Pharmacokinetics of first-line drugs in children with tuberculosis using WHO-recommended weight band doses and formulations

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Summary

We evaluated pharmacokinetics of antituberculosis drugs administered as fixed-dose-combinations
tablets using WHO weight-band dosing tables. Exposures were low for rifampicin and isoniazid in
weight-bands 4.0 <8kg and ≥25kg, rifampicin in the 8kg-12kg weight-band and across all weight-
bands for ethambutol.

ABSTRACT

Background: Dispersible paediatric fixed dose combination (FDCs) tablets delivering higher doses of first-line antituberculosis drugs in WHO-recommended weight-bands were introduced in 2015. We report the first pharmacokinetic data for these FDCs in Zambian and South African children in the treatment-shortening SHINE trial.

Methods: Children weighing 4.0-7.9 kg, 8.0-11.9 kg, 12.0-15.9 kg and 16.0-24.9 kg had 1, 2, 3 and 4 tablets daily (rifampicin/isoniazid/pyrazinamide 75/50/150 mg, with or without 100 mg ethambutol, or rifampicin/isoniazid 75/50 mg), respectively. Children 25.0-36.9 kg received doses recommended for adults <37kg (300, 150, 800, 550 mg daily for rifampicin, isoniazid, pyrazinamide, ethambutol). Pharmacokinetics were evaluated after at least 2 weeks of treatment.

Results: Of 77 children evaluated, median (IQR) age was 3.7 (1.4-6.6) years, 40 (52%) were male and 20 (26%) HIV-positive. AUC_{24} for rifampicin, isoniazid, pyrazinamide and ethambutol were 32.5 (20.1-45.1), 16.7 (9.2 - 25.9), 317 (263 - 399) and 9.5 (7.5 – 11.5) mg.h/L, respectively, and lower in children compared to adults for rifampicin in 4.0-7.9 kg, 8-11.9kg and ≥25kg weight-bands, isoniazid in 4.0-7.9kg and ≥25kg, and ethambutol in all five weight-bands. Pyrazinamide exposures were similar to adults.

Conclusions: Recommended weight-band based FDC doses result in lower drug exposures in children in lower weight-bands and in those ≥25kg (on adult doses). Further adjustments to current doses are needed to match current target exposures in adults. The use of ethambutol at the current WHO-recommended doses requires further evaluation.

Keywords: pharmacokinetics; tuberculosis; antituberculosis drugs; children; dosing.

INTRODUCTION

Tuberculosis treatment regimens in most low-and-middle income countries is standard based on World Health Organization (WHO) recommendations and delivered by national programmes in the public sector. Ensuring optimal treatment is integral to the global strategy to end childhood tuberculosis [1].

Historically, paediatric doses of the first-line antituberculosis drugs were extrapolated from adult doses, employing the same milligram per kilogram of body weight doses. Informed by pharmacokinetic studies demonstrating that this approach does not achieve comparable drug exposures in children[2-5], the WHO revised these recommendations for children weighing less than 25kg in 2010, increasing the daily doses of isoniazid (H) by 100% to 10 (range 7-15) mg/kg, rifampicin (R) by 50% to 15 (range 10-20) mg/kg, and pyrazinamide (Z) to 35 (range 30-40) mg/kg. It was envisaged that using the revised doses, exposures in children would approximate those in adults. Ethambutol (E) doses were unchanged at 20 (range 15-25) mg/kg/day [6, 7], a dose thought to carry minimal risks of ocular toxicity for children [8, 9].

Implementation of the revised first line drug doses was initially challenging as the fixed dose combination (FDC) tablets available at the time did not deliver the revised drug ratios [10]. New child-friendly dispersible FDC tablets of RHZ 75/50/150 mg and RH 75/50 mg became available in 2015 and following prequalification by WHO, have been rolled out globally. These new FDCs are water-dispersible, scored, palatable, and easy to administer[11].

Serum concentrations of antituberculosis drugs predict tuberculosis treatment response and have been reported as surrogate markers for predicting therapeutic success[12, 13]. Hollow fibre models and dose fractionation studies show area under the curve (AUC) to be associated with efficacy of antituberculosis drugs[14, 15]. AUC and serum peak concentration (C_{max}) are closely correlated, and emerging results from pharmacokinetic studies evaluating the revised dosing in children report C_{max}

values below the adult reference values[16], and AUC values lower than those reported in adults, particularly for rifampicin and ethambutol[17-19].

Pharmacokinetic measures of antituberculosis drug exposure vary considerably between study populations. Body size, nutritional status, HIV infection and developing enzyme maturation functions are sources of pharmacokinetic variability in children [20, 21]. *NAT2* acetylator genotype is a key determinant of isoniazid concentrations, and *SLCO1B1* polymorphisms have been associated with rifampicin exposures [4, 5, 22-25]. Other important factors include the type of formulation, dose preparation and administration, drug-drug interactions, and laboratory assay methods used [20, 21].

The revised WHO weight-band dosing, using dispersible child-friendly FDCs, simplifies TB treatment and programmatic implementation but supporting pharmacokinetic evidence in children is lacking. We describe the pharmacokinetics at steady-state in children dosed with this approach in the SHINE trial and sought to identify predictors of exposures of first-line antituberculosis drugs.

METHODS

Study population and design

This pharmacokinetic study was nested in the phase III treatment shortening SHINE trial (SRCTN63579542), a randomised-controlled trial comparing a 4-month vs. standard 6-month antituberculosis drug regimen using revised WHO paediatric weight-band dosing and new FDCs in children with and without HIV. SHINE recruited children aged 0-16 years with non-severe tuberculosis in Zambia, South Africa, Uganda, and India. Non-severe tuberculosis was defined as smear-negative tuberculosis including pulmonary disease confined to one lobe without cavities, intra-thoracic lymph node tuberculosis without significant airway obstruction, and extra-thoracic TB lymphadenitis. Screening, recruitment, clinical care and follow-up procedures are described elsewhere [26]. A subset of children enrolled in the trial were selected consecutively to participate in pharmacokinetic sub-studies. Here we report on African children enrolled in Zambia and in South Africa.

Drugs and dosages

Antituberculosis drugs were administered in weight bands according to the WHO 2015 dosing recommendations[11]. Dispersible paediatric or adult FDC tablets (Macleods Pharmaceuticals) were used. For the 2-month intensive phase, RHZ 75/50/150 mg dispersible tablets with or without ethambutol 100 mg tablets, were administered in four weight bands to children <25 kg. Children ≥25 kg received weight-band based adult doses using RHZE 150/75/400/275 mg tablets [27]. In the continuation phase, RH 75/50 mg tablets for children <24.9 kg and RH 150/75 mg tablets for those ≥25kg were used (table 1). Ethambutol use was guided by local TB treatment recommendations at the time. In South Africa it was indicated for if the child was HIV positive or above 8 years of age[28]. In Zambia all children received ethambutol regardless of age, disease severity or HIV status[29]. Daily drug administration was supervised by the caregiver or parent and drug intake documented on

treatment cards provided by the trial. Children living with HIV initiated antiretroviral therapy (ART) in

accordance with national guidelines.

Pharmacokinetic sampling and laboratory analysis

Intensive pharmacokinetic sampling was scheduled after at least 2 weeks of antituberculosis

treatment. Caregivers were reminded by phone to administer the antituberculosis drugs in the

morning (if evening dosing was preferred by caregiver) for at least a week before the

pharmacokinetic sampling visit. On the sampling day, drug intake was observed by research staff

after an overnight fast, and breakfast was provided at least 2 hours after drug intake, unless the

child was distressed, in which case a snack was permitted. The FDC tablets were dispersed in water

or administered whole, in keeping with the practice at home, while children receiving the adult

formulation swallowed tablets whole.

Serial venous blood samples were obtained pre-dose, and at 1, 2, 4, 6, 8 and 12 hours after drug

intake. Samples were immediately placed on ice before centrifugation within 30 minutes of

collection. Separated plasma samples were stored at -80°C until transportation on dry ice for

analysis at the Pharmacology laboratory, University of Cape Town, South Africa. Drug concentrations

were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays

validated over concentration ranges of 0.117 to 30.0 mg/L for rifampicin, 0.105 to 25.0 mg/L for

isoniazid, 0.200 to 80.0 mg/L for pyrazinamide, and 0.0844 to 5.46 mg/L for ethambutol [18, 19]

according to Food Drug Administration and European Medicines Agency guidelines. The accuracies

(%Nom) of the lower limit of quantification, low, medium, and high-quality controls were between

101% and 107%, 92% and 105%, 101% and 104%, 97% and 107% for rifampicin, isoniazid,

pyrazinamide, and ethambutol, respectively. The precision (%CV) was below 11% for all analytes

during inter- and intra-day validation. External quality control samples were provided by the

University of Nijmegen, Netherlands.

Pharmacokinetic and statistical analysis

Drug concentrations below the lower limit of quantification were imputed by halving the lower limit of quantification (LLQ) for the respective drug. Stata version 16.1 (StataCorp, College Station, Texas, USA) was used to compute the noncompartmental pharmacokinetic measures (including peak concentration [C_{max}], half-life, time to C_{max} and elimination rate constant), and for statistical tests and for regression analyses. The 24-hr concentration for each participant was imputed using a regression equation obtained by regressing log-transformed concentration measurements in the terminal phase of the pharmacokinetic curve against the time of sample. The AUC_{24} was derived using the linear-log trapezoid rule and summarized by weight band for each drug. For rifampicin, the reference AUC_{24} was equal to or greater than the mean AUC_{24} (38.7 mg.h/L) derived from a meta-analysis of adult studies by Stott *et al*. [22]. AUC_{24} median ranges reported for studies included in a systematic review by Daskapan *et al.* were used for isoniazid (11.6-26.3 mg.h/L, excluding one study with outlying results [30]), pyrazinamide (233-429 mg.h/L) and ethambutol (16-28 mg.h/L) [23]. Normal values as described by Alsultan *et al*. were used for the C_{max} reference ranges: rifampicin 8-24 mg/L, isoniazid 3-6 mg/L, pyrazinamide 20-60 mg and ethambutol 2-6 mg/L [16].

Quantile regression was used to evaluate covariate effects on AUC_{24}, after adjusting for the effect of weight-band. HIV status, sex, study site and weight-for-age Z score (WAZ) and weight-for-height Z-score (WHZ), were each tested for their effect on the AUC_{24}, for each drug, in bi-variable models including weight band. All covariates with a p-value <0.2 in the bivariate models were retained in the final model. Drug doses in mg/kg, age, mid-upper arm circumference (MUAC) and the mode of drug administration (dispersed in water, swallowed whole, or other) were not included in these models as they were strongly correlated with weight band.

Ethical and regulatory approvals

The SHINE trial including the pharmacokinetic substudy received regulatory and ethical approvals in Zambia and South Africa. Signed informed consent was obtained from parents/carers for this pharmacokinetic sub-study.

RESULTS

Seventy-seven children (43 Zambian, 34 South African) underwent intensive pharmacokinetic sampling. Their median age was 3.7 (IQR 1.4-6.6) years, 40 (52%) were male and 20 (26%) were living with HIV, with 18 on ART (15 receiving efavirenz- and 3 lopinavir-ritonavir-based regimens) at the time of sampling (after a median [IQR] 7 (6 -19) weeks on antituberculosis treatment). Patient and treatment characteristics are summarised in table 2. All but 2 infants reported an overnight fast before the intensive pharmacokinetic sampling. Most children received dispersible paediatric FDCs (n=63, 82%); 14 (18%) of children ≥25kg received adult FDCs).

All 77 children had samples analysed for rifampicin and isoniazid, while 45 children sampled during the intensive phase of treatment contributed pyrazinamide concentrations. Ethambutol was measured in 22 children (all from Zambia) who received it as part of their regimen.

The median AUC_{24} (IQR) for rifampicin was 32.5 (20.1 - 45.1) mg.h/L. Most children in the 4.0-7.9 kg, 8-11.9 and ≥25.0 kg weight-bands had exposures below the adult reference (tables 3, 4 and figure 1). Regression analysis of factors affecting the AUC_{24} after adjusting for weight band showed a trend to lower exposures with HIV infection (-10.6 mg/h/L, 95% CI -21.9, 0.7; p=0.07) (table 5). Median C_{max} (IQR) was 7.6 (4.9 – 11.4) mg/L (tables 3 and S1) with 40/77 (52%) of the children failing to attain a C_{max} of 8 mg/L, the lower limit of the reference range (Figure 2).

The isoniazid pharmacokinetic profile for one child was not analyzable and was excluded. Median AUC_{24} (IQR) was 16.7 (9.2-25.9) mg.hr/L, within the adult reference range (tables 3 and 4, Figure 1). Children ≥25.0 kg receiving adult formulations had a median of AUC_{24} 5.8 mg.h/L, about half the lowest study median of the adult target range. Low AUC_{24} were also observed in the extreme weight-bands (4.0-7.9 kg and ≥25 kg) compared to adult references. Sex, HIV-status, and anthropometric measures were not associated with AUC_{24} after adjusting for weight band (table 5). Isoniazid median C_{max} (IQR) was 5.1 (2.8 – 7.6) mg/L. C_{max} was below the reference range for 22/76 (29%) children, while 31(41%) had C_{max} higher than 6 mg/L (table 3, table S1, figure 2 and supplementary figure S2).

The median AUC_{24} (IQR) for pyrazinamide was 317 (263 - 399) mg.h/L (table 3) and within the adult reference range across the weight-bands (tables 3, 4; figure 1). AUC_{24} was not significantly associated with sex, HIV-status or anthropometric measures when adjusted for weight band (table 5). The median C_{max} (IQR) was 33.0 (25.9 – 43.1) mg/L with 40/45(89%) of children within the reference range (tables 3, S1 and figure 2).

Ethambutol AUC_{24} (IQR) was 9.5 (7.5 – 11.5) mg.h/L with the median AUC_{24} well below the adult reference range for all weight bands (table 3, 4 and figure 1). Low WAZ (<-2 z-score) was associated with lower AUC_{24} when adjusted for weight-band (table 5). Ethambutol median C_{max} was 1.6 (0.91 – 2.0) mg/L with 16/22 (73%) children having values below the recommended reference range (tables 3, S1 and figure 2).

For all the drugs, exposures increased with increasing weight band, except for children ≥25 kg (Figure 1), and C_{max} and AUC_{24} were strongly correlated (rifampicin r =0.87, p<0.01; isoniazid r=0.79, p<0.01; pyrazinamide r =0.87, p<0.01; ethambutol r =0.88, p<0.01) (figure S1).

DISCUSSION

This is the first pharmacokinetic study to assess the WHO's weight band-based dosing using child-friendly paediatric FDCs that are now widely available in low and middle-income countries as the preferred formulations for young children. We found that rifampicin exposures were low; in the lowest weight band (4.0-7.9 kg), values were around half of those observed in adults and were also low in the 8.0-11.9kg weight band. Ethambutol exposures were low in all weight bands. Exposures of all the drugs increased with weight band, except for children ≥25 kg on adult doses, who had very low rifampicin, isoniazid and ethambutol AUC_{24}. Only 48% and 27% of the children achieved peak concentrations above the lower limit of the recommended adult ranges for rifampicin and ethambutol compared with 70% and 89% for isoniazid and pyrazinamide, respectively.

Our findings are consistent with other studies in children treated with the revised WHO doses who did not achieve recommended concentrations of rifampicin [17-19, 31, 32] and ethambutol [17-19, 32]but were adequate for isoniazid and pyrazinamide [17-19]. In contrast to these studies, we used the child-friendly FDCs with rifampicin to isoniazid ratio of 3:2, currently recommended by the WHO. Our results suggest that higher mg/kg doses should be used in smaller children to achieve current adult drug exposure targets. Lower mg/kg exposures in the 4.0-7.9 kg weight band could be partly because most children weighed near the upper end of the weight band. However, similar observations in a study of Malawian and South African children support our finding that drug concentrations are low in children weighing <8 kg, except in infants under 3 months who have immature metabolic pathways [33]. Young children are most vulnerable to severe forms of TB and may have worse treatment outcomes than older children [34]. Under current dosing guidelines, the smallest children have the lowest drug exposures, and this might be critical in those with severe or extensive disease, including in children with disseminated TB. We also showed low drug exposures in children 25-36.9 kg who receive lower mg/kg of rifampicin and isoniazid dose compared to children weighing <25 kg. These results support proposals to increase the first-line antituberculosis

drug doses currently recommended for adults weighing <55 kg using HRZE 150/75/400/275mg FDC [20].

The SHINE trial results showed that the 4-month regimen was non-inferior to 6 months of treatment, with excellent treatment outcomes in children with non-severe tuberculosis across the randomization arms. In the 1204 children enrolled, unfavourable outcomes were few (7% in intention-to treat population including treatment failure, TB recurrence, lost to follow-up and all-cause mortality), and only 17 grade 3 or more treatment-related adverse events were reported, of which 11 were raised liver enzymes and 10 led to treatment interruption or discontinuation[35] . Notably, SHINE did not include children with severe or extensive disease. Optimised dosing may further improve TB treatment outcomes in children across all severity spectrum of TB disease.

The reference AUC_{24} used for rifampicin should be regarded as a minimum target for the average exposure in each of the paediatric weight bands. The reference is based on the mean AUC_{24} (38.73 mg.h/L) derived by Stott *et al*. in a meta-analysis of pharmacokinetic studies that provides the most comprehensive assessment of exposures in adults [22]. The corresponding mean C_{max} of 5.79 mg/L is well below the widely applied recommended range for C_{max} (8-20 mg/L) on standard treatment [16].

There is growing interest in the use of high-dose rifampicin. Preliminary studies in adults with drug-susceptible tuberculosis suggest that rifampicin doses as high as 35 mg/kg are tolerated well, improved antituberculosis activity and could potentially lead to treatment shortening[36, 37]. Establishing paediatric rifampicin doses that would match the exposures observed in adults dosed at 35 mg/kg is currently under evaluation in the OptiRif study[38]. With optimised doses, it is possible that treatment shortening, shown to be effective, feasible and safe in children with non-severe TB in SHINE, could also be achieved in children with other forms of TB, including those with severe or extensive tuberculosis disease.

Except for the lowest and highest weight bands, adequate isoniazid exposures were in keeping with recent studies evaluating the revised doses [17-19]. One Indian study reported higher than normal AUC and C_{max} in children dosed at 10 mg/kg. In SHINE the pharmacokinetics of the new FDCs in Indian children will be analysed, once the validation process of the assays used in Indian sites is completed. Further pharmacogenomic studies are planned to evaluate impact of slow acetylator status.

For pyrazinamide, the finding of levels comparable to adults is reassuring and consistent with other studies[17, 18, 31].

Ethambutol was used in only a third of the children in this study based on local guidelines. In keeping with other studies in children[17-19, 31], we found low AUC_{24} and C_{max} across all weight bands. With such low systemic exposures, whether ethambutol prevents the development of resistance to other drugs in circumstances of primary isoniazid resistance is uncertain. The fact that, optic neuritis is rarely observed in children may be partly due to low ethambutol exposures[9]. No clinically significant ocular toxicity was reported in the SHINE trial which employed colour vision testing in children aged ≥3 years[39]. The risk of ocular toxicity is dose-dependent, and if the higher doses were used, the risk of ocular toxicity would need to be re-evaluated[9].

The considerable variation in drug exposure by weight band in our study was in part due to the assumption that uniform mg/kg doses are required regardless of body size. Allometric scaling is increasingly used to estimate the higher mg/kg requirements of smaller children to avoid systematic underdosing [40]. There is a wide range of mg/kg doses within a weight-band, most notably in the lower weight-bands, resulting in additional variability[33]. In addition, immaturity of phase I/II drug metabolizing enzymes, leads to higher drug exposures in young infants particularly below the age of 3 months [24, 33, 41]. Due to high correlation of mg/kg dose with weight band, we did not evaluate

the separate impact of mg/kg dose on drug exposure. The average exposure in each weight band is therefore dependent on the distribution of the participants' body weights which may or may not accurately represent the weight distribution of children treated for tuberculosis in other settings. We did not confirm an association between HIV co-infection status and lower antituberculosis drug concentrations[21]. Poor nutritional status has been linked to lower drug concentrations [18, 32, 42]. Except for the association between WAZ score and ethambutol AUC_{24}, we did not find significant associations between the drug exposures and anthropometric measurements or study site. Population pharmacokinetics modelling is planned while genetic polymorphisms have not been assessed in this study.

A growing literature on optimal TB drug exposures based on pharmacokinetic-pharmacodynamic studies, suggest alternative targets in many instances [12, 16, 20, 43]. However, until these are validated as optimal as part of combination treatment, target ranges based on the exposures encountered in adults on standard doses is an accepted approach. Our study was not designed to evaluate whether disease severity affects pharmacokinetics. A recent study found significantly lower antituberculosis drug exposures in adults with severe TB-HIV disease while another found no important pharmacokinetic differences between hospitalized patients and their ambulatory counterparts [44, 45].

In the context of standardized dosing of the antituberculosis drugs for children, drug exposures should match those considered optimal in adults. The revised WHO 2010 recommendations result in improved antituberculosis drug exposures in children, but target exposures are still not achieved across all weight bands. Of particular concern are the relatively low rifampicin exposures in the extreme range weight bands. The role of ethambutol in first-line antituberculosis treatment in children should be investigated as the contribution of ethambutol at the very low exposures found in children with currently used doses is uncertain.

NOTES

Author's contributions

CC and HM prepared the manuscript and coordinated the writing of the paper. CC, HM, and MC analyzed and interpreted the pharmacokinetic analysis. LW supervised the laboratory analysis of the drug assays. HM, RA, AT and DG designed the study and made key revisions to the draft manuscripts. ACH, KZ, MK, VM, MP and MvZ implemented the pharmacokinetic study at the SHINE sites in Zambia and South Africa. All authors reviewed the manuscript for intellectual content and approved the final version of the report.

Trial drugs were manufactured by Macleods Pharmaceuticals Ltd., The funders, the sponsor

(University College, London) and the drug manufacturer had no role in the study design, analysis or

reporting of the findings. ACH reports funding to participate as site in the SHINE trial; contract

through MRC CTU at UCL; prime grant award from BMRC/Wellcome/DFIF; grant award made to

Stellenbosch University, approximately GBP 650 000 over 4 years. AT, KL, LC, and DG report COVID

19 Grant Extension Allocation Award: 181573 from UKRI. VM reports grants or contracts with the

NIH outside of the submitted work.

Competing interests

All authors declare no competing interests

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Figure legends

Figure 1: Rifampicin, isoniazid, pyrazinamide and ethambutol AUC24 boxplots by weight band in children treated for tuberculosis.

Footnote: The horizontal reference lines represent target exposures derived from adult studies. For rifampicin, the estimate AUC24 (38.73 mg.h/L) are derived from a systematic review and meta-analysis by Stott et al. (22). For isoniazid, pyrazinamide and ethambutol, ranges of 11.6-26.3 μg.h/ml, 233-429μg.h/ml and 16-28 μg.h/ml represent the respective ranges of the medians from studies in a systematic review by Daskapan et al.(23).

Figure 2: Rifampicin, isoniazid, pyrazinamide and ethambutol Cmax boxplots by weight band in children treated for tuberculosis.

Footnote: Target reference ranges for Cmax recommended by Alsultan et al. (16); isoniazid 3–6 mg/L, rifampin 8–24 mg/L, pyrazinamide 20–60 mg/L, and ethambutol 2 - 6 mg/L

Table 1: Daily doses of antituberculosis tablets used in children in the SHINE trial based on WHO recommendations

Paediatric dispersible formulations for children weighing 4-24.5kg		
	Intensive Phase	**Continuation Phase**
Weight bands	*HRZ 50/75/150 mg FDC*	*E 100 mg*	*HR 50/75 mg FDC*
4.0-7.9kg	1	1	1
8.0-11.9kg	2	2	2
12.0-15.9kg	3	3	3
16.0-24.9kg	4	4	4
Adult formulation and doses used for children weighing ≥ 25.0kg		
	Intensive phase	**Continuation phase**
Weight-bands	*HRZE 75/150/400/275 mg FDC*	*HR 75/150 mg FDC*
25-36.9kg	2	2

FDC=Fixed dose combination tablet, R=Rifampicin, H=Isoniazid, Z=Pyrazinamide, E=Ethambutol

Table 2. Summary of participant characteristics (N=77) at time of pharmacokinetic sampling by weight band in children treated for tuberculosis.

	4.0-7.9 kg	8.0-11.9 kg	12.0-15.9 kg	16-0-24.9 kg	≥25-36.9 kg	All
N	16	14	16	16	15	77
Age, years (median, IQR)	0.6 (0.4 - 0.8)	1.4 (1.2 - 2.2)	3.7 (2.4 - 4.6)	5.8 (5.5 - 6.7)	11.3 (10.4 - 12.1)	3.7 (1.4 - 6.6)
Males, n	8	7	9	5	11	40 (52%)
HIV positive, n	4	2	5	3	6	20 (26%)
Anthropometric measurements						
Weight, kg (median, IQR)	7.1 (6.8 - 7.7)	9.1 (8.7 - 10.2)	14.0 (12.8 - 14.6)	18.7 (16.6 - 20.6)	28.5 (28.3 - 33.7)	14.0 (8.7 – 20.6)
WAZ	-1.6 (-2.3 - 0.0)	-2.2 (-3.0 - -1.6)	-1.3 (-2.5 - -0.4)	-1.0 (-2.1 – 0.2)	-1.4 (-2.1 - -0.6)	-1.5 (-2.3 - -0.4)
WHZ	0.4 (-0.9 – 1.3)	-0.8 (-1.8 - -0.4)	-0.6 (-0.7 – 1.4)	0.2 (-1.2 – 0.6)	-	-0.2 (-1.2 – 0.8)
MUAC, cm	13.9 (12.5 - 14.3)	14.3 (13.0 - 14.8)	15.5 (14.7 - 16.5)	16.9 (15.7 - 18.1)	18.5 (17.8 - 20.4)	15.3 (14.0 - 17.8)
Duration on TB treatment, weeks	6 (5 - 8)	6 (5 - 7)	14 (7 - 20)	16 (6 - 23)	14 (5 – 14)	7 (6 – 19)
Mode of drug administration						
Dispersed, n	14	11	7	7	1	40 (52%)
Taken whole to mouth, n	1	2	8	9	14	34 (44%)
Other*, n	1	1	1	0	0	3 (4%)
Dosage of anti-TB drugs (median IQR)						
Rifampicin, mg/kg	10.7 (9.8-12.2)	16.4 (15.4-16.7)	16.1 (15.4-17.2)	15.8 (14.5-17.8)	10.3 (8.8-10.7)	14.6 (10.6-16.9)
Isoniazid, mg/kg	7.1 (6.5-8.1)	10.9 (9.8-11.5)	10.7 (10.3-11.7)	10.6 (9.6-11.8)	5.1 (4.4-5.3)	9.7 (6.5 - 11.1)
Pyrazinamide, mg/kg	21.4 (19.5-23.4)	32.3 (26.8-33.7)	31.7 (31.3 – 32.4)	35.1 (26.2-36.1)	28.2 (23.4-28.3)	28.2 (22.7 – 32.3)

| Ethambutol, mg/kg | 14.5 (14.1-15.6) | 18.7 (17.0-21.7) | 21.0 (20.0-22.3) | 23.4 (17.1-25.0) | 17.8 (16.1-19.4) | 18.5 (15.2-21.7) |

WAZ=Weight-for-age z-score, WHZ=Weight-for-age z-score, MUAC=mid-upper- arm circumference

*Two children received drugs by nasogastric tube, for one the mode of administration was not specified

Table 3: Summary of pharmacokinetic parameters for rifampicin, isoniazid, pyrazinamide and ethambutol in children treated for tuberculosis (N=77)

	Rifampicin	Isoniazid	Pyrazinamide	Ethambutol
	n=77	n=76	n=45	n=22
AUC_{24} (mg.hr/L) (IQR)	32.5 (20.1 - 45.1)	16.7 (9.2 - 25.9)	317 (263 - 399)	9.5 (7.5 – 11.5)
C_{max} (mg/L)	7.6 (4.9 – 11.4)	5.1 (2.8 – 7.7)	33.0 (25.9 – 43.1)	1.6 (0.9 – 2.0)
t½ (h)	1.7 (1.5 – 2.3)	3.2 (2.6 – 4.2)	6.3 (5.6 – 7.5)	4.4 (3.3 – 5.7)
t_{max} (h)	2 (1 – 2)	1 (1 – 2)	1 (1 – 2)	2 (2 – 4)
K_e (h^{-1})	0.40 (0.30 – 0.47)	0.22 (0.16 – 0.26)	0.11 (0.09 – 0.12)	0.16 (0.12-0.21)

IQR=Interquartile range, AUC_{24}=area under the concentration-time curve from 0 to 24 h, C_{max}=maximum plasma concentration. T_{max}=time to maximum plasma concentration, $T_{1/2}$=elimination half-life. K_e=elimination rate constant.

The following AUC_{24} reference values were used: For rifampicin, the estimate AUC_{24} (38.73 mg.h/L) are derived from a systematic review and meta-analysis by Stott et al. (22). For isoniazid, pyrazinamide and ethambutol, ranges of 11.6-26.3 µg.h/ml, 233-429µg.h/ml and 16-28 µg.h/ml represent the respective ranges of the medians from studies in a systematic review by Daskapan et al. (23). The target reference ranges for C_{max} recommended by Alsultan *et al* [16]: rifampicin 8-24mg/L, isoniazid 3-6mg/L, pyrazinamide 20-60mg and ethambutol 2-6mg/L.

Table 4: Median (IQR) area under the concentration-time curve (AUC$_{24}$) for rifampicin, isoniazid, pyrazinamide and ethambutol summarized by weight band in children treated for tuberculosis

Weight band, (kg)	Rifampicin (N=77)		Isoniazid (N=76)		Pyrazinamide (N=45)		Ethambutol (N=22)	
	n	AUC$_{24}$, (mg.h/L)	n	AUC$_{24}$, (mg.h/L)	n	AUC$_{24}$, (mg.h/L)	n	AUC$_{24}$, (mg.h/L)
4-7.9	16	20.1 (15.2 - 34.6)	16	11.9 (8.4 – 22.4)	14	242 (174 - 299)	7	7.6 (7.1 – 8.6)
8-11.9	14	28.3(23.4- 40.3)	14	20.9 (16.5 – 28.5)	11	322 (247 – 490)	6	6.9 (4.7 – 11.0)
12-15.9	16	42.0 (27.0 - 54.2)	16	21.0 (17.5 – 33.3)	7	385 (313 – 490)	4	11.6 (10.5 – 13.0)
16-24.9	16	49.8 (34.3 – 70.3)	15	21.5 (14.1 – 32.8)	7	434 (315 – 454)	3	11.7 (11.5 – 16.9)
≥25	15	21.6 (12.4 – 32.8)	15	5.8 (3.5 – 8.8)	6	339 (293 - 369)	2	10.4 (9.2 – 11.5)

IQR=Interquartile range, AUC$_{24}$=area under the concentration-time curve from 0 to 24 h

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Table 5: Rifampicin, isoniazid, pyrazinamide and ethambutol AUC$_{24}$ by patient characteristics in children treated for tuberculosis

| Variable		Number evaluated	Rifampicin		Isoniazid		Pyrazinamide		Ethambutol	
			Coeff (95% CI)	p-value	Coeff	p-value	Coeff	p-value	Coeff	P-value
Sex	Male	40	ref	-	ref	-	ref	-	ref	-
	Female	37	0.8 (-9.3, 10.9)	0.88	1.2 (-4.4, 6.8)	0.67	20.2 (-63.0, 103)	0.63	-1.1 (-5.7, 3.5)	0.62
HIV status	Negative	47	ref	-	ref	-	ref	-	ref	-
	Positive	20	-10.6(-21.9, 0.7)	0.07	-3.9 (-9.7, 1.9)	0.18	-4.8 (226.5,216.9)	0.97	-0.9 (-9.9, 8.1)	0.83
WAZ	<-2	29	ref	-	ref	-	ref	-	ref	-
	>=2	48	3.2 (-8.7, 15.1)	0.59	-1.0 (-7.1, 5.1)	0.75	23.9 (-71.2, 119)	0.61	4.2 (0.2, 8.2)	0.04
WHZ	<-2	52	ref	-	ref	-	ref	-	ref	
	>=2	8	-5.5 (-24.2, 13.2)	0.56	1.0 (-11.0, 13.0)	0.87	52.4 (-120, 226)	0.54	-2.2 (-9.4, 5.0)	0.52

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WAZ=Weight-for-age z-score, WHZ=Weight-for-age z-score

Ref=referent

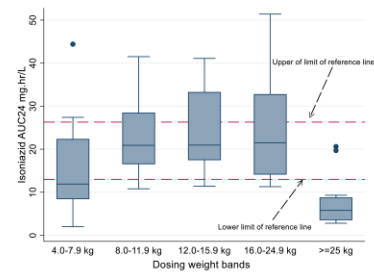Footnote: These were adjusted for the weight-bands

Figure 1

a) **Rifampicin exposures**
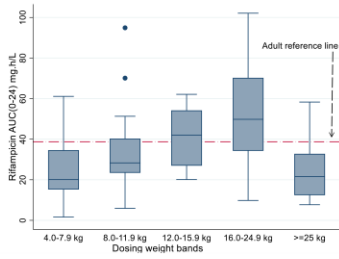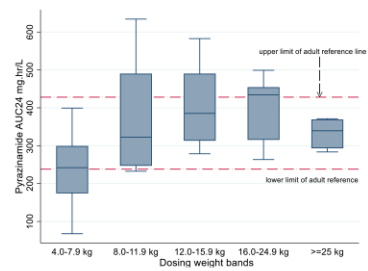
b) **Isoniazid exposures**

c) **Pyrazinamide exposures**

d) **Ethambutol exposures**

Figure 2

a) Rifampicin C$_{max}$

b) Isoniazid C$_{max}$

c) Pyrazinamide C$_{max}$

d) Ethambutol C$_{max}$