Supplementary Materials

Supplemental Methods

Study Site and Capacity

Entebbe General Referral Hospital (EGRH) is a 200-bed public district referral hospital with a catchment area of approximately 3 million persons. In the primary catchment area, HIV prevalence is approximately 6% and malaria is endemic.[9] Representative of a general district hospital in SSA, there is no intensive care unit at EGRH. No vasopressor or inotropic agents are available on the hospital wards and intravenous (IV) fluid is typically delivered as 250-500 milliliter infusions of crystalloid, either normal saline or Ringer's lactate solution. As no piped oxygen was available at EGRH during the study period, oxygen concentrators were provided to hospital wards as part of the study program.

RESERVE-U Enrollment Criteria

Patients were included in the parent RESERVE-U study if they fulfilled the following criteria: (1) age ≥18 years, (2) reported a history of fever or had a recorded axillary temperature of ≥37.5ºC at presentation, (3) had clinical illness severe enough to warrant admission to hospital, and (4) were able to provide informed consent or had a surrogate available to do so. Patients were excluded if they presented following trauma or were admitted to a non-medical ward. During the study period, all admissions to the medical wards were screened for eligibility by study staff. Patients were screened on weekdays during daytime hours and were enrolled as close to admission as possible and no longer than 24 hours afterwards. Although patients 5-17 years of age were also included in the parent RESERVE-U cohort, given variations in pathogen-specific infection risk, normal vital sign parameters, and infection-related host immune responses across age groups, for this analysis we included only adults (age ≥18 years).

Outcomes

The primary outcome of the RESERVE-U study was vital status at 30-days after hospital discharge (obtained via telephone from patients or their surrogates). Secondary outcomes included a composite measure of in-hospital outcome (death in-hospital or transfer to Uganda's national referral hospital due to progressive severity of illness) and functional status at discharge (among patients who survived and were not transferred). Patients transferred to the national hospital were contacted to assess vital status at 30-days.

Clinical Data, Sample Collection, and Rapid Pathogen Diagnostics

Study procedures for the parent RESERVE-U cohort have been described.[9,10] Briefly, enrollment occurred within 24 hours of hospital admission, at which time all patients underwent clinical assessments and had rapid testing performed for malaria, HIV, and influenza; for HIV-infected patients testing for tuberculosis (TB) was also performed. Testing for these pathogens was informed by World Health Organization Integrated Management of Adolescent and Adult Illness guidelines for sepsis and septic shock in resource-limited hospitals in SSA (available at: https://apps.who.int/iris/handle/10665/77751). These guidelines emphasize rapid testing for malaria and HIV, a low threshold for TB testing among HIV-infected patients, and consideration of testing or empiric treatment for influenza. For malaria, rapid testing was performed at EGRH using qualitative detection of histidine-rich protein II and lactate dehydrogenase of *P. falciparum* in whole-blood using the SD Bioline Malaria AG P.f. platform (Alere/Abbott, Abbott Park, IL, USA). For all patients not known to be HIV infected, HIV testing was performed at EGRH using serial diagnostic platforms (Determine HIV-1/2 Ag/Ab, Alere/Abbott); Chembio HIV 1/2 Stat-Pak, Chembio Diagnostic Systems, Medford, NY, USA), Uni-gold Recombigen HIV-1/2, Trinity Biotech, Ireland). For all enrolled HIV-infected patients (known or newly diagnosed), a single urine sample (obtained via urinary catheter or spontaneous void) and a single spontaneously

expectorated sputum sample, if obtainable, were tested for evidence of *Mycobacterium tuberculosis* (MTB) infection at EGRH by the study laboratory technician. For urine samples, 60µL of unconcentrated urine was tested using the Determine™ TB-LAM Ag assay (Alere/Abbott) as per the manufacturer's recommended operating procedure. The intensity of any visible band on the test strip was graded by comparing it with band intensities on the manufacturer's post-2014 reference card scale; results were considered positive using the grade 1 cutoff. For sputum samples, testing was performed using the Xpert MTB/RIF Ultra platform (Cepheid, Sunnyvale, CA, USA). Sputum smear microscopy was performed at the discretion of treating clinicians as was Xpert MTB/RIF Ultra testing of sputum for non-HIV-infected patients. Nasopharyngeal swab samples were tested for influenza A and B viruses via real-time polymerase-chain-reaction (RT-PCR). Influenza PCR, implemented with primers from the U.S. Centers for Disease Control and Prevention, was performed as soon as possible following sample collection at Uganda Virus Research Institute, located approximately 2 kilometers from EGRH.

At enrollment, peripheral blood samples were collected into vacutainer tubes and centrifuged, with resulting serum samples stored at -80ºC. For a subset of consecutively enrolled patients, whole-blood samples were collected in PAXgene blood RNA tubes (PreAnalytiX, Qiagen/BD, Hombrechtikon, Switzerland) and stored at -80ºC.

Serum Immunoassays

Cryopreserved serum samples collected at the time of enrollment were sent to Eve Technologies (Calgary, Alberta, Canada) on dry ice for analysis. Interleukin (IL)-6, IL-8, IL-10, interferon (IFN)-γ, IFN-γ-induced protein-10/C-X-C motif chemokine 10 (IP-10/CXCL10), macrophage inflammatory protein-*1-alpha/chemokine (C-C motif) ligand 3 (MIP*-1α/CCL3),

macrophage inflammatory protein-1-beta/chemokine (C-C motif) ligand 4 (MIP-1β/CCL4), and *tumor necrosis factor-α* (TNF-α), angiopoietin-2 (Ang-2), macrophage migration inhibitory factor (MIF), plasminogen activator inhibitor-1 (PAI-1), soluble TNF-receptor type 1 (sTNFR1) and soluble IL-2 receptor alpha/soluble CD25 (sIL-2RA/sCD25) were quantified using custom Luminex 200 system kits (Luminex, Austin, TX, USA) from MilliporeSigma (Burlington, Massachusetts, USA). Angiopoietin-1 (Ang-1) was quantified using a custom Luminex 200 system kit from R&D Systems (Minneapolis, MN, USA). Values below the lower limit of assay quantification (1.1% of all samples) were replaced with the lowest value that could be reliably quantified for that particular mediator. Values above the upper limit of quantification (1.5% of all samples) were replaced with the highest standard curve value for each particular mediator.

Whole-blood RNA isolation, library preparation, and sequencing

From cryopreserved whole-blood samples collected in PAXgene blood RNA tubes (PreAnalytiX, Qiagen/BD, Hombrechtikon, Switzerland) at the time of study enrollment, RNA was isolated and purified using PAXgene blood RNA kits (Qiagen, Hilden, Germany). RNA sample quantity and integrity were assessed using Qubit 2.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and 4200 TapeStation (Agilent Technologies, Palo Alto, CA, USA) platforms, respectively. DNase treatment was performed using Turbo DNase (Thermo Fisher Scientific) prior to library preparation. rRNA depletion was performed using QIAseq FastSelect – rRNA/Globin Kit (Qiagen). RNA sequencing library preparation was performed using the NEBNext Ultra RNA Library Prep Kit for Illumina following the manufacturer's recommendations (NEB, Ipswich, MA, USA). Sequencing libraries were validated using the 4200 Tapestation (Agilent Technologies) and quantified using the Qubit 2.0 Fluorometer (Invitrogen, Thermo Fisher Scientific) as well as by quantitative PCR (Applied Biosystems, Thermo Fisher Scientific). Sequencing libraries were multiplexed and clustered on one lane of a flowcell and loaded on the

Illumina HiSeq 4000 instrument (Illumina, Inc., San Diego, CA, USA) according to manufacturer's instructions. Samples were sequenced using a 2x150 paired end configuration. Image analysis and base calling were conducted by the HiSeq control software. Raw sequence data were converted into FASTQ files and de-multiplexed using Illumina's bcl2fastq 2.17 software. One mis-match was allowed for index sequence identification**.** Adapters were trimmed from raw reads using Trimmomatic and RNA-sequencing data quality were assessed with FastQC.[11,12] Sequencing reads were aligned to the human genome (GRCh38) using STAR and transcript quantification was performed using the R-subread package's featureCounts utility.[13,14]

Supplemental Tables

Table S1A: Variables included in clinical risk models

Variable	Variable Type	Transformation
Age (years)	Continuous	None
Sex (male/female)	Binary	None
Illness duration prior to hospitalization (days)	Continuous	None
quick Sepsis-related Organ Failure Assessment	Continuous	None
Modified Early Warning Score	Continuous	None
Universal Vital Assessment	Continuous	None

Table S1B: Variables included in clinicomolecular risk models

Variable	Variable Type	Transformation
Age (years)	Continuous	None
Sex (male/female)	Binary	None
Illness duration prior to hospitalization (days)	Continuous	None
quick Sepsis-related Organ Failure Assessment	Continuous	None
Modified Early Warning Score	Continuous	None
Universal Vital Assessment	Continuous	None
sTNFR1 concentration (pg/ml)	Continuous	Log_{10}
Angiopoeitin-2 concentration (pg/ml)	Continuous	Log_{10}

Abbrevations: sTNFR1: soluble TNF-receptor type 1.

Table S2: Biomarker concentrations stratified by vital status at 30-days post-discharge

Biomarker	All patients (N=260)	Alive (N=198)	Dead (N=62)	p-value[a]
IFN-γ, log_{10} pg/ml, median [IQR]	0.16 [-0.25, 0.65]	0.15 [-0.23, 0.60]	0.23 [-0.27, 0.88]	0.199
IL-6, log_{10} pg/ml, median [IQR]	1.16 [0.56, 1.65]	0.98 [0.50, 1.53]	1.58 [1.19, 1.89]	<0.001
IL-8, log_{10} pg/ml, median [IQR]	1.20 [0.84, 1.56]	1.14 [0.83, 1.57]	1.32 [1.00, 1.56]	0.234
IL-10, log_{10} pg/ml, median [IQR]	1.16 [0.55, 1.79]	1.09 [0.41, 1.78]	1.26 [0.96, 1.99]	0.023
IP-10/CXCL10, log_{10} pg/ml, median [IQR]	2.89 [2.38, 3.98]	2.81 [2.39, 3.82]	3.37 [2.34, 4.73]	0.210
MIP-1a/CCL3, log_{10} pg/ml, median [IQR]	1.42 [1.15, 1.75]	1.43 [1.15, 1.75]	1.41 [1.15, 1.75]	0.925
MIP-1b/CCL4, log_{10} pg/ml, median [IQR]	1.78 [1.56, 1.98]	1.77 [1.58, 1.97]	1.82 [1.52, 1.98]	0.975
TNF-α, log_{10} pg/ml, median [IQR]	1.81 [1.57, 2.04]	1.75 [1.53, 2.02]	1.89 [1.70, 2.09]	0.024
Angiopoeitin-1, log_{10} pg/ml, median [IQR]	4.53 [4.29, 4.71]	4.58 [4.38, 4.72]	4.35 [4.14, 4.62]	0.002
Angiopoeitin-2, log_{10} pg/ml, median [IQR]	3.51 [3.29, 3.81]	3.47 [3.27, 3.73]	3.71 [3.41, 4.14]	<0.001
sIL-2Ra/sCD25, log_{10} pg/ml, median [IQR]	3.32 [3.01, 3.64]	3.26 [2.98, 3.57]	3.54 [3.22, 3.88]	<0.001
sTNFR1, log_{10} pg/ml, median [IQR]	3.49 [3.28, 3.68]	3.43 [3.24, 3.62]	3.68 [3.43, 3.89]	<0.001
MIF, log_{10} pg/ml, median [IQR]	2.89 [2.60, 3.20]	2.84 [2.54, 3.16]	3.05 [2.81, 3.30]	0.003
PAI-1, log_{10} pg/ml, median [IQR]	4.97 [4.77, 5.14]	4.95 [4.76, 5.11]	5.03 [4.80, 5.23]	0.090

Abbrevations: IQR: interquartile range, IFN: interferon, IL: interleukin, IP-10/CXCL10: IFN-γ-induced protein-10/C-X-C motif chemokine 10, MIP-1α/CCL3: macrophage inflammatory protein-1-alpha/chemokine (C-C motif) ligand 3, MIP-1β/CCL4: macrophage inflammatory protein-1-beta/chemokine (C-C motif) ligand 4, TNF-α: tumor necrosis factor-α, sIL-2RA/sCD25: soluble IL-2 receptor alpha/soluble CD25, sTNFR1: soluble TNF-receptor type 1, MIF: macrophage migration inhibitory factor, PAI-1: plasminogen activator inhibitor-1.

Legend: [a]Wilcoxon rank-sum test.

Table S3: Performance characteristics of clinical and 2- and 3-biomarker clinicomolecular models for prediction of 30-day mortality (N=260)

Model[a]	Likelihood ratio χ^2 p-value[b]	Optimism-corrected Brier score[c]	NRI (95% CI)	AUC-ROC (95% CI)[d]
qSOFA	Reference	0.171	Reference	0.65 (0.57-0.73)
qSOFA + Ang-2	<0.001	0.161	0.39 (0.11-0.67)	0.71 (0.63-0.79)
qSOFA + sTNFR1	<0.001	0.160	0.50 (0.22-0.77)	0.73 (0.65-0.81)
qSOFA + Ang-2 + sTNFR1	<0.001	0.157	0.69 (0.42-0.96)	0.73 (0.65-0.81)
qSOFA + Ang-2 + sTNFR1 + sIL-2Ra/sCD25	<0.001	0.158	0.67 (0.40-0.94)	0.73 (0.65-0.81)
MEWS	Reference	0.170	Reference	0.68 (0.60-0.76)
MEWS + Ang-2	0.002	0.163	0.40 (0.12-0.68)	0.72 (0.64-0.80)
MEWS + sTNFR1	<0.001	0.161	0.38 (0.09-0.66)	0.74 (0.66-0.82)
MEWS + Ang-2 + sTNFR1	<0.001	0.159	0.60 (0.32-0.87)	0.74 (0.66-0.82)
MEWS + Ang-2 + sTNFR1 + sIL-2Ra/sCD25	<0.001	0.159	0.60 (0.32-0.87)	0.74 (0.66-0.82)
UVA	Reference	0.163	Reference	0.70 (0.62-0.78)
UVA + Ang-2	0.001	0.155	0.43 (0.15-0.71)	0.74 (0.66-0.82)
UVA + sTNFR1	<0.001	0.150	0.53 (0.25-0.80)	0.76 (0.69-0.83)
UVA + Ang-2 + sTNFR1	<0.001	0.149	0.68 (0.41-0.95)	0.76 (0.69-0.83)
UVA + Ang-2 + sTNFR1 + sIL-2Ra/sCD25	<0.001	0.150	0.63 (0.36-0.90)	0.74 (0.66-0.82)

Abbreviations: qSOFA: quick Sepsis-related Organ Failure Assessment; MEWS: Modified Early Warning Score; UVA: Universal Vital Assessment; Ang-2: angiopoietin-2; sTNFR1: soluble tumor necrosis factor receptor-1; sIL-2Ra/sCD25: soluble IL-2 receptor alpha/soluble CD25; NRI: Net reclassification improvement; CI: confidence interval; AUC-ROC: area under the receiver-operating-characteristic curve

Legend: [a]All models adjusted for age, sex, and pre-hospital illness duration; [b]p-value reflects results of likelihood ratio χ^2 test comparing each biomarker-inclusive (i.e., clinicomolecular) model against the corresponding clinical model (qSOFA, MEWS, UVA); [c]Optimism-corrected Brier scores generated using 10,000 bootstraps; [d]AUROC and 95% CIs generated using 100-times-repeated 10-fold cross-validation.

Table S4: Performance characteristics of clinical and clinicomolecular models for prediction of 30-day mortality when adjusted for HIV and malaria status (N=254)

Model[a]	Likelihood ratio χ^2 p-value[b]	Optimism-corrected Brier score[c]	NRI (95% CI)	AUC-ROC (95% CI)[d]
qSOFA	Reference	0.162	Reference	0.71 (0.63-0.79)
qSOFA + Ang-2	0.005	0.157	0.50 (0.21-0.78)	0.74 (0.66-0.82)
qSOFA + sTNFR1	<0.001	0.152	0.44 (0.15-0.72)	0.75 (0.67-0.83)
qSOFA + Ang-2 + sTNFR1	0.001	0.153	0.51 (0.23-0.80)	0.75 (0.67-0.83)
MEWS	Reference	0.162	Reference	0.72 (0.64-0.80)
MEWS + Ang-2	0.011	0.159	0.43 (0.15-0.72)	0.74 (0.66-0.82)
MEWS + sTNFR1	0.001	0.153	0.37 (0.09-0.66)	0.75 (0.67-0.83)
MEWS + Ang-2 + sTNFR1	0.001	0.154	0.48 (0.19-0.76)	0.75 (0.67-0.83)
UVA	Reference	0.159	Reference	0.73 (0.65-0.81)
UVA + Ang-2	0.004	0.154	0.45 (0.17-0.74)	0.75 (0.67-0.83)
UVA + sTNFR1	<0.001	0.148	0.57 (0.29-0.85)	0.77 (0.69-0.85)
UVA + Ang-2 + sTNFR1	<0.001	0.148	0.54 (0.26-0.83)	0.76 (0.68-0.84)

Abbreviations: qSOFA: quick Sepsis-related Organ Failure Assessment; MEWS: Modified Early Warning Score; UVA: Universal Vital Assessment; Ang-2: angiopoietin-2; sTNFR1: soluble tumor necrosis factor receptor-1; NRI: Net reclassification improvement; CI: confidence interval; AUC-ROC: area under the receiver-operating-characteristic curve

Legend: [a]All models adjusted for age, sex, pre-hospital illness duration, HIV status, and malaria status; [b]p-value reflects results of likelihood ratio χ^2 test comparing each biomarker-inclusive (i.e., clinicomolecular) model against the corresponding clinical model (qSOFA, MEWS, UVA); [c]Optimism-corrected Brier scores generated using 10,000 bootstraps; [d]AUROC and 95% CIs generated using 100-times-repeated 10-fold cross-validation.

Table S5: Performance characteristics of clinical and clinicomolecular models for prediction of 30-day mortality when patients with unknown vital status considered deceased (N=288)

Model[a]	Likelihood ratio χ^2 p-value[b]	Optimism-corrected Brier score[c]	NRI (95% CI)	AUC-ROC (95% CI)[d]
qSOFA	Reference	0.211	Reference	0.59 (0.52-0.66)
qSOFA + Ang-2	0.013	0.208	0.20 (-0.05-0.44)	0.63 (0.56-0.70)
qSOFA + sTNFR1	<0.001	0.203	0.32 (0.07-0.57)	0.66 (0.59-0.73)
qSOFA + Ang-2 + sTNFR1	0.001	0.203	0.42 (0.17-0.66)	0.66 (0.59-0.73)
MEWS	Reference	0.210	Reference	0.61 (0.54-0.68)
MEWS + Ang-2	0.027	0.208	0.18 (-0.07-0.42)	0.64 (0.57-0.71)
MEWS + sTNFR1	0.001	0.203	0.22 (-0.03-0.47)	0.67 (0.60-0.74)
MEWS + Ang-2 + sTNFR1	0.002	0.204	0.37 (0.13-0.62)	0.66 (0.59-0.73)
UVA	Reference	0.206	Reference	0.63 (0.56-0.70)
UVA + Ang-2	0.029	0.202	0.22 (-0.02-0.47)	0.66 (0.59-0.73)
UVA + sTNFR1	<0.001	0.196	0.35 (0.10-0.59)	0.68 (0.61-0.75)
UVA + Ang-2 + sTNFR1	0.001	0.197	0.36 (0.12-0.61)	0.68 (0.61-0.75)

Abbreviations: qSOFA: quick Sepsis-related Organ Failure Assessment; MEWS: Modified Early Warning Score; UVA: Universal Vital Assessment; Ang-2: angiopoietin-2; sTNFR1: soluble tumor necrosis factor receptor-1; sIL-2Ra/NRI: Net reclassification improvement; CI: confidence interval; AUC-ROC: area under the receiver-operating-characteristic curve

Legend: [a]All models adjusted for age, sex, and pre-hospital illness duration; [b]p-value reflects results of likelihood ratio χ^2 test comparing each biomarker-inclusive (i.e., clinicomolecular) model against the corresponding clinical model (qSOFA, MEWS, UVA); [c]Optimism-corrected Brier scores generated using 10,000 bootstraps; [d]AUROC and 95% CIs generated using 100-times-repeated 10-fold cross-validation.

Table S6: Logistic regression parameters for the primary Universal Vital Assessment-based clinicomolecular model[a] including sTNFR1 and Ang-2 (N=260)

Variable	Coefficient	Std. Error.	Z-value	Pr(>|z|)
(Intercept)	-11.38668	2.06581	-5.512	<0.001
UVA_score	0.27454	0.06540	4.198	<0.001
age	0.02631	0.01398	1.883	0.060
sex	0.16932	0.34404	0.492	0.622
illnessduration	0.01672	0.03949	0.424	0.672
logstnfr1	1.65522	0.53665	3.084	0.002
logang2	0.60786	0.41255	1.473	0.141

Legend: [a]Full model is as follows: glm(formula = death30d ~ UVA_score + age + sex + illnessduration + logstnfr1 + logang2, family = binomial, data = reserve)

Table S7: Accuracy metrics[a] for the Universal Vital Assessment-based clinicomolecular model including sTNFR1 and Ang-2 over a range of probability cutoffs

Probability cutoff	Accuracy	Sensitivity	Specificity	Positive LR	Negative LR	PPV	NPV
0.3	0.75	0.61	0.80	3.05	0.49	0.50	0.87
0.4	0.80	0.49	0.90	4.90	0.57	0.62	0.85
0.5	0.78	0.31	0.93	4.43	0.74	0.61	0.81
0.6	0.78	0.19	0.97	6.33	0.84	0.67	0.79
0.7	0.77	0.11	0.98	5.50	0.91	0.67	0.78

Abbreviations: LR: likelihood ratio; PPV: positive predictive value; NPV: negative predictive value

Legend: [a]Accuracy metrics derived from confusion matrices generated using 100-times-repeated 10-fold cross-validation.

Supplemental Figures

Figure S1: Study flow diagram