

Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

A. Study Setting

Facility:

The University Teaching Hospital (UTH) is a 1,500-bed national referral hospital for the nation of Zambia and a major primary-level hospital for the city of Lusaka. The emergency department at UTH contains a 4-bed intake room, a 4-bed Acute Bay, and between 35-40 additional beds. The inpatient wards at UTH include over 400 internal medicine beds with an average occupancy above 80% and an average HIV prevalence among internal medicine ward patients of 50%. The 10-bed intensive care unit (ICU) at UTH is an open-admission ICU with 8-10 mechanical ventilators. At the time of the current study, the ICU at UTH was the only ICU in the country (population 13 million) and the average occupancy was above 95%.

Oxygen therapy by nasal cannula is available in most parts of UTH, including in the emergency department, medical wards, and ICU. Noninvasive ventilation was not available at UTH during the time period of the current study, and invasive mechanical ventilation was only available for patients admitted to the ICU. The hemodialysis unit at UTH primarily serves outpatients with end-stage renal disease but is capable of providing intermittent hemodialysis for acute renal failure in the inpatient setting.

Critically ill patients presenting to the emergency department at UTH are most commonly cared for in the Acute Bay. The emergency department length of stay overall ranges between 6 and 48 hours, but length of stay is often longer for patients in the Acute Bay who are considered too unstable for transfer to the medical ward. In the emergency department, physicians conduct bedside rounds twice daily. In the medical inpatient wards, physicians conduct bedside rounds once daily.

Usual Care for Sepsis:

Usual care for sepsis at UTH consists of early empiric antibiotics without blood cultures, and oxygen therapy for patients with hypoxemia. Intravenous (IV) fluid administration during usual care for early sepsis at UTH is variable. The most common orders for IV fluids among patients in the UTH emergency department with sepsis are standing orders for intravenous crystalloid infusion at an hourly rate over 24 hours. In instances in which a provider decides to order an IV fluid bolus for a specific patient, an order will be written for 1-4 liters of IV fluid “fast”, in which case the specified volume of IV crystalloid is infused by a nurse through a peripheral IV over 30-60 minutes. In the emergency department at UTH, weight cannot be measured in non-ambulatory patients and IV fluid is dosed by absolute IV fluid volume rather than weight-based dosing. Central venous catheterization and central venous pressure measurement are not available in the emergency department at UTH. Jugular venous pressure is not routinely measured during the usual care of patients with sepsis in the emergency department at UTH. The only vasopressor available in Zambia during the time period of the study was dopamine. Administration of vasopressors to patients with sepsis and hypotension is uncommon as a part of usual care at UTH. Critically ill patients with sepsis presenting to the emergency department at UTH are rarely admitted to the ICU due to limited ICU capacity, high ICU occupancy, and prioritization of ICU utilization for patients with trauma, cardiovascular disease, poisoning, and post-operative complications.

End-of-Life Care:

A common preference in Zambian culture is for terminally ill family members not to die in the home. Patients with severe or end-stage infection are often brought to the hospital late in their illness. Despite high in-hospital mortality rates, during the time period of the current trial formal palliative care and home hospice resources were unavailable for patients cared for at UTH. Cardiac defibrillators are available in the emergency department and ICU and advanced cardiac life support can be performed in many of the hospital wards at UTH. Limited availability of mechanical ventilation, however, means most patients admitted to UTH who subsequently die do so in the hospital, on an inpatient ward, without receipt of advanced life support.

B. Elements of the Study Design Targeting Patient Safety

The Simplified Severe Sepsis Protocol-2 (SSSP-2) trial was designed in 2012. At the time the SSSP-2 trial was being designed, international recommendations for the care of sepsis patients presenting to the emergency department¹ advocated early protocolized resuscitation with intravenous fluid and vasopressors in a manner similar to the Early Goal Directed Therapy protocol reported by Rivers et al². Two recently completed trials of hemodynamic resuscitation for African patients with severe infection, however, had reported potential findings of harm with early fluid bolus administration. The Fluid Expansion as Supportive Therapy (FEAST) study reported that fluid boluses significantly increased 48-hour mortality in critically ill children in a resource-limited setting³. The Simplified Severe Sepsis Protocol-1 (SSSP-1) trial conducted in the same study setting as the planned SSSP-2 trial had been stopped early by the data and safety monitoring board out of concern for increased mortality in the sepsis protocol group for patients with evidence of respiratory failure at enrollment⁴. Although the extent to which the results of the FEAST trial would generalize to adult patients with sepsis was unclear and the unplanned stoppage of the SSSP-1 trial made the findings hypothesis-generating, we specifically designed the SSSP-2 with safety elements informed by the FEAST and SSSP-2 trials.

First, both FEAST and SSSP-1 enrolled patients with non-specific markers of hypoperfusion^{3,4}. We designed the SSSP-2 trial to only enroll patients with overt hypotension to select a group of patients more likely to benefit from fluid administration and less likely to experience harm.

Second, in the SSSP-1 trial, the signal of increased respiratory failure in the sepsis protocol arm appeared to be limited to patients with tachypnea and hypoxemia at baseline⁴. In the SSSP-2 trial, therefore, we excluded patients with baseline tachypnea and hypoxemia.

Third, in order to increase the level of monitoring for patients in the SSSP-2 trial compared to monitoring in the study setting in usual care, we provided a dedicated study nurse to measure and record vital signs including respiratory rate and oxygen saturation hourly for the six hours after enrollment.

Fourth, given the potentially increased risk of respiratory failure in the group being administered more intravenous fluids as a part of the sepsis protocol, the study nurse also measured respiratory rate and oxygen saturation at the completion of each liter of fluid. This level of monitoring significantly exceeded that which is available as a part of routine care in the study environment.

Fifth, the sepsis protocol intervention was designed to actively monitor for evidence of developing fluid overload or respiratory failure on physical examination (respiratory rate increased by more than 5 breaths per minute, oxygen saturation decreased by 3%, JVP \geq 3 cm) and discontinue fluid administration if these signs developed.

Sixth, the total volume of fluid that could be administered in the first 6 hours after emergency department presentation in the sepsis protocol group in the SSSP-2 trial was limited to 4 liters – less than the average volume received in the intervention group in the original Early Goal Directed Therapy trial and less than the average volume received between presentation and 6 hours after enrollment in any of the subsequently published ProCESS, ARISE, or ProMISe trials⁵⁻⁷.

Seventh, we planned for the trial to be overseen by a data and safety monitoring board experienced with the oversight of critical care trials in a resource-limited setting. The SSSP-1 trial had been stopped early by the data safety and monitoring board based on the concern for increased mortality rates due to respiratory failure, and the current data and safety monitoring board was empowered to do the same in the SSSP-2 trial had such a signal been evident during the conduct of the trial or at the interim analysis.

C. Data Collection

- Baseline characteristics including demographic information and information on pre-existing conditions, organ function and markers of disease severity, and infection were collected and entered into the study database within 24 hours of enrolment.
- Vital signs were collected by study personnel at baseline, two hours and 6 hours as follows:
 - Temperature was measured by axillary thermometer.
 - Heart rate and respiratory rate were measured manually.
 - Blood pressure was measured by manual non-invasive sphygmomanometer. For patients with absent Karotkoff sounds but palpable radial or brachial pulses, systolic blood pressure was measured by palpation.
 - Jugular venous pressure (JVP) was measured by the investigator or study nurses who had been trained for the trial to perform a standardized examination of a JVP using a calibrated spirit level and a metric ruler. The distance from the sternal angle to the jugular venous pulsations at the end of expiration was recorded in centimeters. A JVP value of 3 cm above the sternal angle (approximately 8 cm above the right atrium) was used to approximate a central venous pressure value of 10 mm Hg.
 - Arterial oxygen saturation was measured noninvasively by pulse oximetry.
- At the time ED registration, blood was collected for the following laboratory tests as a part of routine clinical care: serum sodium, potassium, creatinine, blood urea nitrogen, bilirubin, complete blood count with differential, human immunodeficiency virus ELISA, CD4+ lymphocyte count (if HIV positive), aerobic blood culture, and malaria parasite smear. The study staff recorded the results of these routine laboratory studies after they had been analyzed as a part of routine care. The study also provided resources to ensure that these investigations were completed when shortages prevented their completion as a part of routine care.
- In addition to the laboratory studies performed as a part of routine care, study staff also obtained blood for the following additional laboratory tests for enrolled patients, which were not part of routine care: serum bicarbonate level (4 mL), one tube of blood (4 mL) at baseline and an additional tube (4mL) 48 hours later to be stored for further immunologic testing, mycobacterial blood cultures (5 mL) in HIV positive patients.
- Whole blood lactic acid concentrations were measured at enrollment and 6 hours after enrollment using the Lactate Pro (ArkRay, Kyoto City, Japan). The first lactate measurement was made by the study nurse with the first set of vital sign measurements immediately after enrollment using a portable lactate meter and test strips to allow simultaneous assessments of baseline lactate value for all patients prior to receipt of study interventions.
- Admission diagnoses were based on history and physical examination prior to any laboratory or imaging results.
- 0.9% saline and lactated Ringer's were considered isotonic crystalloids.

D. Multivariable Model Development

Among the 209 patients enrolled in the SSSP-2 trial, we assessed the incidence of the primary outcome of in-hospital mortality and the secondary outcome of 28-day mortality. A total of 85 of the 209 patients experienced in-hospital mortality and 109 of the 194 patients with 28-day follow-up experience 28-day all-cause mortality. Based on this number of events, we could include 6-8 degrees of freedom in each multivariable logistic regression model without over-fitting. We pre-specified, prior to initiation of enrollment in the study, the inclusion of two independent co-variables in the model: study group assignment and Simplified Acute Physiology Score 3 (SAPS-3), without the inclusion of an interaction term. We performed no data reduction. We fit a logistic regression model for the primary outcome of in-hospital mortality. Study group assignment was treated as a categorical variable with sepsis protocol group compared with usual care group. SAPS-3 score was treated as a continuous variable (and dichotomized into quartiles in a sensitivity analysis). With the same independent covariates we repeated the model with the outcome of 28-day mortality. Given the size of the dataset and the role of the model as a sensitivity analysis to ensure that any observed differences between groups in univariate analysis were not due to differences in baseline SAPS-3 score, we did not perform an independent validation of the performance of the model.

eTable 1. Suspected diagnoses at the time of hospital admission for enrolled patients

Diagnosis*, No. (%)	Usual care (n=103)	Sepsis Protocol (n=106)
Systemic infection		
Malaria	15 (14.6)	9 (8.5)
Infection of the lungs		
Pneumonia	51 (49.5)	52 (49.1)
Tuberculosis†	63 (61.2)	68 (64.2)
Infection of the gastrointestinal system		
Gastroenteritis	20 (19.4)	15 (14.2)
Enteric fever	9 (8.7)	4 (3.8)
Infection of the central nervous system	17 (16.5)	12 (11.3)
Infection of the urinary tract	2 (1.9)	2 (1.9)

*Each patient could have more than one diagnosis

†Includes both pulmonary and disseminated

eTable 2. Microbial pathogens

Organism, No. (%)	Usual care (n=103)	Sepsis Protocol (n=106)
<i>Mycobacterium tuberculosis</i>		
Blood culture positive	19 (18.4)	24 (22.6)
Sputum culture positive without blood culture positive	6 (5.8)	1 (0.9)
Gram positive		
<i>Staphylococcus aureus</i>	6 (5.8)	2 (1.9)
Coagulase negative <i>Staphylococcus</i> *	2 (1.9)	3 (2.8)
<i>Streptococcus pneumoniae</i>	2 (1.9)	2 (1.9)
Other streptococcus species	1 (1.0)	2 (1.9)
Unspecified gram positive cocci	1 (1.0)	1 (0.9)
Gram positive rods*	4 (3.9)	4 (3.8)
Gram negative		
<i>Escherichia coli</i>	2 (1.9)	1 (0.9)
<i>Pseudomonas aeruginosa</i>	1 (1.0)	1 (0.9)
<i>Klebsiella pneumoniae</i>	2 (1.9)	1 (0.9)
Salmonella species†	1 (1.0)	3 (2.8)
Other gram negatives or unspecified	4 (3.9)	1 (0.9)
Cryptococcus‡	2 (1.9)	1 (0.9)
Malaria (blood smear)§	2 (1.9)	1 (0.9)
Culture-negative	48 (46.6)	58 (54.7)

All organisms were isolated from blood cultures unless otherwise specified. The median time until blood culture results were available was 7.5 days [IQR 4.5-11.5 days] in the usual care group and 7.0 days [IQR 5.0-10.0 days] in the sepsis protocol group. Antimicrobials were added or changed after hospital admission and before hospital discharge for 46 (44.7%) patients in the usual care group and 46 (43.4%) patients in the sepsis protocol group ($P > 0.85$). The change in antimicrobials occurred after the availability of blood culture results for 0 (0.0%) patients in the usual care group and 1 (0.9%) patient in the sepsis protocol group ($P > 0.99$)

*Coagulase negative *Staphylococcus* or Gram positive rods may have represented contamination of blood cultures with skin flora

†Includes *Salmonella typhi* and non-typhi *Salmonella* species

‡Includes diagnoses made by culture, India ink, and cerebral spinal fluid antigen

§Slides for malaria diagnosis were only available for 47 (22.5%) participants

eTable 3. Initial intravenous fluid order for each patient assigned to the usual care group

Fluid order		No. (%) (n=103)
No bolus;	1 liter(s) in 24 hours	3 (2.9)
	2	9 (8.7)
	3	16 (15.5)
	4	11 (10.7)
	5	3 (2.9)
1 liter fast, then	1 liter(s) in 24 hours	1 (1.0)
	2	5 (4.9)
	3	10 (9.7)
	4	7 (6.8)
2 liters fast, then	0 liter(s) in 24 hours	5 (4.9)
	1	1 (1.0)
	2	4 (3.9)
	3	2 (1.9)
	4	1 (1.0)
3 liters fast, then	0 liter(s) in 24 hours	3 (2.9)
	1	1 (1.0)
	2	1 (1.0)
4 liters fast, then	3 liter(s) in 24 hours	1 (1.0)
Other orders*		8 (7.8)
Orders unavailable		10 (9.7)

The initial intravenous fluid orders placed by treating clinicians were available for 93 of the 103 patients in the usual care group. A total of 42 (40.8%) patients were not ordered a fluid bolus.

*Among the 8 patients with “other” initial orders, 7 included an intravenous fluid bolus of at least 1 liter.

eTable 4. Sensitivity analyses

Analysis	n	Odds Ratio	95% CI	P Value
Unadjusted logistic regression model (Primary Analysis) – Odds of in-hospital mortality for patients in the sepsis protocol group versus the usual care group for all 209 patients who received study interventions and were followed.	209	1.88	1.07 – 3.30	0.03
Unadjusted logistic regression model ('worst case' imputation of as-randomized population) – Odds of in-hospital mortality for all patients randomized to the sepsis protocol versus all patients randomized to usual care, assuming that the 1 patient excluded post-randomization from the sepsis protocol group lived and the 2 patients excluded post-randomization from the usual care group died.	212	1.75	1.00 – 3.04	0.047
Adjusted logistic regression model – Odds of in-hospital mortality for patients in the sepsis protocol group versus the usual care group, adjusting for baseline SAPS-3 score and lactic acid level as continuous variables.	209	1.93	1.09 – 3.43	0.03
Unadjusted "As Treated" logistic regression – Odds of in-hospital mortality for patients who received ≥ 3 liters of IV fluid in the six hours after ED registration compared with patients who received < 3 liters of IV fluid in the six hours after ED registration.	209	1.45	0.83 – 2.54	0.20
Adjusted "As Treated" logistic regression – Odds of in-hospital mortality for patients who received ≥ 3 liters of IV fluid in the six hours after ED registration compared with patients who received < 3 liters of IV fluid in the six hours after ED registration adjusting for SAPS-3 score as a continuous variable and suspected site of infection.	209	1.41	0.80 – 2.49	0.24
	n	Hazard Ratio	95% CI	P Value
Unadjusted Cox proportional hazards model -- Comparison of survival between patients in the sepsis protocol group versus the usual care group.	209	1.65	1.12 – 2.44	0.01
Adjusted Cox proportional hazards model -- Comparison of survival between patients in the sepsis protocol group versus the usual care group adjusting for SAPS-3 score at baseline as a continuous variable.	209	1.68	1.14 – 2.49	0.009
Adjusted Cox proportional hazards model -- Comparison of survival between patients in the sepsis protocol group versus the usual care group adjusting for SAPS-3 score at baseline categorized by quartile.	209	1.69	1.14 – 2.51	0.001

An odds ratio greater than 1.0 indicates higher odds of death in the sepsis protocol group. A hazard ratio greater than 1.0 indicates shorter survival in the sepsis protocol group.

SAPS-3 Score = Simplified Acute Physiology Score 3, a severity score and mortality estimation tool with possible values ranging from 0 to 217 in which higher values indicate higher risk of in-hospital mortality; ED = emergency department

eTable 5. Adverse events

Adverse Event*, No. (%)	Usual care (n=103)	Sepsis Protocol (n=106)
Dopamine extravasation, tissue ischemia, or necrosis	0 (0.0)	0 (0.0)
Iatrogenic pulmonary edema	0 (0.0)	2 (1.9)
Transfusion reaction	0 (0.0)	0 (0.0)

*A study nurse prospectively screened for the development of the listed adverse events during the 6 hours after enrollment in both study groups.