

Raised Venous Lactate and Markers of Intestinal Translocation Are Associated With Mortality Among In-Patients With HIV-Associated TB in Rural South Africa

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Introduction: Case fatality among in-patients with HIV-associated tuberculosis (HIV-TB) in Africa is high. We investigated the factors associated with mortality in a rural South African hospital.

Methods: This was a prospective observational study of HIV-TB in-patients, with death by 8 weeks the endpoint.

Results: Of 99 patients (median CD4 count 72 cells/mm³), 32 (32%) died after median 8-day TB treatment. TB was diagnosed microbiologically in 75/99 and clinico-radiologically in 24, with no mortality difference between these groups [31% versus 38% ($P = 0.53$)]. Median venous lactate was 5.5 mmol/L (interquartile range 3.9–6.2) in those who died and 3.1 mmol/L (interquartile range 2.2–4.1) in survivors ($P < 0.001$). In multivariable analysis, lactate ≥ 4 mmol/L [adjusted odds ratio (aOR) 9.8, 95% confidence interval (CI): 3.0 to 32.2], Glasgow Coma Score < 15 (aOR 6.6, 95% CI: 1.5 to 29.6), CD4 count < 50 cells per cubic millimeter (aOR 5.5, 95% CI: 1.6 to 18.5), and age ≥ 50 (aOR 7.7, 95% CI: 1.2 to 46.9) independently

predicted death. In a nested case-control study, comparing those who died versus CD4-matched survivors, median plasma lipopolysaccharide concentrations were 93 and 57 pg/mL ($P = 0.026$) and intestinal fatty acid-binding protein, 132 and 0 pg/mL ($P = 0.002$).

Conclusions: Mortality was high and predicted by elevated lactate, likely reflecting a sepsis-syndrome secondary to TB or bacterial coinfection with intestinal barrier dysfunction appearing to contribute.

Key Words: human immunodeficiency virus, tuberculosis, mortality, lactate, translocation, lipopolysaccharide

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INTRODUCTION

Tuberculosis (TB) is the common cause of death among HIV-infected individuals in sub-Saharan Africa.^{1–3} Public health interventions demonstrated to reduce mortality in

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HIV-associated TB include timely antiretroviral therapy (ART)⁴ and cotrimoxazole prophylaxis.⁵ Despite these, case fatality rates during TB treatment remain high, particularly among hospitalized patients.^{6,7}

The direct cause of death is poorly defined. Possible mechanisms for death include septic shock due to disseminated TB,⁸ bacterial sepsis, other opportunistic infections, or drug-resistant TB.

Autopsy studies have shown that bacterial infections and cytomegalovirus (CMV) end organ disease are common.⁹ The association of bacterial product translocation with mortality seen in patients with chronic liver disease^{10,11} may be relevant in HIV-associated TB. Intestinal CD4 T-cell depletion results in translocation of lipopolysaccharide (LPS) and other bacterial products, contributing to immune activation during chronic HIV infection.^{12,13} We hypothesize that in advanced HIV-associated TB, intestinal immunity and the epithelial barrier may be profoundly impaired, leading to heightened immune activation from bacterial product translocation contributing to a sepsis syndrome.

To improve acute management of in-patients, a greater understanding of the pathophysiology of severe HIV-associated TB is required. We conducted a prospective observational study in a routine care setting in rural South Africa, where the best estimate for national TB incident rates was 993 per 100,000 population and HIV prevalence among incident cases was recorded at 65% in 2011.¹⁴ We investigated factors potentially associated with mortality, including venous lactate and CMV viremia, to assess a novel hypothesis that mortality is associated with biomarkers of intestinal damage and translocation.

MATERIALS AND METHODS

Setting and Study Design

The study took place at Madwaleni Hospital, which is situated in a deeply rural part of the Eastern Cape Province. The hospital has 180 beds and serves approximately 120,000 people. Between May and October 2011, consecutive patients were recruited fulfilling inclusion criteria of hospitalized, age ≥ 18 years, HIV infection and a new diagnosis of active TB on admission or ≤ 14 days before or after admission, and blood samples taken within 14 days of starting TB treatment. TB was confirmed microbiologically or diagnosed clinico-radiologically, reflecting clinical practice in this setting. A nested case-control study assessed associations between plasma LPS and intestinal fatty acid-binding protein (I-FABP) and death: controls were survivors with the closest subsequent enrollment date to each fatal case and CD4 count within 50 cells per cubic millimeter of the case.

Clinical Management and Assessment

A TB symptom enquiry was performed on admission for all patients with confirmed or suspected HIV. If symptomatic, a chest radiograph was performed and sputum sample taken. TB and HIV were treated according to national guidelines.¹⁵ Point-of-care HIV testing was offered to all with

suspected TB. HIV-infected individuals were referred for ART, which was started within 2 weeks of TB treatment if CD4 count $\leq 100/\text{mm}^3$ and within 8 weeks if CD4 count was $100\text{--}350/\text{mm}^3$. First-line ART was tenofovir, lamivudine, and efavirenz. The routine practice in the hospital during the study period was prescription of cotrimoxazole prophylaxis for all patients diagnosed with HIV-associated TB unless a contraindication existed. This was continued after discharge by the community clinics. However, data on this were not systematically recorded for the study. Demographic and clinical data including a performance status measure (see Table S1,^{16,17} Supplemental Digital Content, <http://links.lww.com/QAI/A717>) were recorded. Weight was measured using calibrated electronic scales, and body mass index was calculated. Full blood count and biochemistry were sent to the routine laboratory (see Table S2, Supplemental Digital Content, <http://links.lww.com/QAI/A717>). Venous blood was taken (after fluid resuscitation and without tourniquet) for point-of-care lactate, glucose measurement, and study assays. All patients were given intravenous fluids after admission.

Pulmonary and/or extrapulmonary specimens were taken for TB investigations depending on clinical presentation. If the diagnosis was not confirmed by microscopy, an empirical diagnosis could be made based on clinical and/or radiological features, pending culture results, and according to standard criteria for clinical diagnosis of TB.^{18,19} Ultrasound scans were performed in those with suspected pericardial or abdominal TB.

Laboratory Methods

Specimens were examined using Ziehl-Neelsen staining, locally, within 24 hours and cultured for mycobacteria in liquid medium (MGIT; BD, Sparks, MD). Culture and drug susceptibility testing was performed at the National Health Laboratory Services laboratory in Umtata, South Africa. Venous lactate and glucose were measured using a validated point-of-care meter (Accutrend Plus; Roche Products, Roche Diagnostics Division, United Kingdom) according to manufacturers' instructions.^{20,21} The remainder of the venous blood was centrifuged; plasma was frozen and stored. Assays for HIV and CMV viral loads, LPS, I-FABP, and cryptococcal antigen (CrAg) were performed retrospectively on these plasma specimens at the University of Cape Town. HIV viral load was measured on the Abbott RealTime HIV-1 platform (Abbott Park, IL) and CMV viral load was measured on the Argene CMV R-gene platform (Verniole, France). The lower limit of quantification for HIV was 40 copies per milliliter and for CMV was 150 copies per milliliter. CrAg was measured using a lateral flow assay (IMMY, Norman, OK). LPS was measured using the Limulus amoebocyte lysate assay (using the QCL-1000 kit from Lonza, Walkersville, MD), with modifications as previously described.²² Enzyme-linked immunosorbent assay was used to measure I-FABP (Cusabio, Wuhan, China).

Follow-up and Outcome Ascertainment

Primary outcome was death within 8 weeks of initiating TB treatment. Those discharged from hospital before 8 weeks

were referred to primary care clinics for ongoing management, including ART initiation, and contacted by telephone at 8 weeks. Readmission was a secondary outcome. We attributed cause of death using 3 broad categories: pulmonary TB alone, disseminated TB (evidence of miliary TB on chest x-ray or extrapulmonary TB), or a concomitant diagnosis. This was largely based on clinical diagnosis on admission, but other aetiologies were documented during the study if additional diagnostic information became available.

Definitions

Systemic inflammatory response syndrome (SIRS) was defined by the presence of at least 2 of the following: tympanic temperature >38.3 or $<36^{\circ}\text{C}$; heart rate >90 beats per minute; respiratory rate >20 breaths per minute; Glasgow Coma Score (GCS) <15 and point of care glucose >7.7 mmol/L in the absence of diabetes.²³ White blood cell count was not included in our definition, as it was not measured in all participants on admission. Sepsis-induced tissue hypoperfusion was defined as venous lactate ≥ 4 mmol/L.²⁴

Statistical Methods

Data were analyzed using STATA v11 (Stata Corporation, College Station, TX). Fisher exact test was used for categorical variables and Wilcoxon rank sum for continuous variables. Logistic regression was used for multivariable analysis and calculating adjusted odds ratios (aORs) for death. Variables associated with death in the univariable analysis ($P \leq 0.05$) were considered for inclusion in the multivariable model and retained if a significant association remained after adjustment for other variables. To examine correlation between biological markers, Spearman correlation was performed on data collected for LPS, I-FABP, C-reactive protein (CRP), and lactate.

Ethical Approval

Approval was granted by the University of Cape Town Human Research Ethics Committee (reference number: 136/2011). All participants provided written informed consent. Those temporarily lacking capacity due to illness were invited to consent or withdraw retrospectively once they regained capacity. If such a patient died, then permission was obtained from the Ethics Committee to include that patient's data.

RESULTS

Patient Characteristics

One hundred individuals were enrolled: 99 were included in the analysis and 1 was lost to follow-up (see Figure S1, Supplemental Digital Content, <http://links.lww.com/QAI/A717>). The median age was 32 years [interquartile range (IQR) 28–41], and 40/99 (40%) were male. At the time of presentation, the median time since HIV diagnosis was 3 months (IQR 0–13). Median CD4 count taken <6 months before or on admission was 72 cells per cubic millimeter (IQR 24–148) and median HIV viral load was 147,840 copies per milliliter

(IQR 9174–475,845) ($n = 92$). Of 99 patients, 31 had previously been treated for TB.

Of 99 participants, 22 (22%) were on ART at enrollment: 6 on regimens including stavudine. Additional 7 patients had previously taken ART, but had discontinued. After initiation of TB treatment, data on ART were available on 77/99 patients; 51 of whom were on ART at 8 weeks. Of 99 patients, 35 (35%) received antibiotics (not including cotrimoxazole prophylaxis) other than TB treatment (see Figure S2, Supplemental Digital Content, <http://links.lww.com/QAI/A717>), and 23 patients were prescribed intravenous antibiotics on admission.

TB symptoms were present for >4 weeks in 75/99 patients. TB treatment was administered on the day of admission in 47 patients. Sixty-four patients were diagnosed with pulmonary TB, 23 with extra-pulmonary TB, and 12 with both.

Eighty-six patients had samples from any site examined by microscopy. Of these, 55/86 were smear-negative and of these, 34/55 were *Mycobacterium tuberculosis*-culture positive. Eighty patients had 1 sputum specimen cultured, 26 had an extrapulmonary specimen cultured, and 13 had no specimens cultured. TB was microbiologically confirmed in 75 patients: from sputum (65 patients), pleural fluid (3), cerebrospinal fluid (1), lymph node (4), ascitic fluid (1), and urine (1). Of these 75 patients, 63 were *M. tuberculosis*-culture positive and only 12 were Ziehl-Neelsen-stain positive. The remaining 24 were diagnosed clinico-radiologically: 5 with compatible ultrasounds, 15 with compatible chest radiographs, and 4 on clinical grounds alone. Drug susceptibility testing was performed on 50/63 culture isolates. Forty-four isolates were susceptible to rifampicin and isoniazid, 2 were resistant to isoniazid but not rifampicin, and 4 were resistant to both drugs.

Routine bloods were taken a median of 1 day after admission (IQR 0–3) and 1 day (IQR 0–4) after initiation of TB treatment. Venous blood for study assays was taken a median of 1 day (IQR 1–4) after admission and 1 day after TB treatment initiation (IQR 0–3). Of 99 patients, 83 fulfilled criteria for SIRS, 88/99 patients had a lactate ≥ 2 mmol/L, and 43/99 met the criteria for sepsis-induced hypoperfusion, with a lactate ≥ 4 mmol/L. Of the 23 patients who were commenced intravenous antibiotics, all had a lactate ≥ 2 mmol/L and 11 had a lactate of ≥ 4 mmol/L, suggesting that intravenous antibiotics tended to be prescribed in patients who were more critically ill.

Patient Outcomes and Predictors of Mortality

Of 99 patients, 32 died within 8 weeks, with 28/32 dying during the initial admission. There were 9 readmissions, during which a further 4 patients died. The median duration from TB treatment initiation to death was 8 days (IQR 2.5–38.5). The median duration of initial admission was 16 days (IQR 10–35). Of the 28 who died during the first admission, 25 deaths were attributed to disseminated TB, 1 to Kaposi sarcoma with pulmonary TB, and 2 deaths were unexpected. Of 4 who died during readmission, 2 died of cryptococcal meningitis, 1 died of drug-induced hepatitis, and 1 died of acute gastroenteritis with hypovolemic shock.

The demographic, clinical, and laboratory parameters that are continuous variables are summarized in Table S2 (see Supplemental Digital Content, <http://links.lww.com/QAI/A717>) comparing survivors with those who died.

The median CD4 cell count was lower for those who died compared with those who survived [median 31/mm³ (IQR 13.5–119.5) and 80/mm³ (IQR 34–190), respectively; $P = 0.004$]. CD4 count was associated with mortality in univariable analysis (Table 1), with OR 2.8 [95% confidence interval (CI): 1.2 to 6.7] for CD4 <50 versus ≥ 50 cells per cubic millimeter. The median lactate in those who died was significantly higher at 5.5 versus 3.1 mmol/L in survivors ($P < 0.001$) (Fig. 1) (see Table S2 and Figure S1, Supplemental Digital Content, <http://links.lww.com/QAI/A717>). Venous lactate ≥ 4 mmol/L was strongly associated with death [24/43 (56%) with lactate ≥ 4 mmol/L died, compared with 8/56 (14%) with lactate <4 mmol/L (OR 7.6, 95% CI: 2.9 to 19.8)]. Additional factors associated with death in univariable analysis (Table 1) were age ≥ 50 years (compared with age <30 years, OR 7.7, and 95% CI: 1.2 to 46.9), GCS <15 (OR 4.5, 95% CI: 1.5 to 13.1), performance score 4 compared with score of 2 (OR 19, 95% CI: 5.7 to 64.2), creatinine ≥ 110 mmol/L (OR 3.8, 95% CI: 1.5 to 9.2), CRP ≥ 166 μ mol/L (OR 2.5, 95% CI: 1.0 to 6.0), hypoglycemia or hyperglycemia (OR 3.7, 95% CI: 1.5 to 8.8), and albumin <24 g/L (OR 6.0, 95% CI: 2.2 to 16.6). There was a significant correlation between CRP and lactate concentrations ($r = 0.28$, $P = 0.005$).

Of 6 individuals taking stavudine, 3 died (lactate range: 5.2–6.1 mmol/L). All had culture-confirmed TB and their death was believed to be due to disseminated TB. Twelve of 35 (35%) who received antibiotics other than TB treatment and 20/64 (31%) who did not receive antibiotics died ($P = 0.66$). There was no significant difference in HIV viral load between those who died and those who survived. Plasma CrAg was measured retrospectively: 4/99 were positive and 3 of these individuals died. Two of these 3, both of whom had culture-confirmed TB, died during readmission from cryptococcal meningitis. Further, an individual, whose plasma CrAg was negative at enrollment, was readmitted with cryptococcal meningitis 53 days after initiation of empiric TB treatment and survived with treatment.

Of 97 patients, 23 (24%) had a detectable CMV viral load with a median viral load of 500 copies per milliliter (IQR 250–1450) among these 23 patients. Eleven of 23 (48%) with a detectable CMV viral load died compared with 21/74 (28%) without a detectable CMV viral load (unadjusted OR 2.3, 95% CI: 0.88 to 6.1, $P = 0.087$).

In multivariable analysis (Table 2), venous lactate ≥ 4 mmol/L (aOR 9.8, 95% CI: 3.0 to 32.2; $P < 0.001$), age ≥ 50 years compared with age <30 (aOR 7.7, 95% CI: 1.2 to 46.9; $P = 0.006$), GCS <15 (aOR 6.6, 95% CI: 1.5 to 29.6; $P = 0.014$), and CD4 <50 cells per cubic millimeter (aOR 5.5, 95% CI: 1.6 to 18.5; $P = 0.006$) were independently associated with death. Performance status on admission was not included in the model because of collinearity with low GCS. Sensitivity analysis examining factors associated with mortality in those with microbiologically proven TB ($n = 75$) continued to demonstrate that lactate ≥ 4 mmol/L was independently associated with death (aOR 6.6, 95% CI: 1.7 to 25.4; $P < 0.006$).

Intestinal Damage and Microbial Translocation

The 32 individuals who died were matched to 32 survivors with similar CD4 cell counts (± 50 cells/mm³). For 28 of the controls, the CD4 cell count was matched within 20 cells per cubic millimeter of the corresponding case. The median plasma LPS concentration for those who died was significantly higher than that of survivors, at 93 pg/mL (IQR 65.8–163.0) versus 57 pg/mL (IQR 0.0–100.3) ($P = 0.026$) (Fig. 2). Median I-FABP in those who died was 131.5 pg/mL (IQR 0.0–2033.0) compared with 0 pg/mL (IQR 0.0–6751.0) in survivors ($P = 0.002$) (Fig. 3). Among these 64 patients who had LPS and I-FABP assayed, there were no statistically significant correlations between LPS and lactate ($r = 0.22$, $P = 0.079$), between I-FABP and CRP ($r = 0.21$, $P = 0.089$), between LPS and I-FABP ($r = 0.03$, $P = 0.82$), nor between LPS and CRP ($r = -0.01$, $P = 0.93$).

DISCUSSION

In this study, mortality was 32% at 8 weeks. Factors independently associated with mortality were raised venous lactate, reduced admission GCS, low CD4 count, and older age. The low median CD4 count at enrollment (median 72 cells/mm³) demonstrates profound immunosuppression; this is consistent with a Ugandan study in which low baseline CD4 count in HIV-associated TB predicted mortality.²⁵ Reduced GCS is likely to reflect severe systemic illness or central nervous system TB. Venous lactate ≥ 4 mmol/L was the strongest predictor of death, with OR for death of 9.8 (95% CI: 3.0 to 32.2).

In a South African study of 1006 hospitalized HIV-associated TB patients before widespread ART availability, 190/1006 (19%) died during hospital admission (half within the first 2 weeks) and further 60/1006 patients (6%) died after discharge.⁶ The reasons for the high mortality of patients hospitalized with HIV-associated TB despite TB treatment and ART availability observed in our study and others^{6,7,26,27} have yet to be fully elucidated. Postmortem studies have suggested that TB itself and bacterial and other coinfections are important contributors.^{9,28} Opportunistic coinfections in those with HIV-associated TB have been associated with mortality.²⁹ In our study, 4 individuals had detectable plasma CrAg; 3 of whom died.

The prognostic role of venous lactate in HIV-associated TB has not been studied previously. Bekker et al³⁰ demonstrated a rise in serum lactate and tumor necrosis factor- α in the first week of TB treatment in HIV-uninfected patients in the 1990s. In our study, 83/99 presented with SIRS and 88/99 had a lactate ≥ 2 mmol/L, with higher venous lactate concentration among those who died (median 5.5 mmol/L) than survivors (median 3.1 mmol/L). Moreover, 43/99 patients had evidence of tissue hypoperfusion with lactate ≥ 4 mmol/L. In a previous study of bacterial sepsis in the United States, increased lactate was linearly associated with mortality risk.³¹ We hypothesize that, in certain patients, hyperlactatemia is a reflection of sepsis related to disseminated TB or superadded bacterial infection. Based on these lactate findings, we suggest that the severity of illness of

TABLE 1. Univariable Analysis of Categorical Exposure Variables and Association With Death

Variable	Number in Category*	Number (%) Died	OR for Death	95% CI	P
Age, yrs					
18–29	31	7 (23%)	1	—	—
30–39	41	14 (34%)	1.8	0.62 to 5.1	0.288
40–49	16	3 (19%)	0.8	0.17 to 3.6	0.761
≥50	11	8 (73%)	9.1	1.9 to 44.0	0.006
Gender					
Male	40	16 (40%)	1	—	—
Female	59	16 (27%)	0.6	0.24 to 1.3	0.181
CD4 cell count, cells/mm ³					
0–49	45	20 (44%)	2.8	1.2 to 6.7	0.020
50–650	54	12 (22%)	1	—	—
ART status					
Currently not on ART†	77	26 (34%)	1	—	—
Currently on ART	22	6 (27%)	0.7	0.28 to 2.1	0.567
Site of TB disease					
Pulmonary	64	22 (34%)	1	—	—
Extrapulmonary ± pulmonary	35	10 (29%)	0.8	0.31 to 1.9	0.556
Diagnosis confirmed by culture and/or microscopy					
Yes	75	23 (31%)	1	—	—
No (clinical/radiological diagnosis)	24	9 (38%)	1.2	0.72 to 1.9	0.534
Weeks from onset of TB symptoms to admission					
≤4	24	6 (25%)	1	—	—
>4	75	26 (35%)	1.59	0.56 to 4.5	0.381
Performance status score					
1 and 2	47	12 (26%)	1	—	—
3	26	5 (19%)	1.4	0.38 to 4.8	0.633
4	26	20 (77%)	19	5.7 to 64.2	<0.001
GCS					
15	81	21 (26%)	1	—	—
≤14	18	11 (61%)	4.5	1.5 to 13.1	0.006
Serum glucose (mmol/L) * abnormal glucose defined by hypoglycemia (≤3.9 mmol/L) or hyperglycemia (≥7.8 mmol/L)					
Abnormal	41	20 (49%)	3.7	1.5 to 8.8	0.004
4.0–7.7	58	15 (15%)	1	—	—
Serum creatinine, mmol/L					
39–109	64	14 (22%)	1	—	—
110–1166	35	18 (51%)	3.8	1.5 to 9.2	0.003
Serum albumin, g/L‡					
12–23	54	26 (48%)	6.0	2.2 to 16.6	<0.001
24–42	45	6 (13%)	1	—	—
Venous lactate, mmol/L					
1.1–3.9	56	8 (14%)	1	—	—
4.0–7.8	43	24 (56%)	7.6	2.9 to 19.8	<0.001
Serum CRP, μmol/L§					
2.3–165.9	49	11 (22%)	1	—	—
166–497	50	21 (42%)	2.5	1 to 6	0.040

*Total number = 99 unless otherwise specified.

†This category includes 70 individuals naive to ART and 7 who had previously been on ART but stopped.

‡The median values of 23 g/L and 166 μmol/L, respectively, were used as a cut-off for comparison.

§Clinically relevant cut-offs were used for other parameters.

hospitalized HIV-associated TB patients is more akin to patients with bacterial sepsis than out-patients with pulmonary TB. We did not observe any association between

antibiotic prescription and mortality, but this analysis is confounded by indication given the observational design of our study. Therefore, we cannot conclude that antibiotics are

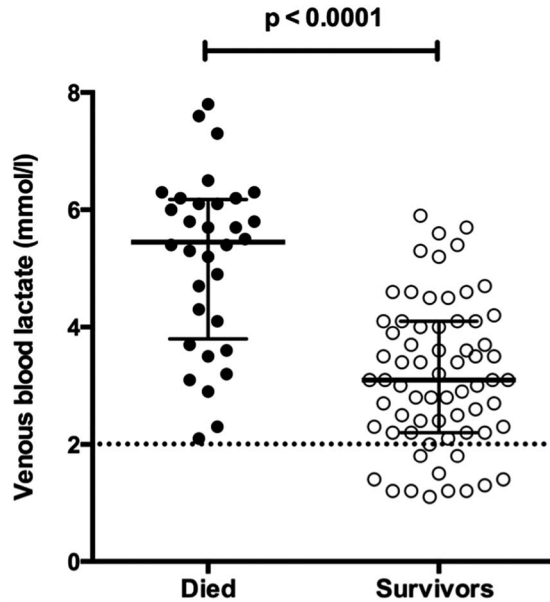


FIGURE 1. Venous lactate concentrations, comparing those who died with those who survived. Median lactate for those who died was 5.5 mmol/L (IQR 3.8–6.2) compared with the median lactate for survivors, 3.1 mmol/L (IQR 2.2–4.1, $P < 0.001$, Wilcoxon rank-sum test).

not beneficial in this patient group. This question can only be adequately addressed by a randomized controlled trial.

In the nested case-control study, LPS and I-FABP concentrations were higher in those who died than in survivors, reflecting bacterial product translocation and intestinal damage, respectively. The gastrointestinal tract is a major site for HIV-1 replication and depletion of CD4 cells.³² Higher concentrations of plasma LPS and peptidoglycan have been demonstrated in HIV-infected versus uninfected individuals,³² providing evidence for microbial product translocation, which has been associated with immune activation during chronic HIV infection.¹² Microbial translocation in HIV-TB has not been extensively studied. In a surprising finding from Uganda, circulating LPS concentrations were lower among HIV-TB patients with CD4 cell counts <350 cells per cubic millimeter compared with healthy HIV-infected controls.³³ The investigators attributed this to elevations of LPS-binding proteins in HIV-TB but were unable to confirm this hypothesis. In the Ugandan study, the association of LPS with mortality was not investigated.

In our study, the elevation of I-FABP and LPS levels in individuals who died compared with those of survivors supports the hypothesis that gut damage and microbial translocation, respectively, may have resulted in immune activation and contributed to sepsis syndrome and death. We did not find a significant correlation between LPS and CRP. However, measuring circulating LPS is challenging and may not accurately quantify microbial translocation.³⁴ The correlation between LPS and other inflammatory markers was not explored.

No significant association was observed between CMV viremia and mortality. A significant association between

TABLE 2. Multivariable Model of Factors Associated With Death

Variable	Category	Adjusted OR for Death*	95% CI	P (Wald Test)
Age, yrs	18–29	1		
	30–39	1.9	0.5 to 7.1	0.350
	40–49	0.63	0.1 to 4.1	0.631
	≥ 50	7.7	1.2 to 46.9	0.028
Gender	Male	1		
	Female	0.9	0.29 to 2.7	0.810
CD4 cell count, cells/mm ³	0–49	5.5	1.6 to 18.5	0.006
	50–650	1		
GCS	15	1		
	≤ 14	6.6	1.5 to 29.6	0.014
Venous lactate, mmol/L	1.1–3.9	1		
	4.0–7.8	9.8	3.0 to 32.2	<0.001

*All ORs adjusted for age, gender, CD4 cell count, GCS, and venous lactate. Creatinine, CRP, hemoglobin, albumin, and abnormal blood glucose were considered in developing the final model but were not statistically significant in the multivariable analysis and thus excluded from the final model.

CMV viremia and mortality in HIV-infected out-patients has been demonstrated previously.³⁵ Our negative findings may have been due to sample size and other, stronger, factors driving mortality. Larger studies are warranted to assess the contribution of CMV viremia to mortality in HIV-TB.

There were limitations. We did not include a control group of patients with HIV but without active TB. Because

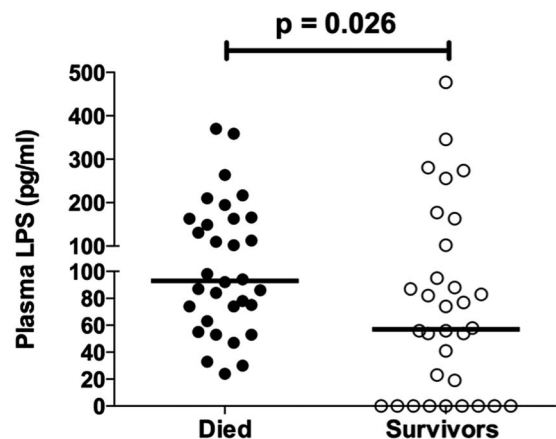


FIGURE 2. Nested case-control study comparing plasma LPS concentrations of those who died ($n = 32$) with survivors ($n = 32$), matched for CD4 count (± 50 cells/mm³). Median plasma LPS for those who died was 93 pg/mL (IQR 65.8–163) and in survivors, 57 pg/mL (IQR 0–100.3, $P = 0.026$, Wilcoxon rank-sum test).

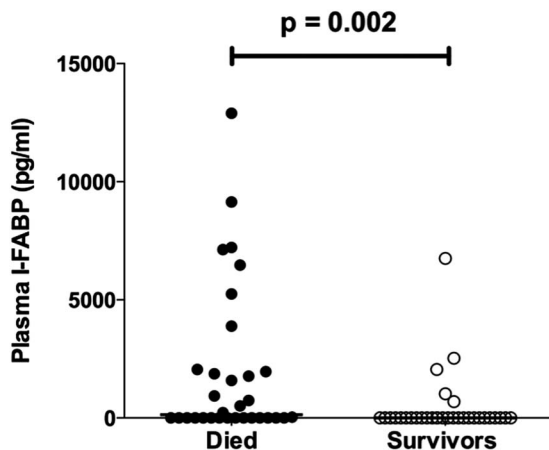


FIGURE 3. Nested case-control study comparing the I-FABP concentrations of those who died ($n = 32$) with survivors ($n = 32$), matched for CD4 T cell count (± 50 cells/mm³). Median I-FABP value in those who died was 131.5 pg/mL (IQR 0–2033) and in survivors, 0 pg/mL (IQR 0–6751, $P = 0.002$, Wilcoxon rank-sum test).

blood cultures were not available, we were unable to study whether translocation of viable bacteria occurred or to diagnose mycobacteraemia and its contribution to mortality. The marker of monocyte response to bacterial LPS, the coreceptor soluble CD14 (sCD14),²² has been shown in previous studies to predict mortality in HIV²² and HIV-associated TB.³⁶ Toossi et al³³ demonstrated that HIV-associated TB patients with low CD4 counts did not display a fall in circulating sCD14 levels after initiation of TB treatment although patients with high CD4 counts did show a fall. We could not measure this or other markers of microbial product translocation, such as core endotoxin antibody,²² because of limited sample volumes. Assessment for additional coinfections relied mainly on clinical examination because of the paucity of diagnostic investigations available. Furthermore, nearly a quarter of cases did not have microbiological confirmation of TB; however, raised venous lactate continued to be an independent predictor of mortality after sensitivity analysis of those individuals with proven TB. Despite these limitations, these latter points reflect the health service constraints in many high HIV-prevalence settings to which our results are applicable.

CONCLUSIONS

Mortality after initiation of TB treatment in hospitalized HIV-infected patients remains very high even in the era of ART availability. Profound immunosuppression, impaired consciousness, raised venous lactate, and older age were independently associated with mortality. Plasma LPS and I-FABP were higher in those who died than those who survived, suggesting a role for gut barrier dysfunction and microbial translocation. Larger studies further defining the pathophysiology and immediate causes of death in hospitalized HIV-associated TB patients are required. Additionally, clinical trials of novel interventions aimed at improving acute

management of these severely ill patients, many of whom have sepsis syndrome, are required.

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REFERENCES

1. Ansari NA, Kombe AH, Kenyon TA, et al. Pathology and causes of death in a group of 128 predominantly HIV-positive patients in Botswana, 1997–1998. *Int J Tuberc Lung Dis.* 2002;6:55–63.
2. Lucas SB, De Cock KM, Hounnou A, et al. Contribution of tuberculosis to slim disease in Africa. *BMJ.* 1994;308:1531–1533.
3. Rana FS, Hawken MP, Mwachari C, et al. Autopsy study of HIV-1-positive and HIV-1-negative adult medical patients in Nairobi, Kenya. *J Acquir Immune Defic Syndr.* 2000;24:23–29.
4. Havlir DV, Kendall MA, Ive P, et al. Timing of antiretroviral therapy for HIV-1 infection and tuberculosis. *N Engl J Med.* 2011;365:1482–1491.
5. Grimwade K, Swingle G. Cotrimoxazole prophylaxis for opportunistic infections in adults with HIV. *Cochrane Database Syst Rev.* 2003;3: CD003108.
6. Edginton ME, Wong ML, Phofa R, et al. Tuberculosis at Chris Hani Baragwanath Hospital: numbers of patients diagnosed and outcomes of referrals to district clinics. *Int J Tuberc Lung Dis.* 2005;9:398–402.
7. Kyeyune R, den Boon S, Cattamanchi A, et al. Causes of early mortality in HIV-1 infected TB suspects in an East African referral hospital. *J Acquir Immune Defic Syndr.* 2010;55:446–450.
8. Vadillo M, Corbella X, Carratala J. AIDS presenting as septic shock caused by Mycobacterium tuberculosis. *Scand J Infect Dis.* 1994;26:105–106.
9. Martinson NA, Karstaedt A, Venter WD, et al. Causes of death in hospitalized adults with a premortem diagnosis of tuberculosis: an autopsy study. *AIDS.* 2007;21:2043–2050.
10. Frances R, Benlloch S, Zapater P, et al. A sequential study of serum bacterial DNA in patients with advanced cirrhosis and ascites. *Hepatology.* 2004;39:484–491.
11. Zapater P, Frances R, Gonzalez-Navajas JM, et al. Serum and ascitic fluid bacterial DNA: a new independent prognostic factor in noninfected patients with cirrhosis. *Hepatology.* 2008;48:1924–1931.
12. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med.* 2006;12:1365–1371.
13. Kamat A, Ancuta P, Blumberg RS, et al. Serological markers for inflammatory bowel disease in AIDS patients with evidence of microbial translocation. *PLoS One.* 2010;5:e15533.
14. WHO. *Global Tuberculosis Control Report.* 2012. Available at: http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf. Accessed March 2013.
15. Department of Health, Republic of South Africa (2009). *National tuberculosis management guidelines.* Available at: http://familymedicine.ukzn.ac.za/Libraries/Guidelines_Protocols/TB_Guidelines_2009.sflb.ashx. Accessed October 2012.
16. de Valliere S, Barker RD. Poor performance status is associated with early death in patients with pulmonary tuberculosis. *Trans R Soc Trop Med Hyg.* 2006;100:681–686.

17. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol*. 1982;5: 649–655.
18. Wilson D, Nachege J, Morroni C, et al. Diagnosing smear-negative tuberculosis using case definitions and treatment response in HIV-infected adults. *Int J Tuberc Lung Dis*. 2006;10:31–38.
19. WHO. *Improving the Diagnosis and Treatment of Smear-Negative Pulmonary and Extrapulmonary Tuberculosis Among Adults and Adolescents: Recommendations for HIV-Prevalent and Resource-Constrained Settings*. 2006. Available at: http://www.who.int/tb/publications/2006/tbhiv_recommendations.pdf. Accessed October 2012.
20. Baldari C, Bonavolonta V, Emerenziani GP, et al. Accuracy, reliability, linearity of Accutrend and Lactate Pro versus EBIO plus analyzer. *Eur J Appl Physiol*. 2009;107:105–111.
21. Perez EH, Dawood H, Chetty U, et al. Validation of the Accutrend lactate meter for hyperlactatemia screening during antiretroviral therapy in a resource-poor setting. *Int J Infect Dis*. 2008;12: 553–556.
22. Sandler NG, Wand H, Roque A, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. *J Infect Dis*. 2011;203: 780–790.
23. Daniels R. Surviving the first hours in sepsis: getting the basics right (an intensivist's perspective). *J Antimicrob Chemother*. 2011;66(suppl 2): ii11–ii23.
24. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med*. 2013;39:165–228.
25. Moore D, Liechty C, Ekwaru P, et al. Prevalence, incidence and mortality associated with tuberculosis in HIV-infected patients initiating antiretroviral therapy in rural Uganda. *AIDS*. 2007;21:713–719.
26. Crump JA, Ramadhani HO, Morrissey AB, et al. Bacteremic disseminated tuberculosis in sub-saharan Africa: a prospective cohort study. *Clin Infect Dis*. 2012;55:242–250.
27. Mugusi SF, Ngaimisi E, Janabi MY, et al. Risk factors for mortality among HIV-positive patients with and without active tuberculosis in Dar es Salaam, Tanzania. *Antivir Ther*. 2012;17:265–274.
28. Wong EB, Omar T, Setlhako GJ, et al. Causes of death on antiretroviral therapy: a post-mortem study from South Africa. *PLoS One*. 2012;7:e47542.
29. Cain KP, Anekthananon T, Burapat C, et al. Causes of death in HIV-infected persons who have tuberculosis, Thailand. *Emerg Infect Dis*. 2009;15:258–264.
30. Bekker LG, Maartens G, Steyn L, et al. Selective increase in plasma tumor necrosis factor-alpha and concomitant clinical deterioration after initiating therapy in patients with severe tuberculosis. *J Infect Dis*. 1998;178:580–584.
31. Trzeciak S, Dellinger RP, Chansky ME, et al. Serum lactate as a predictor of mortality in patients with infection. *Intensive Care Med*. 2007;33:970–977.
32. Brenchley JM, Douek DC. HIV infection and the gastrointestinal immune system. *Mucosal Immunol*. 2008;1:23–30.
33. Toossi Z, Funderburg NT, Sirdeshmuk S, et al. Systemic immune activation and microbial translocation in dual HIV/tuberculosis-infected subjects. *J Infect Dis*. 2013;207:1841–1849.
34. Ketchum PA, Novitsky TJ. Assay of endotoxin by limulus amoebocyte lysate. *Methods Mol Med*. 2000;36:3–12.
35. Fielding K, Koba A, Grant AD, et al. Cytomegalovirus viremia as a risk factor for mortality prior to antiretroviral therapy among HIV-infected gold miners in South Africa. *PLoS One*. 2011;6:e25571.
36. Ravimohan S, Tamuhla N, Steenhoff AP, et al. Early immunologic failure is associated with early mortality among advanced HIV-infected adults initiating antiretroviral therapy with active tuberculosis. *J Infect Dis*. 2013;208:1784–1793.