

Sulfadiazine plasma concentrations under pyrimethamine and sulfadiazine therapy in pregnant women with acquired vs ocular toxoplasmosis: a case-control study.

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Received Date : June 06, 2024

Accepted Date : June 08, 2024

Published Date : July 08, 2024

ABSTRACT

Context : Empirical guidelines, mostly grounded in experimental data, are utilized to treat pregnancy-acquired toxoplasmosis. Our study aims to close this gap in the literature by providing pharmacological data on pregnant women treated with pyrimethamine (PY) and sulfadiazine (SA) for acute *Toxoplasma gondii* infection.

Techniques : 89 pregnant women with primary *Toxoplasma* infection (PT) who were treated with PY (50 mg first dose, then 25 mg/day), SA (50 mg/kg of body weight/day), and folic acid (10–15 mg per week) were included in this retrospective case-control study. These were contrasted with a group of 17 women who had been treated for acute ocular toxoplasmosis (OT) with a 75 mg PY dose at first, followed by a 25 mg dose twice daily, while adhering to the same SA and folic acid regimen.

There was no documentation of the precise time between drug administration, blood work, and co-medication. Using liquid chromatography-mass spectrometry, the plasma levels of PY and SA were measured 14±4 days following treatment initiation. The Mann-Whitney U test was used to compare the results at a $p < 0.05$ level.

Results : SA levels were less than 20 mg/l in 23 PT patients (26%). Out of the 23 patients, 15 of them (17%) had PY levels that were lower than 700 µg/l. Despite the same SA dosage method, there were notable differences in the drug concentrations between individuals and groups (PY: PT

median 810 µg/l, 95% CI for the median [745; 917] vs. OT 1230 µg/l [780; 1890], $p = 0.006$; SA: PT 46.2 mg/l [39.9; 54.4] vs. OT 70.4 mg/l [52.4; 89], $p = 0.015$).

Conclusions : When compared to OT patients, pregnant women with PT had median SA plasma concentrations that were 34% lower, and in a significant number of PT patients, these concentrations were below a lower reference value of 50 mg/l. Thus, a still-unsupportable transmission risk may be explained by the interindividual variability of plasma concentrations combined with consistently reduced medication levels and potentially decreased compliance in pregnant women. It is worthwhile to take into account systematic drug-level testing in PT receiving PY/SA treatment.

Keywords : Primary toxoplasmosis, Ocular toxoplasmosis, Pyrimethamine, Sulfadiazine, Plasma concentration, Liquid chromatography-mass spectrometry

INTRODUCTION

Context

When a mother contracts *Toxoplasma gondii* while she is pregnant, the infection may spread to the developing baby. Congenital *Toxoplasma* infections in children can cause severe clinical signs such as hydrocephalus, retinochoroiditis, or cerebral calculi, or they might be entirely asymptomatic (with subclinical infection).

Later in life, the parasite can reactivate in children with subclinical infection and cause retinochoroiditis, often known as ocular toxoplasmosis (OT). Early treatment of newly infected pregnant women is justified to lower the risk of transmission and congenital toxoplasmosis [1–5]. The combination of pyrimethamine (PY) and sulfadiazine (SA) is thought to be the most effective since the two medications work in concert, cross the placenta, and build up in the tissues of the mother and the fetus. Studies using observational data have shown a link between prenatal care and the avoidance of symptomatic illness in newborns [6].

The late 1950s saw the first descriptions of the parasitostatic effect of SA and the parasitocidal effect of PY, together with the initial dosing techniques. Later, the in vitro actions of both medications were confirmed for various strains of

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T. gondii [10, 11]. The effectiveness of this medication combination in preventing the parasite's replication process has been confirmed by investigations on experimentally infected animals, in vitro studies, and trials involving immunocompromised people [7, 12–15]. However, demonstrating the effectiveness of these medications in immunocompetent people and fetuses has proven challenging. Research conducted on rhesus monkeys suggests that the combination of drugs, when given promptly after infection, can lower the parasite burden in fetal tissue to levels that cannot be detected [13].

It is still unclear if treatment failures in human congenital toxoplasmosis are caused by inadequate medication concentrations in the fetal tissue or by treatment starting later than planned following maternal infection [16]. Studies conducted in vitro have shown that the medications function in a concentration-dependent manner. When combined, mice's plasma concentrations for PY and SA should be at least 100 µg/l and 25 mg/l, respectively [17]. Maximum concentrations of 220 µg/l for PY and 58.7 mg/l for SA were achieved in rhesus monkeys using a medication regimen that was also used on people [13].

Therapeutic medication monitoring in patients with *Toxoplasma* infection has shown that plasma concentrations are not only variable among individuals and different patient groups, but also unpredictable.

even with conventional treatment [2, 18–21]. Thus far, it can be presumed that plasma concentrations in the range of 700–1300 µg/l (PY) and 50–150 mg/l (SA) are effective in humans [14, 22]. It is necessary to give folinic acid concurrently to avoid bone marrow suppression, a hazardous side effect of PY. There are data on the pharmacokinetics of PY and SA mostly for children with congenital toxoplasmosis [2, 18, 19, 21, 23] and males who are HIV-positive [15]. However, pharmacological information from expectant patients receiving PY and SA for an acute *Toxoplasma* infection is currently lacking [18]. A pharmacological explanation for the combined treatment's unsatisfactory efficacy in preventing vertical transmission is still warranted.

We reasoned that comparing the plasma concentrations of PY and SA in OT-affected women and pregnant women with pregnancy-acquired toxoplasmosis would shed light on the potential contributions of pregnancy-associated pharmacological variables. Our case-control study sought to determine potential differences in PY and SA plasma concentrations between pregnant and non-pregnant women based on similar patient characteristics and a comparable treatment strategy.

Techniques

Patients From 1997 to 2011, plasma samples from 89 consecutive series of pregnant women aged 18.8 to 43.8

years (mean 29.6±6.0, [95% confidence interval: 28.4; 30.9]) receiving anti-parasitic treatment for confirmed or suspected primary *Toxoplasma* infection (PT) were submitted for drug-level testing in this retrospective case-control study (Table 1). When the combo therapy was administered to the ladies, they had all reached or passed the 16th week of pregnancy, with the following normal dosages applied: According to the 1988 Robert Koch Institute protocol (https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Toxoplasmose.html), the German federal health authorities recommended PY 50 mg on the first day, followed by 25 mg/day and SA 50 mg/kg of body weight/d up to a maximum dosage of 4.0 g/day, divided into three to four doses per day. Each obstetrician had the authority to prescribe drugs and oversee patients. Treatment started as soon as a toxoplasma infection diagnosis was confirmed and lasted for at least four weeks.

In order to measure the plasma concentrations of PY and SA, blood or plasma samples were drawn about 14 days following the start of the treatment and sent to the Southern German reference laboratory (Laboratory Harold Hlobil, Sindelfingen, Germany).

For comparison, serum samples from 17 HIV-negative women with comparable ages (17–35, mean 26.1±5.3 [23.6; 28.5] years) who received treatment for acute symptomatic *Toxoplasma* retinochoroiditis at the University Hospital Bern (Inselspital) between 1992 and 2001 were available. (Tables 1 and 2). The OT patients received the same SA dosage as the PT group for a minimum of six weeks, but their treatment began with a loading dose of 75 mg PY, followed by 25 mg PY given twice daily.

Every person had blood samples taken for side effect control on a regular basis, usually 14 days (range 11–17 days) after treatment started. The leftover samples were kept in a biobank at -18 °C until their analysis in 2011. In order to confirm the stability of PY and SA in plasma during long-term storage at -18 °C, we also included samples from ten male HIV-negative patients who were treated for acute OT during the same period and who underwent the identical methods for sampling, storage, and analysis. Table 2 presents the group's baseline characteristics. Because the treatment procedure had shifted from PY/SA to the more recent standard, there were no more recent blood samples from patients treated after 2001.

2001 saw the introduction of the Fansidar® fx combination, which was pyrimethamine and sulfadoxine; by 2004, it had changed to a fixed-dose combination of trimethrim 160 mg and sulfamethoxazole 800 mg twice a day. Since each patient was an outpatient, precise timing of medicine administration and blood sample collection was unknown. Consequently, figuring out specific trough-to-peak ratios was not feasible.

Finding the concentrations of plasma

Chromatography was used at the same advisory laboratory to measure the plasma concentrations of PY and SA either on the day of blood collection (PT) or after the frozen samples were thawed (OT). Liquid chromatography-mass spectrometry (HPLC-MS/MS 3200 Q Trap, Sciex, Germany) was used for the analysis. For this, 225 µl of methanol (MeOH; 50 µl of plasma/serum sample) The mixture was homogenized for one minute using Rotisolv HPLC-Grade, Roth, Germany, 25 µl of acetonitrile (Rotisolv Pestilyse, Roth, Germany), and an internal standard (droperidol) as a systematic test performance control. It was then precipitated for ten minutes at 13,000 rpm. The manufacturer's instructions called for using 100 µl of the supernatant for LC-MS. Four more specific internal standards (ISs) were made by adding known concentrations of PY (Sigma Aldrich, Germany, P-7771) or SA (LGC Standards) to drug-free serum. For PY, the concentrations of the ISs were 200 µg/l, 400 µg/l, 1000 µg/l, and 2000 µg/l; similarly, for SA, the values were 10 mg/l, 20 mg/l, 50 mg/l, and level 4 (100 mg/l).

Both high and low positive controls (PY 1200 µg/l and SA 60 mg/l) were added. Low positive controls were PY 500 µg/l and SA 25 mg/l. The presented technique, which will be referred to as LC-MS from now on, was validated and shown to be sensitive, accurate, and selective for measuring PY and SA in human serum/plasma samples. The following were the intra-assay coefficients of variation (CVs): CV=2.0% for PY control 1 (500 µg/l), CV=0.9% for control 2 (1200 µg/l), and CV=2.10% and 0.9% for SA control 1 (25 mg/l) and control 2 (60 mg/l). The ISs were verified to be stable after a year of storage at ≤16 °C. No peaks that interfered with the quantification process were found during the validation procedure. Lower detection limits for PY and SA in the test were 30 µg/l and 9 mg/l, respectively.

The computed intra- and interday CVs were still less than 10%. Data analysis was done using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

The text provides parametric data (age, days from therapy commencement) together with range, mean±standard deviation (SD), and associated 95% confidence intervals (CIs). The text displays the medians and associated 95% confidence intervals for the non-parametric serum concentrations of PY and SA. Bootstrapping was used in SPSS to obtain the 95% confidence intervals for the medians. The Mann-Whitney U-test for the serum concentration of PY and SA and the t-test for independent samples for age and days following treatment commencement were used to analyze differences between OT and PT. A p-value of less than 0.05 was deemed significant.

Outcomes

The two groups' initial features are shown within Table 1. We observed no difference in plasma concentrations by grouping OT samples based on when they were sampled (Group 1: 10–12 days, Group 2: 13–15 days, and Group 3: 16–18 days after treatment initiation). This indicates that by the time blood was sampled, both PY and SA concentrations had already reached a steady-state (Fig. 1a, b). The OT group's steady-state findings led us to believe that the PT group would likewise reach its PY and SA steady-state plasma concentrations following this period. The PY levels (Fig. 2a) were found to be 34% higher in women with OT (1230 [780; 1890] µg/l) compared to those in pregnant women with PT (810 [745; 917] µg/l; $p=0.006$), which is consistent with the difference in dosing (50 mg/day vs. 25 mg/day). This was determined by comparing the median values of both drugs for both groups. Even though both groups used the same SA dosage regimen, the SA levels differed by 34% (PT 46.2 [39.9; 54.4] mg/l vs. OT 70.4 [52.4; 89] mg/l; $p=0.015$).

The majority of PT patients were, by the reference values, underdosed for SA if we assume, based on published research, an upper concentration limit for PY of 1700 µg/l and a lower concentration limit for SA of 50 mg/l. In contrast, PY concentrations were above the desired value in the majority of OT samples (Fig. 2b). SA levels fell below 20 mg/l in 23 of the 89 PT patients (26%) that were evaluated. Parallel to this, 15 of these 23 individuals (17% of total patients) had PY levels below the desired 700 µg/l concentration.

Similar values for both medications were found when the serum concentrations of male and female patients with acute OT receiving PY and SA treatment were compared (Table 2). Since the concentrations for males after the immediate work-up of unfrozen plasma samples are in good agreement with published pharmacokinetic results [23, 24], the observed differences in the plasma concentrations of either drug cannot be explained by the different storage conditions for PT and OT samples.

Concentrations of 50–150 mg/l of SA are thought to be therapeutic for the majority of infections [22]. However, in 26% of our PT patients, the concentration of SA was less than 20 mg/l, and in 17% of the patients, the concentrations of both PY and SA were not reached. This is quite consistent with the 5-to 13% clinically documented transmission rates in Europe [3, 25]. Pregnant individuals had reduced medication levels even with the same SA treatment plan.

The median SA plasma concentration in PT patients was more than 34% (46.2 vs. 70.4 mg/l) lower than that in the OT group when compared to those in non-pregnant women with OT, suggesting that the treatment procedure itself is not insufficient.

The reduced SA concentrations during pregnancy may be explained by physiological and metabolic changes. It has

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been discovered that maternal antibiotic concentrations are typically between 10% and 50% lower than those of non-pregnant women [26]. The SA plasma concentrations may be impacted by changes in body weight, a greater clearance rate, or the pregnancy-related increase in total body fluid [27, 28]. The fact that two-thirds of patients with pregnancy experience medication turnover and physiological alterations in metabolism are not explained by these changes [13].

Parallel to this, SA concentrations below anticipated values showed significantly lower PY levels ($<700 \mu\text{g/l}$). Therefore, one possibility for these results could have been low medication compliance, which is a documented condition during pregnancy in various diseases [29, 30].

The clinically detected SA plasma values are similar to those observed in rhesus monkeys during experimental toxoplasmosis, where peak concentrations were observed to be 58.7 mg/l [13]. It is evident from this that the recommended SA goal levels ($50\text{--}150 \text{ mg/l}$) [22] are either unfeasible or unnecessary when PY concentrations are higher than $700 \mu\text{g/l}$. SA enters the stomach quickly and is mostly excreted in the urine by acetylation. The half-life of SA elimination is between 6 and 12 hours in people with normal hepatic and renal function [24].

The fact that the outpatient clinics had not kept track of the amount of time that had passed between the oral medication consumption and the blood sample was an evident but inevitable restriction for our retrospective analysis. According to research, SA's elimination half-life in monkeys is around 5 hours, meaning that the estimated difference between observed and peak values is 50% [13].

This was addressed in our series by significantly lowering the SA cut-off value to 20 mg/l . In this research, an estimated 30% variation between trough and peak concentrations must be weighed against a comparatively high sample size of 89 patients with PT. However, we must acknowledge that information regarding potential co-medications and how they can affect drug levels is lacking.

Variations in the interval between drug ingestion, blood sample, and co-medication may not provide a complete explanation for the significant interindividual variation in plasma concentrations (by a factor of five) [13]. Furthermore, variations in the interval between drug ingestion and blood sample do not easily account for the significant inter-group variation in mean plasma concentrations.

Our study's retrospective methodology is another significant drawback, albeit it might be offset by the sizeably big patient sample. The measurement of plasma concentrations in pregnant women who were past the 16th week of gestation was carried out as a clinical routine analysis throughout a number of years, and it was initiated by suspected or confirmed *Toxoplasma* seroconversion.

The interpretation of results for a single patient is limited by the consequent absence of more specific information regarding body size, weight, general health, comorbidities, and their influence in pregnant patients; however, we believe that the tendency in the large patient group of pregnant women is robust. The clinical data of the second group of OT patients, whose sample size was noticeably smaller, revealed an age range that is reasonably comparable to that of the pregnant women. Significant comorbidities or equivalent treatments were absent from all of these patients, and no underlying hepatic or renal illness was found to be present.

A modification in the treatment regimen for OT after 2001 made it impossible to increase the sample size of the second group, as previously mentioned. The plasma concentrations were ascertained using blood samples obtained roughly 14 days into the treatment. This assumption was based on the subgroup analysis of non-pregnant (OT) women, which suggested that both medications would have achieved a steady state by then (Fig. 1a, b). Our study is one of the few in this field of research, despite the fact that pregnant women are typically disqualified from pharmacokinetic studies because of ethical issues. The majority of the time, anti-parasitic medication dosage during pregnancy has been empirical, with the noteworthy exception of a recent study on antimalarial medication in African women. While receiving Fansidar® care, although the pharmacological behaviors of the two medications differ, there was an overall three-fold greater clearance for sulfadoxine in pregnant women compared to postpartum women [31–33]. This finding is consistent with our results for SA.

The effect of long-term storage at $\leq -18^\circ\text{C}$ on the stability of PY and SA has not been studied before, as was the case for the samples taken from individuals who had OT but not PT. According to certain statistics, PY can remain stable for several months at -20°C and for at least 91 days at room temperature or 4°C [34, 35].

Given the lack of published data on females receiving standard PY and SA therapy [18] and the need to rule out the significant influence of sample storage conditions, we decided to include samples from a small group of males with OT who had undergone the same procedures for sampling, storing, and analyzing data (Table 2). We have no evidence that freezing could have affected any of the measured concentrations in OT patients, and our findings are therefore robust and reliable. The median plasma levels from these males (PY $1321 [962; 2140] \mu\text{g/l}$; SA: $82.4 [53.5; 115.0] \text{ mg/l}$) are in good agreement with published values for males (PY $1887 \pm 1161 \mu\text{g/l}$; SA $42.26 \pm 12.28 \text{ mg/l}$ up to $84.9 \pm 23.5 \mu\text{g/ml}$) [15, 36].

CONCLUSION

According to our data, every sixth patient with pregnancy-

acquired toxoplasmosis had insufficient drug levels for both drugs. This finding could only be partially explained by missing data regarding co-medication and pregnancy-associated pharmacologic changes, as well as the time lapse between drug intake and blood sampling not being recorded. Pregnant patients' median PY and SA concentrations were found to be 34% lower than those of non-pregnant patients receiving treatment for active OT. We need to establish how these concentrations may be explained and to what extent the observed lower end ranges of plasma levels for PY and SA in pregnant women can be explained, given the lengthy debate around the effectiveness of prenatal Toxoplasma therapy with relation to clinical outcomes in neonates.

Future research may find that the efficacy of the medications in the foetus and infant is influenced by the presence of women and a plasma concentration in the foetus that is one-third of the mother level [35]. In order to objectively monitor compliance and other relevant parameters before implementing a treatment plan, it is crucial to measure plasma medication concentrations systematically [37]. When applied prospectively, these could be able to reduce the discrepancy between the predicted and actual results of pregnancy in human PT.

REFERENCES

- McLeod R, Mack D, Foss R, et al. Levels of pyrimethamine in sera and cerebrospinal and ventricular fluids from infants treated for congenital toxoplasmosis. Toxoplasmosis Study Group. *Antimicrob Agents Chemother.* 1992;36:1040–8.
- Gras L, Wallon M, Pollak A, et al. Association between prenatal treatment and clinical manifestations of congenital toxoplasmosis in infancy: a cohort study in 13 European centres. *Acta Paediatr.* 2005;94:1721–31.
- Hotop A, Hlobil H, Groß U. Efficacy of rapid treatment initiation following primary Toxoplasma gondii infection during pregnancy. *Clin Infect Dis.* 2012;54:1545–52.
- Wallon M, Peyron F, Cornu C, et al. Congenital Toxoplasma infection: monthly prenatal screening decreases transmission rate and improves clinical outcome at age 3 years. *Clin Infect Dis.* 2013;56:1223–31.
- Mandelbrot L, Kiefer F, Sitta R, et al. Prenatal therapy with pyrimethamine + sulfadiazine vs spiramycin to reduce placental transmission of toxoplasmosis: a multicenter, randomized trial. *Am J Obstet Gynecol.* 2018;219(386):e1–9.
- Maldonado YA, Read JS. Diagnosis, treatment, and prevention of congenital toxoplasmosis in the United States. *Pediatrics.* 2017;139:e20163860.
- Eyles D, Coleman N. Synergistic effect of sulphadiazine and daraprim against experimental toxoplasmosis in the mouse. *Antibiot Chemother.* 1953;3:483–90.
- Eyles DE, Coleman N. An evaluation of the curative effects of pyrimethamine and sulfadiazine, alone and in combination, on experimental mouse toxoplasmosis. *Antibiot Chemother.* 1955;5:529–39.
- Frenkel JK, Weber RW, Lunde MN. Acute toxoplasmosis. Effective treatment with pyrimethamine, sulfadiazine, leucovorin calcium, and yeast. *JAMA.* 1960;173:1471–6.
- Derouin F, Chastang C. In vitro effects of folate inhibitors on Toxoplasma gondii. *Antimicrob Agents Chemother.* 1988;33:1753–9.
- Meneceur P, Bouldouyre MA, Aubert D, et al. In vitro susceptibility of various genotypic strains of Toxoplasma gondii to pyrimethamine, sulfadiazine, and atovaquone. *Antimicrob Agents Chemother.* 2008;52:1269–77.
- Sheffield HG, Melton ML. Effect of pyrimethamine and sulfadiazine on the fine structure and multiplication of Toxoplasma gondii in cell cultures. *J Parasitol.* 1975;61:704–12.
- van de Schoondermark van E, Galama J, Vree T, et al. Study of treatment of congenital Toxoplasma gondii infection in rhesus monkeys with pyrimethamine and sulfadiazine. *Antimicrob Agents Chemother.* 1995;39:137–44.
- Weiss LM, Harris C, Berger M, Tannowitz HB, Wittner M. Pyrimethamine concentrations in serum and cerebral fluid during treatment of acute toxoplasma encephalitis in patients with AIDS. *J Infect Dis.* 1988;157:580–3.
- Klinker H, Langmann P, Richter E. Pyrimethamine alone as prophylaxis for cerebral toxoplasmosis in patients with advanced HIV infection. *Infection.* 1996;24:324–7.
- Montoya JG, Remington JS. Management of Toxoplasma gondii infection during pregnancy. *Clin Infect Dis.* 2008;47:554–66.
- Weiss LM, Luft BJ, Tanowitz HB, Wittner M. Pyrimethamine concentrations in serum during treatment of acute murine experimental toxoplasmosis. *Am J Trop Med Hyg.* 1992;46:288–91.
- Corvaisier S, Charpiat B, Mounier C, et al. Population pharmacokinetics of pyrimethamine and sulfadoxine

- in children treated for congenital toxoplasmosis. *Antimicrob Agents Chemother.* 2004;48:3794–800.
19. Trenque T, Simon S, Villena I, et al. Population pharmacokinetics of pyrimethamine and sulfadoxine in children with congenital toxoplasmosis. *Br J Clin Pharmacol.* 2004;57:735–41.
 20. Schmidt DR, Høgh B, Andersen O, Hansen SH, Dalhoff K, Petersen E. Treatment of infants with congenital toxoplasmosis: tolerability and plasma concentrations of sulfadiazine and pyrimethamine. *Eur J Pediatr.* 2006;165:19–25.
 21. Lipka B, Milewska-Bobula B, Filipek M. Monitoring of plasma concentration of pyrimethamine (PYR) in infants with congenital *Toxoplasma gondii* infection—own observations. *Wiad Parazytol.* 2011;57:87–92.
 22. WHO: Second meeting of the subcommittee of the expert committee on the selection and use of essential medicines, Geneva 29 September to 3 October 2008. https://www.who.int/selection_medicines/committees/subcommittee/2/sulfadiazine_rev.pdf.
 23. Langmann P, Schirmer D, Zilly M, Klincker H. Drug monitoring of pyrimethamine during maintenance therapy of toxoplasmic encephalitis in patients with advanced HIV infection during HAART. *Med Sci Monit.* 2004;10:65–9.
 24. Costantine MM. Physiologic and pharmacokinetic changes in pregnancy. *Front Pharmacol.* 2014;5:65.
 25. Prusa AR, Kasper DC, Pollak A, Gleiss A, Waldhoer T, Hayde M. The Austrian Toxoplasmosis Register, 1992–2008. *Clin Infect Dis.* 2015;60:e4-10.
 26. Newton E. Global library of women's medicine. (ISSN: 1756–2228) 2008. <https://doi.org/10.3843/GLOWM.10175>
 27. Levison ME, Levison JH. Pharmacokinetics and pharmacodynamics of antibacterial agents. *Infect Dis Clin North Am.* 2009;23:791–7.
 28. Cook IF, Cochrane JP, Edstein MD. Race-linked differences in serum concentrations of dapsone, monoacetyldapsone and pyrimethamine during malaria prophylaxis. *Trans R Soc Trop Med Hyg.* 1986;80:897–901.
 29. Julsgaard M. Adherence to medical treatment in relation to pregnancy, birth outcome & breastfeeding behavior among women with Crohn's disease. *Dan Med J.* 2016;63:B5263.
 30. Martelli L, Lopez A, Strobel S, Danese S, Roblin X, Baumann C, Peyrin-Biroulet L. Adherence to infliximab therapy in inflammatory bowel disease patients in a real-life setting. *J Dig Dis.* 2017;18:566–73.
 31. Odongo CO, Kuteesa RB, Muhammad N, Gordon O, Francis WO, Josaphat B, et al. Trimester-specific population pharmacokinetics and other correlates of variability in sulphadoxine-pyrimethamine disposition among Ugandan pregnant women. *Drugs R D.* 2015;15:351–62.
 32. Salman S, Baiwog F, Page-Sharp M, Grifn S, Karunajeewa HA, Mueller I, et al. Optimal antimalarial dose regimens for sulfadoxine-pyrimethamine with or without azithromycin in pregnancy based on population pharmacokinetic modeling. *Antimicrob Agents Chemother.* 2017;61:e02291-2316.
 33. De Kock M, Tarning J, Workman L, Nyunt MM, Adam I, Barnes KI, et al. Pharmacokinetics of sulfadoxine and pyrimethamine for intermittent preventive treatment of malaria during pregnancy and after delivery. *CPT Pharmacomet Syst Pharmacol.* 2017;6:430–8.
 34. Nahata MC, Morosco RS, Hipple TF. Stability of pyrimethamine in a liquid dosage formulation stored for three months. *Am J Health Syst Pharm.* 1997;54:2714–6.
 35. Peytavin G, Leng JJ, Forestier F, Saux M, Hohlfeld P, Farinotti R. Placental transfer of pyrimethamine studied in an ex vivo placental perfusion model. *Biol Neonate.* 2000;78:83–5.
 36. Jacobson JM, Davidian M, Rainey PM, Hafner R, Raasch RH, Luft BJ. Pyrimethamine pharmacokinetics in human immunodeficiency virus-positive patients seropositive for *Toxoplasma gondii*. *Antimicrob Agents Chemother.* 1996;40:1360–5.
 37. Pleyer U, Gross U, Schlüter D, Wilking H, Seeber F. Toxoplasmosis in Germany. *Dtsch Arztebl Int.* 2019;116:435–44.