

# Delayed Sputum Culture Conversion in Tuberculosis–Human Immunodeficiency Virus–Coinfected Patients With Low Isoniazid and Rifampicin Concentrations

Christine Sekaggya-Wiltshire,<sup>1,a</sup> Amrei von Braun,<sup>2,a</sup> Mohammed Lamorde,<sup>1</sup> Bruno Ledergerber,<sup>2</sup> Allan Buzibye,<sup>1</sup> Lars Henning,<sup>2,3</sup> Joseph Musaaazi,<sup>1</sup> Ursula Gutteck,<sup>4</sup> Paolo Denti,<sup>5</sup> Miné de Kock,<sup>5</sup> Alexander Jetter,<sup>6</sup> Pauline Byakika-Kibwika,<sup>1,7</sup> Nadia Eberhard,<sup>2</sup> Joshua Matovu,<sup>1</sup> Moses Joloba,<sup>8</sup> Daniel Muller,<sup>4</sup> Yukari C. Manabe,<sup>9</sup> Moses R. Kanya,<sup>7</sup> Natascia Corti,<sup>6</sup> Andrew Kambugu,<sup>1</sup> Barbara Castelnuevo,<sup>1,b</sup> and Jan S. Fehr,<sup>2,10,b</sup>

<sup>1</sup>Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda; <sup>2</sup>Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Switzerland; <sup>3</sup>Division of Tropical Health and Medicine, James Cook University, Queensland, Australia; <sup>4</sup>Department of Clinical Chemistry, University Hospital Zurich, University of Zurich, Switzerland; <sup>5</sup>Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, South Africa; <sup>6</sup>Department of Clinical Pharmacology and Toxicology, University Hospital Zurich, University of Zurich, Switzerland; <sup>7</sup>School of Medicine, and <sup>8</sup>School of Biomedical Sciences, College of Health Sciences, Makerere University, Kampala, Uganda; <sup>9</sup>Division of Infectious Diseases, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore; and <sup>10</sup>Department of Public Health at Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Switzerland

(See the Editorial Commentary by Pasipanodya and Gumbo on pages 717–8.)

**Background.** The relationship between concentrations of antituberculosis drugs, sputum culture conversion, and treatment outcome remains unclear. We sought to determine the association between antituberculosis drug concentrations and sputum conversion among patients coinfecting with tuberculosis and human immunodeficiency virus (HIV) and receiving first-line antituberculosis drugs.

**Methods.** We enrolled HIV-infected Ugandans with pulmonary tuberculosis. Estimation of first-line antituberculosis drug concentrations was performed 1, 2, and 4 hours after drug intake at 2, 8, and 24 weeks of tuberculosis treatment. Serial sputum cultures were performed at each visit. Time-to-event analysis was used to determine factors associated with sputum culture conversion.

**Results.** We enrolled 268 HIV-infected patients. Patients with low isoniazid and rifampicin concentrations were less likely to have sputum culture conversion before the end of tuberculosis treatment (hazard ratio, 0.54; 95% confidence interval, .37–.77;  $P = .001$ ) or by the end of follow-up (0.61; .44–.85;  $P = .003$ ). Patients in the highest quartile for area under the rifampicin and isoniazid concentration-time curves for were twice as likely to experience sputum conversion than those in the lowest quartile. Rifampicin and isoniazid concentrations below the thresholds and weight <55 kg were both risk factors for unfavorable tuberculosis treatment outcomes. Only 4.4% of the participants had treatment failure.

**Conclusion.** Although low antituberculosis drug concentrations did not translate to a high proportion of patients with treatment failure, the association between low concentrations of rifampicin and isoniazid and delayed culture conversion may have implications for tuberculosis transmission.

**Clinical Trials Registration:** NCT01782950.

**Keywords.** Tuberculosis; outcomes; pharmacokinetics; anti-tuberculosis drugs.

Tuberculosis infected >10 million persons in 2015 with approximately 1.5 million deaths, of whom 400 000 occurred among persons coinfecting with human immunodeficiency virus (HIV) [1]. Clearance of mycobacteria from sputum is necessary to achieve individual cure and decrease tuberculosis transmission; however, up to 50% of patients remain sputum culture positive after 1 month of directly observed therapy, and 20% after 2 months, a fact that has major public health implications for curbing the tuberculosis pandemic [2–4]. Earlier studies reported that a

positive sputum culture after 2 months of treatment is a predictor of treatment failure and tuberculosis relapse [5]. However, more recent studies have demonstrated this finding to be a poor predictor of tuberculosis treatment outcome [6, 7]. Despite this, the sputum culture result at month 2 is used to monitor treatment response and as a marker of the sterilizing activity of antituberculosis drugs [7, 8]. Delayed sputum culture conversion has been associated with high bacillary load at treatment initiation, lung cavities, and drug resistance [9, 10].

Rifampicin and isoniazid display concentration-dependent killing of mycobacteria, leading to a decrease in bacillary load within the first few days of treatment [11]. Several studies have demonstrated low antituberculosis drug concentrations in patients receiving tuberculosis treatment [12–14]. At present, there is conflicting evidence about whether there is an association between antituberculosis drug concentrations, time to sputum conversion, and tuberculosis treatment outcome [14–16].

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<sup>a</sup>C. S. W. and A. v. B. contributed equally to this work.

<sup>b</sup>B. C. and J. S. F. contributed equally to this work.

Correspondence: C. Sekaggya-Wiltshire, Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda (csekaggya@idi.co.ug).

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The aim of the current study was to determine the association between antituberculosis drug concentrations and sputum conversion in a cohort of tuberculosis-HIV-coinfected patients receiving first-line antituberculosis drugs at the standard dosage.

## METHODS

### Setting

We carried out a prospective observational study entitled Study on Outcomes Related to TB and HIV Drug Concentrations among tuberculosis-HIV-coinfected patients from May 2013 to November 2015 at the Infectious Diseases Institute (IDI) in Kampala, Uganda.

### Study Design and Population

Tuberculosis was diagnosed using a combination of clinical symptoms (cough for  $\geq 2$  weeks, a history of fever as reported by the patient, unintended weight loss of  $\geq 10\%$  in the previous 3 months, and night sweats) and findings of chest radiography, sputum florescent microscopy, cultures, and Xpert MTB/RIF assay. All patients had a documented Uni-Gold test result confirming HIV infection. HIV-infected patients with pulmonary tuberculosis diagnosed were included in the study if they were  $\geq 18$  years old, willing to comply with study visits, and had no prior history of tuberculosis treatment. Details of the study methods were published elsewhere [17].

Patients were excluded if they (1) required tuberculosis treatment for  $>6$  months (eg, for tuberculous meningitis); (2) had been previously treated for infections due to mycobacteria other than *Mycobacterium tuberculosis*; (3) were pregnant; (4) had an elevated alanine aminotransferase level  $>5$  times the upper limit of normal; (5) had a glomerular filtration rate  $<50$  mL/min, which could affect drug elimination; or (6) had comorbid conditions that reduce life expectancy to  $<1$  year (eg, visceral Kaposi sarcoma). Patients were withdrawn from the study on request, based on medical judgment of the study physician, if they became pregnant, experienced a toxic effect necessitating a change or interruption of tuberculosis treatment, had a drug sensitivity test result showing resistance to any first-line antituberculosis drug (which could delay sputum conversion), or had only mycobacteria other than *M. tuberculosis* identified in the baseline culture.

Tuberculosis treatment was provided according to World Health Organization (WHO) recommendations [14] using fixed-dose combinations, which included 2 months (intensive phase) of rifampicin, isoniazid, ethambutol, and pyrazinamide (RHEZ), followed by 4 months (continuation phase) of isoniazid and rifampicin with dosages based on weight bands: 3 tablets of RHEZ or isoniazid-rifampicin for weight  $<55$  kg, 4 tablets for weight 55–69 kg, and 5 tablets for weight  $\geq 70$  kg. Each tablet contained 150 mg of rifampicin, 75 mg of isoniazid for both RHEZ and isoniazid-rifampicin combinations plus 400 mg of pyrazinamide and 275 mg of ethambutol for RHEZ.

Antiretroviral treatment (ART) consisting of tenofovir, lamivudine, and efavirenz was started  $\geq 2$  weeks after initiation

of tuberculosis treatment, as long as the patient was willing to commence treatment. Patients who were already receiving ART at the time of tuberculosis diagnosis continued their treatment, and patients taking nevirapine were switched to efavirenz to reduce drug-drug interactions. Patients receiving protease inhibitors received rifabutin instead of rifampicin and were therefore excluded from this analysis.

### Pharmacokinetic Measurements

Patients were followed up at 2, 8, and 24 weeks after initiation of tuberculosis treatment and fasted for  $\geq 8$  hours before the study visit. At each follow-up visit, blood sampling was performed at 0 hours (before drug ingestion) and 1, 2, and 4 hours after witnessed ingestion of antituberculosis drugs. A standardized meal was provided after the 1-hour blood collection. Adherence counseling was performed at every follow-up visit. Adherence was assessed through pill counts and self-report using 7-day and 1-month recall [18].

Blood samples were collected in serum Vacutainer test tubes, and serum was separated by centrifuging within 1 hour of the blood sampling. Serum concentrations of rifampicin, isoniazid, pyrazinamide, and ethambutol were measured at the IDI translational laboratory in Kampala using UV high-performance liquid chromatography, as described elsewhere [19]. Additional information on the methods are available in the [Supplementary Material](#).

### Microbiological Assessment

A spot sputum sample was requested at every follow-up visit for fluorescent microscopy, as well as culture using both Löwenstein-Jensen medium and the BACTEC Mycobacteria Growth Indicator Tube 960 system. In accordance with the standard of care, sputum microscopy was also performed after 5 months of tuberculosis treatment. Patients who could not spontaneously provide a sputum sample underwent sputum induction where possible.

After a substudy protocol amendment was approved, the last 108 study participants underwent intensive sputum culture monitoring. This subset of patients provided sputum samples for microscopy and culture every 2 weeks for the first 12 weeks of treatment. All other procedures were identical to those performed in the rest of the study population.

### Ethical Considerations

Ethical approval was received from Joint Clinical and Research Centre Institutional Review Board, Uganda National Council for Science and Technology (reference No. HS1303) and the National Drug Authority. This study is registered in Clinicaltrials.gov (NCT01782950). Written informed consent was obtained from all study participants.

### Assessment of Outcome

Tuberculosis treatment outcomes were defined according to the WHO treatment guidelines [14]. Sputum culture conversion was defined as conversion of sputum cultures from positive to negative with no subsequent positive cultures in patients with

a positive culture at baseline. Participants were considered lost to follow-up if their tuberculosis treatment outcome or clinical status was unknown by the end of the follow-up period (6 months after completion of tuberculosis treatment).

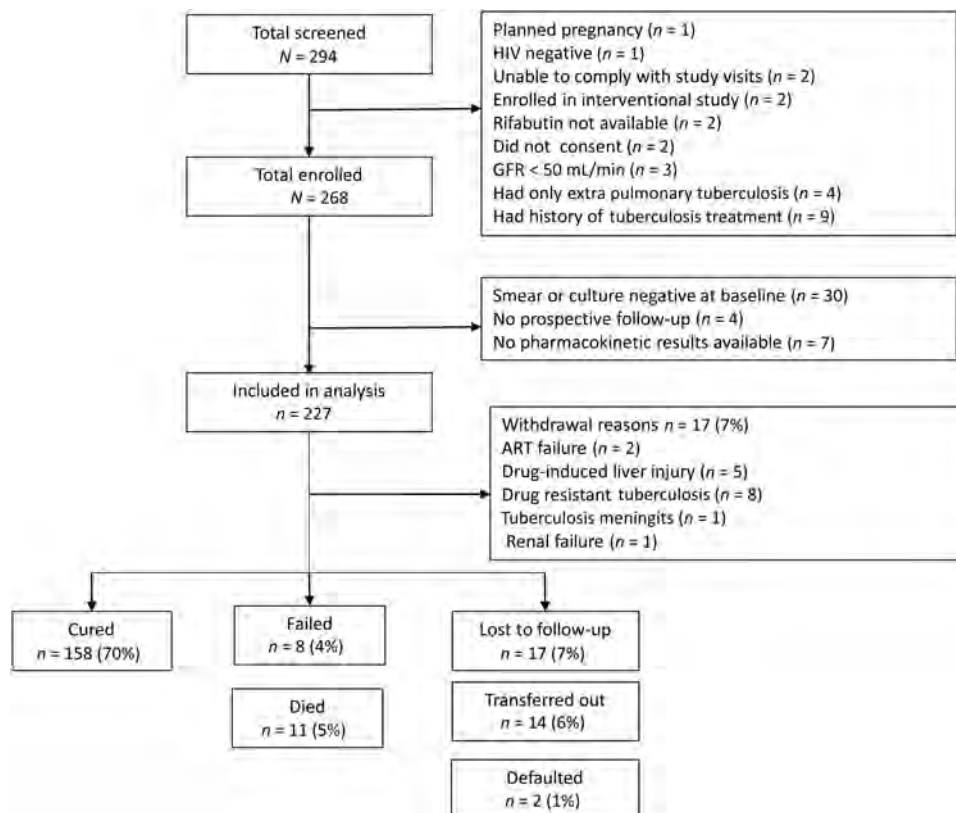
### Data Analysis

We included patients who had a positive sputum culture at baseline, were followed up prospectively, and had pharmacokinetic data available on  $\geq 1$  occasion. The estimated maximum drug concentration ( $eC_{max}$ ) for each drug was defined as the highest concentration of the 1-, 2-, and 4-hour blood samples at each study visit. Population pharmacokinetic models were developed using Monolix software [20] and individual area under the concentration-time curves (AUC) over 24 hours were derived from these models. Details of these models can be found in the [Supplementary Material](#).

We used time-to-event analyses (Kaplan-Meier curves and Cox proportional hazards regression) to determine factors associated with sputum culture conversion. Predictors of low drug concentrations and the status of cured versus not cured were determined with logistic general estimation equations accounting for multiple time points with pharmacokinetic determinations per patient. WHO weight bands were divided into 3 equal tertiles (upper, middle, and lower) as one predictor

of low concentrations. Wilcoxon rank-sum tests were used to assess associations between the  $eC_{max}$  of each drug and baseline characteristics. We determined the number of drugs and proportions of patients with an  $eC_{max}$  below the lower limit of the reference ranges for each participant, using thresholds from Peloquin et al [21]: 3 mg/L for isoniazid, 8 mg/L for rifampicin, 20 mg/L for pyrazinamide, and 2 mg/L for ethambutol [21, 22]. We also evaluated the recent maximum drug concentration ( $C_{max}$ ) and AUC thresholds from Pasipanodya et al [23]: 8.8 mg/L and 52 mg·h/L to isoniazid, 6.6 mg/L and 13 mg·h/L for rifampicin, 58.3 mg/L and 363 mg·h/L for pyrazinamide.

A drug was classified as below the cutoff if the  $eC_{max}$  was below the reference range on the study visit when sputum culture conversion was established or below the reference range on  $\geq 1$  study visit before sputum culture conversion. For patients without conversion by the end of tuberculosis treatment, we used the  $eC_{max}$  from the last study visit, and for those conversion, we used the lowest  $eC_{max}$  among the values below the threshold. For analysis of AUCs, we used the lowest AUC before or at the time of sputum conversion or the AUC from the last study visit for those who without conversion by the end of tuberculosis treatment. Our hypothesis was that patients with antituberculosis drug concentrations below the reference ranges were more



**Figure 1.** Flow diagram shows patients excluded and treatment outcomes in patients followed up. Abbreviations: ART, antiretroviral therapy; GRF, glomerular filtration rate; HIV, human immunodeficiency virus.

likely to have delayed sputum culture conversions than those with normal concentrations. All analyses were performed using Stata software (version 14.2; StataCorp).

## RESULTS

### Patient Demographics

From April 2013 to May 2015, a total of 294 HIV-infected patients were screened for the study, and 268 were enrolled. Forty-one patients (15.3%) were excluded from the analysis: 30 had negative sputum smears and cultures throughout the study, 4 had no prospective follow-up data, and 7 had no pharmacokinetic data (3 of whom were taking rifabutin) (Figure 1). Patients who were excluded had a lower median CD4 cell count (190/ $\mu$ L vs 76/ $\mu$ L;  $P = .02$ ). The other characteristics of excluded patients are provided in the Supplementary Data.

We included 227 patients with a median follow-up of 182 person-years in our final analysis; 134 patients (59%) were male, the median age (interquartile range [IQR]) was 34 (29–40) years, the median body mass index (BMI), 19.2 (17.6–21.5)  $\text{kg}/\text{m}^2$ , and the median CD4 cell count, 190/ $\mu$ L (IQR, 66–339/ $\mu$ L). Forty-nine patients (21.6%) were receiving ART at baseline (Table 1). Patients weighing >70 kg received a slightly lower dose per kilogram weight for all drugs (Table 2).

**Table 1. Clinical and Demographic Characteristics of Study Population**

Baseline Characteristics	Patients, No. (%) <sup>a</sup> (N = 227)
Male sex	134 (59)
Age, median (IQR), y	34 (29–40)
BMI, median (IQR), $\text{kg}/\text{m}^2$	19.2 (17.7–21.5)
BMI <18 $\text{kg}/\text{m}^2$	74 (32.6)
Time since HIV diagnosis, median (IQR), mo	6 (0.5–11)
Chest radiographic findings	
Cavities	48 (21.1)
Miliary	29 (12.8)
WHO stage III	200 (88.1)
WHO stage IV	18 (7.9)
CD4 cell count, median (IQR), cells/ $\mu$ L <sup>b</sup>	190 (66–340)
CD4 cell count subgroup <sup>b</sup>	
>200/ $\mu$ L	91 (48.4)
50–200/ $\mu$ L	56 (29.8)
<50/ $\mu$ L	41 (21.8)
First-line ART	46 (20.2)
Zidovudine, lamivudine, and efavirenz	23 (10.1)
Tenofovir, lamivudine, and efavirenz	20 (8.8)
Zidovudine, lamivudine, and nevirapine <sup>c</sup>	3 (1.3)
Second-line ART	3 (1.3)
Tenofovir, lamivudine, lopinavir, and ritonavir	2 (0.9)
Tenofovir, lamivudine, atazanavir, and ritonavir	1 (0.4)

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; WHO, World Health Organization.

<sup>a</sup>Data represent No. (%) of patients unless otherwise specified.

<sup>b</sup>CD4 cell counts were available for only 188 patients

<sup>c</sup>Nevirapine was replaced by efavirenz before initiation of tuberculosis treatment.

### Attainment of Therapeutic Targets for Antituberculosis Drugs

The overall median  $eC_{\text{max}}$  for isoniazid and rifampicin were below the lower limits of the reference ranges [21] (2.23 [IQR, 1.57–3.03] and 7.07 mg/L [5.22–8.95] mg/L, respectively), whereas the median  $eC_{\text{max}}$  values for pyrazinamide and ethambutol were normal (40.9 [35.55–46.4] and 3.03 [2.12–4.08] mg/L, respectively). Pharmacokinetic results according to tuberculosis treatment outcomes are shown in Table 3. The proportions of participants with concentrations below the reference ranges were 190 of 227 (84%) for isoniazid, 176 of 227 (78%) for rifampicin, 6 of 227 (3%) for pyrazinamide, and 70 of 227 (31%) for ethambutol. Male patients were more likely to have lower  $eC_{\text{max}}$  values for rifampicin and isoniazid (hazard ratio [HR], 0.54; 95% confidence interval [CI], .35–.86;  $P = .009$ ).

There was no association between the  $eC_{\text{max}}$  of rifampicin and isoniazid and BMI, age, WHO weight bands, or tertiles within the weight bands (Table 4). However, for isoniazid, we observed that patients in the upper tertile of the WHO weight bands had lower median AUCs (9.48 [IQR, 6.09–14.87] mg·h/L) than those in the middle (10.80 [6.83–15.76] mg·h/L) or lower (10.94 [6.98–17.01] mg·h/L) tertiles. The clearance of rifampicin was higher at weeks 8 and 24 than at week 2, with a median drop from week 2 AUC values of 10.07 mg·h/L at week 8 and 5.12 mg·h/mL at week 24.

We compared our median  $eC_{\text{max}}$  and AUC findings to new targets set by Pasipanodya et al [23]. All but 1 participant had an isoniazid  $eC_{\text{max}}$  and AUC below those recommended by Pasipanodya et al. In the case of rifampicin, none of the participants had an AUC below the target recommended by Pasipanodya et al, but 136 of 222 (61.3%) had an  $eC_{\text{max}}$  below the new recommended target. The majority of the participants (222 of 224) had a pyrazinamide  $eC_{\text{max}}$  less than the new recommended target, and 59 of 224 (26.3%) had a lower pyrazinamide AUC (Figure 2).

### Pharmacokinetics and Sputum Culture Conversion

Using thresholds published by Peloquin et al [21], patients with low  $eC_{\text{max}}$  for isoniazid or rifampicin were less likely to have sputum culture conversion before the end of tuberculosis treatment (HR, 0.54; 95% CI, .37–.77;  $P = .001$ ) or by the end of follow-up (0.61; .44–.85;  $P = .003$ ) (Table 5). Patients with  $\geq 1$  drug below the cutoff were also less likely to have sputum conversion than those who had no drug below the cutoff, after adjustment for age, BMI, and sex (Table 3).

**Table 2. Dose Given According to Weight Bands**

Weight Band, kg	Dose, mg/kg		
	Rifampicin	Isoniazid	Pyrazinamide
25–39	11.68	5.81	30.76
40–54	9.38	4.69	25.13
55–69	10.08	5.04	27.12
>70	8.33	4.16	22.22

**Table 3. Median Concentrations of Isoniazid, Rifampicin, and Pyrazinamide and Tuberculosis Treatment Outcomes**

Treatment Outcome (N = 210)	Median Value (IQR)					
	Isoniazid		Rifampicin		Pyrazinamide	
	AUC, mg·h/L	eC <sub>max</sub> , mg/L	AUC, mg·h/L	eC <sub>max</sub> , mg/L	AUC, mg·h/L	eC <sub>max</sub> , mg/L
Cure (n = 158)	10.70 (6.44–16.46)	2.42 (1.65–3.43)	36.36 (27.21–46.67)	6.98 (5.0–9.60)	423.22 (369.83–499.28)	40.93 (35.25–46.85)
Death (n = 11)	7.79 (4.04–10.23)	1.33 (1–2.47)	38.45 (31.54–46.21)	5.73 (1.48–8.48)	497.48 (283.20–565.67)	41.96 (31.04–45.13)
Failure (n = 8)	10.32 (6.82–12.71)	2.73 (1.43–3.79)	45.70 (32.29–65.19)	7.55 (6.20–11.08)	503.54 (386.84–632.87)	41.1 (35.79–49.85)
Default (n = 2)	8.51 (6.51–10.79)	1.82 (1.81–2.09)	40.30 (31.04–74.99)	6.98 (6.22–11.08)	478.37 (406.22–550.52)	41.43 (36.93–42.52)
Lost to follow-up (n = 17)	11.70 (8.27–19.25)	2.80 (1.96–3.67)	33.14 (24.51–48.03)	6.22 (5.11–8.04)	426.0 (370.47–476.98)	39.96 (34.75–45.23)

Abbreviations: AUC, area under the concentration-time curve; eC<sub>max</sub>, estimated maximum concentration; IQR, interquartile range.

In the subgroup analysis of 108 patients in whom sputum smears and cultures were obtained every 2 weeks, 69% were male, with a median (IQR) age of 35 (30–41) years and a median BMI of 19.03 (17.7–21.4) kg/m<sup>2</sup>. Patients with low isoniazid and rifampicin concentrations had a <10% probability of remaining culture positive by the end of follow-up (Figure 3). The median times to sputum

conversion among those with no drug, 1, or >1 drug below the cut-off were 1 (IQR, 1–1), 5 (13–7), and 9 (7–11) weeks, respectively.

Every 1-mg·h/L increment in AUC of rifampicin or isoniazid led to a 2%–4% increase in the likelihood of having sputum conversion (HR, 1.02 [95% CI, 1.01–1.02] *P* = .01] for rifampicin and 1.04 [1.02–1.06; *P* < .001] for isoniazid). Patients

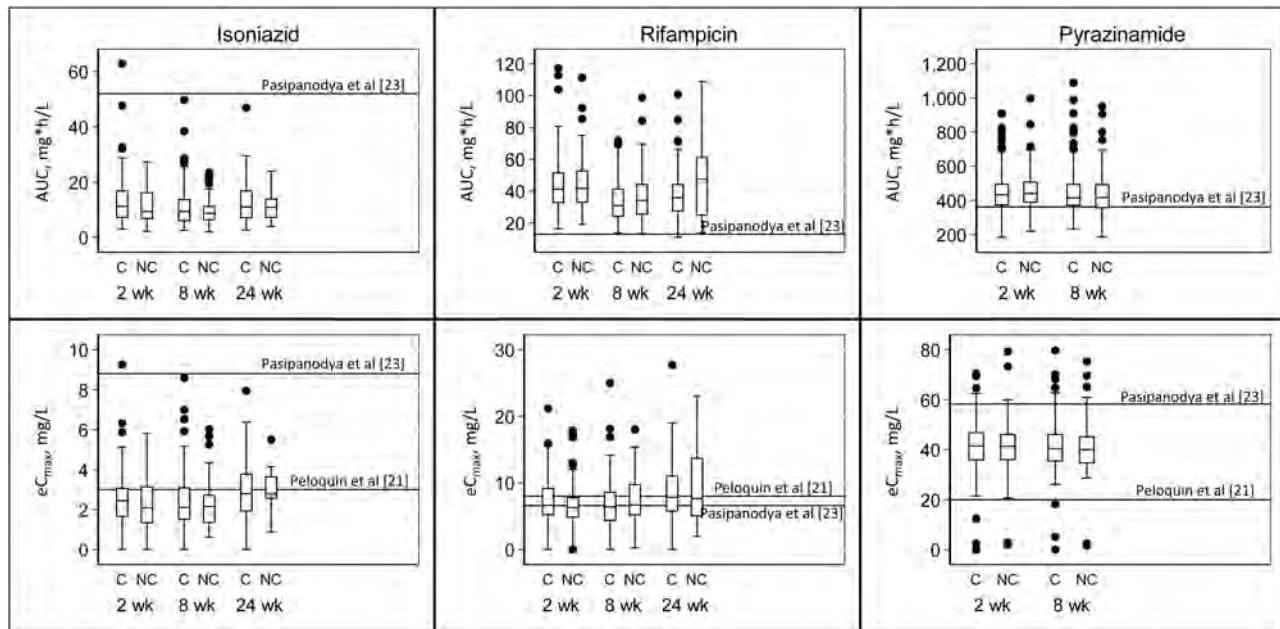
**Table 4. Predictors of Low Maximum Concentrations of Both Rifampicin and Isoniazid and Unfavorable Treatment Outcomes<sup>a</sup>**

Characteristic (N = 227)	Predictors of Low Rifampicin and Isoniazid eC <sub>max</sub>						Predictors of Unfavorable Treatment Outcomes					
	Unadjusted Model			Adjusted Model			Unadjusted Model			Adjusted Model		
	OR	95% CI	<i>P</i> Value	OR	95% CI	<i>P</i> Value	OR	95% CI	<i>P</i> Value	OR	95% CI	<i>P</i> Value
<b>Age, y</b>												
<30 y	Reference	...	...	Reference	...	...	Reference	...	...	Reference	...	...
30–39 y	1.32	.85–2.06	.22	1.29	.82–2.02	.28	0.85	.44–1.67	.64	0.90	.45–1.77	.76
≥40 y	1.04	.62–1.74	.88	1.01	.59–1.72	.97	0.81	.37–1.75	.59	0.88	.41–1.91	.76
Male sex	2.03	1.38–2.98	<.001	1.82	1.18–2.79	.006	0.62	.35–1.10	.10	1.62	.90–2.90	.10
CD4 cell count (per 100 cells/μL) <sup>b</sup>	1.00	.91–1.11	.87	...	...	...	1.10	.96–1.25	.17	...	...	...
<b>BMI</b>												
Underweight	1.19	.81–1.74	.37	1.37	.86–2.18	.19	0.66	.36–1.21	.178	0.59	.32–1.09	.09
Normal	Reference	...	...	Reference	...	...	Reference	...	...	Reference	...	...
Overweight	0.65	.25–1.69	.38	0.67	.26–1.73	.41	1.00	.24–4.22	>.99	0.89	.22–3.61	.87
Obese	0.35	.78–1.58	.17	0.32	.05–1.89	.21	0.51	.05–4.72	.55	0.43	.05–4.13	.47
<b>Weight band</b>												
25–39 kg	1.04	.40–2.76	.92	0.62	.18–2.14	.45	1.28	1.03–1.59	.024	1.35	1.10–1.65	.004
40–54 kg	1.24	.83–1.88	.29	0.88	.54–1.45	.62	1.17	1.05–1.31	.005	1.21	1.08–1.36	.001
55–69 kg	Reference	...	...	...	...	...	Reference	...	...	...	...	...
>70 kg	0.86	.38–1.95	.72	1.46	.58–3.70	.42	0.96	.82–1.12	.60	0.97	.85–1.10	.63
<b>Tertile within weight band</b>												
Lower	0.93	.62–1.38	.70	0.84	.55–1.29	.42	0.98	.92–1.05	.59	...	...	...
Middle	Reference	...	...	Reference	...	...	Reference	...	...	Reference	...	...
Upper	1.33	.87–2.01	.18	1.39	.89–2.18	.15	1.00	.94–1.05	.90	...	...	...
<b>eC<sub>max</sub> below cutoff</b>												
None	...	...	...	...	...	...	Reference	...	...	Reference	...	...
Isoniazid <3 mg/L	...	...	...	...	...	...	1.02	.95–1.10	.55	1.03	.95–1.11	.51
Rifampicin <8 mg/L	...	...	...	...	...	...	1.04	.97–1.11	.25	1.05	.98–1.12	.17
Isoniazid <3 mg/L and rifampicin <8 mg/L	...	...	...	...	...	...	1.07	1.00–1.15	.06	1.09	1.01–1.17	.02

Abbreviations: BMI, body mass index; CI, confidence interval; eC<sub>max</sub>, estimated maximum concentration; OR, odds ratio.

<sup>a</sup>Table depicts logistic regression analysis for predictors of low maximum concentrations for both rifampicin and isoniazid, as well as predictors of unfavorable treatment outcomes, which include failure, death, loss to follow-up, and default.

<sup>b</sup>CD4 cell counts were available for only 188 patients.

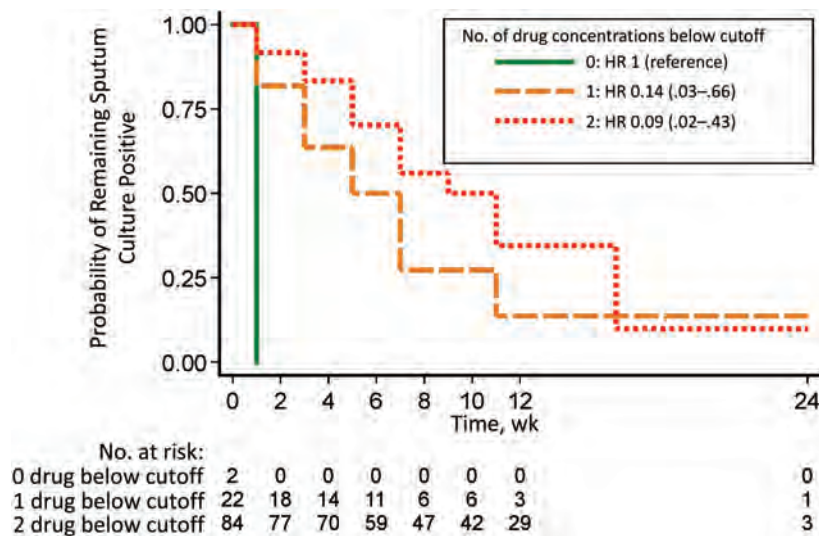


**Figure 2.** Association between concentrations of rifampicin, isoniazid, and pyrazinamide and tuberculosis treatment outcome. Box plots demonstrating area under the concentration-time curve (AUC) and estimated maximum concentrations ( $eC_{max}$ ) for isoniazid, rifampicin, and pyrazinamide at weeks 2, 8 and 24 and stratified by tuberculosis treatment outcome: cured (C) and not cured (NC). The top of each box represents the 75th percentile (Q3); the bottom, the 25th percentile (Q1); and the horizontal line within each box, the 50th percentile (median) concentration. The top whisker represents  $Q3 + (1.5 \times \text{the interquartile range [IQR]})$ , and the bottom whisker,  $Q1 - (1.5 \times \text{IQR})$ ; dots represent outliers.

**Table 5. Cox Proportional Hazards Model Showing Predictors of Sputum Culture Conversion**

Characteristic	Unadjusted HRs			Adjusted HRs		
	HR	95% CI	PValue	HR	95% CI	PValue
Age >40 y	0.79	.54–1.16	.24	0.82	.55–1.21	.31
BMI						
Underweight	Reference	...	...	...	...	...
Normal	1.05	.79–1.40	.74	1.03	.76–1.38	.87
Overweight	1.05	.49–2.29	.90	0.97	.44–2.15	.93
Obese	0.75	.28–2.06	.58	0.75	.26–2.11	.58
Male sex	0.94	.71–1.24	.65	1.07	.80–1.45	.64
No. of drugs below cutoff						
0	Reference	...	...	...	...	...
1	0.42	.22–0.77	.006	0.40	.22–0.74	.003
2	0.27	.15–0.49	<.001	0.26	.14–0.47	<.001
$eC_{max}$						
Isoniazid <3 mg/L	0.54	.37–.77	.001	...	...	...
Rifampicin <8 mg/L	0.61	.44–.84	.003	...	...	...
Pyrazinamide <20 mg/L	0.98	.40–2.38	.97	...	...	...
Ethambutol <2 mg/L	0.78	.58–1.06	.11	...	...	...
Isoniazid AUC quartiles						
<5.7	Reference	...	...	...	...	...
5.7–8.9	1.13	.75–1.69	.56	...	...	...
9.0–13.3	1.29	.87–1.90	.21	...	...	...
>13.4	1.89	1.27–2.81	.002	...	...	...
Rifampicin AUC quartiles						
<25.5	Reference	...	...	...	...	...
25.5–32.3	1.25	.85–1.85	.25	...	...	...
32.4–44.5	1.53	1.02–2.29	.04	...	...	...
>44.6	2.00	1.34–3.00	.001	...	...	...

Abbreviations: AUC, area under the concentration-time curve; BMI, body mass index; CI, confidence interval;  $eC_{max}$ , estimated maximum concentration; HR, hazard ratio.



**Figure 3.** Association between number of drugs below the cutoff and time to sputum culture conversion in 108 patients with intensive sputum culture monitoring. Kaplan-Meier curves for these patients demonstrate the probability of remaining sputum culture positive over time, stratified by the number of drug concentrations below the lower limit of the normal reference range for rifampicin (<8 mg/L) and isoniazid (<3 mg/L). Hazard ratios (HRs) are shown with 95% confidence intervals in parentheses.

in the highest AUC quartile for rifampicin and for isoniazid were approximately twice as likely to experience sputum conversion by the end of follow-up as those in the lowest AUC quartile (Table 5). Using target concentrations for pyrazinamide described by Pasipanodya et al [23], we found no association between the AUC of pyrazinamide and time to sputum conversion (HR, 0.9; 95% CI, .7–1.32;  $P = .86$ ) (Table 3).

#### Tuberculosis Treatment Outcomes

Of the 227 patients, 158 (70%) were cured, 10 (4.4%) had treatment failure, and 17 (7.5%) were withdrawn from the study (Figure 1). All patients lost to follow-up had experienced sputum culture conversion before the end of follow-up. Of those who were withdrawn or transferred out, 20 of 31 (64.5%) had experienced sputum conversion, and 8 (72%) of the 11 patients who died had sputum culture conversion.

Patients with  $eC_{max}$  values below the cutoff for both isoniazid and rifampicin had a moderately increased risk of unfavorable treatment outcomes, including death, failure, loss to follow-up, and default (odds ratio, 1.09; 95% CI, 1.01–1.17;  $P = .02$ ). Those weighing <55 kg were also more likely to have unfavorable treatment outcomes (Table 4).

#### DISCUSSION

We found an association between low concentrations of isoniazid and rifampicin and delayed sputum culture conversion. Our findings are consistent with recent studies, which have demonstrated that higher exposures of rifamycins are associated with faster sputum clearance and a higher proportion of patients with negative cultures after 8 weeks of treatment [24, 25]. Delayed sputum culture conversion may have implications for tuberculosis transmission, especially in settings such as ours in

sub-Saharan Africa, because patients are not isolated during tuberculosis treatment.

Others have also reported the association between drug concentrations and sputum conversion. In a retrospective study in predominantly HIV-uninfected participants [26] and a prospective study in which >50% of participants were HIV infected [27], patients with a low isoniazid  $eC_{max}$  were more likely to remain culture positive at week 8. Contrary to this finding, Chang and colleagues [15] and van Crevel and colleagues [16] found no association between  $C_{max}$  of rifampicin and sputum culture conversion. The conflicting evidence may be attributed to the variability in study population and study design, including different sampling time points. In addition, when correlating concentrations with treatment response, the fact that drug concentrations in blood may not be reflective of concentrations at the site of activity (eg, lung tissue) should be taken into account.

Despite the high proportion of patients with low isoniazid and rifampicin concentrations, few patients had tuberculosis treatment failure (4.4%). However, low concentrations of both isoniazid and rifampicin were associated with unfavorable outcomes, which included death, failure, loss to follow-up, and default. Other prospective observational studies reported an association between low concentrations of pyrazinamide [28], isoniazid [27, 29], and rifampicin [29] and unfavorable tuberculosis treatment outcome. Although patients in the lower weight bands were not at risk of lower drug concentrations and actually received a relatively higher dose per kilogram than those in the higher weight bands (who were slightly underdosed), they had worse outcomes. This finding may be attributed to several factors, including the fact that patients in lower weight band may be sicker than those in higher weight bands.

We evaluated other thresholds, including those proposed by Pasipanodya et al [23]. The proposed targets for isoniazid were achieved in almost none of our participants, whereas all of them had AUCs above the proposed targets for rifampicin. Although the target AUC for pyrazinamide was achieved in 74% of our participants, only 21% achieved this target in a study by Alsultan et al [30]. These proposed thresholds still need to be evaluated prospectively. Different thresholds for rifampicin (<3.01 mg/L) and pyrazinamide (<38.1 mg/L) were associated with treatment failure and death in children with mostly extrapulmonary tuberculosis. Therefore, the site of tuberculosis may contribute to the difference in study findings and high variability in pharmacokinetic parameters [31].

The limitations of our study include our use of the total drug concentrations, and yet it is the free drug that is pharmacologically active. The AUC/minimum inhibitory concentration (MIC) and  $C_{max}/MIC$  have been shown by others to be useful predictors of culture conversion [32, 33]. Lack of MIC data limits our ability to further evaluate pharmacokinetic/pharmacodynamic markers of sputum conversion and tuberculosis treatment outcomes. Sturkenboom and colleagues [34] described variability in the  $C_{max}$  of rifampicin (0.4–5.7 hours), and several studies have demonstrated variability in the  $C_{max}$  of isoniazid based on N-acetyltransferase polymorphisms; therefore, sampling up to 4 hours after dosing may have limited the accuracy of the pharmacokinetic parameter estimates [34, 35]. We did not evaluate the effect of drug concentrations on relapse, which has been associated with low pyrazinamide concentrations [23, 28].

The merits of our study include its large sample size, its prospective design with a large body of clinical data, and the pharmacokinetic sampling done on 3 occasions (at 2, 8, and 24 weeks) and with 3 time points (1, 2, and 4 hours), which enabled us to have a close approximation of the  $eC_{max}$  and develop models for AUCs. With intensive monitoring of sputum cultures we were able to give a more accurate estimate of the time to sputum conversion in a subset of the population. In conclusion, although low isoniazid and rifampicin concentrations in this HIV-tuberculosis-coinfected population did not translate into high proportions of patients with treatment failure, the association between low concentrations of isoniazid and rifampicin and prolonged sputum culture positivity has implications for tuberculosis transmission and warrants further investigation.

### Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

### Notes

**Author contributions.** M. L., P. B. K., M. J., Y. C. M., N. C., C. S. W., and J. S. F. contributed to the conceptualization of this protocol. C. S. W. and A. v. B. contributed equally to writing this article. C. S. W., A. v. B., A. B., L. H., J. Ma., U. G., N. E., and D. M. contributed to the data collection, C. S.

W., B. L., P. D., A. J., J. Mu., and M. R. K. contributed to the data management and analysis, M. d. K., A. K., B. C., and J. S. F. supervised the writing of this article, and all authors read and approved the final version.

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