Pharmacokinetics of antitubercular drugs in patients hospitalized with HIV-associated tuberculosis: a population modeling analysis [version 2; peer review: 2 approved with reservations]

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Abstract

Background. Early mortality among hospitalized HIV-associated tuberculosis (TB/HIV) patients is high despite treatment. The pharmacokinetics of rifampicin, isoniazid, and pyrazinamide were investigated in hospitalized TB/HIV patients and a cohort of outpatients with TB (with or without HIV) to determine whether drug exposures differed between groups.

Methods. Standard first-line TB treatment was given daily as per national guidelines, which consisted of oral 4-drug fixed-dose combination tablets containing 150 mg rifampicin, 75 mg isoniazid, 400 mg pyrazinamide, and 275 mg ethambutol. Plasma samples were drawn on the 3rd day of treatment over eight hours post-dose. Rifampicin, isoniazid, and pyrazinamide in plasma were quantified and NONMEM was used to analyze the data.

Results. Data from 60 hospitalized patients (11 of whom died within 12 weeks...
of starting treatment) and 48 outpatients were available. Median (range) weight and age were 56 (35 - 88) kg, and 37 (19 - 77) years, respectively. Bioavailability and clearance of the three drugs were similar between TB/HIV hospitalized and TB outpatients. However, rifampicin's absorption was slower in hospitalized patients than in outpatients; mean absorption time was 49.9% and 154% more in hospitalized survivors and hospitalized deaths, respectively, than in outpatients. Higher levels of conjugated bilirubin correlated with lower rifampicin clearance. Isoniazid's clearance estimates were 25.5 L/h for fast metabolizers and 9.76 L/h for slow metabolizers. Pyrazinamide's clearance was more variable among hospitalized patients. The variability in clearance among patients was 1.70 and 3.56 times more for hospitalized survivors and hospitalized deaths, respectively, than outpatients.

Conclusion. We showed that the pharmacokinetics of first-line TB drugs are not substantially different between hospitalized TB/HIV patients and TB (with or without HIV) outpatients. Hospitalized patients do not seem to be underexposed compared to their outpatient counterparts, as well as hospitalized patients who survived vs who died within 12 weeks of hospitalization.

Keywords rifampicin, isoniazid, pyrazinamide, hospitalization, tuberculosis

This article is included in the Wellcome Centre for Infectious Diseases Research in Africa (CIDRI-Africa) gateway.
Amendments from Version 1
We addressed the comments by the reviewers. The title was changed to better reflect the main objective of the analysis where we focused on the hospitalization status of TB patients and whether the hospitalized participants were underexposed compared to their outpatients counterparts. The hospitalized column in the participant characteristics table was further stratified into hospitalized survivors and hospitalized deaths. A table was added summarizing the AUC0-24h and Cmax values for all three drugs.

Any further responses from the reviewers can be found at the end of the article

Introduction
The mortality rate among treated hospitalized HIV-associated tuberculosis (TB/HIV) patients is high, ranging from 11% to 32%1,2. Hospitalized TB/HIV patients usually have some features of bacterial sepsis, with elevated venous lactate levels, and impaired intestinal barrier function, resulting in microbial translocation and high levels of circulating lipopolysaccharide, which mediates an inflammatory response3,4. Delayed gastric emptying and changes in gastric pH have been observed in severely ill patients5. The gastrointestinal changes in severely ill patients could lead to differences in the rate and amount of drug absorption, and therefore affect drug exposure6. Other changes in severely ill patients may include increased volume of distribution, changes in plasma protein binding, and changes in the intrinsic activity of drug metabolizing enzymes or in the hepatic blood flow that may affect drug clearance6-7. These changes could negatively affect the treatment outcome in vulnerable patients. These changes could affect the levels of drug concentrations. Lower antitubercular drug concentrations have been shown to be associated with delayed sputum culture conversion times as shown by Sekaggya-Wiltshire et al.8 and Mah et al.9.

In addition to the extent of disease that could result in variable absorption, rifampicin’s extent and rate of absorption is highly variable8. Rifampicin is mainly cleared by the liver and undergoes extensive first-pass metabolism9. Saturable elimination has been reported for rifampicin at higher doses due to saturation of the biliary transport mechanisms10,11,12. After repeated administration, rifampicin exhibits autoinduction, in which it increases its own metabolism partly by activating the pregnane X receptor13, which in turn induces the B-estrases in liver microsomes, which are responsible for the biotransformation of rifampicin to 25-desacetyl rifampicin14,15.

Isoniazid also has highly variable pharmacokinetics, mainly due to genetic polymorphism in N-acetyltransferase 2 (NAT2), the enzyme responsible for metabolizing the drug; the elimination of isoniazid in fast-acetylators is up to six times faster than the slow acetylators16. Body composition parameters such as weight and fat-free mass (FFM) are usually good predictors of clearance and volume of distribution for many drugs. FFM is generally advised as a better scalar than bodyweight since it accounts for both the difference in body size and composition, unlike weight, which accounts for body size only17,18.

The aim of the study was to compare the pharmacokinetics of rifampicin, isoniazid, and pyrazinamide between hospitalized patients and outpatients recruited from the same hospital catchment area.

Methods
Study population
The study population is made up of two groups: the hospitalized patients and outpatients recruited as controls. The hospitalized study population for this pharmacokinetic (PK) sub-study was a subset from participants enrolled for an observational cohort study investigating the mortality causes in hospitalized TB/HIV patients carried out between November 2014 and November 201616. Patients presenting to Khayelitsha Hospital in Cape Town, South Africa with TB/HIV who needed hospitalization due to tuberculosis (i.e., who were too ill to receive initial treatment as outpatients) and who survived to the third day of TB treatment were enrolled sequentially, as long as they still needed inpatient care and did not require transfer to a tertiary care facility for intensive care or investigations. The study team invited eligible hospitalized patients in the parent study to take part and discussed with them the study. Tuberculosis (TB) outpatients with or without HIV were recruited from around the same hospital catchment area as controls. The study team liaised with the clinic staff to ask any new patients when they were started on TB treatment if they would like to discuss taking part in the PK sub-study.

Study design
All participants received a once daily dosing of antitubercular drugs that were given as 4-drug fixed-dose combination (FDC) tablets containing rifampicin-isoniazid-pyrazinamide-ethambutol at 150/75/400/275 mg, which were either Rifafour e-275 tablets (SANOFI) or Ritib tablets (PHARMACARE)). The number of tablets to be given to each participant is determined based on their weights according to the weight-based dosing of the South African national TB management guidelines outlined in Table 1. Clinical data and baseline blood tests...
were obtained at enrolment. The 12-week mortality outcome was documented for hospitalized patients.

**Ethics and consent**

The study was approved by University of Cape Town Human Research Ethics Committee (UCT HREC reference: 057/2013) on 12 April 2013. All participants signed an informed consent form.

**Data collection**

For each patient, age, sex, weight, height and details of concomitant medications were collected, and a complete medical history was recorded. Serum chemistry and a complete blood picture were carried out at the Groote Schuur National Health Laboratory Services on each participant on samples taken at enrolment for the PK study.

Participants were scheduled for a PK visit during their 3rd day of treatment, when blood samples were drawn just before and 1, 2.5, 4, 6, and 8 hours after dose. Participants were required to fast overnight and they were given a standardized breakfast after the 1-hour sample and a standardized lunch between the 4- and 6-hour sample. Immediately following their collection, samples were put in an ice bath until being centrifuged in a cooling centrifuge and later stored at -80°C.

**Drug quantification**

Plasma rifampicin, isoniazid and pyrazinamid concentrations were determined by validated liquid chromatography with tandem mass spectrometry assays at the Division of Clinical Pharmacology, University of Cape Town. The lower limit of quantification (LLOQ) was 0.117 mg/L for rifampicin, 0.105 mg/L for isoniazid, and 0.203 mg/L for pyrazinamide. The accuracy of the low-, medium-, and high-quality control samples ranged between 99.7% - 100.8% for rifampicin, 98.3% - 100.4% for isoniazid, and 88.1% and 92.3% for pyrazinamide. The precision of the quality control samples ranged from 4.7 - 7.7%, 3.0% - 5.1%, and 2.9% - 3.6% for rifampicin, isoniazid, and pyrazinamide, respectively.

**Pharmacokinetic and statistical analyses**

A population pharmacokinetic model was developed for each of the three drugs using nonlinear mixed-effects modeling in NONMEM® version 7.4 and the algorithm first-order conditional estimation with eta-epsilon interaction (FOCE) Pirana was used for model management, Perl-speaks-NONMEM (PsN) 4.9.0 was used for post-processing of NONMEM® results and and R version 3.6.2 was used for generating the figures. Different disposition models with first-order elimination were evaluated. The use of a lag time and transit compartments were tried to capture the delay in the first-order absorption process. Between-subject variability was evaluated for all disposition parameters and between-occasion variability was assessed for bioavailability, and other absorption parameters. Censored below the lower limit of quantification (BLQ) concentration values were handled as per Beal’s M6 method, in which the first BLQ values in series were replaced with LLOQ/2 and the subsequent BLQs were discarded. Residual unexplained variability was described using a combination of additive and proportional error components. The additive error was bound to be at least 20% of the LLOQ. Allometric scaling of clearance and volume parameters was tested as suggested by Anderson and Holford using the fixed power exponents of 0.75 for clearance and 1 for volume. Body weight and FFM, calculated based on the formula in Jannmahasatian et al., were both tested for allometric scaling as body size descriptors. Since no NAT2 genotyping data were available, mixture modeling was used for the isoniazid pharmacokinetic model to distinguish between the clearances of different groups of metabolizers.

Following the development of a basic model, covariate testing was done. Various effects, including hospitalization, patient status (outpatients or hospitalized who survived or hospitalized who died within 12 weeks), drug formulation, and various biomarkers which indicate general organ dysfunction e.g. aspartate transaminase (AST), alanine transaminase (ALT), serum creatinine, serum urea, albumin, and procalcitonin, were tested on clearance, bioavailability, and absorption parameters for all three drugs. Trefoil factor-3, which is a marker of gut barrier integrity was tested as a covariate on the absorption parameters. The effect of concurrent administration of HIV drugs was tested.

The model development process and covariate inclusion were guided by physiological plausibility, model fit diagnostics including drop in the objective function value (OFV) and inspection of diagnostic plots. Comparison between nested models was done using the likelihood ratio test for the drop in OFV, assumed to be approximately χ² distributed with n degrees of freedom, where n is the number of additional estimated parameters. A p-value of 0.05 was generally used for inclusion and 0.01 for retention. Visual predictive checks (VPCs) were used to assess compatibility of the model with the observed data. Weakly-informative priors on the ratio of the volume of the central compartment (Vc) to the volume of the peripheral compartment (Vp), Vratio (Vc/Vp) with 30% uncertainty were used to stabilize the model for isoniazid PK. The typical values were obtained from a previously published model.

The precision of the parameter estimates, expressed as the 95% confidence intervals, was assessed by applying a non-parametric bootstrap with 500 iterations. A non-compartmental pharmacokinetic (PK) analysis of the same participants in this report has previously been published.

**Variability correlation across the three drugs**

Correlations of unexplained variability in the pharmacokinetic parameters: clearance, bioavailability, area under the curve from time 0 to 24 hours (AUC₀₋₂₄) and absorption between each of the three drugs were assessed to check if there was any relation between the unexplained variabilities in each pharmacokinetic parameter between the three drugs. There were two occasions per patient. An occasion is defined as any dosing event followed by at least one sample. When checking the correlation between variabilities, only the variability from the primary occasion was included i.e. the occasion associated with the predose was excluded.
Results
Study data
A total of 108 patients completed the study; 60 were hospitalized TB/HIV patients, and 48 were TB outpatients, of whom 29 were HIV-positive. Table 2 provides a summary of the participants characteristics. Four hospitalized patients (n=4, 3.7%) had missing height values, which were replaced by the regression-imputed values based on sex. Two hospitalized patients with renal impairment had individual tablets for each drug instead of the FDC to allow adjustment of the ethambutol dose, and one hospitalized patient had the tablets crushed, mixed with water, then inserted via a nasogastric tube. All patients had blood samples collected on the 3rd day of treatment, except for one participant, in whom it was found out during the study that there was an earlier dose, so the collected samples were on the 4th day of treatment. Nine participants were on efavirenz, 9 on tenofovir, 1 on lopinavir/ritonavir, and 1 on lamivudine.

A total of 108 pharmacokinetic profiles with 632 concentration-time observations for each of the three drugs were available for analysis. The number of observations that were BLQ were 33 (5.2%), 88 (13.9%), and 1 (0.2%) for rifampicin, isoniazid, and pyrazinamide, respectively, most of which were predose samples. The 12-week mortality rate of hospitalized patients was 11/60 (18%). One participant was lost to follow up after 2 months; the participant’s results were included in the survived group. We chose to stratify the analysis of the hospitalized patients into those who survived and those who died within 12 weeks as an indicator of severity of the patients’ sickness.

Rifampicin pharmacokinetics
Rifampicin pharmacokinetics was best characterized by a one-compartment disposition model with first-order elimination, and absorption described by a chain of transit compartments. The parameter values of the final model are shown in Table 3. The model fit the data well as shown in the VPC in Figure 1. Apparent clearance (CL) and apparent volume of distribution (V) were allometrically scaled using FFM as a body size descriptor. Allometric scaling by FFM (difference in OFV, dOFV = -30, df = 2, p-value = <0.001) resulted in a more significant drop than scaling by total body weight (dOFV = -7.7, df = 2, p-value = 0.02). The typical CL and V values for a participant with the median FFM were 8.82 L/h and 56.8 L, respectively.

No difference in CL or bioavailability was found between hospitalized survivors, hospitalized deaths, and outpatients. Nonetheless hospitalized patients, and even more so those who died in the first 12 weeks, were found to absorb rifampicin slower than outpatients (dOFV = -16.1, df = 2, p-value = <0.001). The effect of patient group (outpatients, hospitalized survivors and hospitalized deaths) was modeled on the absorption rate constant (ka) and 1/mean absorption

<table>
<thead>
<tr>
<th>Table 2. Participants baseline characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median (1st – 3rd Quartile) or no. (%) of participants given</strong></td>
</tr>
<tr>
<td><strong>Sex: Females</strong></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
</tr>
<tr>
<td><strong>Fat-free mass (kg)</strong></td>
</tr>
<tr>
<td><strong>Total bilirubin (μmol/L)</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Conjugated bilirubin (μmol/L)</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Lactate (mmol/L)</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Aspartate aminotransferase, AST (U/L)</strong>&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Alanine aminotransferase, ALT (U/L)</strong>&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>HIV-positive</strong></td>
</tr>
<tr>
<td><strong>Current antiretroviral therapy</strong></td>
</tr>
<tr>
<td><strong>CD4 Count (cells μL&lt;sup&gt;-1&lt;/sup&gt;)</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup> Total bilirubin was missing for 1 hospitalized patient and 1 outpatient  
<sup>b</sup> Conjugated bilirubin was missing for 5 hospitalized patients and 3 outpatients  
<sup>c</sup> Lactate was missing for 2 hospitalized patients and 13 outpatients  
<sup>d</sup> AST was missing for 8 hospitalized patients and 3 outpatients  
<sup>e</sup> ALT was missing for 1 hospitalized patient and 1 outpatient
Table 3. Final population pharmacokinetic parameter estimates for rifampicin, isoniazid and pyrazinamide.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical value (95% CI)</th>
<th>[Shrinkage]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rifampicin</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>Clearances (L/h)</td>
<td>8.82 (8.10 – 9.48)</td>
<td>-</td>
</tr>
<tr>
<td>Clearances of fast metabolizers (L/h)</td>
<td>-</td>
<td>25.5 (22.7 – 28.7)</td>
</tr>
<tr>
<td>Clearances of slow metabolizers (L/h)</td>
<td>-</td>
<td>9.76 (8.28 – 11.2)</td>
</tr>
<tr>
<td>Proportion of fast NAT2 metabolizers (%)</td>
<td>-</td>
<td>64.5 (54.4 – 75.8)</td>
</tr>
<tr>
<td>Volume of distribution (L)</td>
<td>56.8 (53.9 – 61.2)</td>
<td>59.0 (54.7 – 64.2)</td>
</tr>
<tr>
<td>Intercompartmental clearance, Q (L/h)</td>
<td>-</td>
<td>1.43 (0.874 – 2.14)</td>
</tr>
<tr>
<td>Peripheral volume, Vp (L)</td>
<td>-</td>
<td>30.7 (25.9 – 37.1)</td>
</tr>
<tr>
<td>Absorption rate constant, ka (h⁻¹)</td>
<td>1.38 (1.04 – 1.70)</td>
<td>2.43 (1.80 – 6.50)</td>
</tr>
<tr>
<td>Mean transit time, MTT (h)</td>
<td>0.342 (0.259 – 0.534)</td>
<td>0.442 (0.266 – 0.781)</td>
</tr>
<tr>
<td>No. of absorption transit compartments (.)</td>
<td>12 fixed</td>
<td>8 fixed</td>
</tr>
<tr>
<td>Bioavailability, F (%)</td>
<td>100 fixed</td>
<td>100 fixed</td>
</tr>
<tr>
<td>% difference in mean absorption time (MAT) for hospitalized survivors²</td>
<td>+49.9 (+2.80 – +80.9)</td>
<td>-</td>
</tr>
<tr>
<td>% difference in MAT for hospitalized deaths³</td>
<td>+154 (+63.9 – 351)</td>
<td>-</td>
</tr>
<tr>
<td>Exponent of power relationship between Clearance and conjugated bilirubin⁴</td>
<td>-0.333 (-0.474 – -0.194)</td>
<td>-</td>
</tr>
<tr>
<td>Between-subject variability for clearance (BSVCL) (%)</td>
<td>42.4 (37.3 – 49.4) [7%]</td>
<td>25.3 (17.2 – 33.4) [24%]</td>
</tr>
<tr>
<td>Fold change in BSVCL for hospitalized survivors¹</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fold change in BSVCL for hospitalized deaths¹</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Between-occasion variability (BOV) (%) for:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioavailability</td>
<td>21.3 (16.4 – 27.6) [41%]</td>
<td>34.9 (26.6 – 40.3) [31%]</td>
</tr>
<tr>
<td>Absorption rate constant, ka</td>
<td>119 (100 – 137) [32%]</td>
<td>122 (84.0 – 186) [51%]</td>
</tr>
<tr>
<td>Mean transit time, MTT</td>
<td>93.8 (67.4 – 111) [49%]</td>
<td>99.7 (45.9 – 172) [56%]</td>
</tr>
<tr>
<td>Proportional error (%)</td>
<td>17.2 (14.9 – 18.5)</td>
<td>13.9 (12.0 – 16.4)</td>
</tr>
<tr>
<td>Additive error (mg/L)</td>
<td>0.0234 fixed¹</td>
<td>0.021 fixed¹</td>
</tr>
</tbody>
</table>

¹ Values in parentheses are empirical 95% confidence intervals, obtained with a 500-sample nonparametric bootstrap ² The values of CL/F and V/F were allometrically scaled, so the typical values reported here refer to the median body weight of 66 kg and the median FFM of 43 kg of the cohort included in the model. ³ The peripheral volume, Vp, was calculated from the estimated Vratio (Vc/Vp) and Vc. A prior of 2.02 was included on Vratio with 30% uncertainty. ⁴ Patient status effect was modeled on ka and 1/MTT simultaneously using the same effect parameter, theta (θ). ka for hospitalized = TVka × θ; MTT for hospitalized survived or hospitalized deaths group = TVMTT / θ. Mean absorption time = MTT+1/ka calculated for each group was 1.1 h for outpatients, 1.6 h for hospitalized survivors, and 3.2 h for hospitalized deaths. ⁵ Conjugated bilirubin (BRC) Effect on CL = (BRC/median BRC)⁻⁰.₃³³; CL = TVCL x BRC Effect on CL. ⁶ BSVCL for hospitalized = BSVCL × fold change. i.e BSVCL is 33.8% for hospitalized survivors and 70.8% for hospitalized deaths. ⁷ The estimate of the additive component of the error was not significantly different from its lower bound of 20% of LLOQ, so it was fixed to this value.

The time (MTT) simultaneously using the same effect parameter, theta (θ), as outlined in the formulae below.

\[
\text{MTT}_{\text{group}} = \frac{\text{MTT}_{\text{outpatients}}}{\theta_{\text{patient group effect}}} \quad k_{\text{group}} = \theta_{\text{patient group effect}} \cdot k_{\text{outpatients}}
\]

Where \( \text{MTT}_{\text{outpatients}} \) is the typical mean transit time for the outpatients in hours, \( \text{MTT}_{\text{group}} \) is the mean transit time for hospitalized survived or hospitalized deaths group in hours, \( k_{\text{group}} \) is the absorption rate constant for hospitalized survivors or hospitalized deaths group in hour⁻¹, and \( k_{\text{outpatients}} \) is the typical absorption rate constant for outpatients in hour⁻¹.

On average, hospitalized patients who survived had a mean absorption time (MAT) of 1.6 h (accounting for both MTT and...
ka), while the value was 2.7 h for hospitalized patients who died in the first 12 weeks, compared to 1.1 h for outpatients.

Additionally, we found that higher values of conjugated bilirubin (BRC) were correlated with lower values of rifampicin CL (dOFV = -17.3, df = 1, p-value = <0.001), according to the power relationship outlined below

$$CL_i = CL_{\text{typical}} \left( \frac{BRC_i}{BRC_{\text{median}}} \right)^{\theta_{\text{bac}}}$$

Figure 1. Visual predictive check (VPC) (n=1000) showing plasma drug concentration versus time after dose for the final models of each drug: a) for rifampicin stratified by patient group; b) for isoniazid stratified by metabolizer status; c) for pyrazinamide stratified by patient group. The circles are the original observations; the solid line and the dashed lines are the median, 5th and 95th percentiles of the observed data; the shaded areas are the 95% confidence intervals of the same percentiles as simulated by the model. A suitably fitting model will have most of the observed percentiles within the simulated confidence intervals.
Where $CL_i$ is the clearance for patient $i$, $CL_{typical}$ is the typical clearance, which is 8.82 L/h, $BRC_i$ is the BRC for patient $i$, $BRC_{median}$ is the BRC median in all patients (6 µmol/L) and $\beta_{BRC}$ is the exponent of power relationship between $CL$ and $BRC$, estimated to be -0.333. The power function was a better fit for the relationship between BRC and CL compared to linear, piece-wise linear. The relationship is depicted in Figure 2. Both total bilirubin (BRT) and BRC were found to correlate significantly with CL; however, the two covariates (i.e. BRT and BRC) are highly positively correlated ($r = 0.860$), so only one of them was included in the final model. BRC was chosen over BRT because it resulted in a more significant drop in OFV. We tried incorporating saturation of elimination of rifampicin and the first-pass metabolism into the model. However, both models resulted in a marginal improvement of the fit. Therefore, we decided to keep the model more straightforward and not include either saturation or first-pass metabolism in the final model. Also, the inclusion of autoinduction of rifampicin clearance was tested in the model, but did not result in an improved fit for the data.

None of the biomarkers tested were found to correlate significantly to CL, except for the level of venous lactate and AST. However, the correlation between clearance and lactate or AST was less significant than the correlation with BRC, so only the effect of BRC was included in the final model.

An effect for the formulation was found to be statistically significant ($dOFV = -12.9$, $df =1$, $p$-value < 0.001), with the individual tablets having 21.8% of the bioavailability of FDC. However, only two participants were on individual tablets ($n = 2$, 1.85%) instead of the FDC, one of whom vomited during the study.

Isoniazid pharmacokinetics

A two-compartment disposition model with first-order absorption with a chain of transit compartments and first-order elimination proved to fit the data best. The final parameter estimates are shown in Table 3.

A 2-compartment model was a better fit than the 1-compartment in terms of a significant drop in OFV, which was about 42 points, and by a VPC, but the model was unstable and Vp could not be reliably estimated. To stabilize the estimate of the Vp, a prior was included on the Vratio ($Vc/Vp$) with a value of 3.728 with 30% uncertainty. Allometric scaling of CL and Vc using FFM was used because it caused a more significant drop in the OFV of 24.9 points instead of weight which caused a drop of only 15.5 points.

Mixture modeling was used to account for the differences in CL between fast and slow metabolizers in place of NAT2 genotype testing ($dOFV = -15.5$, $df =2$, $p$-value < 0.0005). The proportion of fast metabolizers was estimated to be 64.5%. The typical clearance values were estimated to be 25.5 L/h and 9.76 L/h for fast and slow metabolizers. A three-component mixture distinguishing into fast, intermediate, and slow metabolizers was examined but was not supported by the data.
Figure 1 includes a VPC for the final isoniazid model stratified by metabolizer type, indicating that the model fit the data well. Isoniazid pharmacokinetics were not different in hospitalized patients compared to outpatients. The final parameter estimates are shown in Table 3.

**Pyrazinamide pharmacokinetics**
A one-compartment disposition model with first-order elimination and first-order absorption with transit absorption compartments best fit the data. Allometry with FFM was applied to CL and V (dOFV = - 32.6 points for FFM, better than total body weight, dOFV = -28.3). Final parameter values are displayed in Table 3.

No significant differences were found in the CL, bioavailability, or absorption between hospitalized survivors, hospitalized deaths, and outpatients. The between-subject variability in CL was significantly higher among hospitalized patients, i.e. 20% for outpatients vs 33.8% for hospitalized patients who survived vs 70.8% for hospitalized patients who died within 12 weeks (dOFV = -27, df=2, p-value < 0.001). A VPC showing that the model correctly captures the data for pyrazinamide is shown in Figure 1.

Neither the HIV status nor the CD4+ cell count influenced the pharmacokinetics of any of the three drugs. The effect of efavirenz co-administration (n = 9) was tested on the CL and bioavailability of all three drugs. No significant effect for the co-administration of efavirenz was found.

The model-derived individual \(\text{AUC}_{0-24h}\) and \(C_{max}\) for all three drugs are shown in Table 4 and are represented graphically in Figure 3 in a box and whisker plots.

**Variability correlation across the three drugs**
The correlations of the remaining unexplained variability in clearance, bioavailability, \(\text{AUC}_{0-24h}\), and absorption among the three drugs were assessed and the results are shown in the correlation matrix in Figure 4. The equations used to calculate the unexplained variabilities for each parameter are shown below.

**Discussion**
The main finding of our analysis is that the overall drug exposures for rifampicin, isoniazid, and pyrazinamide are similar between hospitalized TB/HIV patients who died with 12 weeks, hospitalized TB/HIV patients who survived and TB outpatients. For rifampicin, our model showed that absorption was slower in hospitalized patients, even slower among hospitalized patients who died within 12 weeks, and that higher levels of bilirubin were associated with lower rifampicin clearance. For pyrazinamide, the between-subject variability in CL was higher among hospitalized patients, and higher among hospitalized patients who died compared to hospitalized patients who survived. While rifampicin absorption is slower in hospitalized patients, the bioavailability is similar between hospitalized who died, hospitalized who survived and outpatients, which indicates that intravenous rifampicin administration may be not be necessary for hospitalized TB patients.

There are limited data comparing the pharmacokinetics of first-line anti-TB drugs between hospitalized patients and outpatients, especially during the first few days of starting treatment. A non-compartmental analysis (NCA) of the data from this pharmacokinetic study was published by Schutz et al. The NCA show that the overall exposures of all three drugs among hospitalized patients and outpatients were similar, which is in line with our findings.

For all three models, we found allometric scaling by FFM to be better than by total body weight in capturing the differences in \(\text{CL/F}\) and \(\text{V/F}\) due to differences in body size. FFM is more physiologically plausible because it takes into account not just body size but also body composition. Since FFM is a more accurate predictor for \(\text{CL/F}\) and \(\text{V/F}\), but dosing is done based on weight, patients with lower weights end up being more underestimated. This is supported by Muliaiditan et al., Rockwood et al., and McIliveron et al. who have shown that patients with lower body weights are underexposed relative to patients.

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**Table 4.** \(C_{max}\) and \(\text{AUC}_{0-24h}\) for rifampicin, isoniazid, and pyrazinamide stratified into outpatients, hospitalized survivors, and hospitalized deaths.

<table>
<thead>
<tr>
<th></th>
<th>Median (1st - 3rd Quartile)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(C_{max}) (mg/L)</td>
</tr>
<tr>
<td></td>
<td>Outpatients</td>
</tr>
<tr>
<td><em>Rifampicin</em></td>
<td></td>
</tr>
<tr>
<td>8.05</td>
<td>7.41</td>
</tr>
<tr>
<td>(6.77 – 9.48)</td>
<td>(5.52 – 9.15)</td>
</tr>
<tr>
<td><em>Isoniazid</em></td>
<td>3.62</td>
</tr>
<tr>
<td>(2.55 – 4.31)</td>
<td>(2.75 – 5.05)</td>
</tr>
<tr>
<td><em>Pyrazinamide</em></td>
<td>45.4</td>
</tr>
<tr>
<td>(40.7 – 53.6)</td>
<td>(42.7 – 50.0)</td>
</tr>
</tbody>
</table>
with higher body weight. Therefore, we agree with their conclusions that the clinical recommendation for the use of weight-banded dosing regimens should be reconsidered to take into account the variability in body size and composition.

The pharmacokinetic parameters from our models for rifampicin, isoniazid and pyrazinamide were comparable to those from other similar studies\textsuperscript{10,28–31}

**Rifampicin PK model**

The structural model we developed for rifampicin was similar to previously developed rifampicin models\textsuperscript{10,32}. However, CL values are lower in this analysis because autoinduction was not included in the model. Regarding the differences in absorption, published articles report that critically ill patients tend to have a more impaired absorption of drugs through a decreased barrier gut function and delayed gastric emptying, which lead to reduced perfusion of the gastrointestinal tract\textsuperscript{5–7}. We reason that only rifampicin’s absorption out of the three drugs was affected by these gastrointestinal changes because of rifampicin’s low solubility\textsuperscript{35}, whereas both isoniazid and pyrazinamide have high solubility according to the biopharmaceutics classification system\textsuperscript{36,37}. Rifampicin’s absorption is mainly from the stomach and proximal intestine\textsuperscript{38} and is more likely to be easily affected by changes in the gastric pH\textsuperscript{11}. As a result, the \(C_{\text{max}}\) for hospitalized patients tends to be lower than that of the outpatients, while the \(AUC_{0-24}\) does not seem to be affected as shown in Figure 3. A systemic review by Muda et al.\textsuperscript{32} of rifampicin pharmacokinetic models concludes that the sources of variability in rifampicin’s pharmacokinetics are still not fully understood and could be due to factors such as drug-drug interactions, and different formulations.

Rifampicin and its major metabolite are mostly excreted through the biliary tract, the same tract that excretes bilirubin. Therefore, higher bilirubin levels correlate with lower rifampicin clearances since bilirubin and rifampicin compete for the same elimination pathway\textsuperscript{11,39}.

While marked differences have been reported in the rate and extent of absorption with different formulations\textsuperscript{40}, only two patients in our study were on individual tablets, and one of them vomited during the study, therefore the effect of formulation was not included in the final model. Saturation of clearance and first-pass metabolism have been reported previously for rifampicin\textsuperscript{11}. While there was no significant effect for either

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Box and whisker plots of the model-derived individual \(C_{\text{max}}\) and \(AUC_{0-24}\) for the three drugs. The dots are individual values, and the whiskers represent the 2.5\textsuperscript{th} and 97.5\textsuperscript{th} percentiles. The dashed line represents the currently recommended minimum threshold: 8 mg/L for rifampicin, 3 mg/L for isoniazid, and 20 mg/L for pyrazinamide. The yellow shaded areas represent the exposure targets based on Scott et al. for rifampicin\textsuperscript{33} and Daskapan et al. for isoniazid and pyrazinamide\textsuperscript{34}. This is only for visualization purposes; no statistical tests can be carried out here since dose amounts are not accounted for.}
\end{figure}
Figure 4. Correlation matrix for the unexplained variability in a) clearance, b) bioavailability, c) area under the curve (AUC$_{0-24h}$), and d) absorption between the three drugs. The correlation coefficient is shown in the lower panel. Only the variability from the main occasion was included (not the predose). Variability in clearance = BSVCL + BOVCL. Variability in bioavailability = BSVBIO + BOVBIO. Variability in AUC = BSVBIO + BOVBIO - BSVCL. Variability in absorption = BSVKA + BOVKA - BSVMTT - BOVMTT.

HIV status or efavirenz co-administration, previous studies have reached contrasting results regarding both. Some studies found no significant difference in rifampicin concentrations, while others found decreased rifampicin levels in HIV-positive TB patients. Nevertheless, a meta-analysis by Stott et al. concluded that HIV positivity had no effect on rifampicin exposure.

Isoniazid PK model
The estimated proportion of fast acetylators/metabolizers of 64.5% is in line with the proportion of fast/intermediate acetylators in South Africans from previous publications which ranges between 48% - 60.4% - 46. In previously published pharmacokinetic studies in adults, isoniazid’s CL ranged between 22 and 26 L/h in fast metabolizers and between 10 and 16 L/h in slow metabolizers, which are similar to this study’s results.

We opted for adding a prior on the ratio of the two volumes (Vc/Vp) instead of the Vp because this is expected to be more consistent across studies which may be characterized by different body size and/or differences in bioavailability.

Isoniazid has been observed in fast metabolizers across the three patient groups as shown in Figure 3; the AUC$_{0-24h}$ levels on average were below the recommended targets. This effect has been previously reported by Sundell et al.

Pyrazinamide PK model
The values reported for the pyrazinamide model are in line with the values from previously published models. While there were no significant differences in pharmacokinetic parameters between the patient groups, we found a difference in the between-subject variability in CL (BSV-CL). The BSV-CL in outpatients was 19.9, 33.8% among hospitalized patients who survived and 70.8% among hospitalized patients who died. The differences in variability could be explained by the severity of the illness of the different patient groups. More critically sick patients have factors such as degree of hepatic impairment, sepsis that may lead to more variability.

Variability correlation across the three drugs
There was no strong correlation between the unexplained variability in clearance, AUC$_{0-24h}$ and bioavailability across the three
drugs. The moderate correlation in the unexplained variability in absorption could be explained by the fact that most of the participants were taking an FDC formulation. Therefore, the factors affecting the tablet disintegration and dissolution e.g., manufacturing variables, and drug absorption, e.g., gastrointestinal contents, will be the same across the three drugs in any particular patient.

One limitation of the study is that NAT2 genotype testing was not carried out for the participants’ samples, but this was resolved by using a mixture model to assign each participant to either being a fast or a slow metabolizer. Another limitation is that blood samples were collected on the 3rd day (4th day for one hospitalized participant) of treatment, which did not allow for the inclusion of autoinduction of rifampicin’s clearance in the model. However, hospitalized patients are at substantial risk of death within 7 days of admission before autoinduction is established, so the exposures we report here are relevant for these patients. The models cannot be extended to TB patients who are hospitalized for other causes such as renal failure, liver failure, which could significantly alter the antitubercular drugs pharmacokinetics. The study design did not allow us to characterize the possible drug-drug interactions between antitubercular and antiretroviral drugs. However, our study cohort is representative of the general South African population who gets hospitalized for tuberculosis, and the study was conducted on the 3rd day of treatment since those hospitalized patients are at a substantial risk of death within 7 days of admission. Another limitation is that ethambutol concentrations were not determined, and we could not investigate if its pharmacokinetics was different in hospitalised patients. However, unlike the other three drugs which are bactericidal, ethambutol is a bacteriostatic drug that is mainly added to tuberculosis treatment regimens to prevent against the development of rifampicin resistance. For this reason, its contribution in the very first days of treatment is expected to be minimal.

In summary, no important differences in any of the exposures of the three drugs: rifampicin, isoniazid, and pyrazinamide between hospitalized TB/HIV patients and TB outpatients were observed. The main findings of the analysis were that rifampicin’s absorption is slower in hospitalized patients (and slower in hospitalized patients who died compared to those who survived) and that patients with higher levels of bilirubin had lower rifampicin clearance. Pyrazinamide’s clearance was more variable among hospitalized patients (and more variable in hospitalized patients who died compared to those who survived).

Data availability
Underlying data
The data that support this research cannot be adequately de-identified in accordance with the Safe Harbor method since the dataset contains full treatment dates. The data including the drug concentrations, dosing and sampling dates and times, plus the covariates tested can be made available upon reasonable request to bona fide researchers by contacting Paolo Denti (paolo.denti@uct.ac.za)

Model code files
The final NONMEM model code files are publicly available at the GitHub repository (https://github.com/noha-abdelgawad/PMX-TB-Models/tree/main/Project.KDHTB.Hospitalized).

Author contributions
Gr.M. conceptualized the study with input from GaM, RJW, HM, and PD. CS and AW recruited patients and took samples with help from SJ and DB, with clinical oversight from GrM and RB. RJW, MS and LW contributed to drug quantification and laboratory oversight. NA analyzed and interpreted the data, and drafted the paper. All authors approved of the final version.

Acknowledgments
Computations were performed using facilities provided by the University of Cape Town’s ICTS High Performance Computing team: hpc.uct.ac.za

References


Open Peer Review

Current Peer Review Status: ❓❓

Version 1

Reviewer Report 08 August 2022

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Jotam Pasipanodya
Quantitative Preclinical & Clinical Sciences Department, Praedicare Inc., Dallas, TX, USA

The manuscript by Abdelgawad et al., which compares pharmacokinetics (PK) of first-line anti-tuberculosis drugs in hospitalized patients with concurrent HIV to ambulatory patients, does not give any new meaningful information in its current form.

Professors' McIlleron, Wilkinson and Maartens groups has led the field of PK and pharmacodynamics (PD) of anti-TB drugs in patients with/out HIV. A careful review of past studies from the group could have improved the introduction and discussion, and therefore possibly sharpen the hypothesis of whether PK variability impacts early TB mortality. As an example, several studies from the group have identified low rifampin/pyrazinamide exposures and some of those studies, including that by Sekaggya-Wiltshire et al., (2018)¹ revealed that those low exposures associated with slow culture conversion in TB/HIV patients. Therefore, this reader is surprised that the authors only sought to determine if PKs of hospitalized [inpatients] patients were different from outpatients. The group already have enough data to answer that question. However, the comparison of PKs of hospitalized deaths to survivors and outpatients [shown in Figure 1], is indeed an interesting question which was not fully explored by the authors. Importantly, a table of comparison of baseline characteristics, including both anti-TB and HAART drug doses, stratified by outpatients, hospitalized survivors and hospitalized deaths, would help readers. Vinnard et al., (2017)² has published PKs of anti-TB drugs in HIV patients, including examining effect of viraemia on rifampin absorption.

Finally, drug-drug interactions in the PKs of TB/HIV patients must not only be qualitatively described, but rather investigated quantitatively. Concentration-dependent antagonism has been reported with anti-TB drugs in animal models: it would be interesting to see if those correlate with outcomes in TB/HIV.

References
Full Text

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Infectious diseases, pharmacometrics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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**Author Response 02 Nov 2022**

**Noha Abdelgawad**, University of Cape Town, Observatory, South Africa

We thank the reviewer for his comments and suggestions. We include below a point-by-point response to the comments, outlining the changes we made following the suggestions.

“The manuscript by Abdelgawad et al., which compares pharmacokinetics (PK) of first-line antituberculosis drugs in hospitalized patients with concurrent HIV to ambulatory patients, does not give any new meaningful information in its current form.

Professors’ McIlleron, Wilkinson and Maartens groups has led the field of PK and pharmacodynamics (PD) of anti-TB drugs in patients with/out HIV. A careful review of past studies from the group could have improved the introduction and discussion, and therefore possibly sharpen the hypothesis of whether PK variability impacts early TB mortality. As an example, several studies from the group have identified low rifampin/pyrazinamide exposures and some of
those studies, including that by Sekaggya-Wiltshire et al., (2018) revealed that those low exposures associated with slow culture conversion in TB/HIV patients. Therefore, this reader is surprised that the authors only sought to determine if PKs of hospitalized [inpatients] patients were different from outpatients. The group already have enough data to answer that question.”

Response: In this paper, we report the pharmacokinetic analysis of rifampicin, isoniazid, and pyrazinamide within a pharmacokinetic study in hospitalized TB/HIV patients with TB patients with or without HIV enrolled as controls in South African patients. The paper addresses a knowledge gap due to the lack of previous publications comparing rifampicin, isoniazid, and pyrazinamide pharmacokinetics between hospitalized and non-hospitalized patients during early days of treatment. None of the previous studies, to our knowledge, looked at acutely ill patients on day 3 of TB treatment. Although multiple studies describe the pharmacokinetics of first-line TB drugs once the patients are established on treatment, there is a paucity of data looking at the pharmacokinetics during early treatment among patients hospitalized for acute illness, for whom the mortality rates are very high during the first 1 - 2 weeks of hospitalization.

In this study, we investigate if there is a difference in the PK parameters and drug concentrations between hospitalized and non-hospitalized patients. Our report highlights that hospitalized TB patients' mortality is not due to underexposure of antitubercular drugs. Although these patients may benefit from higher doses, exposure in hospitalized patients was similar to outpatients and not lower in inpatients who died. The report also introduces some new findings, particularly, the slower rifampicin absorption and higher between-subject variability in pyrazinamide clearance in hospitalized deaths compared to hospitalized survivors and in hospitalized vs outpatients. So, while rifampicin absorption is slower in hospitalized patients, the bioavailability is similar between hospitalized who died, hospitalized who survived and outpatients, which means that intravenous rifampicin administration may not be necessary for hospitalized TB patients. A section was added to the discussion to further clarify this point.

We did not have any detailed pharmacodynamic (PD) measures such as culture conversion times to explore PK-PD relationships, and the study was not powered to investigate PK-PD relationships. The only PD data collected was whether the participant died within 12-weeks of hospitalization or not. The patients hospitalized were critically ill patients, who usually die within the first week or two of hospitalization. In such a cohort, mortality is more likely due the severity of patient's illness and their critical condition at presentation to hospital. The effect of anti-TB drugs concentrations will be very difficult to extricate from those other factors, especially if all patients receive similar doses and in such a small cohort. For the same reasons, the data in this study does not allow exploration of concentration-dependent antagonism.

We thank the reviewer for the chance to improve the manuscript. A section was added to the introduction to highlight that lower antitubercular drug concentrations have been shown to be associated with worsened sputum measures of mycobacterial load as shown by Sekaggya-Wiltshire et al. [1] and Mah et al. [2].

“Finally, drug-drug interactions in the PKs of TB/HIV patients must not only be qualitatively
described, but rather investigated quantitatively. Concentration-dependent antagonism has been reported with anti-TB drugs in animal models: it would be interesting to see if those correlate with outcomes in TB/HIV.”

**Response:** Drug-drug interactions of first-line anti-TB drugs with antiretroviral drugs were not the main question of this analysis. The main objective was to see what the differences in the pharmacokinetics are between hospitalized (survivors and deaths) and outpatients. However, as part of the analysis, the effect of ART drugs (efavirenz, lopinavir/ritonavir, lamivudine) was tested on the different pharmacokinetic parameters of the 3 drugs and was not significant. Only 25/108 (23%) participants were on ARTs. Only 9 were on efavirenz, 9 on tenofovir, 1 on lopinavir/ritonavir, and 1 on lamivudine. The concurrent ART therapy is now highlighted in the manuscript.

“However, the comparison of PKs of hospitalized deaths to survivors and outpatients [shown in Figure 1], is indeed an interesting question which was not fully explored by the authors. Importantly, a table of comparison of baseline characteristics, including both anti-TB and HAART drug doses, stratified by outpatients, hospitalized survivors and hospitalized deaths, would help readers.”

**Response:** Table 2 has been replaced with one stratified into outpatients, hospitalized survivors, hospitalized deaths instead of outpatients and hospitalized patients. Details regarding the HIV status, CD4 count, viral loads, and current antiretroviral (ART) therapy were added to the table as well. Thanks for this suggestion.

“Vinnard et al., (2017) 2 has published PKs of anti-TB drugs in HIV patients, including examining effect of viraemia on rifampin absorption.”

**Response:** Trefoil factor-3, a marker of gut barrier integrity, was tested as a covariate on the absorption and bioavailability of rifampicin but its effect was not found to be statistically significant. This is now clarified in the manuscript. This agrees with Vinnard et al. who quantified intestinal damage and microbial translocation by measuring levels of I-FABP and sCD14. They concluded that variability in markers of gut damage and microbial translocation did not contribute to variability in the oral bioavailability of rifampicin [3].

**References:**
Competing Interests: No competing interests were disclosed.

Reviewer Report 06 June 2022

https://doi.org/10.21956/wellcomeopenres.19537.r48936

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Oscar Della Pasqua
Clinical Pharmacology & Therapeutics, School of Life and Medical Sciences, University College London, London, UK

In this manuscript Abdelgawad and colleagues propose to compare the population pharmacokinetics of rifampicin, isoniazid, and pyrazinamide between hospitalized and non-hospitalized tuberculosis patients with or without HIV.

I congratulate the authors for considering drug exposure as a potential determinant of the clinical deterioration and TB-associated complications in HIV+ patients. However, the manuscript has some important limitations, which need to be considered carefully to ensure accurate interpretation of the results. Given the heterogeneity of the patient population and available first and second line antiretroviral therapies for HIV, the conclusions drawn by the authors cannot be generalised.

The most important limitation is the complete lack of details on the background therapy used for the treatment of HIV (i.e., which drugs, which doses and dosing regimens). The rationale for the current investigation is that hospitalised TB/HIV+ patients are more likely to have a poor prognosis. There are numerous publications highlighting that some, but not all, antiretroviral drugs show clinically relevant interaction with rifampicin. On the other hand, rifampicin is known to be a strong CYP3A4 inducer. For instance, both pre-systemic and systemic induction by efavirenz-based ART has been shown to affect rifampicin pharmacokinetics (See e.g., Sundell et al., 2021¹). For the HIV-1 protease inhibitors (PIs) lopinavir, atazanavir (ATV), and darunavir, intestinal absorption and hepatic elimination are largely governed by CYP3A4/5 activity, and as such affected by co-administration of metabolic inducers such as rifampicin (See e.g., Montanha et al., 2022²).

Apparenetly, the focus of the data analysis presented here was to assess whether significant differences exist in the pharmacokinetics of first line therapy drugs, and consequently differences in drug exposure in TB/HIV+ patients who are hospitalised, relative to those who do not require hospitalisation. Their analysis does not consider the possibility of a pharmacokinetic drug-drug interaction with anti-retrovirals.

Therefore, to ensure appropriate interpretation of the results, further details should have been
provided on the use of concurrent HIV co-medications. In addition, readers would benefit from an overview of the clinical and demographic baseline characteristics of the patients who died within 12 weeks from the start of the treatment with anti-tubercular drugs, including an outline of their viral load and CD4 counts, as compared to those who are not hospitalised or do not deteriorate during the course of therapy.

**Minor comments:**

1. **Study objectives.** The authors state that the aim of the study was to compare the pharmacokinetics of rifampicin, isoniazid, and pyrazinamide between hospitalised patients and outpatients recruited from the same hospital catchment area. However this does not fully reflect the title of the manuscript. What is the relevance of HIV co-infection? There is no emphasis on the role of concurrent disease; the comparison seems to be limited to the patient condition (i.e., hospitalised vs. outpatients).

2. In addition, the method section is not very clear. First, it is stated that the study population is made of two groups (hospitalised vs. outpatients (control group)). Then details are given about the inclusion of HIV+ and HIV – subjects to the outpatient cohort. There is no explanation why HIV+ and HIV- subjects were not included in the hospitalised group. Apparently, the hospitalised group consists only of TB/HIV+ patients.

3. **Inclusion criteria.** The authors should have listed the cause of hospitalisation and explain why no specific antiretroviral therapy has been defined as inclusion/exclusion criteria. Restricting the inclusion of patients on regimens with high probability of drug-drug interaction would have made this investigation more relevant to clinicians and healthcare providers.

4. **First line therapy in drug susceptible tuberculosis consists of four drugs.** The authors do not provide any explanation for the exclusion of ethambutol from this analysis. The authors should also highlight the potential implications of variable exposure to ethambutol.

5. **Assuming that the analysis focuses primarily on the effect of hospitalisation on the pharmacokinetic properties of anti-tubercular drugs, justification is missing for the sample size of the groups.** Given the role of demographic baseline characteristics on the pharmacokinetic disposition of both antiretroviral and antitubercular drugs, it would be important to understand which parameters will be of primary interest during the data analysis.

6. **There are many publications on the population pharmacokinetics of antitubercular drugs (e.g., Muliaditan et al., 2019; Muda et al., 2022), including investigations where findings contrast with results and conclusions drawn by the authors (Sundell et al., 2021; Rao et al., 2021).** Unfortunately, these references were not included or considered for discussion. Consequently, the work is presented as if this was the first time population pharmacokinetic models were being developed. Prior distributions and structural model(s) could have been used for the purpose of the current analysis. This would have provided an opportunity to optimise sampling times and focus on features such as absorption rate. Similarly, the use of priors describing parameter distributions would have allowed the authors to assess the pharmacokinetics of anti-retroviral drugs.

7. **Covariate model building.** The authors do not explain the lack of a time varying clearance for rifampicin, as autoinduction is a well-known process in the elimination of this drug. Under
autoinduction conditions, the elimination half-life of rifampicin decreases significantly, as clearance increases. It seems that the authors have disregarded these mechanisms and focused on the assumption of stochastic processes to explain variability, making the analysis of the data descriptive, rather than mechanistic or mechanism-based.

8. Interestingly, the model includes between-occasion variability but relies on fixed point estimates for the bioavailability. This should be better explained and justified. Fixing a parameter suggests that no reference group was considered. By using interoccasion variability, differences in clearance (i.e., metabolic clearance) due to specific processes. This would have provided a more biologically plausible description of the underlying metabolic autoinduction.

9. Mean transit time, absorption rate constants. The authors should present a more detailed outline of the rationale for the use of transit compartments. It is unclear whether the use of this approach was due to intrinsic drug absorption properties or due to extrinsic factors (e.g., formulation, food effect).

10. This analysis would benefit from a summary including secondary pharmacokinetic parameters (i.e., post-hoc estimates of the individual curves and subsequent calculation of AUC, $C_{\text{max}}$, $C_{\text{ss}}$, $C_{\text{min}}$). These parameters are shown graphically in Figure 3, but were not summarised numerically. Readers will find it much easier to compare and interpret these results.

11. It has been previously established that there is a rise in serum bilirubin during treatment with rifampicin. This is due to the competition between rifampicin and bilirubin for hepatic uptake and excretion. However, this is relevant primarily in individuals who have hepatic impairment or other comorbidities such as cirrhosis. The authors need to explain the inclusion criteria and cause of hospitalisation, as the normal range for conjugated bilirubin is $< 5.1 \, \mu\text{mol/L}$. The values depicted in figure 2 should not be applicable to HIV or TB patients who do not experience hepatic toxicity or have other co-morbidities (i.e., hepatic disease).

12. There is no explanation why interindividual variability was not identified for the volume of distribution. There seems to be a sufficiently large interindividual variation in body weight and fat-free mass. In addition, the authors did not report the shrinkage for ETA on clearance.

13. The discussion should present further details on the limitations outlined in the previous points. In particular, the authors need to make clear that their results cannot be generalised given the lack of details on the inclusion criteria and background antiretroviral therapy of the patients included in this analysis.

14. I would recommend that the authors share the NONMEM control stream files as supplementary material.

References
Publisher Full Text


**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
No

**Are the conclusions drawn adequately supported by the results?**
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Clinical pharmacology, modelling and simulation, tuberculosis, paediatrics, biomarkers, clinical drug development

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

**Author Response 02 Nov 2022**

**Noha Abdelgawad**, University of Cape Town, Observatory, South Africa

We thank the reviewer for the useful input on our manuscript. We include below a point-by-point response to the comments. The changes we made following the suggestions are outlined.
“In this manuscript Abdelgawad and colleagues propose to compare the population pharmacokinetics of rifampicin, isoniazid, and pyrazinamide between hospitalized and non-hospitalized tuberculosis patients with or without HIV.

I congratulate the authors for considering drug exposure as a potential determinant of the clinical deterioration and TB-associated complications in HIV+ patients. However, the manuscript has some important limitations, which need to be considered carefully to ensure accurate interpretation of the results. Given the heterogeneity of the patient population and available first and second line antiretroviral therapies for HIV, the conclusions drawn by the authors cannot be generalised.

The most important limitation is the complete lack of details on the background therapy used for the treatment of HIV (i.e., which drugs, which doses and dosing regimens). The rationale for the current investigation is that hospitalised TB/HIV+ patients are more likely to have a poor prognosis. There are numerous publications highlighting that some, but not all, antiretroviral drugs show clinically relevant interaction with rifampicin. On the other hand, rifampicin is known to be a strong CYP3A4 inducer. For instance, both pre-systemic and systemic induction by efavirenz-based ART has been shown to affect rifampicin pharmacokinetics (See e.g., Sundell et al., 2021). For the HIV-1 protease inhibitors (PIs) lopinavir, atazanavir (ATV), and darunavir, intestinal absorption and hepatic elimination are largely governed by CYP3A4/5 activity, and as such affected by co-administration of metabolic inducers such as rifampicin (See e.g., Montanha et al., 2022).

Apparently, the focus of the data analysis presented here was to assess whether significant differences exist in the pharmacokinetics of first line therapy drugs, and consequently differences in drug exposure in TB/HIV+ patients who are hospitalised, relative to those who do not require hospitalisation. Their analysis does not consider the possibility of a pharmacokinetic drug-drug interaction with anti-retrovirals.”

Response: Indeed, the focus of this manuscript is comparing the pharmacokinetics of rifampicin, isoniazid, and pyrazinamide between tuberculosis patients in need of hospitalization vs tuberculosis outpatients. The objectives of this study did not specifically include exploring the pharmacokinetics of anti-retrovirals or drug-drug interactions between antitubercular and antiretroviral drugs. The concentrations of antiretroviral drugs were not quantified, so we cannot explore the effect of the antitubercular drugs on their pharmacokinetics. Details regarding the concomitant HIV drugs used are now added to the manuscript.

We agree that the concomitant use of efavirenz with rifampicin is expected to have an effect on rifampicin's pharmacokinetics, as shown by Sundell et al. However, when we tested efavirenz as a covariate on rifampicin PK parameters, there was no significant effect. We think this could be due to a number of reasons. First, possible non-compliance of patients to their ART regimens prior to hospitalisation (since efavirenz plasma levels were not quantified, it is not possible to ascertain whether they took their efavirenz doses or not). Secondly, Sundell et al. looked at rifampicin PK on the first day of treatment, when no rifampicin autoinduction is present and the difference between patients taking efavirenz or
not is expected to be maximal. In our study, rifampicin PK was observed on day 3, when some of rifampicin’s autoinduction has already happened, thus possibly reducing the expected difference between patients taking efavirenz or not. Consistently with this hypothesis, several previous studies looking into rifampicin-efavirenz interaction found no effect at steady-state, when rifampicin auto-induction is complete $^2,^3$.

Table 1 is now stratified into outpatients, hospitalized survivors, hospitalized deaths instead of outpatients and hospitalized patients. Details regarding the HIV status, CD4 count, and concurrent HIV comedications have been added to the table as well. Thanks for the suggestion.

**Minor comments:**

1. **Study objectives.** The authors state that the aim of the study was to compare the pharmacokinetics of rifampicin, isoniazid, and pyrazinamide between hospitalised patients and outpatients recruited from the same hospital catchment area. However, this does not fully reflect the title of the manuscript. What is the relevance of HIV co-infection? There is no emphasis on the role of concurrent disease; the comparison seems to be limited to the patient condition (i.e., hospitalised vs. outpatients).

**Response:** The title was changed to “Pharmacokinetics of antitubercular drugs in patients hospitalized with HIV-associated tuberculosis: a population modelling analysis” to better reflect the study objectives.

2. **In addition, the method section is not very clear. First, it is stated that the study population is made of two groups (hospitalised vs. outpatients (control group)). Then details are given about the inclusion of HIV+ and HIV – subjects to the outpatient cohort. There is no explanation why HIV+ and HIV- subjects were not included in the hospitalised group. Apparently, the hospitalised group consists only of TB/HIV+ patients.**

**Response:** HIV+ patients that were newly diagnosed with tuberculosis requiring hospitalization were enrolled as part of a large observational cohort study and the PK sub-study was performed in selected patients on the 3rd day of treatment. The outpatient controls were enrolled after the completion of the hospital cohort study and for logistical reasons, both HIV+ and HIV- were included in the outpatients group. The focus of the PK sub-study was the comparison of antitubercular drug concentrations at a very early time point (the 3rd day of treatment). Thus, for the outpatients control group, the inclusion criteria were not restricted to HIV+ patients only but rather to adults newly diagnosed with tuberculosis who can be enrolled prior to receiving their 3rd dose of TB therapy.

3. **Inclusion criteria.** The authors should have listed the cause of hospitalisation and explain why no specific antiretroviral therapy has been defined as inclusion/exclusion criteria. Restricting the inclusion of patients on regimens with high probability of drug-drug interaction would have made this investigation more relevant to clinicians and healthcare providers.

**Response:** Thanks for giving us the chance to clarify this point in the manuscript. The main focus of the PK study was to compare the pharmacokinetics of rifampicin, isoniazid and pyrazinamide in patients with different illness severity i.e., patients who presented to care in
need of hospitalization due to tuberculosis (who were too ill to receive initial treatment as outpatients) vs those with tuberculosis that do not require hospitalization (the outpatients control group). The study was not designed to explore the drug-drug interactions of the first-line antitubercular drugs with anti-retrovirals. Therefore, patients were enrolled regardless of their ART therapy status. At the time of study design, the publications showing possible drug-drug interactions such as **Sundell et al.** \(^1\) with anti-retrovirals had not been published yet.

4. **First line therapy in drug susceptible tuberculosis consists of four drugs.** The authors do not provide any explanation for the exclusion of ethambutol from this analysis. The authors should also highlight the potential implications of variable exposure to ethambutol.

**Response:** We thank the reviewer for pointing this out and reminding us to explain this more in detail. In this study, we decided to focus on the PK of rifampicin, isoniazid, and pyrazinamide, because they are reported to be more effective and faster-acting during the early days of TB treatment. So, these drugs are thought to be more important for the hospitalised patients in the study, who usually die within the first few weeks of hospitalization. Unlike the other three drugs, ethambutol's standard plasma-based pharmacokinetic-pharmacodynamic profile suggests that it may be of limited clinical value. Ethambutol is a bacteriostatic drug that lacks sterilizing activity and that should not be used alone for TB treatment because it has limited potency against slow-growing and nonreplicating bacteria \(^4\). Its inclusion in the TB regimens is mainly justified because of its protective role against rifampicin resistance development. Therefore, due to limited funding available we preferred to analyze the three bactericidal drugs in a larger number of patients, rather than to measure all four drugs in fewer patients. The exclusion of ethambutol from the analysis was added as a limitation to the discussion section.

5. Assuming that the analysis focuses primarily on the effect of hospitalisation on the pharmacokinetic properties of anti-tubercular drugs, justification is missing for the sample size of the groups. Given the role of demographic baseline characteristics on the pharmacokinetic disposition of both antiretroviral and antitubercular drugs, it would be important to understand which parameters will be of primary interest during the data analysis.

**Response:** The analysis focuses on the differences between patients with HIV-associated tuberculosis who are ill enough to be hospitalized at the time of TB diagnosis and ambulant outpatients. The hospitalised group has an extremely high mortality rate and the rationale of the study is that the severity of their illness may lead to underexposure of antitubercular drugs which may contribute to their mortality. Sample size calculation was done for the main prospective observational cohort study to investigate factors associated with mortality among hospitalized patients with tuberculosis, whose primary outcome was dichotomized into survivor vs fatal outcome factoring in a cumulative mortality of 15%. However, since the PK study was exploratory, no formal sample size calculation was made.

6. **There are many publications on the population pharmacokinetics of antitubercular drugs** (e.g., *Muliaditan et al.*, 2019; *Muda et al.*, 2022), including investigations where findings contrast with results and conclusions drawn by the authors (Sundell et al., 2021; Rao et al., 2021). Unfortunately, these references were not included or considered for discussion. Consequently, the
work is presented as if this was the first time population pharmacokinetic models were being developed. Prior distributions and structural model(s) could have been used for the purpose of the current analysis. This would have provided an opportunity to optimise sampling times and focus on features such as absorption rate. Similarly, the use of priors describing parameter distributions would have allowed the authors to assess the pharmacokinetics of anti-retroviral drugs.

Response: Thank you for the chance to enrich the discussion section. As the reviewer mentions, there are indeed many publications describing the popPK of these drugs, but large differences are sometimes reported between studies, possibly due to issues such as drug formulations, drug assays, etc. For this reason, it would have not been straightforward to choose the priors to use in the analysis and therefore make an “external” comparison. Rather, we recruit an “ad hoc” control group of patients from the same area, sampled at the same time after treatment initiation, and receiving the same drug formulations. The collected data was enough to support estimation of parameters without using priors and allow an “internal” comparison between the hospitalised and non-hospitalised patients, which we believe provides stronger results than using priors or historical data as a comparison. However, we agree that the discussion should be extended to and some of the references the reviewer suggested had not been published at the time of writing the manuscript. The discussion section has now been expanded to elaborate more on previous publications.

We have decided not to include the models by Rao et al. \(^5\) in our discussion, because their findings are very odd to reconcile with all previous literature. They report pharmacokinetics in patients with sepsis from TB bloodstream infection or meningitis after 2 weeks of treatment. Their models are one-compartment for rifampicin and pyrazinamide and two-compartments for isoniazid, which is in line with ours. However, their rifampicin clearance parameter estimate of 0.1 L/h is puzzling and unlike any previously published models, as summarized in the review by Muda et al. \(^6\). For their isoniazid model, they included mid-upper-arm circumference (MUAC) on central volume, but MUAC has not been proven to be an accurate body size descriptor. Allometric scaling using descriptors that take into account both body size and composition (e.g. fat-free mass) are the gold standard and have been established as more accurate predictors for differences in pharmacokinetics \(^7\). Moreover, they conclude that the patients were underexposed to the anti-TB drugs due to their critical illness, but no comparison was done with a control group (outpatients). In our study, exposures were often also below the generally accepted targets, but importantly the hospitalized patients were not underexposed compared to the outpatients control group.

As for the pyrazinamide model by Sundell et al. \(^8\), they found relative bioavailability to be ~50% higher in females compared to males, but they used no allometry to adjust for body size. We found fat-free mass to be better than total body weight when included in the model using allometric scaling in capturing the effect of body size on differences in apparent clearance and volume. Allometric scaling by fat-free mass ascribes PK differences to changes in body size and composition. It is more physiologically plausible and more parsimonious than using sex as a categorical covariate. It is also a well-established concept \(^7\). This is also supported by findings from Mulaiditan et al. \(^9\), Rockwood et al. \(^10\), and McIlleron et al. \(^3\) who have shown that patients with lower body weights are underexposed.
relative to patients with higher body weight. Since fat-free mass is the best body size descriptor for clearance and volume, but dosing is determined based on total weight, patients with lower weights end up being underexposed. Therefore, we agree that the clinical recommendation for the use of weight-banded dosing regimens should be reconsidered to account for the variability in body size and composition. This point has been elaborated in the discussion section.

Additionally, Sundell et al. 8 report a significant effect for efavirenz co-administration on the volume of distribution, but there is no mechanistic basis for this interaction. Other studies specifically looking at HIV e.g., Rockwood et al. 10 have looked into the effect of efavirenz on pyrazinamide PK and found no significant effect.

Regarding the ART comment, we agree that using prior models we could have detected large differences in PK, but we have to reiterate that the objective of the main observational study was to investigate factors relating to the high mortality of hospitalized tuberculosis patients and the PK sub-study was conducted to only compare the pharmacokinetics of antitubercular drugs in TB patients who are critically ill in need of hospitalization vs TB outpatients, during the early days of treatment. The concentrations of the anti-retroviral drugs were not assayed as their pharmacokinetics was not the objective of this investigation.

7. Covariate model building. The authors do not explain the lack of a time varying clearance for rifampicin, as autoinduction is a well-known process in the elimination of this drug. Under autoinduction conditions, the elimination half-life of rifampicin decreases significantly, as clearance increases. It seems that the authors have disregarded these mechanisms and focused on the assumption of stochastic processes to explain variability, making the analysis of the data descriptive, rather than mechanistic or mechanism-based.

Response: The inclusion of auto-induction of clearance in the model was tested but did not improve the model fit, which could be due to the fact that all participants were sampled on the same day (the 3rd day of treatment). This is now clarified in the results section.

8. Interestingly, the model includes between-occasion variability but relies on fixed point estimates for the bioavailability. This should be better explained and justified. Fixing a parameter suggests that no reference group was considered. By using inter-occasion variability, differences in clearance (i.e., metabolic clearance) due to specific processes. This would have provided a more biologically plausible description of the underlying metabolic autoinduction.

Response: As illustrated by Lavielle & Aarons11, bioavailability cannot be identified in such a cohort since all participants were administered oral drugs and none intravenously, therefore the population value for bioavailability was fixed to 1 but was allowed to be different between the occasions. The occasions in this model are occasion 1 for day 2 (for the pre-dose sample) and occasion 2 for Day 3 of treatment. Significant day-to-day changes are expected in absorption parameters (absorption rate constant, bioavailability)12. While rifampicin's autoinduction is expected to have an effect on clearance, only one sample was available from the day before the day of the PK sampling (pre-dose sample), plus the time of the dose may not have been accurately reported for some of the patients. For these reasons, it is unlikely to be able to detect day-to-day changes in clearance. Moreover, the
majority of pre-dose samples were below the limit of quantification for rifampicin.

9. Mean transit time, absorption rate constants. The authors should present a more detailed outline of the rationale for the use of transit compartments. It is unclear whether the use of this approach was due to intrinsic drug absorption properties or due to extrinsic factors (e.g., formulation, food effect).

Response: Thanks for giving the chance to clarify. For all three drugs, a delay in absorption was observed in the profiles. The use of the flexible transit compartment model describes the delay in absorption better than the standard absorption model (in terms of drop in the objective function and the visual predictive check) because it is more physiologically plausible as shown by Savic et al.\textsuperscript{13} The drop in OFV with the transit model (df = 1) was 9.22 points for rifampicin, 107 points for isoniazid, and 23.7 points for pyrazinamide. All participants were fasted overnight, so there is no food effect. And no significant formulation effect was found, only a trend (only 2 participants had a different formulation (individual tablets instead of the fixed-dose combination tablets).

10. This analysis would benefit from a summary including secondary pharmacokinetic parameters (i.e., post-hoc estimates of the individual curves and subsequent calculation of AUC, C\text{max}, C_{\text{SS}}, C_{\text{min}}). These parameters are shown graphically in Figure 3, but were not summarised numerically. Readers will find it much easier to compare and interpret these results.

Response: Thank you for the suggestion. The AUC\textsubscript{0-24h} and C\text{max} values shown graphically in Figure 3 have now been added in a table (Table 4).

11. It has been previously established that there a rise in serum bilirubin during treatment with rifampicin. This is due to the competition between rifampicin and bilirubin for hepatic uptake and excretion. However, this is relevant primarily in individuals who have hepatic impairment or other comorbidities such as cirrhosis. The authors need to explain the inclusion criteria and cause of hospitalisation, as the normal range for conjugated bilirubin is < 5.1 \mu mol/L. The values depicted in figure 2 should not be applicable to HIV or TB patients who do not experience hepatic toxicity or have other co-morbidities (i.e., hepatic disease).

Response: The main inclusion criteria for the hospitalized group was patients presenting to care in need of hospitalization due to tuberculosis and who are too ill to receive initial treatment as outpatients. These patients were hospitalized due to TB infection and many of these patients would have had disseminated TB with liver involvement, which means they had abnormal liver function tests including elevated bilirubin levels at the time of hospitalization. The bilirubin values presented in Table 2 and in Figure 2 are values measured during screening, i.e., before the start of treatment, which means that the high bilirubin levels were not due to rifampicin treatment.

12. There is no explanation why interindividual variability was not identified for the volume of distribution. There seems to be a sufficiently large interindividual variation in body weight and fat free mass. In addition, the authors did not report the shrinkage for ETA on clearance.

Response: All three models included allometric scaling of the volume of distribution by fat-
free mass as a way to explain the differences between the participants with different body sizes. The values for interindividual variability in the volume of distribution were very small (2.5% for rifampicin) and its inclusion did not result in a better fit of the model (dOFV = -0.34, df = 1), therefore, it was not included. The shrinkage for the ETAs for the three drugs has now been added to Table 3.

13. The discussion should present further details on the limitations outlined in the previous points. In particular, the authors need to make clear that their results cannot be generalised given the lack of details on the inclusion criteria and background antiretroviral therapy of the patients included in this analysis.

Response: Thank you for raising this point. The limitations are now better mentioned in the discussion. The models cannot be extended to TB patients who are hospitalized for other causes such as renal failure, liver failure, which could significantly alter the antitubercular drugs pharmacokinetics. The study design did not allow us to characterize the possible drug-drug interactions between antitubercular and antiretroviral drugs. However, our study cohort is representative of the general South African population who gets hospitalized for tuberculosis, and the study was conducted on the 3rd day of treatment since those hospitalized patients are at a substantial risk of death within 7 days of admission.

14. I would recommend that the authors share the NONMEM control stream files as supplementary material.


References:


9. Muliaditan M, Pasqua O Della. How long will treatment guidelines for TB continue to overlook variability in drug exposure?


**Competing Interests:** No competing interests were disclosed.