

ORIGINAL ARTICLE

Cellular host response sepsis test for risk stratification of patients in the emergency department: A pooled analysis

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Abstract

Objectives: Sepsis is one of the most common, costly, and misdiagnosed conditions in U.S. emergency departments (EDs). ED providers often treat on nonspecific signs, subjective suspicion, or presumption of infection, resulting in over- and undertreatment. An increased understanding of host response has opened a new direction for sepsis diagnostics. The IntelliSep test is a U.S. Food and Drug Administration–cleared cellular host response diagnostic that could help distinguish sepsis in ED settings. Our objective was to evaluate the potential of the cellular host response test to expedite appropriate care for patients who present with signs of infection.

Methods: We performed a pooled analysis of five adult (≥ 18 years) cohorts enrolled at seven geographically diverse U.S. sites in separate studies. Structured blinded adjudication was used to classify presence or absence of sepsis, and only patients with high confidence in the adjudicated label were included ($n=1002$), defined as patients for whom there was consensus in the determination of sepsis per the Sepsis-3 and severe sepsis per the Sepsis-2 definitions between both the independent adjudication panel and the site-level physician.

Results: Among patients with signs or suspicion of infection, the test achieved similar or better performance compared to other indicators in identifying patients at high risk for sepsis (specificity $> 83\%$) and significantly superior performance in identifying those at low risk (sensitivity $> 92\%$; 0% sepsis-associated mortality). The test also stratified severity of illness, as shown by 30-day in-hospital mortality ($p < 0.001$), hospital length of stay ($p < 0.01$), and use of hospital resources ($p < 0.001$).

Conclusions: Our data suggest that the cellular host response test provides clinically actionable results for patients at both high and low risk for sepsis and provides a rapid, objective means for risk stratification of patients with signs of infection. If integrated into standard of care, the test may help improve outcomes and reduce unnecessary antibiotic use.

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KEY WORDS

cellular host response, emergency medicine, hospital resource utilization, immune dysregulation, risk stratification, sepsis, severity of illness, survival

INTRODUCTION

Sepsis is one of the most common, costly, and misdiagnosed conditions in U.S. emergency departments (EDs).^{1,2} The historical lack of a reliable diagnostic marker and nonspecific presentation leads to variability in sepsis treatment,³ outcomes,^{4,5} and cost⁶ among institutions and providers. To encourage early, standardized care for patients with suspected sepsis,^{7–9} regulatory bodies have imposed performance metrics. Recently, the Centers for Disease Control and Prevention released new guidelines¹⁰ to improve management and outcomes of patients with sepsis, which includes the SEP-1 core measure. Under these pressures, ED providers base the decision to treat on nonspecific signs and subjective suspicion of infection or, in some cases, presumption of infection in patients with evidence of organ dysfunction.¹¹ These strategies can result in both over- and undertreatment of sepsis.¹²

Until recently, there has been little success in developing a reliable biomarker for sepsis.¹³ Complicating development of a sepsis diagnostic is the lack of a reference standard for determining the presence or absence of the condition,¹⁴ and so retrospective adjudication often serves as the comparator for candidate diagnostics. The process of adjudication is subjective and influenced by a number of factors^{15,16} resulting in variability among providers in diagnosing sepsis, which can impact assessment of diagnostic performance.^{17,18}

An increased understanding of the host response has opened a new frontier in sepsis diagnostics.¹⁹ An early, rapid diagnostic to assess host response may aid ED providers in distinguishing the pathobiology of sepsis from a simple infection, possibly prior to manifestation of organ dysfunction, thus expediting delivery of appropriate care to those at highest risk of adverse outcomes. The IntelliSep test is the first U.S. Food and Drug Administration–cleared cellular host response in vitro diagnostic test for sepsis. The novel diagnostic uses microfluidics and machine learning to rapidly quantify structural changes^{20–22} that human leukocytes undergo upon activation.^{23–25}

Multiple prospective observational studies involving adult cohorts at several U.S. sites have been conducted to develop and validate the test for early diagnosis of sepsis.^{24,26–28} This study is a pooled analysis of five distinct but similar adult cohorts to evaluate the potential of the test to improve outcomes for patients who present to the ED with signs or suspicion of infection. To mitigate potential biases that could be introduced from misclassification,^{17,18} we included only cases in which there was high confidence in the adjudicated label.

METHODS

Study population

We included multiple prospective adult (≥ 18 years) cohorts enrolled at several U.S. sites in separate studies, all conducted to develop and validate the cellular host response test for sepsis in those who present to the ED with signs or suspicion of infection (Tables S1 and S2). All studies were approved by national, local, or hospital-specific institutional review boards as appropriate.

Data collection

Study coordinators collected demographic, clinical, and laboratory data through chart review using the most current available data. In addition, coordinators calculated baseline Sequential Organ Failure Assessment (SOFA) scores (from nearest to normal values available from the previous 6 months; if unavailable, baseline function was considered normal) and daily SOFA scores²⁹ for each of the first 3 days of hospitalization. Outcome data, including hospitalization, level of care, and disposition, were gathered from the medical record. Hospital mortality was censored at 30 days. Acute Physiology And Chronic Health Evaluation II (APACHE II) scores were calculated as described.³⁰ In calculating APACHE II scores, the value was considered normal for patients for whom a lab value was not collected per standard of care.

Confirmation of diagnosis

A structured adjudication process was used to classify the presence or absence of sepsis by Sepsis-3 criteria. To be considered positive for Sepsis-3, patients must have each of three components: (1) infection (present on presentation to the ED), (2) organ dysfunction (manifesting within 3 days of the ED visit), and (3) organ dysfunction caused by a dysregulated host response to the infection. A rigorous three-tiered process was used to adjudicate each of these components. First, coordinators extracted data from the electronic health record to complete an objective evaluation for infection³¹ and organ dysfunction.³² Subsequently, a site investigator with access to the medical record completed a clinical review and recorded pertinent clinical information, including any treatment the patient received and the clinical response. All cases were reviewed by at least two independent physicians for each of the required components of sepsis. If these physicians agreed on the presence of all three components, the diagnosis of sepsis was

confirmed. Upon disagreement, the case went to a third adjudicator for review. A final determination on the presence or absence of sepsis was necessary for all cases. For the diagnosis of severe sepsis per the Sepsis-2 consensus definition, enrolled patients were examined for meeting any two systemic inflammatory response syndrome criteria during the ED visit. Among these patients, those who were adjudicated to have infection and who also met prespecified criteria for organ dysfunction received the diagnosis of severe Sepsis-2.³³

It is well established that retrospective physician adjudication is the best but flawed criterion standard^{15,16} because it is subjective and influenced by (1) biases in each adjudicator's training, clinical background, and experience; (2) decisions of the treating physicians and of the local physician; and (3) inherent variability between adjudicators. Moreover, misclassification by a clinical nonreference method introduces biases into the estimates of the performance measures,¹⁷ and as low as 5% misclassification rate by the comparator can lead to significant underestimation of true test performance.¹⁸ Therefore, for the purpose of this analysis, we included only evaluable patients with high confidence in the adjudicated label, defined as patients for whom there was consensus in the determination of sepsis per the Sepsis-3 definition and severe sepsis per the Sepsis-2 definition between both the independent adjudication panel and the site-level physician ($n=1002$). A detailed flow chart for how evaluable patients were selected is presented in Figure S1.

Performance of the test

The IntelliSep test (Cytovale) is a semiquantitative in vitro test that assesses cellular host response via deformability cytometry of leukocyte biophysical properties from a 100- μ L whole blood specimen in less than 10 min.²⁸ It yields the IntelliSep Index (ISI), a single score between 0.1 and 10.0 that represents the probability of the clinical syndrome of sepsis (Table S3). To facilitate clinical interpretation, the index range is stratified into three discrete interpretation bands based on probability of sepsis: Band 1 (low probability), Band 2, and Band 3 (high probability). Details on the development of the test algorithm and the selection of the prespecified Interpretation band cutoffs are presented elsewhere.^{24,26,27}

Where possible, the test was run on an aliquot of blood obtained from the first EDTA-anticoagulated whole blood sample collected for clinical purposes per standard care upon ED presentation. Otherwise, per study protocol study, coordinators obtained EDTA-anticoagulated blood samples for the test. Study personnel completed the test within 5 h of venipuncture. When authorized per study protocol, the remnant of the EDTA sample was utilized for procalcitonin measurements.

Statistical analysis

Unless otherwise stated, p -values were derived from an unpaired two-sample Welch's t-test, where the null hypothesis is that the

mean of two samples is equal. Descriptive statistics are presented as means, standard deviations, medians, and first and third quartiles (Q1–Q3) for continuous variables and as counts and percentages for categorical variables. An alpha level of 5% is used for all analyses, unless otherwise stated. Two-sided CIs for proportions are provided using the Clopper-Pearson method, where appropriate. Receiver operating characteristic curves were constructed to illustrate the performance of the ISI for classification of patients as septic or not septic. Time-to-event analyses are depicted with Kaplan-Meier plots, and the log-rank test was used for comparison between groups. Unless otherwise noted, missing values are not imputed.

RESULTS

Study population

Adjudicators determined that 317 (31.6%) of the evaluable population ($n=1002$) had an infection, of whom 201 (63.4% of infected) had or developed at least one organ failure. Of these 201 patients, adjudicators determined 192 (19.2% of evaluable population) to have sepsis. The test performance in the total population ($n=1196$) is shown in Figure S2. The median ISI for all patients was 4.9 (Q1–Q3, 3.7–6.2). Using prespecified cutoffs,²⁸ 521 (53.8%) were in Band 1, 243 (24.8%) in Band 2, and 238 (20.7%) in Band 3. Distribution of the total population stratified by ED diagnosis of "sepsis" or "no sepsis" as abstracted from the medical record and adjudicated sepsis status is presented in Figure S3.

Baseline characteristics and comparative statistical descriptions for the 1002 evaluable patients, stratified by interpretation band, are shown in Table 1. Characteristics of evaluable patients by adjudicated outcome are presented in Table S4. When compared with patients adjudicated as not septic, those with sepsis were older ($p<0.0001$) and had significantly higher ISI values ($p<0.0001$). There was no difference in sex or race. Patients with sepsis were more likely to have hypertension, congestive heart failure, chronic kidney disease, human immunodeficiency virus, hepatitis C, and/or autoimmune disease; however, there was no difference in the presence of diabetes, obesity, hepatitis B, malignancy, or end-stage renal disease between those with sepsis and those without. In septic patients, the most common sources of infection were urinary (28.7%) and respiratory (25.6%). Clinical characteristics of the subpopulation excluded from the final analysis ($n=194$), mirroring that of the analysis population, are presented in Table S5.

Performance versus adjudicated sepsis diagnosis

Diagnostic characteristics of the test and receiver operating characteristic curve analysis demonstrating the capacity of the ISI to differentiate patients with adjudicated sepsis from those without are presented in Figure 1. The positive percent agreement (sensitivity) with the Band 1 cutoff was 93.2% (95% CI 88.7%–96.3%) and the

TABLE 1 Characteristics of study patients, including selected ED interventions, by ISI interpretation band.

Characteristic	Total (n = 1002)	ISI interpretation band			p-value ^a
		1 (n = 521)	2 (n = 243)	3 (n = 238)	
Age (years)					
Median (Q1–Q3)	57 (41–71)	55 (39–69)	62 (43–73)	61 (46–75)	<0.01, <0.001, ns
Subjects ≥65	362 (36.1)	167 (32.1)	96 (39.5)	99 (41.6)	<0.05, <0.05, ns
Biological sex					
Male	496 (49.5)	259 (49.7)	118 (48.6)	119 (50.0)	ns, ns, ns
Female	506 (50.5)	262 (50.0)	125 (51.4)	119 (50.0)	ns, ns, ns
Race					
Black or African American	363 (36.2)	198 (38.0)	77 (31.7)	88 (37.0)	ns, ns, ns
White	575 (57.4)	293 (56.2)	149 (61.3)	133 (55.9)	ns, ns, ns
Other	64 (6.4)	30 (5.8)	17 (7.0)	17 (7.1)	ns, ns, ns
Comorbidities					
Autoimmune disease	40 (4.0)	19 (3.7)	7 (2.9)	14 (5.9)	ns, ns, ns
Cancer	104 (10.4)	44 (8.5)	30 (12.4)	30 (12.4)	ns, ns, ns
Congestive heart failure	173 (17.3)	89 (17.1)	47 (19.3)	37 (15.6)	ns, ns, ns
Diabetes	294 (29.3)	155 (29.8)	59 (24.3)	80 (33.6)	ns, ns, <0.05
HIV	20 (2.0)	4 (0.8)	6 (2.5)	10 (4.2)	ns, <0.01, ns
Hepatitis B	6 (0.6)	1 (0.2)	3 (1.2)	2 (0.8)	ns, ns, ns
Hepatitis C	68 (6.8)	29 (5.6)	18 (7.4)	21 (8.8)	ns, ns, ns
Hypertension	565 (56.4)	282 (54.13)	142 (58.4)	141 (59.2)	ns, ns, ns
Obesity (BMI ≥ 30)	113 (11.3)	58 (11.1)	32 (13.2)	23 (9.7)	ns, ns, ns
End-stage renal disease	29 (2.9)	15 (2.9)	8 (3.3)	6 (2.5)	ns, ns, ns
Chronic kidney disease ^b	161 (16.1)	75 (14.4)	41 (16.9)	45 (18.9)	ns, ns, ns
Home medications upon ED presentation					
Antibiotics	86 (8.6)	47 (9.0)	21 (8.6)	18 (7.6)	ns, ns, ns
Immunosuppressant or biologics	26 (2.6)	10 (1.9)	9 (3.7)	7 (2.9)	ns, ns, ns
Corticosteroids	104 (10.4)	56 (10.8)	26 (10.7)	22 (9.2)	ns, ns, ns
Nursing home resident	99 (9.9)	29 (5.6)	33 (13.6)	37 (15.6)	<0.001, <0.001, ns
2+ SIRS criteria met	861 (85.9)	414 (79.5)	219 (90.1)	228 (95.8)	<0.001, <0.001, <0.05
Infected by adjudication	317 (31.6)	75 (14.4)	83 (34.2)	159 (66.8)	<0.001, <0.001, <0.001
Source of infection ^c					
Central nervous system	7 (2.2)	1 (1.3)	5 (6.0)	1 (0.6)	ns, ns, <0.05
Cardiovascular system	28 (8.8)	5 (6.7)	8 (9.6)	15 (9.4)	ns, ns, ns
Gastrointestinal/abdominal	54 (17.0)	16 (21.3)	14 (16.9)	24 (15.1)	ns, ns, ns
Respiratory system	81 (25.6)	12 (16.0)	20 (24.1)	49 (30.8)	ns, <0.05, ns
Skin and skin structure or bone and joint	75 (23.7)	15 (20.0)	21 (25.3)	39 (24.5)	ns, ns, ns
Urine and genitourinary	91 (28.7)	20 (26.7)	17 (20.5)	54 (34.0)	ns, ns, <0.05
Other	16 (5.1)	5 (6.7)	5 (6.0)	6 (3.8)	ns, ns, ns
Organ dysfunction in subjects adjudicated as infected, n (% of infected)	201 (63.4)	19 (25.3)	48 (57.8)	134 (84.3)	<0.001, <0.001, <0.001
Systems with organ dysfunction, n (% of infected) ^d					
Central nervous system	97 (30.6)	7 (9.3)	19 (22.9)	71 (44.7)	<0.05, <0.001, <0.00
Cardiovascular system	129 (40.7)	9 (12.0)	25 (30.1)	95 (59.8)	<0.01, <0.001, <0.001
Gastrointestinal	60 (18.9)	5 (6.7)	13 (15.7)	42 (26.4)	ns, <0.001, ns
Hemodynamic	55 (17.4)	4 (5.3)	9 (10.8)	42 (26.4)	ns, <0.001, <0.01

TABLE 1 (Continued)

Characteristic	Total (n = 1002)	ISI interpretation band			p-value ^a
		1 (n = 521)	2 (n = 243)	3 (n = 238)	
Renal	117 (36.9)	9 (12.0)	25 (30.1)	83 (52.2)	<0.01, <0.001, <0.01
Respiratory	93 (29.3)	12 (16.0)	16 (19.3)	65 (40.9)	ns, <0.001, <0.001
Other	8 (2.5)	0 (0.0)	0 (0.0)	8 (5.0)	ns, <0.05, <0.05
Sepsis, by adjudication	192 (19.2)	13 (2.5)	46 (18.9)	133 (55.9)	<0.001, <0.001, <0.001
All-cause cumulative in-hospital mortality					
3-day	20 (2.0)	9 (1.7)	1 (0.4)	10 (4.2)	ns, <0.05, <0.01
7-day	41 (4.1)	12 (2.3)	7 (2.9)	22 (9.2)	ns, <0.001, <0.01
30-day	59 (5.9)	15 (2.9)	14 (5.8)	30 (12.6)	ns, <0.001, <0.01
APACHE II	11 (7–17)	9 (5–15)	12 (8–17)	14 (10–19)	<0.001, <0.001, <0.01
SOFA, 3-day max (baseline subtracted)	2 (1–4)	2 (0–3)	2 (1–4)	4 (2–6)	<0.001, <0.001, <0.001
Infected (by adjudication) and worsening	100 (10.0)	19 (3.7)	30 (12.4)	51 (21.4)	<0.001, <0.001, <0.01
ED diagnosis of sepsis					
Yes	179 (17.9)	38 (7.3)	42 (17.3)	99 (41.6)	<0.001, <0.001, <0.001
No	823 (82.1)	483 (92.7)	20 (82.7)	139 (58.4)	<0.001, <0.001, <0.001
Admitted to hospital	681 (68.0)	300 (57.6)	175 (72.0)	206 (86.6)	<0.001, <0.001, <0.001
Admitted to ICU	161 (16.1)	59 (11.3)	40 (16.5)	62 (26.1)	<0.05, <0.001, <0.05
Transferred from noncritical unit to ICU during hospital stay	29 (2.9)	8 (1.5)	11 (4.5)	10 (4.2)	<0.05, <0.05, ns
Hospital-free days	25 (21–28)	26 (24–28)	25 (21–28)	22 (14–25)	<0.001, <0.001, <0.001
Blood culture					
Number ordered	490 (48.9)	175 (33.6)	129 (53.1)	186 (78.2)	<0.001, <0.001, <0.001
Number positive (of ordered)	105 (21.4)	18 (10.3)	20 (15.5)	67 (36.0)	<0.01, <0.001, <0.001
Number positive (of total)	105 (10.5)	18 (3.5)	20 (8.2)	67 (28.2)	<0.01, <0.001, <0.001
Antibiotics prescribed	459 (45.8)	165 (31.7)	112 (46.1)	182 (76.5)	<0.001, <0.001, <0.001
Lactate measured	589 (58.8)	232 (44.5)	163 (67.1)	194 (81.5)	<0.001, <0.001, <0.001
Lactate	1.8 (1.2–3.0)	1.6 (0.8–2.4)	1.7 (1.1–3.3)	2.2 (1.4–3.1)	<0.05, <0.001, <0.05
WBC (10 ³ cells/µL)	12.8 (8.3–16.1)	10.6 (6.9–14.0)	13.7 (10.9–17.2)	15.9 (11.9–19.9)	<0.001, <0.001, <0.001
ISI	4.9 (3.7–6.2)	3.8 (3.1–4.3)	5.5 (5.2–5.8)	7.4 (6.7–8.1)	<0.001, <0.001, <0.001

Note: Data are reported as n (%) or median (Q1–Q3), unless otherwise specified. Cancer refers to those with history or current cancer that did not meet the study exclusion criteria of history of hematologic malignancies and/or receipt of cytotoxic chemotherapy within 3 months of the ED encounter. Worsening is defined as an increase in SOFA score in the 2 subsequent days compared to the day of presentation.

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; HIV, human immunodeficiency virus; ICU, intensive care unit; ISI, IntelliSep Index; ns, not significant; Q1–Q3, interquartile range; SIRS, systemic inflammatory response syndrome; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell count.

^ap-values for two-by-two comparisons between Band 1 and Band 2 (first), Band 1 and Band 3 (second), and Band 2 and Band 3 (third).

^bInclusive of end-stage renal disease.

^cIn subjects adjudicated as infected, multiple sources may be included per subject.

^dIn subjects adjudicated as infected, multiple systems may be included per subject.

negative percent agreement (specificity) with the Band 3 cutoff was 87.0% (95% CI 81.4%–91.4%). The prevalence of sepsis in Band 3 was 133 of 238 (positive predictive value 55.9%, 95% CI 48.4%–62.9%); in Band 2, 46 of 243; and in Band 1, 13 of 521 (negative predictive value 97.5%, 95% CI 94.0%–99.1%). Relationships between adjudicated infection, organ dysfunction and sepsis within each interpretation band are presented in Figure S4.

Demographics, comorbidities, and home medications

The test provided appropriate risk stratification across interpretation bands for demographics groups (age, sex, and race; Figure 2) and in the presence or absence of comorbidities (Figure S5) and select home medications (Figure S6). Band 1 patients were significantly younger than those in Bands 2 and 3. There were no significant

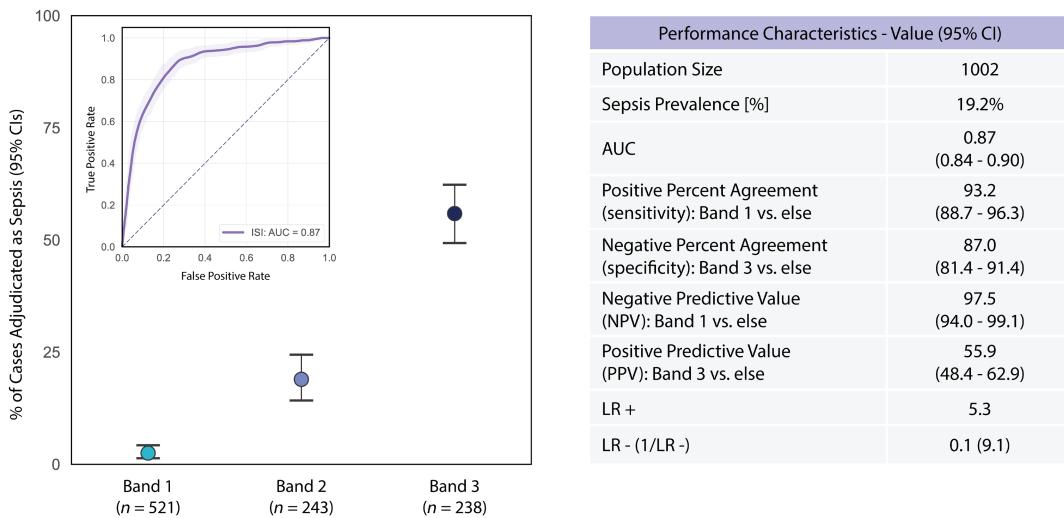


FIGURE 1 Test performance on the study population ($n=1002$). (Left) Performance of the ISI in the diagnosis of sepsis, including prevalence of sepsis within each interpretation band (error bars represent 95% CI) as well as receiver operating characteristics curve (inset) of the ISI. (Right) Diagnostic test characteristics of the ISI compared to adjudicated sepsis. LR+ is defined as sensitivity/(1 - specificity) with a Band 3 (high) cutoff (i.e., Band 3 [high] vs. else) and LR- is defined as (1 - sensitivity)/specificity with a Band 1 (low) cutoff (i.e., Band 1 [low] vs. else). AUC, area under the receiver operating characteristic curve; ISI, IntelliSep Index; LR+, positive likelihood ratio; LR-, negative likelihood ratio.

differences in sex, race, or comorbidities across interpretation bands, except for diabetes and human immunodeficiency virus infection, which were more common in Band 3 (Table 1).

Severity of illness risk stratification

Band 3 patients had higher APACHEII and 3-day maximum SOFA scores, were more commonly admitted to the hospital and intensive care unit (ICU), and more commonly had adverse outcomes (Table 1). Band 2 and Band 3 patients were more likely to worsen following presentation (as measured by an increase in SOFA score compared to day of presentation) than Band 1 patients (Band 1 vs. Band 2 $p<0.05$, Band 1 vs. Band 3 $p<0.001$).

Kaplan-Meier estimates revealed that Band 1 patients had a 7-day all-cause mortality of 2.3%, compared to 2.9% in Band 2 and 9.2% in Band 3 (Figure 3). These mortality trends continue, with 2.9% of Band 1 suffering in-hospital mortality at 30 days, compared to 12.6% in Band 3. Additionally, 30-day in-hospital mortality with adjudication of sepsis for Band 1 was 0.0%, compared to 1.2% and 8.8% in Bands 2 and 3, respectively (Figure 3, right panel). Sepsis was the presumed cause of death for zero of 15 Band 1 patients that suffered in-hospital mortality during the first 30 days of hospitalization, compared to three of 14 in Band 2, and 21 of 30 in Band 3 (Table S6).

Differences in utilization of hospital resources were also observed between interpretation bands (Figure 4). Hospital admissions were highest for Band 3 patients (86.6%), compared to Band 2 (72.0%) and Band 1 (57.6%). Rates of ICU admission showed similar trends, with Band 3 patients more likely to be admitted to the ICU (26.1%), compared to Band 2 (16.5%, $p<0.05$) and Band 1 (11.3%,

$p<0.0011$). Additionally, patients in Bands 2 and 3 were more likely to need escalation of care from noncritical to critical during their hospital stay (Table 1, $p<0.05$). Among patients who did not suffer 30-day in-hospital mortality, hospital length of stay was significantly different among interpretation bands (Figure 4, right panel), with 29.0% (87 of 300) of those admitted in Band 1 discharged within 2 days, compared to 22.9% (40 of 175) in Band 2 and 8.7% (18 of 206) in Band 3.

Other indicators of sepsis

Table S7 compares the performance of the test and other commonly assessed indicators of sepsis, namely, white blood cell count, procalcitonin, lactate, blood cultures, SOFA, and quick SOFA. Among patients with signs or suspicion of infection, the test achieved comparable or better performance in identifying patients at high risk for sepsis. Notably, the test achieved significantly superior performance in correctly identifying those at low risk for sepsis. Among the 21 patients who suffered sepsis-associated in-hospital mortality in any of assessed low-risk groups across all assessed variables, none were in Band 1, three were in Band 2, and 18 in Band 3 (Table S8).

Observed standard of care

Observed trends in antibiotics prescribed and orders for blood cultures and lactate across interpretation bands is presented in Figure 5. While the percentage of patients within each interpretation band that receive care increases across the bands, a similar number of

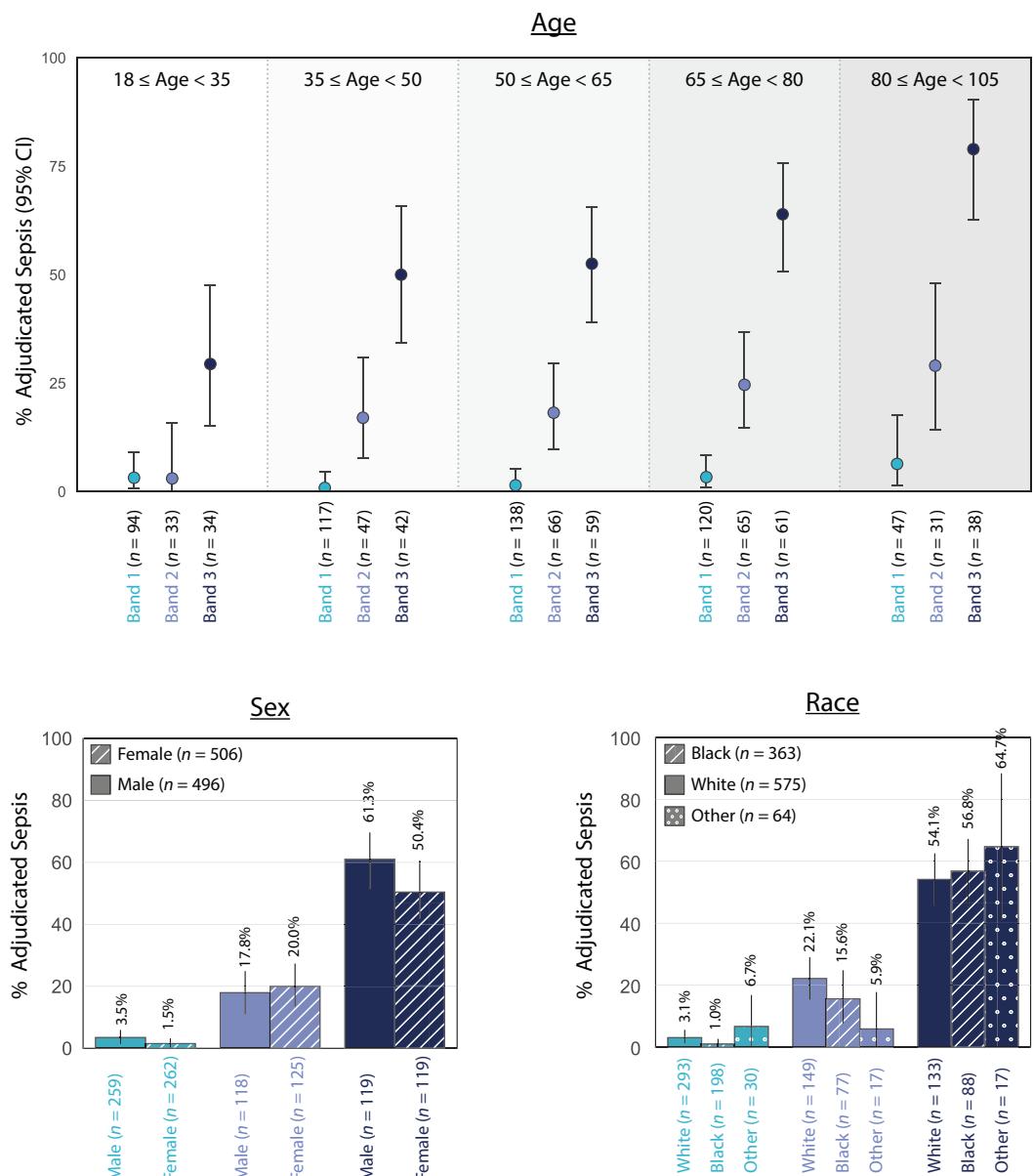


FIGURE 2 Performance of the ISI (across interpretation bands) in risk stratification of sepsis (per retrospective physician adjudication) for various age groups (top panel; error bars represent 95% CI), by sex (bottom left panel) and race (bottom right panel). Bars, percentages; error bars, 95% CIs. ISI, IntelliSep Index.

patients receive each care metric independent of the interpretation band categorization.

DISCUSSION

This pooled analysis of 1002 patients for whom there was high confidence in the adjudicated label found that the test demonstrated increasing likelihood of sepsis across three statistically distinct interpretation bands. The test also appropriately risk stratified for severity of illness, as shown by 30-day in-hospital mortality, discharge disposition, and hospital length of stay, suggesting that the test yields clinically actionable results that if integrated into standard of

care, may have the potential to alert providers to patients who are at risk for adverse outcomes, and may warrant immediate intervention. The data were collected across several years and from geographically diverse institutions with a heterogeneous patient population, indicating that the test yields effective risk stratification across time and among a diverse group of patients. Finally, this analysis shows that the test performs favorably compared to other commonly assessed indicators of sepsis, including white blood cell count, procalcitonin, lactate, blood culture, SOFA, and quick SOFA.

The test may provide a diagnostic aid for sepsis similar to commonly used diagnostics for myocardial ischemia, around which providers can protocolize care. For example, the test achieved 97.5% negative predictive value in Band 1 patients, superior to the negative

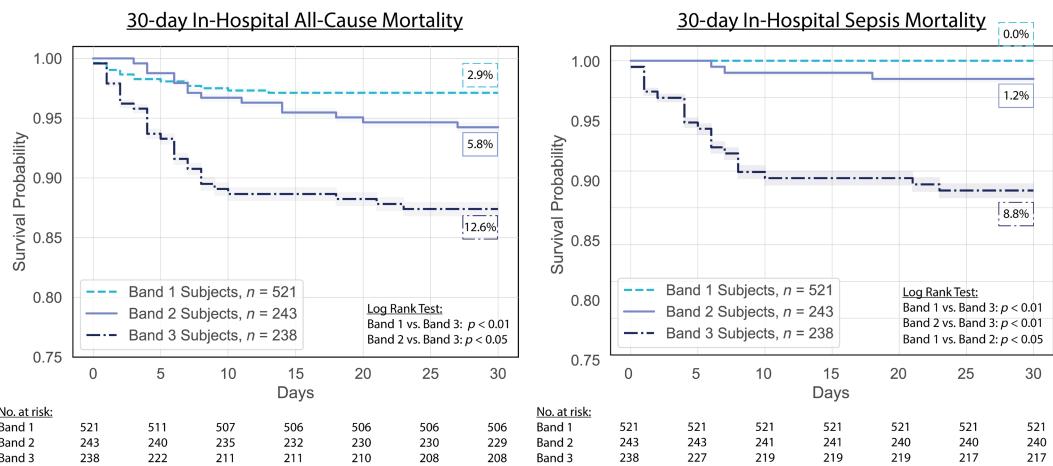


FIGURE 3 Probability of survival during the first 30 days of hospitalization for all evaluable patients, (left) all-cause in-hospital mortality, and (right) in-hospital mortality with adjudication of sepsis, stratified by ISI interpretation band. Plots were created with right-censoring, with the assumption that patients discharged from the ED or hospital survived ≥ 30 days in the absence of evidence to the contrary (e.g., return to the ED, discharge to hospice, or other indication in the electronic health record, which was reviewed after 30 days, that the subject died). Shading depicts 95% CIs. At each time point, the number at risk, per interpretation band, are noted below each figure panel. Presumed cause of death for all patients that suffered in-hospital mortality during the first 30 days of hospitalization is provided in Table S6.

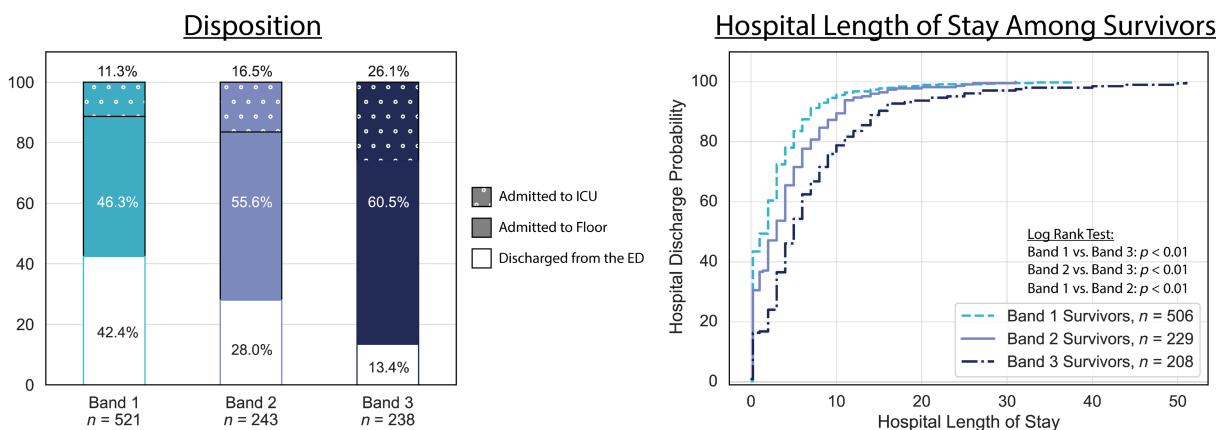


FIGURE 4 ED disposition of evaluable patients (discharge from the ED, floor, or ICU admission) across ISI interpretation bands (left). Hospital length of stay among patients that did not suffer for review only 30-day in-hospital mortality, depicted as discharge probability curves, stratified by ISI interpretation band (right). ICU, intensive care unit; ISI, IntelliSep Index.

predictive value of a normal electrocardiogram for excluding acute myocardial infarction.³⁴ The 55.9% positive predictive value in Band 3 is superior to the positive predictive value for high-sensitivity cardiac troponin for diagnosing acute myocardial infarction in patients presenting to EDs with chest pain.³⁵ Finally, Band 2 represents a discrete population with an intermediate risk of sepsis.

In addition to performing as a diagnostic aid for sepsis, the test provides a rapid, objective means for risk stratification for severity of illness in those with signs or suspicion of infection. Increasing interpretation bands correlated with APACHEII and 3-day maximum SOFA scores as well as hospital resource utilization (Table 1, Figure 4). Though more than half of Band 1 patients are admitted to the hospital, they have shorter stays compared to Bands 2 and 3, with many discharged after only one night. Interestingly, of patients admitted to the hospital, Band 2 includes the highest percentage requiring

escalation in level of care, suggesting that these patients might be in evolution of disease at time of ED presentation. These findings suggest that the test has the potential to aid ED throughput by assisting providers in identifying low-risk patients who may benefit from close outpatient follow-up or observation status as well as high risk patients who may require admission to higher levels of care.

The test is especially effective in risk stratification of patients with signs or suspicion of infection for risk of death prior to discharge. Figure 3 shows Band 1 patients have a higher probability of survival to discharge at 30 days than Band 3. Furthermore, increasing interpretation bands were associated with increasing mortality in those with sepsis, and Band 3 patients were at the highest risk for death earlier in their hospital course. Together, these findings suggest that Band 1 patients who do not survive hospitalization are at risk for mortality from nonsepsis conditions; thus, attention should be focused on alternative diagnoses in this population.

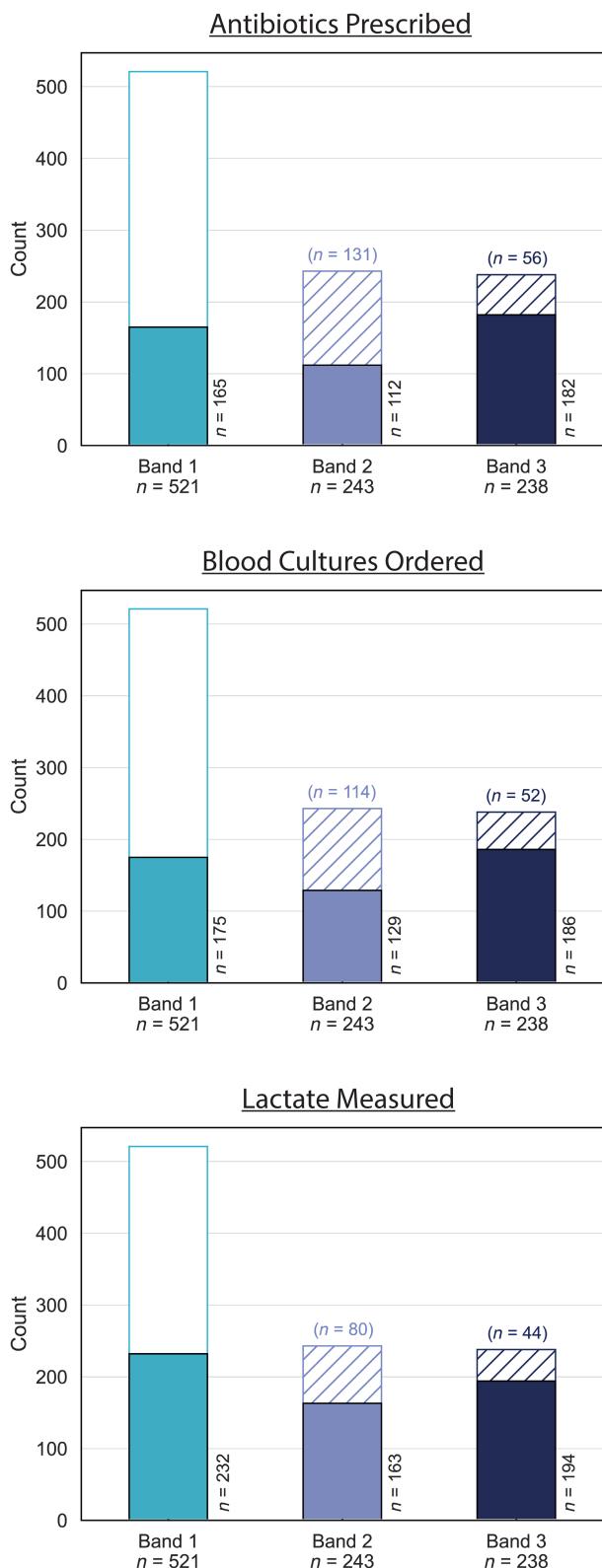


FIGURE 5 Observed trends in prescription of antibiotics, and orders for blood cultures and lactate in the ED, across interpretation bands. Solid bars denote the number of patients in each band that received the care metric; outlined (for Band 1) and hashed bars (for Bands 2 and 3) denote the remainder of the patients in each band that did not receive the care metric.

Importantly, test results are reported as a single Index and interpretation band that is immediately available to providers; it does not require complex calculations on multiple data points collected over time. As such, the test could fill the need for a rapid sepsis host response diagnostic in the ED setting as suggested in a recent modified Delphi study.³⁶ Such a test was considered valuable for improving sepsis outcomes and reducing rates of unnecessary antibiotic use even if it did not identify the specific pathogen.³⁶ More than half of patients in our study were classified as Band 1. The very low mortality in this group suggests these patients, even if adjudicated to be sepsis, have a low risk for adverse outcomes. Despite this, IV antibiotics were prescribed for nearly one-third of Band 1 patients. By comparison, antibiotics were not prescribed for over half of Band 2 patients or for nearly one in four Band 3 patients. Furthermore, nearly one-third of Band 1 patients had blood cultures ordered and, though 10% of these were positive, they were equally likely to result in a contaminant as opposed to a true pathogen. By contrast, blood cultures were ordered in over 75% of Band 3 patients and more than 30% of these were positive. These results suggest that the test may assist providers in identifying a low-risk population in whom blood cultures and IV antibiotics may not be necessary, while identifying a group in whom these interventions may be beneficial. For example, judicious use of blood cultures and IV antibiotics to only those patients in Band 1 with risk factors or high suspicion for bacteremia could reduce blood culture contamination and unnecessary antibiotic use.

As an objective diagnostic marker for sepsis, the test may help improve compliance with care delivery metrics. SEP-1 compliance among U.S. hospitals varies widely.³⁷ Among the challenges hospitals face in achieving SEP-1 compliance is the variable nature of sepsis¹⁶ as well as the complex and arbitrary Time0 criteria of SEP-1.¹² By integrating the test early in the ED workflow, providers could have a fast, objective, patient-centered measure of sepsis risk to help them identify patients who should be included in the metric and directing appropriate care toward those patients.

LIMITATIONS

Our study has limitations. Though the patient population was collected from EDs in a geographically diverse group of hospitals, not all studies had the same enrollment criteria, resulting in the exclusion of some enrolled patients. Also, all patient data were obtained from observational studies, and so we are unable to demonstrate the clinical utility of the test when used as part of clinical practice.

CONCLUSIONS

Our pooled analysis of 1002 adult patients from geographically diverse U.S. sites for whom there was high confidence in the adjudicated label suggests that the cellular host response test provides clinically

actionable results for both patients at high risk for sepsis and those at low risk and delivers a rapid, objective means for risk stratification of patients with signs of infection. If implemented into an ED workflow and process for sepsis care delivery, the test could help ensure that the right care is delivered to the right patient at the right time.

AUTHOR CONTRIBUTIONS

H. R. O'Neal and R. Sheybani had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: H. R. O'Neal, R. Sheybani, A. M. Shah, C. B. Thomas, and R. Scoggins. Acquisition of data: data was collected by study sites personnel. Analysis and interpretation of data: H. R. O'Neal, R. Sheybani, R. Scoggins. Drafting of the manuscript: H. R. O'Neal, R. Sheybani. Critical revision of the manuscript for important intellectual content: all authors. Statistical expertise: R. Sheybani. Acquisition of funding: R. Sheybani, H. T. K. Tse, A. M. Shah.

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CONFLICT OF INTEREST STATEMENT

R. Sheybani, A.M. Shah, H.T.K. Tse, and R. Scoggins are affiliated with Cytovale and have an equity interest in the company whose device is the subject of this study. C.K. Kraus and W.H. Self received funding personally from Cytovale for consulting. The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Supplementary Material

Cellular host response sepsis test for risk stratification of patients in the emergency department: A pooled analysis

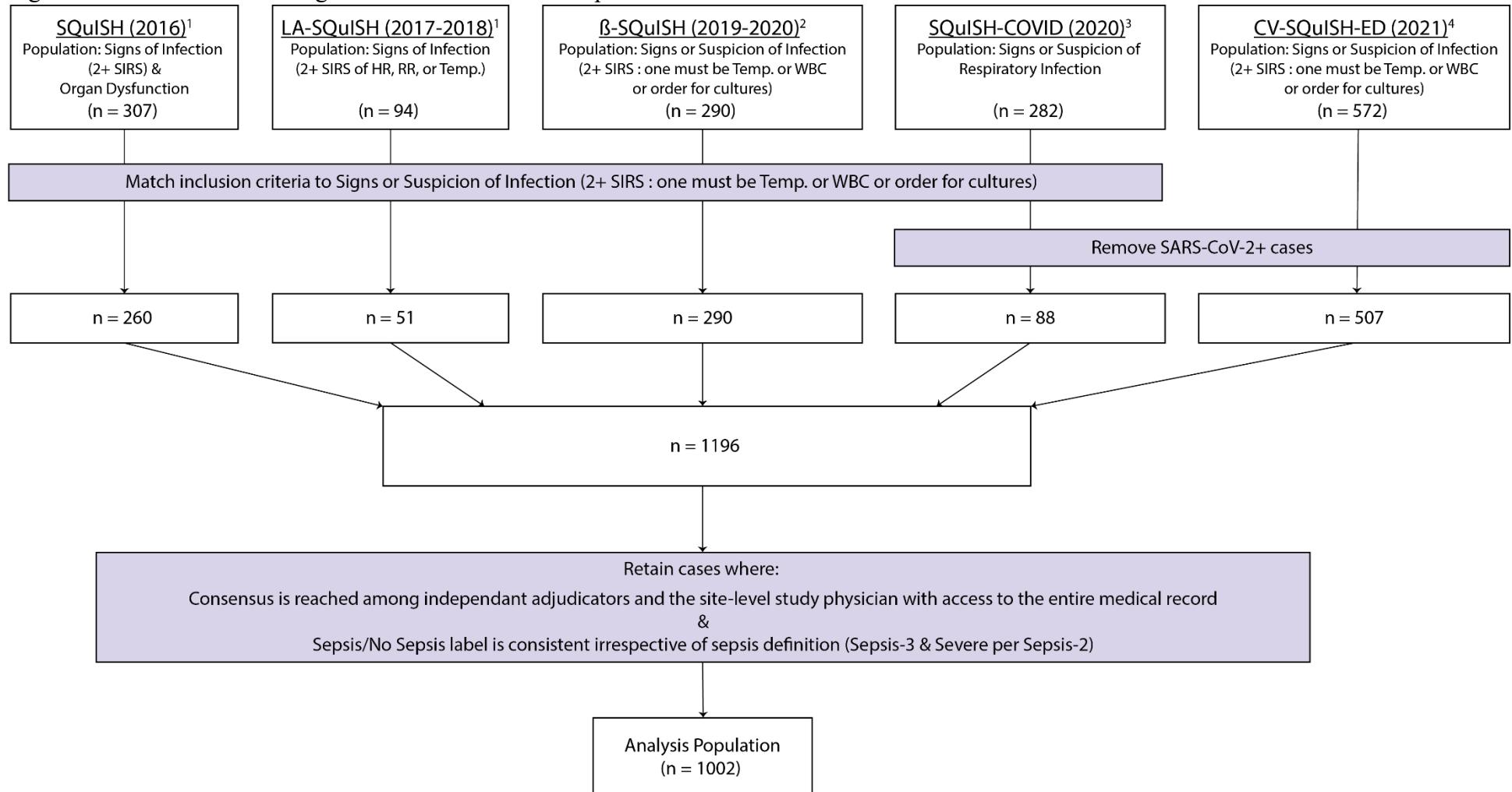
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Cellular host response sepsis test for patients in the ED

Figures

Figure S1. Flow Chart showing the selection of evaluable patients.

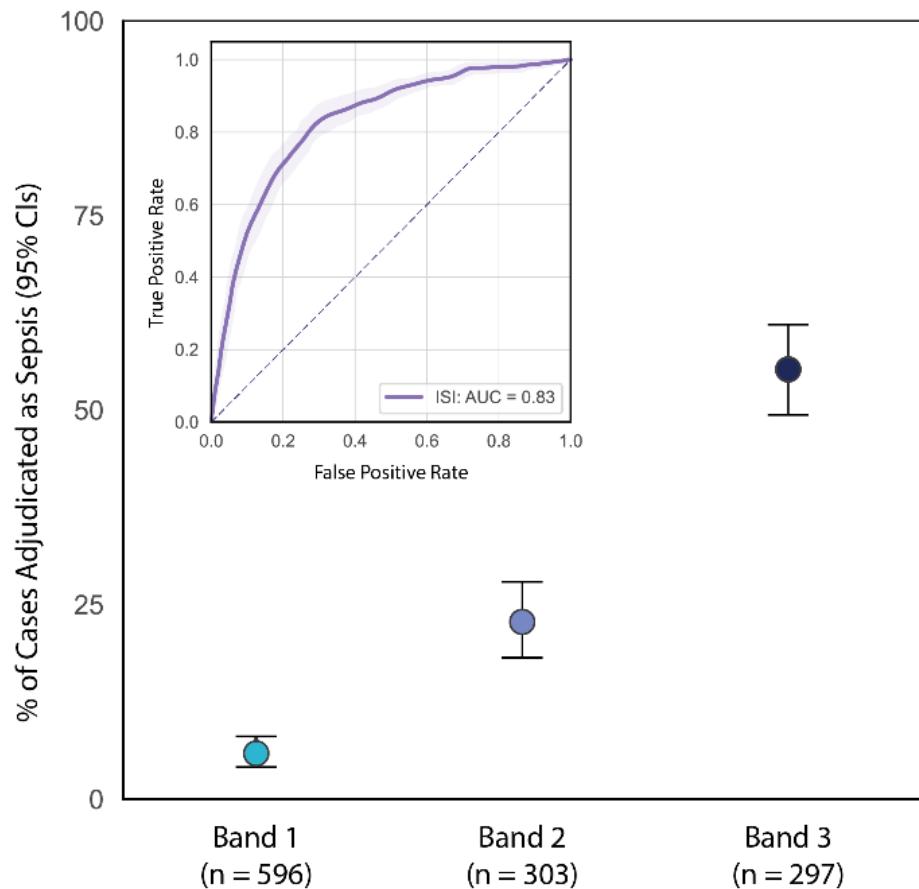


Studies include SQuISH (2016),¹ LA-SQuISH (2017-2018),¹ β-SQuISH (2019-2020),² SQuISH-COVID (2020),³ and CV-SQuISH-ED (2021).⁴

Abbreviations: SIRS, Systemic Inflammatory Response Syndrome; HR, heart rate; RR, respiratory rate; Temp, temperature; WBC, white blood cell count;

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Figure S2. Test Performance on the total population ($n = 1,196$).



Performance Characteristics - Value (95% CI)	
Population Size	1196
Sepsis Prevalence [%]	22.4%
AUC	0.83 (0.80 - 0.86)
Positive Percent Agreement (sensitivity): Band 1 vs. else	86.9 (81.9 - 90.4)
Negative Percent Agreement (specificity): Band 3 vs. else	85.7 (80.6 - 89.4)
Negative Predictive Value (NPV): Band 1 vs. else	94.1 (90.5 - 96.5)
Positive Predictive Value (PPV): Band 3 vs. else	55.2 (48.7 - 60.9)
LR +	4.3
LR - (1/LR -)	0.2 (4.5)

Left Panel: Performance of the ISI in the diagnosis of sepsis, including prevalence of sepsis within each Interpretation Band (error bars represent 95% CI) as well as Receiver Operating Characteristics curve (inset) of the ISI. Right B: Diagnostic test characteristics of the ISI as compared to adjudicated sepsis. LR+ is defined as sensitivity/(1-specificity) with a Band 3 cutoff (i.e., Band 3 vs. else) and LR- is defined as (1-sensitivity)/specificity with a Band 1 cutoff (i.e., Band 1 vs. else). Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; ISI; IntelliSep Index; LR+, positive likelihood ratio; LR-, negative likelihood ratio.

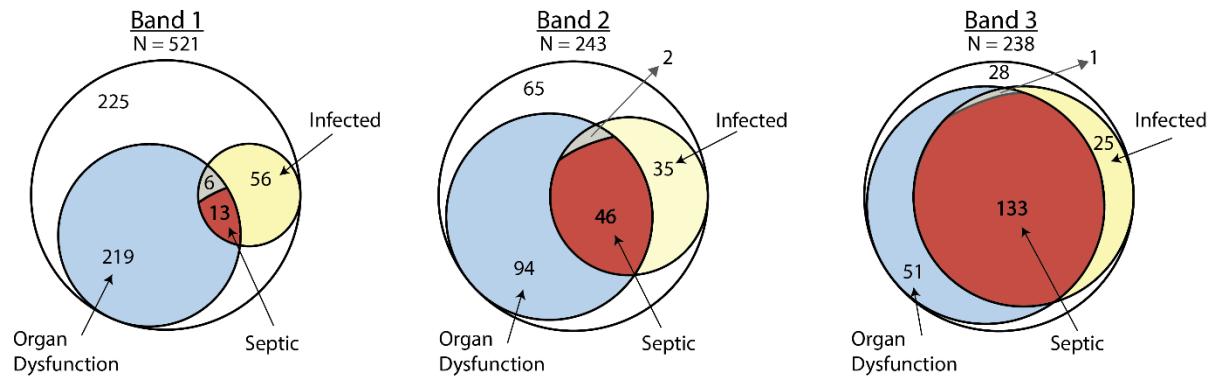
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Figure S3. Distribution of patients stratified by ED diagnosis of ‘sepsis’ or ‘no sepsis’ as abstracted from the medical record and retrospective physician adjudicated sepsis disease status.

	<u>Adjudicated Sepsis</u>	<u>Adjudicated No Sepsis</u>
<u>ED Diagnosis:</u> Sepsis	112	67
<u>ED Diagnosis:</u> No Sepsis	80	743

Cellular host response sepsis test for patients in the ED

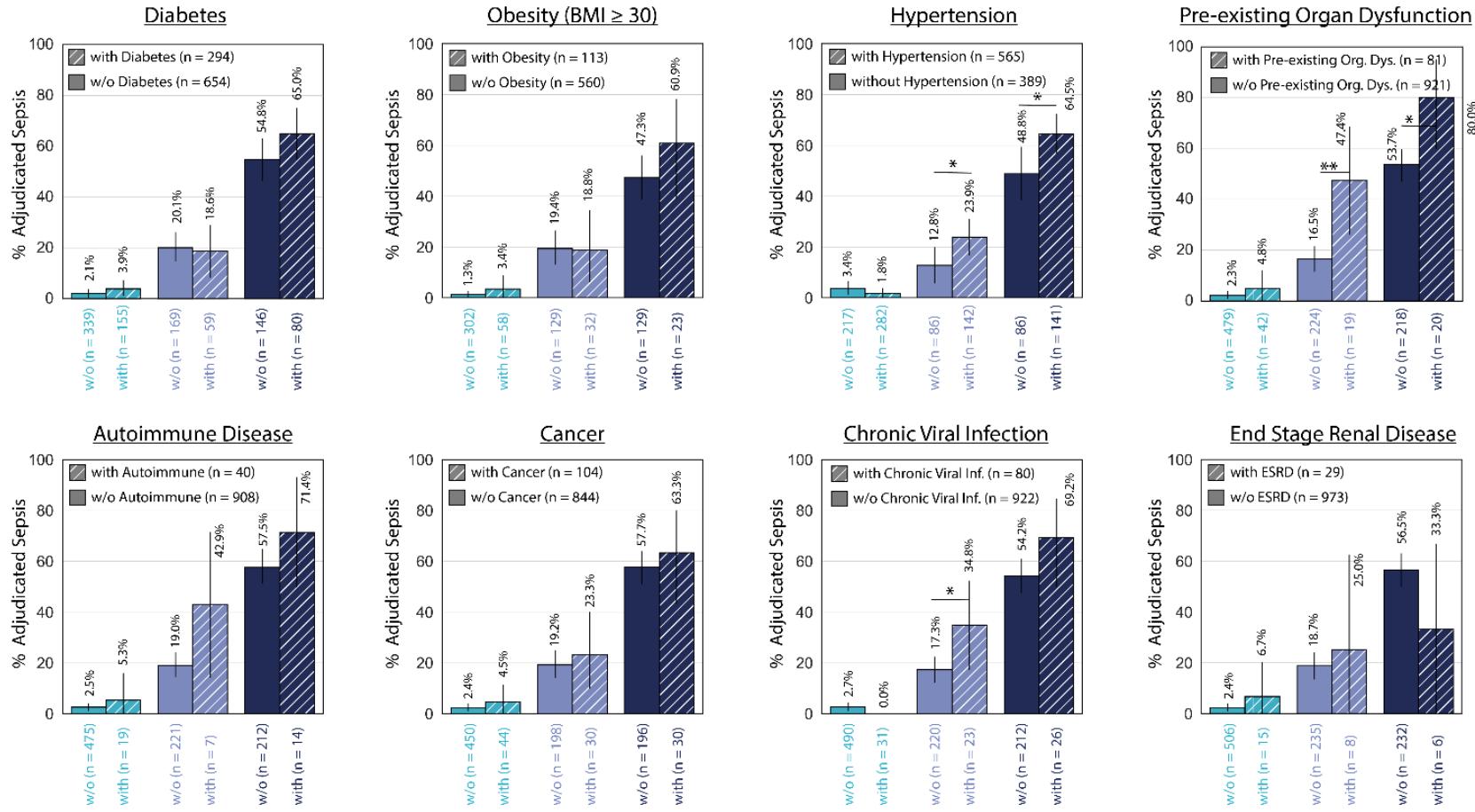
Figure S4. Relationships between infection, organ dysfunction and sepsis, as determined by adjudication, within each Interpretation Band.



White areas represent patients in each Band with neither infection nor organ dysfunction. Yellow areas represent those with infection without organ dysfunction while blue areas represent those with organ dysfunction without infection. Gray areas represent those with organ dysfunction and infection; however, the organ dysfunction was adjudicated to be due to a process other than a dysregulated host response to the infection. Finally, red areas indicate those with organ dysfunction due to a dysregulated host response to infection (sepsis). The proportion of patients with infection, organ dysfunction, both of these, and sepsis increased across Interpretation Bands.

Cellular host response sepsis test for patients in the ED

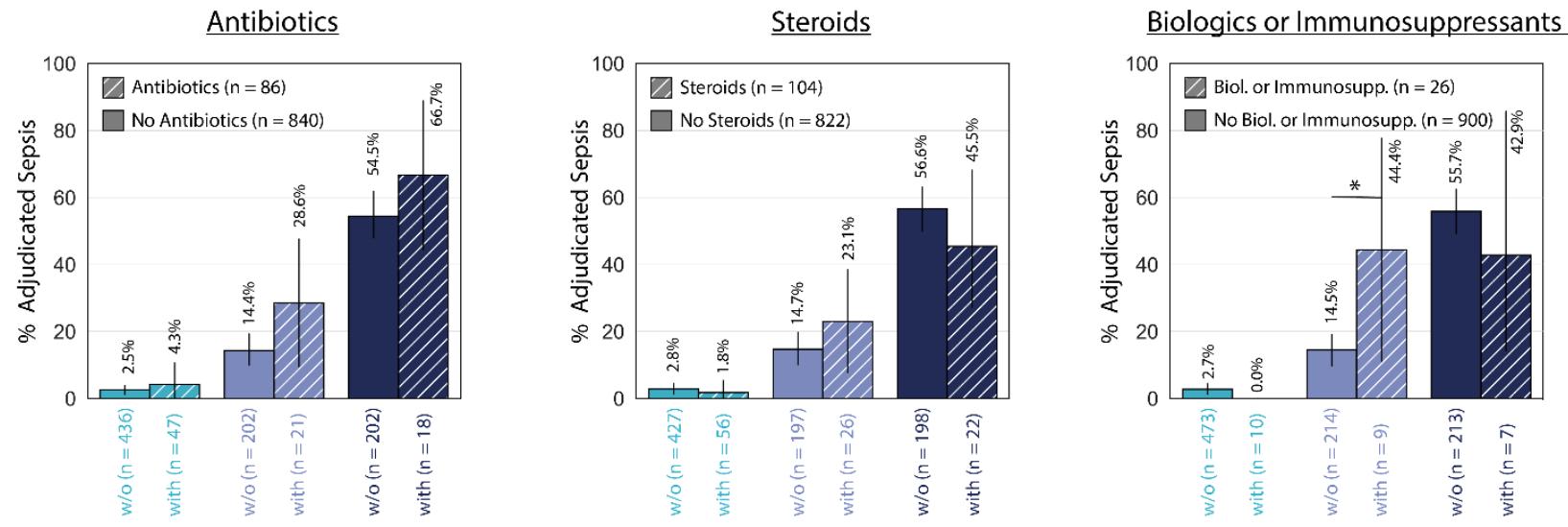
Figure S5. Performance of the ISI (across its Interpretation Bands) in risk stratification of sepsis (per retrospective physician adjudication) in the presence or absence of various common comorbidities and pre-existing organ dysfunction.



Pre-existing organ dysfunction was defined as baseline SOFA score of greater than 2 for up to six-months prior to the ED presentation for which an ISI was obtained). Bars, percentages; error bars, 95% confidence intervals. p -values reported as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Cellular host response sepsis test for patients in the ED

Figure S6. Performance of the ISI (across its Interpretation Bands) in risk stratification of sepsis (per retrospective physician adjudication) for patients taking or not taking select home medications prior to the ED presentation for which an ISI was obtained.



Bars, percentages; error bars, 95% confidence intervals. p -values reported as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Cellular host response sepsis test for patients in the ED

Tables

Table S1. Prospective Studies.

Study	Dates	Enrollment Criteria*	Sites	IRB Approval
SQuISH	Feb 2016 – Dec 2016	SIRS 2+ and at least one sign of potential organ dysfunction, defined as serum lactate >2 mmol/L, altered mental status, hypoxia (peripheral capillary oxygen saturation < 90% on room air), hypotension (systolic blood pressure < 90 mmHg), acute kidney injury, total bilirubin > 2.5 mg/dL, platelet count < 100,000 cells/ μ L, INR > 1.5, and no history of vitamin k antagonist use	(1) Our Lady of the Lake Regional Medical Center (Baton Rouge, LA) (2) Baton Rouge General Medical Center - Bluebonnet Campus (Baton Rouge, LA)	LSUHSC IRB #8964
LA-SQuISH	July 2017 – Jan 2018	SIRS 2+ with or without evidence of organ dysfunction. Of note, this cohort excluded abnormal WBC from SIRS criteria in an effort to enroll patients either before laboratory data were available or those who did not have laboratory studies performed	(1) Our Lady of the Lake Regional Medical Center (Baton Rouge, LA) (2) Baton Rouge General Medical Center - Bluebonnet Campus (Baton Rouge, LA)	LSUHSC IRB #9749
β -SQuISH-ED	April 2019 – Feb 2020	SIRS 2+ (with at least one being aberration of temperature or WBC), or a clinician order for culture of a body fluid (e.g., blood, urine, sputum, or sterile bodily fluids)	(1) Our Lady of the Lake Regional Medical Center (Baton Rouge, LA) (2) Baton Rouge General Medical Center - Bluebonnet Campus (Baton Rouge, LA) (3) Froedtert Hospital (Milwaukee, WI)	LSUHSC IRB #19-019, Franciscan Missionaries of Our Lady University IRB #2019-012, Baton Rouge General IRB #2018-017, and Medical College of Wisconsin/Froedtert Hospital IRB #PRO00034253
SQuISH-COVID**	April 2020	patients presenting with respiratory symptoms and a CBC collected	(1) Our Lady of the Lake Regional Medical Center (Baton Rouge, LA)	LSUHSC IRB #20-048
CV-SQuISH-ED	May 2021 – Oct 2021	SIRS 2+ (with at least one being aberration of temperature or WBC) or a clinician order for culture of a body fluid (e.g., blood, urine, sputum, or sterile bodily fluids).	(1) Our Lady of the Lake Regional Medical Center (Baton Rouge, LA) (2) Harborview Medical Center (Seattle, WA) (3) University of Washington Medical Center - Montlake (Seattle, WA) (4) Wake Forest Baptist Medical Center (Winston-Salem, NC) (5) University of Missouri University Hospital (Columbia, MO)	WCG IRB #20203901

Abbreviations: CBC, complete blood count; INR, international normalized ratio; IRB, Institutional Review Board; LSUHSC, Louisiana State University Health Sciences Center – New Orleans, Human Patients Research Protection Program; SIRS 2+, two or more criteria for Systemic Inflammatory Response Syndrome; WBC, white blood cell count, WCG, WIRB Copernicus Group (formerly known as Western Institutional Review Board (WIRB®))

* In all studies, patients with any of the following characteristics were excluded: expected palliative course, history of hematologic malignancies or myeloproliferative diseases, receipt of cytotoxic chemotherapy within 3 months of the ED encounter, patients transferred from other acute care facilities.

** Due to inherent difficulties with the retrospective sepsis adjudication of patients that are SARS-CoV-2+, most notably assessing whether organ dysfunction is caused by dysregulated host response rather than directly by the infectious organism, this subpopulation was excluded from analysis. Test performance in risk stratification of those with COVID-19 is presented elsewhere.³

Cellular host response sepsis test for patients in the ED

Table S2. Study Sites.

Study Site	Location	Description	No. Staffed Beds	No. ER Visits
Our Lady of the Lake Regional Medical Center	Baton Rouge, LA	Level II state trauma center, affiliated with LSU Health Sciences Center School of Medicine in New Orleans and Tulane University School of Medicine	777	~113,343
Baton Rouge General Medical Center - Bluebonnet Campus	Baton Rouge, LA	Private hospital	335	~74,670
Froedtert Hospital	Milwaukee, WI	ACS level I trauma center, academic medical center affiliated with Medical College of Wisconsin	685	~56,479
Harborview Medical Center	Seattle, WA	Level I state trauma center, academic medical center affiliated with University of Washington School of Medicine	321	~58,121
University of Washington Medical Center - Montlake	Seattle, WA	Academic medical center affiliated with University of Washington School of Medicine	591	~26,994
Wake Forest Baptist Medical Center	Winston-Salem, NC	Level I state I trauma center, academic medical center affiliated with Wake Forest School of Medicine	808	~110,755
University of Missouri University Hospital	Columbia, MO	ACS level I trauma center, academic affiliated with University of Missouri	552	~ 73,401

Cellular host response sepsis test for patients in the ED

Table S3. Distribution of patients stratified by IntelliSep Index within the three Interpretation Bands vs. retrospective physician adjudicated sepsis disease status.

IntelliSep Interpretation Band	IntelliSep Index Range	Adjudicated Sepsis	Adjudicated No Sepsis	Sepsis Prevalence
Band 1 (ISI Range: 0.1 – 4.9) <i>n = 521 (52%)</i>	0.1 – 1.9	0	38	0.0%
	2.0 – 3.5	4	171	2.3%
	3.6 – 4.9	9	299	2.9%
Band 2 (ISI Range: 5.0 – 6.2) <i>n = 243 (24%)</i>	5.0 – 5.6	20	130	13.3%
	5.7 - 6.2	26	67	28.0%
Band 3 (ISI Range: 6.3 – 10.0) <i>n = 238 (24%)</i>	6.3 – 7.4	63	71	47.0%
	7.5 – 8.6	45	23	66.2%
	8.7 – 10.0	25	11	69.4%
Total <i>n = 1002</i>	0.1 – 10.0	192	810	19%

Cellular host response sepsis test for patients in the ED

Table S4. Characteristics of study patients, including selected ED interventions, by sepsis adjudication status.

Characteristic	Total n = 1,002	Adjudication		<i>p</i> -value
		No Sepsis n = 810	Sepsis n = 192	
Age (years)				
Median (Q1-Q3)	57 (41-71)	55 (39-69)	66 (53-78)	<0.001
Subjects ≥ 65, n (%)	362 (36.1)	261 (32.2)	101 (52.6)	<0.001
Biological Sex, n (%)				
Male	496 (49.5)	393 (48.5)	103 (53.7)	ns
Female	506 (50.5)	417 (51.5)	89 (46.4)	ns
Race, n (%)				
Black or African American	363 (36.2)	299 (36.9)	64 (33.3)	ns
White	575 (57.4)	461 (56.9)	114 (59.4)	ns
Other	64 (6.4)	64 (6.4)	14 (7.3)	ns
Comorbidities				
Autoimmune disease	40 (4.0)	26 (3.2)	14 (7.3)	<0.05
Cancer	104 (10.4)	76 (9.4)	28 (14.6)	ns
Congestive Heart Failure	173 (17.3)	130 (16.1)	43 (22.4)	<0.05
Diabetes	294 (29.3)	225 (27.8)	69 (35.9)	ns
HIV	20 (2.0)	11 (1.4)	9 (4.7)	<0.01
Hepatitis B	6 (0.6)	4 (0.5)	2 (1.0)	ns
Hepatitis C	68 (6.8)	47 (5.8)	21 (10.9)	<0.05
Hypertension	565 (56.4)	435 (53.7)	130 (67.7)	<0.01
Obesity (BMI ≥ 30)	113 (11.3)	91 (11.2)	22 (11.5)	ns
End Stage Renal Disease	29 (2.9)	24 (3.0)	5 (2.6)	ns
Chronic Kidney Disease (inclusive of end stage renal disease)	161 (16.1)	112 (13.8)	49 (25.5)	<0.001
Home Medications upon ED presentation, n (%)				
Antibiotics	86 (8.6)	66 (8.2)	20 (10.4)	ns
Immunosuppressant or Biologics	26 (2.6)	19 (2.4)	7 (3.7)	ns
Corticosteroids	104 (10.4)	87 (10.7)	17 (8.9)	ns
Nursing Home Resident, n (%)	99 (9.9)	51 (6.3)	48 (25.0)	<0.001
2+ SIRS criteria met, n (%)	861 (85.9)	669 (82.6)	192 (100.0)	<0.001
Infected by adjudication, n (%)	317 (31.6)	125 (15.4)	192 (100.0)	<0.001
Source of infection in subjects as infected (multiple sources included per subject), n (%)				
Central nervous system	7 (2.2)	2 (1.6)	5 (2.6)	ns
Cardiovascular system	28 (8.8)	3 (2.4)	25 (13.0)	<0.01
Gastrointestinal/Abdominal	54 (17.0)	21 (16.8)	33 (17.2)	ns
Respiratory system	81 (25.6)	25 (20.0)	56 (29.2)	ns
Skin & Skin Structure or Bone & Joint	75 (23.7)	34 (27.2)	41 (21.4)	ns
Urine & Urine system/Genitourinary	91 (28.7)	29 (23.2)	62 (32.3)	ns
Other	16 (5.1)	12 (9.6)	4 (2.1)	<0.01
Organ dysfunction in subjects adjudicated as infected, n (% of infected)	201 (63.4)	9 (7.2)	192 (100.0)	<0.001
Systems with organ dysfunction in subjects adjudicated as infected (multiple systems included per subject), n (% of infected)				
Central nervous system	97 (30.6)	0 (0.0)	97 (50.5)	<0.001
Cardiovascular system	129 (40.7)	0 (0.0)	129 (67.2)	<0.001
Gastrointestinal	60 (18.9)	0 (0.0)	60 (31.3)	<0.001
Hemodynamic	55 (17.4)	0 (0.0)	55 (28.7)	<0.001
Renal	117 (36.9)	2 (1.6)	115 (59.9)	<0.001
Respiratory	93 (29.3)	7 (5.6)	86 (44.8)	<0.001

Cellular host response sepsis test for patients in the ED

Characteristic	Total <i>n</i> = 1,002	Adjudication		<i>p</i> -value
		No Sepsis <i>n</i> = 810	Sepsis <i>n</i> = 192	
Other	8 (2.5)	0 (0.0)	8 (4.2)	<0.05
All-cause cumulative in-hospital mortality, <i>n</i> (%)				
3-day	20 (2.0)	14 (1.7)	6 (3.1)	ns
7-day	41 (4.1)	24 (3.0)	17 (8.9)	<0.001
30-day	59 (5.9)	35 (4.3)	24 (12.5)	<0.001
APACHE II, Median (Q1-Q3)	11 (7-17)	10 (6-16)	16 (12-20)	<0.001
SOFA, 3-day max (baseline subtracted), Median (Q1-Q3)	2 (1-4)	2 (0-3)	5 (3-7)	<0.001
Infected (by adjudication) & worsening, <i>n</i> (%)	100 (10.0)	25 (3.1)	75 (39.1)	<0.001
ED diagnosis of sepsis, <i>n</i> (%)				
Yes	179 (17.9)	67 (8.3)	112 (58.3)	<0.001
No	823 (82.1)	743 (91.7)	80 (41.7)	<0.001
Admitted to hospital, <i>n</i> (%)	681 (68.0)	492 (60.7)	189 (98.4)	<0.001
Admitted to ICU, <i>n</i> (%)	161 (16.1)	99 (12.2)	62 (32.4)	<0.001
Transferred from non-critical unit to ICU during hospital stay, <i>n</i> (%)	29 (2.9)	17 (2.1)	12 (6.3)	<0.01
Hospital free days, Median (Q1-Q3)	25 (21-28)	26 (23-28)	20 (14-24)	<0.001
Blood culture, <i>n</i> (%)				
Number ordered	490 (48.9)	313 (38.6)	177 (92.2)	<0.001
Number positive (of ordered)	105 (21.4)	22 (7.0)	83 (46.9)	<0.001
Number positive (of total)	105 (10.5)	22 (2.7)	83 (43.2)	<0.001
Antibiotics prescribed, <i>n</i> (%)	459 (45.8)	290 (35.8)	169 (88.0)	<0.001
Lactate measured, <i>n</i> (%)	589 (58.8)	405 (50.0)	184 (95.8)	<0.001
Lactate, Median (Q1-Q3)	1.8 (1.2-3.0)	1.6 (1.0-2.5)	2.5 (1.5-3.6)	<0.001
WBC (10^3 cells/ μ l), Median (Q1-Q3)	12.8 (8.3-16.1)	12.3 (7.9-15.0)	16.4 (12.4-20.0)	<0.001
ISI, Median (Q1-Q3)	4.9 (3.7-6.2)	4.4 (3.5-5.5)	7.1 (6.0-8.0)	<0.001

Note: Cancer refers to those with history or current cancer which did not meet the study exclusion criteria of history of hematologic malignancies, and/or receipt of cytotoxic chemotherapy within 3 months of the ED encounter. Worsening is defined as an increase in SOFA score in the 2 subsequent days compared to the day of presentation.

Abbreviations: APACHE II, acute physiology and chronic health evaluation II; BMI, body mass index; ED, emergency department; HIV, human immunodeficiency virus; ICU, intensive care unit; ISI, IntelliSep Index; *n*, number; ns, not significant; Q1-Q3, interquartile range; SIRS, Systemic Inflammatory Response Syndrome; SOFA, sequential organ failure assessment; WBC, white blood cell count.

Cellular host response sepsis test for patients in the ED

Table S5. Characteristics of the subpopulation with low-confidence adjudicated labels that were excluded from the final analysis ($n = 194$), including selected ED interventions, by ISI Interpretation Bands.

Characteristic	Total <i>n</i> = 194	ISI Interpretation Band			<i>p</i> -value ^a
		1 <i>n</i> = 75	2 <i>n</i> = 60	3 <i>n</i> = 59	
Age (years)					
Median (Q1-Q3)	62 (49-73)	61 (48-73)	67 (49-73)	61 (52-71)	ns, ns, ns
Subjects ≥ 65 , <i>n</i> (%)	90 (46.4)	31 (41.3)	34 (56.7)	25 (42.4)	ns, ns, ns
Biological Sex, <i>n</i> (%)					
Male	105 (54.1)	36 (48.0)	38 (63.3)	31 (52.5)	ns, ns, ns
Female	89 (45.9)	39 (52.0)	22 (36.7)	28 (47.5)	ns, ns, ns
Race, <i>n</i> (%)					
Black or African American	74 (38.1)	36 (48.0)	17 (28.3)	21 (35.6)	<0.05, ns, ns
White	111 (57.2)	35 (46.7)	40 (66.7)	36 (61.0)	ns, ns, ns
Other	9 (4.6)	4 (5.3)	3 (5.0)	2 (3.4)	<0.05, ns, ns
Comorbidities					
Autoimmune disease	10 (5.2)	4 (5.3)	2 (3.3)	4 (6.8)	ns, ns, ns
Cancer	20 (10.3)	8 (10.7)	6 (10.2)	6 (10.2)	ns, ns, ns
Congestive Heart Failure	28 (14.4)	11 (14.7)	13 (21.7)	4 (6.8)	ns, ns, <0.05
Diabetes	70 (36.1)	26 (34.7)	22 (36.7)	22 (37.3)	ns, ns, ns
HIV	4 (2.1)	2 (2.7)	0 (0.0)	2 (3.4)	ns, ns, ns
Hepatitis B	1 (0.5)	1 (1.3)	0 (0.0)	0 (0.0)	ns, ns, ns
Hepatitis C	13 (6.7)	8 (10.7)	1 (1.7)	4 (6.8)	<0.05, ns, ns
Hypertension	131 (67.5)	53 (70.7)	35 (58.3)	43 (72.9)	ns, ns, <0.05
Obesity (BMI ≥ 30)	29 (15.0)	8 (10.7)	10 (16.7)	11 (18.6)	ns, ns, ns
End Stage Renal Disease	6 (3.1)	2 (2.7)	4 (6.7)	0 (0.0)	ns, ns, <0.05
Chronic Kidney Disease ^b	29 (15.0)	13 (17.3)	13 (21.7)	3 (5.1)	ns, <0.05, <0.05
Home Medications upon ED presentation, <i>n</i> (%)					
Antibiotics	22 (11.3)	8 (10.7)	6 (10.0)	8 (13.6)	ns, ns, ns
Immunosuppressant or Biologics	7 (3.6)	2 (2.7)	3 (5.0)	2 (3.4)	ns, ns, ns
Corticosteroids	21 (10.8)	13 (17.3)	3 (5.0)	5 (8.5)	<0.05, ns, ns
Nursing Home Resident, <i>n</i> (%)	27 (13.9)	8 (10.7)	11 (18.3)	8 (13.6)	ns, ns, ns
2+ SIRS criteria met, <i>n</i> (%)	171 (88.1)	62 (82.7)	54 (90.0)	55 (93.2)	<0.001, <0.001, <0.001
Infected by adjudication, <i>n</i> (%)	183 (94.3)	68 (90.7)	57 (95.0)	58 (98.3)	ns, ns, ns
Source of infection^c, <i>n</i> (%)					
Central nervous system	8 (4.4)	3 (4.4)	3 (5.3)	2 (3.5)	ns, ns, ns
Cardiovascular system	12 (6.6)	6 (8.8)	1 (1.8)	5 (8.6)	ns, ns, ns
Gastrointestinal/Abdominal	25 (13.7)	8 (11.8)	9 (15.8)	8 (13.8)	ns, ns, ns
Respiratory system	60 (32.8)	20 (29.4)	20 (35.1)	20 (34.5)	ns, ns, ns
Skin & skin structure or Bone&Joint	50 (27.3)	17 (25.0)	15 (26.3)	18 (31.0)	ns, ns, ns
Urine & Genitourinary	54 (29.5)	21 (30.9)	14 (24.6)	19 (32.8)	ns, ns, ns
Other	11 (6.0)	2 (2.9)	4 (7.0)	5 (8.6)	ns, ns, ns
Organ dysfunction in subjects adjudicated as infected, <i>n</i> (% of infected)					
Systems with organ dysfunction, <i>n</i> (% of infected) ^d					
Central nervous system	40 (21.9)	20 (29.4)	10 (17.2)	10 (17.2)	ns, ns, ns
Cardiovascular system	44 (24.0)	16 (23.5)	12 (21.1)	16 (27.6)	ns, ns, ns
Gastrointestinal	23 (12.6)	9 (13.2)	6 (10.5)	18 (13.8)	ns, ns, ns

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Characteristic	Total <i>n</i> = 194	ISI Interpretation Band			<i>p</i> -value ^a
		1 <i>n</i> = 75	2 <i>n</i> = 60	3 <i>n</i> = 59	
Hemodynamic	26 (14.2)	14 (20.6)	6 (10.5)	6 (10.3)	ns, ns, ns
Renal	56 (30.6)	22 (32.4)	13 (22.8)	21 (36.2)	ns, ns, ns
Respiratory	65 (35.5)	24 (35.3)	17 (29.8)	24 (41.4)	ns, ns, ns
Other	10 (5.5)	5 (7.4)	3 (5.3)	2 (3.5)	ns, ns, ns
Sepsis, by adjudication, <i>n</i> (%)					
Sepsis-3 Consensus Definition	76 (39.2)	22 (29.3)	23 (38.3)	31 (52.5)	ns, <0.01, ns
Severe Sepsis-2 Consensus Definition	127 (65.5)	44 (58.7)	43 (71.7)	40 (67.8)	ns, ns, ns
All-cause cumulative in-hospital mortality, <i>n</i> (%)					
3-day	1 (0.5)	0 (0.0)	0 (0.0)	1 (1.7)	ns, ns, ns
7-day	2 (1.0)	1 (1.3)	0 (0.0)	1 (1.7)	ns, ns, ns
30-day	5 (2.6)	3 (4.0)	0 (0.0)	2 (3.4)	ns, ns, ns
APACHE II, Median (Q1-Q3)	13 (10-17)	13 (9-17)	13 (10-16)	13 (10-19)	ns, ns, ns
SOFA, 3-day max (baseline subtracted), Median (Q1-Q3)	2 (2-4)	2 (2-4)	2 (1-4)	2 (2-4)	ns, ns, ns
ED diagnosis of sepsis, <i>n</i> (%)					
Yes	48 (24.7)	15 (20.0)	15 (25.0)	18 (30.5)	ns, ns, ns
No	146 (75.3)	60 (80.0)	45 (75.0)	41 (69.5)	ns, ns, ns
Admitted to hospital, <i>n</i> (%)	166 (85.6)	63 (84.0)	52 (86.7)	51 (86.4)	ns, ns, ns
Admitted to ICU, <i>n</i> (%)	20 (10.3)	9 (12.0)	6 (10.0)	5 (8.5)	ns, ns, ns
Transferred from non-critical unit to ICU during hospital stay, <i>n</i> (%)	8 (4.1)	0 (0.0)	7 (11.7)	1 (1.7)	<0.01, ns, <0.05
Hospital free days, Median (Q1-Q3)	24 (21-25)	24 (21-26)	24 (20-25)	23 (21-25)	ns, ns, ns
Blood culture, <i>n</i> (%)					
Number ordered	150 (77.3)	53 (70.7)	45 (75.0)	52 (88.1)	ns, <0.05, ns
Number positive (of ordered)	36 (24.0)	4 (7.6)	11 (24.4)	21 (40.4)	<0.05, <0.001, <0.05
Number positive (of total)	36 (18.6)	4 (5.3)	11 (18.3)	21 (35.6)	<0.05, <0.001, <0.05
Antibiotics prescribed, <i>n</i> (%)	154 (79.4)	56 (74.7)	45 (75.0)	53 (89.8)	ns, <0.05, ns
Lactate measured, <i>n</i> (%)	150 (77.3)	58 (77.3)	47 (78.3)	45 (76.3)	ns, ns, ns
Lactate, Median (Q1-Q3)	1.5 (1.1-2.4)	1.6 (1.1-2.3)	1.4 (1.2-2.6)	1.4 (1.2-2.6)	ns, ns, ns
WBC (10^3 cells/ μ l), Median (Q1-Q3)	13.3 (8.5-17.4)	9.9 (6.5-13.6)	13.6 (11.5-17.0)	16.8 (13.3-21.1)	<0.001, <0.001, <0.01
ISI, Median (Q1-Q3)	5.4 (4.4-6.5)	4.1 (3.6-4.5)	5.6 (5.2-5.9)	7.2 (6.6-7.8)	<0.001, <0.001, <0.001

Note: Cancer refers to those with history or current cancer which did not meet the study exclusion criteria of history of hematologic malignancies, and/or receipt of cytotoxic chemotherapy within 3 months of the ED encounter. Worsening is defined as an increase in SOFA score in the 2 subsequent days compared to the day of presentation.

Abbreviations: APACHE II, acute physiology and chronic health evaluation II; BMI, body mass index; ED, emergency department; HIV, human immunodeficiency virus; ICU, intensive care unit; ISI, IntelliSep Index; *n*, number; ns, not significant; Q1-Q3, interquartile range; SIRS, Systemic Inflammatory Response Syndrome; SOFA, sequential organ failure assessment; WBC, white blood cell count.

^a *p*-values for 2 by 2 comparisons between Band 1 vs Band 2 (first), Band 1 vs Band 3 (second), and Band 2 vs Band 3 (third)

^b inclusive of end stage renal disease

^c in subjects adjudicated as infected, multiple sources may be included per subject

^d in subjects adjudicated as infected, multiple systems may be included per subject

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Table S6. Presumed cause of death for all patients that suffered in-hospital mortality during the first 30 days of hospitalization.

Subject	Cause of Death	Received Antibiotics in the ED	ISI Interpretation Band
1	sepsis	Yes	3
2	sepsis	Yes	3
3	sepsis	Yes	3
4	sepsis	Yes	3
5	sepsis	Yes	3
6	sepsis	Yes	3
7	sepsis	Yes	3
8	sepsis	No	3
9	sepsis	Yes	3
10	sepsis	Yes	3
11	sepsis	Yes	3
12	sepsis	No	3
13	sepsis	No	3
14	sepsis	Yes	3
15	sepsis	Yes	3
16	sepsis	No	3
17	sepsis	Yes	3
18	sepsis	Yes	3
19	sepsis	No	3
20	sepsis	Yes	3
21	sepsis	Yes	3
22	hemorrhagic shock secondary to surgery on non-healing wounds	Yes	3
23	ascites, anasarca, complications of stage 5 chronic kidney disease, possible abdominal infection	No	3
24	hypoglycemia following insulin overdose	Yes	3
25	brain death following 30 ft fall	No	3
26	metastatic intra-abdominal adenocarcinoma	Yes	3
27	cardiac arrest	No	3
28	retropharyngeal mass	Yes	3
29	alcoholic dementia	Yes	3
30	ischemic colitis and substance abuse	Yes	3
31	sepsis	Yes	2
32	sepsis	Yes	2
33	sepsis	Yes	2
34	cardiac arrest during hemodialysis	Yes	2

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Subject	Cause of Death	Received Antibiotics in the ED	ISI Interpretation Band
35	cardiac arrest	Yes	2
36	alcohol-induced hepatic failure with hepatorenal syndrome	No	2
37	CHF complication with pacemaker malfunction	No	2
38	end stage CHF complication	No	2
39	NSTEMI	No	2
40	COPD exacerbation leading to respiratory failure	Yes	2
41	CHF complication, COPD exacerbation, gangrene	No	2
42	complications with LVAD device and stroke	No	2
43	NSTEMI	Yes	2
44	stroke	No	2
45	hypoglycemia following insulin overdose	Yes	1
46	respiratory failure due to status epilepticus and stroke	No	1
47	CHF complications	No	1
48	cardiac arrest secondary to drug overdose	Yes	1
49	cardiac arrest	Yes	1
50	metabolic encephalopathy	Yes	1
51	CHF complications	No	1
52	stroke	No	1
53	respiratory failure due to status epilepticus and stroke	No	1
54	COPD exacerbation leading to respiratory failure	Yes	1
55	respiratory failure due to end-stage pulmonary fibrosis	No	1
56	stroke	No	1
57	atrial fibrillation	Yes	1
58	complications of small bowel obstruction and subsequent surgery	No	1
59	pulmonary embolism and deep brain thrombosis	No	1

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; LVAD, left ventricular assist device; NSTEMI, Non-ST-Elevation Myocardial Infarction

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Table S7. Performance comparison between the ISI and a selection of other commonly-assessed indicators of sepsis compared to adjudicated sepsis.

Population (n); % Adjudicated Sepsis	Indicator of sepsis	PPA (95% CI)	NPV (95% CI)	LR- (1/LR-)	Mortality (n)^a	AUC (95% CI)	NPA (95% CI)	PPV (95% CI)	LR+
997; 19.3%	IntelliSep High: Band 3 Low: Band 1	93.2 (88.1 – 96.0)	97.5 (94.0 – 99.1)	0.1 (10)	0	0.87 (0.84 – 0.90)	87.0 (80.8 – 91.0)	55.9 (48.4 – 62.9)	5.3
	WBC High: > 12 or < 4 Low: $\geq 4 \& \leq 12$	79.2 (72.2 – 84.2)	88.7 (83.2 – 92.7)	0.5 (2)	3	0.59 (0.56 – 0.62)	39.0 (31.6 – 45.8)	23.6 (17.6 – 30.1)	1.3
847; 20.1%	IntelliSep High: Band 3 Low: Band 1	92.4 (87.3 – 95.9)	97.0 (92.5 – 98.7)	0.1 (10)	0	0.87 (0.83 – 0.90)	87.1 (81.1 – 91.7)	56.5 (48.7 – 64.0)	5.2
	PCT High: ≥ 2 Low: < 0.5	74.7 (67.5 – 81.0)	93.0 (88.0 – 96.3)	0.3 (3)	3	0.87 (0.83 – 0.90)	94.1 (88.7 – 96.7)	68.8 (60.7 – 75.2)	8.8
589; 31.2%	IntelliSep High: Band 3 Low: Band 1	92.9 (88.2 – 96.2)	94.4 (89.6 – 97.0)	0.1 (10)	0	0.84 (0.81 – 0.88)	83.7 (77.5 – 88.7)	66.0 (58.4 – 72.6)	4.3
	Lactate High: ≥ 4 Low: < 2	65.8 (58.4 – 72.6)	80.5 (74.0 – 85.9)	0.5 (2)	3	0.67 (0.62 – 0.71)	87.9 (81.8 – 91.9)	44.3 (36.7 – 51.5)	1.8
566; 32.5%	IntelliSep High: Band 3 Low: Band 1	92.9 (87.6 – 95.8)	94.3 (89.6 – 97.0)	0.1 (10)	0	0.84 (0.81 – 0.87)	83.5 (76.9 – 88.3)	66.8 (59.0 – 73.1)	4.2
	Blood Cultures High: Positive Low: Negative	45.1 (37.8 – 52.6)	78.1 (71.0 – 83.5)	0.6 (2)	12	0.70 (0.66 – 0.73)	94.2 (89.6 – 97.0)	79.0 (72.2 – 84.5)	7.8
978; 19.4%	IntelliSep High: Band 3 Low: Band 1	93.2 (88.6 – 96.3)	97.4 (94.0 – 99.1)	0.1 (10)	0	0.87 (0.84 – 0.90)	86.8 (80.6 – 90.9)	55.7 (47.9 – 62.5)	5.2
	qSOFA High: ≥ 2 Low: < 2	34.2 (27.5 – 41.4)	84.9 (78.8 – 89.5)	0.7 (1)	12	0.67 (0.63 – 0.71)	89.2 (83.6 – 93.0)	43.3 (36.0 – 50.5)	3.2
1000; 19.1%	IntelliSep High: Band 3 Low: Band 1	93.2 (88.6 – 96.3)	97.5 (94.0 – 99.1)	0.1 (10)	0	0.87 (0.85 – 0.90)	87.0 (81.3 – 91.3)	55.7 (48.1 – 62.7)	5.3
	SOFA (day of presentation) High: ≥ 2 Low: < 2	84.3 (78.3 – 89.1)	93.3 (88.6 – 96.3)	0.3 (3)	1	0.75 (0.71 – 0.79)	52.0 (44.5 – 59.1)	29.3 (23.0 – 36.3)	1.8

^aSepsis-associated mortality in low-risk group (n)

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Note for Table S7: Missing values were not imputed, and so each biomarker/scoring system is compared to the ISI in the population for which values were available. For each variable, high and low risk cutoffs are indicated. LR+ is defined as sensitivity/(1-specificity) with a high-risk cutoff (i.e., High vs. else) and LR- is defined as (1-sensitivity)/specificity with a low-risk cutoff (i.e., Low 1 vs. else). SOFA was calculated for the worst values on the day of ED presentation.

Abbreviations: LR+, positive likelihood ratio; LR-, negative likelihood ratio; WBC, white blood cell count; PCT, procalcitonin; qSOFA, quick SOFA; SOFA, sequential organ failure assessment.

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Table S8. The risk category of patients who suffered in-hospital sepsis-associated mortality across all variables of commonly-assessed indicators of sepsis compared to the cellular host response (IntelliSep) test.

Subject	WBC	PCT	Lactate	Blood Cultures	qSOFA	SOFA (Day of Presentation)	IntelliSep Interpretation Band
1	> 12 or < 4	≥ 2	≥ 4	Positive	≥ 2	≥ 2	3
2	> 12 or < 4	≥ 2	≥ 4	Positive	≥ 2	≥ 2	3
3	> 12 or < 4	Not Collected	Not Collected	Not Collected	≥ 2	≥ 2	3
4	> 12 or < 4	< 0.5	< 2	Negative	< 2	≥ 2	3
5	> 12 or < 4	≥ 0.5 and < 2	≥ 2 and < 4	Negative	< 2	≥ 2	3
6	> 12 or < 4	≥ 2	≥ 2 and < 4	Negative	≥ 2	≥ 2	3
7	> 12 or < 4	≥ 2	≥ 2 and < 4	Negative	< 2	≥ 2	3
8	> 12 or < 4	≥ 0.5 and < 2	< 2	Negative	< 2	≥ 2	3
9	> 12 or < 4	≥ 2	≥ 4	Negative	< 2	≥ 2	3
10	> 12 or < 4	≥ 2	≥ 4	Negative	< 2	≥ 2	3
11	> 12 or < 4	≥ 2	≥ 2 and < 4	Negative	≥ 2	≥ 2	3
12	> 12 or < 4	≥ 0.5 and < 2	≥ 2 and < 4	Negative	≥ 2	≥ 2	3
13	≥ 4 and ≤ 12	≥ 0.5 and < 2	≥ 4	Negative	≥ 2	≥ 2	3
14	> 12 or < 4	≥ 2	≥ 4	Negative	≥ 2	≥ 2	3
15	> 12 or < 4	≥ 0.5 and < 2	≥ 2 and < 4	Negative	< 2	≥ 2	3
16	> 12 or < 4	≥ 2	≥ 2 and < 4	Positive	< 2	< 2	3
17	> 12 or < 4	Not Collected	≥ 2 and < 4	Positive	< 2	≥ 2	3
18	> 12 or < 4	≥ 2	≥ 4	Positive	< 2	≥ 2	3
19	> 12 or < 4	≥ 2	≥ 2 and < 4	Positive	< 2	≥ 2	3
20	≥ 4 and ≤ 12	≥ 2	≥ 4	Positive	≥ 2	≥ 2	3
21	> 12 or < 4	≥ 0.5 and < 2	≥ 2 and < 4	Positive	< 2	≥ 2	3
22	> 12 or < 4	< 0.5	< 2	Not Collected	≥ 2	≥ 2	2
23	> 12 or < 4	< 0.5	≥ 2 and < 4	Positive	≥ 2	≥ 2	2
24	≥ 4 and ≤ 12	≥ 2	≥ 2 and < 4	Positive	≥ 2	≥ 2	2

Abbreviations: WBC, white blood cell count, PCT; procalcitonin; qSOFA, quick SOFA; SOFA, sequential organ failure assessment.

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