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Title Page

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

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

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21

22 **Abstract**

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

33

34 **Introduction**

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

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

46 By contrast, evidenced-based recommendations on the number, timing, and volume of blood
47 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/mm³, 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/mm³, 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**

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.

173

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185 design, data collection and interpretation, or the decision to submit the work for publication.

186

187

188 Table 1. **Additional MTB BSI diagnoses made by second Myco/F Lytic culture in KHTB and JHTB**
189 **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)

190

Notes:

191

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

192

(chronologically).

193

+ = positive; - = negative

194

95%CI = 95% confidence interval by binomial distribution

195

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.**

201

202

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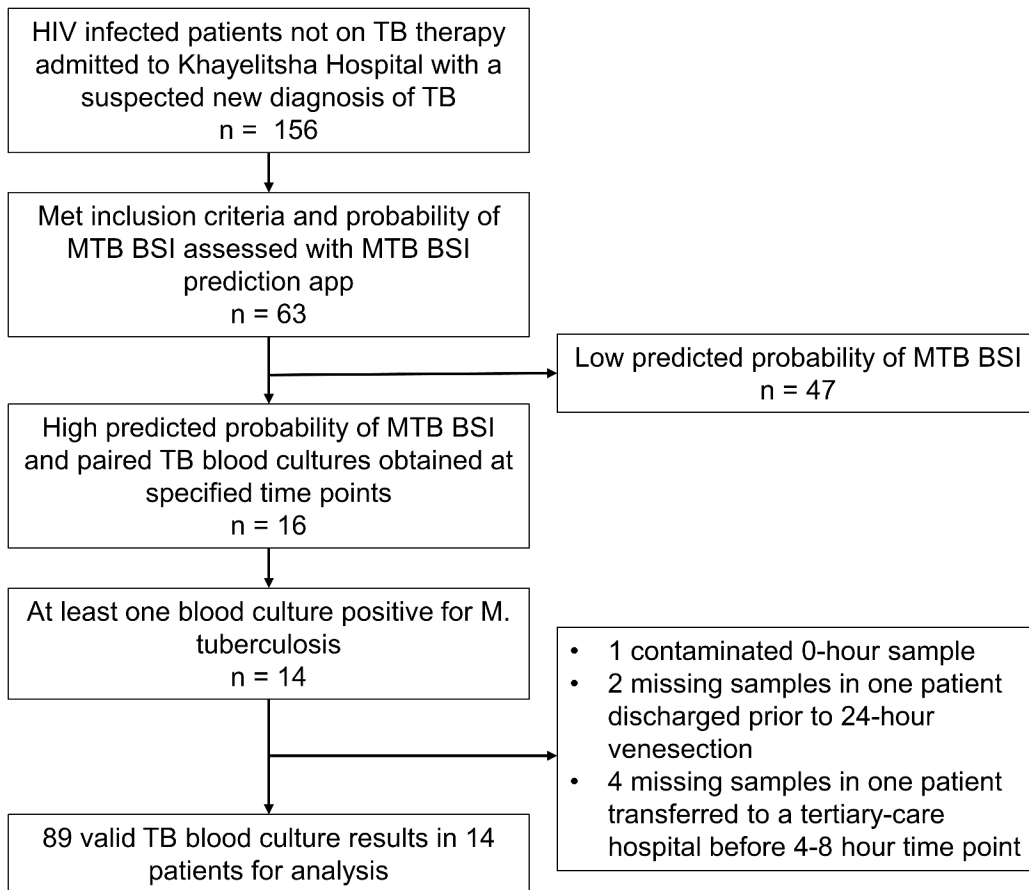
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263



Cumulative proportion MTB BSI identified

1.00
0.75
0.50
0.25
0.00

1 2 3 4 5 6

Cumulative number blood cultures

