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

Word count: 1483

Authors: David A. Barr; Andrew D. Kerkhoff; Charlotte Schutz; Amy M. Ward; Gerry R. Davies; Robert J. Wilkinson; Graeme Meintjes.

1. Wellcome Liverpool Glasgow Centre for Global Health Research, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK.
2. Wellcome Centre for Infectious Diseases Research in Africa, Institute of Infectious Disease and Molecular Medicine and Department of Medicine, University of Cape Town, South Africa.
3. Division of Infectious Disease, Department of Medicine, University of California San Francisco School of Medicine, San Francisco, CA, USA.
4. Department of Clinical Infection, Microbiology and Immunology, Institute of Global Health, University of Liverpool, UK.
5. Department of Medicine, Imperial College London W2 1PG
6. Francis Crick Institute, Midland Road, London, NW1 2AT

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

# Address Correspondence to: Dr David Barr, david.barr@liverpool.ac.uk
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
73% and four samples will detect 90–95% of patients with documented bacteraemia.\(^{(10, 11)}\) Almost all published studies of MTB BSI have performed a single 3-5ml liquid mycobacterial culture, and the proportion of MTB BSI missed by this strategy is unknown. We estimated the diagnostic yield of additional (>1) blood cultures for MTB in two ways:

1. retrospectively, in two large cohort studies of HIV-associated TB conducted in hospital settings; and
2. in a prospective evaluation of serial blood cultures in HIV-infected patients at high risk of MTB BSI in a hospital setting.

**Materials and methods**

Ethical approval was granted by the Human Research Ethics Committee University of Cape Town (Ref 001/2012, Ref 057/2013, and 057/2013 amendment 24/04/2016). Both the cohort studies and the prospective evaluation were carried out in the Western Cape, South Africa, a setting in which HIV and TB are the most common causes of death among adults, despite a well-functioning anti-retroviral programme.\(^{(12)}\) The GF Jooste Hospital TB (JHTB) study recruited unselected HIV-infected patients newly admitted to acute medical services at GF Jooste Hospital without a known TB diagnosis and not on anti-TB therapy. These patients underwent extensive microbiological screening for TB including a single 5ml BD Bactec Myco/F Lytic blood culture (BD, Sparks, MD) on the day of admission.\(^{(13)}\) The Khayelitsha Hospital (KHTB) study recruited HIV-infected patients admitted with symptoms suggestive of active tuberculosis and a CD4 count less than 350 cells/mm\(^3\), and also performed a routine 3-5ml Bactec Myco/F Lytic blood culture prior to start of anti-TB therapy.\(^{(14)}\) Both these hospitals had access to mycobacterial blood culture investigations through the National Health Laboratory Service (NHLS). A subset of patients in both cohorts had an additional MTB blood culture which was requested by their admitting medical team if clinically indicated (local guidelines recommend TB blood culture if CD4 count is less than 100 cells/mm\(^3\), in a patient with TB symptoms where there is difficulty obtaining sputum samples for TB testing or the sputum Xpert MTB/RIF assay is negative, and cultures are generally sent before start of anti-TB therapy). By interrogating the NHLS electronic
database we identified the subset of patients in both cohorts who had a second BD Bactec Myco/F Lytic blood culture carried out as part of routine care during the same admission as their study recruitment.

To enrich recruitment to the prospective study of serial blood cultures, we used data from n=350 KHTB patients to develop a model predicting MTB BSI in patients using only clinical variables available on day of admission to Khayelitsha Hospital.(15) This model used an ensemble machine learning approach combining logistic regression, random forest, and support vector machine methods, and gave Receiver Operator Characteristic (ROC) curve area under curve 0.86 in a test data set comprising 66 KHTB patients not used in model training. This ensemble model was packaged in a web-based application available at the patient bedside via a smart phone.(16)

Between 21 June 2016 and 19 October 2016, on weekdays Monday-Thursday, all HIV-infected patients newly admitted to Khayelitsha Hospital with CD4 count < 350 cells/uL and suspected TB but not yet started on anti-TB therapy, were screened using the MTB BSI prediction app. Patients with predicted probability greater than 0.56 who gave informed consent, underwent 3 venesections over a 24-hour period: immediately before (0 hours), 4-8 hours after, and 22-24 hours after first dose of anti-TB therapy. At each of these venesections, 5ml of peripheral blood was directly inoculated into a Myco/F Lytic BACTEC (BD, Sparks, MD) bottle, while 5ml was collected in a sodium heparin tube, immediately centrifuged for 25 minutes at 3000 G, and the resulting cell pellet (red cells and buffy coat) inoculated into a Myco/F Lytic bottle. Samples were transported to an NHLS TB laboratory in Cape Town for incubation the same day. Isolate identity was confirmed in all cases by secondary Löwenstein–Jensen slope culture, auramine acid-fast microscopy, and PCR / line probe assay.

**Results**

Using data from two independent cohort studies - the GF Jooste Hospital TB (JHTB) study, and the Khayelitsha Hospital TB (KHTB) study – we identified HIV-infected inpatients who had multiple mycobacterial blood culture performed during a single admission to hospital with
suspected TB. More than one blood culture was recorded for 59/410 JHTB patients and 169/680 KHTB patients, giving \( n=228 \) total for analysis. Of these patients, 99/228 (43\%) had at least one blood culture positive for \( M. \) tuberculosis (20/59 in JHTB, and 79/169 in KHTB).

Overall, 72/99 (0.73; 95\%CI = 0.64 to 0.82) of MTB BSIs were identified on the first culture, while 27/99 (0.27; 95\%CI = 0.18 to 0.36) had negative first culture but grew \( M. \) tuberculosis on the second (table 1).

To further investigate the yield of additional mycobacterial blood cultures, we carried out a prospective evaluation of multiple blood cultures in sixteen HIV-infected inpatients at Khayelitsha Hospital. Based on baseline clinical variables and a machine learning algorithm, these patients were selected to have a high predicted probability of MTB BSI (see methods section). A set of 2x 5ml blood cultures were performed at time 0 hours, 4-8 hours and 22-24 hours after first dose of anti-TB medication, with a total of 6 cultures over a 24-hour period. Because of the potential for antimicrobial carry-over in blood, the second sample from each pair had plasma removed before inoculation by centrifuge pelleted cells.

In total, 89 blood culture results were available in the 16 patients, with 7 results missing (figure 1). Of these 32/89 (36\%) were positive for \( M. \) tuberculosis. Pelleted samples were more likely to recover \( M. \) tuberculosis (19/44; 43\%) than directly inoculated samples (13/45; 29\%), but the difference may have been due to chance (\( p = 0.189 \) by Fisher’s exact test).

Two independent blood culture samples were obtained at each time point. At least one blood culture was positive in 14/16 patients (87.5\%). All isolates were identified as \( M. \) tuberculosis. Nine (9/14, 64\%) of these patients were culture positive on the first sample from the pair of samples taken at 0-hours. A further 3/14 (21\%) were culture negative on the first sample but grew \( M. \) tuberculosis on the second sample taken at 0-hour timepoint. This meant that 12/14 (86\%) of MTB BSI patients were identified by performing 2 independent cultures at the same time-point before antibiotic therapy. The remaining two patients were identified on the 4\(^{th}\) and 6\(^{th}\) blood culture respectively (both pelleted before inoculation) (Figure 2).

**Discussion**
Using two independent data sets, and a dedicated prospective evaluation, we estimate that approximately two-thirds of MTB BSI is identified by one Myco/F Lytic blood culture (55% and 73% in the data sets, and 64% in the prospective evaluation). To our knowledge, this is the first investigation of the additional yield associated with number of TB blood cultures. One previous study randomised patients to 6 blood cultures at a single time point or 3 blood cultures at 2 time points (but the same total number of cultures), and found no difference in recovery of *M. tuberculosis* between these arms.(8) This agrees with our prospective study finding that two blood culture at the same time point increases yield compared to a single culture.

The importance of blood stream infection in HIV-associated tuberculosis disease is increasingly recognised. ‘Disseminated’ tuberculosis causes 2 out of 5 inpatient deaths amongst HIV infected inpatients in low-resource settings, and is undiagnosed prior to death in half of these cases.(17) This dissemination is assumed to occur via the blood stream, and, despite practical limitations, blood culture can be considered the gold-standard diagnostic test.(18) TB blood culture positivity is associated with significantly higher mortality than blood culture negative HIV-associated TB.(5-8) In the context of a generalised HIV epidemic, *M. tuberculosis* is the most frequent blood culture isolate in hospitalised patients with severe sepsis.(2-5)

With few exceptions,(7, 8) reports characterising MTB BSI have relied on a single blood culture for diagnosis; our results show this will have substantially under-estimated the true point prevalence. This has implications for studies of HIV-associated TB pathogenesis, and supports calls for increased clinical research focused on MTB BSI, including development of blood based rapid diagnostics.(6) Where resources currently allow, an additional TB blood culture will increase culture diagnosis in seriously unwell HIV-infected inpatients, particularly when sputum is unobtainable.

Although several independent data sets have been used in this study, our findings are not generalisable outside of high HIV-TB burden settings. Most high HIV-TB burden settings do not have routine access to TB blood cultures. The findings are, however, useful to inform research studies carried out in those settings. In this study we were unable to assess the relative cost-effectiveness of additional blood cultures compared to other diagnostics – like induced sputum
or urine Xpert® MTB/RIF Ultra, and the urine-lipoarabinomannan assay – which are potentially more accessible in low resource settings. The data presented in this report do, however, give an opportunity to improve the reference standard in diagnostic performance studies assessing these novel diagnostics in the most critically unwell HIV-associated TB patients.

**Conclusion**

We estimate that a single TB blood culture underestimates the point prevalence of MTB BSI by approximately one-third. Additional blood cultures – even within the same 24-hour period – increase diagnostic yield by a proportion similar to that seen for non-mycobacterial BSI. We recommend, where resources allow, at least 2 blood cultures are taken when MTB BSI is suspected in unwell HIV-infected adults, particularly when sputum is unobtainable. These can be collected at the same time-point, prior to anti-TB treatment, in patients starting urgent empirical therapy.

**Acknowledgements**

GM and DB were funded by Wellcome (grant numbers 098316 and 105165/Z/14/A). GM was also supported by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation (NRF) of South Africa (Grant No 64787), NRF incentive funding (UID: 85858) and the South African Medical Research Council through its TB and HIV Collaborating Centres Programme with funds received from the National Department of Health (RFA# SAMRC-RFA-CC: TB/HIV/AIDS-01-2014). CS is a recipient of South African Medical Research Council (SAMRC) scholarship under the National Health Scholarship Programme. RJW is supported by the Francis Crick Institute which receives its core funding from Cancer Research UK (FC00110218), the UK Medical Research Council (FC00110218), and Wellcome (FC00110218); and by Wellcome (104803, 203135). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.
Table 1. Additional MTB BSI diagnoses made by second Myco/F Lytic culture in KHTB and JHTB cohort studies.

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

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
FIGURE LEGENDS

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

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


of acute hospital admission by systematic testing of urine samples using Xpert MTB/RIF: a

Associated With Increased Mortality Rates in Hospitalized Patients With HIV-Associated


studies of HIV-infected adults and children in resource-limited settings: a systematic review and

tuberculosis among hospitalised HIV patients in South Africa: a common condition that can be
HIV infected patients not on TB therapy admitted to Khayelitsha Hospital with a suspected new diagnosis of TB  
\[ n = 156 \]

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  
\[ n = 14 \]

- 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

89 valid TB blood culture results in 14 patients for analysis