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# Xpert MTB/RIF Cycle Threshold as a Marker of Tuberculosis (TB) Disease Severity: Implications for TB Treatment Stratification

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***Background.*** Recent trials have demonstrated that shortened 4-month treatment durations are effective for the majority of people with tuberculosis (TB). However, there is a population of patients with TB who require longer treatment durations. Prospectively identifying those who require shorter versus longer treatment durations would support evaluation and implementation of optimized regimens.

***Methods.*** We analyzed data from the RIFASHORT TB treatment-shortening noninferiority trial to define a TB phenotype classification. The RIFASHORT trial primary outcome was reanalyzed using the protocol-defined noninferiority criterion of 8 percentage points, stratifying by those classified as having limited or extensive disease.

***Results.*** Xpert MTB/RIF semiquantitative bacterial burden in combination with TB disease involvement grading on chest X-ray achieved the strongest differentiation between relapse and nonrelapse. The extensive disease TB phenotype (high semiquantitative bacterial burden and extensive TB disease on X-ray) accounted for one-quarter of the RIFASHORT trial population and more than half of all posttreatment TB relapses (13/23). For the limited TB disease phenotype (a semiquantitative bacterial burden other than high or no extensive TB disease on X-ray), the experimental 4-month 1200-mg rifampicin–containing regimen met the protocol-defined noninferiority criterion in both modified intention-to-treat (adjusted risk difference: −1.3%; 95% CI, −6.7% to 4.0%) and per protocol analyses (1.7%; 95% CI, −3.8% to 7.1%).

***Conclusions.*** The TB phenotype classification derived here successfully identified three-quarters of RIFASHORT trial participants for whom a 4-month 1200-mg rifampicin regimen was noninferior to the 6-month standard of care. A definitive phase III randomized trial of disease-stratified rifampicin-based TB treatment is justified.

**Keywords.** sputum culture; sputum smear; chest X-ray; TB phenotype; relapse.

Studies of drug-susceptible tuberculosis (TB) treatment regimens shorter than 6 months have demonstrated that, for most people, treatment duration can be reduced to less than 6 months with no adverse impact on outcomes [1–4]. However, all but one of these trials failed to show noninferiority of the shorter regimens in the overall study population. The question remains how to identify people with TB who may be adequately treated with shorter treatment durations as opposed to those who require a longer duration of treatment.

Established measures of TB disease severity, such as sputum smear grade and abnormalities on chest X-ray, are known to be associated with TB treatment outcomes but are poor prognostic tools for individual responses with known interoperator variability [5]. Previous attempts to individualize TB treatment duration according to sputum smear and culture status have proven unsuccessful [6, 7].

Cepheid Xpert MTB/RIF and Xpert MTB/RIF Ultra are 2 widely used diagnostic tools for TB disease [8, 9]. These assays output quantitative cycle threshold values and a semiquantitative assessment (high, medium, low, very low, trace) of bacterial burden, which are being explored as correlates of disease severity [10, 11].

Xpert MTB/RIF semiquantitative bacterial burden presents a near-patient measure of TB disease severity that is easily obtained and interpreted with results available within 2 hours, reducing potential interoperator variability. An accessible test that accurately differentiates TB disease severity and poor treatment outcome risk is highly desirable and may enable individualized TB treatment durations [12]. Here we assessed the performance of Xpert MTB/RIF semiquantitative bacterial burden in TB treatment stratification strategies.

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This study assessed Xpert MTB/RIF and Xpert MTB/RIF Ultra cycle threshold as a measure of TB disease severity in comparison with other established measures. We defined a TB phenotype classification of limited and extensive disease based on baseline measures of TB disease severity. Further, we reanalyzed data from a recently completed phase III trial of 4-month TB treatment regimens, stratifying the analysis by the TB phenotype classification.

## METHODS

### Study Population and Data

We analyzed data from the RIFASHORT trial, which assessed two 4-month TB treatment regimens both containing an increased dose of rifampicin (1200 mg or 1800 mg) provided once daily as a flat dose together with standard doses of isoniazid, plus ethambutol and pyrazinamide for 2 months, compared with the 6-month standard of care containing rifampicin at 10 mg/kg (300 mg to 750 mg once daily, according to weight band). Participants were enrolled in Botswana, Guinea, Nepal, Pakistan, Peru, and Uganda, with the primary outcome defined at 12 months postrandomization. The trial composite primary unfavorable outcome included culture-confirmed TB relapse, retreatment for TB, death or loss to follow-up during treatment, and permanent treatment change due to adverse events. Details on the study design are available in the main trial results publication [3]. In brief, 672 human immunodeficiency virus (HIV-1)–negative adults with pulmonary TB were randomized 1:1:1 to the 3 study arms. Neither experimental regimen demonstrated noninferiority in comparison to the control, based on a noninferiority margin of 8 percentage points. However, a high proportion of participants had a successful treatment outcome in all 3 trial arms (control: 93%; 1200 mg rifampicin: 90%; 1800 mg rifampicin: 87%).

Baseline data were collected on sputum smear grade, sputum culture grade (defined below), chest X-ray parameters, and cycle threshold from either Xpert MTB/RIF (Xpert) or Xpert MTB/RIF Ultra (Ultra), depending on which test platform was available at each site. The Xpert and Ultra platforms differ with respect to their gene targets. Xpert has 5 probes targeting sequences of the *rpoB* (RNA polymerase beta) gene, whereas Ultra includes an additional gene signature (IS1081/IS6110) as well as 4 probes targeting *rpoB* gene sequences [8, 9]. For both tests, a quantitative cycle threshold output is derived from the *rpoB* gene probe outputs. A lower cycle threshold, the number of cycles required to detect TB, is indicative of a higher bacterial burden. Both tests also output a semiquantitative interpretation of the cycle threshold value, indicating whether the bacterial burden is high, medium, low, very low, or trace (the latter for Ultra only). Interpretation of the semiquantitative bacterial burden is consistent between Xpert and Ultra as the quantitative cycle threshold cut-points defining the semiquantitative bacterial burden was calibrated independently for each test (manufacturer's cycle threshold cut-points for Xpert: high $<16 \leq$ medium $< 22 \leq$ low $< 28 \leq$ very low; Ultra: high $< 18.9 \leq$ medium $< 24.9 \leq$ low $< 28.9 \leq$ very low) [8, 9].

Baseline chest X-rays were collated digitally and read centrally by an independent expert at St George's University of London. All sputum cultures were performed on solid LJ-slope medium at local site laboratories. Recurrence samples were stored locally at least −20 °C and shipped to St George's University of London for whole-genome sequencing.

### Statistical Methods

The study population included all RIFASHORT trial participants who tested positive on Xpert or Ultra and had baseline cycle threshold data. Posttreatment TB relapse was defined by 2 consecutive positive sputum cultures with no evidence of exogenous reinfection by whole-genome sequencing of matched baseline and relapse samples. Individuals with a single, positive last sputum culture who reinitiated TB treatment (therefore without a confirmatory culture) were also defined as TB relapses.

### Xpert MTB/RIF Cycle Threshold as a Measure of TB Disease Severity

The distribution of Xpert and Ultra cycle threshold probe values at baseline was described using boxplots, pooling data from the whole study population. Quantitative cycle threshold values were computed as the minimum of the *rpoB* probe outputs. The association between Xpert and Ultra cycle threshold quantitative output and other measures of disease severity (sputum smear, sputum culture, and chest X-ray) was described using boxplots and assessed formally using linear regression. For sputum smear, the Ziehl-Neelsen grading system was used (no acid-fast bacilli seen, scanty, 1+, 2+, 3+). Sputum cultures were graded according to the number of bacterial colonies seen on LJ slopes (1+, 20–100; 2+, 100–200; 3+, >200).

The likelihood ratio test assessed for the general association between each measure of disease severity and Xpert or Ultra cycle threshold quantitative output. The coefficient of determination ($R^2$) was used to describe the proportion of variability in Xpert and Ultra cycle threshold quantitative output explained by other disease severity markers. To understand if there were inherent differences in the populations tested with Xpert and Ultra, we also compared TB disease severity markers between these 2 groups.

### TB Phenotype Classification

In determining optimal markers for classifying limited and extensive TB disease, data were pooled from the two 4-month study arms. The control arm was excluded to avoid treatment efficacy biasing this analysis as there were fewer TB relapses in the control arm. The sensitivity and specificity of each disease severity marker in identifying TB relapse were estimated along with 2-way combinations of each disease severity marker combined with Xpert and Ultra semiquantitative bacterial burden.

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

| Population Characteristics | No. (%) (n = 667) | Relapse, No. (Row %) (n = 25) |
|---|---|---|
| Study site | | |
| Botswana | 54 (8.1) | 2 (3.7) |
| Peru | 119 (17.8) | 0 |
| Uganda | 224 (33.6) | 15 (6.7) |
| Nepal | 70 (10.5) | 3 (4.3) |
| Guinea | 175 (26.2) | 5 (2.9) |
| Pakistan | 25 (3.7) | 0 |
| Sex | | |
| Male | 490 (73.5) | 22 (4.5) |
| Female | 177 (26.5) | 3 (1.7) |
| Missing | 8 (1.2) | 0 |
| Age, median (IQR), y | 29 (23–39) | 38 (24–46) |
| BMI, median (IQR), kg/m$^2$ | 18.7 (17.0–20.9) | 18.1 (16.3–19.9) |
| Chest X-ray extent of lung disease | | |
| Normal | 2 (0.3) | 0 |
| Minimal disease | 18 (2.7) | 0 |
| Moderately advanced disease | 294 (44.1) | 7 (2.4) |
| Far advanced | 343 (51.4) | 18 (5.2) |
| Missing | 4 (0.6) | 0 |
| Cavitation on chest X-ray | | |
| Yes | 578 (86.7) | 23 (4.0) |
| No | 85 (12.7) | 2 (2.4) |
| Bilateral disease on chest X-ray | | |
| Yes | 446 (66.9) | 22 (3.3) |
| No | 219 (32.8) | 3 (0.5) |
| Missing | 2 (0.3) | 0 |
| Chest X-ray lung zone involvement score | | |
| 0 | 6 (0.9) | 0 |
| 1 | 28 (4.2) | 0 |
| 2 | 119 (17.8) | 1 (0.8) |
| 3 | 171 (25.6) | 6 (3.5) |
| 4 | 176 (26.4) | 9 (5.1) |
| 5 | 98 (14.7) | 7 (7.1) |
| 6 | 65 (9.7) | 2 (3.1) |
| Extensive disease on chest X-ray[a] | | |
| Yes | 274 (41.1) | 4 (1.5) |
| No | 389 (58.3) | 21 (5.4) |
| Missing | 4 (0.6) | 0 |
| Baseline sputum smear grade | | |
| No AFB seen | 45 (6.7) | 1 (2.2) |
| Scanty/1+ | 250 (37.5) | 4 (1.6) |
| 2+ | 148 (22.2) | 11 (7.4) |
| 3+ | 224 (33.6) | 9 (4.0) |
| Missing | 7 (1.0) | 0 |
| Baseline sputum culture grade | | |
| Negative | 27 (4.0) | 2 (7.4) |
| 1+ | 140 (21.0) | 3 (2.1) |
| 2+ | 160 (24.0) | 5 (3.1) |
| 3+ | 333 (49.9) | 15 (4.5) |
| Baseline Xpert MTB/RIF semiquantitative bacterial burden (n = 456) | | |
| High | 138 (30.3) | 8 (5.8) |
| Medium | 218 (47.8) | 6 (2.8) |
| Low | 76 (16.7) | 2 (2.6) |
| Very low | 24 (5.3) | 0 |
| Baseline Xpert MTB/RIF Ultra semiquantitative bacterial burden (n = 211) | | |
| High | 132 (62.6) | 8 (6.1) |
| Medium | 60 (28.4) | 1 (1.7) |
| Low | 14 (6.6) | 0 |
| Very low | 7 (3.3) | 0 |

Abbreviations: AFB, acid-fast bacilli; BMI, body mass index; IQR, interquartile range.

[a]Extensive disease on chest X-ray was defined as ≥50% lung involvement, bilateral disease, and cavitation. A total of 99.3% (667/672) of RIFASHORT trial participants were Xpert or Ultra positive and included in the analysis.

For chest X-ray, we explored 3 grading systems. The first used a study-specific classification system graded by an independent expert as follows: far advanced disease, moderate disease, minimal disease, or normal (defined in Supplementary Section 1). The second grading combined 3 chest X-ray observations (≥50% lung involvement, cavitation, and bilateral disease) into a binary classification. The third grading was a binary classification based on lung involvement alone (≥50%).

### Reanalysis of RIFASHORT Trial Data by TB Phenotype Classification

Reanalysis of RIFASHORT trial data followed the same methods as the primary analysis of the trial, including modified intention-to-treat (mITT) and per-protocol analyses (further details in Supplementary Section 2). We evaluated the risk difference in the proportion with an unfavorable treatment outcome at 12 months postrandomization using Cochran-Mantel-Haenszel weights and the protocol-defined noninferiority criterion of 8 percentage points, with stratification by study site. The analysis was performed separately for those classified as having limited and extensive TB disease.

## RESULTS

### Relapse Characteristics

Baseline TB disease severity measures of the study population are shown in Table 1. Ultra was used for 21% of participants in Peru, 37% of participants in Uganda, and 59% of participants in Guinea. Xpert was used exclusively in Botswana, Nepal, or Pakistan. A total of 30.3% of participants tested with Xpert and 62.6% of participants tested with Ultra had a high semiquantitative bacterial burden.

There were 25 TB relapses during posttreatment follow-up, 22 of which were culture confirmed, giving an overall relapse risk of 3.7% (25/667). Two relapses occurred in the control arm, with 11 and 12 in the 1200-mg and 1800-mg rifampicin treatment arms, respectively.

There was no difference in the distribution of chest X-ray lung grading scores between participants tested with Xpert or Ultra ($P = .16$), or lung involvement zone scores ($P = .34$), or sputum smear grading ($P = .13$). However, a higher proportion

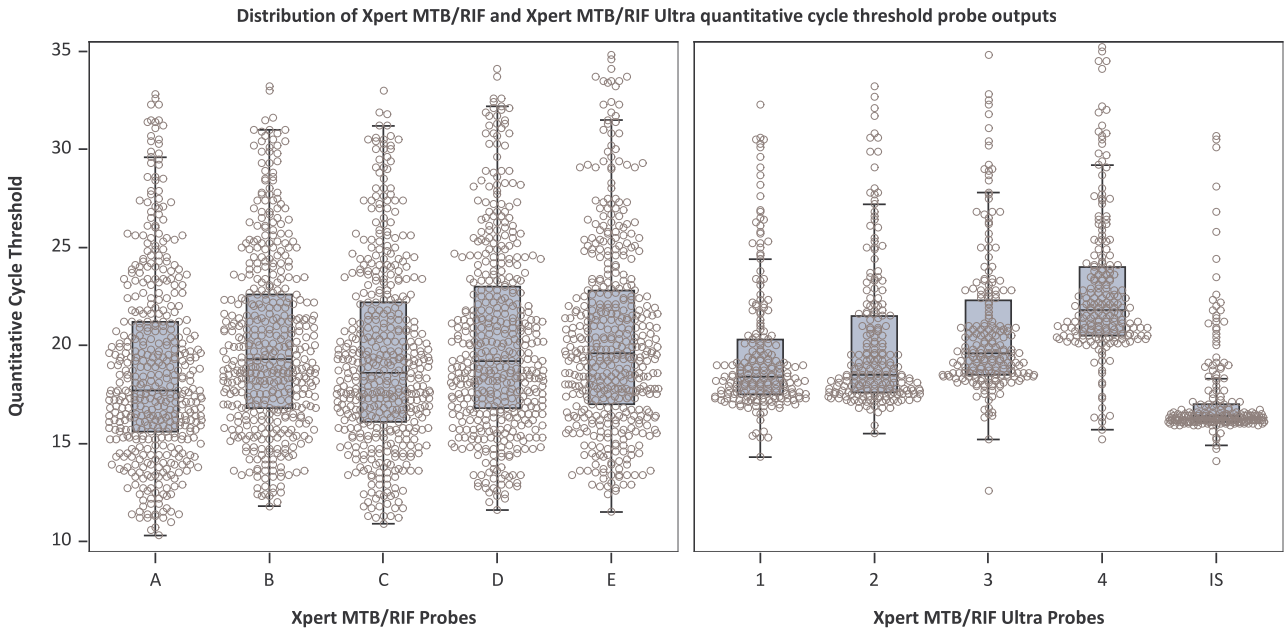


**Figure 1.** Boxplots showing the distribution of baseline quantitative cycle threshold outputs for each probe of Xpert MTB/RIF (*left panel*) and Xpert MTB/RIF Ultra (*right panel*) for the RIFASHORT study population.

of those tested with Ultra had cavitation on chest X-ray (91.5% vs 84.4%; $P = .03$) and had 3+ on sputum culture (56.8% vs 46.7%; $P < .0001$), compared with those tested with Xpert.

The risk of TB relapse during follow-up was highest in Uganda (6.7%) and Nepal (4.3%). Men had a higher relapse risk than woman (4.5% vs 1.7%) and those who experienced relapse were older (median: 38 vs 28 years). There were no TB relapses among people with normal or minimal disease on chest X-ray, although these groups were small (n = 20 for both combined). Relapse risk was 2.4% and 5.2% among those with moderately advanced disease and far advanced disease on chest X-ray, respectively. According to sputum smear, the highest relapse risk was seen for those with a 2+ grading (7.4%). For sputum culture, the highest relapse risk was seen for those with a 3+ grading (4.5%). For Xpert and Ultra semiquantitative bacterial burden, the highest relapse risk was in the high grading (5.8% and 6.1%, respectively).

#### Xpert MTB/RIF and Ultra Cycle Threshold and Disease Severity

There was higher variability in the distribution of quantitative cycle threshold values from Xpert compared with Ultra (Figure 1). Ultra IS probe (IS1081/IS6110) cycle threshold values had very low variability, clustering around the median value (median, 16.4; IQR, 16.1–17.0), emphasizing that the IS probe is not designed to be interpreted in terms of disease burden.

There was strong evidence of an association between Xpert quantitative cycle threshold and sputum culture grade, sputum smear grade, and chest X-ray grade (all $P < .001$) (Figure 2, top panels). However, the coefficient of determination varied considerably. The chest X-ray extent of lung disease explained 6.3% of the variability in Xpert quantitative cycle threshold, but had no association with Ultra. Sputum smear grade explained 23.6% and 29.8% of variability in Xpert and Ultra, respectively.

#### TB Phenotype Classification

The sensitivity and specificity of each baseline disease severity marker in identifying relapse is shown in Table 2. A high semiquantitative bacterial burden from Xpert or Ultra had a sensitivity of 65.2%, identifying 15 of 23 relapse cases. However, 160 of 423 (37.8%) of those who did not relapse also had a high semiquantitative bacterial burden, meaning that semiquantitative bacterial burden alone had low specificity (62.2%). Similar patterns were observed for baseline chest X-ray (far advanced: 69.6% sensitivity, 48.1% specificity) and sputum culture (3+: 56.5% sensitivity, 50.5% specificity).

The highest sensitivity for a single marker was seen for a combined definition of extensive TB disease involvement on chest X-ray. A chest X-ray grading definition requiring 50% or greater lung involvement, bilateral disease, and presence of cavities had 82.6% sensitivity (capturing 19/23 relapses); however, specificity was low (41.5%).

A high Xpert or Ultra semiquantitative bacterial burden in combination with this definition of extensive TB disease involvement on chest X-ray at baseline gave the strongest differentiation between relapse and nonrelapse, achieving 56.5% sensitivity (13/23 relapses) and 75.4% specificity, meaning that over half of all relapses were identified in one-quarter of

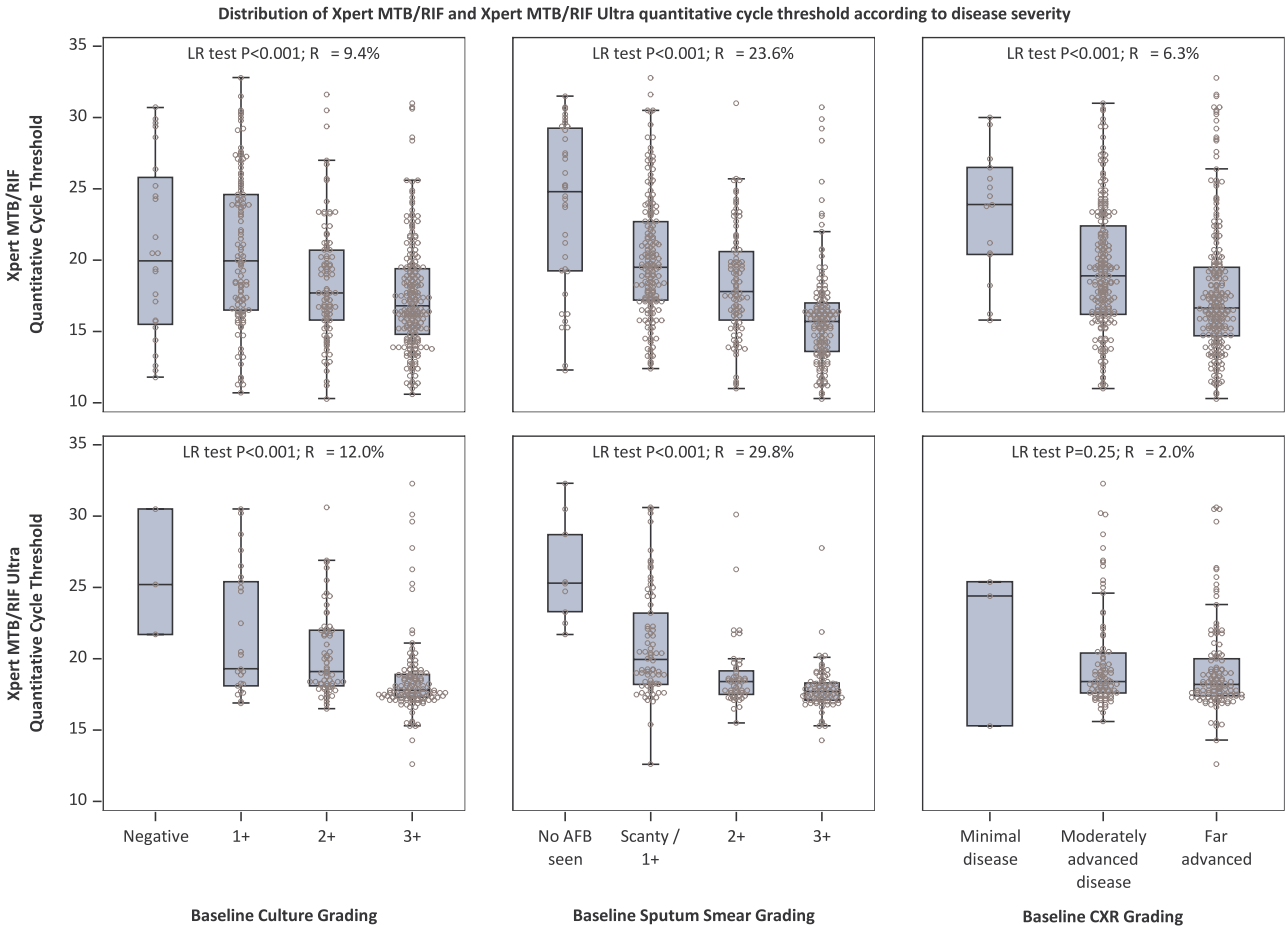


**Figure 2.** Boxplots showing the distribution of baseline quantitative cycle threshold output for Xpert MTB/RIF (*top row*) and Xpert MTB/RIF Ultra (*bottom row*) according to culture grading (first column), sputum smear grading (second column), and CXR grading (third column). Abbreviations: AFB, acid-fast bacilli; CXR, chest X-ray; LR, likelihood ratio.

the study population (26.2% [116/442]) meeting the extensive disease classification (Table 2). This combination was therefore selected as the classification criteria for defining limited and extensive TB disease (limited disease: less than high bacterial burden or no extensive disease on X-ray; extensive disease: high bacterial burden and extensive disease on X-ray).

#### Reanalysis of RIFASHORT Trial Data by TB Phenotype Classification

Among those with limited TB disease, the 4-month experimental regimen with 1200 mg rifampicin met the protocol-defined noninferiority criterion in both the mITT analysis (adjusted risk difference [aRD], −1.3%; 95% CI, −6.7% to 4.0%) and the per protocol (PP) analysis (aRD, 1.7%; 95% CI, −3.8% to 7.1%) (Figure 3). The full disaggregation of outcome events is available in Supplementary Table 1.

Among those with extensive TB disease, the 1200-mg 4-month experimental regimen performed poorly in comparison to the control. In both mITT (aRD, 16.0%; 95% CI, 5.5% to 26.4%) and PP analysis (aRD, 14.2%; 95% CI, 4.0% to 24.4%) the point estimate of the risk difference to control was greater than 14%.

Similar patterns were observed for the 1800-mg 4-month experimental regimen, except it did not achieve noninferiority among those with limited disease and performed somewhat better than the 1200-mg regimen among those with extensive disease (Figure 3). In the limited disease stratum, the underperformance of the 1800-mg rifampicin regimen compared with the 1200-mg rifampicin regimen was partially explained by changes in treatment due to adverse events (Supplementary Table 1).

In a simplified analysis stratified by semiquantitative bacterial burden only (limited disease: less than high; extensive disease: high), 39.2% were classified as having extensive disease, accounting for 15 of 23 relapses. In the limited disease stratum, the 1200-mg rifampicin regimen met the protocol-defined noninferiority criterion in mITT analysis (aRD, 0.0%; 95% CI, −6.2% to 6.2%) but not in PP analysis (aRD, 3.0%; 95% CI, −3.2% to 9.2%) (Supplementary Figure 1 and Supplementary Table 2).

**Table 2. Baseline semiquantitative bacterial burden in combination with other TB disease severity markers for identifying those who will relapse**

| | Relapse n. (Sensitivity %) Semiquantitative Bacterial Burden | | | | | No Relapse n. (100-Specificity %) Semiquantitative Bacterial Burden | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | High n (%) | Medium n (%) | Low n (%) | Very Low n (%) | Total n (%) | High n (%) | Medium n (%) | Low n (%) | Very Low n (%) | Total n (%) |
| Sputum smear | | | | | | | | | | |
| 3+ | 6 (26.1) | 1 (4.4) | 0 | 0 | 7 (30.4) | 89 (21.0) | 44 (10.4) | 5 (1.2) | 2 (0.5) | 140 (33.1) |
| 2+ | 5 (21.7) | 5 (21.7) | 1 (4.4) | 0 | 11 (47.8) | 31 (7.3) | 40 (9.5) | 12 (2.8) | 1 (0.2) | 84 (19.9) |
| Scanty/1+ | 3 (13.0) | 0 | 1 (4.4) | 0 | 4 (17.4) | 36 (8.5) | 90 (21.3) | 38 (9.0) | 6 (1.4) | 170 (40.2) |
| No AFB seen | 1 (4.4) | 0 | 0 | 0 | 1 (4.4) | 4 (1.0) | 9 (2.1) | 6 (1.4) | 10 (2.4) | 29 (6.9) |
| Total | 15 (65.2) | 6 (26.1) | 2 (8.7) | 0 | 23 | 160 (37.8) | 183 (43.3) | 61 (14.4) | 19 (4.5) | 423 |
| CXR | | | | | | | | | | |
| Far advanced | 12 (52.2) | 3 (13.0) | 1 (4.4) | 0 | 16 (69.6) | 99 (23.8) | 90 (21.6) | 16 (3.9) | 10 (2.4) | 215 (51.9) |
| Moderate disease | 3 (13.0) | 3 (13.0) | 1 (4.4) | 0 | 7 (30.4) | 58 (13.9) | 86 (20.7) | 40 (9.6) | 6 (1.4) | 190 (45.7) |
| Minimal disease | 0 | 0 | 0 | 0 | 0 | 0 | 3 (0.7) | 4 (1.0) | 2 (0.5) | 9 (2.2) |
| Normal | 0 | 0 | 0 | 0 | 0 | 0 | 2 (0.5) | 0 | 0 | 2 (0.5) |
| Total | 15 (65.2) | 6 (26.9) | 2 (8.7) | 0 | 23 | 157 (37.7) | 181 (43.5) | 60 (14.4) | 18 (4.3) | 416 |
| Culture | | | | | | | | | | |
| 3+ | 9 (39.1) | 3 (13.0) | 1 (4.4) | 0 | 13 (56.5) | 100 (23.9) | 85 (20.3) | 17 (4.1) | 5 (1.2) | 207 (49.5) |
| 2+ | 4 (17.4) | 1 (4.4) | 0 | 0 | 5 (21.7) | 35 (8.4) | 56 (13.4) | 12 (2.9) | 2 (0.5) | 105 (25.1) |
| 1+ | 1 (4.4) | 2 (8.7) | 0 | 0 | 3 (13.0) | 18 (4.3) | 34 (8.1) | 30 (7.2) | 8 (1.9) | 90 (21.5) |
| Negative | 1 (4.4) | 0 | 1 (4.4) | 0 | 2 (8.7) | 5 (1.2) | 7 (1.7) | 1 (0.2) | 3 (0.7) | 16 (3.8) |
| Total | 15 (65.2) | 6 (26.1) | 2 (8.7) | 0 | 23 | 158 (37.8) | 182 (43.5) | 60 (14.4) | 18 (4.3) | 418 |
| CXR combination | | | | | | | | | | |
| ≥50% Lung activity + cavities + bilateral disease | 13 (56.5) | 5 (21.7) | 1 (4.4) | 0 | 19 (82.6) | 103 (24.6) | 108 (25.8) | 26 (6.2) | 8 (1.9) | 245 (58.5) |
| Normal to moderate disease | 2 (8.7) | 1 (4.4) | 1 (4.4) | 0 | 4 (17.4) | 55 (13.1) | 74 (17.7) | 35 (8.4) | 10 (2.4) | 174 (41.5) |
| Total | 15 (65.2) | 6 (26.1) | 2 (8.7) | 0 | 23 | 158 (37.7) | 182 (43.4) | 61 (14.6) | 18 (4.3) | 419 |
| CXR lung activity | | | | | | | | | | |
| ≥50% Lung activity | 14 (60.9) | 6 (26.1) | 2 (8.7) | 0 | 22 (95.6) | 135 (32.2) | 137 (32.7) | 38 (9.1) | 10 (2.4) | 320 (76.4) |
| <50% Lung activity | 1 (100) | 0 | 0 | 0 | 1 (4.4%) | 23 (5.5) | 45 (10.7) | 23 (5.5) | 8 (1.9) | 99 (23.6) |
| Total | 15 (65.2) | 6 (26.1) | 2 (8.7) | 0 | 23 | 158 (37.7) | 182 (43.4) | 61 (14.6) | 18 (4.3) | 419 |

The table shows the number of participants who are included in each 2-way combination of TB disease burden markers, stratified by TB relapse. In the relapse column, the percentages correspond to the sensitivity of each combination of markers for identifying relapse. In the no-relapse column, the percentages correspond to 100-specificity of each combination of markers for no relapse. Total columns show the sensitivity and 100-specificity for individual disease burden markers.

Abbreviations: AFB, acid-fast bacilli; CXR, chest X-ray; TB, tuberculosis.

## DISCUSSION

A combination of baseline chest X-ray and semiquantitative bacterial burden was used to define a binary classification of limited and extensive TB disease. The extensive disease classification accounted for one-quarter of RIFASHORT trial participants but more than half of all TB relapses. Reanalysis of the RIFASHORT trial data showed that outcomes in the 4-month 1200-mg rifampicin arm met the predefined criteria for noninferiority among the three-quarters of the study population with the limited TB disease phenotype. These findings

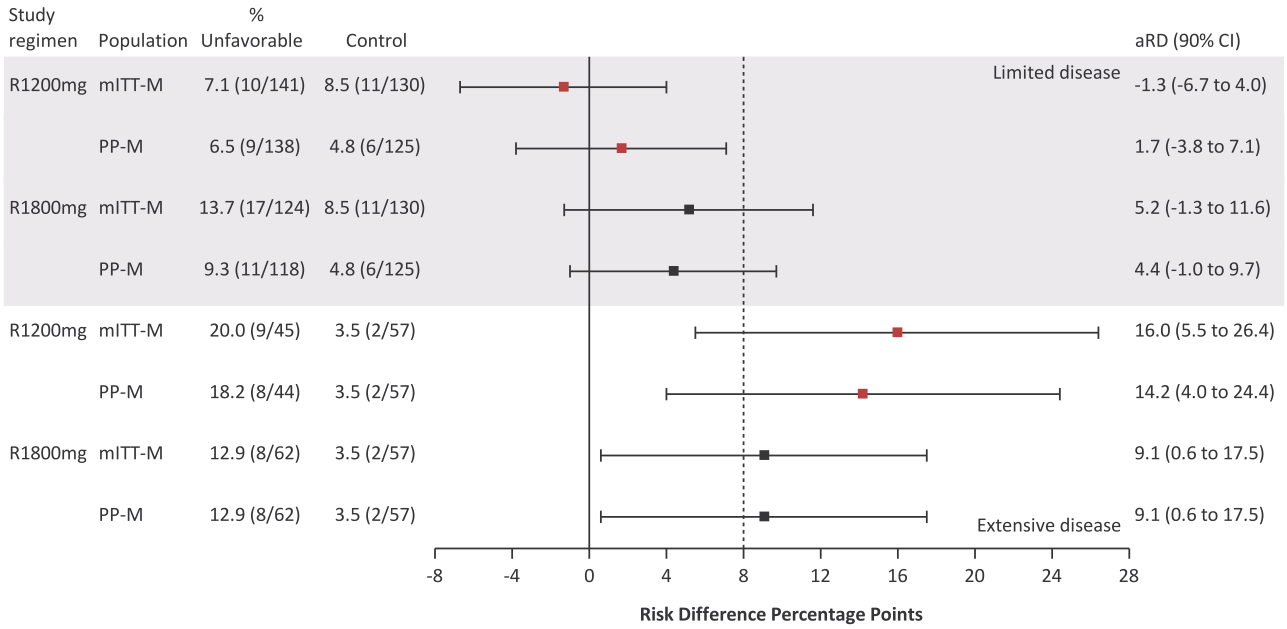


**Figure 3.** Forest plot showing the efficacy of the RIFASHORT 4-month treatment regimens compared with the 6-month control, stratified by limited disease (top shaded area) and extensive disease (unshaded area). Abbreviations: aRD, adjusted risk difference; CI, confidence interval; mITT-M, modified intention-to-treat microbiologically positive study population; PP-M, per protocol microbiologically positive study population; R1200mg, RIFASHORT study regimen using a 1200-mg dose of rifampicin; R1800mg, RIFASHORT study regimen using a 1800-mg dose of rifampicin.

demonstrate the potential for a stratified treatment strategy in determining the duration of TB treatment.

A noninferior 4-month rifapentine-based regimen was reported by Dorman et al in 2021 [1]; however, implementation has been hindered by limited access to rifapentine, excessive pill burden, and poor tolerability. A reduction in treatment duration from 6 to 4 months for three-quarters of all patients with drug-susceptible TB using a rifampicin-based regimen would be a major advancement in the field, overcoming challenges faced by the Study 31 regimen and realizing cost savings for TB programs [13, 14]. In the limited disease stratum presented here, the 4-month 1200-mg rifampicin arm had similar efficacy to the current 6-month standard of care, meeting the predefined margin of noninferiority for both mITT and PP analyses. In contrast, the 4-month 1800-mg rifampicin arm did not demonstrate noninferiority due to a small number of additional adverse events and treatment withdrawals, suggesting a more favorable tolerability profile for the 1200-mg rifampicin arm, as discussed in the RIFASHORT trial results [3].

Xpert cycle threshold has been shown to correlate with sputum smear grading [15], and other studies have shown an association between Xpert cycle threshold and poor treatment outcomes [16]. A meta-analysis of 3 TB treatment duration–shortening trials demonstrated the potential for risk-stratification tools in the allocation of treatment duration [17]. However, the risk-stratification tool previously proposed required 6 data inputs, including a sputum culture at 2 months post–treatment initiation. More recently, Chang et al [18] published a TB disease classification based on the combination of chest X-ray and Xpert quantitative cycle threshold. Our classification aligns with this, but has 1 important distinction: using semiquantitative bacterial burden instead of quantitative cycle threshold. The interpretation of semiquantitative bacterial burden is logistically preferable as it is an automatic readout of the machine and requires no interpretation of the quantitative cycle threshold.

The TB phenotype classification developed here requires just 2 baseline data inputs, Xpert or Ultra semiquantitative bacterial burden and chest X-ray. While chest X-ray is only required in those with a high semiquantitative bacterial burden, chest X-ray will not always be available in the field. Importantly, the 1200-mg rifampicin arm had similar efficacy when limited disease was classified based on semiquantitative bacterial burden alone. This means our disease classification may be implemented in settings where chest X-ray is not available, offering potential for individualized TB treatment durations using readily available tools at the point of treatment initiation.

The RIFASHORT trial data are limited in that they exclude people with HIV, those under 18 years old, and pregnant women. Additional analysis in these groups is recommended along with validation of the TB phenotype classification in other populations with different distributions of disease severity. Further, there were few TB relapses in the trial. The limited number of TB relapses restricted our analysis of TB phenotype

classification to 2-way combinations of disease burden markers. Additional data are required to develop a more sophisticated model accounting for other characteristics. However, the simplicity of the TB phenotype classification may also be seen as a strength regarding ease of implementation. Additional data would also be required to assess the relative sensitivity for TB relapse of Ultra compared with Xpert, given that a higher proportion of participants had a high semiquantitative bacterial burden with Ultra. This apparent disparity between Xpert and Ultra is potentially due to the reduced ability of Ultra to differentiate between medium and high bacterial burden [9].

Better tools for identifying who will experience TB relapse are still needed. The tools assessed in this study were imprecise individual predictors of TB relapse, with each disease severity marker having modest sensitivity and specificity. However, as relapse is an uncommon outcome, baseline disease-stratification methods with modest sensitivity may be sufficient to close the efficacy gap among the majority who may be treated with shorter regimens, as demonstrated here.

In conclusion, the TB phenotype classification derived here successfully identified three-quarters of RIFASHORT trial participants for whom a 4-month 1200-mg rifampicin regimen was noninferior to the 6-month standard of care. However, this analysis was conducted after completion of the study; treatment stratification strategies have yet to demonstrate success in prospective randomized controlled trials [6, 7, 19]. A 4-month rifampicin-based treatment duration for three-quarters of people with TB would be a major advancement in the field and this stratified treatment approach should be assessed in a phase III trial.

## 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.*** D. J. G. and K. F. devised the study, D. J. G. performed all statistical analysis and drafted the initial manuscript, J. D., P. D. B., A. A. W., K. G., and K. L. performed laboratory analysis for the RIFASHORT trial; all other authors contributed to refinements of the analysis and manuscript.

***Data availability.***The RIFASHORT trial data are available upon reasonable request via an application to the TB treatment individual patient data platform (TB-IPD). A collaborative initiative to support the generation of reliable evidence on the treatment of TB to inform future TB treatment guidelines. It is supported by the WHO Global TB Programme and maintained by UCL.

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