1   **Title Page**

2   **Title:** HIV-associated *M. tuberculosis* blood stream infection is under-diagnosed by a single

3   blood culture.

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18 **Running head:** TB blood stream infections missed by a single blood culture

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Abstract

We assessed the additional diagnostic yield for *M. tuberculosis* blood stream infection (MTB BSI) from doing more than one TB blood culture in HIV-infected inpatients. In a retrospective analysis of two cohorts based in Cape Town, South Africa, 72/99 (73%) patients with MTB BSI were identified by the first of two blood cultures during the same admission, with 27/99 (27%, 95%CI 18 to 36%) negative on first culture but positive on second. In a prospective evaluation of up to 6 blood cultures over 24 hours, 9 out of 14 patients with MTB BSI (65%) grew *M. tuberculosis* on their first blood culture; 3 more patients (21%) were identified by a second independent blood culture at the same time point, and the remaining 2 diagnosed only on 4th and 6th blood cultures respectively. Additional blood cultures increase the yield for MTB BSI, similar to what is reported for non-mycobacterial BSI.

Introduction

Mycobacterium tuberculosis blood stream infection (MTB BSI) is a frequent and life-threatening presentation of tuberculosis in high HIV burden settings. Published cohorts of HIV-1-infected inpatients with suspected tuberculosis show a point prevalence ranging from 9%[1] to 38%[2] on a single blood culture. MTB BSI has been associated with severe sepsis,[2-5] and high risk of death,[5-8] in people living with HIV.

Several methods for recovery of mycobacteria from blood exist, including a manual solid-media based lysis-centrifugation system (Wampole™ Isostat®/Isolator ™ Microbial System, BioMerieux, Durham, NC, USA), and automated liquid-media systems (MB BacT/Alert®, Inverness, Waltham, MA, USA; Bactec Myco/F Lytic®, BD Microbiology Systems, Sparks, MD). Broth-based systems are probably more sensitive than agar.[8, 9] Beyond this, there is limited data on how to optimise blood culture for diagnosis of MTB BSI.

By contrast, evidenced-based recommendations on the number, timing, and volume of blood cultures are available for non-mycobacterial BSI, where a single 10-mL blood culture will detect

48   73% and four samples will detect 90–95% of patients with documented bacteraemia.(10, 11)

49   Almost all published studies of MTB BSI have performed a single 3-5ml liquid mycobacterial

50   culture, and the proportion of MTB BSI missed by this strategy is unknown. We estimated the

51   diagnostic yield of additional (>1) blood cultures for MTB in two ways:

52   1.   retrospectively, in two large cohort studies of HIV-associated TB conducted in hospital

53   settings; and

54   2.   in a prospective evaluation of serial blood cultures in HIV-infected patients at high risk

55   of MTB BSI in a hospital setting.

56   **Materials and methods**

57   Ethical approval was granted by the Human Research Ethics Committee University of Cape

58   Town (Ref 001/2012, Ref 057/2013, and 057/2013 amendment 24/04/2016). Both the

59   cohort studies and the prospective evaluation were carried out in the Western Cape, South

60   Africa, a setting in which HIV and TB are the most common causes of death among adults,

61   despite a well-functioning anti-retroviral programme.(12) The GF Jooste Hospital TB (JHTB)

62   study recruited unselected HIV-infected patients newly admitted to acute medical services

63   at GF Jooste Hospital without a known TB diagnosis and not on anti-TB therapy. These

64   patients underwent extensive microbiological screening for TB including a single 5ml BD

65   Bactec Myco/F Lytic blood culture (BD, Sparks, MD) on the day of admission.(13) The

66   Khayelitsha Hospital (KHTB) study recruited HIV-infected patients admitted with symptoms

67   suggestive of active tuberculosis and a CD4 count less than 350 cells/mm^3, and also

68   performed a routine 3-5ml Bactec Myco/F Lytic blood culture prior to start of anti-TB

69   therapy.(14) Both these hospitals had access to mycobacterial blood culture investigations

70   through the National Health Laboratory Service (NHLS). A subset of patients in both cohorts

71   had an additional MTB blood culture which was requested by their admitting medical team

72   if clinically indicated (local guidelines recommend TB blood culture if CD4 count is less than

73   100 cells/mm^3, in a patient with TB symptoms where there is difficulty obtaining sputum

74   samples for TB testing or the sputum Xpert MTB/RIF assay is negative, and cultures are

75   generally sent before start of anti-TB therapy). By interrogating the NHLS electronic

76 database we identified the subset of patients in both cohorts who had a second BD Bactec

77 Myco/F Lytic blood culture carried out as part of routine care during the same admission as

78 their study recruitment.

79 To enrich recruitment to the prospective study of serial blood cultures, we used data from

80 n=350 KHTB patients to develop a model predicting MTB BSI in patients using only clinical

81 variables available on day of admission to Khayelitsha Hospital.(15) This model used an

82 ensemble machine learning approach combining logistic regression, random forest, and

83 support vector machine methods, and gave Receiver Operator Characteristic (ROC) curve

84 area under curve 0.86 in a test data set comprising 66 KHTB patients not used in model

85 training. This ensemble model was packaged in a web-based application available at the

86 patient bedside via a smart phone.(16)

87 Between 21 June 2016 and 19 October 2016, on weekdays Monday-Thursday, all HIV-

88 infected patients newly admitted to Khayelitsha Hospital with CD4 count < 350 cells/uL and

89 suspected TB but not yet started on anti-TB therapy, were screened using the MTB BSI

90 prediction app. Patients with predicted probability greater than 0.56 who gave informed

91 consent, underwent 3 venesections over a 24-hour period: immediately before (0 hours), 4-

92 8 hours after, and 22-24 hours after first dose of anti-TB therapy. At each of these

93 venesections, 5ml of peripheral blood was directly inoculated into a Myco/F Lytic BACTEC

94 (BD, Sparks, MD) bottle, while 5ml was collected in a sodium heparin tube, immediately

95 centrifuged for 25 minutes at 3000 G, and the resulting cell pellet (red cells and buffy coat)

96 inoculated into a Myco/F Lytic bottle. Samples were transported to an NHLS TB laboratory

97 in Cape Town for incubation the same day. Isolate identity was confirmed in all cases by

98 secondary Löwenstein–Jensen slope culture, auramine acid-fast microscopy, and PCR / line

99 probe assay.

100 **Results**

101 Using data from two independent cohort studies - the GF Jooste Hospital TB (JHTB) study, and

102 the Khayelitsha Hospital TB (KHTB) study – we identified HIV-infected inpatients who had

103 multiple mycobacterial blood culture performed during a single admission to hospital with

104   suspected TB. More than one blood culture was recorded for 59/410 JHTB patients and

105   169/680 KHTB patients, giving n=228 total for analysis. Of these patients, 99/228 (43%) had at

106   least one blood culture positive for *M. tuberculosis* (20/59 in JHTB, and 79/169 in KHTB).

107   Overall, 72/99 (0.73; 95%CI = 0.64 to 0.82) of MTB BSIs were identified on the first culture,

108   while 27/99 (0.27; 95%CI = 0.18 to 0.36) had negative first culture but grew *M. tuberculosis* on

109   the second (table 1).

110   To further investigate the yield of additional mycobacterial blood cultures, we carried out a

111   prospective evaluation of multiple blood cultures in sixteen HIV-infected inpatients at

112   Khayelitsha Hospital. Based on baseline clinical variables and a machine learning algorithm,

113   these patients were selected to have a high predicted probability of MTB BSI (see methods

114   section). A set of 2x 5ml blood cultures were performed at time 0 hours, 4-8 hours and 22-24

115   hours after first dose of anti-TB medication, with a total of 6 cultures over a 24-hour period.

116   Because of the potential for antimicrobial carry-over in blood, the second sample from each

117   pair had plasma removed before inoculation by centrifuge pelleted cells.

118   In total, 89 blood culture results were available in the 16 patients, with 7 results missing (figure

119   1). Of these 32/89 (36%) were positive for *M. tuberculosis*. Pelleted samples were more likely to

120   recover *M. tuberculosis* (19/44; 43%) than directly inoculated samples (13/45; 29%), but the

121   difference may have been due to chance (p = 0.189 by Fisher's exact test).

122   Two independent blood culture samples were obtained at each time point. At least one blood

123   culture was positive in 14/16 patients (87.5%). All isolates were identified as *M. tuberculosis*.

124   Nine (9/14, 64%) of these patients were culture positive on the first sample from the pair of

125   samples taken at 0-hours. A further 3/14 (21%) were culture negative on the first sample but

126   grew *M. tuberculosis* on the second sample taken at 0-hour timepoint. This meant that 12/14

127   (86%) of MTB BSI patients were identified by performing 2 independent cultures at the same

128   time-point before antibiotic therapy. The remaining two patients were identified on the 4th and

129   6th blood culture respectively (both pelleted before inoculation) (Figure 2).

130

131   **Discussion**

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132 Using two independent data sets, and a dedicated prospective evaluation, we estimate that

133 approximately two-thirds of MTB BSI is identified by one Myco/F Lytic blood culture (55% and

134 73% in the data sets, and 64% in the prospective evaluation). To our knowledge, this is the first

135 investigation of the additional yield associated with number of TB blood cultures. One previous

136 study randomised patients to 6 blood cultures at a single time point or 3 blood cultures at 2

137 time points (but the same total number of cultures), and found no difference in recovery of *M.*

138 *tuberculosis* between these arms.(8) This agrees with our prospective study finding that two

139 blood culture at the same time point increases yield compared to a single culture.

140 The importance of blood stream infection in HIV-associated tuberculosis disease is increasingly

141 recognised. 'Disseminated' tuberculosis causes 2 out of 5 inpatient deaths amongst HIV

142 infected inpatients in low-resource settings, and is undiagnosed prior to death in half of these

143 cases.(17) This dissemination is assumed to occur via the blood stream, and, despite practical

144 limitations, blood culture can be considered the gold-standard diagnostic test.(18) TB blood

145 culture positivity is associated with significantly higher mortality than blood culture negative

146 HIV-associated TB.(5-8) In the context of a generalised HIV epidemic, *M. tuberculosis* is the

147 most frequent blood culture isolate in hospitalised patients with severe sepsis.(2-5)

148 With few exceptions,(7, 8) reports characterising MTB BSI have relied on a single blood culture

149 for diagnosis; our results show this will have substantially under-estimated the true point

150 prevalence. This has implications for studies of HIV-associated TB pathogenesis, and supports

151 calls for increased clinical research focused on MTB BSI, including development of blood based

152 rapid diagnostics.(6) Where resources currently allow, an additional TB blood culture will

153 increase culture diagnosis in seriously unwell HIV-infected inpatients, particularly when sputum

154 is unobtainable.

155 Although several independent data sets have been used in this study, our findings are not

156 generalisable outside of high HIV-TB burden settings. Most high HIV-TB burden settings do not

157 have routine access to TB blood cultures. The findings are, however, useful to inform research

158 studies carried out in those settings. In this study we were unable to assess the relative cost-

159 effectiveness of additional blood cultures compared to other diagnostics – like induced sputum

160 or urine Xpert® MTB/RIF Ultra, and the urine-lipoarabinomannan assay – which are potentially

161 more accessible in low resource settings. The data presented in this report do, however, give an

162 opportunity to improve the reference standard in diagnostic performance studies assessing

163 these novel diagnostics in the most critically unwell HIV-associated TB patients.

164

165 **Conclusion**

166 We estimate that a single TB blood culture underestimates the point prevalence of MTB BSI by

167 approximately one-third. Additional blood cultures – even within the same 24-hour period –

168 increase diagnostic yield by a proportion similar to that seen for non-mycobacterial BSI. We

169 recommend, where resources allow, at least 2 blood cultures are taken when MTB BSI is

170 suspected in unwell HIV-infected adults, particularly when sputum is unobtainable. These can

171 be collected at the same time-point, prior to anti-TB treatment, in patients starting urgent

172 empirical therapy.

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Table 1. **Additional MTB BSI diagnoses made by second Myco/F Lytic culture in KHTB and JHTB**

cohort studies.

Cohort	Either culture positive	BC1+/BC2+	BC1+/BC2-	BC1-/BC2+	Proportion identified only by 2nd culture
KDHTB	79	44	17	18	0.23 (95%CI 0.14 to 0.32)
JHTB	20	7	4	9	0.45 (95%CI 0.23 to 0.67)
Combined	99	51	21	27	0.27 (95%CI 0.18 to 0.36)

Notes:

BC1 = 1st Myco/F Lytic blood culture; BC2 = 2nd Myco/F Lytic blood culture

(chronologically).

+ = positive; - = negative

95%CI = 95% confidence interval by binomial distribution

196

197 **FIGURE LEGENDS**

198 Figure 1. **Patient recruitment and blood culture availability in prospective study.**

199 Figure 2. **Cumulative yield for identifying MTB BSI with up to 6 serial Myco/F Lytic blood**
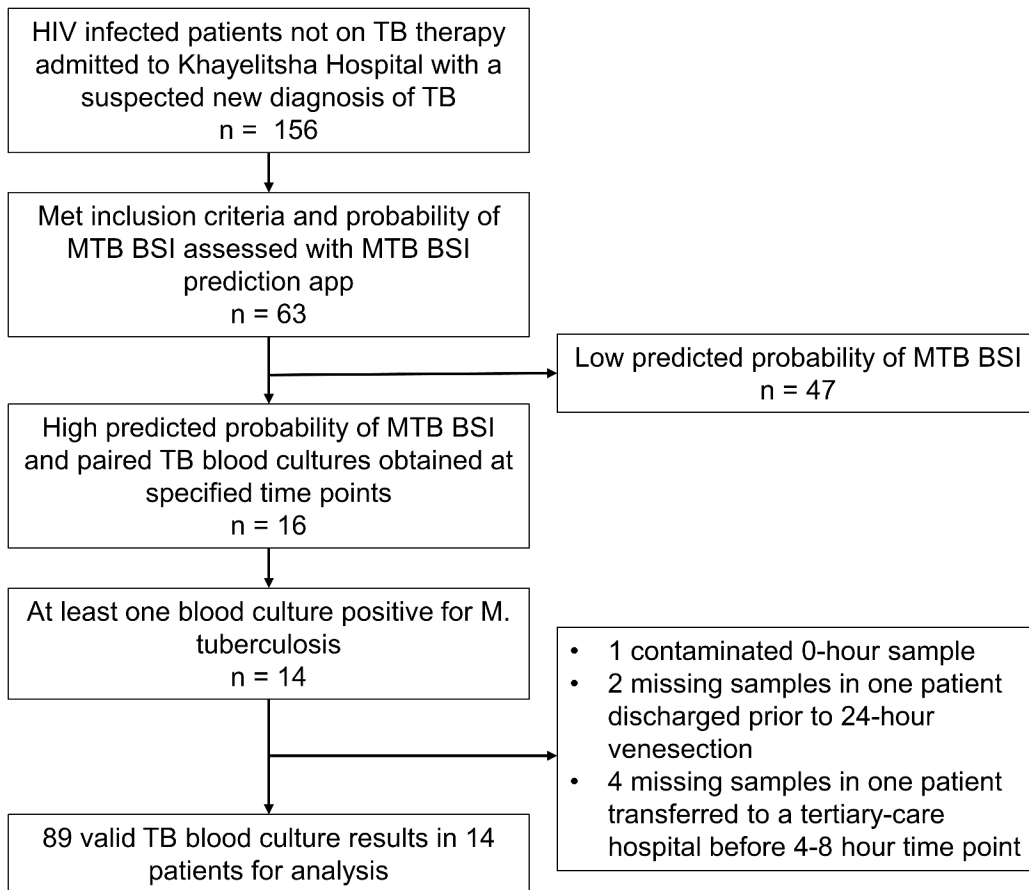200 **cultures.**

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┌─────────────────────────────────┐
│ HIV infected patients not on TB │
│ therapy admitted to Khayelitsha │
│ Hospital with a suspected new   │
│ diagnosis of TB                 │
│ n =  156                        │
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                │
                ▼
┌─────────────────────────────────┐
│ Met inclusion criteria and      │
│ probability of MTB BSI assessed │
│ with MTB BSI prediction app     │
│ n = 63                          │
└─────────────────────────────────┘
                │
                │              ┌───────────────────────────────────┐
                ├─────────────▶│ Low predicted probability of MTB  │
                │              │ BSI                               │
                │              │ n = 47                            │
                ▼              └───────────────────────────────────┘
┌─────────────────────────────────┐
│ High predicted probability of   │
│ MTB BSI and paired TB blood     │
│ cultures obtained at specified  │
│ time points                     │
│ n = 16                          │
└─────────────────────────────────┘
                │
                ▼
┌─────────────────────────────────┐
│ At least one blood culture      │
│ positive for M. tuberculosis    │
│ n = 14                          │
└─────────────────────────────────┘
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- 1 contaminated 0-hour sample
- 2 missing samples in one patient discharged prior to 24-hour venesection
- 4 missing samples in one patient transferred to a tertiary-care hospital before 4-8 hour time point

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┌─────────────────────────────────┐
│ 89 valid TB blood culture       │
│ results in 14 patients for      │
│ analysis                        │
└─────────────────────────────────┘
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