









RESEARCH ARTICLE

A comparison of the population pharmacokinetics of rifampicin, isoniazid and pyrazinamide between hospitalized and non-hospitalized tuberculosis patients with or without HIV [version 1; peer review: 2 approved with reservations]

Noha Abdelgawad ¹, Maxwell Chirehwa¹, Charlotte Schutz^{2,3}, David Barr⁴, Amy Ward^{2,3}, Saskia Janssen⁵, Rosie Burton^{3,6}, Robert J. Wilkinson ^{2,3,7,8}, Muki Shey ², Lubbe Wiesner ¹, Helen McIlleron ^{1,2}, Gary Maartens ^{1,2}, Graeme Meintjes^{2,3}, Paolo Denti¹

¹Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Observatory, 7925, South Africa

²Wellcome Centre for Infectious Diseases Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Observatory, 7925, South Africa

³Department of Medicine, University of Cape Town, Observatory, 7925, South Africa

⁴Wellcome Trust Liverpool Glasgow Centre for Global Health Research, University of Liverpool, Liverpool, L3 5QA, UK

⁵Amsterdam University Medical Centre, University of Amsterdam, Amsterdam, 19268, The Netherlands

⁶Khayelitsha Hospital, Department of Medicine, Khayelitsha, 7784, South Africa

⁷Department of Infectious Diseases, Imperial College London, London, W12 0NN, UK

⁸The Francis Crick Institute, London, NW1 1AT, UK

V1 First published: 28 Feb 2022, 7:72
<https://doi.org/10.12688/wellcomeopenres.17660.1>

Latest published: 28 Feb 2022, 7:72
<https://doi.org/10.12688/wellcomeopenres.17660.1>

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

Open Peer Review

Approval Status  

| | 1 | 2 |
|--------------------------|---|---|
| version 1 28 Feb 2022 |  view |  view |

1. Oscar Della Pasqua , University College London, London, UK
2. Jotam Pasipanodya , Praedicare Inc., Dallas, USA

Any reports and responses or comments on the article can be found at the end of the article.

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.

Keywords

Modelling & Simulation, Population pharmacokinetics, Tuberculosis, Hospitalization, TB/HIV



This article is included in the [Wellcome Centre for Infectious Diseases Research in Africa \(CIDRI-Africa\)](#) gateway.

Corresponding authors: Graeme Meintjes (graeme.meintjes@uct.ac.za), Paolo Denti (paolo.denti@uct.ac.za)

Author roles: **Abdelgawad N:** Formal Analysis, Methodology, Software, Visualization, Writing – Original Draft Preparation; **Chirehwa M:** Software, Supervision, Writing – Review & Editing; **Schutz C:** Investigation, Writing – Review & Editing; **Barr D:** Data Curation, Writing – Review & Editing; **Ward A:** Investigation; **Janssen S:** Data Curation, Writing – Review & Editing; **Burton R:** Data Curation; **Wilkinson RJ:** Conceptualization, Resources, Writing – Review & Editing; **Shey M:** Methodology; **Wiesner L:** Methodology, Writing – Review & Editing; **McIlleron H:** Conceptualization, Writing – Review & Editing; **Maartens G:** Conceptualization, Writing – Review & Editing; **Meintjes G:** Conceptualization, Data Curation, Writing – Review & Editing; **Denti P:** Conceptualization, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Wellcome ([098316 <https://doi.org/10.35802/098316>], [214321 <https://doi.org/10.35802/214321>], 203135 <https://doi.org/10.35802/203135>], 211360 <https://doi.org/10.35802/211360>], and 105165 <https://doi.org/10.35802/105165>). CS was funded by the South African Medical Research Council under the National Health Scholars Programme. GrM was supported by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation (NRF) of South Africa (Grant No 64787), NRF incentive funding (UID: 85858) and the South African Medical Research Council through its TB and HIV Collaborating Centres Programme with funds received from the National Department of Health (RFA# SAMRC-RFA-CC: TB/HIV/AIDS-01-2014). GaM was supported in part by the NRF (Grant Number 119078). RJW is supported by the Francis Crick Institute, which receives funding from Wellcome (FC00110218), Cancer Research UK (FC00110218), the UK Medical Research Council (FC00110218). RJW also receives support from the National Institutes of Health (U01AI115940) and EDCTP (SRIA 2015-1065). PD received NRF incentive funding UID 109056. The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of this report. The opinions, findings and conclusions expressed in this manuscript reflect those of the authors alone. The University of Cape Town Clinical PK Laboratory is supported in part via the Adult Clinical Trial Group (ACTG), by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health under award numbers UM1 AI068634, UM1 AI068636, and UM1 AI106701; as well as the Infant Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT), funding provided by National Institute of Allergy and Infectious Diseases (U01 AI068632), The Eunice Kennedy Shriver National Institute of Child Health and Human Development, and National Institute of Mental Health grant AI068632.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2022 Abdelgawad N *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Abdelgawad N, Chirehwa M, Schutz C *et al.* **A comparison of the population pharmacokinetics of rifampicin, isoniazid and pyrazinamide between hospitalized and non-hospitalized tuberculosis patients with or without HIV [version 1; peer review: 2 approved with reservations]** Wellcome Open Research 2022, 7:72 <https://doi.org/10.12688/wellcomeopenres.17660.1>

First published: 28 Feb 2022, 7:72 <https://doi.org/10.12688/wellcomeopenres.17660.1>

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 response^{3,4}. 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 clearance⁵⁻⁷. These changes could negatively affect the treatment outcome in vulnerable patients.

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 mechanisms^{9,10}. After repeated administration, rifampicin exhibits autoinduction, in which it increases its own metabolism partly by activating the pregnane X receptor¹¹, which in turn induces the B-esterases in liver microsomes, which are responsible for the biotransformation of rifampicin to 25-desacetyl rifampicin^{12,13}.

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 acetylators¹⁴. 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 only^{15,16}.

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 2016². Patients presenting to Khayelitsha Hospital in Cape Town, South Africa with TB/HIV who needed hospitalization 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

Table 1. Summary of weight-based dosing.

| Pre-treatment body weight (kg) | No. of RHZE FDC ^a (150/75/400/275 mg) during intensive treatment phase (daily dose for 2 months) |
|--------------------------------|---|
| 30–37 | 2 |
| 38–54 | 3 |
| 55–70 | 4 |
| >70 | 5 |

^a RHZE, rifampin, isoniazid, pyrazinamide, and ethambutol fixed-dose combination tablets

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 pyrazinamide concentrations were determined by validated liquid chromatography with tandem mass spectrometry assays at the Division of Clinical Pharmacology, University of Cape Town¹⁷. 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, respectively².

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 (FOCEI) Pirana was used for model management, Perl-speaks-NONMEM (PsN) 4.9.0 was used for post-processing of NONMEM[®] results and R version 3.6.2 was used for generating the figures¹⁸. 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 discarded¹⁹. 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 Holford²⁰ using the fixed power exponents of 0.75 for clearance and 1 for volume. Body weight and FFM, calculated based on the formula in Janmahasatian *et al.*²¹, 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, trefoil factor-3, and procalcitonin, were tested on clearance, bioavailability, and absorption parameters for all three drugs.

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 χ^2 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 (V_c) to the volume of the peripheral compartment (V_p), V_{ratio} (V_c/V_p) with 30% uncertainty were used to stabilize the model for isoniazid PK. The typical values were obtained from a previously published model²².

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 published².

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 (AUC_{0-24h}) 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.

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

Table 2. Participants baseline characteristics.

| | Median (interquartile range) or no. (%) of participants given | | |
|--|---|----------------------|--------------------|
| | Hospitalized (n = 60) | Outpatients (n = 48) | Total (n = 108) |
| No. (%) of: | | | |
| Females | 31 (52%) | 12 (25%) | 43 (40%) |
| Age (yr) | 38 (32 – 41) | 36 (32 – 42) | 37 (32 – 41) |
| Weight (kg) | 55 (48 – 60) | 58 (52 – 63) | 56 (49 – 62) |
| Fat-free mass (kg) | 40 (36 – 47) | 47 (41 – 51) | 43 (38 – 49) |
| No. (%) of: | | | |
| HIV-positive | 60 (100%) | 29 (60%) | 89 (82%) |
| Total bilirubin (µmol/L) ^a | 10.0 (6.00 – 14.5) | 10.0 (7.00 – 14.0) | 10.0 (6.00 – 14.0) |
| Conjugated bilirubin (µmol/L) ^b | 6.00 (3.00 – 9.00) | 6.00 (4.00 – 8.00) | 6.00 (3.00 – 9.00) |
| Lactate (mmol/L) ^c | 1.55 (1.13 – 2.30) | 1.60 (1.20 – 1.95) | 1.60 (1.20 – 2.00) |
| Aspartate aminotransferase, AST (U/L) ^d | 50.0 (34.0 – 78.9) | 30.0 (21.0 – 50.0) | 38.0 (27.0 – 69.0) |
| Alanine aminotransferase, ALT (U/L) ^e | 27.0 (18.0 – 47.0) | 20.0 (13.5 – 30.0) | 24.0 (16.0 – 40.8) |

^a Total bilirubin was missing for 1 hospitalized patient and 1 outpatient

^b Conjugated bilirubin was missing for 5 hospitalized patients and 3 outpatients

^c Lactate was missing for 2 hospitalized patients and 13 outpatients

^d AST was missing for 8 hospitalized patients and 3 outpatients

^e ALT was missing for 1 hospitalized patient and 1 outpatient

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. The final parameter estimates are shown in Table 3.

No difference in CL or bioavailability was found between hospitalized patients 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 (*k_a*) and 1/mean absorption time (MTT) simultaneously using the same effect parameter, theta (θ), as outlined in the formulae below.

$$MTT_{group} = \frac{MTT_{outpatients}}{\hat{\epsilon}_{patient\ group\ effect}} \quad k_{a_{group}} = \hat{\epsilon}_{patient\ group\ effect} \cdot k_{a_{outpatients}}$$

Where $MTT_{outpatients}$ is the typical mean transit time for the outpatients in hours, MTT_{group} is the mean transit time for hospitalized survived or hospitalized deaths group in hours, $k_{a_{group}}$ is the absorption rate constant for hospitalized survivors or hospitalized deaths group in hour⁻¹, and $k_{a_{outpatients}}$ 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 *k_a*), 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

Table 3. Final population pharmacokinetic parameter estimates for rifampicin, isoniazid and pyrazinamide.

| Parameter | Typical value (95% CI) ^a | | |
|--|-------------------------------------|--------------------------|-----------------------|
| | Rifampicin | Isoniazid | Pyrazinamide |
| Clearance (L/h) ^b | 8.82 (8.10 – 9.48) | - | 2.61 (2.48 – 2.75) |
| Clearance of Fast metabolizers (L/h) | - | 25.5 (22.7 – 28.7) | - |
| Clearance of Slow metabolizers (L/h) | - | 9.76 (8.28 – 11.2) | - |
| Proportion of fast NAT2 metabolizers (%) | - | 64.5 (54.4% – 75.8%) | - |
| Volume of distribution (L) ^b | 56.8 (53.9 – 61.2) | 59.0 (54.7 – 64.2) | 36.0 (34.4 – 37.9) |
| Intercompartmental clearance, Q (L/h) | - | 1.43 (0.874 – 2.14) | - |
| Peripheral volume, V _p (L) ^c | - | 30.7 (25.9 – 37.1) | - |
| Absorption rate constant, k _a (h ⁻¹) | 1.38 (1.04 – 1.70) | 2.43 (1.80 – 6.50) | 1.92 (1.53 – 2.59) |
| Mean transit time, MTT (h) | 0.342 (0.259 – 0.534) | 0.442 (0.266 – 0.781) | 0.379 (0.220 – 0.566) |
| No. of absorption transit compartments (.) | 12 fixed | 8 fixed | 4 fixed |
| Bioavailability, F (%) | 100 fixed | 100 fixed | 100 fixed |
| % difference in mean absorption time (MAT) for hospitalized survivors ^d | +49.9% (+2.80% – +80.9%) | - | - |
| % difference in MAT for hospitalized deaths ^d | +154% (+63.9% – 351%) | - | - |
| Exponent of power relationship between Clearance and conjugated bilirubin ^e | -0.333 (-0.474 – -0.194) | - | - |
| Between-subject variability for clearance (BSVCL) (%) | 42.4 (37.3 – 49.4) | 25.3 (17.2 – 33.4) | 19.9 (13.2 – 25.0) |
| Fold change in BSVCL for hospitalized survivors ^f | - | - | 1.70 (1.26 – 2.65) |
| Fold change in BSVCL for hospitalized deaths ^f | - | - | 3.56 (1.64 – 6.53) |
| Between-occasion variability (BOV) (%) for: | | | |
| Bioavailability | 21.3 (16.4 – 27.6) | 34.9 (26.6 – 40.3) | 10.5 (4.13 – 15.1) |
| Absorption rate constant, k _a | 119 (100 – 137) | 122 (84.0 – 186) | 75.3 (40.3 – 95.3) |
| Mean transit time, MTT | 93.8 (67.4 – 111) | 99.7 (45.9 – 172) | 102 (61.9 – 145) |
| Proportional error (%) | 17.2 (14.9 – 18.5) | 13.9 (12.0 – 16.4) | 11.4 (7.59– 13.9) |
| Additive error (mg/L) | 0.0234 fixed ^g | 0.021 fixed ^g | 2.48 (1.47 – 3.44) |

^a Values in parentheses are empirical 95% confidence intervals, obtained with a 500-sample nonparametric bootstrap

^b 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.

^c The peripheral volume, V_p, was calculated from the estimated Vratio (V_c/V_p) and V_c. A prior of 2.02 was included on Vratio with 30% uncertainty.

^d Patient status effect was modeled on k_a and 1/MTT simultaneously using the same effect parameter, theta (θ). k_a for hospitalized = TVk_a × θ; MTT for hospitalized = TVMTT / θ. Mean absorption time = MTT+1/k_a calculated for each group was 1.1 h for outpatients, 1.6 h for hospitalized survivors, and 3.2 h for hospitalized deaths.

^e $BRC \text{ Effect on CL} = (BRC/\text{median BRC})^{-0.326}$; $CL = TVCL \times BRC \text{ Effect on CL}$

^f BSVCL for hospitalized = BSVCL × fold change. i.e. BSVCL is 33.8% for hospitalized survivors and 70.8% for hospitalized deaths.

^g The estimate of the additive component of the error was not significantly different from its lower boundary of 20% of LLOQ, so it was fixed to this value.

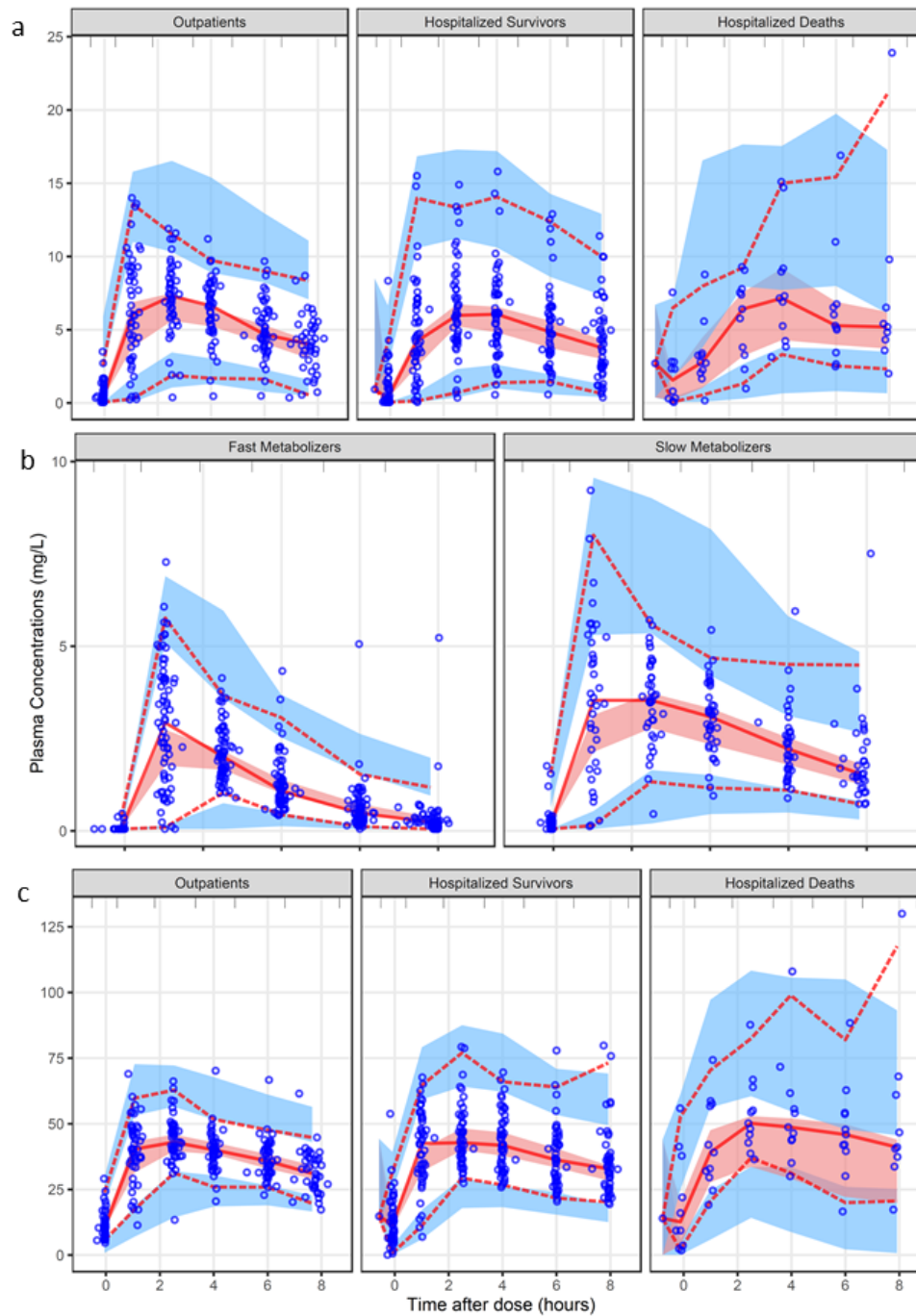


Figure 1. Visual predictive check (VPC) ($n=1000$) showing plasma drug concentration versus time after dose for the final models of each drug: **a)** for rifampicin stratified by patient group; **b)** for isoniazid stratified by metabolizer status; **c)** for pyrazinamide stratified by patient group. 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.

$$CL_i = CL_{typical} \cdot \left(\frac{BRC_i}{BRC_{median}} \right)^{\beta_{BRC}}$$

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 $\mu\text{mol/L}$) and β_{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, piecewise linear. The relationship is depicted in [Figure 2](#). Both total

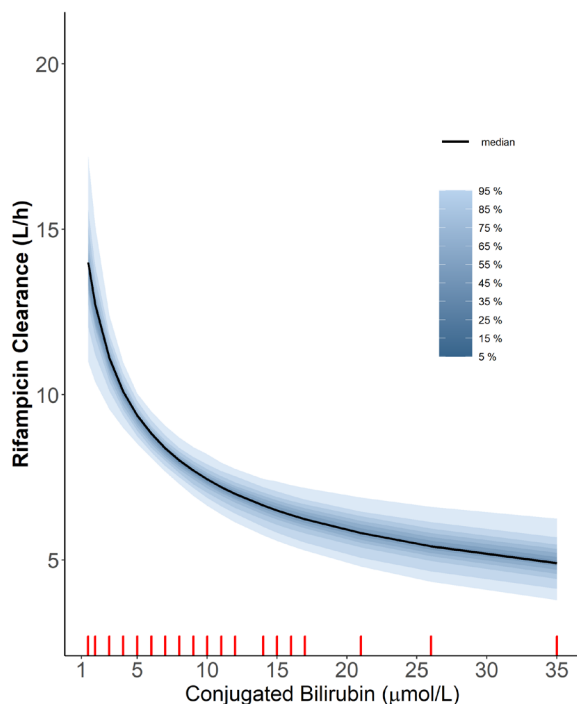


Figure 2. The relationship of rifampicin clearance vs the level of conjugated bilirubin using the power function. The red ticks represent the values of conjugated bilirubin observed in our cohort. The solid line represents the median and the shaded areas represent the uncertainty distribution around the effect due to the precision of parameter estimates.

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.

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 ($\text{dOFV} = -12.9$, $\text{df} = 1$, $p\text{-value} < 0.001$), 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 V_p could not be reliably estimated. To stabilize the estimate of the V_p , a prior was included on the V_{ratio} (V_c/V_p) with a value of 3.728 with 30% uncertainty²². Allometric scaling of CL and V_c 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 ($\text{dOFV} = -15.5$, $\text{df} = 2$, $p\text{-value} < 0.0005$). 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.

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 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.

Variability correlation across the three drugs

The correlations of the remaining unexplained variability in clearance, bioavailability, 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 [Figure 4](#). Moderate correlations were found for all, except for absorption, which ranged between 68.4% - 84.6%.

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 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.

There are limited data comparing the pharmacokinetics of first-line anti-TB drugs between hospitalized patients and outpatients. A non-compartmental analysis (NCA) of the data from this pharmacokinetic study was published by Schutz *et al.*². 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. The pharmacokinetic parameters from our models for rifampicin, isoniazid and pyrazinamide were comparable to those from other similar studies^{8,23-26}

Rifampicin PK model

The structural model we developed for rifampicin was similar to previously developed rifampicin models⁸. However, CL values are lower in this analysis because sampling was done on the third day of treatment, where autoinduction is still not significant. 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 tract⁵⁻⁷. We reason that only rifampicin's absorption out of the three drugs was affected by these gastrointestinal changes because of rifampicin's low solubility²⁷, whereas both isoniazid and pyrazinamide have high solubility according to the biopharmaceutics classification system^{28,29}. Rifampicin's absorption is mainly from the stomach and proximal intestine³⁰ and is more likely to be easily affected by changes in the gastric pH⁹. As a result, the C_{max} for hospitalized patients tends to be lower than that of the outpatients, while the AUC_{0-24h} does not seem to be affected as shown in [Figure 3](#).

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^{9,31}.

While marked differences have been reported in the rate and extent of absorption with different formulations³², 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 rifampicin³³. While there was no significant effect for either HIV status or efavirenz co-administration, previous studies have reached contrasting results regarding both. Some studies found no significant difference in rifampicin concentrations³⁴, while others found decreased rifampicin levels in HIV-positive TB patients³⁵⁻³⁷. Nevertheless, a meta analysis by Stott *et al.*³⁸ 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%³⁹⁻⁴¹. 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^{23,24}.

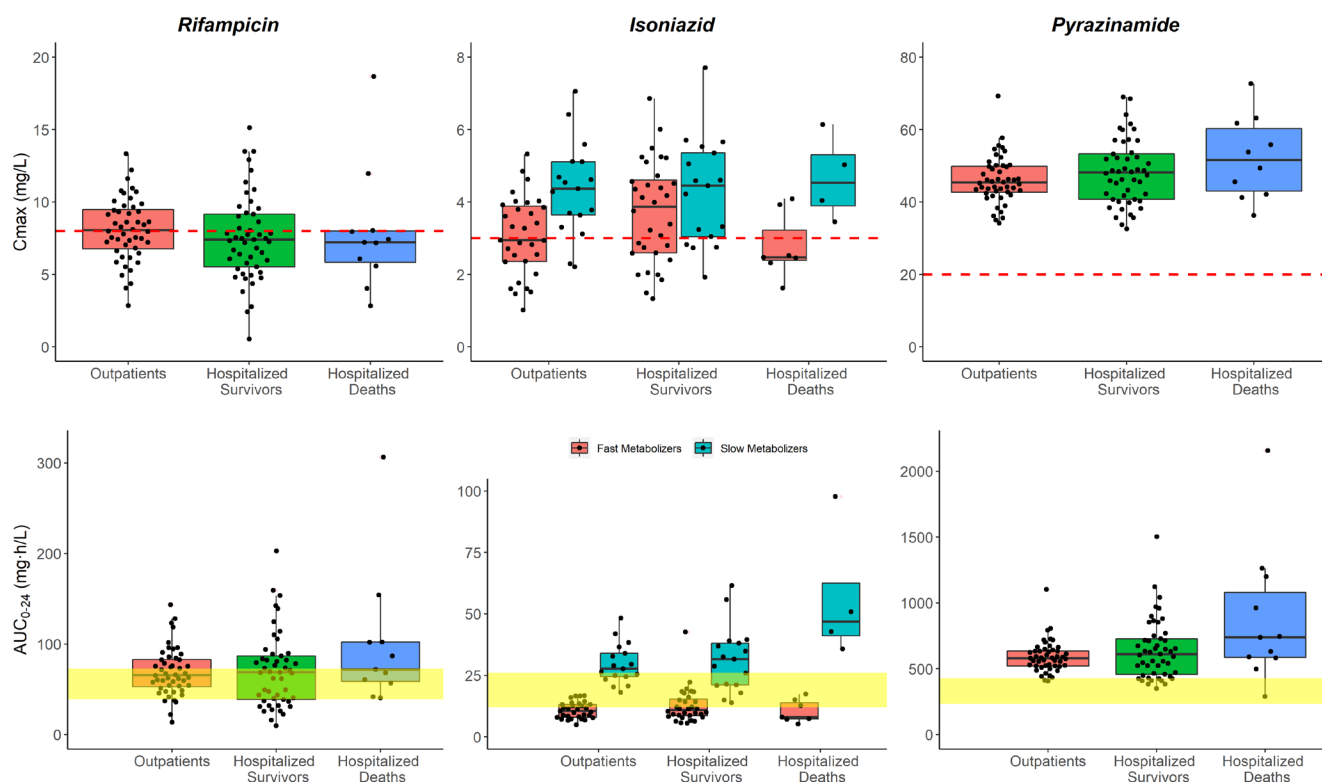


Figure 3. Box and whisker plots of the model-derived individual C_{max} and AUC_{0-24} for the three drugs. The dots are individual values, and the whiskers represent the 2.5th and 97.5th percentiles. The dashed line represents the currently recommended minimum threshold: 8 mg/L for rifampicin, 3 mg/L for isoniazid, and 20 mg/L for pyrazinamide. The yellow shaded areas represent the exposure targets based on Stott *et al.* for rifampicin³⁸ and Daskapan *et al.* for isoniazid and pyrazinamide³⁷. This is only for visualization purposes; no statistical tests can be carried out here since dose amounts are not accounted for.

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

Inadequate exposure of 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.*⁴².

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.

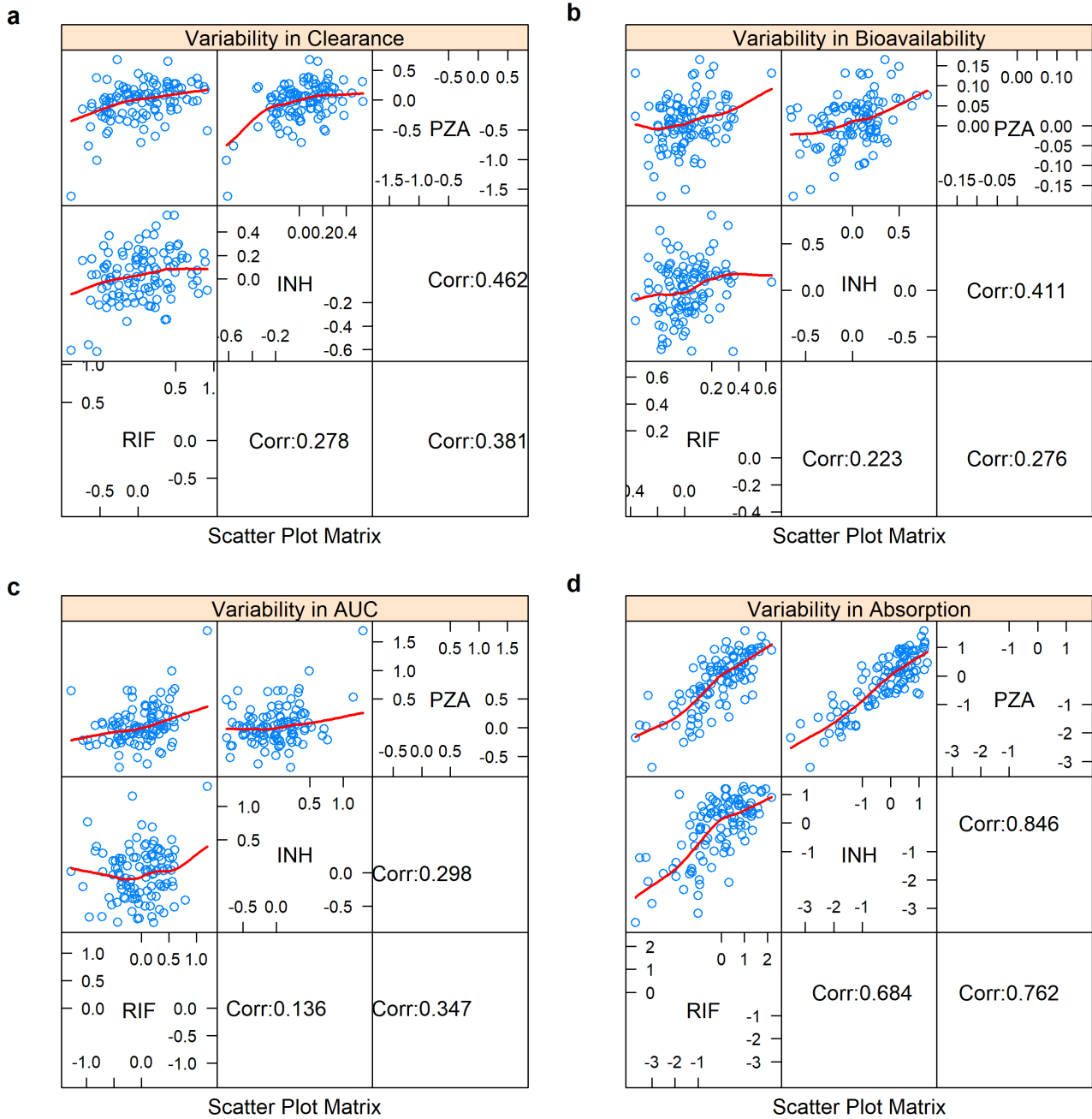


Figure 4. Correlation matrix for the unexplained variability in **a)** clearance, **b)** bioavailability, **c)** area under the curve (AUC_{0-24h}), and **d)** absorption between the three drugs. The correlation coefficient is shown in the lower panel. Only the variability from the main occasion was included (not the predose). Variability in clearance = $BSVCL + BOVCL$. Variability in bioavailability = $BSVBIO + BOVBIO$. Variability in AUC = $BSVBIO + BOVBIO - BSVCL - BOVCL$. Variability in absorption = $BSVKA + BOVKA - BSVMTT - BOVMTT$.

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)

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

- Kyeyune R, Den Boon S, Cattamanchi A, *et al.*: **Causes of early mortality in HIV-infected TB suspects in an East African referral hospital.** *J Acquir Immune Defic Syndr.* 2010; **55**(4): 446–50. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Schutz C, Chirehwa M, Barr D, *et al.*: **Early antituberculosis drug exposure in hospitalized patients with human immunodeficiency virus-associated tuberculosis.** *Br J Clin Pharmacol.* 2020; **86**(5): 966–78. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Subbarao S, Wilkinson KA, Van Halsema CL, *et al.*: **Raised venous lactate and markers of intestinal translocation are associated with mortality among in-patients with HIV-associated TB in rural South Africa.** *J Acquir Immune Defic Syndr.* 2015; **70**(4): 406–13. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Brenchley JM, Price DA, Schacker TW, *et al.*: **Microbial translocation is a cause of systemic immune activation in chronic HIV infection.** *Nat Med.* 2006; **12**(12): 1365–71. [PubMed Abstract](#) | [Publisher Full Text](#)
- Roberts DJ, Hall RI: **Drug absorption, distribution, metabolism and excretion considerations in critically ill adults.** *Expert Opin Drug Metab Toxicol.* 2013; **9**(9): 1067–84. [PubMed Abstract](#) | [Publisher Full Text](#)
- Parsons RL: **Drug Absorption in Gastrointestinal Disease With Particular Reference to Malabsorption Syndromes.** *Clin Pharmacokinet.* 1977; **2**(1): 45–60. [PubMed Abstract](#) | [Publisher Full Text](#)
- Blot SI, Pea F, Lipman J: **The effect of pathophysiology on pharmacokinetics in the critically ill patient—concepts appraised by the example of antimicrobial agents.** *Adv Drug Deliv Rev.* Elsevier; 2014; **77**: 3–11. [PubMed Abstract](#) | [Publisher Full Text](#)
- Wilkins JJ, Savic RM, Karlsson MO, *et al.*: **Population pharmacokinetics of rifampin in pulmonary tuberculosis patients, including a semimechanistic model to describe variable absorption.** *Antimicrob Agents Chemother.* American Society for Microbiology Journals; 2008; **52**(6): 2138–48. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Acoella G: **Clinical Pharmacokinetics of Rifampicin.** *Clin Pharmacokinet.* 1978; **3**(2): 108–27. [PubMed Abstract](#) | [Publisher Full Text](#)
- Kenny MT, Strates B: **Metabolism and pharmacokinetics of the antibiotic rifampin.** *Drug Metab Rev.* Informa Healthcare; 1981; **12**(1): 159–218. [PubMed Abstract](#) | [Publisher Full Text](#)
- Chen Y, Ferguson SS, Negishi M, *et al.*: **Induction of Human CYP2C9 by Rifampicin, Hyperforin, and Phenobarbital Is Mediated by the Pregnane X Receptor.** *J Pharmacol Exp Ther.* American Society for Pharmacology and Experimental Therapeutics; 2004; **308**(2): 495–501. [PubMed Abstract](#) | [Publisher Full Text](#)
- Loos U, Musch E, Jensen JC, *et al.*: **Pharmacokinetics of oral and intravenous rifampicin during chronic administration.** *Klin Wochenschr.* Springer-Verlag; 1985; **63**(23): 1205–11. [PubMed Abstract](#) | [Publisher Full Text](#)
- Jamis-Dow CA, Katki AG, Collins JM, *et al.*: **Rifampin and rifabutin and their metabolism by human liver esterases.** *Xenobiotica.* Taylor & Francis; 1997; **27**(10): 1015–24. [PubMed Abstract](#) | [Publisher Full Text](#)
- Ellard GA, Gammon PT: **Pharmacokinetics of isoniazid metabolism in man.** *J Pharmacokinet Biopharm.* Kluwer Academic Publishers-Plenum Publishers; 1976; **4**(2): 83–113. [PubMed Abstract](#) | [Publisher Full Text](#)
- Morgan DJ, Bray KM: **Lean Body Mass as a Predictor of Drug Dosage.** *Clin Pharmacokinet.* 1994; **26**(4): 292–307. [PubMed Abstract](#) | [Publisher Full Text](#)
- McLeay SC, Morrish GA, Kirkpatrick CMJ, *et al.*: **The Relationship between Drug Clearance and Body Size.** *Clin Pharmacokinet.* 2012; **51**(5): 319–30. [PubMed Abstract](#) | [Publisher Full Text](#)
- Smith PJ, van Dyk J, Fredericks A: **Determination of rifampicin, isoniazid and pyrazinamide by high performance liquid chromatography after their simultaneous extraction from plasma.** *Int J Tuberc Lung Dis.* 1999; **3**(11 Suppl 3): S325–8; discussion S351–2. [PubMed Abstract](#)
- Keizer RJ, Karlsson MO, Hooker A: **Modeling and simulation workbench for NONMEM: Tutorial on Pirana, PsN and Xpose.** *CPT Pharmacometrics Syst Pharmacol.* 2013; **2**(6): e50. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Beal SL: **Ways to fit a PK model with some data below the quantification limit.** *J Pharmacokinet Pharmacodyn.* 2001; **28**(5): 481–504. [PubMed Abstract](#) | [Publisher Full Text](#)
- Anderson BJ, Holford NHG: **Mechanism-based concepts of size and maturity in pharmacokinetics.** *Annu Rev Pharmacol Toxicol.* 2008; **48**: 303–32. [PubMed Abstract](#) | [Publisher Full Text](#)
- Janmahasatian S, Duffull SB, Ash S, *et al.*: **Quantification of Lean Bodyweight.** *Clin Pharmacokinet.* 2005; **44**(10): 1051–65. [PubMed Abstract](#) | [Publisher Full Text](#)
- Gausi K, Ignatius EH, Sun X, *et al.*: **A semi-mechanistic model of the bactericidal activity of high-dose isoniazid against multi-drug-resistant tuberculosis.** *Am J Respir Crit Care Med.* 2021; **204**(11): 1327–1335. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Denti P, Jeremiah K, Chigutsa E, *et al.*: **Pharmacokinetics of isoniazid, pyrazinamide, and ethambutol in newly diagnosed pulmonary TB patients in Tanzania.** *PLoS One.* 2015; **10**(10): e0141002. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wilkins JJ, Langdon G, McIlleron H, *et al.*: **Variability in the population pharmacokinetics of isoniazid in South African tuberculosis patients.** *Br J Clin Pharmacol.* 2011; **72**(1): 51–62. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chirehwa MT, McIlleron H, Rustomjee R, *et al.*: **Pharmacokinetics of pyrazinamide and optimal dosing regimens for drug-sensitive and -resistant tuberculosis.** *Antimicrob Agents Chemother.* 2017; **61**(8): e00490–17. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wilkins JJ, Langdon G, McIlleron H, *et al.*: **Variability in the population pharmacokinetics of pyrazinamide in South African tuberculosis patients.** *Eur J Clin Pharmacol.* 2006; **62**(9): 727–35. [PubMed Abstract](#) | [Publisher Full Text](#)
- Becker C, Dressman JB, Junginger HE, *et al.*: **Biowaiver monographs for**

- immediate release solid oral dosage forms: Rifampicin.** *J Pharm Sci.* John Wiley and Sons Inc.; 2009; **98**(7): 2252–67.
[PubMed Abstract](#) | [Publisher Full Text](#)
28. Becker C, Dressman JB, Amidon GL, *et al.*: **Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Isoniazid.** **project of the International Pharmaceutical Federation FIP, Groupe BCS. www.fip.org/bcs. *J Pharm Sci.* John Wiley and Sons Inc.; 2007; **96**(3): 522–31.
[PubMed Abstract](#) | [Publisher Full Text](#)
29. Becker C, Dressman JB, Amidon GL, *et al.*: **Biowaiver monographs for immediate release solid oral dosage forms: Pyrazinamide.** *J Pharm Sci.* John Wiley and Sons Inc.; 2008; **97**(9): 3709–20.
[PubMed Abstract](#) | [Publisher Full Text](#)
30. Mariappan TT, Singh S: **Regional gastrointestinal permeability of rifampicin and isoniazid (alone and their combination) in the rat.** *Int J Tuberc Lung Dis.* 2003; **7**(8): 797–803.
[PubMed Abstract](#)
31. Lal S, Singhal SN, Burley DM, *et al.*: **Effect of Rifampicin and Isoniazid on Liver Function.** *Br Med J.* 1972; **1**(5793): 148–50.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
32. McIlleron H, Wash P, Burger A, *et al.*: **Widespread distribution of a single drug rifampicin formulation of inferior bioavailability in South Africa.** *Int J Tuberc Lung Dis.* 2002; **6**(4): 356–61.
[PubMed Abstract](#)
33. Chirehwa MT, Rustomjee R, Mthiyane T, *et al.*: **Model-Based Evaluation of Higher Doses of Rifampin Using a Semimechanistic Model Incorporating Autoinduction and Saturation of Hepatic Extraction.** [Erratum appears in *Antimicrob Agents Chemother.* 2016 May; **60**(5): 3262; PMID: 27107106]. *Antimicrob Agents Chemother.* 2015; **60**(1): 487–94.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
34. McIlleron H, Rustomje R, Vahedi M, *et al.*: **Reduced antituberculosis drug concentrations in HIV-infected patients who are men or have low weight: Implications for international dosing guidelines.** *Antimicrob Agents Chemother.* American Society for Microbiology; 2012; **56**(6): 3232–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
35. Jeremiah K, Denti P, Chigutsa E, *et al.*: **Nutritional supplementation increases rifampin exposure among tuberculosis patients coinfecting with HIV.** *Antimicrob Agents Chemother.* American Society for Microbiology; 2014; **58**(6): 3468–74.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
36. McIlleron H, Wash P, Burger A, *et al.*: **Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients.** *Antimicrob Agents Chemother.* American Society for Microbiology Journals; 2006; **50**(4): 1170–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
37. Daskapan A, Idrus LR, Postma MJ, *et al.*: **A Systematic Review on the Effect of HIV Infection on the Pharmacokinetics of First-Line Tuberculosis Drugs.** *Clin Pharmacokinet.* Springer International Publishing; 2019; **58**(6): 747–66.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Stott KE, Pertinez H, Sturkenboom MGG, *et al.*: **Pharmacokinetics of rifampicin in adult TB patients and healthy volunteers: A systematic review and meta-analysis.** *J Antimicrob Chemother.* Oxford University Press; 2018; **73**(9): 2305–13.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
39. Mthiyane T, Millard J, Adamson J, *et al.*: **N-Acetyltransferase 2 Genotypes among Zulu-Speaking South Africans and Isoniazid and N-Acetyl-Isoniazid Pharmacokinetics during Antituberculosis Treatment.** *Antimicrob Agents Chemother.* 2020; **64**(4): e02376-19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
40. Adams CH, Werely CJ, Victor TC, *et al.*: **Allele frequencies for glutathione S-transferase and N-acetyltransferase 2 differ in African population groups and may be associated with oesophageal cancer or tuberculosis incidence.** *Clin Chem Lab Med.* 2003; **41**(4): 600–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
41. Loktionov A, Moore W, Spencer SP, *et al.*: **Differences in N-acetylation genotypes between Caucasians and Black South Africans: Implications for cancer prevention.** *Cancer Detect Prev.* 2002; **26**(1): 15–22.
[PubMed Abstract](#) | [Publisher Full Text](#)
42. Sundell J, Bienvenu E, Janzén D, *et al.*: **Model-Based Assessment of Variability in Isoniazid Pharmacokinetics and Metabolism in Patients Co-Infected With Tuberculosis and HIV: Implications for a Novel Dosing Strategy.** *Clin Pharmacol Ther.* 2020; **108**(1): 73–80.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 08 August 2022

<https://doi.org/10.21956/wellcomeopenres.19537.r51522>

© 2022 Pasipanodya J. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



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

1. Sekaggya-Wiltshire C, von Braun A, Lamorde M, Ledergerber B, et al.: Delayed Sputum Culture Conversion in Tuberculosis–Human Immunodeficiency Virus–Coinfected Patients With Low Isoniazid and Rifampicin Concentrations. *Clinical Infectious Diseases*. 2018; **67** (5): 708-716 [Publisher](#)

[Full Text](#)

2. Vinnard C, Ravimohan S, Tamuhla N, Pasipanodya J, et al.: Markers of gut dysfunction do not explain low rifampicin bioavailability in HIV-associated TB. *Journal of Antimicrobial Chemotherapy*. 2017; **72** (7): 2020-2027 [Publisher 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.

Reviewer Report 06 June 2022

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

© 2022 Della Pasqua O. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



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).

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.

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.*, 2019³; Muda *et al.*, 2022⁴), including investigations where findings contrast with results and conclusions drawn by the authors (Sundell *et al.*, 2021⁵; Rao *et al.*, 2021⁶). 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_{max} , C_{ss} , C_{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 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

1. Sundell J, Bienvenu E, Äbelö A, Ashton M: Effect of efavirenz-based ART on the pharmacokinetics of rifampicin and its primary metabolite in patients coinfecting with TB and HIV. *Journal of Antimicrobial Chemotherapy*. 2021; **76** (11): 2950-2957 [Publisher Full Text](#)
2. Montanha MC, Fabrega F, Howarth A, Cottura N, et al.: Predicting Drug-Drug Interactions between Rifampicin and Ritonavir-Boosted Atazanavir Using PBPK Modelling. *Clin Pharmacokinet*. **61** (3): 375-386 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Muliaditan M, Della Pasqua O: How long will treatment guidelines for TB continue to overlook variability in drug exposure?. *Journal of Antimicrobial Chemotherapy*. 2019; **74** (11): 3274-3280 [Publisher Full Text](#)
4. Muda MR, Harun SN, Syed Sulaiman SA, Sheikh Ghadzi SM: Population pharmacokinetics analyses of rifampicin in adult and children populations: A systematic review. *Br J Clin Pharmacol*. 2022. [PubMed Abstract](#) | [Publisher Full Text](#)
5. Sundell J, Wijk M, Bienvenu E, Äbelö A, et al.: Factors Affecting the Pharmacokinetics of Pyrazinamide and Its Metabolites in Patients Coinfecting with HIV and Implications for Individualized Dosing. *Antimicrobial Agents and Chemotherapy*. 2021; **65** (7). [Publisher Full Text](#)
6. Rao PS, Moore CC, Mbonde AA, Nuwagira E, et al.: Population Pharmacokinetics and Significant Under-Dosing of Anti-Tuberculosis Medications in People with HIV and Critical Illness. *Antibiotics (Basel)*. 2021; **10** (6). [PubMed Abstract](#) | [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.
