Influence of Body Weight, Ethnicity, Oral Contraceptives and Pregnancy on the Pharmacokinetics of Azithromycin in Women of Child-Bearing Age

Running Title: **Pharmacokinetics of Azithromycin in Pregnancy**

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ABSTRACT

Women of child-bearing age commonly receive azithromycin for treatment of community-acquired infections, including during pregnancy. This study determined azithromycin pharmacokinetics (PKs) in pregnant and nonpregnant women and identified covariates contributing to PK variability. Plasma samples were collected using a sparse sampling strategy in pregnant women, 12-40 weeks gestational age, and nonpregnant women of child bearing age receiving oral azithromycin for treatment of infection. PK data from extensive sampling conducted on 12 healthy women were also included. Plasma samples were assayed for azithromycin by high performance liquid chromatography. Population data were analyzed by nonlinear mixed effects modeling. The population analysis included 53 pregnant and 25 non-pregnant women. A three compartment model with first order absorption and a lag time provided the best fit of the data. Lean body weight, pregnancy, ethnicity and co-administration of oral contraceptives were covariates identified as significantly influencing the oral clearance of azithromycin and, except for oral contraceptive use, intercompartmental clearance between the central and second peripheral compartment. No other covariate relationships were identified. Compared to non-pregnant women not receiving oral contraceptives, a 21% to 42% higher dose-adjusted, azithromycin area under the plasma concentration-time curve (AUC) occurs in non-African American women who are pregnant or receiving oral contraceptives. Conversely, azithromycin AUC is similar between pregnant, African American women and non-pregnant women not receiving oral contraceptives. Although higher maternal and fetal azithromycin exposure suggests that lower doses be administered to non-African American women during pregnancy, consideration of azithromycin pharmacodynamics during pregnancy should guide any dose adjustments.

INTRODUCTION

Drug therapy in pregnant women must take into account the physiological changes accompanying pregnancy. These physiological changes can impact drug disposition by altering plasma protein binding, hepatic clearance and renal excretion (6, 24). Depending on the type and extent of any alterations, adjustment in dose may be needed to ensure the desired outcome is achieved and mother or fetus are not exposed to excess drug. For agents cleared by drug metabolizing enzymes or transport proteins, insufficient understanding of how pregnancy affects the activity of these pathways often hampers devising appropriate dosing strategies.

Azithromycin is among the drugs most commonly prescribed to pregnant women (7). Its frequent use in pregnancy reflects its established safety and efficacy in non-pregnant women and men for outpatient treatment of respiratory (23, 39, 44), skin (30) and gynecological infections (45) as well as the lack of association between maternal administration of azithromycin and increased occurrence of major congenital malformations (14, 50). Pregnant women receive the dose of azithromycin determined to be safe and effective for non-pregnant women and men (45, 50). This extrapolation of dose requirements assumes that the clinical consequences of any pregnancy-related changes in azithromycin pharmacokinetics are negligible. It also ignores the impact that functional changes in the immune system during pregnancy may have on antibiotic responsiveness and dose requirements (25).

Azithromycin exhibits several distinct pharmacokinetic characteristics. It is incompletely absorbed following oral administration (43), extensively distributed into tissues (9) and primarily eliminated by hepatobiliary excretion (34, 43). Not surprisingly, dose-adjusted azithromycin exposure varies widely among individuals (33). Limited data are available regarding the influence of pregnancy on azithromycin pharmacokinetics. Interpretation of the two studies

which have examined azithromycin pharmacokinetics in pregnancy is confounded by their conflicting results and unique populations (46, 49). Women undergoing caesarean section were evaluated in one study (46), and women in Papua New Guinea receiving antimalarial treatment in the other (49). Intrinsic or environmental differences between these subjects and pregnant women receiving azithromycin for community-acquired infections in the United States hinder generalizing their findings.

This study investigated the population pharmacokinetics of azithromycin in women receiving treatment for an infection during the second and third trimester of pregnancy. Women of child bearing age who were not pregnant were included for comparison. To ensure a representative population, subjects were recruited from four university-based obstetrical practices. The inter-individual variability of the pharmacokinetic parameters was determined and factors contributing to this variability identified.

MATERIALS AND METHODS

Performance sites and subjects. This research was conducted at Brigham & Women's Hospital (Boston, MA), Meriter Hospital (Madison, WI), University of Illinois at Chicago (Chicago, IL) and University of Michigan (Ann Arbor, MI). The study consisted of two components, an initial pilot trial in 12 healthy women to establish the structural model and baseline pharmacokinetic parameter estimates and a population pharmacokinetic analysis in pregnant and nonpregnant women receiving azithromycin for treatment of an infection. Institution Review Board approval was obtained from each of the institutions above as well as the University of Wisconsin at Madison and U.S Food and Drug Administration's Research Involving Human Subjects Committee. Subjects provided written informed consent prior to participation in the study.

The pilot trial was performed solely at the University of Illinois at Chicago. Women of child bearing age, based on menstrual history, at least 18 years of age, not pregnant or breastfeeding and within 25% of their acceptable range of weight as referenced by the Table of Desirable Body Weights and Heights (1983 Metropolitan Life Insurance Company) were recruited. Subjects were judged healthy by medical history, physical examination and screening laboratory testing (complete blood count, serum chemistries, and urine pregnancy test). Women were required to use either a barrier or hormonal form of contraception throughout the study. Exclusion criteria included a history of tobacco use or alcohol or drug abuse in the last 12 months and administration within 28 days before starting the study of any medication known to interact with azithromycin. Subjects were required to be free of all medications, except oral contraceptives, within 1 week and alcohol within 48 hours prior to the start of the study and continuing until 96 hours after the last dose of azithromycin.

Participants in the population study included women of child bearing potential (based on menstrual history) greater than 18 years of age who were receiving azithromycin for treatment of an infection and were either a). at least 12 weeks gestational age or b). nonpregnant and, if previously pregnant, were at least 3 months postpartum.

Study design for the pilot trial. The pilot trial utilized a single period, open-label, multiple-dose design. Eligible women received oral azithromycin 500 mg on day 1 and 250 mg daily on days 2-5. Azithromycin, as the 250 mg tablets (Pfizer, New York, NY), was dispensed into bottles and caps equipped for electronically recording administration times (MEMS, AARDEX Ltd., Union City, CA 94557). Participants were admitted to the University of Illinois at Chicago Clinical Research Center on the evening of day 4. Following an overnight fast, subjects received the last dose of oral azithromycin on the morning of day 5. Standardized meals

were provided at 4 and 10 hours after the final dose. Approximately 5 ml of blood was collected through an indwelling catheter into a heparinized evacuated tube prior to and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, 72, and 96 hours following the last azithromycin dose. Participants were discharged from the Clinical Research Center after the 12 hour sample and, subsequently, returned each morning for the next 4 days.

Study design for the population pharmacokinetic analysis. This phase of the study was conducted as a prospective, open-label, multicenter population pharmacokinetic analysis. A sparse sampling scheme guided data collection. Participants received the dose regimen of azithromycin prescribed by their treating physician. Blood samples of approximately 5 mL were collected from participants within each of 4 sampling windows: pre-dose (if not the first dose), 10 minutes to 1.5 hours after any dose, 2 to 5 hours after any dose and 24 to 96 hours after the last dose. A single sample was obtained within each sampling window, except the 24 to 96 hour window where 2 samples at least 2 hours apart were collected. Also, if sampling occurred with the first dose, no pre-dose sample was obtained and two samples at least 1 hour apart were collected during the 2 to 5 hour window. Sampling windows were constructed from the D-optimal sample times computed for two- and three-compartment models with first order absorption and elimination. Calculations were performed using estimates of azithromycin pharmacokinetics from non-pregnant women and men (4, 9, 48) and ADAPT II software (15, 16). An additional 2 to 3 ml of blood was collected at a single sampling time for determination of serum creatinine. Demographic and clinical characteristics were recorded for each participant along with the azithromycin oral formulation and dosing, meal and sampling times. Medication compliance was assessed from patient interviews and drug administration records. Women

having at least one quantifiable azithromycin plasma concentration, documented dosing and sampling times and judged to be compliant were included in the pharmacokinetic dataset.

Laboratory analysis. Following collection, blood samples were centrifuged, and plasma separated and stored at -70^{o}C until shipped to the University of Illinois at Chicago on dry ice for analysis.

Azithromycin plasma concentrations were assayed by a high performance liquid chromatographic procedure with electrochemical detection derived from Shepard *et al* (51) and Patel *et al* (41). Briefly, internal standard, clarithromyin, and 0.1M sodium carbonate solution were added to each plasma sample. Samples were then extracted with tert-methyl-butyl ether, and the ether layer evaporated and reconstituted in mobile phase. The reconstituted sample was washed with hexane, and 50-µl injected onto a Waters XTerra C_{18} 5-µm, 4.6 by 150-mm column. Samples were eluted with a mobile phase consisting of 0.01M ammonium acetate at pH 10 and 50% (v/v) acetonitrile at a flow rate of 1 ml/min. The analytes were detected with a Coulochem II electrochemical detector (ESA, Inc., Bedford, MA) with applied potentials set at + 600 and + 850 mV.

The assay was linear in the range of 10.1 to 505 ng/ml. For plasma concentrations above the upper limit, samples were diluted with blank plasma to fall within the range of the standard curve. Mean accuracy ranged from 97.1% to 104.8% of the theoretical concentration and precision (relative standard deviation) less than 4% for back-calculated calibration standards (n=5 assay runs). The between run accuracy and precision for quality control samples were 98.2% and 3.2% at 302 ng/ml, 101.4% and 3.8% at 101 ng/ml, 102.7% and 5.8% at 30.2 ng/ml and 103.1% and 13.1% at the lower limit of quantitation. Owing to logistical problems at the University of Illinois at Chicago laboratory, 12 plasma samples were analyzed by a proprietary

high performance liquid chromatographic assay at the Pharmacokinetics Laboratory, National Jewish Health (Denver, CO). Cross validation of 15 patient samples showed reasonable correspondence between laboratories with an average relative deviation of +3.9% for concentrations in the mid to upper range (> 150 ng/ml) of the standard curve and -4.7% for concentrations in the lower range.

Pharmacokinetic analysis. Data from both parts of the study were analyzed using the nonlinear mixed effects modeling software, NONMEM (version VI 2.0, ICON Development Solutions, Ellicott City, MD), with a Compaq Visual Fortran 6.6 compiler.

The plasma concentration-time data from the pilot study in healthy women were fit separately for each individual using the first order method in NONMEM. Several alternative models were assessed, including two and three compartments, first-, zero- or mixed first-zero order absorption and inclusion of a lag time prior to onset of absorption. Models were parameterized as clearances and distribution volumes. As data were only collected following oral administration, clearance and volume parameters were expressed as apparent values, i.e., uncorrected for bioavailability. A proportional residual error was incorporated. Model selection criteria included visual inspection of diagnostic plots, standard error of the parameter estimates and the minimum value of the objective function (OFV). The OFV is a NONMEM goodness of fit criteria, and provides a statistical test for comparing competing models. The difference in OFV (Δ OFV) between hierarchical models is approximately χ^2 distributed with degrees of freedom (df) equal to the number of additional model parameters.

The data from the population study were analyzed using the first order conditional estimation method of NONMEM. The azithromycin plasma concentrations from the healthy women were pooled with the patients in the population database. Prior to pooling, the plasma

concentration data from the healthy women were condensed (from 17 to 5 samples) to emulate the data sets from the patients by randomly selecting plasma concentrations within the sampling windows using the RAND function in Microsoft Excel. This approach allowed the performance of the sparse sampling design to be evaluated, with the goal of informing the design of future pharmacokinetic studies in pregnancy. The impact of condensing the dataset was assessed by re-analyzing the data with inclusion of the full profile data from the healthy volunteers.

The structural model selected from the fitting of the individual data served as the starting point for development of the population pharmacokinetic model. The appropriateness of the structural (base) model was verified by evaluating the fitting criteria as described above and comparing the fit with alternative models. The parameters from the individual data provided initial estimates for the population fitting. A log normal distribution was assumed for the pharmacokinetic parameters, and inter-individual variability (IIV) modeled as exponential error. The IIV was initially determined for all pharmacokinetic parameters, and retained for a parameter in the final model only if its inclusion produced a significant decrease in OFV (Δ OFV > 3.84, χ^2, $p<0.05$). Covariance between parameters was also explored by estimating the full variance-covariance matrix. Residual variability was described as proportional error. Drug analysis laboratory was evaluated as an independent factor influencing residual error.

After the structural and error models were defined, covariates explaining the inter-individual variability in pharmacokinetic parameter estimates were identified. In addition to the previously described criteria, the covariate analysis was guided by the reduction in the IIV and biological plausibility of any covariate relationship. First, relationships between body size measures and the clearance and volume terms were separately examined as linear, power and proportional functions. Body size measure included total body weight, lean body weight (26),

body surface area (18) and body mass index (29). The body size measure producing the greatest reduction in OFV for each parameter, providing the minimum drop was at least 6.6 (χ^2, $p<0.01$, df=1), was included in the model. Next, other variables were evaluated, including age, pregnancy status, gestational age (confirmed by ultrasound), estimated creatinine clearance (13), ethnicity, concurrent medications, significant hepatic or renal impairment, healthy volunteer or patient, type of infection, azithromycin dose, administration of drug fasting (> 3 hours after a meal) or with a meal, and study site. Concurrent medication was analyzed as the presence or absence of any drug, drugs suspected to interact with azithromycin and specific agents received by 7 or more patients. Based on their similar pharmacokinetic behavior during the graphical analysis, the Asian, Caucasian, Hispanic and Pacific Islander ethnic groups were combined, and ethnicity re-expressed as a categorical variable, indicating whether or not the subject was African American.

Individual empirical Bayesian estimates of the pharmacokinetic parameters were obtained from the base pharmacokinetic model with any body size covariates included. Relationships between the Bayesian estimates and covariates were screened by graphical and generalized additive modeling procedures (S-Plus version 6.1, Insightful Corporation, Seattle WA). Covariates identified in the screening analysis were first added alone to expressions for the pharmacokinetic parameters in the base model. Those producing a decrease in OFV > 3.84 ($p<0.05$, χ^2, df=1) were entered in a stepwise fashion into an intermediate model and retained if their addition decreased the OFV by > 3.84. A backward elimination step followed with covariates entered during the forward addition step individually eliminated and only retained if their removal increased the OFV by > 6.6 ($p<0.01$, χ^2, df=1). Continuous covariates were normalized to an accepted standard e.g., (70 kg for total body weight, 50 kg for lean body

208 weight) or population median (e.g., 29 weeks gestational age) and modeled as linear or power
209 functions of the pharmacokinetic parameter. Categorical covariates were input as indicator
210 variables with a value of 1 if the trait was present and 0 otherwise.

211 **Model Validation.** The validity of the final population pharmacokinetic model was
212 evaluated by bootstrap analysis using Wings for NONMEM (http://wfn_sourceforge.net/)(40).
213 Resampling with replacement from the dataset was used to construct 1000 bootstrap datasets.
214 Each data set was fit to the final population model, and the median and 2.5th and 97.5th
215 percentiles determined for the fixed and random effect parameters. The performance of the final
216 population model was also evaluated by visual predictive check (54). Briefly, 250 datasets were
217 simulated for an oral azithromycin dosage regimen of 500 mg on day 1 and 250 mg daily on days
218 2 through 5. The simulations employed covariate values from the patient dataset and the final
219 population estimates for the fixed and random effect parameters. The median and 80%
220 prediction intervals for the simulated azithromycin plasma concentrations partitioned by
221 ethnicity, pregnancy and oral contraceptive use were plotted against the observed values. To
222 adjust for the varying azithromycin dosage regimens among patients, observed values were
223 normalized to reflect the simulated dose prior to plotting. The assumption of a linear relationship
224 between azithromycin dose and plasma concentration is supported by others (17, 20, 33).

225 **Statistical analysis**. Based on the results of the population analysis, subjects were
226 categorized into the following groups: 1). pregnant African American women, 2). pregnant
227 women of non-African American (i.e., Asian, Caucasian not Hispanic, Hispanic or Pacific
228 Islander) ethnicity, 3). nonpregnant women of any ethnicity who were not receiving oral
229 contraceptives and 4). nonpregnant and non-African American women who were receiving oral
230 contraceptives. Empirical Bayesian estimates of the pharmacokinetic parameters for each

231 woman were derived from NONMEM, and used to estimate the area under the plasma
232 concentration time curve from time 0 to infinity (AUC) for an oral azithromycin dosage regimen
233 of 500 mg on day 1 and 250 mg daily on days 2 through 5. One-way ANOVA was used to
234 compare the individual estimates of azithromycin AUC among groups. Differences between
235 groups were identified by Tukey's Honestly Significant Difference (HSD) method.

236 **RESULTS**

237 Seventeen healthy women (pilot study) and 72 pregnant or non-pregnant women
238 receiving azithromycin for treatment of an infection were enrolled. Five healthy volunteers did
239 not meet the eligibility criteria, and, thus, did not continue to the drug administration phase. Six
240 subjects in phase 2 were excluded from the pharmacokinetic dataset as a result of incomplete
241 dose administration information in 3, no evaluable azithromycin plasma concentrations in 2 and
242 withdrawal from the study in 1. Accordingly, the population pharmacokinetic database consisted
243 of 344 azithromycin plasma concentrations collected from 78 women. Azithromycin plasma
244 concentrations ranged from 10.3 ng/ml to 1270 ng/ml. For healthy volunteers, only five
245 randomly selected concentrations per subject were integrated in the population database. An
246 isolated plasma sample from 11 patients was excluded from the dataset for being below the
247 quantifiable limit of the assay.

248 The demographic and clinical characteristics of the participants included in the
249 population analysis are summarized in Table 1. The 3 groups displayed similar ages, lean body
250 weights, heights and azithromycin dosage regimens. An imbalance in ethnicity occurred among
251 groups, with only two African Americans found among the non-pregnant patients and none
252 among the healthy volunteers. As expected, total body weights, creatinine clearances, infection
253 types and concomitant medications differed between the pregnant women and the other two

groups. No subjects with clinically significant renal (creatinine clearance < 30 ml/min/1.73m^2) or hepatic disease were enrolled. Fifteen women reported azithromycin-related adverse effects. The adverse effects were mild to moderate in intensity, and included nausea, vomiting, diarrhea and abdominal cramping.

A triexponential decline in azithromycin plasma concentrations with a lag time preceding absorption was consistently observed following oral administration of azithromycin in the pilot study. Based on these observations, a three compartment model with elimination from the central compartment, first order absorption and a lag time was selected for the population pharmacokinetic (base) model. The fit with the three-compartment model provided a statistically significant improvement compared to a two-compartment model (Δ OFV= -113, $p<0.001$, χ^2, df=2). The suitability of the three compartment model is further supported by the diagnostic plots in Figure 1.

The population parameters for the base model were lag time (t_{lag}) of 1.3 hours, oral clearance (CL/F) of 94 l/hr, apparent intercompartmental clearance from the central to first peripheral compartment ($CL_{D\text{-}P1}$ /F) of 485 l/hr, apparent intercompartmental clearance from the central to second peripheral compartment ($CL_{D\text{-}P2}$ /F) of 63 l/hr, apparent volume of distribution of the central compartment (V_c/F) of 415 L, apparent volume of distribution of the first peripheral compartment (V_{P1}/F) of 1900 L and apparent volume of distribution of the second peripheral compartment (V_{P2}/F) of 13800 L. Data were insufficient to allow estimation of the absorption rate constant (k_a). Consequently, a fixed value of 0.8 hr^{-1} was selected based on the median value from the pilot study and literature (9, 36, 49). The insensitivity of the parameter estimates to this fixed value was verified by varying the k_a between 0.2 hr^{-1} to 8 hr^{-1}. Estimates of IIV were available for CL/F, $CL_{D\text{-}P2}$ /F, V_c/F and V_{P1}/F. The model was unable to

accommodate IIV terms for t_{lag}, $CL_{D\text{-}P1}$ /F and V_{P2}/F. The use of a full variance-covariance matrix did not improve the model fit, and, therefore, a diagonal matrix was employed. A proportional error best described residual variability. Drug assay laboratory was not found to influence residual error.

Several body size descriptors, including total body weight, lean body weight, body surface area and body mass index, were evaluated as potential covariates. A significant decrease in OFV was observed following the incorporation of lean body weight (Δ OFV=13, $p<0.001$, χ^2, df=1) in the model as a covariate of CL/F. A direct proportional relationship between lean body weight and CL/F provided a comparable fit to a linear function and better fit than a power function. After incorporating the proportional relationship between lean body weight and CL/F, IIV decreased by 15%. No significant relationships were identified between descriptors of body size and other pharmacokinetic parameters.

The screening analysis identified: 1). clinical site, gestational age, oral contraceptive use, pregnancy, ethnicity, and ethnicity-pregnancy interaction as potential covariates for weight–adjusted CL/F, 2). pregnancy, race, and ethnicity-pregnancy interaction as potential covariates for $CL_{D\text{-}P2}$ /F and 3). gestational age and pregnancy as potential covariates for Vc/F. Following the forward inclusion and backward elimination processes, the model retained only pregnancy in non-African American women (Δ OFV= 21.4, $p<0.001$, χ^2, df=1) and oral contraceptive use (Δ OFV= 6.9, $p<0.01$, χ^2, df=1) as covariates of CL/F and pregnancy in non-African American women (Δ OFV= 29.9) as a covariate for $CL_{D\text{-}P2}$ /F. Co-administration of oral contraceptives occurred only in nonpregnant women (Table 1). The covariates for CL/F modestly reduced the IIV from 41% to 36% and the residual error from 40% to 32%. The IIV for $CL_{D\text{-}P2}$ /F decreased

299 from 101% to 86% with inclusion of the covariate. Other covariates did not produce a
300 statistically significant change in OFV, and were not included in the final model.
301 By reason of the similar magnitude of the coefficients for the two covariates of CL/F and
302 potential mediation of both effects through the actions of female sex hormones, the assignment
303 of a single coefficient to describe the impact of each covariate on CL/F was evaluated.
304 Following the substitution of a single coefficient to describe the effect of pregnancy in non-
305 African American women and oral contraceptive use on CL/F, no deterioration in the fit was
306 observed. Also, the coefficient, OFV and IIV remained unchanged despite the loss of a
307 parameter. The more parsimonious approach was adopted for the final model, with CL/F
308 expressed as:

309 $$CL/F = (\theta 1 + Race \times Preg \times \theta 2 + OC \times \theta 2) \times (LBW/50)$$

310 where θ1 represents CL/F in non-pregnant women not receiving oral contraceptives, Race = 0
311 for African American women and 1 for non-African American women, Preg = 0 if not pregnant
312 and 1 if pregnant, OC = 1 for women receiving oral contraceptives and 0 for women not
313 receiving oral contraceptives, θ2 = change in CL/F for pregnancy in non-African Americans or
314 use of oral contraceptives and LBW = lean body weight. The $CL_{D\text{-}P2}$ /F was expressed as:

315 $$CL_{D\text{-}P2}/F = \theta 3 + Race \times Preg \times \theta 4$$

316 where θ3 represents $CL_{D\text{-}P2}$ /F in non-pregnant women, Race and Preg are defined above, and θ4
317 = change in $CL_{D\text{-}P2}$ /F for pregnancy in non-African Americans.
318 Parameter estimates for the final model are presented in Table 2. The typical value for
319 azithromycin CL/F in a 50 kg lean body weight woman of any race who was not pregnant and
320 not receiving oral contraceptives was 134 l/h. Pregnancy in non-African American women or
321 co-administration of oral contraceptives lowered the CL/F of azithromycin by approximately

38%. For the azithromycin $CL_{D\text{-}P2}$ /F, an approximate 65% decrease occurred during pregnancy in non-African American women. Pregnancy in African American women had no effect on either CL/F or $CL_{D\text{-}P2}$ /F. Even after incorporation of the covariates, a high degree of IIV remained for the clearance and volume terms.

The close agreement, $\pm$ 15%, between the population parameters from the final model and bootstrap medians support the stability of the model and accuracy of the parameter estimates. The 2.5^{th} – 97.5^{th} percentiles from the bootstrap and the relative standard errors from the model fitting indicate that the fixed and random effect parameters were estimated with reasonable precision. An exception is the coefficient for the effect of pregnancy in non-African American women on $CL_{D\text{-}P2}$ /F, where the bootstrap confidence interval overlapped zero. However, despite the imprecision, the parameter was retained in the model as a result of the significant improvement in the model fitting following its addition and relatively narrow asymptotic standard error.

The visual predictive checks adjusted for pregnancy, race and oral contraceptive use are shown in Figure 2, and indicate acceptable predictive performance by the model. The number of observed plasma concentrations within the 80% prediction intervals was 84 of 92 (91%) in Figure 2A and 163 of 189 (86%) in Figure 2B.

Condensing the full profile data in the healthy women to provide a complete sparse sampling dataset for the population analysis did not affect the parameter estimates or covariate selection. Re-analysis of the population data with inclusion of the full profile data produced similar estimates, $\pm$ 15%, for the fixed and random effect parameters as the sparse dataset. The only exceptions were a 37% difference for Vc/F and 20% difference for $CL_{D\text{-}P2}$ /F. Interestingly, the relative standard errors for the fixed and random effects parameters averaged

21% lower when the analysis was performed with the sparse dataset, indicating a modestly improved precision compared to the hybrid dense/sparse sampling dataset.

The azithromycin AUC for each individual was derived from the individual Bayesian estimates of the pharmacokinetic parameters. The AUCs are summarized in Figure 3. Compared to nonpregnant women not receiving oral contraceptives, AUC was significantly lower during pregnancy in non-African American women (mean difference: 4.5, 95% simultaneous confidence interval: 0.1 – 8.8 mg-h/l) or with co-administration of oral contraceptives (mean difference: 11.4, simultaneous confidence interval: 5.7 – 17.2 mg-h/l). The AUC during pregnancy in non-African American women (mean difference: 6.4, 95% simultaneous confidence interval: 2.3 – 10.5 mg-h/l) or with co-administration of oral contraceptives (13.3, 95% simultaneous confidence interval: 7.8 – 18.9 mg-h/l) was also significantly lower than in African American women during pregnancy.

DISCUSSION

This study represents the first report describing how pregnancy affects the pharmacokinetics of a drug cleared by hepatobiliary excretion in an ethnically diverse population. Pregnancy significantly impacted the pharmacokinetics of azithromycin. Uniquely, the influence of pregnancy on azithromycin pharmacokinetics depended on ethnicity. Compared to women who were not pregnant and not receiving oral contraceptives, azithromycin CL/F and $CL_{D\text{-}P2}$ /F were significantly lower during pregnancy in women of Asian, Caucasian, Hispanic and Pacific Islander ethnicity. On the contrary, CL/F and $CL_{D\text{-}P2}$ /F in African American women during pregnancy were nearly identical to the values in non-pregnant women. Unfortunately, the small number of African American women in the non-pregnant group did not provide an adequate sample to definitively determine whether CL/F and $CL_{D\text{-}P2}$ /F are the same for non-

368 pregnant women of African American and non-African American ancestry. Bayesian estimates
369 of CL/F and $CL_{D\text{-}P2}$ /F for the two non-pregnant African American women in the study, neither
370 of whom were receiving oral contraceptives, fell within the 25^{th} and 75^{th} percentiles of the values
371 seen among all non-pregnant women. Although this limited sample suggests that CL/F and $CL_{D\text{-}P2}$ /F
372 are not impacted by ethnicity in nonpregnant women, further data is needed to establish this
373 point.

374 Co-administration of oral contraceptives in nonpregnant women also influenced
375 azithromycin CL/F, producing a decrease comparable to that observed during pregnancy in
376 women of non-African American ancestry. The occurrence of analogous alterations in drug
377 clearance from oral contraceptive administration and pregnancy are reported with other agents
378 (24, 35, 38, 42). Neither presence of an infection, type of infection, renal or hepatic disease,
379 creatinine clearance, concurrent medications other than oral contraceptives nor dose was found
380 to affect the pharmacokinetics of azithromycin.

381 The similar effects of oral contraceptives and pregnancy in non-African Americans on
382 azithromycin CL/F suggest a common estrogen or progesterone mediated mechanism. Likely
383 possibilities for the mechanism include an increase in bioavailability or reduction in
384 hepatobiliary excretion of azithromycin (3, 4, 34). The oral absorption and hepatobiliary
385 elimination of azithromycin are mediated in part by the drug efflux transporters, multidrug
386 resistance protein 2 (MRP2) and P-glycoprotein (3, 5, 8, 21, 52). Decreased expression of MRP2
387 on the canalicular membrane of hepatocytes during pregnancy and following administration of
388 ethinyl estradiol in rats suggest a role for MRP2 in the hormone-mediated changes in the
389 hepatobiliary clearance of azithromycin (11, 12, 32, 53). As distribution clearance depends on
390 blood flow and permeability of the drug from the vasculature to the tissues, the changes in $CL_{D\text{-}}$

391 $_{P2}$ /F most likely represent pregnancy-related alterations in tissue binding or intracellular
392 concentrations of azithromycin.
393 The reduced oral clearance results in an increased systemic exposure to azithromycin
394 with the administration of standard doses in pregnant women of non-African American ethnicity
395 compared to nonpregnant women. While a proportional decrease in dose would offset the
396 increased maternal and fetal drug exposure in these populations, limited understanding of the
397 pharmacodynamics of azithromycin during pregnancy hampers the ability to make an informed
398 decision for altering dose. As the antimicrobial efficacy of azithromycin best relates to the ratio
399 of area under the plasma concentration-time curve over minimum inhibitory concentration (2),
400 administering a lower dose during pregnancy in non-African American women would not be
401 expected to adversely impact therapeutic response. A potential factor complicating this
402 inference is the immune system changes reported to accompany pregnancy, generally enhanced
403 humoral and suppressed cell-mediated immunity (25). These changes may alter bacterial
404 responsiveness to azithromycin and the AUC required for therapeutic effectiveness. Uncertainty
405 on how to alter the target AUC complicates adjusting doses. The greater exposure must also be
406 considered from the viewpoint of maternal and fetal safety. Limited passage of azithromycin
407 across the placenta observed in *in vitro* (22) and *in vivo* (46) studies and good safety profile of
408 azithromycin administration in pregnancy from observational reports (14, 45, 50) suggest that the
409 increased exposure is unlikely to enhance harm to the fetus. However, systematic investigations
410 are needed to confirm safe and effective levels of azithromycin exposure in pregnancy.
411 Interestingly, our findings in African American women are consistent with those reported
412 by Salman et al. (49), where pregnancy was found not to influence either CL/F or $CL_{D\text{-}P2}$ /F in an
413 investigation of azithromycin pharmacokinetics in pregnant and age-matched non-pregnant

414 Papua New Guinean women. The only significant relationship identified by Salman *et al* in their
415 population pharmacokinetic analysis was between pregnancy and Vc/F (49), with Vc/F being
416 86% higher during pregnancy. Ramsey *et al* reported an elimination half life of approximately
417 12 hours for azithromycin in 20 women near term and scheduled for a cesarean section (46).
418 However, estimation of this value from plasma concentrations collected at 6 to 24 hours after the
419 dose indicates that the values actually represent the distribution half lives and are consistent with
420 values reported by Salman *et al* (49) for the distribution half life of azithromycin in non-pregnant
421 and pregnant women.

422 A secondary aim was to confirm the ability of the sparse sampling strategy to provide
423 appropriate estimates of the pharmacokinetic parameters and identify factors contributing to
424 pharmacokinetic variability in pregnancy. The collection of a small number of samples per
425 individual offers several advantages including facilitating implementation of the study in a
426 clinical setting, allowing informative pharmacokinetic data to be obtained in a population
427 representative of the patients typically receiving treatment and minimizing the impact of the
428 research on the subject (28). However, reliance on sparse data for the population
429 pharmacokinetic analysis may potentially reduce the precision of the parameter estimates and
430 power of the covariate analysis (10, 16, 27, 47). These drawbacks reflect the influence that the
431 number of samples per individual has on the standard error of the parameter estimates (16, 37),
432 inter-individual and residual variability (1, 27), and shrinkage of individual parameter estimates
433 (10, 27). These issues were not found to have a noticeable impact on the outcome of the current
434 study. Validation by goodness of fit plots, bootstrap analysis, visual predictive checks and re-
435 analysis using a combined dense/sparse dataset supported the ability of the limited sampling
436 model to accurately and precisely characterize the population pharmacokinetics of azithromycin

and appropriately identify important covariates. Similar to other reports, the addition of some subjects with full profiles did not improve performance (10, 31). In fact, the sparse dataset estimated the parameters with greater precision than the hybrid dense/sparse dataset. The good performance of our sparse sampling strategy likely relates to the use of d-optimally constructed sampling windows to guide the sampling times. Others have shown that the collection of sparse samples at optimal times compensates for the analytical problems cited above (10, 19).

A limitation of this study is the small number of African American women in the nonpregnant group. Although the values for CL/F in the 2 nonpregnant African American patients provide preliminary evidence of similar CL/F as observed in the non-African American patients, the data did not allow us to definitively establish this value. The influence of ethnicity on the action of oral contraceptives was also unable to be assessed due to the lack of African Americans among the women receiving oral contraceptives.

The population pharmacokinetic analysis identified several factors contributing to pharmacokinetic variability in women of child-bearing age, including lean body weight, pregnancy and co-administration of oral contraceptives. Ethnicity influenced the changes in azithromycin pharmacokinetics seen during pregnancy. The environmental and genetic causes for these ethnically-related differences are important considerations for future studies. The pharmacokinetic changes during pregnancy predict increased maternal and fetal exposure to azithromycin when non-African American women receive standard (i.e., those given to non-pregnant women) doses during pregnancy. Potential immunological changes in pregnant women and limited understanding of safe levels of fetal azithromycin exposure warrant further investigation to determine the clinical implications of these pharmacokinetic changes.

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Table 1

Characteristics of Patients and Healthy Volunteers*

Parameter	Pregnant Patients	Non-Pregnant Patients	Healthy Women
Number	53	13	12
Age (years)	28 (18-41)	33 (28-49)	24 (21-32)
Gestational Age (weeks)	29.1 (11.9-39)	---	---
Total body weight (kg)	76 (47-178)	67 (47-112)	61 (45-84)
Lean Body Weight (kg)	45 (32-68)	43 (33-57)	40 (30-51)
Height (cm)	163 (138-175)	160 (155-173)	160 (150-176)
Creatinine Clearance (ml/min)	127 (45-229)	83 (37-115)	91 (69-109)
Ethnicity			
African American	17	2	0
Asian	3	1	1
Caucasian (non-Hispanic)	28	6	8
Hispanic	5	3	1
Pacific Islander	0	1	2
Infection, number			
Upper or lower RTI	30	11	---
PROMs	14	0	---
Chlamydia	8	0	---
Skin	1	2	---
Azithromycin oral regimen, number			
500 or 1000 mg day 1, then 250 mg daily for 4 days	34	11	12
1000 mg single dose	8	0	0
Other	11	2	0
Concurrent Medication,† number			
Albuterol	3	1	0
Amoxicillin	14	0	0
Ampicillin	13	0	0
Betamethasone	6	0	0
Ceftriaxone	7	0	0
Fluoxetine	3	0	0
Fluticasone	3	0	0
Gabapentin	0	2	0
Insulin	4	1	0
Oral contraceptives	0	4	6
Prednisone	2	1	0

*median (range) unless indicated otherwise

† Excludes vitamins, oral iron and medications received by 2 or less participants

Abbreviations: PROMS: premature rupture of membranes, RTI: respiratory tract infection

Table 2

Population Pharmacokinetic Parameters and Bootstrap Results from the Final Covariate Model

Parameter	Final Model		Bootstrap (n=1000)	
	Estimate	RSE (%)	Median	2.5^{th} - 97.5^{th} Percentiles
k_a (h^{-1})	0.8	---	---	---
t_{lag} (h)	1.3	0.1	1.3	1.0 – 1.6
CL/F (l/h/50 kg LBW)	134	12	135	85 - 176
Effect of pregnancy in non-African Americans **Or** Effect of co-administration of oral contraceptives	-51	28	-44	-78 - -4
CL_{D-P1} /F (l/h)	401	14	398	235 - 609
CL_{D-P2} /F (l/h)	120	15	115	35 - 208
Effect of pregnancy in non-African Americans	-78	31	-72	-140 - 29
Vc/F (l)	456	11	436	189 - 716
V_{P1}/F (l)	1560	29	1630	925 - 3629
V_{P2}/F (l)	16100	16	17400	6124 - 31837
Inter-Individual Variability (CV, %)				
CL/F	36	39	34	16 - 49
CL_{D-P2} /F	86	48	86	3 - 133
Vc/F	114	29	116	75 - 161
V_{P1}/F	60	48	60	0.5 - 110
Residual error (CV, %)	32	34	32	18 - 42

Abbreviations: RSE: relative standard error, CV: coefficient of variation, k_a: absorption rate constant, t_{lag}: lag time, CL/F: oral clearance, CL_{D-P1} /F: apparent intercompartmental clearance from the central to peripheral compartment 1, CL_{D-P2} /F: apparent intercompartmental clearance from the central to peripheral compartment 2, Vc/F: apparent volume of the central compartment, V_{P1}/F: apparent volume of peripheral compartment 1, V_{P2}/F: apparent volume of peripheral compartment 2.