Accepted Manuscript Posted Online

Journal of Clinical Microbiology

JCM

1 **A Prospective Cohort Study on the Performance of CSF Xpert**

2 **MTB/RIF, CSF and Urine LAM Lateral Flow Assay for the Diagnosis of**

3 **Tuberculous Meningitis in Zambia**

4 [1,2,3]Omar K Siddiqi MD MPH, [4]Gretchen L Birbeck MD MPH DTMH, [5]Musie Ghebremichael

5 PhD, [3]Eugene Mubanga, [6]Shawn Love MD, [4]Clayton Buback, [7,8]Barry Kosloff, [7,8]Helen Ayles

6 MBBS MRCP DTMH PhD, [3]Masharip Atadzhanov MD PhD FRCP, [9]Keertan Dheda MBBcH FCP

7 FCCP PhD FRCP, [10]Igor J Koralnik MD

8 [1]Global Neurology Program, Department of Neurology, Beth Israel Deaconess Medical Center,

9 Boston, MA, [2]Center for Vaccines and Virology Research, Department of Internal Medicine, Beth

10 Israel Deaconess Medical Center, Boston, MA, [3]University of Zambia School of Medicine, Department

11 of Internal Medicine, Lusaka, Zambia, [4]Department of Neurology, University of Rochester, Rochester,

12 NY, [5]Harvard Medical School and Ragon Institute of MGH, MIT and Harvard, Boston, MA,

13 [6]Department of Neurology, University of California Los Angeles, Los Angeles, CA, [7]Zambart, Lusaka,

14 Zambia, [8]London School of Hygiene and Tropical Medicine, London, UK [9]University of Cape Town,

15 Cape Town, South Africa [10]Department of Neurological Sciences, Rush University Medical Center,

16 Chicago IL

17 **Keywords**: Tuberculous Meningitis, Xpert MTB/RIF, LAM, HIV, Zambia, Africa

18 **Corresponding author**: Dr. Igor J. Koralnik: Rush University Medical Center, POB1, Suite 1106, 1725

19 W. Harrison Street, Chicago, IL, 60612, phone 312-563-1022, fax 312-942-2380, igor_koralnik@rush.

20 edu

21 **Alternate author**: Dr. Omar K. Siddiqi: Beth Israel Deaconess Medical Center, E/CLS – 1005, 330

22 Brookline Avenue, Boston, MA, 02215, phone +260979365956, fax 617-735-4527,

23 osiddiqi@bidmc.harvard.edu

24   **Background**: Tuberculous meningitis (TBM) is a devastating infection of the central nervous

25   system lacking an adequate point-of-care diagnostic test.

26   **Methods**: We conducted a prospective cohort study of 550 Zambian adults with suspected

27   TBM to determine the diagnostic accuracy of cerebrospinal fluid (CSF) Xpert MTB/RIF, CSF

28   lipoarabinomannan (LAM), Urine LAM, CSF total protein, and CSF glucose compared to the

29   gold standard of CSF culture. We categorized patients with a positive CSF tuberculosis (TB)

30   culture as definite TBM. We also assessed inpatient and one-year mortality on definite TBM

31   patients when CSF Xpert MTB/RIF results were available in real-time to treating physicians

32   relative to a historical comparison cohort in whom Xpert results were not available in real-

33   time.

34   **Results**: Of the 550 patients, 474 (86.2%) were HIV-infected and 105/550 (19.1%) had

35   definite TBM based on a positive CSF culture. The sensitivity/specificity of the diagnostic

36   tests were CSF Xpert MTB/RIF 52.9%/94.2%, CSF LAM 21.9%/94.2%, urine LAM

37   24.1%/76.1%, CSF glucose < 40 mg/dL and total protein > 100 mg/dL 66.3%/90%. A model

38   including CSF Xpert MTB/RIF, CSF LAM, CSF glucose, and CSF total protein demonstrated an

39   area under the receiver operating curve of 0.90. The inpatient and one-year mortality for

40   definite TBM was 43% and 57%.

41   **Conclusions**: There was low sensitivity for the diagnosis of TBM across all diagnostics tests.

42   CSF Xpert MTB/RIF and CSF LAM are highly specific for the diagnosis of TBM. Despite the use

43   of Xpert MTB/RIF for diagnostic purpose in real-time, TBM was still associated with a high

44   mortality in Zambian patients.

45

46

47

48

49   **Introduction**

50   TBM is a devastating infection of the central nervous system often leading to severe

51   neurological impairment and death. Its effects are even more profound in sub-Saharan

52   Africa where HIV is endemic. The diagnosis of TBM is problematic regardless of setting. CSF

53   acid-fast bacteria staining has a sensitivity of 10-20% that varies greatly based on technical

54   expertise.(1) CSF *Mycobacterium tuberculosis (M. tuberculosis)* culture is widely considered

55   to be an imperfect gold standard with a sensitivity from 50-70%(2, 3) and can take as long as

56   six weeks to become positive. Neither of these techniques is practical in many low and

57   middle-income countries (LMIC) due to the complexity of widely instituting a specialized

58   laboratory skill and the lack of effective systems to communicate results to patients in the

59   community. As a result, treatment is often initiated empirically, with a lack of supportive

60   diagnostic testing.

61   Xpert MTB/RIF is an automated real-time PCR platform that provides a diagnosis of TBM

62   and rifampicin resistance through analysis of CSF in under 2 hours. In 2013, the World

63   Health Organization endorsed Xpert MTB/RIF as the initial test for the diagnosis of TBM after

64   a review of multiple studies which demonstrated a sensitivity of 60% and specificity of 95%

65   in comparison to CSF culture.(4) Interestingly, no study to date has demonstrated that the

66   use of Xpert MTB/RIF leads to improved survival in patients with TBM. Indeed, Xpert

67   MTB/RIF on sputum has reduced the time to diagnosis of pulmonary TB but has not

68   improved morbidity or mortality.(5, 6) Additionally, Xpert MTB/RIF requires electricity,

69   disposable cartridges, and servicing that prevents its scale-up to rural areas.

70   In contrast to TBM, cryptococcal meningitis diagnosis is facilitated by a simple low-cost

71   lateral flow assay. One possible point-of-care candidate for the diagnosis of TBM is the LAM

72   lateral flow assay (LFA) which requires < 100 μl of urine applied to a small test strip at room

73   temperature. It has been used to detect disseminated TB with its greatest utility in HIV-

74   infected patients with severe immunosuppression. A large multicenter randomized

75   controlled trial of urine LAM-guided initiation of anti-tuberculosis treatment in sub-Saharan

76   on inpatients with suspected pulmonary TB showed a reduction in 8-week inpatient

77   mortality.(7) In a proof-of-concept study, LAM ELISA testing of CSF on a cohort of South

78   African adults with suspected TBM had a sensitivity of 64% and a specificity of 69% for the

79   diagnosis of culture positive TBM.(8) An autopsy study of LAM LFA for the diagnosis of

80   definite TBM showed a sensitivity of 75% and a specificity of 87%.(9) There has been no

81   report of LAM LFA on urine or CSF in living patients for the diagnosis of TBM.

82   We evaluated the diagnostic accuracy of Xpert MTB/RIF on CSF, LAM LFA on fresh urine

83   and/or CSF, and current standard of care for TBM diagnosis in Zambia which combines

84   clinical presentation with CSF glucose and total protein values. *CSF M. tuberculosis* culture

85   served as the gold standard. We also studied whether use of Xpert MTB/RIF improved

86   survival of TBM patients in a tertiary care facility in Zambia.

87   **Methods**

88   **Study Design and Patient Population**

89   We conducted a prospective cohort study of TBM at the University Teaching Hospital (UTH)

90   in Lusaka, Zambia between April 4, 2014 and August 31, 2017. We enrolled adults (age ≥ 18

91   years) who presented with signs and symptoms concerning for TBM and already received a

92   lumbar puncture as part of routine care. Patients with unknown HIV status were offered HIV

93   testing as part of routine clinical care and not as part of the study. All potential study

94   subjects were examined by a study neurologist. After a lumbar puncture was completed,

95   study staff identified patients from the UTH microbiology laboratories who had ≥ 3 ml of

4

96   excess CSF remaining after routine testing composed of gram stain, India Ink stain,

97   Cryptococcoal antigen testing, and bacterial culture on a blood agar plate. Mycobacteria

98   growth indicator tube (MGIT) culture is currently not part of routine testing on all CSF.

99   Patients with a positive CSF bacterial gram stain, India Ink stain, Cryptococcal antigen test, or

100   positive bacterial culture were excluded from enrollment. A study nurse obtained written

101   informed consent from the patient or health care proxy for study enrollment to use excess

102   CSF from the lumbar puncture for additional *M. tuberculosis* testing. No lumbar puncture

103   was performed soley for study purposes or at the recommendation of study staff. The nurse

104   also documented demographic data, presenting symptoms, past medical history, and

105   medications through formal interview with the patient or health care proxy and review of

106   the medical record as explicity requested in the consent form. Study subjects then provided

107   an additional sample of whole blood and urine. A study neurologist conducted a structured

108   neurologic assessment with particular attention to focal abnormalities. We recorded

109   Medical Research Council severity using the established grading system: grade I (GCS score

110   15; no focal neurological signs), grade II (GCS score 11–14 or 15 with focal neurological

111   signs), or grade III (GCS score ≤10).(10) Study staff recorded inpatient mortality. A study

112   coordinator called patients or members of the household quarterly for one year to ask only

113   about patient survival. The study was approved by the University of Zambia School of

114   Medicine's Biomedical Research Ethics Committee and Beth Israel Deaconess Medical Center

115   Institutional Review Board.

116   **Laboratory testing**

117   Routine CSF testing at the UTH microbiology and biochemistry laboratories includes cell

118   count, gram stain, India Ink stain, bacterial culture, fungal culture, total protein and glucose

119   concentration. When the UTH biochemistry laboratory did not have necessary reagents to

5

120   run CSF total protein, samples were processed at a private medical laboratory in Lusaka.

121   Both the UTH laboratory and private laboratory are locally accredited. CSF Xpert MTB/RIF,

122   CSF LAM, urine LAM, and CSF TB culture were performed in the Zambart research

123   laboratories.

124   Study staff delivered 3 ml of CSF and 3 ml of urine in plain sterile tubes in a cooler filled with

125   ice packs to the Zambart research laboratories. In the laboratory, staff would bring the

126   samples to room temperature 1 hour prior to use. Using a sterile filtered tip pipette, a lab

127   technician removed 60 µl of CSF and urine and transferred the specimens to a LAM LFA.

128   When possible, two investigators blinded to clinical details and trained in LAM LFA

129   interpretation read the samples after 25-35 minutes in comparison to a kit reference card

130   according to manufacturer's instructions, and scored the test as positive, negative, or

131   indeterminate. If there was disagreement, the lowest reading was taken as the final result.

132   For 20% of the samples only one LAM LFA reader was available. Indeterminate samples were

133   treated as negative for purposes of the analysis.

134   The remaining CSF was centrifuged at 3500 g for 20 minutes. Supernatant was removed

135   leaving a pellet and 500 µl of residual fluid. The samples were then vortexed. One hundred

136   µl each was used to inoculate two separate MGIT tubes that were placed in a Bactec960

137   instrument for culture. Samples postive on MGIT culture were confirmed as *M. tuberculosis*

138   with the Capilia TB MPT64 Ag assay (Tauns Laboratories, Japan). Phosphate-buffered saline

139   was added to the remaining CSF to bring it to a volume of 500 µl. Laboratory staff then

140   added 1.5 ml of Xpert sample reagent. The specimen was incubated for 10 minutes at room

141   temperature. The specimen was then either shaken vigorously for 20 seconds or vortexed

142   for 10 seconds and was left to incubate for an additional 5 minutes. A laboratory technician

143   then transferred 2 ml of specimen/sample reagent mixture to an Xpert cartridge for testing.

144   Results were rated as negative or positive for *M. tuberculosis* with or without rifampicin

145   resistance according to manufacturer's instructions. Samples found to be rifampicin resistant

146   by Xpert MTB/RIF had confirmatory drug susceptibility testing (DST) for rifampicin and

147   isoniazid conducted separately. Xpert MTB/RIF results were placed in the patient's file by

148   study staff within 24 hours of the test being completed.

149   **Diagnostic categorization**

150   We classified patients as having definite or probable TBM to evaluate the performance of

151   the Xpert MTB/RIF, CSF LAM, and Urine LAM. Definite TBM was defined as a CSF sample that

152   was MGIT culture positive. Probable TBM was defined as patients with a CSF white blood

153   cell count between 10-500, CSF total protein > 100 mg/dl, and CSF glucose < 40 mg/dl. This

154   was adapted from a uniform case definition of probable TBM for use in clinical research.(11)

155   **Statistical analyses**

156   Descriptive measures (such as median, interquartile range, frequencies, and percentages)

157   were used to summarize the data. Wilcoxon rank sum and Fisher exact tests were used to

158   compare continuous and categorical, respectively, study variables between groups.

159   The primary outcome of interest was the performance of Xpert MTB/RIF, CSF LAM, and urine

160   LAM for the diagnosis of TBM relative to CSF MGIT culture as the gold standard. Sensitivity

161   and specificity together with their corresponding 95% confidence intervals were calculated

162   to assess the predictive accuracy of the diagnostic tests (CSF Xpert MTB/RIF, CSF LAM, urine

163   LAM, CSF total protein and glucose) at specific thresholds. Exact binomial confidence

164   intervals were used to estimate confidence intervals for sensitivities, and specificities.

165   Receiver operating characteristic (ROC) curves and the area under the ROC curves were used

166   to evaluate and compare the overall predictive accuracy of the diagnostic tests. Secondary

167   outcomes of interest were factors associated with inpatient and one-year mortality.

7

168   Univariate and multivariate logistic regression models were utilized to assess the clinical

169   predictors of inpatient and one-year mortality. A significance level of <0.10 was used to

170   select variables for the multivariate analyses. The inpatient mortality rate for the HIV-

171   infected TBM patients were compared to the inpatient mortality rate of HIV-infected TBM

172   controls from a prior study at the same facility where PCR results were not readily available

173   (12) to assess the impact of real-time use of CSF Xpert MTB/RIF. All P values were 2-sided

174   and considered statistically significant if <0.05.

175   **Results**

176   **Patients characteristics**

177   Five-hundred and fifty patients were enrolled into the study as shown in Figure-1. Of those,

178   474 (86.2%) were HIV-infected. Eleven patients (2%) had an unknown HIV status because

179   they either declined testing or died prior to testing. The incidence of TB culture positive CSF

180   was higher among HIV-infected patients (20.5% vs 12.3%) though this difference was not

181   statistically significant.

182   Table 1 shows the patient demographics stratified by HIV status. The median age of patients

183   who were TBM culture positive was significantly higher in HIV-positive patients compared to

184   HIV-negative patients (P = 0.005). There were significantly more HIV-infected men 64/190

185   (33.7%) than women 33/188 (17.6%) diagnosed with TBM (P = 0.006). There was no

186   significant difference in CD4+ T-cell counts or age between these two groups. In HIV-

187   negative patients with TBM, the median CD4+ T-cell count was < 200 cells/µl and

188   significantly less than HIV-negative patients without TBM. Overall, more patients with a

189   negative CSF culture had received a prior diagnosis of TB though this difference was only

190   significant in the HIV-infected population. For those patients taking ART at the time of

191   enrollment, TBM patients were on ART for a significantly shorter period than those who did

8

192   not have TBM. Fifty-one percent of HIV-infected men with TBM received their HIV diagnosis

193   within one week of enrollment compared with 27% of females (P = 0.03). Among HIV-

194   infected patients with TBM, there was no significant difference between men and women

195   for a prior diagnosis of TB, taking ART, or inpatient mortality.

196   **Accuracy of diagnostic tests for TBM**

197   The sensitivity/specificity for the diagnostic tests were CSF Xpert MTB/RIF 52.9%/94.2%, CSF

198   LAM 21.9%/94.2%, urine LAM 24.1%/76.1%. The combination of CSF glucose < 40 mg/dL

199   and total protein > 100 mg/dL had sensitivity/specificity of 66.3%/90%. Figure-2 shows the

200   received operating characteristic (ROC) curves with area under the curve (AUC) for CSF Xpert

201   MTB/RIF, CSF LAM, Urine LAM, CSF glucose, and CSF total protein in comparison to the gold

202   standard of CSF TB culture. The AUCs were as follows: CSF Xpert MTB/RIF 0.75 (0.69 - 0.81; P

203   < 0.0001), CSF LAM 0.59 (0.54 - 0.64; P = 0.001), Urine LAM 0.53 (0.47 - 0.58; P = 0.94), CSF

204   total protein 0.81 (0.76 - 0.87; P = 0.001), CSF glucose 0.84 (0.78 - 0.90; P < 0.0001). Based

205   on the Youden Index, the optimal cut off values when equally weighting for sensitivity and

206   specificity for the diagnostic tests with continuous variables were as follows: CSF glucose <

207   36 mg/dl (sensitivity 75% specificity 87%), CSF total protein > 110 mg/dl (sensitivity 82%

208   specificity 75%). Figure-2 also shows the performance of a diagnostic model that includes

209   CSF Xpert MTB/RIF, CSF LAM, CSF total protein, and CSF glucose with an AUC of 0.90 (0.84 -

210   0.94; P<0.0001).

211   CSF LAM detected 5 cases missed by Xpert MTB/RIF. When both tests were used in

212   combination in HIV-infected patients the sensitivity was 59.1% with a specificity of 93.2%.

213   We identified 14 cases of probable TBM. The inclusion of these cases did not significantly

214   change the performance of Xpert MTB/RIF, CSF LAM, or urine LAM for the diagnosis of TBM.

215   Of the CSF LAM indeterminate samples, 8% (1/13) were CSF TB culture positive. Of the urine

216   LAM indeterminate samples 19% (3/16) were CSF TB culture positive. A sensitivity analysis

217   treating these 4 indeterminate samples as positive did not significantly change the overall

218   test performance for CSF or urine LAM.

219   **Inpatient and one-year mortality**

220   Table-2 demonstrates the risk factors for inpatient and one-year mortality. No patients were

221   lost during the period they were hospitalized but 113/550 (20.5%) were lost to follow-up

222   after 1 year post-discharge. One year mortality outcomes among those who could be

223   tracked was 58.9%. A sensitivity analysis treating all lost to follow-ups as having survived

224   showed a one year mortality rate of 46.7%. A sensitivity analysis treating all lost to follow-

225   ups as having died showed a one year mortality rate of 67.3%. Longer time period since HIV

226   diagnosis, longer duration of treatment with ART, and a higher CD4+ T-cell count at the time

227   of hospitalization were associated with surviving to discharge. Positive CSF TB culture and a

228   higher Medical Research Council (MRC) TBM severity grade (10) were associated with

229   inpatient mortality. Higher CD4+ T-cell count was associated with lower odds of one-year

230   mortality. TB positive CSF culture and higher MRC TBM severity grade were associated with

231   higher odds of one-year mortality. In the multivariate analysis in Table-2 only the MRC grade

232   remained significant for both inpatient mortality and one-year mortality. Higher CD4 count

233   was associated with one-year survival on multivariate regression analysis.

234   The cumulative inpatient and one-year mortality for TBM patients that were diagnosed as

235   CSF Xpert MTB/RIF positive was 49% and 76%, respectively. This is in comparison to an

236   inpatient and one-year mortality of 37% and 62% for patients that were Xpert MTB/RIF

237   negative but CSF TB culture positive. These differences were not statistically significant.

238   There was also no significant difference in MRC grade between CSF Xpert MTB/RIF positive

239   and negative TBM patients. Xpert MTB/RIF detected rifampicin resistance on 6/55 (11%)

240   positive samples. The cumulative inpatient and one-year mortality for these patients was

241   67% and 83%, respectively. The inpatient mortality of 49% in this study was not significantly

242   different from the inpatient mortality of 46% from a prior study at the same institution

243   where PCR results from TBM patients were not shared with the treating team in real

244   time.(12)

245   **Discussion**

246   **Xpert MTB/RIF performance and impact on mortality**

247   Xpert MTB/RIF performed similarly compared to what has been previously reported for the

248   diagnosis of TBM.(13, 14) It misses almost half of definite TBM cases which results in

249   frequent empiric treatment based on diagnostic algorithms or provider suspicion. There

250   was no difference in inpatient mortality among HIV-positive patients in this study when

251   Xpert MTB/RIF results were turned over to the treating team within 24 hours compared to a

252   prior PCR study in the same institution when results were not immediately available for

253   clinical care purposes.(12) This may reflect the high rate of empiric treatment in cases that

254   were Xpert negative.

255   **Utility of LAM and CSF Biochemistry**

256   CSF LAM testing was highly specific and detected 5 cases that were missed by Xpert

257   MTB/RIF. The additional detection of cases by CSF LAM comes with little additional

258   resources given LAM's cost and ease of use. These findings highlight that there are novel

259   CSF biomarkers for TBM that can be used to enhance the diagnosis.

260   Based on the performance of urine LAM, it does not appear to be of value as a stand-alone

261   test for the diagnosis of TBM. However, urine LAM was positive in 51 HIV-infected patients

262   with CD4+ T-cell count < 200 who had a negative CSF culture. In a prior study, urine LAM had

263   a sensitivity of 46% and a specificity of 93% for the diagnosis of disseminated TB in HIV

264   patients with advanced immunosuppression.(15) Thus, it is likely that the majority of these

265   51 patients had disseminated TB with unclear CNS involvement. In a recently developed

266   uniform case definition of TBM, clinical research patients have been assigned to categories

267   of probable or possible TBM based on a numerical score derived from a combination of

268   clinical presentation, CSF findings, neuroimaging, and evidence of TB elsewhere.(11) Urine

269   LAM is currently not factored into this scoring system as evidence of TB elsewhere. Based on

270   our findings, we feel it deserves strong consideration for inclusion.

271   CSF glucose and total protein demonstrated the highest AUC compared with all of the other

272   diagnostic tests. The optimal cutoff values of a CSF glucose < 36 mgl/dL and CSF total protein

273   > 110 mg/dL established by the Youden Index when providing equal weight to sensitivity and

274   specificity are consistent with the cutoff values established in the consensus paper for the

275   diagnosis of TBM for clinical research.(11) It is important to note, that cryptococcal

276   meningitis and bacterial meningitis patients were screened out in our population.

277   **High mortality**

278   The inpatient and one-year mortality among TBM patients was extremely high. MRC grade

279   was the only significant factor associated with both inpatient and one-year mortality in the

280   multivariate model suggesting that the major driver of mortality is the advanced stage of

281   illness at the time of initial medical evaluation. Additionally, there were significantly more

282   men than women diagnosed with TBM in this study. These findings support numerous

283   research studies that document poor health seeking behavior among HIV-infected males

284   resulting in presentation during advanced stages of illness.(16)

285   **HIV-negative TBM patients**

286   There was a relatively small number of HIV-negative patients diagnosed with TBM. These

287   patients were younger than the HIV-positive population and had similarly low CD4+ T-cell

288   counts. The etiology for the CD4+ lymphocytopenia in this population was likely TB infection

289   itself rather than another source of immunodeficiency.(17)

290   **Limitations**

291   This study has a number of limitations. There is stigma around lumbar punctures in Zambia

292   that leads to a high refusal rate of nearly 25%.(18) As a result, this cohort may not be

293   representative of all patient with TBM but rather only those who provided CSF. This study

294   used approximately 3 ml of centrifuged CSF for Xpert MTB/RIF testing. It has been shown

295   that larger volumes of centrifuged CSF can increase Xpert MTB/RIF sensitivity.(19)

296   Additionally, CSF TB culture is widely accepted to be an imperfect gold standard for the

297   diagnosis of TBM especially in patients with paucibacillary disease in whom false negative

298   cultures are likely problematic. As a result, the true performance of Xpert MTB/RIF, CSF LAM,

299   and urine LAM are likely better than reported here. A consensus definition of probable and

300   possible TBM was developed for clinical research in 2010.(11) Data collection in this study

301   did not provide sufficient information to use the recommended definitions. An adapted

302   definition of probable TBM was used and we did not classify patients with possible TBM.

303   **Future research**

304   GeneXpert MTB/RIF Ultra (Xpert Ultra) is the next generation Xpert MTB/RIF test that has

305   shown increased sensitivity for the diagnosis of TBM. It has been endorsed by the WHO as

306   the test of choice for the diagnosis of TBM.(20) A larger study that incorporates Xpert Ultra

307   would be beneficial to see if it results in a decrease in TBM-associated mortality. In TB

308   endemic settings, many TBM patients are empirically commenced on TB medication based

309   on clinical presentation and limited laboratory data. It is possible that Xpert Ultra will make

310   no difference on mortality in these settings. This is an important question given the

311   significant financial resources Xpert Ultra requires for scale up in LMIC.

13

312

313 **Conflict of Interest Statements**

314 Igor J. Koralnik has served on scientific advisory boards for Hoffman La Roche, Glaxo Smith

315 Klyne and Merck Serono and received consulting fees from Bristol Myers Sqibb, Ono

316 Pharmaceuticals, Merck Serono, Hoffman La Roche, GlaxoSmithKline, Perseid Therapeutics,

317 Vertex Pharmaceutical, Johnson & Johnson. He is an editorial board member for the Journal

318 of NeuroVirology and receives royalties from UpToDate for topics on the management of

319 HIV, CNS mass lesions, progressive multifocal leukoencephalopathy.

320 Gretchen L. Birbeck has served as a consultant for GlaxoSmithKline. She is on the Advisory

321 Board for the US NIH Fogarty International Center, the Board of Directors for the American

322 Neurological Association, and Editorial Board for BMC Medicine and Neurology.

323 Keertan Dheda has received speaker fees from Alere.

324 Omar K. Siddiqi, Musie Ghebremichael, Eugene Mubanga, Shawn Love, Clayton Buback,

325 Barry Kosloff, Helen Ayles, and Masharip Atadzhanov report no conflict of interest.

326

327

328 **Acknowledgements**

329 The authors thank the UTH microbiology lab for technical assistance as well as the patients

330 and their families.

331

332

333

334

335

336

337

338

References

340

341 1. Clarridge JE, 3rd, Shawar RM, Shinnick TM, Plikaytis BB. 1993. Large-scale use of
342   polymerase chain reaction for detection of Mycobacterium tuberculosis in a routine
343   mycobacteriology laboratory. J Clin Microbiol 31:2049-56.
344 2. Garg RK. 1999. Tuberculosis of the central nervous system. Postgrad Med J 75:133-
345   40.
346 3. Bahr NC, Boulware DR. 2014. Methods of rapid diagnosis for the etiology of
347   meningitis in adults. Biomark Med 8:1085-103.
348 4. WHO. 2013. Automated real-time nucleic acid amplification technology
349   for rapid and simultaneous detection of tuberculosis and rifampicin
350   resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary
351   and extrapulmonary tuberculous in adults and children. Policy update. World Health
352   Organizaton Press, Geneva.
353 5. Churchyard GJ, Stevens WS, Mametja LD, McCarthy KM, Chihota V, Nicol MP, Erasmus
354   LK, Ndjeka NO, Mvusi L, Vassall A, Sinanovic E, Cox HS, Dye C, Grant AD, Fielding KL.
355   2015. Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for
356   tuberculosis: a cluster-randomised trial embedded in South African roll-out of Xpert
357   MTB/RIF. Lancet Glob Health 3:e450-e457.
358 6. Theron G, Zijenah L, Chanda D, Clowes P, Rachow A, Lesosky M, Bara W, Mungofa S,
359   Pai M, Hoelscher M, Dowdy D, Pym A, Mwaba P, Mason P, Peter J, Dheda K, team T-
360   N. 2014. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF
361   testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised,
362   controlled trial. Lancet 383:424-35.
363 7. Peter JG, Zijenah LS, Chanda D, Clowes P, Lesosky M, Gina P, Mehta N, Calligaro G,
364   Lombard CJ, Kadzirange G, Bandason T, Chansa A, Liusha N, Mangu C, Mtafya B, Msila
365   H, Rachow A, Hoelscher M, Mwaba P, Theron G, Dheda K. 2016. Effect on mortality of
366   point-of-care, urine-based lipoarabinomannan testing to guide tuberculosis
367   treatment initiation in HIV-positive hospital inpatients: a pragmatic, parallel-group,
368   multicountry, open-label, randomised controlled trial. Lancet 387:1187-97.
369 8. Patel VB, Singh R, Connolly C, Kasprowicz V, Zumla A, Ndungu T, Dheda K. 2010.
370   Comparison of a clinical prediction rule and a LAM antigen-detection assay for the
371   rapid diagnosis of TBM in a high HIV prevalence setting. PLoS One 5:e15664.
372 9. Cox JA, Lukande RL, Kalungi S, Van Marck E, Lammens M, Van de Vijver K, Kambugu
373   A, Nelson AM, Colebunders R, Manabe YC. 2015. Accuracy of Lipoarabinomannan
374   and Xpert MTB/RIF Testing in Cerebrospinal Fluid To Diagnose Tuberculous
375   Meningitis in an Autopsy Cohort of HIV-Infected Adults. J Clin Microbiol 53:2667-73.

376 10. Wilkinson RJ, Rohlwink U, Misra UK, van Crevel R, Mai NTH, Dooley KE, Caws M, Figaji
377   A, Savic R, Solomons R, Thwaites GE, Tuberculous Meningitis International Research
378   C. 2017. Tuberculous meningitis. Nat Rev Neurol 13:581-598.
379 11. Marais S, Thwaites G, Schoeman JF, Torok ME, Misra UK, Prasad K, Donald PR,
380   Wilkinson RJ, Marais BJ. 2010. Tuberculous meningitis: a uniform case definition for
381   use in clinical research. Lancet Infect Dis 10:803-12.
382 12. Siddiqi OK, Ghebremichael M, Dang X, Atadzhanov M, Kaonga P, Khoury MN, Koralnik
383   IJ. 2014. Molecular diagnosis of central nervous system opportunistic infections in
384   HIV-infected Zambian adults. Clin Infect Dis 58:1771-7.
385 13. Patel VB, Theron G, Lenders L, Matinyena B, Connolly C, Singh R, Coovadia Y, Ndung'u
386   T, Dheda K. 2013. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for
387   tuberculous meningitis in a high burden setting: a prospective study. PLoS Med
388   10:e1001536.
389 14. Nhu NT, Heemskerk D, Thu do DA, Chau TT, Mai NT, Nghia HD, Loc PP, Ha DT, Merson
390   L, Thinh TT, Day J, Chau N, Wolbers M, Farrar J, Caws M. 2014. Evaluation of
391   GeneXpert MTB/RIF for diagnosis of tuberculous meningitis. J Clin Microbiol 52:226-
392   33.
393 15. Shah M, Hanrahan C, Wang ZY, Dendukuri N, Lawn SD, Denkinger CM, Steingart KR.
394   2016. Lateral flow urine lipoarabinomannan assay for detecting active tuberculosis in
395   HIV-positive adults. Cochrane Database Syst Rev
396   doi:10.1002/14651858.CD011420.pub2:CD011420.
397 16. Mills EJ, Beyrer C, Birungi J, Dybul MR. 2012. Engaging men in prevention and care
398   for HIV/AIDS in Africa. PLoS Med 9:e1001167.
399 17. Skogmar S, Schon T, Balcha TT, Jemal ZH, Tibesso G, Bjork J, Bjorkman P. 2013. CD4
400   cell levels during treatment for tuberculosis (TB) in Ethiopian adults and clinical
401   markers associated with CD4 lymphocytopenia. PLoS One 8:e83270.
402 18. Elafros MA, Belessiotis C, Birbeck GL, Bond V, Sikazwe I, Kvalsund MP. 2017. Barriers
403   to Lumbar Puncture in Zambia: Insights into the 'Tap Gap', abstr 350332. American
404   Neurological Association Annual Meeting, San Diego, CA,
405 19. Bahr NC, Marais S, Caws M, van Crevel R, Wilkinson RJ, Tyagi JS, Thwaites GE,
406   Boulware DR, Tuberculous Meningitis International Research C. 2016. GeneXpert
407   MTB/Rif to Diagnose Tuberculous Meningitis: Perhaps the First Test but not the Last.
408   Clin Infect Dis 62:1133-5.
409 20. Bahr NC, Nuwagira E, Evans EE, Cresswell FV, Bystrom PV, Byamukama A, Bridge SC,
410   Bangdiwala AS, Meya DB, Denkinger CM, Muzoora C, Boulware DR, Team A-CT. 2018.
411   Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-
412   infected adults: a prospective cohort study. Lancet Infect Dis 18:68-75.
413

414

415

416

417

418

419

420

421

422   Figure Legends

423

424   **Figure 1 -** Flow sheet of patient recruitment and percentages with positive CSF

425   culture, CSF Xpert, CSF LAM, and urine LAM testing based on HIV status.

426

427   Figure-1 - Abbreviations: TBM, tuberculous meningitis; MTB, *Mycobacterium*

428   *tuberculosis*; Cx, culture; CSF, cerebrospinal fluid; Xpert, GeneXpert MTB/RIF; RIF,

429   rifampicin; LAM, lipoarabinomannan.

430

431   **Figure-2:** Receiver operating curves (ROC) for diagnostic tests to distinguish TBM

432   from non-TBM cases. The model (blue line) demonstrates the area under curve

433   incorporating all variables except for urine LAM.

434

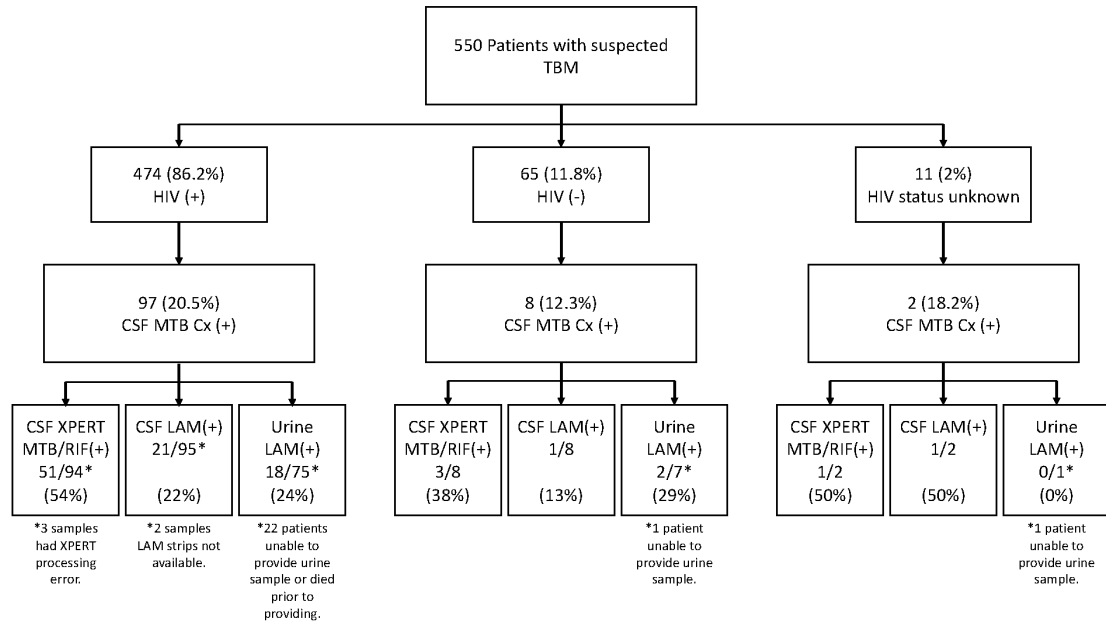435   Figure-2 - Abbreviations: TP, total protein.

436

437

```
                        ┌──────────────────────┐
                        │ 550 Patients with     │
                        │ suspected TBM         │
                        └──────────────────────┘
```

| 474 (86.2%) HIV (+) | 65 (11.8%) HIV (-) | 11 (2%) HIV status unknown |

| 97 (20.5%) CSF MTB Cx (+) | 8 (12.3%) CSF MTB Cx (+) | 2 (18.2%) CSF MTB Cx (+) |

| CSF XPERT MTB/RIF(+) 51/94* (54%) | CSF LAM(+) 21/95* (22%) | Urine LAM(+) 18/75* (24%) | CSF XPERT MTB/RIF(+) 3/8 (38%) | CSF LAM(+) 1/8 (13%) | Urine LAM(+) 2/7* (29%) | CSF XPERT MTB/RIF(+) 1/2 (50%) | CSF LAM(+) 1/2 (50%) | Urine LAM(+) 0/1* (0%) |

*3 samples had XPERT processing error.

*2 samples LAM strips not available.

*22 patients unable to provide urine sample or died prior to providing.

*1 patient unable to provide urine sample.

*1 patient unable to provide urine sample.

Table 1 - Patient Demographics

	HIV-positive TBM culture positive (n=97)	TBM culture negative (n=378)	P value	HIV-negative TBM culture positive (n=8)	TBM culture negative (n=57)	P value
Age y, median (IQR)	35 (30-41)	36 (30-43)	0.36	25 (24-27)	41 (28-55)	0.006
Gender, male	64 (66%)	190 (50%)	0.006	3 (38%)	27 (47%)	0.88
CD4+ T cells/µL, median (IQR)	104 (45-167)	129 (42-346)	0.06	165 (142-264)	593 (498-842)	0.003
Prior TB diagnosis	20 (21%)	163 (43%)	< 0.0001	0 (0%)	5 (9%)	0.38
Days since HIV Diagnosis, median days (IQR)	14 (3-152)	270 (30-1825)	< 0.0001	-	-	-
Taking ART (%)	33 (34%)	231 (61%)	< 0.0001	-	-	-
Duration of ART, median days (IQR)	105 (19-723)	730 (60-1825)	< 0.0001	-	-	
MRC TBM severity grade						
Grade 1	13 (13%)	-	-	0 (0%)	-	-
Grade 2	44 (46%)			3 (37.5%)		
Grade 3	32 (33%)			3 (37.5%)		
Unknown*	8 (8%)			2 (25%)		
Inpatient Mortality	42 (43%)	83 (22%)	0.0002	2 (25%)	14 (25%)	0.68
One-year Morality	59 (61%)	165 (44%)	0.003	2 (25%)	26 (46%)	0.47

Abbreviations: MRC, Medical Research Council

* incomplete neurological examination due to patient's inability to cooperate or death prior to examination.

ROC	AUROC
CSF GLUCOSE	0.84
CSF LAM	0.59
CSF TP	0.81
GeneXpert MTB/RIF	0.75
Model	0.90
URINE LAM	0.53
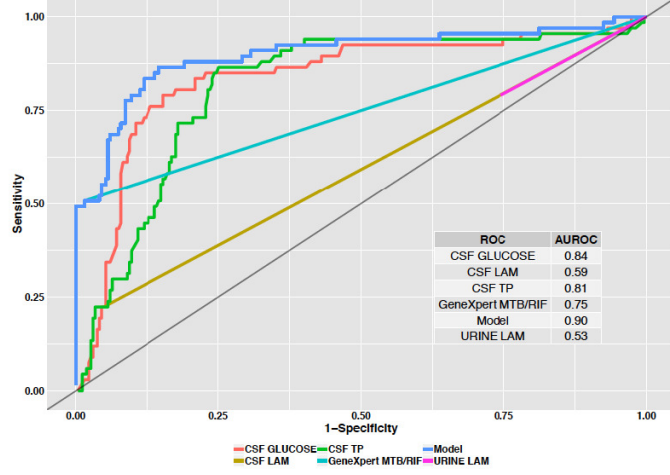
Table 2 - Variables Associated with Inpatient and One-Year Mortality
in Univariate and Multivariate Analysis

Variable (N=550)	Univariate Analysis Odds Ratio (95% CI)	P Value	Multivariate Analysis Odds Ratio (95% CI)	P Value
Inpatient Mortality Categorical (n)				
Female (261)	0.81 (0.56 - 1.19)	0.29		
HIV (475)	1.02 (0.57 - 1.85)	0.93		
Taking ART (190)	0.85 (0.57 -1.26)	0.42		
History of TB (189)	0.75 (.50 - 1.12)	0.17		
TB positive CSF culture (107)	2.51 (1.61 - 3.90)	<0.0001	2.38 (0.92 - 6.14)	0.07
Continuous				
Age	0.99 (.97 - 1.0005)	0.19		
Duration of HIV diagnosis (days)	0.9995 (0.9993 - 0.9998)	0.001	0.999 (.998 - 1.000)	0.20
Duration of ART (days)	0.9996 (0.9994 - 0.9999)	0.02	1.0002 (0.9994 - 1.000)	0.60
CD4	0.9990 (0.9982 - 0.9998)	0.02	0.9996 (0.9981 - 1.0012)	0.70
MRC severity	2.933 (2.103 - 4.091)	<0.001	2.40 (1.34 - 4.27)	0.003
One-Year Mortality Categorical (n)				
Female (261)	0.82 (0.56 – 1.21)	0.32		
HIV (475)	1.37 (.77 – 2.42)	0.28		
Taking ART (190)	1.25 (.83 – 1.86)	0.28		
History of TB (189)	1.47 (.97 – 2.24)	0.07	0.62 (0.35 - 1.10)	0.10
TB positive CSF culture (107)	1.68 (1.03 - 2.74)	0.04	1.28 (0.67 - 2.43)	0.45
Continuous				
Age	1.01 (.99 – 1.03)	0.33		
Duration of HIV diagnosis (days)	0.9998 (0.9996 – 0.9999)	0.05	0.9999 (0.9997 – 1.0001)	0.68
Duration of ART (days)	0.9999 (.9996 – 1.0001)	0.45		
CD4	0.9985 (0.9978 – 0.9993)	0.0001	0.998 (0.997 - 0.999)	0.04

MRC severity	2.08 (1.52 - 2.86)	<0.0001	1.72 (1.16 - 2.55)	0.006