**Supplementary material: CID 123838**

**S1. Urine Xpert MTB/RIF Ultra analysis method**

Xpert MTB/RIF Ultra testing was performed by a trained technician as follows: 15 ml of urine was concentrated by centrifugation (15 minutes at 3000 rpm), the pellet resuspended in 1ml of sterile saline, then 700ul of pellet suspension was mixed with 1.4ml of Ultra sample reagent, shaken vigorously, and then tested using the Xpert Ultra assay according to the manufacturer’s instructions.

**S2. TB reference standard and case definitions:**

1. **Definite/microbiological TB:** specimen positive by smear microscopy, culture or Xpert MTB/RIF Ultra on sputum or extra-pulmonary samples. The urine Ultra and urine TB-LAM were excluded from this reference standard.
2. **Probable TB:**  patient did not fulfill the criteria for definite/ microbiological TB but diagnosed with active TB by a clinician who decided to give the patient a full course of TB treatment. This definition includes cases diagnosed on the basis of X-ray abnormalities or suggestive histology and extrapulmonary cases without laboratory confirmation and having good response to TB treatment at 12 weeks follow-up.
3. **Not TB**: No culture or molecular evidence of *M. tuberculosis.* An alternative diagnosis may be made. No clinical deterioration on 12-week follow-up despite not being prescribed TB treatment.
4. **Unclassifiable**: Unable to assign to any of the abovementioned groups due to death of unknown cause, on-going but uncharacterised symptoms at follow-up, or loss-to-follow-up at 12 weeks.

**S3. Post-hoc Tb definition reclassification:**

|  |
| --- |
| **Probable tuberculosis** |
| Requires all of the following:   1. Tuberculosis not microbiologically confirmed 2. Treated for TB 3. No alternative diagnosis made 4. plus at least one of the features below: |
| **Pleural effusion**  Exudative pleural effusion with adenosine deaminase >30g/dL in fluid |
| **Pericardial effusion** |
| **Chest X-ray features of pulmonary TB:**   * Miliary pattern * Hilar adenopathy * Breakdown/ cavitation * Patchy opacification or consolidation |
| **Abdominal ultrasound features**: (at least one)   * Multi-region lymph nodes ≥ 1 cm diameter * Splenic micro-abscesses * Ascites * Psoas abscess |
| **Tuberculous meningitis (TBM)**  Cerebrospinal fluid (CSF) score ≥2 out of 4 [1]) |
| **Other central nervous system TB:**  Compatible features on Computerized tomography (CT) of brain |
| **Peripheral lymph nodes**  Peripheral lymph nodes >2cm in diameter |

1. Marais S, Thwaites G, Schoeman JF, et al. Tuberculous meningitis: a uniform case definition for use in clinical research. Lancet Infect Dis **2010**; 10(11): 803-12.

|  |
| --- |
| **Not tuberculosis** |
| Tuberculosis not microbiologically confirmed; and disseminated *cryptococcosis* |
| Tuberculosis not microbiologically confirmed; and culture proven bacterial bloodstream infection |
| Tuberculosis not microbiologically confirmed; and clinical diagnosis of community acquired pneumonia with no criteria for clinical tuberculosis and improved without anti-tuberculosis treatment prior to discharge |
| Tuberculosis not microbiologically confirmed; and treated for *Pneumocystis jiroveci* pneumonia, not treated for tuberculosis and improved prior to discharge |
| Tuberculosis not microbiologically confirmed; and no criteria for clinical tuberculosis with other alternative diagnosis confirmed (examples: malignancy, venous thromboembolism, cardiac failure) |

**S4. Patient flow diagram:**

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Note. A screening register was not used in this study.

**S5. Details of ‘Unclassifiable TB’ cases (according to post-hoc modified classification), n=34:**

|  |  |
| --- | --- |
| Outcome  Demised | 31 |
| Uncontactable | 3 |
| TB blood culture result available  Negative | 32/34  32 |
| Contaminated | 0 |
| Urine TB culture result available  Negative | 32/34  29 |
| Contaminated | 3 |
| Sputum TB culture result available | 9/34 |
| Negative | 8 |
| Contaminated | 1 |

**Figure S1. LOESS regression line of diagnostic yield of sputum Ultra, Urine Ultra and LF-LAM for definite TB stratified by CD4 count**



Scatter plot of individual patients' CD4 count (on log scale, x-axis) versus TB test result (0=negative, 1=positive, y axis) with LOESS line of best fit shown with 95%CI in shaded area. The LOESS line of best fit is analogous to a moving average for the proportion of patients with positive test result for given CD4 count. Annotated with results of a logistic regression predicting test positivity by log CD4 count (OR = Odds Ratio; 95%CI = 95% confidence interval for OR, and associated p value).

**Figure S2. LOESS regression line of diagnostic yield of sputum Ultra, Urine Ultra and LF-LAM for definite TB stratified by haemoglobin.**



Scatter plot of individual patients' haemoglobin (g/dl) ( x-axis) versus TB test result (0=negative, 1=positive, y axis) with LOESS line of best fit shown with 95%CI in shaded area. The LOESS line of best fit is analogous to a moving average for the proportion of patients with positive test result for given haemoglobin. Annotated with results of a logistic regression predicting test positivity by haemoglobin (OR = Odds Ratio; 95%CI = 95% confidence interval for OR, and associated p value).

**Table S1. Analysis of association between diagnostic yield of urine Ultra (for definite TB) and other baseline variables and outcomes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Urine Ultra positive** | **Urine Ultra negative** | **All patients** | **p.value** |
| Urine LAM positive | 29 (54.7%) | 11 (22.0%) | 40 (38.8%) | 0,001 |
| Sputum Ultra positive | 9 (17.0%) | 12 (24.0%) | 21 (20.4%) | 0,465 |
| Age |  |  |  |  |
| median | 36,797 | 36,601 | 36,665 | 0,984 |
| IQR | 31.384, 42.209 | 31.845, 44.746 | 31.611, 43.399 |  |
| Female | 30 (56.6%) | 23 (46.0%) | 53 (51.5%) | 0,327 |
| Study Site |  |  |  | 0,251 |
| CMH | 43 (81.1%) | 35 (70.0%) | 78 (75.7%) |  |
| Frere | 10 (18.9%) | 15 (30.0%) | 25 (24.3%) |  |
| Not on ART | 35 (66.0%) | 29 (58.0%) | 64 (62.1%) | 0,423 |
| Cough | 38 (71.7%) | 41 (82.0%) | 79 (76.7%) | 0,250 |
| Weight Loss | 49 (92.5%) | 45 (91.8%) | 94 (92.2%) | 1,000 |
| Night sweats | 36 (67.9%) | 30 (60.0%) | 66 (64.1%) | 0,420 |
| Fever | 32 (60.4%) | 26 (52.0%) | 58 (56.3%) | 0,431 |
| RR >30 breaths pm | 37 (69.8%) | 29 (58.0%) | 66 (64.1%) | 0,226 |
| HR> 120 beats pm | 26 (49.1%) | 24 (48.0%) | 50 (48.5%) | 1,000 |
| Temperature >390 Celsius | 18 (34.0%) | 12 (24.0%) | 30 (29.1%) | 0,287 |
| Non ambulant | 39 (75.0%) | 32 (64.0%) | 71 (69.6%) | 0,283 |
| CD4 (cells/mm3) |  |  |  | 0,009 |
| Median | 38 | 79 | 48 |  |
| IQR | 15.500, 92.500 | 33.000, 172.250 | 20.000, 129.000 |  |
| Haemoglobin (g/dl) |  |  |  | 0,029 |
| Median | 8,5 | 10,1 | 9,2 |  |
| IQR | 7.000, 10.350 | 8.225, 11.700 | 7.400, 11.400 |  |
| CXR abnormal | 43 (82.7%) | 42 (85.7%) | 85 (84.2%) | 0,086 |
| CXR adenopathy | 14 (26.9%) | 13 (26.5%) | 27 (26.7%) | 1,000 |
| CXR nodular | 13 (25.0%) | 6 (12.2%) | 19 (18.8%) | 0,129 |
| CXR cavitation | 15 (28.8%) | 9 (18.4%) | 24 (23.8%) | 0,249 |
| CXR consolidation | 12 (23.1%) | 12 (24.5%) | 24 (23.8%) | 1,000 |
| CXR pleural effusion | 8 (15.4%) | 10 (20.4%) | 18 (17.8%) | 0,606 |
| CXR miliary | 10 (19.2%) | 0 (0.0%) | 10 (9.9%) | 0,001 |
| Urine Xpert volume |  |  |  | 1,000 |
| 1-10ml | 7 (13.5%) | 6 (12.0%) | 13 (12.7%) |  |
| 11-15ml | 45 (86.5%) | 44 (88.0%) | 89 (87.3%) |  |
| USS adenopathy | 13 (59.1%) | 6 (30.0%) | 19 (45.2%) | 0,072 |
| USS absent | 5 (22.7%) | 1 (5.0%) | 6 (14.3%) | 0,187 |
| USS splenic microabscesses | 14 (63.6%) | 13 (65.0%) | 27 (64.3%) | 1,000 |
| TB blood culture positive | 24 (50.0%) | 4 (8.7%) | 28 (29.8%) | 0,000 |
| TB urine culture positive | 13 (26.5%) | 1 (2.2%) | 14 (14.9%) | 0,001 |
| Inpatient death | 11 (20.8%) | 3 (6.0%) | 14 (13.6%) | 0,043 |
| End of study death | 16 (33.3%) | 9 (20.0%) | 25 (26.9%) | 0,167 |

CMH: Cecilia Makiwane Hospital; ART: Antiretroviral therapy; RR: respiratory rate; pm: per minute; CXR: Chest X-ray; ml: millilitres; USS: Abdominal ultrasound findings.

**Table S2. Analysis of association between diagnostic yield of urine LF-LAM (for definite TB)**

**and other baseline variables and outcomes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Urine LAM positive** | **Urine LAM negative** | **All patients** | **p.value** |
| Urine Ultra positive | 29 (72.5%) | 24 (38.1%) | 53 (51.5%) | 0,001 |
| Sputum Ultra positive | 9 (22.5%) | 12 (19.0%) | 21 (20.4%) | 0,803 |
| Age |  |  |  | 0,547 |
| median | 37,017 | 36,613 | 36,665 |  |
| IQR | 29.146, 43.248 | 33.188, 44.182 | 31.611, 43.399 |  |
| Female | 24 (60.0%) | 29 (46.0%) | 53 (51.5%) | 0,225 |
| Study Site |  |  |  | 0,485 |
| CMH | 32 (80.0%) | 46 (73.0%) | 78 (75.7%) |  |
| Frere | 8 (20.0%) | 17 (27.0%) | 25 (24.3%) |  |
| Not on ART | 28 (70.0%) | 36 (57.1%) | 64 (62.1%) | 0,216 |
| Cough | 34 (85.0%) | 45 (71.4%) | 79 (76.7%) | 0,152 |
| Weight Loss | 37 (92.5%) | 57 (91.9%) | 94 (92.2%) | 1,000 |
| Night sweats | 28 (70.0%) | 38 (60.3%) | 66 (64.1%) | 0,401 |
| Fever | 28 (70.0%) | 30 (47.6%) | 58 (56.3%) | 0,041 |
| RR >30 breaths pm | 25 (62.5%) | 41 (65.1%) | 66 (64.1%) | 0,835 |
| HR >120 beats pm | 21 (52.5%) | 29 (46.0%) | 50 (48.5%) | 0,550 |
| T >39 degrees Celsius | 9 (22.5%) | 21 (33.3%) | 30 (29.1%) | 0,272 |
| Non ambulant | 35 (87.5%) | 36 (58.1%) | 71 (69.6%) | 0,002 |
| CD4 (cells/mm3) |  |  |  | 0,001 |
| Median | 23,5 | 73 | 48 |  |
| IQR | 12.000, 77.750 | 33.000, 160.000 | 20.000, 129.000 |  |
| Haemoglobin (g/dl) |  |  |  | 0,247 |
| Median | 9,1 | 9,25 | 9,2 |  |
| IQR | 7.000, 10.350 | 7.675, 11.775 | 7.400, 11.400 |  |
| CXR abnormal | 34 (87.2%) | 51 (82.3%) | 85 (84.2%) | 0,592 |
| CXR adenopathy | 12 (30.8%) | 15 (24.2%) | 27 (26.7%) | 0,496 |
| CXR nodular | 7 (17.9%) | 12 (19.4%) | 19 (18.8%) | 1,000 |
| CXR cavitation | 9 (23.1%) | 15 (24.2%) | 24 (23.8%) | 1,000 |
| CXR consolidation | 10 (25.6%) | 14 (22.6%) | 24 (23.8%) | 0,812 |
| CXR pleural effusion | 6 (15.4%) | 12 (19.4%) | 18 (17.8%) | 0,790 |
| CXR miliary | 6 (15.4%) | 4 (6.5%) | 10 (9.9%) | 0,179 |
| USS adenopathy | 9 (56.2%) | 10 (38.5%) | 19 (45.2%) | 0,344 |
| USS absent | 5 (31.2%) | 1 (3.8%) | 6 (14.3%) | 0,023 |
| USS splenic micro abscesses | 11 (68.8%) | 16 (61.5%) | 27 (64.3%) | 0,746 |
| TB blood culture positive | 19 (52.8%) | 9 (15.5%) | 28 (29.8%) | 0,000 |
| TB urine culture positive | 11 (30.6%) | 3 (5.2%) | 14 (14.9%) | 0,002 |
| Inpatient death | 7 (17.5%) | 7 (11.1%) | 14 (13.6%) | 0,388 |
| End of study death | 10 (28.6%) | 15 (25.9%) | 25 (26.9%) | 0,812 |

CMH: Cecilia Makiwane Hospital; ART: Antiretroviral therapy; RR: respiratory rate; pm: per minute; CXR: Chest X-ray; ml: millilitres; USS: Abdominal ultrasound findings.

**Table S3. Number of participants with valid results for individual microbiological reference standard (“definite TB”) tests**

|  |  |
| --- | --- |
| **TB Test** | **Number (%)** |
| TB blood culture | 218 (92) |
| Sputum TB culture | 62 (26) |
| Sputum Xpert Ultra | 88 (37) |
| Urine TB culture | 216 (91) |
| Other extrapulmonary sample TB culture | 6\* (2) |
| Other extrapulmonary sample Xpert Ultra | 4\* (2) |
| Sputum smear | 3 (1) |

\* Only positive TB results were captured for other extrapulmonary samples on study database, therefore these are under-estimates