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# Safety and efficacy of rifabutin among HIV/TB-coinfected children on lopinavir/ritonavir-based ART

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**Background:** TB is the leading cause of death among HIV-infected children, yet treatment options for those who require PI-based ART are suboptimal. Rifabutin is the preferred rifamycin for adults on PI-based ART; only one study has evaluated its use among children on PIs and two of six children developed treatment-limiting neutropenia.

**Methods:** Since 2009, rifabutin has been available for HIV/TB-coinfected children requiring PI-based ART in the Harvard/APIN programme in Nigeria. We retrospectively analysed laboratory and clinical toxicities at baseline and during rifabutin therapy, and examined HIV/TB outcomes.

**Results:** Between 2009 and 2015, 48 children received rifabutin-containing TB therapy with PI (lopinavir/ritonavir)-based ART: 50% were female with a median (IQR) baseline age of 1.7 (0.9–5.0) years and a median (IQR) CD4+ cell percentage of 15% (9%–25%); 52% were ART experienced. Eighty-five percent completed the 6 month rifabutin course with resolution of TB symptoms and 79% were retained in care at 12 months. Adverse events (grade 1–4) were more common at baseline (27%) than during rifabutin treatment (15%) ($P$=0.006). Absolute neutrophil count was lower during rifabutin compared with baseline (median=1762 versus 2976 cells/mm$^3$, respectively), but only one instance (2%) of grade 3 neutropenia occurred during rifabutin treatment.

**Conclusions:** With clinical and laboratory monitoring, our data suggest that rifabutin is a safe option for TB therapy among children on PI-based ART. By contrast with the only other study of this combination in children, severe neutropenia was rare. Furthermore, outcomes from this cohort suggest that rifabutin is effective, and a novel option for children who require PI-based ART. Additional study of rifabutin plus PIs in children is urgently needed.

## Introduction

TB is the leading cause of death among HIV-infected children globally.[1] Of the estimated 2.1 million children living with HIV, 20%–50% in endemic areas will acquire TB infection.[1–4] Despite improved access to ART, mortality among HIV/TB-coinfected children is over five times that for TB infection alone.[4]

Treatment of HIV/TB coinfection is particularly challenging in children who require PI-based ART due to drug–drug interactions and lack of optimized treatment regimens. Lopinavir/ritonavir-based ART is the preferred first-line treatment regimen for all children under 3 years of age and for older children who have failed first-line ART.[1,5] Identifying efficacious TB treatment options for children requiring PI-based ART is essential for those requiring cotreatment.

Lopinavir is metabolized by cytochrome P450 (CYP) 3A4 isoenzymes and is a substrate for the P-glycoprotein (P-gp) efflux transporter. Rifampicin, a key component of first-line TB therapy, potently induces CYP3A4 and P-gp, and, when given in combination, reduces systemic lopinavir exposure by ∼75%.[6] Accordingly, the WHO recommends that HIV/TB-coinfected children who require PI-based ART be treated with (i) a triple-nucleoside (3NRTI) regimen, (ii) a 'super-boosted' lopinavir/ritonavir-based regimen (i.e. additional ritonavir to provide a 1:1 dose ratio instead of the

standard 4:1 lopinavir/ritonavir ratio) or (iii) for children <3 years of age, a nevirapine-based regimen; each along with standard rifampicin-containing TB therapy.[1] All of these options are suboptimal. Compared with NNRTI- or PI-based regimens among adults, 3NRTI regimens result in higher rates of virological failure, and observational studies show only 47% virological suppression among children treated with a 3NRTI regimen.[7–10] While pharmacokinetic data in children suggest that super-boosting lopinavir with additional ritonavir may overcome the interaction with rifampicin, this strategy is clinically and logistically problematic in resource-limited settings due to frequent gastrointestinal and hepatic toxicity, poor palatability, added expense and unavailability of ritonavir as a single-drug product.[11–13]

Among coinfected adults requiring PI-based ART, rifabutin is recommended in place of rifampicin.[1] Rifabutin is a significantly less potent inducer of CYP3A4, allowing standard PI dosing, with minimal effect on lopinavir concentrations when given in combination with lopinavir/ritonavir.[14,15] Since all PIs increase serum concentrations of rifabutin, the dose must be decreased when given with PI-based ART. Pharmacokinetic studies of dosing strategies of 150 mg daily or 150 mg thrice weekly in combination with lopinavir/ritonavir found that the target maximum rifabutin concentration was achieved in the daily but not the thrice weekly strategy among adults,[14,16] but studies in children receiving lopinavir/ritonavir are inadequate. The single study published to date evaluating safety and pharmacokinetics of rifabutin in combination with lopinavir/ritonavir-based ART among children was stopped early after two of six children developed treatment-limiting neutropenia.[17]

Since 2004, the AIDS Prevention Initiative in Nigeria (APIN) has provided care and treatment for adults and children living with HIV in Nigeria with funding support from the President's Emergency Plan for AIDS Relief (PEPFAR) and the Global Fund in collaboration with the Harvard T.H. Chan School of Public Health. Recognizing that treatment options were deficient for HIV/TB-coinfected adults and children who require concurrent PI-based ART, APIN began providing rifabutin for adults in 2008 and children in 2009.

## Methods

### Study population

This retrospective cohort analysis evaluates the safety and efficacy of rifabutin-containing TB treatment combined with lopinavir/ritonavir-based ART among children 15 years of age and younger. From 2009 to 2015, 4459 HIV-infected children received care at the six APIN programme sites that contributed patients to this study; 48 of them received rifabutin as a component of TB therapy between March 2009 and October 2015. Coinfected children were candidates for rifabutin-containing TB treatment if they were <3 years of age (for such children lopinavir/ritonavir-based ART is the recommended first-line ART) or if they were aged ≥3 years and failing first-line, NNRTI-based ART (for such children a switch to lopinavir/ritonavir-based second-line ART is required). The guidelines for lopinavir/ritonavir use in children evolved during this study period (lopinavir/ritonavir was not recommended for all children <3 years regardless of NNRTI exposure until the 2013 WHO guidelines); therefore, the 48 children included in this analysis represent a small subset of those potentially eligible for its use. Specifically, of the 4459 children enrolled at these sites (2009–15), ~510 (11.4%) would have been eligible for rifabutin (319 were <3 years and TB coinfected, and 191 were ≥3 years, failing first-line ART and newly TB coinfected) based on current guidelines.

All HIV-infected children enrolled in the APIN paediatric ART programme undergo screening for TB as part of routine care at baseline and when clinically indicated: this screening comprises a clinical evaluation for symptoms or signs of TB infection, chest radiograph, tuberculin skin testing and evaluation by Ziehl–Neelsen staining and/or Xpert MTB/RIF (since 2013; Cepheid) for children with productive chronic cough capable of providing sputum samples. Antiretroviral drugs were dosed according to WHO weight bands and cotrimoxazole was given to all children with active TB.

### Formulation and dosing

While no paediatric formulation currently exists, a rifabutin 20 mg/mL oral suspension can be compounded from capsules in sweetening vehicles and is stable for at least 12 weeks at temperatures up to 40°C.[18] APIN staff pharmacists compounded the suspension with Ora-Plus and Ora-Sweet.

US guidelines for treatment of opportunistic infections in HIV-infected children recommend rifabutin at 10–20 mg/kg once daily for treatment of drug-susceptible TB, but no dosing guidelines exist for children on concurrent PI-based ART.[19] Based on extrapolations from adult data and consideration of the unique developmental pharmacology of infants and children, APIN dosed rifabutin 5 mg/kg once daily for infants <12 months of age and 2.5 mg/kg daily for children aged ≥12 months, concurrently receiving lopinavir/ritonavir-based ART. Infants potentially require a higher mg/kg dosage because CYP3A4 activity does not achieve adult levels until at least 12 months of age and P-gp expression is lower.[20,21]

### Monitoring and toxicity grading

Laboratory and clinical evaluations conducted among children initiating rifabutin therapy were in accordance with APIN guidance. Given the known association between rifabutin use and neutropenia, anaemia, thrombocytopenia and liver function abnormalities, the following four laboratory evaluations were examined for the adverse event (AE) analyses: absolute neutrophil count (ANC), haemoglobin, platelet count and ALT. Laboratory values were assessed at baseline (prior to starting concurrent rifabutin + lopinavir/ritonavir therapy) and during rifabutin + lopinavir/ritonavir cotreatment. Frequency of laboratory monitoring was at the discretion of the treating physician and typically followed the Nigerian national guideline schedule of baseline, 3 and 6 months after starting or changing ART and then every 6 months or more frequently if clinically indicated.[22,23]

Records were also retrospectively reviewed for evidence of clinical toxicities [i.e. uveitis (pain or redness of the eye or visual changes), arthralgias, myalgias, stomatitis, jaundice, nausea or other complaints] present at baseline or during rifabutin + lopinavir/ritonavir therapy. Laboratory or clinical abnormalities were graded based on the 2014 Division of AIDS tables for grading severity of adult and paediatric AEs.[24] An AE was defined as any grade 1–4 abnormality and a serious AE (SAE) was defined as any grade 3 or 4 abnormality. During rifabutin + lopinavir/ritonavir therapy, laboratory abnormalities were reported if the severity grade increased from the baseline value.

### Statistical analyses

Statistical analyses were performed using Stata version 13.1 (StataCorp, College Station, TX, USA). The non-parametric Wilcoxon signed rank test was used for paired samples due to non-normal distribution of continuous variables. McNemar's test was used for paired categorical data; for patients with repeated toxicity occurrences during concurrent rifabutin + lopinavir/ritonavir treatment, the highest severity grade was used. Since neutropenia is a potential dose-limiting toxicity associated with rifabutin in children, we used Spearman's correlation to examine associations between the lowest measured ANC and total daily rifabutin dosage (mg) or daily rifabutin dosage by weight (mg/kg). *P* values <0.05 were considered statistically significant.

### Ethical considerations

All patients/caregivers enrolled in the APIN programme provided consent for care and were given the option to allow their de-identified data to be used for future evaluations. This retrospective study involving secondary use of de-identified data was approved by the Institutional Review Board (IRB) of the Harvard T.H. Chan School of Public Health (IRB15-0141) and APIN Regulatory Affairs under a Nigerian Institute of Medical Research IRB-approved data repository.

## Results

### Study population

Between 2009 and 2015, 48 HIV/TB-coinfected children on lopinavir/ritonavir-based ART received rifabutin as part of their TB treatment regimen. For baseline patient characteristics, see Table 1. Fifty percent were female and at rifabutin initiation the median age was 1.7 (IQR=0.9–5.0) years, baseline CD4+ cell count was 455 (IQR=237–809) cells/mm$^3$ and CD4+ cell percentage was 14.5 (IQR=9.4–24.5). Most children were significantly underweight, with a median weight-for-age *z* score of −2.6 (IQR=<−3 to −1.1) for those aged <5 years and a median BMI *z* score of −1.4 (IQR=−2.4 to −0.5) for those aged ≥5 years. Twenty-three percent were categorized as WHO clinical stage 4 due to suspected disseminated or abdominal TB. Thirteen (27%) children were <12 months of age and thus received the higher rifabutin dosage by weight (5 mg/kg daily).

**Table 1.** Baseline characteristics of children initiated on rifabutin-containing TB treatment in combination with lopinavir/ritonavir-based ART

| Characteristics at rifabutin initiation | *n* (%) (*N*=48[a]) | Median (IQR) |
|---|---|---|
| Age (years) | | 1.7 (0.9–5.0) |
| Age category (years) | | |
| 0 to <1 | 13 (27%) | 0.7 (0.5–0.8) |
| 1 to <3 | 19 (40%) | 1.6 (1.3–2.0) |
| ≥3 | 16 (33%) | 8.5 (5–11) |
| Female | 24 (50%) | |
| Anthropometrics | | |
| age 0 to <5 years | 36 (75%) | |
| weight-for-age *z* score (*N*=36) | | −2.6 (<−3 to −1.1) |
| weight-for-length *z* score (*N*=32) | | −1.6 (−2.8 to −0.3) |
| age ≥5 years | 12 (25%) | |
| BMI *z* score (*N*=12) | | −1.4 (−2.4 to −0.5) |
| WHO clinical stage | | |
| 3 | 37 (77%) | |
| 4 | 11 (23%) | |
| CD4+ cell count (cells/mm$^3$) (*N*=47) | | 455 (237–809) |
| CD4+ cell percentage (*N*=38) | | 14.5 (9.4–24.5) |
| Viral load (copies/mL) (*N*=38) | | 297432 (92911–1009309) |
| Receiving ART at start of rifabutin + lopinavir/ritonavir | 25 (52%) | |
| WHO 5 TB screening questions, positivity rate | 39 (81%) | |
| Cough ≥2 weeks | 36 (75%) | |
| Subsequent ART regimen: NRTI backbone in addition to lopinavir/ritonavir | | |
| zidovudine + lamivudine | 27 (56%) | |
| abacavir + lamivudine | 16 (33%) | |
| stavudine + lamivudine | 2 (4%) | |
| ART not recorded | 3 (6%) | |

[a]Number available for analysis is 48, unless otherwise indicated.

Over half (52%) of the children were already receiving ART, for a median duration of 14.2 (IQR=3.5–29.8) months, prior to initiating rifabutin. Furthermore, 10 (21%) children received lopinavir/ritonavir-based ART for a median duration of 10.4 (IQR=2.0–16.2) months prior to rifabutin. All children subsequently received lopinavir/ritonavir-based ART in combination with rifabutin-containing TB treatment. Two-thirds (32 of 48 children) were <36 months of age at rifabutin initiation and thus required lopinavir/ritonavir-based ART as the preferred first-line ART regimen. The most commonly prescribed NRTI backbone was zidovudine + lamivudine (56%), followed by abacavir + lamivudine (33%). Children who received zidovudine-containing regimens were significantly younger than those receiving abacavir (median age=1.3 versus 6.9 years; $P$<0.0001). The regimen was not recorded for three children as they died or were lost to follow-up (LTFU) after the initial visit.

### TB and HIV treatment outcomes

Forty-one (85%) patients completed the expected 6 month rifabutin course with resolution of TB symptoms (Table 2). Of the seven patients who did not complete the 6 month course, patient death or LTFU was recorded for three after the first clinical visit (two died, one LTFU), three after the second visit (one died, two LTFU) and one was transferred to another clinic just prior to completion. At 12 months (6 months after completion), 38 (79%) patients were retained in care with no symptoms to suggest recurrent/relapsed TB.

Regarding HIV treatment outcomes, the median change in CD4+ cell count (CD4+ cell percentage) from baseline to the 6 and 12 month follow-up visits was an increase of 499 cells/mm$^3$ (7) and 428 cells/mm$^3$ (14), respectively. HIV-1 RNA values were only available for 33 (69%) and 26 (54%) patients at the 6 and 12 month follow-up visits, of which 22 (67%) and 21 (81%) were virologically suppressed (viral load <1000 copies/mL), respectively.

### Laboratory toxicities at baseline

At baseline, most patients (79%; 38 patients) had one or more laboratory AE and in 15 (31%) an SAE had occurred (Tables 3 and 4). Of 164 laboratory values available at baseline, 45 (27%) were characterized as AEs, while 15 (9%) were characterized as SAEs. Anaemia (any grade, 1–4) was the most common laboratory abnormality: 35 of 45 (78%) abnormal values at baseline and 12 of 15 (80%) SAEs. Neutropenia was identified in five patients at baseline (grade 2, *n*=3; grade 4, *n*=2). Other baseline laboratory abnormalities included thrombocytopenia in one patient and elevated ALT in four patients.

**Table 2.** TB and HIV treatment outcomes among children who received rifabutin-containing TB treatment in combination with lopinavir/ritonavir-based ART

| | 6 months after starting rifabutin + lopinavir/ritonavir | | 12 months after starting rifabutin + lopinavir/ritonavir | |
|---|---|---|---|---|
| TB and HIV treatment outcomes | *N*[a] | median (IQR) or *n* (%) | *N*[a] | median (IQR) or *n* (%) |
| TB treatment outcomes | 48 | | 48 | |
| completed rifabutin course with no TB symptoms | | 41 (85%) | | 38 (79%) |
| TB relapse or recurrence | | — | | 0 |
| died | | 3 (6%) | | 3 (6%) |
| LTFU | | 3 (6%) | | 6 (13%) |
| transferred | | 1 (2%) | | 1 (2%) |
| HIV treatment outcomes | | | | |
| change in CD4+ cell count (cells/mm$^3$)[b] | 39 | 499 (199–850) | 36 | 428 (178–969) |
| change in CD4+ cell percentage[b] | 33 | 7 (0.3–13) | 33 | 14 (6–27) |
| viral load <1000 copies/mL | 33 | 22 (67%; 46% overall) | 26 | 21 (81%; 44% overall) |

[a]Number available for analysis.
[b]Denotes change from baseline to 6 or 12 months after starting rifabutin + lopinavir/ritonavir.

**Table 3.** Laboratory AEs at baseline and during rifabutin + lopinavir/ritonavir cotreatment

| | Prior to rifabutin + lopinavir/ritonavir initiation (*N*=48; total number of laboratory values=164), *n* (%) | During rifabutin + lopinavir/ritonavir cotreatment[a] (*N*=48; total number of laboratory values=232), *n* (%) | *P* |
|---|---|---|---|
| Laboratory AEs | 45 (27%) | 34 (15%) | **0.006** |
| Subjects with AEs[b] | 38 (79%) | 20 (42%) | **0.0009** |
| Laboratory SAEs | 15 (9%) | 10 (4%) | 0.19 |
| Subjects with SAEs[b] | 15 (31%) | 9 (19%) | 0.17 |
| AEs by severity[c] | | | |
| grade 1 | 13 (29%) | 13 (38%) | |
| grade 2 | 17 (38%) | 11 (32%) | |
| grade 3 | 12 (27%) | 8 (24%) | |
| grade 4 | 3 (7%) | 2 (6%) | |
| Subjects with AEs by severity[d,e] | | | |
| grade 1 | 11 (23%) | 12 (25%) | |
| grade 2 | 16 (33%) | 10 (21%) | |
| grade 3 | 12 (25%) | 8 (17%) | |
| grade 4 | 3 (6%) | 2 (4%) | |

AE, any grade 1–4; SAE, grade 3 or 4.
[a]Any laboratory abnormality at least one severity grade higher than the baseline value.
[b]Subjects who experience one or more AEs or SAEs are counted only once.
[c]Percentages are based on number of AEs reported for each group.
[d]Subjects are counted only once within a particular severity grade.
[e]Percentages are based on number for each group.

## Laboratory toxicities during concurrent rifabutin + lopinavir/ritonavir cotreatment

During rifabutin + lopinavir/ritonavir cotreatment, 232 individual laboratory values (including ANC, haemoglobin, platelet count and ALT) were obtained among the 48 patients. Thirty-four (15%) of these values were at least one grade higher in severity than at baseline (grade 1, *n*=13; grade 2, *n*=11; grade 3, *n*=8; grade 4, *n*=2) (Table 3). Anaemia was again the most common AE: 17 (50%) of the 34 laboratory abnormalities. During treatment, haemoglobin was evaluated 59 times and 45 values (76%) were abnormal, but only 17 values (29%) were increased in severity from baseline. Also, 14 (29%) children developed anaemia that increased in severity from baseline, of which 7 (15%) had grade 3 or 4 anaemia; 7 patients developed neutropenia while on

**Table 4.** Comparison of baseline and follow-up laboratory toxicity values among 48 children receiving rifabutin-containing TB treatment

| Laboratory parameter | Prior to rifabutin + lopinavir/ritonavir initiation | During rifabutin + lopinavir/ritonavir cotreatment | | $P^a$ |
|---|---|---|---|---|
| | | AEs (*N*=number of laboratory measurements available) | subjects with AEs (*N*=48) | |
| ANC | | | | |
| value (cells/mm$^3$), median (IQR) | 2976 (2037–4353) | 1762 (1370–2639) | | **0.0004** |
| patients with available value, *n* (%) | 43 (90%) | 43 (90%) | | |
| number of values during rifabutin + lopinavir/ritonavir, median (IQR) | – | 1 (1–2) | | |
| months from rifabutin + lopinavir/ritonavir start to first value, median (IQR) | – | 2.3 (1.5–5.1) | | |
| AEs by severity | (*N*=43) | (*N*=57) | (*N*=43) | |
| grade 1, *n* (%) | 0 | 3 (5%) | 3 (7%) | |
| grade 2, *n* (%) | 3 (8%) | 3 (5%) | 3 (7%) | |
| grade 3, *n* (%) | 0 | 1 (2%) | 1 (2%) | |
| grade 4, *n* (%) | 2 (5%) | 0 | 0 | |
| Haemoglobin | | | | |
| haemoglobin (g/dL), median (IQR) | 9.3 (8.1–9.9) | 9.3 (8.8–10.2) | | 0.25 |
| patients with available value, *n* (%) | 42 (88%) | 43 (90%) | | |
| number of values during rifabutin + lopinavir/ritonavir, median (IQR) | – | 1 (1–2) | | |
| months from rifabutin + lopinavir/ritonavir start to first value, median (IQR) | – | 2.3 (1.5–5.1) | | |
| AEs by severity | (*N*=42) | (*N*=59) | (*N*=43) | |
| grade 1, *n* (%) | 10 (24%) | 4 (7%) | 2 (5%) | |
| grade 2, *n* (%) | 13 (31%) | 5 (8%) | 5 (12%) | |
| grade 3, *n* (%) | 11 (26%) | 6 (10%) | 5 (12%) | |
| grade 4, *n* (%) | 1 (2%) | 2 (3%) | 2 (5%) | |
| Platelet count | | | | |
| value ($\times 10^9$/L), median (IQR) | 360 (279–472) | 299 (224–388) | | **0.02** |
| patients with available value, *n* (%) | 43 (90%) | 43 (90%) | | |
| number of values during rifabutin + lopinavir/ritonavir, median (IQR) | – | 1 (1–2) | | |
| months from rifabutin + lopinavir/ritonavir start to first value, median (IQR) | – | 2.3 (1.5–5.1) | | |
| AEs by severity | (*N*=43) | (*N*=58) | (*N*=43) | |
| grade 1, *n* (%) | 0 | 2 (3%) | 1 (2%) | |
| grade 2, *n* (%) | 1 (2%) | 2 (3%) | 1 (2%) | |
| grade 3, *n* (%) | 0 | 1 (2%) | 1 (2%) | |
| grade 4, *n* (%) | 0 | 0 | 0 | |
| ALT | | | | |
| value (IU/L), median (IQR) | 20 (11–36) | 23 (18–33) | | 0.93 |
| patients with available value, *n* (%) | 36 (75%) | 43 (90%) | | |
| number of values during rifabutin + lopinavir/ritonavir, median (IQR) | – | 1 (1–2) | | |
| months from rifabutin + lopinavir/ritonavir start to first value, median (IQR) | – | 2.3 (1.4–5.4) | | |
| AEs by severity | (*N*=36) | (*N*=58) | (*N*=43) | |
| grade 1, *n* (%) | 3 (8%) | 4 (7%) | 3 (7%) | |
| grade 2, *n* (%) | 0 | 1 (2%) | 1 (2%) | |
| grade 3, *n* (%) | 1 (3%) | 0 | 0 | |
| grade 4, *n* (%) | 0 | 0 | 0 | |

[a]*P* value for difference between paired samples.

rifabutin + lopinavir/ritonavir (grade 1, *n*=3; grade 2, *n*=3; grade 3, *n*=1); five instances of thrombocytopenia occurred among 3 patients; and five instances of elevated ALT occurred among 4 patients. Although creatinine was additionally measured, and no instances of abnormal creatinine occurred at baseline or during follow-up, this was not included in the overall AE analyses.

Significantly more AEs were present at baseline compared with during therapy (Table 3). The overall proportion of AEs at baseline compared with during rifabutin + lopinavir/ritonavir treatment was 27% (45 of 164 laboratory values) and 15% (34 of 232 values), respectively (*P*=0.006). Furthermore, the proportion of children with an AE at baseline was significantly greater than during rifabutin + lopinavir/ritonavir treatment: 79% versus 42%, respectively (*P*<0.001).

### *Trends in neutropenia over time*

There was a statistically significant decline in ANC from a median baseline value of 2976 (IQR=2037–4353) to 1762 (IQR=1370–2639) cells/mm$^3$ during concurrent rifabutin + lopinavir/ritonavir therapy (*P*=0.0004) (Table 4), though the threshold for grade 1 neutropenia is <1000 cells/mm$^3$. Among the five patients with neutropenia at baseline, including two patients with grade 4, the ANC normalized during rifabutin + lopinavir/ritonavir cotreatment. Among the seven patients who developed neutropenia during treatment (grade 1, *n*=3; grade 2, *n*=3; grade 3, *n*=1), all continued on rifabutin with resolution of neutropenia either during or shortly after completing therapy, with a median time to repeat ANC evaluation of 5.8 (IQR=5.1–6.7) months.

Six of the seven neutropenic events occurred among children <3 years of age. Specifically, among patients with ANC measured during rifabutin + lopinavir/ritonavir, any neutropenia occurred in 6 of 29 (21%) patients aged <3 years compared with 1 of 14 (7%) aged ≥3 years, though this difference was not statistically significant. Of the 10 patients who were LTFU or died at any time during study follow-up, all had normal baseline ANC, but follow-up ANC was not available for four who died or were LTFU within 2 weeks of the initial visit; for the remaining six patients, the final ANC available before LTFU was normal in all but one patient, who had grade 1 neutropenia.

Of the 13 patients aged <12 months who received rifabutin dosed at 5 mg/kg daily, the ANC during rifabutin therapy was available for 12 as one patient was LTFU after the first visit. One experienced grade 3 neutropenia 1 month following rifabutin initiation, which normalized by month 4 despite ongoing rifabutin treatment; one patient developed grade 1 neutropenia at month 2, which resolved by month 8 after rifabutin treatment was complete. Of the 35 children aged ≥12 months, who mostly received rifabutin at 2.5 mg/kg daily (three patients just over 12 months of age received a dose near 5 mg/kg daily), 3 developed grade 2 neutropenia and 2 developed grade 1. No association between ANC during therapy and total daily rifabutin (mg) dosage (Figure 1a) or daily rifabutin (mg/kg) dosage by weight (Figure 1b) was observed.

### *SAEs*

There were no serious clinical toxicities reported during rifabutin therapy and no medication discontinuations due to clinical or laboratory SAEs. Approximately 15% of patients experienced mild to moderate clinical AEs (three with nausea/vomiting, three with rash and one with dizziness) during rifabutin + lopinavir/ritonavir treatment, but all continued therapy with resolution of these events. The total number of laboratory SAEs present at baseline (9%; 15 of 164 laboratory values) was higher than during therapy (4%; 10 of 232 values) and the proportion of children with a laboratory SAE at baseline (31%) was higher than during therapy (19%), but these trends did not achieve statistical significance. Overall, the number of children with an SAE was low, so no significant trends in individual laboratory toxicities were observed. While grade 3 or 4 anaemia was more common at baseline (29%; 12 of 42 patients) compared with during rifabutin therapy (16%; 7 of 43 patients), this difference was not significant (*P*=0.12). Other than anaemia, only three SAEs were identified at baseline (grade 4 neutropenia; *n*=2; grade 3 ALT elevation, *n*=1), of which all resolved during rifabutin + lopinavir/ritonavir cotreatment, and only two SAEs other than anaemia were identified during therapy (grade 3 neutropenia, *n*=1; grade 3 thrombocytopenia, *n*=1).

## Discussion

This is the first report on rifabutin safety and efficacy among HIV/TB-coinfected children requiring lopinavir/ritonavir-based ART. With clinical and laboratory monitoring, our data suggest that rifabutin is a safe option for TB therapy among coinfected children on lopinavir/ritonavir-based ART. We observed favourable treatment outcomes, with 85% of children retained in care with resolution of TB symptoms by 6 months and 79% of patients retained in care at 12 months with no symptoms to suggest TB relapse. SAEs were infrequent and occurred more commonly prior to rather than during treatment and there were no discontinuations due to medication toxicities.

Only one other study has examined rifabutin among children on lopinavir/ritonavir-based ART and that prospective pharmacokinetic study was stopped early after one-third of patients developed treatment-limiting neutropenia.[17] By contrast, in our cohort there were no occurrences of grade 4 neutropenia during rifabutin treatment and just one (2%) instance of grade 3. While the median ANC in our cohort was lower during rifabutin + lopinavir/ritonavir cotreatment than at baseline, the decline was mild, did not reach a clinically significant threshold (i.e. ANC ≤1000 cells/mm$^3$) in most patients and was associated with neither adverse clinical outcomes nor total (mg/kg) rifabutin dosage. Furthermore, severe neutropenia was more common at baseline than during cotreatment and resolved despite rifabutin therapy, suggesting a potential association with the underlying severity of HIV/TB disease rather than rifabutin.

Reasons for the discrepancy in rates of severe neutropenia between the two studies are unclear, though differences in patient characteristics and rifabutin dosing strategies are notable. Patients in the Moultrie *et al.*[17] study did not have active TB: children had recently completed a course of therapy for TB (range=3.9–5.7 weeks prior) and were receiving rifabutin as a single anti-TB agent, for study purposes only, along with lopinavir/ritonavir-based ART. It has been observed that rifabutin-associated neutropenia occurs more frequently in healthy volunteers than HIV-infected adults and, although the reason for this is unknown, a proposed

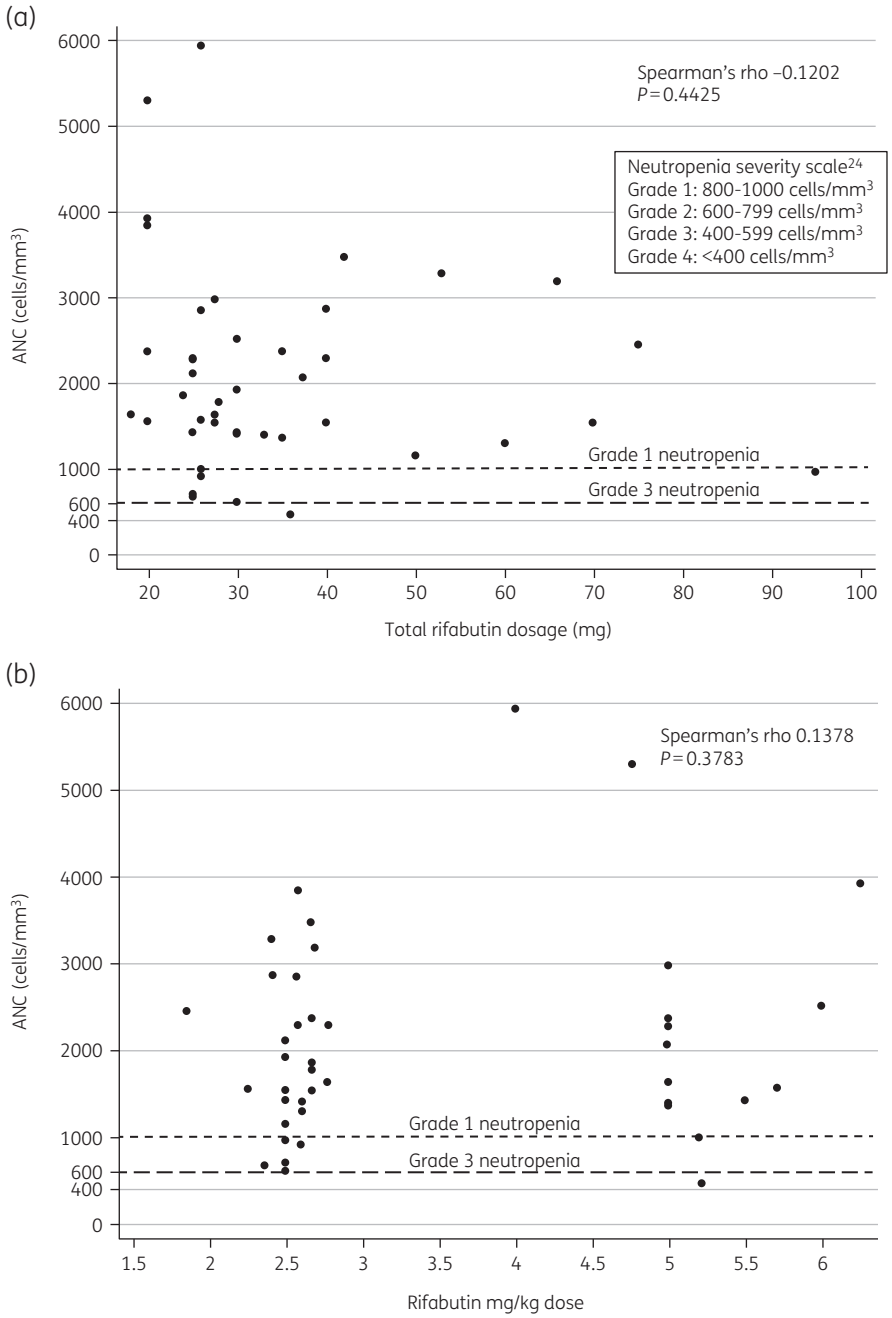


**Figure 1.** No association between ANC during rifabutin + lopinavir/ritonavir cotreatment and (a) total daily rifabutin (mg) and (b) mg/kg dosage.

mechanism is via immune response dysregulation compared with those with intact immunity.[25] Based on immunological, virological and anthropometric criteria, children in our study were sicker than those in the Moultrie *et al.*[17] study, all having active TB, lower baseline CD4+ cell percentage (median=14.5% versus 24.9%, respectively), higher baseline HIV-1 RNA values (median=297432 copies/mL versus <400 copies/mL for four of six children for whom results were available) and more severe malnutrition (weight-for-age *z* score=−2.6 versus −1.1, among those <5 years), which may impact pharmacokinetic/pharmacodynamic indices, including side effect profiles.[26]

Moreover, rifabutin dosing strategies differed between the two studies: 5 mg/kg thrice weekly in Moultrie *et al.*[17] compared with 2.5 mg/kg daily for children aged ≥12 months and 5 mg/kg daily for infants aged <12 months in our study. Among adults, two studies conducted in Vietnam and South Africa compared rifabutin at 150 mg daily with 150 mg thrice weekly in combination with lopinavir/ritonavir and found that the target maximum rifabutin concentration was achieved with the daily but not the thrice weekly strategy.[14,16] Moreover, in the South African cohort, increasing from thrice weekly to daily dosing of rifabutin 150 mg resulted in a >2-fold increase in $AUC_{0-24}$. Thus, the effect of dosing rifabutin

daily is more than simply additive. While Moultrie *et al.*[17] state that rifabutin dosed 5 mg/kg three times per week resulted in lower $AUC_{0-48}$ and $AUC_{0-24}$ values compared with adults receiving 150 mg daily, the $AUC_{0-24}$ (5.36 μg·h/mL) approximated that of adults receiving rifabutin 300 mg daily without ART in the studies from South Africa (3.05 μg·h/mL) and Vietnam (5.64 μg·h/mL), often considered a more standard comparator in rifabutin pharmacokinetic studies. Clinically, it is notable that 27% of the patients in our cohort were <12 months of age and so received more than double the total weekly mg/kg rifabutin dosage used in Moultrie *et al.*[17] (5 mg/kg daily versus 5 mg/kg thrice weekly), with only one instance of grade 3 neutropenia.

This retrospective study raises a valid concern as to whether the low incidence of severe neutropenia could reflect inadequate rifabutin concentrations, but most patients (85%) completed rifabutin-containing TB therapy with resolution of TB symptoms at 6 months, with 79% retained in care without evidence of TB relapse at 12 months of follow-up. While reports on TB-related mortality among HIV-infected children are limited, favourable TB outcomes in this cohort were equivalent to or better than those reported in the literature. In a recent study from Nigeria, TB infection was associated with a >4-fold increased risk of death, with HIV/TB-associated mortality varying widely among studies from 5% to 30%.[27,28] While our pharmacokinetic study of rifabutin among children on lopinavir/ritonavir-based ART is ongoing in Nigeria, we believe our positive experience justifies a continued role for rifabutin use in children requiring PI-based ART and ongoing study of this combination is needed. Although the WHO has designated the evaluation of newer HIV treatment options for coinfected children, such as dolutegravir, a research priority,[29] PI-based ART will remain a mainstay for those most likely to become TB coinfected (i.e. children <36 months of age and those requiring second-line ART) for the foreseeable future. Thus, a strategy to treat TB in children who require PI-based ART is imperative.

As a retrospective cohort analysis, this study is limited by the laboratory data available, with a median of 1 (IQR=1–2) laboratory evaluation during rifabutin-containing TB treatment, which corresponded to the programmatic protocol for monitoring in Nigeria.[26] The diagnosis of TB was primarily limited to clinical and/or radiographic criteria rather than microbiological or PCR confirmation of TB, so it is possible that TB was over-diagnosed in this cohort, but rates are consistent with most studies of TB in HIV-infected children, for whom microbiological diagnosis of TB is limited. Finally, while missing viral load data limit assessment of HIV treatment outcomes, a CD4+ cell count was available for 75% of patients at the 12 month visit, with a median increase of 428 cells/$mm^3$.

In summary, in our cohort of 48 coinfected children receiving lopinavir/ritonavir-based ART, rifabutin-containing TB treatment was safe and efficacious. During rifabutin therapy, only one (2%) instance of grade 3 neutropenia arose and 85% of children completed the 6 month TB treatment course with resolution of TB symptoms. While a pharmacokinetic study of rifabutin dosed at 2.5 mg/kg daily is ongoing in Nigeria, these data provide support for rifabutin use, in combination with close clinical and laboratory monitoring, in children on lopinavir/ritonavir-based ART who otherwise lack available and effective treatment options.

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## Transparency declarations

None to declare.

## Disclaimer

The contents are solely the responsibility of the authors and do not necessarily represent the official views of the funding institutions.

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