	13/35 (37.1%)
Asian	5/35 (14.3%)
Multiple/other	1/35 (2.9%)
Diagnosis	
SLE	33/35 (94.3%)
Non-SLE	2/35 (5.7%)
Undifferentiated CTD	1/35 (2.9%)
Linear scleroderma	1/35 (2.9%)
Disease activity at each visit	
SLE PGA (0–3 scale)	0.5 (0–1)
SLE PGA>1	19/90 (21.1%) visits 13/35 (37.1%) patients
History of lupus nephritis	12/35 (34.3%)
Active lupus nephritis	6/35 (17.1%)
Antiphospholipid antibody syndrome	4/35 (11.4%)
Azathioprine	
Total daily dose (mg/day), visit	112.5 (100–150)
Weight-based (mg/kg) dosage	1.56 (1.17–1.96)
Hydroxychloroquine prescribed	96/108 (88.9%)
Prednisone prescribed	63/108 (58.3%)

CTD, connective tissue disease; PGA, Physician Global Assessment.

non-adherent patients are not reported due to very low sample sizes and inability to generate the range across most dosages. Altogether, 28/108 (25.9%) visits had a metabolite concentration BQL.

AZA concentrations throughout pregnancy and prepartum/postpartum

Dose-adjusted mean 6-TGN and 6-MMPN levels by trimester are shown in figures 1 and 2. Overall, a poor linear correlation was observed between pregnancy duration in weeks and dose-adjusted 6-TGN (r=0.11, p=0.45, df=50) and 6-MMPN (r=0.05, p=0.73, df=50) concentrations. We observed minimal changes in dose-adjusted 6-TGN concentrations throughout pregnancy and prepartum/postpartum with the exception of a slight decrease in the third trimester. However, dose-adjusted 6-MMPN concentrations appeared higher in pregnancy compared with pre-pregnancy or postpartum, but the data did not permit formal statistical testing. Similarly, the ratio of 6-MMPN to 6-TGN also appeared higher during pregnancy compared with pre-pregnancy and postpartum,

Table 2 Metabolite concentrations in pregnant patients adherent by MASRI score

Metabolite	Metabolite concentrations (pmol/8×10^8 RBC)				
	Azathioprine total daily dosage				
	50 (n=1)	100 (n=16)	125 (n=3)	150 (n=14)	200 (n=1)
6-TGN	104	69.5 (23-334)	73 (68–81)	102.5 (52-432)	110
6-MMPN	710	1349.5 (250–5087)	3959 (3639–4073)	4282 (274–13 184)	12 859

Concentrations reported as median (range). Sample size represents the number of visits.
6-MMPN, 6-methylmercaptopurine nucleotide; 6-TGN, 6-thioguanine nucleotide.

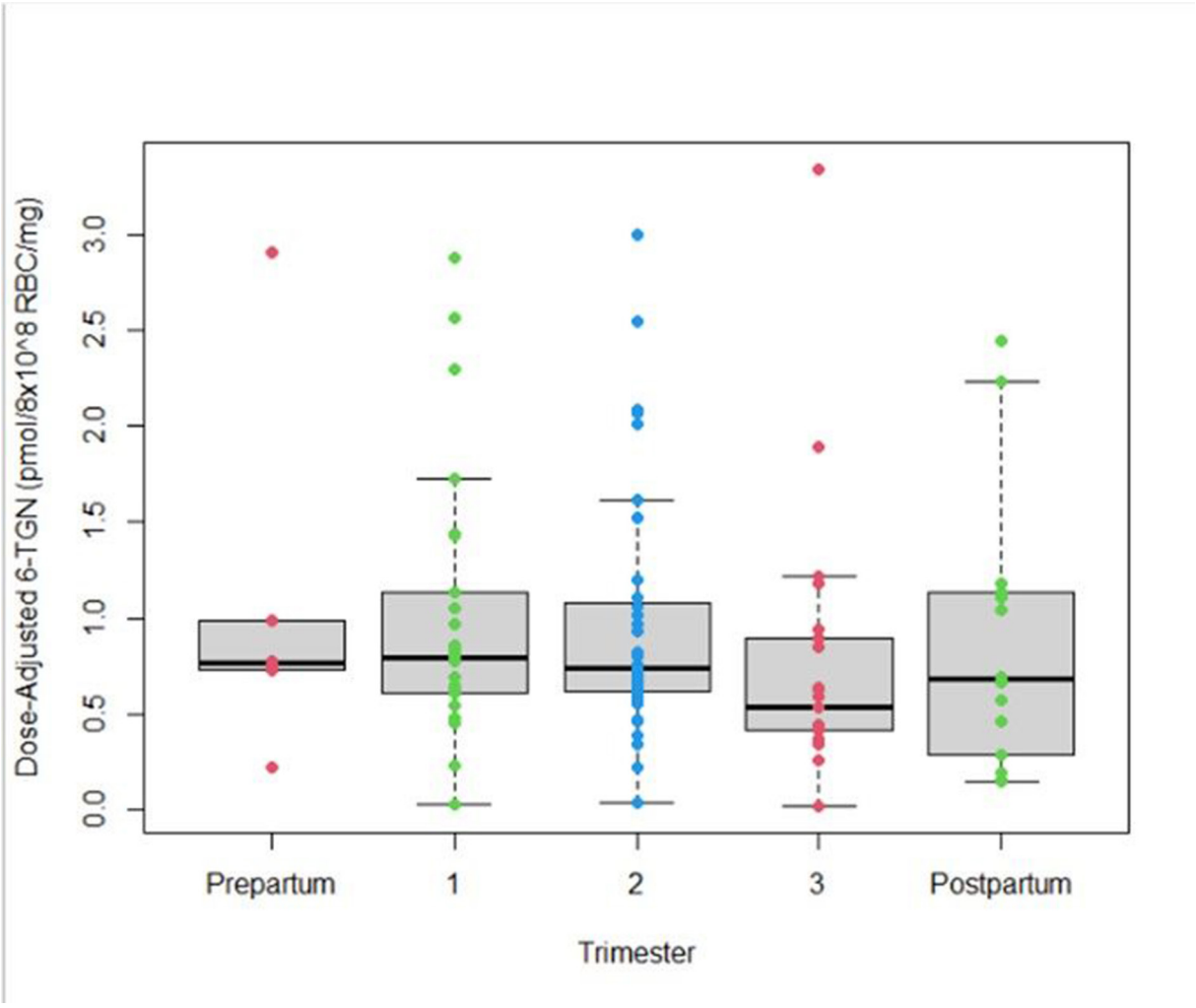

Figure 1 Dose-adjusted 6-TGN throughout pregnancy and peripartum. Grey area represents the IQR, whereas the solid black line represents the median. Coloured dots represent individual data points. 6-TGN, 6-thioguanine nucleotide.

but relatively stable once pregnancy commenced (online supplemental figure 1).

Wide interindividual and intraindividual variability in metabolite concentrations was observed, even accounting for different AZA dosages. As expected, patients with intermediate metabolising phenotypes for TPMT function had higher 6-TGN concentrations compared with those with normal function. These higher concentrations were still observed after adjusting for AZA total daily dosage (online supplemental figure 2).

Individual patient trajectories for 6-TGN and 6-MMPN in patients without dose changes are noted in online supplemental figure 3.

Relationship between AZA dose, TPMT and metabolite concentrations

There were significant, moderate associations between the total daily dosage of AZA and 6-TGN (r=0.49, p<0.001, df=70) and 6-MMPN concentrations (r=0.54, p<0.001, df=70). There was also a significant, but weaker correlation between weight-based dosage and 6-TGN (r=0.37, p=0.002, df=66) and 6-MMPN (r=0.47, p≤0.001, df=66).

Of pregnancies with known TPMT enzyme measurement, 26/30 (86.7%) had normal activity and 4/30 (13.3%) had intermediate activity. Subjects with intermediate baseline TPMT activity had higher dose-adjusted 6-TGN compared with those with normal baseline TPMT (median 2.23 vs 0.71 pmol/8×10^8 RBC/mg). Dose-adjusted 6-MMPN was lower in those with intermediate metabolising phenotypes (median 12.09 vs 16.97 pmol/8×10^8 RBC/mg). As expected, those with intermediate metabolising phenotypes also had lower ratios of 6-MMPN to 6-TGN (median 6.8 vs 24.6 pmol/8×10^8 RBC).

Sensitivity analyses for AZA concentrations

No clear difference was observed in dose-adjusted 6-TGN concentrations throughout pregnancy and pre-pregnancy/postpartum when examined in several subgroups, excluding (1) visits with metabolite concentrations BQL; (2) patients with dose changes and (3) patients with a recent history of lupus nephritis. The slight decrease in dose-adjusted 6-TGN in the third trimester was less apparent when excluding patients who had a dosage change, but more pronounced in the small subgroup of

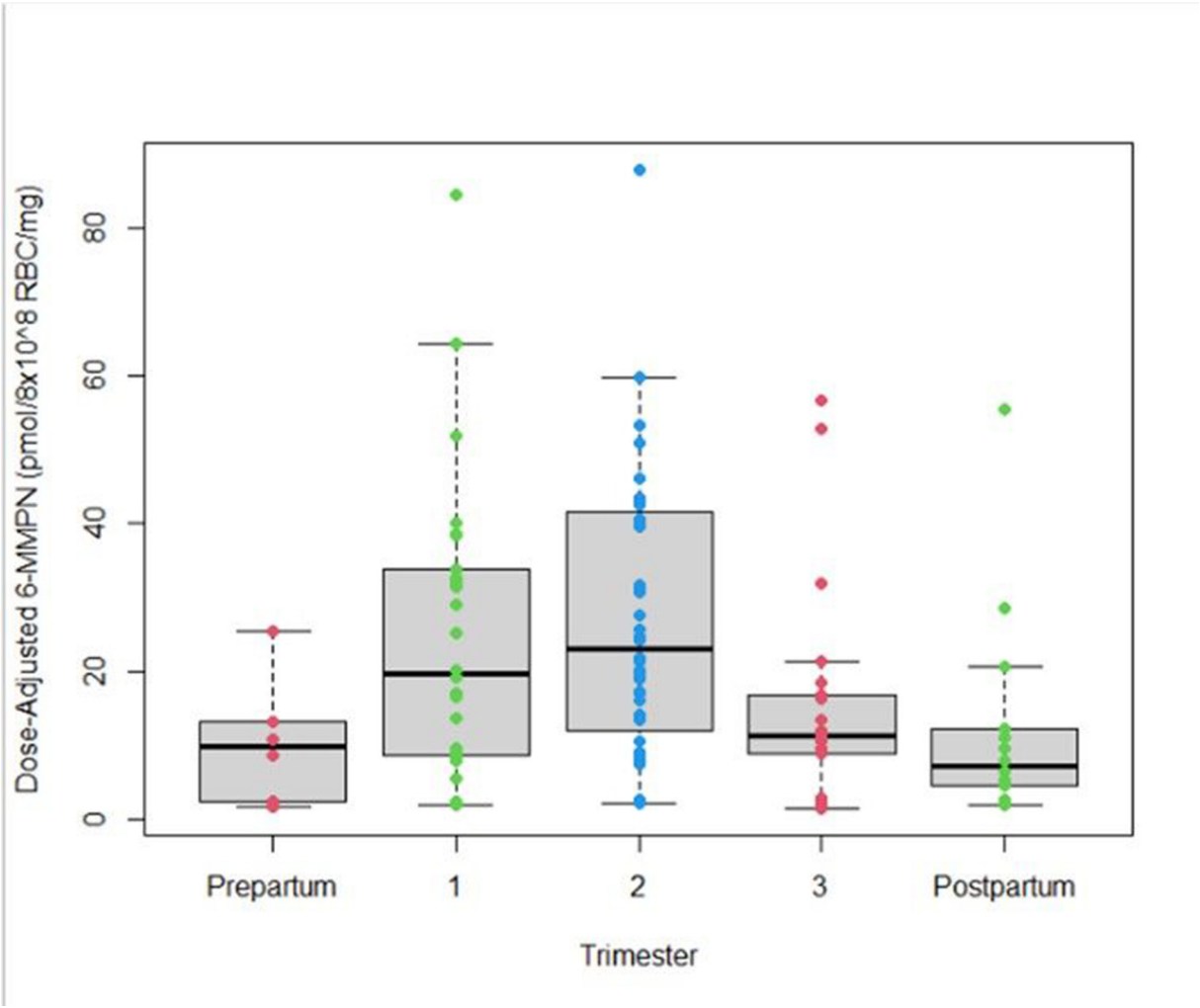

Figure 2 Dose-adjusted 6-MMPN throughout pregnancy and peripartum. Grey area represents the IQR, whereas the solid black line represents the median. Coloured dots represent individual data points. 6-MMPN, 6- methylmercaptopurine nucleotide.

patients with a recent history of lupus nephritis. Among these subgroups, dose-adjusted 6-MMPN still appeared higher during pregnancy compared with pre-pregnancy or postpartum, although the data did not permit formal statistical testing among subgroups. There were too few observations of patients with active lupus nephritis during pregnancy to evaluate changes in metabolite concentrations across pregnancy duration.

Overall, 96/108 (88.9%) visits were expected to have achieved steady state concentrations, defined as having been on a stable dose of AZA for at least 1 month. When excluding patients who had not achieved steady state, the results were overall similar; dose-adjusted 6-TGN was relatively stable throughout pregnancy except for a small decrease in the third trimester, and dose-adjusted 6-MMPN remained higher during pregnancy.

Safety

Ten patients with 13 pregnancy visits (12.0% of visits) had MMPN concentrations in ranges concerning for hepatotoxicity (>5700 pmol/8×10^8 RBC). Eight of the ten patients had normal TPMT activity, one patient had intermediate activity and one patient had unknown activity. The median (IQR) weight-based dosage at these visits was 2.1 mg/kg (1.8–2.1). Reassuringly, none of the patients with elevated 6-MMPN had elevated aspartate aminotransferase (AST) or alanine transaminase (ALT) values concurrently with high 6-MMPN, and none of the patients were diagnosed with cholestasis during pregnancy. However, most patients had reductions in their AZA dosage once high 6-MMPN was observed, possibly preventing the development of hepatotoxicity (online supplemental table 1).

Maternal SLE disease activity

For patients with SLE, the PGA was available for 90/101 (89.1%) visits. No linear association was observed between SLE PGA and 6-TGN concentration (r=0.04, p=0.75, df=54) or 6-MMPN concentration (r=−0.05, p=0.71, df=54). Across all pregnancy and non-pregnancy visits, SLE PGA appeared lower in those achieving 6-TGN≥159 pmol/8×10^8 RBC (figure 3) and >234 pmol/8×10^8 RBC (online supplemental figure 4), but formal hypothesis testing could not be conducted due to the unequal number of

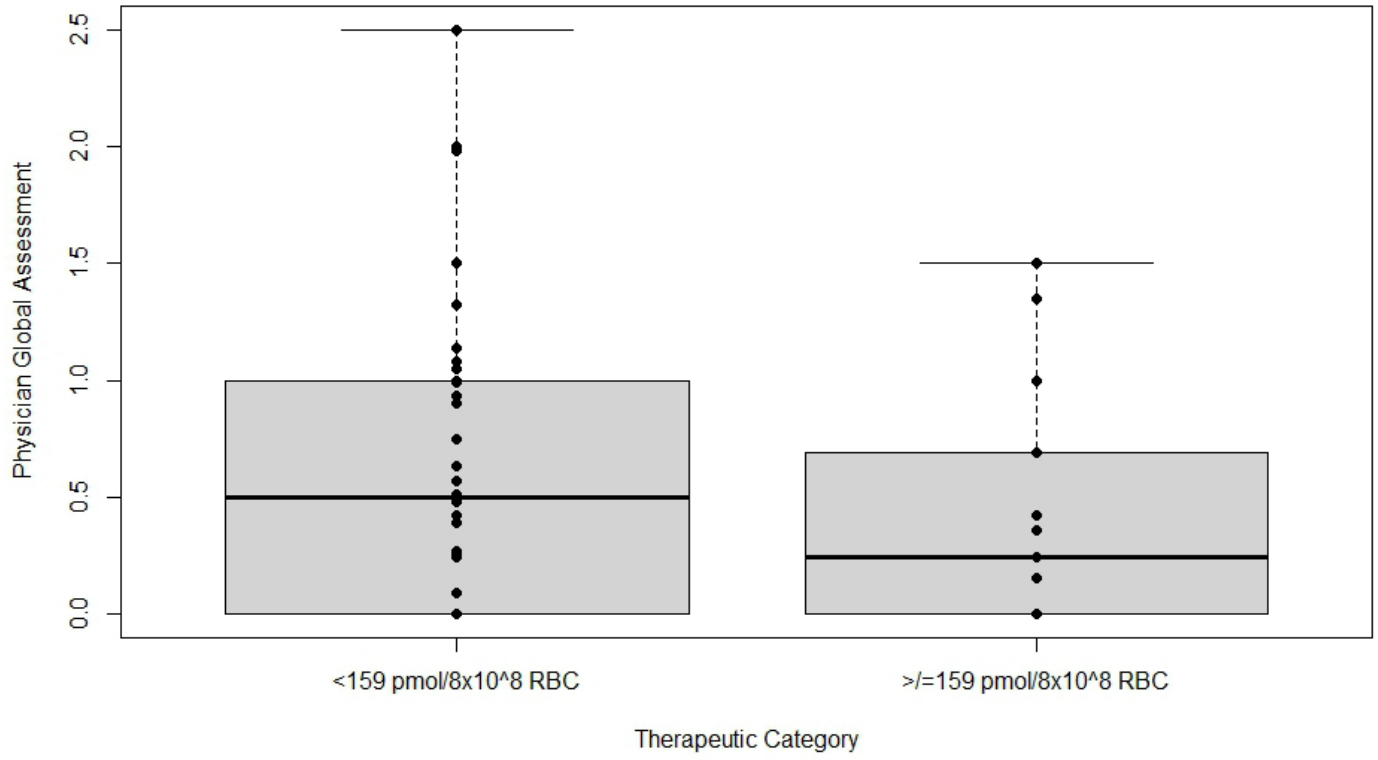

Figure 3 SLE Physician Global Assessment and 6-TGN therapeutic category. Grey area represents the IQR, whereas the solid black line represents the median. 6-TGN, 6-thioguanine nucleotide.

observations per patient. The median (IQR) SLE PGA was 0.5 (0–1) in those with 6-TGN<159 pmol/8×10^8 RBC and 0.24 (0–0.69) in those with 6-TGN≥159 pmol/8×10^8 RBC. The median (IQR) SLE PGA was 0.5 (0–1) in those with 6-TGN<234 pmol/8×10^8 RBC and 0.15 (0–0.71) in those with 6-TGN>234 pmol/8×10^8 RBC. However, only 15/101 (14.9%) visits for women with SLE had a 6-TGN concentration ≥159 pmol/8×10^8 RBC.

The SLEPDAI was available for 90/101 (89.1%) visits. No linear association was observed between SLEPDAI and 6-TGN concentration or 6-MMPN concentration (r=0.02, p>0.8, df=54 for both). Across all pregnancy and non-pregnancy visits, SLEPDAI appeared lower in those achieving 6-TGN≥159 pmol/8×10^8 RBC (figure 4) and >234 pmol/8×10^8 RBC, consistent with the PGA analysis.

Neonatal outcomes

Of the 35 SLE pregnancies, 33 (94.3%) had live births and 11/32 (34.4%) delivered preterm. Of the two pregnancies (four visits) without live births, all 6-TGN levels were subtherapeutic (61, 68, 73 and 113 pmol/8×10^8 RBC). Among those with live births, pregnancy average 6-TGN was higher in pregnancies that did not deliver preterm with a mean of 128.7 vs 76 pmol/8×10^8 RBC. Overall, the median (IQR) gestational age among those with live births and available birth data (n=32) was 37 (35–37) weeks.

Analysed as a continuous variable, there was a non-significant increase in neonatal gestational age at birth with pregnancy average 6-TGN concentrations; the results were not significantly different when controlling for active lupus nephritis or SLE PGA, and concomitant prednisone usage. Using categorical cutoffs of therapeutic exposure, the average gestational age was higher in those with pregnancy average 6-TGN ≥159 pmol/8×10^8 RBC (35.9 vs 38 weeks), but the results were not statistically significant. However, there were only four pregnancies with average 6-TGN ≥159, none of which delivered preterm. The results were similar using a pregnancy average 6-TGN of >234 pmol/8×10^8 RBC.

DISCUSSION

In this observational study of pregnant women with rheumatic disease, we only observed a statistically significant association between AZA dose and metabolite concentrations. However, we made several important observations including minimal changes in 6-TGN concentrations throughout pregnancy and peripartum. Because there were no clinically relevant changes in exposure, our data suggest that routine dosage adjustments to maintain AZA efficacy may not be required in pregnancy. However, 6-MMPN was higher in pregnancy compared with non-pregnancy, although reassuringly, none of the 10 patients with high 6-MMPN developed clinically evident hepatotoxicity. Because most patients with high 6-MMPN also had their dosages reduced, it is possible that the development of hepatotoxicity was attenuated in our cohort. Moreover, there was high variability in AZA metabolite concentrations within and between patients. Accordingly,

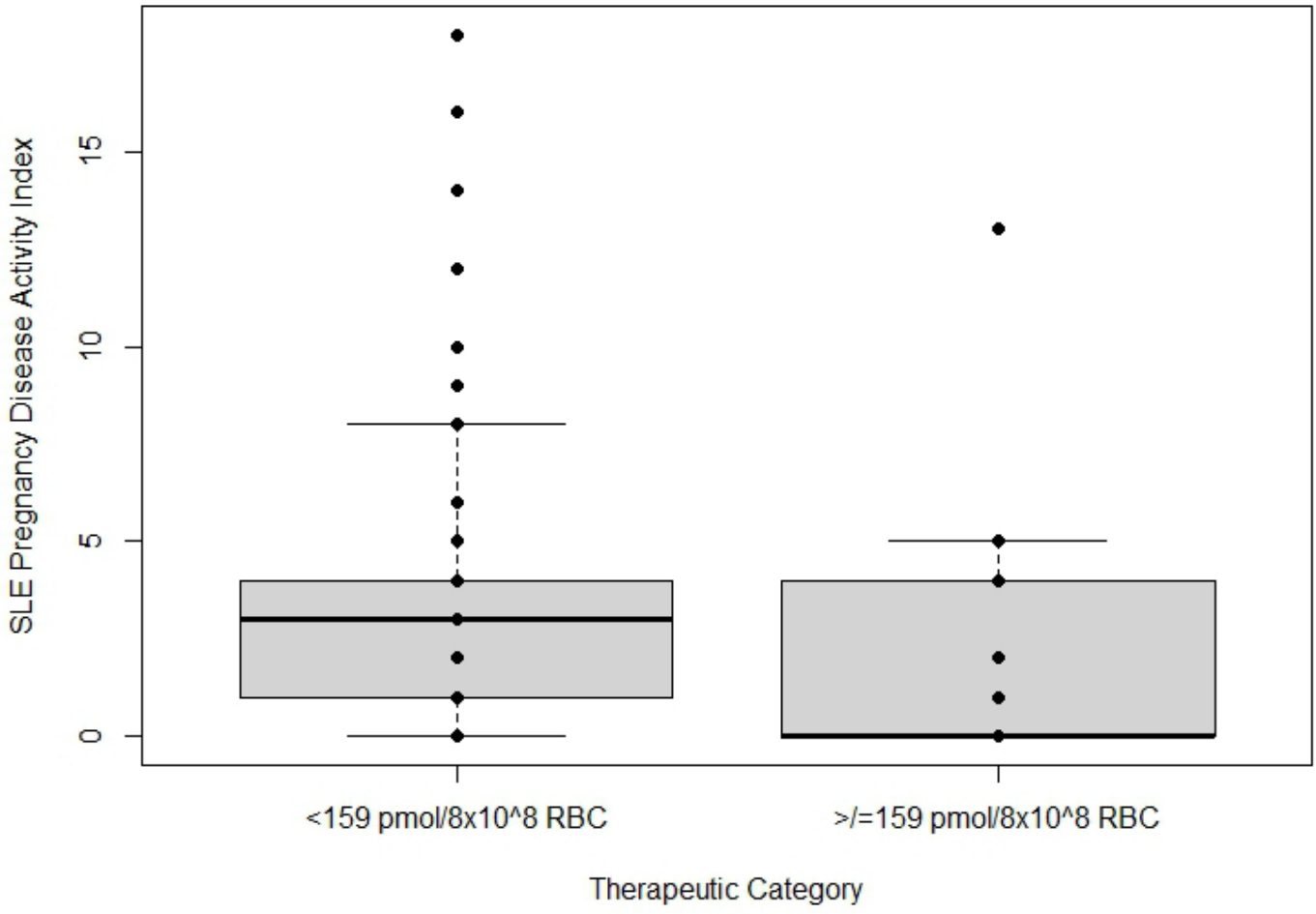

Figure 4 SLE Pregnancy Disease Activity Index and 6-TGN therapeutic category. Grey area represents the IQR whereas the solid black line represents the median. 6-TGN, 6-thioguanine nucleotide.

measuring metabolite concentrations during pregnancy is a potential tool to identify patients in whom closer monitoring of liver function tests could optimise safety.

Additionally, we found a significant, but weak-to-moderate correlation between weight-based AZA dosage and metabolite concentrations in pregnancy, as well as higher 6-TGN concentrations in patients who had intermediate baseline TPMT activity. Although the relationship between TPMT activity and 6-TGN is well characterised in non-pregnant adults, it is unknown whether TPMT activity changes during pregnancy. Reassuringly, we observed that intermediate baseline TPMT activity (measured while non-pregnant) was still associated with 6-TGN concentrations even during pregnancy. Therefore, our study confirms the importance of using total body weight to guide initial AZA dosing and using lower doses for patients with low or intermediate TPMT activity.

Medication adherence in pregnant women with lupus is challenging, with up to one in four pregnancy visits being complicated by severe medication non-adherence.[22] Although limited by a small number of visits with MASRI scores, we identified a range of 6-TGN and 6-MMPN concentrations in patients classified as adherent by the MASRI score. Available measures of medication adherence (eg, MASRI, pill count, refill records, drug concentrations) each have individual strengths and limitations, but it is likely that low AZA drug concentrations may better identify severe medication non-adherence.[18] Accordingly, monitoring AZA concentrations during pregnancy may help clinicians identify pregnant women whose concentrations are concerning for non-adherence and provide an opportunity to counsel patients in order to optimise pregnancy outcomes.[23] However, due to the small number of patients in our study with MASRI scores and the potential for missing data at non-random, studies leveraging non-biological measures of medication adherence (eg, pharmacy refill data, electronic pill bottle caps) will be useful to confirm the metabolite concentrations most suggestive of non-adherence.

In prior reports of pregnant women with inflammatory bowel disease on stable dosing of AZA, it was reported that 6-TGN decreased and 6-MMPN increased during pregnancy.[14] In our cohort, we similarly observed an increase in 6-MMPN and an increase in the ratio of 6-MMPN to 6-TGN during pregnancy compared with non-pregnancy, but minimal changes in 6-TGN. The reason for higher 6-MMPN during pregnancy is unclear, but could be due to changes to the metabolite's PK (eg, clearance or volume of distribution), or less likely alterations in TPMT activity (given the lack of change with

6-TGN) or other drug-metabolising enzyme or transporter. Certain patients did have a notable increase in the ratio of 6-MMPN to 6-TGN, suggesting the possibility of individual patient alterations in drug PK. Ultimately, a formal PK study, along with longitudinal measurements of TPMT activity, are needed to definitively characterise changes to AZA disposition during pregnancy. Based on the available data at this time, it seems reasonable for non-pregnant women who become pregnant on AZA to continue their current dosage. Clinicians could then consider increasing the dosage to control SLE disease activity as needed while measuring 6-TGN and 6-MMPN concentrations to monitor for individual PK changes.

Data from a prospective dose-escalation study of AZA in non-pregnant adults with SLE suggest that patients may respond at a lower concentration than traditionally targeted in inflammatory bowel disease (159 vs 235 pmol/8×10^8 RBC).[5] In pregnant women with SLE achieving average 6-TGN≥159 pmol/8×10^8 RBC, we observed a trend towards improved PGA, but the study was not designed to definitively evaluate efficacy. Additionally, it is worth noting that we only had PGA and SLEPDAI data at approximately 89% of visits, so it is possible that missing data (including while hospitalised) or unmeasured confounders impacted our observations. With regard to neonatal outcomes, we also observed a trend towards higher gestational ages with rising 6-TGN concentrations. Reassuringly, none of the pregnancies whose average 6-TGN≥159 pmol/8×10^8 RBC delivered preterm, although only four pregnancies consistently achieved these concentrations. Due to the low number of pregnancies achieving average 6-TGN≥159, regression analyses were underpowered to explore the relationship between 6-TGN and neonatal outcomes. Additionally, we did not have outcome data for all patients, and it is possible that unintentional bias could be introduced if missing data did not occur at random. It is also possible that changes in concomitant medications or medication adherence could have caused further variability in disease activity or neonatal outcome assessments. Lastly, our analysis was limited to preterm birth and gestational age at outcomes, which although are highly clinically relevant, do not capture the full spectrum of neonatal outcomes. Nevertheless, the trend towards improving disease activity and neonatal gestational age with higher 6-TGN concentrations highlights the need for further study.

There are important limitations with any opportunistic, registry-based study. On rare occasion, there were discrepancies between the study database and medical records; for example, medication dosages reported by patients in study surveys, written in the clinic note by the provider or on the prescription itself. Accordingly, we adjudicated AZA dosing and other data as noted in the methods. The lower range of AZA dose in this study is likely multifactorial due to (1) dose reductions in patients with elevated 6-MMPN; and (2) relatively mild disease activity (median PGA of 0.5). Second, we did not have measures of medication adherence for all patients, and it is possible that nausea or other illness impacted medication adherence. It is also possible that the MASRI under-classified non-adherence, as nearly a quarter of visits had very low metabolite concentrations. Third, we were unable to account for the time elapsed since the last AZA administration. Despite this potential limitation, the within-day variations for the metabolite concentrations are very low due to their long half-lives in red blood cells (~5 days).[21] Moreover, medication adherence is unlikely to significantly alter study conclusions as we conducted a sensitivity analysis where we excluded very low concentrations. Additionally, some data were either missing or were not available at the same time that the AZA metabolite was measured, and we therefore imputed the closest value within 30 days based on available medical records. Lastly, because SLE in pregnancy is complex and heterogeneous, disease misclassification is possible and may be due to either pregnancy complications (eg, differentiating between lupus nephritis and preeclampsia) or evolving disease manifestations over time.

Despite potential limitations, there are several notable strengths of the study. First, to our knowledge, we present the largest study of AZA metabolites in pregnancies with rheumatic disease. Second, we had longitudinal drug measurements in patients before, during and after pregnancy, which uniquely enabled us to evaluate for changes in metabolite concentrations across patients and within an individual patient. Lastly, we were able to quantify AZA concentrations in a subgroup of patients classified as adherent using the MASRI, providing preliminary data concerning a range of concentrations consistent with medication adherence without relying on drug concentrations alone.

To optimise the dose of AZA for pregnant women with rheumatic disease, formal PK/pharmacodynamic (PD) studies are needed. To optimise PK studies, researchers should prospectively collect the date/time of AZA administration along with metabolite concentrations, measures of medication adherence and metabolising enzyme activity longitudinally throughout pregnancy. PK models can then be linked with disease activity or other outcome (pharmacodynamic) measures, accounting for potential confounders, and the combined PK/PD model could then be used to definitively characterise the optimal dosage of AZA.

Conclusions

In this exploratory study, we observed minimal changes in 6-TGN concentrations throughout pregnancy and peripartum, whereas 6-MMPN concentrations appeared higher during pregnancy. Accordingly, monitoring metabolite concentrations in pregnancy is a potential tool to help identify patients with medication non-adherence, as well as those with high 6-MMPN in whom more frequent liver enzyme monitoring or dosage adjustment may optimise safety.

Contributors The study was designed by SB. The acquisition of data was performed by SB, MC, AE and CAS. The interpretation and analysis of the data was performed by all authors. The work was drafted by SB. MC, as guarantor, accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish. All authors critically reviewed or revised the manuscript, approved the submitted version and agree to be accountable for all aspects of the work.

Funding Support from the study was provided by the Rheumatology Research Foundation's Scientist Development Award, the Thrasher Research Fund and a Duke Health ENABLE grant.

Competing interests SB receives support from the National Institutes of Health, the US Food and Drug Administration, the Childhood Arthritis and Rheumatology Research Alliance, Purdue Pharma, and consulting for UCB. SB serves on an NIH DSMB. AE receives grant support from GlaxoSmithKline. MC is a consultant to and has grants from UCB and GSK.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by The Duke Institutional Review Board (IRB #Pro00000756, #Pro00084014, #Pro00000775). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. A limited dataset may be made available upon reasonable request of the corresponding author.

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