Presentation and outcome of suspected sepsis in a high-HIV burden, high antiretroviral coverage setting

Wendy Chaka[a,1], **Christopher Berger**[b,1], **Stella Huo**[b], **Valerie Robertson**[a], **Chipo Tachiona**[a], **Marcelyn Magwenzi**[a], **Trish Magombei**[a], **Chengetai Mpamhanga**[c], **David Katzenstein**[d], **John Metcalfe**[b,1,*]

[a]University of Zimbabwe College of Health Sciences, Department of Medical Microbiology, Box A178 Avondale, Harare, Zimbabwe

[b]Zuckerberg San Francisco General Hospital, Division of Pulmonary and Critical Care Medicine, University of California, San Francisco, USA

[c]Parirenyatwa Group of Hospitals, Public Health Microbiology Laboratory, Mazowe Street, Harare, Zimbabwe

[d]Stanford University Department of Medicine/Infectious Diseases, Stanford, CA 94305-5107, USA

Abstract

Objective: To define sepsis syndromes in high-HIV burden settings in the antiretroviral therapy (ART) era. Methods: We characterized a prospective cohort of adults presenting to a tertiary emergency department in Harare, Zimbabwe with suspected community-acquired sepsis using blood and urine cultures, urine tuberculosis lipoarabinomannan (TB LAM), and serum cryptococcal antigen (CrAg) testing. The primary outcome was 30-day all-cause mortality.

Results: Of 142 patients enrolled 68% ($n = 96/142$, 95% confidence interval (CI) [60–75%]) were HIV-positive, 41% ($n = 39/96$, 95% CI [31–50%]) of whom were ART-naïve. Among HIV-positive patients, both opportunistic pathogens (TB LAM-positivity, 36%, 95% CI [24–48%]; CrAg-positivity, 15%, 95% CI [7–23%]) and severe non-AIDS infections (*S. pneumoniae* urine antigen-positivity 12%, 95% CI [4–20%]; bacteraemia 17% ($n = 16/96$, 95% CI [9–24%]), of which 56% ($n = 9/16$, 95% CI [30–80%]) were gram-negative organisms) were common. *Klebsiella pneumoniae* recovered from blood and urine was uniformly resistant to ceftriaxone, as

*Corresponding author at: University of California, San Francisco, Division of Pulmonary and Critical Care Medicine, Zuckerberg San Francisco General Hospital, 1001 Potrero Avenue, Rm 5K1, San Francisco, CA 94110-0111, USA. john.metcalfe@ucsf.edu (J. Metcalfe).
[1]These authors are contributed equally to this work.
Authors' information
Please note that CB and WC contributed equally to this manuscript and should be identified as co-first authors.

were most *Escherichia coli* isolates. Acknowledging the power limitations of our study, we conclude that relative to HIV-negative patients, HIV-positive patients had modestly higher 30-day mortality (adjusted hazard ratio (HR) 1.88, 95% CI [0.78–4.55]; $p = 0.16$, and 3.59, 95% CI [1.27–10.16], $p = 0.02$) among those with and without viral suppression, respectively.

Conclusion: Rapid point-of-care assays provide substantial clinically actionable information in the setting of suspected sepsis, even in areas with high ART coverage. Antimicrobial resistance to first-line antibiotics in high burden settings is a growing threat.

Keywords

HIV-associated sepsis; Sepsis in low-income countries; Antimicrobial resistance

Background

Sepsis is a major global health priority (Reinhart et al., 2017), affecting some 30 million people annually and resulting in six million deaths. While high quality data guiding management has been generated in high-income countries, the overwhelming majority of disease burden occurs in low-and-middle-income countries (LMICs) (American College of Chest Physicians, 1992 Fleischmann et al., 2016), where aetiologies, management guidelines, and outcomes may differ significantly from high-income settings. In particular, patient presentation, microbial aetiologies, and outcomes in high HIV burden populations are poorly defined in the context of successful scale-up of antiretroviral therapy (ART).

Provision of primary and HIV-specific care in sub-Saharan Africa is challenged by poor governance, poverty, ineffective national health insurance programs, and substandard quality of care. Zimbabwe, among the world's highest HIV prevalence (14.6%) (Zimbabwe Ministry of Health and Child Care, 2014) nations, is ranked 154th of 188 countries in human development. Despite major challenges, significant progress over the past five years in national ART coverage has been achieved, reaching 86.8% of known HIV-positive persons (Zimbabwe Ministry of Health and Child Care, 2014). Previous studies of sepsis, septic shock, and mortality from the southern Africa region describe ART-naïve individuals and early mortality related to ART initiation. This has limited the generalizability of these studies to chronic ART-treated individuals presenting with suspected sepsis in a region of hyperendemic HIV and increasing ART coverage (Carugati et al., 2018; Koss et al., 2015; Lawn et al., 2008; Marazzi et al., 2008).

We consecutively recruited adults with suspected sepsis admitted to the medical wards in an urban tertiary care centre in Harare, Zimbabwe. Our objective was to describe clinical presentation, HIV status and reported ART treatment, and comorbid opportunistic infections (Kaukonen et al., 2015).

Methods

Study population

This was a prospective cohort study of patients admitted to Parirenyatwa Group of Hospitals (PGH), the largest tertiary teaching hospital in Zimbabwe, serving a population of 1.25

million. A convenience sample of adult patients (age 18 years) presenting to the PGH Casualty Department (CD) with suspected sepsis and admitted to the medical ward during the study period February–November 2016 were screened consecutively from 0700 to 1700 h. Sepsis was defined as suspected or documented infection plus the presence of at least one systemic inflammatory response syndrome (SIRS) criteria (body temperature <36°C or >38°C; heart rate >90 beats per minute; respiratory rate >20 breaths per minute; white blood cell (WBC) count <4×10^9 cells/L or >12×10^9 cells/L, or presence of >10% immature neutrophils) (Nisula et al., 2015; Singer et al., 2016) based on prior evidence indicating no clear cut point exists for the number of SIRS criteria in defining the severity of sepsis (Kaukonen et al., 2015). Patients presenting with primary surgical or obstetric conditions according to the treating clinician on duty were excluded from screening. Test results were supplied to treating clinicians, who coordinated all patient management and were not co-investigators in the study. Written informed consent was obtained from each patient, or, if they were unable to do so, from accompanying relatives or friends. Ethical approval for the study was obtained from the Joint Research Ethics Committee (JREC) for The University of Zimbabwe College of Health Sciences (UZCHS) and PGH and The Medical Research Council of Zimbabwe (MRCZ).

We collected socio-demographic and clinical data using a standardized data collection tool. For patients whose HIV status was unknown or not documented at enrolment, HIV counselling and testing was provided according to national guidelines (Zimbabwe Ministry of Health and Child Care, 2014). A rapid HIV test was performed (Determine HIV-1/2, Alere Medical Co., Ltd, Chiba, Japan) followed by a confirmatory test (First response HIV 1–2, Premier Medical Corporation Ltd, Daman, India) for positive results. A third enzyme immunoassay (EIA) (ChemBio HIV 1/2, Diagnostic Systems, Inc., Medford, New York, USA) was used to evaluate discordant serologic results.

Microbiologic investigations

A single 10 mL blood sample was obtained aseptically in the CD prior to empiric antibiotic administration and a single aerobic blood culture was performed (BacT/ALERT; Biomérieux, Durham, North Carolina, USA) (see supplementary text). Coagulase-negative *Staphylococcus epidermidis, Staphylococcus hominis, and Micrococcus* spp. were considered contaminants and excluded from final analyses. For quality assurance and to assess for the possibility of false negative results, a random subset of 5 blood cultures found to be negative by our BacT/ALERT system were processed in parallel through a private commercial laboratory in Harare and demonstrated no difference in results.

All patients provided a midstream urine sample that was examined macroscopically for appearance, cultured, underwent dipstick urinalysis (Siemens Multistix 10 SG reagent strips – Siemens Healthcare GmbH, Henkestr, Erlangen, Germany), and the remainder examined for cells, ova, and casts. Colony counts of 10^5 ml^{-1} indicated infection and isolates were Gram stained and further characterized (Vitek 2 – BioMérieux Vitek, Inc. Hazelwood, Missouri, USA) to obtain species identification and antimicrobial susceptibility patterns. We recorded antimicrobial susceptibility data for both blood and urine cultures (Supplementary Tables 1 and 2).

Several point-of-care (POC) tests were done including urine streptococcal antigen using the BinaxNOW *Streptococcus pneumoniae* Antigen Card (Abbott, Waltham, Massachusetts, USA) (Alere, 2020a,b; Olofsson et al., 2019; Roson et al., 2004; Shoji et al., 2018) for all study patients. For all HIV-positive patients, urine was tested with a lateral flow assay for lipoarabinomannan using Determine TB LAM kits (Abbott, Waltham, Massachusetts, USA) (Alere, 2020a, b; Lawn et al., 2013), and, if CD4+ cell count was <200 cells/mm^3, serum was tested for Cryptococcal antigen (CrAg) using a lateral flow assay (IMMY, Inc, Oklahoma, USA) (Drain et al., 2019; Hansen et al., 2013; Jarvis et al., 2009; Rajasingham et al., 2012). Due to the low incidence of malaria in Harare province and its relatively high altitude (World Health Organization, 2018), investigations for malaria were not routinely carried out unless patients reported a history of travel outside of Harare.

HIV (immunologic and virologic testing)

CD4+ cell count testing was performed by flow cytometry (Partec cyflow, Sysmex Partec GmbH, Görlitz, Germany) according to manufacturer instructions and Zimbabwe national guidelines (Zimbabwe Ministry of Health and Child Care, 2014). HIV viral load testing was performed using GeneXpert HIV-1 Quant Viral load kits (Cepheid, Sunnyvale, California, USA) (see supplementary text). For patients on ART with viral loads >1000 copies/ml ($n = 20$) genotyping and subtyping was completed (REGA HIV-1 subtyping tool) (Liu and Shafer, 2006; Manasa et al., 2014) (see supplementary text).

Definitions

Regardless of reported linkage to ART programs, we classified patients as HIV-negative; HIV-positive, virally suppressed (<1000 copies/ml); and HIV-positive, high viral load (1000 copies/ml). Clinical assessment of sepsis was done using the quick Sequential Organ Failure (qSOFA) score which includes (1) respirations >22 breaths/minute, (2) altered mentation, (3) systolic blood pressure 100 mmHg, with two or more considered 'high risk' (Singer et al., 2016). The qSOFA score ranges from 0 to 3, with each criterion being worth one point. For the 48% of patients ($n = 68/142$) for whom clinical and laboratory data was complete, the SOFA score (Vincent et al., 1996), including (1) creatinine >110 µmoL/L, (2) platelets <150×10^3 µL^{-1}, and (3) total bilirubin >20 µmol/L was also calculated.

Statistical analysis

The primary outcome was death from any cause at 30 days following enrolment. Descriptive analyses of baseline variables were performed to summarize patient characteristics. Differences between groups were assessed using Chi Square for categorical variables, and Kruskal–Wallis or Mann–Whitney tests for continuous variables. Bonferroni's adjustment was used to correct for multiple testing. Kaplan–Meier (KM) curves were plotted to compare time to death from time of admission between patient groups (HIV-negative; HIV-positive ART-naïve; high viral load; and virally suppressed).

Multivariate associations with 30-day mortality among patients admitted with suspected sepsis were examined by using a Cox proportional hazard model and robust standard of errors (SEs) to generate relative risk (RR) estimates; the model was *a priori* specified to include HIV, age, sex, and severity of sepsis (i.e., qSOFA). Missing values from the qSOFA

calculation were imputed using the Multiple Imputation (MI) method, generating 20 imputed datasets. For each covariate with missing information, we used variables that could potentially explain the missing data as predictors of missing values. Cox model analyses were conducted on each imputed dataset. The results obtained from the imputed datasets were combined using Rubin's rules. We used the MICE package in R for multiple imputation. However, on further consideration, severity of sepsis was noted to likely be a causal mediator of the effect of HIV on death, as illustrated in our initial conceptual directed acyclic graph (DAG) (Supplementary Figure 1). Therefore, we decided not to include severity of sepsis in our final model. Rather, and because severity of sepsis was considered to be a potentially strong predictor of death, we have included a separate model including severity of sepsis in the supplement (Supplementary Table 4). The proportional hazard assumption was checked using scaled Schoenfeld residuals. None of the covariates nor the global test were found to be statistically significant. All analyses were performed using Stata (Version 13.0; College Station, Texas, USA) and graphics done in R (Version 3.5.3; Vienna, Austria) (R Core Team, 2016).

Results

Patient cohort

From February to November 2016, 155 adult patients were admitted from the casualty department to the medical ward with one or more SIRS criteria and suspected infection (Figure 1). Of 142 (92%, $n = 142/155$, 95% confidence interval (CI) [87–96%]) patients enrolled during the study period, 68% ($n = 96/142$, 95% CI [60–75%]) were HIV-positive. Of HIV-positive patients, 41% ($n = 39/96$, 95% CI [31–50%]) were ART-naïve and 59% ($n = 57/96$, 95% CI [50–69%]) reported current use of ART. Median time on ART was 14 months (interquartile range (IQR) 1–60 months), though only 51% ($n = 21/41$, 95% CI [36–67%]) of those with available viral load data (72%; $n = 41/57$, 95% CI [60–84%]) were virally suppressed (<1000 copies/ml) (<200 copies/ml, $n = 18$; 200–500 copies/ml, $n = 3$). Twenty patients (49%) reporting current ART use had high viral loads (median 42,900 copies/ml; IQR 4908–99,600 copies/ml). Median CD4+ cell counts among patients with high viral loads were low regardless of ART status (26 cells/mm^3; IQR 10–93). Men were more likely than women to be ART-naïve with known status (37%, $n = 15/41$, 95% CI [22–51%] vs. 14%, $n = 5/37$, 95% CI [2–25%]; $p = 0.02$).

Clinical presentation

There were no differences between groups (HIV-negative; HIV-positive, virally suppressed; HIV-positive, high viral load) in median pulse, respiratory rate, arterial pressure, WBC count, platelet count, sodium, potassium, urea, or creatinine (Table 1). At presentation 25% ($n = 35/141$, 95% CI [18–32%]) of patients were hypotensive (mean arterial pressure (MAP) <65 mmHg). Risk of poor outcome was predicted to be high for 16% ($n = 19/121$, 95% CI [9–22%]) of patients by qSOFA and for 51% ($n = 35/68$, 95% CI [40–63%]) of patients using full SOFA scores. HIV-positive patients (66%, $n = 53/80$, 95% CI [56–77%]) were more likely to have moderate or high qSOFA scores (i.e., qSOFA score 1) than HIV-negative patients (46%, $n = 19/41$, 95% CI [31–62%]; $p = 0.048$). Of the 142 patients enrolled, 47% ($n = 67/142$, 95% CI [39–55%]), 27% ($n = 38/142$, 95% CI [19–34%]), and

9% (*n* = 13/142, 95% CI [4–14%]) had two, three, and four SIRS criteria, respectively. Using a combination of the SOFA criteria and laboratory data, HIV-positive patients (60%, *n* = 30/50, 95% CI [46–74%]) were more likely to have two or more life-threatening organ dysfunctions than HIV-negative patients (28%, *n* = 5/18, 95% CI [10–53%]; *p* = 0.02) (Table 1).

Aetiologic results

Overall, 20% (*n* = 28/142, 95% CI [13–26%]) of blood cultures and 24% (*n* = 29/120, 95% CI [17–32%]) of urine cultures were positive (Table 2), without significant variation by HIV status. HIV-positive patients more often had bacteriologic evidence of infection (72%, *n* = 41/57, 95% CI [60–84%] vs 26%, *n* = 9/34, 95% CI [12–41%], *p* < 0.001), and POC tests frequently gave actionable clinical information: TB (urine TB LAM-positivity, 36%, 95% CI [24–48%]), cryptococcal disease (CrAg positivity 15%, 95% CI [7–23%]), and *S. pneumoniae* sepsis (urine antigen, 12%, 95% CI [4–20%]). Of these patients, 1 had both TB and cryptococcal infection, 3 tested positive for both TB and *S. pneumoniae*, and 10 had evidence of either TB or Cryptococcus and a concurrent bacterial infection (blood or urine). For *S. pneumoniae* specifically, HIV-positive patients were more likely to screen positive as compared to HIV-negative patients (12%, *n* = 8/65, 95% CI [4–20%] vs 0%, *n* = 0/34, *p* < 0.05). ART-naïve patients and patients on ART with high viral loads had higher rates of opportunistic infections including TB and cryptococcal infections (51%, *n* = 20/39, 95% CI [36–67%] and 60%, *n* = 12/20, 95% CI [36–81%], respectively) relative to those on ART who were virally suppressed (19%, *n* = 4/21, 95% CI [5–42%]) (Table 2).

Microbiologic drug susceptibility testing (DST)

Microbiologic DST demonstrated a notable uniform resistance to ceftriaxone for *Klebsiella pneumoniae* (blood and urine), and significant resistance to ceftriaxone among *E. coli* isolated from urine. Both *S. typhi* isolates were resistant to fluoroquinolones, and *Enterococcus faecalis* specimens were uniformly susceptible to vancomycin (Supplementary Tables 1 and 2).

HIV RNA sequencing

We sequenced RNA from 8 of 18 patients with high viral loads; all eight were HIV-1 sub-type C. Of the six samples from ART-naïve patients, five exhibited no resistance mutations; one had evidence of non-nucleoside reverse-transcriptase inhibitor (NNRTI) (K103N, V106M, Y181C and G190A) and nucleotide reverse transcriptase inhibitor (NRTI) resistance (D67N, K70E, L74I, Y115F and M184V), as did two patients reporting current use of full dose combination efavirenz (EFV) or nevirapine (NVP) and tenofovir df/lamivudine (EFV/NVP/TDF/3TC) with an NNRTI backbone.

Mortality

Among all patients, 31.6% (*n* = 45/142, 95% CI [24–39%]) died within 30 days of hospital admission (Figure 2). This included 13.4% (*n* = 19/142, 95% CI [8–19%]) who died in-hospital within three days, 14.8% (*n* = 21/142, 95% CI [9–21%]) who died in-hospital after three days, and 3.5% (*n* = 5/142, 95% CI [4–7%]) who died post-hospital discharge but

within 30 days of admission. Patients were additionally traced in December 2016, anywhere from 1 to 10 months after their initial discharge. Among those confirmed to be alive at 30 days (68.3%; $n = 97/142$), seven ($n = 7/97$, 7.2%, 95% CI [2–12]) were traced and confirmed to have died after their hospital admission. Relative to HIV-negative patients, and after adjustment for age and sex the 30-day mortality hazard ratio (HR) for HIV-positive patients with high-viral load, viral suppression, and ART-naïve, was 3.59 (95% CI [1.27–10.16]; $p = 0.02$), 1.88 (95% CI [0.78–4.55]; $p = 0.16$), and 1.61 (95% CI [0.64–4.09]; $p = 0.31$), respectively (Table 3). In a secondary analysis considering time on ART, compared to HIV-negative patients, higher mortality (HR 3.70, 95% CI [1.34–10.17], $p = 0.01$) was noted among those HIV-positive patients on ART <6 months relative to those on ART >6 months (HR 1.98, 95% CI [0.92–4.27], $p = 0.08$) or those who were ART-naïve (HR 1.40, 95% CI [0.57–3.40], $p = 0.46$) (Supplementary Table 3). Disease-specific mortality among HIV-positive patients was 25% ($n = 2/8$, 95% CI [3–65%]) for *S. pneumoniae*, 36% ($n = 5/14$, 95% CI [13–65%]) for cryptococcal disease, and 32% ($n = 7/22$, 95% CI [14–55%]) for TB. For bacteraemic patients, HIV-positive patients had mortality rates of 60% ($n = 3/5$, [15–95%]) and 33% ($n = 3/9$, [7–70%]) for gram-positive and gram-negative infections, respectively.

Discussion

In a consecutive sample of adults hospitalized with community-acquired suspected sepsis in Harare, Zimbabwe, we found that morbidity and mortality were dominated by HIV and associated opportunistic pathogens, consistent with prior studies from sub-Saharan Africa (Lewis et al., 2019). Routine use of available POC diagnostic assays for TB and Cryptococcus identified over one-half of aetiologies in the CD. Our study was significant in examining blood and urine cultures with antimicrobial susceptibilities, identifying substantial resistance to first-line antibiotics for gram-negative organisms. While our cohort demonstrated higher mortality among HIV-positive as compared with HIV-negative patients, consistent with prior studies (Lewis et al., 2019), it is notable that HIV-positive patients on ART less than 6 months had relatively higher mortality than ART-naïve patients as did those with high viral loads.

Opportunistic and severe non-AIDS infections remain important causes of morbidity and mortality among HIV-infected patients despite the widespread use of ART in sub-Saharan Africa (Etard et al., 2006; Jacob et al., 2009; Lawn et al., 2008; Ndadane and Maharaj, 2019). The aetiologies and outcomes of sepsis among the HIV-positive population in our cohort are similar to other sub-Saharan African studies conducted in the pre- and early ART eras (Andrews et al., 2017; Boillat-Blanco et al., 2018; Jacob et al., 2009; Moore et al., 2019). A recent metanalysis of sepsis among adults in sub-Saharan Africa reported that HIV and TB predominated as leading causes of both sepsis and resulting mortality (Lewis et al., 2019). Similarly, TB and cryptococcal disease were commonly associated with sepsis in our cohort. Key differences from previous studies, notably the predominance of gram-negative sepsis (especially *E. coli*), are likely related to regional or setting-specific differences in prevalence of specific pathogens (for example, predominance of non-typhoidal *Salmonella* and *S. pneumoniae* in Malawi (Peters et al., 2004; Waitt et al., 2015). We did find, however, that HIV-positive patients were more likely to screen positive as compared to their HIV-

negative counterparts (12%, $n = 8/65$, 95% CI [4–20%] vs 0%, $n = 0/34$, p 0.05) for *S. pneumoniae* (Roson et al., 2004). The incidence of invasive pneumococcal disease has been reported to be higher in HIV-positive patients in Africa both prior to (Jones et al., 1998) and following the widespread scale-up of ART (Conklin et al., 2016; Gill et al., 2008; Meiring et al., 2016).

TB (Archibald et al., 1998; Huerga et al., 2017; Peter et al., 2016) and cryptococcal infection (Eshun-Wilson et al., 2018) are common causes of sepsis and death (Archibald et al., 1998; Cox et al., 2012; Powell et al., 2016) in sub-Saharan Africa, and were likewise the two most common diagnoses in our cohort. Importantly, urine TB-LAM and serum CrAg together identified an aetiology for sepsis that would alter traditional antibiotic management in nearly half of all patients in our study. These data point for the urgent need for these tests to become a part of the routine management and care of sepsis in high HIV burden settings.

The global burden of antimicrobial resistance (AMR) is heterogenous and poorly characterized in many settings, in particular in sub-Saharan Africa (Review on Antimicrobial Resistance, 2016). However, there is evidence of increasing AMR in clinical bacterial isolates to the available and commonly used antibiotics in Africa (Ekwanzala et al., 2018; Leopold et al., 2014), including in neighbouring Malawi (Musicha et al., 2017). While our study lacks a time dimension, we found high levels of resistance to first-line antibiotics in Zimbabwe, including β-lactam antibiotics and ciprofloxacin, with a notable greater degree of resistance among urine as compared to blood isolates. Ceftriaxone, often administered empirically in the CD for suspected sepsis due to its low cost and ostensible broad-spectrum, was found to be ineffective against most *E. Coli* and *K. pneumoniae* isolates in our study. Consistent with reports of high prevalence of extended spectrum beta-lactamase (ESBL) producing organisms on the African continent (Storberg, 2014), we found evidence of resistance among *E. Coli* and *K. pneumoniae* isolates to both amoxicillin-clavulanate and piperacillin-tazobactam in both urine and blood specimens. Although our sample size was small, given the widespread reliance on ceftriaxone as a life-saving antibiotic in the setting of incipient critical illness, our results suggest urgent validation in larger studies to determine its current efficacy.

Many patients in the study (27%) presenting with sepsis had undiagnosed HIV infection and were not receiving treatment. Among those who were on treatment, only 50% had achieved virologic suppression. Additionally, although genotyping was possible in only a small number of the subjects, 3/8 had high level drug resistance to first-line therapy. This may represent the use of the CD as an important safety net and contact point for HIV patients who are developing life-threatening complications from their HIV. Certainly critical/severe illness impairs access and full adherence to ART regimens leading to virologic failure (high viral load) without drug resistance mutations. Mortality has also been shown to be greatest during the initial months of ART initiation (Etard et al., 2006; Lawn et al., 2008), consistent with our finding of an increased 30-day mortality for HIV-positive patients on ART for <6 months. We hypothesize that this high mortality may be in part explained by Immune Reconstitution Inflammatory Syndrome (IRIS) (Muller et al., 2010; Murdoch et al., 2007). However, the high mortality among HIV-positive patients on ART with high viral loads may

also be explained by inadequate clinical monitoring of ART, transmitted and acquired resistance, and virologic failure (50%) on first-line treatment.

Our study has several important limitations. First, the modest sample size and variety of potential sepsis aetiologies limit pathogen-specific conclusions. Additionally, our sample size limits the strength of the conclusions we can make regarding the effect of HIV-relevant parameters on sepsis outcomes. Second, respiratory rate in the CD was inconsistently reported, and as in a number of high-income settings (Cretikos et al., 2008; Hogan, 2006), when recorded was often reported but not properly measured. Multiple imputation was therefore used to impute missing values for day 0 respiratory rate when qSOFA scores were utilized. Third, ours was a single-centre study, and the degree to which a tertiarycare centre in Harare, Zimbabwe is generalizable tothe region or continent is unknown.

In conclusion, HIV and opportunistic pathogens, especially TB and cryptococcal infection, dominated the clinical presentation of patients presenting to the CD with suspected sepsis, and were associated with high mortality. However, severe non-AIDS infections were also important contributors, with AMR observed to be a growing public health problem, in particular for gram-negative organisms. Low-cost POC testing to rapidly identify HIV and opportunistic pathogens in the CD should be routinely employed in patient management and therapeutic decisions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

Alere. https://www.alere.com/en/home/product-details/determine-tb-lam.html.

Alere Determine TB LAM Ag;.

Alere;. https://www.alere.com/en/home/product-details/binaxnow-streptococcus-pneumoniae.html. Alere Binaxnow Streptococcus Pneumoniae Antigen Card.

American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992;20(6):864–74. [PubMed: 1597042]

Andrews B, Semler MW, Muchemwa L, Kelly P, Lakhi S, Heimburger DC, et al. Effect of an early resuscitation protocol on in-hospital mortality among adults with sepsis and hypotension: a randomized clinical trial. JAMA 2017;318 (13):1233–40. [PubMed: 28973227]

Archibald LK, den Dulk MO, Pallangyo KJ, Reller LB. Fatal Mycobacterium tuberculosis bloodstream infections in febrile hospitalized adults in Dar es Salaam, Tanzania. Clin Infect Dis 1998;26(2):290–6. [PubMed: 9502444]

Boillat-Blanco N, Mbarack Z, Samaka J, Mlaganile T, Mamin A, Genton B, et al. Prognostic value of quickSOFA as a predictor of 28-day mortality among febrile adult patients presenting to emergency departments in Dar es Salaam, Tanzania. PLoS ONE 2018;13(6):e0197982.

Carugati M, Zhang HL, Kilonzo KG, Maze MJ, Maro VP, Rubach MP, et al. Predicting mortality for adolescent and adult patients with fever in resource-limited settings. Am J Trop Med Hyg 2018;99(5):1246–54. [PubMed: 30226134]

Conklin LM, Bigogo G, Jagero G, Hampton L, Junghae M, da Gloria Carvalho M, et al. High Streptococcus pneumoniae colonization prevalence among HIV-infected Kenyan parents in the year before pneumococcal conjugate vaccine introduction. BMC Infect Dis 2016;16:18. [PubMed: 26774803]

Cox JA, Lukande RL, Nelson AM, Mayanja-Kizza H, Colebunders R, Van Marck E, et al. An autopsy study describing causes of death and comparing clinicopathological findings among hospitalized patients in Kampala, Uganda. PLoS ONE 2012;7(3):e33685.

Cretikos MA, Bellomo R, Hillman K, Chen J, Finfer S, Flabouris A. Respiratory rate: the neglected vital sign. Med J Aust 2008;188(11):657–9. [PubMed: 18513176]

Drain PK, Hong T, Hajat A, Krows M, Govere S, Thulare H, et al. Integrating hypertension screening at the time of voluntary HIV testing among adults in South Africa. PLoS ONE 2019;14(2):e0210161.

Ekwanzala MD, Dewar JB, Kamika I, Momba MNB. Systematic review in South Africa reveals antibiotic resistance genes shared between clinical and environmental settings. Infect Drug Resist 2018;11:1907–20. [PubMed: 30425540]

Eshun-Wilson I, Okwen MP, Richardson M, Bicanic T. Early versus delayed antiretroviral treatment in HIV-positive people with cryptococcal meningitis. Cochrane Database Syst Rev 2018;7:CD009012.

Etard JF, Ndiaye I, Thierry-Mieg M, Gueye NF, Gueye PM, Laniece I, et al. Mortality and causes of death in adults receiving highly active antiretroviral therapy in Senegal: a 7-year cohort study. AIDS 2006;20(8):1181–9. [PubMed: 16691070]

Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, et al. Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. Am J Respir Crit Care Med 2016;193(3):259–72. [PubMed: 26414292]

Gill CJ, Mwanakasale V, Fox MP, Chilengi R, Tembo M, Nsofwa M, et al. Impact of human immunodeficiency virus infection on Streptococcus pneumoniae colonization and seroepidemiology among Zambian women. J Infect Dis 2008;197(7):1000–5. [PubMed: 18419536]

Hansen J, Slechta ES, Gates-Hollingsworth MA, Neary B, Barker AP, Bauman S, et al. Large-scale evaluation of the immuno-mycologics lateral flow and enzyme-linked immunoassays for detection of cryptococcal antigen in serum and cerebrospinal fluid. Clin Vaccine Immunol 2013;20(1):52–5. [PubMed: 23114703]

Hogan J. Why don't nurses monitor the respiratory rates of patients?. Br J Nurs 2006;15(9):489–92. [PubMed: 16723921]

Huerga H, Ferlazzo G, Bevilacqua P, Kirubi B, Ardizzoni E, Wanjala S, et al. Incremental yield of including determine-TB LAM assay in diagnostic algorithms for hospitalized and ambulatory HIV-positive patients in Kenya. PLoS ONE 2017;12(1):e0170976.

Jacob ST, Moore CC, Banura P, Pinkerton R, Meya D, Opendi P, et al. Severe sepsis in two Ugandan hospitals: a prospective observational study of management and outcomes in a predominantly HIV-1 infected population. PLoS ONE 2009;4(11): e7782.

Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, Harrison TS. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. Clin Infect Dis 2009;48(7):856–62. [PubMed: 19222372]

Jones N, Huebner R, Khoosal M, Crewe-Brown H, Klugman K. The impact of HIV on Streptococcus pneumoniae bacteraemia in a South African population. AIDS 1998;12(16):2177–84. [PubMed: 9833859]

Kaukonen K-M, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. N Engl J Med 2015;372 (17):1629–38. [PubMed: 25776936]

Koss CA, Jarlsberg LG, den Boon S, Cattamanchi A, Davis JL, Worodria W, et al. A clinical predictor score for 30-day mortality among HIV-infected adults hospitalized with pneumonia in Uganda. PLoS ONE 2015;10(5):e0126591.

Lawn SD, Harries AD, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. AIDS 2008;22(15):1897–908. [PubMed: 18784453]

Lawn SD, Dheda K, Kerkhoff AD, Peter JG, Dorman S, Boehme CC, et al. Determine TB-LAM lateral flow urine antigen assay for HIV-associated tuberculosis: recommendations on the design and reporting of clinical studies. BMC Infect Dis 2013;13:407. [PubMed: 24004840]

Leopold SJ, van Leth F, Tarekegn H, Schultsz C. Antimicrobial drug resistance among clinically relevant bacterial isolates in sub-Saharan Africa: a systematic review. J Antimicrob Chemother 2014;69(9):2337–53. [PubMed: 24879668]

Lewis JM, Feasey NA, Rylance J. Aetiology and outcomes of sepsis in adults in sub-Saharan Africa: a systematic review and meta-analysis. Crit Care 2019;23 (1):212. [PubMed: 31186062]

Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. Clin Infect Dis 2006;42(11):1608–18. [PubMed: 16652319]

Manasa J, Danaviah S, Pillay S, Padayachee P, Mthiyane H, Mkhize C, et al. An affordable HIV-1 drug resistance monitoring method for resource limited settings. J Vis Exp 2014;(85).

Marazzi MC, Liotta G, Germano P, Guidotti G, Altan AD, Ceffa S, et al. Excessive early mortality in the first year of treatment in HIV type 1-infected patients initiating antiretroviral therapy in resource-limited settings. AIDS Res Hum Retroviruses 2008;24(4):555–60. [PubMed: 18366314]

Meiring S, Cohen C, Quan V, de Gouveia L, Feldman C, Karstaedt A, et al. HIV infection and the epidemiology of invasive pneumococcal disease (IPD) in South African adults and older children prior to the introduction of a pneumococcal conjugate vaccine (PCV). PLoS ONE 2016;11(2):e0149104.

Moore CC, Jacob ST, Banura P, Zhang J, Stroup S, Boulware DR, et al. Etiology of sepsis in Uganda using a quantitative polymerase chain reaction-based TaqMan array card. Clin Infect Dis 2019;68(2):266–72. [PubMed: 29868873]

Muller M, Wandel S, Colebunders R, Attia S, Furrer H, Egger M, et al. Immune reconstitution inflammatory syndrome in patients starting antiretroviral therapy for HIV infection: a systematic review and meta-analysis. Lancet Infect Dis 2010;10(4):251–61. [PubMed: 20334848]

Murdoch DM, Venter WD, Van Rie A, Feldman C. Immune reconstitution inflammatory syndrome (IRIS): review of common infectious manifestations and treatment options. AIDS Res Ther 2007;4:9. [PubMed: 17488505]

Musicha P, Cornick JE, Bar-Zeev N, French N, Masesa C, Denis B, et al. Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998–2016): a surveillance study. Lancet Infect Dis 2017;17 (10):1042–52. [PubMed: 28818544]

Ndadane N, Maharaj RC. The epidemiology of sepsis in a district hospital emergency centre in Durban, KwaZulu natal. Afr J Emerg Med 2019;9(3):123–6. [PubMed: 31528529]

Nisula S, Yang R, Poukkanen M, Vaara ST, Kaukonen KM, Tallgren M, et al. Predictive value of urine interleukin-18 in the evolution and outcome of acute kidney injury in critically ill adult patients. Br J Anaesth 2015;114(3):460–8. [PubMed: 25472925]

Olofsson E, Ozenci V, Athlin S. Evaluation of the Sofia S. pneumoniae FIA for detection of pneumococcal antigen in patients with bloodstream infection. J Clin Microbiol 2019;57(8).

Peter JG, Zijenah LS, Chanda D, Clowes P, Lesosky M, Gina P, et al. Effect on mortality of point-of-care, urine-based lipoarabinomannan testing to guide tuberculosis treatment initiation in HIV-positive hospital inpatients: a pragmatic, parallel-group, multicountry, open-label, randomised controlled trial. Lancet 2016;387 (10024):1187–97. [PubMed: 26970721]

Peters RP, Zijlstra EE, Schijffelen MJ, Walsh AL, Joaki G, Kumwenda JJ, et al. A prospective study of bloodstream infections as cause of fever in Malawi: clinical predictors and implications for management. Trop Med Int Health 2004;9 (8):928–34. [PubMed: 15304000]

Powell MK, Benkova K, Selinger P, Dogosi M, Kinkorova Lunackova I, Koutnikova H, et al. Opportunistic infections in HIV-infected patients differ strongly in frequencies and spectra

between patients with low CD4+ cell counts examined postmortem and compensated patients examined antemortem irrespective of the HAART era. PLoS ONE 2016;11(9):e0162704.

Rajasingham R, Meya DB, Boulware DR. Integrating cryptococcal antigen screening and pre-emptive treatment into routine HIV care. J Acquir Immune Defic Syndr 2012;59(5):e85–91. [PubMed: 22410867]

Reinhart K, Daniels R, Kissoon N, Machado FR, Schachter RD, Finfer S. Recognizing sepsis as a global health priority – a WHO resolution. N Engl J Med 2017;377 (5):414–7. [PubMed: 28658587]

Review on Antimicrobial Resistance. Tackling drug-resistant infections globally: final report and recommendations. 2016.

Roson B, Fernandez-Sabe N, Carratala J, Verdaguer R, Dorca J, Manresa F, et al. Contribution of a urinary antigen assay (Binax NOW) to the early diagnosis of pneumococcal pneumonia. Clin Infect Dis 2004;38(2):222–6. [PubMed: 14699454]

Shoji H, Domenech A, Simonetti AF, Gonzalez A, Garcia-Somoza D, Cubero M, et al. The Alere BinaxNOW pneumococcal urinary antigen test: diagnostic sensitivity for adult pneumococcal pneumonia and relationship to specific serotypes. J Clin Microbiol 2018;56(2).

Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). JAMA 2016;315(8):801–10. [PubMed: 26903338]

Storberg V. ESBL-producing Enterobacteriaceae in Africa – a non-systematic literature review of research published 2008–2012. Infect Ecol Epidemiol 2014;4:, doi:10.3402/iee.v4.20342.

R Core Team. A language and environment for statitical computing. 2016 Available from: http://www.r-project.org/.

Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalfof the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 1996;22(7):707–10. [PubMed: 8844239]

Waitt PI, Mukaka M, Goodson P, SimuKonda FD, Waitt CJ, Feasey N, et al. Sepsis carries a high mortality among hospitalised adults in Malawi in the era of antiretroviral therapy scale-up: a longitudinal cohort study. J Infect 2015;70 (1):11–9. [PubMed: 25043393]

World Health Organization. World malaria report: Zimbabwe country profile. Geneva: WHO Press; 2018.

Zimbabwe Ministry of Health and Child Care. In: Mo H, editor. Zimbabwe national guidelines on HIV testing and counselling. Zimbabwe: Harare; 2014.
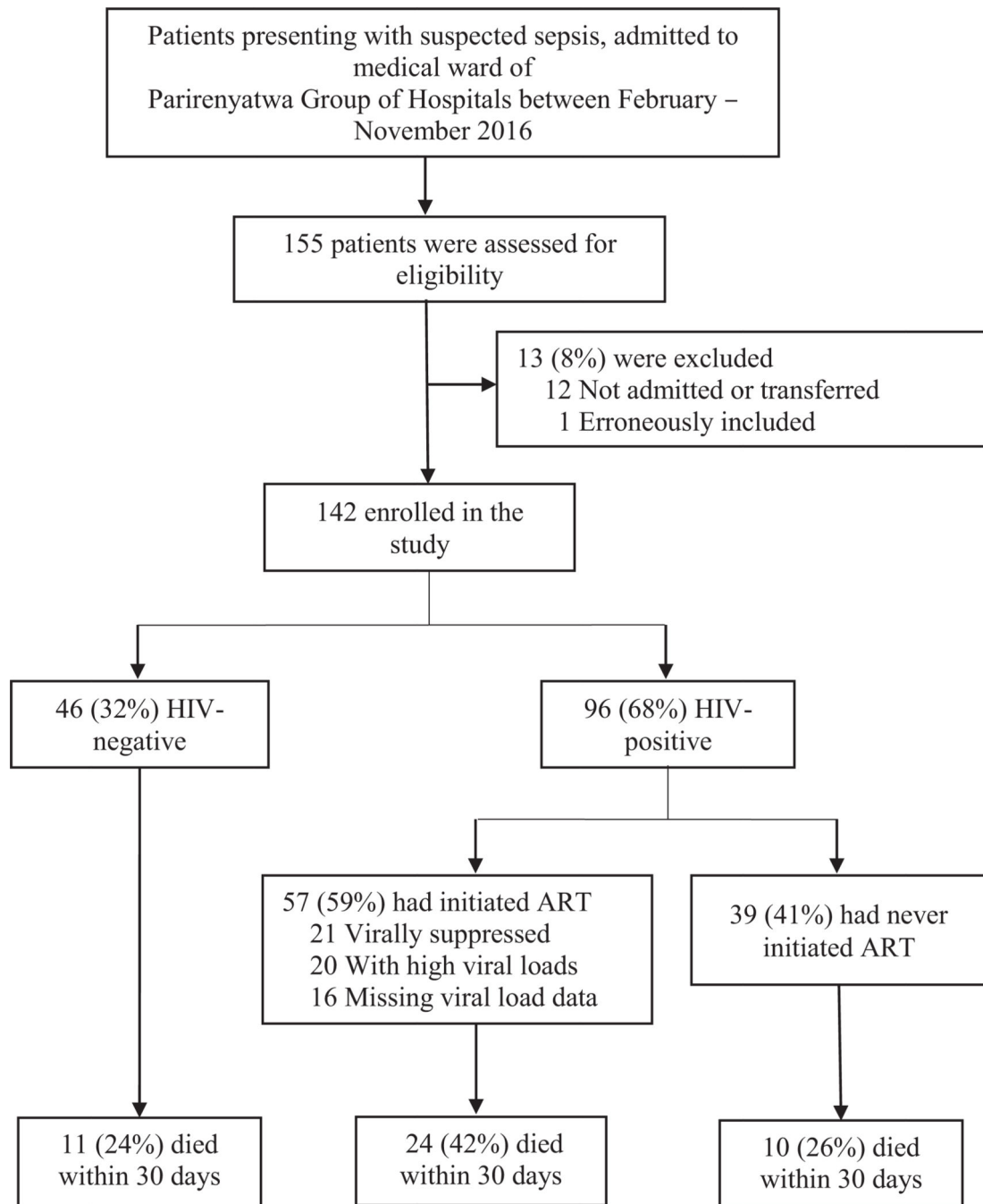
Figure 1.
Participant enrolment and outcomes.
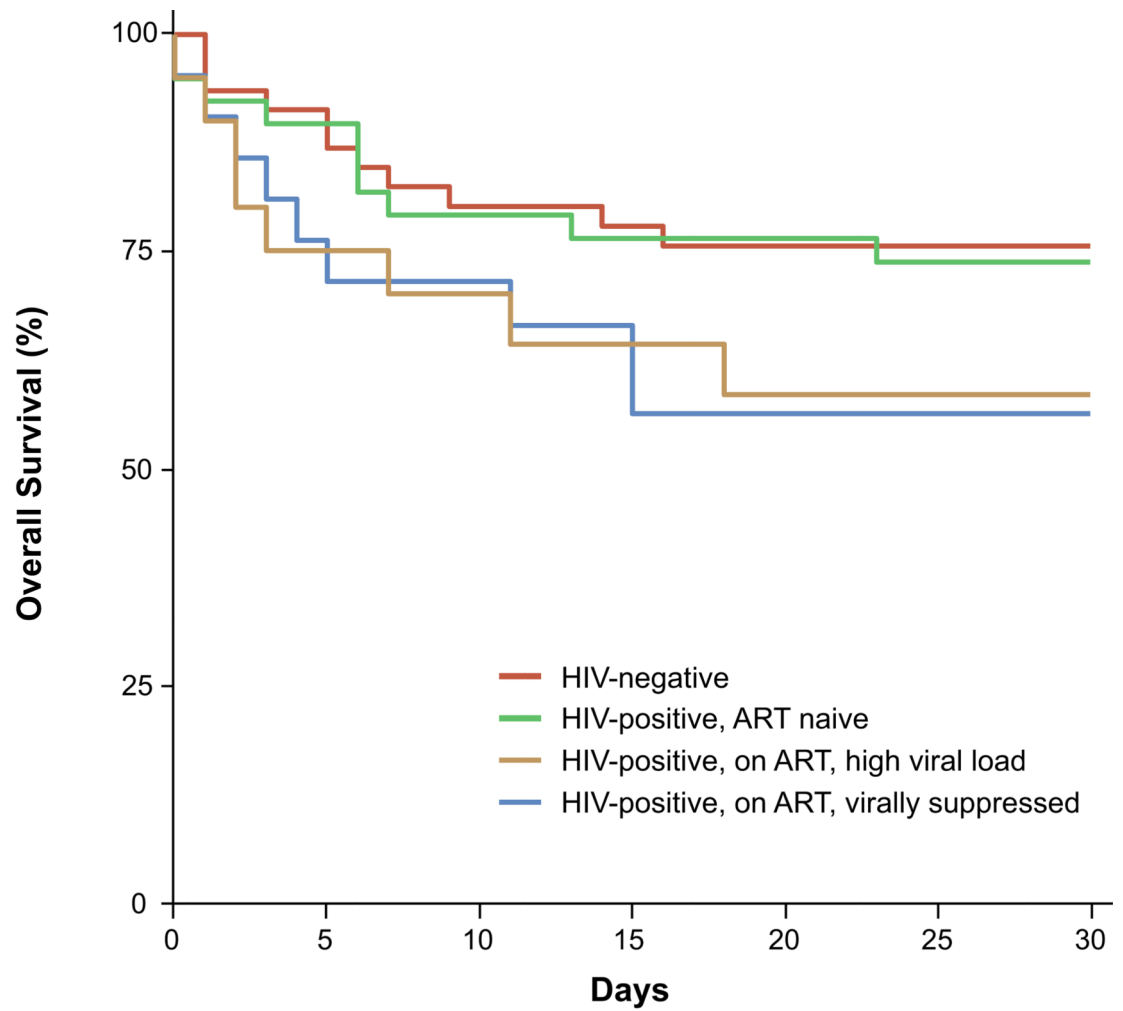
Figure 2.
Kaplan–Meier estimates of time to death from time of admission among HIV-negative, HIV-positive, ART-naïve, and HIV-positive, on ART virally suppressed/high viral load patients.

Table 1

Baseline characteristics of study participants.

Variable	HIV-negative (n = 46)	HIV-positive		ART-naïve (n = 39)
		On ART (n = 57[a])		
		Virally suppressed (n = 21)	High viral load (n = 20)	
Demographic and HIV characteristics				
Age – years	39 (28–58)	49 (41–59)	33 (31–41)	38 (31–45)
Male sex – no. (%)	26 (57%)	9 (43%)	12 (60%)	22 (56%)
CD4 count – no. of cells/µl	n/a	134 (46–169)	18 (10–38)	28 (9–98)
Geometric mean CD4+ count – no. of cells/µl	n/a	102	23	28
HIV-1 RNA viral load – copies/ml	n/a	0 (0–40)	42,900 (4908–99,600)	323,600 (175,750–973,000)
Time on ART – months	n/a	48 (4–108)	7 (1–48)	n/a
TMP/SMX prophylaxis – no. (%)	n/a	13 (62%)	12 (60%)	7 (18%)
Clinical characteristics at presentation				
Pulse rate – beats/minute	95 (88–109)	103 (90–118)	116 (106–128)	100 (95–113)
Respiratory rate – breaths/minute	21 (19–22)	20 (19–20)	20 (10–20)	21 (20–23)
Mean arterial pressure – mmHg	82 (67–94)	71 (60–83)	80 (66–88)	74 (67–84)
White-cell count – × 10^9/litre	9 (6–13)	9 (6–16)	4 (3–7)	8 (5–11)
Haemoglobin level – g/dl	11.8 (9.2–13.2)	8.2 (6.0–9.8)	8.4 (7.3–10.3)	9.0 (6.8–10.8)
Platelet count – × 10^3 $µl^{-1}$	206 (109–276)	192 (101–284)	165 (107–257)	209 (153–341)
Sodium level – mmol/litre	132 (129–136)	133 (130–135)	130 (125–132)	132 (128–135)
Potassium level – mmol/litre	3.9 (3.6–4.4)	4.3 (4.0–4.6)	4.2 (4.0–4.8)	3.9 (3.3–4.6)
Urea level – mmol/litre	5.9 (4.3–9.2)	8.2 (4.8–15.2)	7.1 (4.7–8.5)	7.0 (3.8–15.0)
Creatinine – µmol/litre	86 (72–112)	87 (62–124)	82 (64–134)	86 (53–204)
Fulfilling SOFA criteria[b] – no. (%)	5/18 (28%)	8/14 (57%)	5/9 (56%)	13/22 (59%)
qSOFA Score[c]				
0 (Low)	21/41 (51%)	6/20 (30%)	5/15 (33%)	5/34 (15%)
1 (Moderate)	14/41 (34%)	12/20 (60%)	6/15 (40%)	21/34 (62%)
2 (High)	6/41 (15%)	2/20 (10%)	4/15 (27%)	8/34 (24%)

Values are medians (interquartile range (IQR)), unless otherwise specified.

[a] 16 have missing data regarding viral load.

[b] Sequential Organ Failure Assessment (SOFA) criteria: 2 of respiratory rate >22 breaths/minute, altered mentation, systolic blood pressure 100 mmHg, creatinine >110 μmol/L, platelets <150 × 10^3 μL^{-1} or total bilirubin >20 μmol/L (74 participants do not have complete data for SOFA criteria).

[c] Low (0), moderate (1), or high (2) qSOFA score (range, 0 [best] to 3 [worst]). (19 participants do not have complete data for qSOFA score.)

Table 2

Positive bacteriologic data of study participants.

| | HIV-negative (n = 46) | HIV-positive | | | | p-Value[c] | Overall |
| | | On ART (n = 57[a]) | | ART-naïve (n = 39) | | HIV-positive vs. HIV-negative | |
		Virally suppressed (n = 21)	High viral load (n = 20)				
S. pneumoniae antigen – no./total no. (%)	0/34 (0%)	0/15 (0%)	1/18 (6%)	7/27 (26%)		<0.05	0.001
Serum CrAg[b] – no./total no. (%)	Not tested	1/18 (6%)	5/19 (26%)	7/35 (20%)		–	0.27
Urine TB LAM – no./total no. (%)	Not tested	3/14 (21%)	9/18 (50%)	8/25 (32%)		–	0.39
Aerobic blood culture – no./total no. (%)	7/46 (15%)	4/21 (19%)	2/20 (10%)	10/39 (26%)		0.35	0.53
Gram-positive pathogens – no.	3	0	0	4		–	–
Streptococcus pneumoniae	0	0	0	2		–	–
Streptococcus pyogenes	1	0	0	0		–	–
Actinomycetes	1	0	0	1		–	–
Kocuria rosea	1	0	0	0		–	–
Unidentified	0	0	0	1		–	–
Gram-negative pathogens – no.	4	3	1	1		–	–
Salmonella typhi	2	0	0	0		–	–
Escherichia coli	2	2	0	1		–	–
Klebsiella pneumonia	0	0	0	0		–	–
Unidentified	0	1	1	0		–	–
Others – no.	0	1	1	5		–	–
Urine culture – no./total no. (%)	7/39 (18%)	5/16 (31%)	3/18 (17%)	9/34 (26%)		0.40	0.61
Gram-positive pathogens – no.	3	0	0	6		–	–
Enterococcus faecalis	2	0	0	4		–	–
Unidentified	1	0	0	1		–	–
Staphylococcus gallinarum	0	0	0	1		–	–
Gram-negative pathogens – no.	4	4	3	1		–	–
Escherichia coli	2	1	2	1		–	–
Klebsiella pneumoniae	2	3	0	0		–	–
Pseudomonas aeruginosa	0	0	1	0		–	–

	HIV-negative (n = 46)	HIV-positive			p-Value[c]	
		On ART (n = 57[a])		ART-naïve (n = 39)	HIV-positive vs. HIV-negative	Overall
		Virally suppressed (n = 21)	High viral load (n = 20)			
Others – no.	0	1	0	2	–	–

[a] Among the 16 with missing viral load data: 1, 2, 1, and 1 was/were noted to have positive blood culture result in *S. aureus*, *E. coli*, *Brevundimonas diminuta*, and *K. pneumoniae*, respectively. 1 and 1 was noted to have positive urine culture result in *E. coli*, and *K. pneumoniae*, respectively. 0, 1, and 2 was/were noted to have positive *S. pneumoniae* antigen, Serum CrAg, and Urine TB LAM result, respectively.

[b] CrAg refers to serum cryptococcal antigen.

[c] For group comparisons, the chi-square test was used, unless the expected cell frequency was less than 6, in which case Fisher's exact test was used.

Table 3

Multivariate Cox proportional-hazards analysis of 30-day mortality by HIV and ART status.

Risk factor	Hazard ratio (95% CI)	p-Value	β regression coefficient
Group			
HIV−	Reference	–	–
HIV+, ART-naïve	1.61 (0.64–4.09)	0.31	0.48
HIV+, on ART, virally suppressed	1.88 (0.78–4.55)	0.16	0.63
HIV+, on ART, high viral load	3.59 (1.27–10.16)	0.02	1.28
Age–per 10 yrs.	1.47 (1.19–1.82)	<0.001	0.39
Male sex	1.21 (0.63–2.32)	0.56	0.19