OBSERVATIONAL STUDY

OPEN

Validation of a Novel, Rapid Sepsis Diagnostic for Emergency Department Use

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OBJECTIVES: To assess the in vitro IntelliSep test, a microfluidic assay that quantifies the state of immune activation by evaluating the biophysical properties of leukocytes, as a rapid diagnostic for sepsis.

DESIGN: Prospective cohort study.

SETTING: Five emergency departments (EDs) in Louisiana, Missouri, North Carolina, and Washington.

PATIENTS: Adult patients presenting to the ED with signs (two of four Systemic Inflammatory Response Syndrome criteria, where one must be temperature or WBC count) or suspicion (provider-ordered culture) of infection.

INTERVENTIONS: All patients underwent testing with the IntelliSep using ethylene diamine tetraacetic acid-anticoagulated whole blood followed by retrospective adjudication for sepsis by sepsis-3 criteria by a blinded panel of physicians.

MEASUREMENTS AND MAIN RESULTS: Of 599 patients enrolled, 572 patients were included in the final analysis. The result of the IntelliSep test is reported as the IntelliSep Index (ISI), ranging from 0.1 to 10.0, divided into three interpretation bands for the risk of sepsis: band 1 (low) to band 3 (high). The median turnaround time for ISI results was 7.2 minutes. The ISI resulted band 1 in 252 (44.1%), band 2 in 160 (28.0%), and band 3 in 160 (28.0%). Sepsis occurred in 26.6% (152 of 572 patients). Sepsis prevalence was 11.1% (95% CI, 7.5–15.7%) in band 1, 28.1% (95% CI, 21.3–35.8%) in band 2, and 49.4% (95% CI, 41.4–57.4%) in band 3. The Positive Percent Agreement of band 1 was 81.6% and the Negative Percent Agreement of band 3 was 80.7%, with an area under the receiver operating characteristic curve of 0.74. Compared with band 1, band 3 correlated with adverse clinical outcomes, including mortality, and resource utilization.

CONCLUSIONS: Increasing ISI interpretation band is associated with increasing probability of sepsis in patients presenting to the ED with suspected infection.

KEYWORDS: diagnosis; emergency service, hospital; leukocytes; microfluidics; sepsis

Sepsis, life-threatening organ dysfunction due to a dysregulated host response to infection (1), is one of the most common (2) and costly (3) medical conditions in the United States, accounting for a large proportion of hospital readmissions (4), morbidity, and mortality (5–7). Most cases arise in the community (8) and present to the emergency department (ED), where both early diagnosis and efficient treatment are challenging (9, 10).

Hospitals frequently implement guideline-based processes (11) to facilitate early diagnosis (12) and optimize treatment of sepsis (13–16). Despite these efforts, sepsis ranks as the sixth most misdiagnosed condition in EDs, with high rates of both under-diagnosis and over-diagnosis (17). A rapid, objective test targeting the pathophysiology of sepsis may decrease misdiagnosis and improve care for patients presenting to the ED with signs or suspicion of infection.

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KEY POINTS

Question: Can the IntelliSep test, a microfluidic assay that characterizes the state of innate immune activity by measuring and quantifying structural changes that occur upon neutrophil and monocyte activation, serve as a rapid means of diagnosing sepsis risk stratifying for adverse events and resource utilization in a population of patients presenting to the emergency department (ED) with signs or suspicion of infection?

Findings: In this study of 572 patients from five EDs in four states, we found that the IntelliSep test can serve as a rapid (< 10 min) diagnostic aid for sepsis as adjudicated by an expert panel using sepsis-3 criteria. The test achieved a positive percent agreement of 81.6%, negative percent agreement of 80.7%, and area under the receiver operating characteristic curve of 0.74; increasing scores were associated with increasing resource utilization and adverse outcomes, including in-hospital mortality.

Meaning: The IntelliSep may serve as a diagnostic aid for sepsis for ED providers and has potential to improve the efficiency and efficacy of sepsis care.

Upon activation, neutrophils and monocytes undergo structural changes that result in altered deformability (18). Detecting these changes may provide an early sign of a dysregulated host response underlying the clinical syndrome of sepsis (19). The IntelliSep is a rapid (< 10 min) in vitro test that assesses the viscoelastic properties of these leukocytes and quantifies these properties in the form of the IntelliSep Index (ISI) (20). In a preliminary single-center observational study of adults presenting to the ED with signs or suspicion of infection, the ISI showed a high degree of discrimination between patients with and without sepsis (21).

We performed a multicentered, prospective cohort study to test the hypothesis that ISI is associated with an increased risk of sepsis in a population of patients with signs or suspicion of infection. We also assessed the utility of the ISI in the risk stratification of patients by the adverse outcome and resource utilization. Some of the results of this study have been previously reported in the form of an abstract (22).

MATERIALS AND METHODS

Study Population

After approval by the western institutional review board - Copernicus Group (WCG) interquartile review board (Title: "CV-SQuISH-ED: A Clinical Validation Solving the Question of Inflammation or Sepsis Hastily in the Emergency Department," WCG number 20203901, December 7, 2020, NCT 04933760), patients were enrolled from five EDs in Louisiana, Missouri, North Carolina, and Washington between May and October 2021. Eligible patients were 18 years or older with signs of infection (two of four Systemic Inflammatory Response Syndrome (SIRS) criteria, where at least one must have been temperature or leukocyte criteria) or suspicion of infection (orders placed for culture of blood, urine, sputum, or sterile body fluid). Subjects had K-2 ethylene diamine tetraacetic acid (EDTA)-anticoagulated whole blood collected within 4 hours of the first vital sign measurement. Exclusion criteria are delineated in the protocol, which is included within the **Supplementary Material** (http://links.lww.com/CCX/B289). Study performance was in accordance with the ethical standards of the responsible committee on human experimentation and the Helsinki Declaration of 1975. Research personnel actively screened for eligible patients, and a waiver of informed consent was granted. Clinical and research personnel were blinded to the ISI result.

Data Collection

Research personnel abstracted demographic data, including age, sex, ethnicity, race, comorbidities, and outpatient medications from the electronic health record (EHR). Baseline Sequential Organ Failure Assessment (SOFA) scores were recorded from the nearest normal values available from the previous 6 months; if unavailable, baseline function was considered normal. Laboratory and physiologic data were collected for the first 3 days of hospitalization. Radiographic, microbiologic, and molecular pathogen detection data were collected if performed during the first 2 days of hospitalization. Outcome data, including hospitalization, level of care, and disposition data were recorded from the EHR.

Confirmation of the Diagnosis

The primary objective was to assess the performance of the IntelliSep test as a diagnostic marker of sepsis using

the sepsis-3 definition. The protocol also includes evaluation for sepsis-2 and severe sepsis-2; however, these analyses are not included in the present article. To be considered positive for sepsis, patients must meet three criteria: 1) infection (present on presentation to the ED), 2) organ dysfunction (manifesting within 3 days of the ED visit), and 3) causation of organ dysfunction by a dysregulated host response to the infection.

We developed a rigorous process to adjudicate for each component. First, coordinators extracted data from the EHR to complete an objective evaluation for infection using prespecified criteria (23) and organ dysfunction using SOFA (24). Subsequently, a site investigator with access to the entire EHR and blinded to the ISI result completed a clinical review and recorded pertinent clinical information. The adjudication process made no distinction between viral or bacterial sepsis.

Compiled data were transmitted electronically to external adjudicators with access only to these data, not the ISI result. If two adjudicators agreed on all three criteria, the case was considered a "unanimous" adjudication. Upon disagreement, a third adjudicator reviewed the case and all discussed it in an open forum. If they came to an agreement, the case was considered a "consensus" adjudication; otherwise, a majority vote resulted in a "forced" adjudication label.

Severity of Illness, Resource Utilization, and Mortality

The severity of illness was assessed by both Acute Physiology And Chronic Health Evaluation (APACHE)-II (calculated from data available during the first hospital day) and SOFA scores (maximum score in the first 3 d, baseline subtracted). Coordinators recorded order and administration times for antibiotics, blood culture orders, and lactic acid. Admit dispositions and escalations of care during the first 48 hours of hospitalization were recorded, as were discharge dispositions. Hospital mortality was assessed at 3-, 7-, and 30-day intervals.

Performance of the IntelliSep

The IntelliSep test was performed on an aliquot of blood from the K-2 EDTA-anticoagulated whole blood sample. On-shift clinical laboratory personnel trained on IntelliSep equipment were responsible for test performance in addition to other standard responsibilities. To prevent sample degradation, IntelliSep testing was required to be completed within four hours of sample collection. Details of performing the IntelliSep test (20) can be found in the Supplemental Material (http://links.lww.com/CCX/B289). The IntelliSep result is reported as the ISI, a single number ranging from 0.1 to 10.0, divided into three prespecified interpretation bands using limits of less than equals to 4.9 (band 1) and greater than or equal to 6.3 (band 3), with band 2 intervening (25).

Statistical Analysis

The primary endpoint required for regulatory review was to achieve nonoverlapping 80% CIs for the prevalence of sepsis between bands 1 and 3; for this publication, 95% CIs are included. Based on disease prevalence per band in a preliminary study (21), power analysis indicated the need to enroll 55 total patients to achieve the primary endpoint with 80% power. To ensure the study included a diverse array of patients and comorbid states, we planned to enroll up to 600 total patients. In addition, we assessed the Positive Percent Agreement and Negative Percent Agreement of the ISI as compared with the adjudicated standard for sepsis as well as a receiver operating characteristic (ROC) curve analysis.

Baseline characteristics and descriptive statistics are presented as means, standard deviations, medians, and first and third quartiles. Unless otherwise stated, p values are derived from an unpaired two-sample Welch's t test. An alpha level of 5% is used for all analyses. Two-sided CIs for proportions are provided using the Clopper-Person method.

RESULTS

A total of 599 patients were enrolled, with 572 in the final analysis, 245 (42.8%) enrolled with modified SIRS criteria, 93 (16.3%) with culture criteria, and 234 (40.9%) with both (**S1-Fig. 1**, http://links.lww.com/CCX/B289). **Table 1** includes baseline characteristics of patients by the presence or absence of adjudicated sepsis. The median age was 56 years (Q1–Q3, 40–68); 250 (43.7%) patients were female and 172 (30.1%) were Black. Patients with adjudicated sepsis were older (63 vs. 53 yr, $p < 0.001$) and more likely to have cancer, diabetes, and HIV infection.

TABLE 1.
Selected Characteristics of Patients, Including Selected Emergency Department Interventions, by Adjudication Status for Sepsis-3

Category	Subcategory	Total, *n* = 572	Sepsis-3		*p*
			No, *n* = 420	Yes, *n* = 152	
Age	Median (Q1–Q3)	56.0 (40.0–68.0)	53.0 (37.0–66.0)	63.0 (46.0–73.0)	< 0.0001
	Subjects ≥ 65. *n* (%)	187 (32.7)	118 (28.1)	69 (45.4)	< 0.0001
Biological sex, *n* (%)	Male	322 (56.3)	227 (54.1)	95 (62.5)	ns
	Female	250 (43.7)	193 (46.0)	57 (37.5)	ns
Race, *n* (%)	Black	172 (30.1)	130 (31.0)	42 (27.6)	ns
	White	356 (62.2)	255 (60.7)	101 (66.5)	ns
	Other	44 (7.7)	35 (8.3)	9 (5.9)	ns
Comorbidities, *n* (%)	Autoimmune Disease	23 (4.0)	16 (3.8)	7 (4.6)	ns
	Diabetes	165 (28.9)	111 (26.4)	54 (35.5)	< 0.05
	HIV	11 (1.9)	5 (1.2)	6 (4.0)	< 0.05
	Hepatitis C	46 (8.0)	32 (7.6)	14 (9.2)	ns
	Hypertension	290 (50.7)	200 (47.6)	90 (59.2)	ns
	Obesity	98 (17.1)	65 (15.5)	33 (21.7)	ns
	End-stage renal disease	18 (3.2)	14 (3.3)	4 (2.6)	ns
Outpatient medications, *n* (%)	Antibiotics	67 (11.7)	43 (10.2)	24 (15.8)	ns
	Anti-inflammatory	179 (31.3)	121 (28.8)	58 (38.2)	< 0.05
	Corticosteroids	41 (7.2)	25 (5.6)	16 (10.5)	ns
Infected by adjudication, *n* (%)	Yes	286 (50.0)	134 (31.9)	152 (100.0)	< 0.0001
Sepsis adjudication, *n* (%)	Unanimous	450 (78.7)	334 (79.5)	116 (76.3)	ns
	Consensus	120 (21.0)	84 (20.0)	36 (23.7)	ns
	Forced	2 (0.4)	2 (0.5)	0 (0.0)	ns
Lactate measured, *n* (%)	Yes	294 (51.4)	178 (42.4)	116 (76.3)	< 0.0001
Lactate, median (Q1–Q3)		1.7 (1.3–2.6)	1.6 (1.3–2.6)	1.8 (1.3–2.7)	ns
IntelliSep Index, median (Q1–Q3)		5.2 (4.1–6.5)	4.8 (3.9–5.9)	6.4 (5.3–7.5)	< 0.0001

ns = not significant, Q1–Q3 = interquartile range.

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 emergency department encounter. "Anti-inflammatory" outpatient medications included acetaminophen (Tylenol), celecoxib (Celebrex), ibuprofen (Advil), indomethacin (Indocin), and naproxen (Aleve). S1-Table 1 (http://links.lww.com/CCX/B289) contains the complete table of characteristics.

ISI Values

A valid ISI was obtained in greater than 98% of tested subjects with a median turnaround time (receipt by technician to reporting of result) of 7.2 minutes (Q1–Q3, 6.8–7.9). **Table 2** includes baseline characteristics of patients by ISI interpretation band. The median ISI for all patients was 5.2 (Q1–Q3, 4.10–6.50). There were 252 (44%) patients in band 1, 160 (28%) in band 2, and 160 (28%) in band 3.

TABLE 2.
Selected Characteristics of Patients, Including Selected Emergency Department Interventions, by Interpretation Band

Category	Subcategory	Interpretation Band			*p*
		Band 1, *n* = 252	Band 2, *n* = 160	Band 3, *n* = 160	
Age	Median (Q1–Q3)	54.0 (39.8–67.0)[a,b]	56.0 (39.0–68.0)[a,c]	58.00 (41.8–71.0)[b,c]	ns[a], ns[b], ns[c]
	≥ 65, *n* (%)	77 (30.6)[a,b]	54 (33.8)[a,c]	56 (35.0)[b,c]	ns[a], ns[b], ns[c]
Biological sex, *n* (%)	Male	146 (57.9)[a,b]	92 (57.5)[a,c]	84 (52.5)[b,c]	ns[a], ns[b], ns[c]
	Female	106 (42.1)[a,b]	68 (42.5)[a,c]	76 (47.5)[b,c]	ns[a], ns[b], ns[c]
Race, *n* (%)	Black or African American	76 (30.2)[a,b]	47 (29.4)[a,c]	49 (30.6)[b,c]	ns[a], ns[b], ns[c]
	White	154 (61.1)[a,b]	104 (65.0)[a,c]	98 (61.3)[b,c]	ns[a], ns[b], ns[c]
	Other	22 (8.7)[a,b]	9 (5.6)[a,c]	13 (8.1)[b,c]	ns[a], ns[b], ns[c]
Comorbidities, *n* (%)	Autoimmune disease	9 (3.6)[a,b]	7 (4.4)[a,c]	7 (4.4)[b,c]	ns[a], ns[b], ns[c]
	Diabetes	71 (28.2)[a,b]	40 (25.0)[a,c]	54 (33.8)[b,c]	ns[a], ns[b], ns[c]
	HIV	2 (0.8)[a,b]	3 (1.9)[a,c]	6 (3.8)[b,c]	ns[a], $p < 0.05$[b], ns[c]
	Hepatitis C	20 (7.9)[a,b]	11 (6.9)[a,c]	15 (9.4)[b,c]	ns[a], ns[b], ns[c]
	Hypertension	121 (48.0)[a,b]	82 (51.3)[a,c]	87 (54.4)[b,c]	ns[a], ns[b], ns[c]
	Obesity	44 (17.5)[a,b]	32 (20.0)[a,c]	22 (13.8)[b,c]	ns[a], ns[b], ns[c]
	End-stage renal disease	6 (2.4)[a,b]	9 (5.6)[a,c]	3 (1.9)[b,c]	ns[a], ns[b], ns[c]
Outpatient medications, *n* (%)	Antibiotics	30 (11.9)[a,b]	17 (10.6)[a,c]	20 (12.5)[b,c]	ns[a], ns[b], ns[c]
	Anti-inflammatory	79 (31.3)[a,b]	45 (28.1)[a,c]	55 (28.1)[b,c]	ns[a], ns[b], ns[c]
	Corticosteroids	18 (7.1)[a,b]	10 (6.3)[a,c]	13 (8.1)[b,c]	ns[a], ns[b], ns[c]
Adjudicated sepsis-3	Yes	28 (11.1)[a,b]	45 (28.1)[a,c]	79 (49.4)[b,c]	$p < 0.0001$[a], $p < 0.0001$[b], $p < 0.0001$[c]
Sepsis adjudication, *n* (%)	Unanimous	206 (81.8)[a,b]	120 (75.0)[a,c]	124 (77.5)[b,c]	ns[a], ns[b], ns[c]
	Consensus	44 (17.5)[a,b]	40 (25.0)[a,c]	36 (22.5)[b,c]	ns[a], ns[b], ns[c]
	Forced	2 (0.8)[a,b]	0 (0.0)[a,c]	0 (0.0)[b,c]	ns[a], ns[b], ns[c]
WBC (10^3 cells/μL), median (Q1–Q3)		8.9 (6.7–12.9)[a,b]	12.6 (9.4–15.7)[a,c]	15.7 (11.8–19.1)[b,c]	$p < 0.0001$[a], $p < 0.0001$[b], $p < 0.0001$[c]
Lactate measured, *n* (%)	Yes	93 (36.9)[a,b]	91 (56.9)[a,c]	110 (68.8)[b,c]	$p < 0.0001$[a], $p < 0.0001$[b], $p < 0.05$[c]
Lactate, median (Q1–Q3)		1.6 (1.2–2.6)[a,b]	1.6 (1.2–2.7)[a,c]	1.8 (1.3–2.6)[b,c]	ns[a], ns[b], ns[c]
IntelliSep Index, median (Q1–Q3)		4.0 (3.3–4.4)[a,b]	5.5 (5.2–5.9)[a,c]	7.4 (6.7–7.9)[b,c]	$p < 0.0001$[a], $p < 0.0001$[b], $p < 0.0001$[c]

ns = not significant, Q1–Q3 = interquartile range.

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 emergency department encounter. "Anti-inflammatory" outpatient medications included acetaminophen (Tylenol), celecoxib (Celebrex), ibuprofen (Advil), indomethacin (Indocin), and naproxen (Aleve). S1-Table 2 (http://links.lww.com/CCX/B289) contains the complete table of characteristics.

Sepsis Diagnosis

ISI values were higher in patients with sepsis (median 6.4; Q1–Q3, 5.3–7.5) than those without (median 4.8; Q1–Q3, 3.9–5.9, $p < 0.0001$). The prevalence of sepsis increased across ISI interpretation bands (**Fig. 1**), each having nonoverlapping 95% CIs (band 1, 11.1%, 95% CI, 7.5–15.7%; band 2, 28.1%, 95% CI, 21.3–35.8%; band 3, 49.4%, 95% CI, 41.4–57.4%). The negative predictive value for band 1 was 89.9% (95% CI, 84.3–92.5%) and the positive predictive value for band 3 was 49.4% (95% CI, 41.4–57.4%). The area under the ROC curve for discriminating adjudicated sepsis was 0.74 (95% CI, 0.69–0.78) (Fig. 1). For band 1 (vs. else) the positive percent agreement (sensitivity) was 81.6% (95% CI, 74.5–87.4%), and for band 3 (vs. else), the negative percent agreement (specificity) was 80.7% (95% CI, 73.0–86.3%).

Infection Sources and Organ Dysfunction

Adjudicators determined 286 patients (50%) had infection, with 117 (20.5%) having multiple sources (**S1-Tables 1** and **2**, http://links.lww.com/CCX/B289). Respiratory infections were most common (59.8%), followed by urinary (40.6%), gastrointestinal (31.5%), and skin (29.0%). The organ dysfunction criterion was met by 473 patients (82.7%), with respiratory (50%), cardiovascular (36.4%), and renal (35%) dysfunction most common. Both infection and organ dysfunction were present in 187 subjects (32.7%). In 152 subjects (81.3%), adjudicators determined the cause of organ dysfunction was a dysregulated host response to infection, resulting in a sepsis prevalence of 26.6%. The determination of sepsis was unanimous in 116 cases, consensus in 36 cases, and forced in two cases.

The prevalence of both infection and organ dysfunction increased across bands (**Fig. 2**), with infection present in 76 of 252 (30.2%) in band 1, 93 of 160 (58.1%) in band 2, and 117 of 160 (73.1%) in band 3. The prevalence of organ dysfunction increased from 139 of 252 (55.2%) to 100 of 160 (62.5%) and 122 of 160 (76.3%) in bands 1, 2, and 3, respectively. Both infection and organ dysfunction were present in 40 of 175 (22.3%) in band 1, 57 of 136 (41.9%) in band 2, and 90 of 149 (60.4%) in band 3. The causation criterion was more frequently met in band 3 (79 of 90, 87.8%) compared with band 2 (45 of 57, 78.9%) and band 1 (28 of 40, 70%). Figure 2 includes clinical outcomes for patients with adjudicated sepsis in each interpretation band. The study period correlated with the Delta surge of the COVID-19 pandemic (26). Details of severe acute respiratory syndrome coronavirus 2 testing and results are in S1-Tables 1 and 2 (http://links.lww.com/CCX/B289) and outcomes of this population are in **S1-Table 3** (http://links.lww.com/CCX/B289). Microorganisms recovered by various methods are detailed in **S1-Table 6** (http://links.lww.com/CCX/B289).

Severity of Illness and Mortality

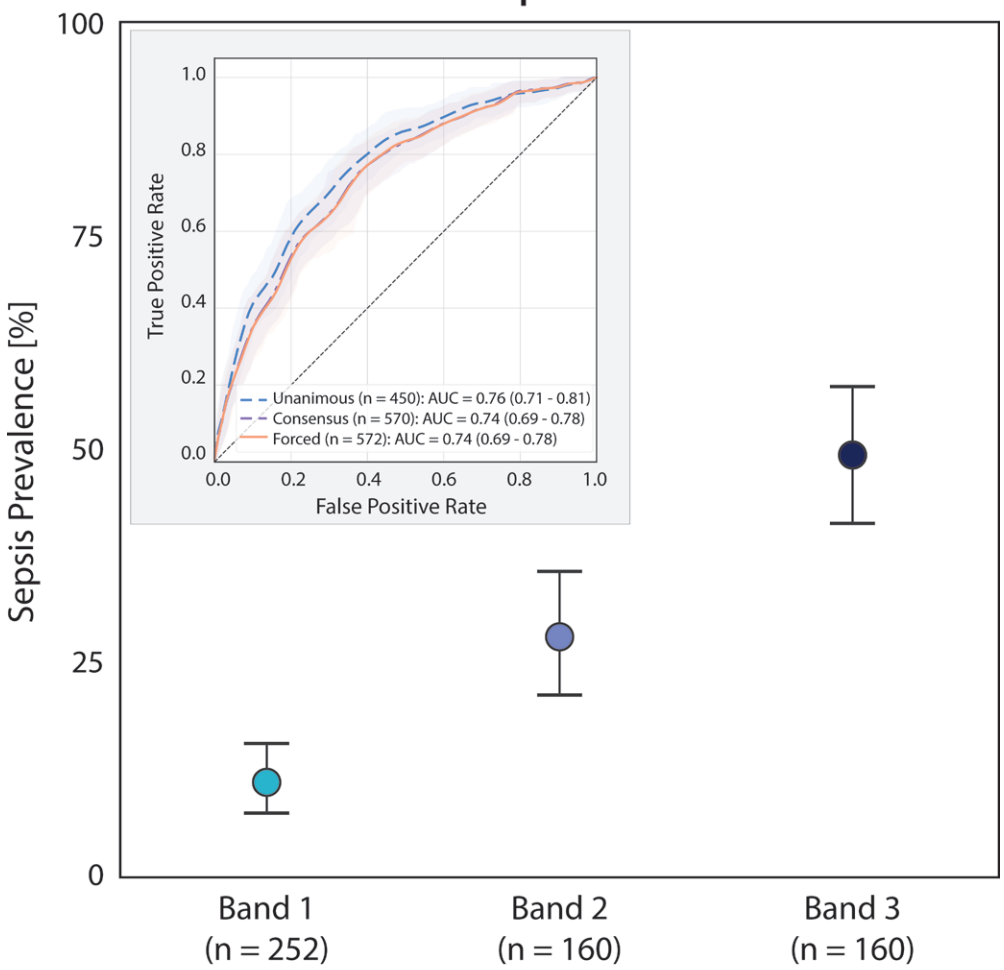
Band 3 had the highest severity of illness scores as assessed by both APACHE-II (calculated from data available during the first hospital day) and SOFA scores (maximum score over baseline in the first 3 d) (S1-Table 2, http://links.lww.com/CCX/B289). In total, 24 patients (4.2 %) died within 30 days of enrollment before hospital discharge, including 12 patients (1.6%) adjudicated to have sepsis. Sepsis-associated mortality increased across interpretation bands from 0.4% in band 1 to 2.5% in band 2 and 4.4% in band 3. All-cause and infection-associated in-hospital mortality had similar trends across bands (**Fig. 3**; S1-Table 2, http://links.lww.com/CCX/B289). The presumed cause of death for all patients with 30-day in-hospital mortality is provided in **S1-Table 4** (http://links.lww.com/CCX/B289).

Resource Utilization

Resource utilization increased across interpretation bands (S1-Table 2, http://links.lww.com/CCX/B289). A total of 360 patients (63.0%) were admitted to the hospital: 48.8% of patients with band 1 results, compared with 66.9% with band 2 ($p < 0.001$) and 81.3% with band 3 ($p < 0.01$ vs. band 2; $p < 0.0001$ vs. band 1). ICU admission increased across bands, with 7.9% of patients in band 1 admitted to the ICU compared with 14.4% in band 2 ($p < 0.05$) and 17.5% in band 3 ($p < 0.01$). In total, 25 of 289 patients (8.7%) who were admitted to a noncritical care unit required escalation of care within the first 3 hospital days, with 1% (1 of 103) in band 1, 17.9% (15 of 84) in band 2, and 8.8% (9 of 102) in band 3.

S1-Tables 1 and 2 (http://links.lww.com/CCX/B289) include selected sepsis-specific resource utilization (such as antibiotic administration and cultures ordered) by adjudicated diagnosis of sepsis and interpretation band, respectively. Also included are individual components of the Centers for Medicare and Medicaid

A



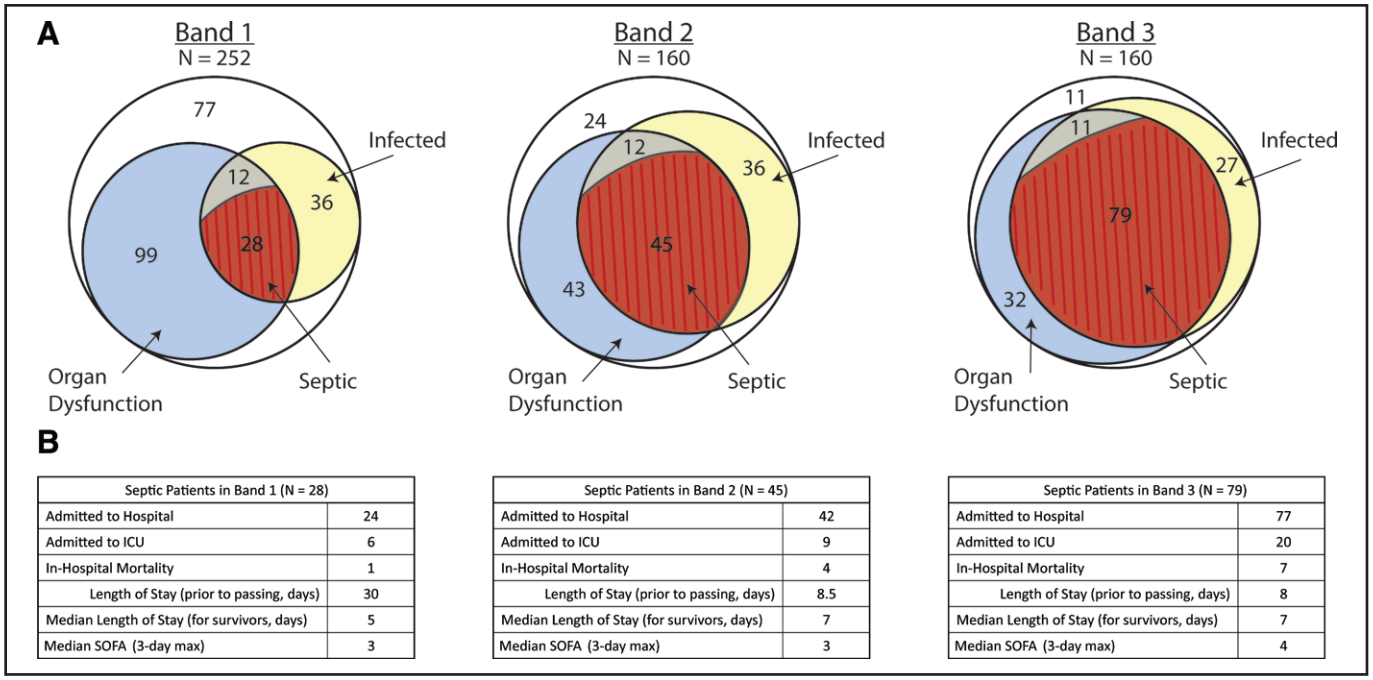
B

Positive Percent Agreement (Sensitivity): Band 1 vs. else	81.6% (74.5%, 87.4%)
Negative Percent Agreement (Specificity): Band 3 vs. else	80.7% (73.0%, 86.3%)

IntelliSep Result	Adjudicated +	Adjudicated -	Sepsis Prevalence (95% CI)
Band 1	28	224	11.1% (7.5%, 15.7%)
Band 2	45	115	28.1% (21.3%, 35.8%)
Band 3	79	81	49.4% (41.4%, 57.4%)
Total	152	420	26.6%

Figure 1. Selected performance characteristics of IntelliSep for the diagnosis of Sepsis, as defined by Sepsis-3 criteria. **A**, Performance of the IntelliSep Index (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 with respect to unanimous, consensus, and forced adjudication. **B**, Diagnostic test characteristics of the ISI as compared with the adjudicated endpoint of sepsis, as well as prevalence of the adjudicated endpoint of sepsis within each interpretation band. AUC = area under the receiver operating characteristic curve.

Services 3-hour Severe Sepsis and Septic Shock Management Bundle (antibiotics within 3 hr of presentation, cultures collected before antibiotics, and lactate measured). **S1-Table 5** (http://links.lww.com/CCX/B289) includes these measures in patients with no infection, infection (without sepsis), and sepsis. Providers prescribed antibiotics in 52.6% (301 of 572) of patients, including 44.1% (185 of 420) without sepsis and 76.3% (116 of 152) of patients with sepsis. Of the 161 (28.2% of the total population) who received antibiotics within 3 hours of presentation, 95 (59%) were not adjudicated to have sepsis. Observed trends in the prescription of antibiotics within 3 hours across ISI interpretation bands are presented in S1-Table 2 (http://links.lww.com/CCX/B289). The percentage of patients within each Interpretation Band that received antibiotics within 3 hours of presentation increases across interpretation bands (19.8% of band 1, 28.1% of band 2, and 41.3% of band 3; $p < 0.05$ vs. band 2; $p < 0.0001$ vs. band 1). Across interpretation bands, a similar number of patients received antibiotics within 3 hours of presentation.



B

Septic Patients in Band 1 (N = 28)	
Admitted to Hospital	24
Admitted to ICU	6
In-Hospital Mortality	1
Length of Stay (prior to passing, days)	30
Median Length of Stay (for survivors, days)	5
Median SOFA (3-day max)	3

Septic Patients in Band 2 (N = 45)	
Admitted to Hospital	42
Admitted to ICU	9
In-Hospital Mortality	4
Length of Stay (prior to passing, days)	8.5
Median Length of Stay (for survivors, days)	7
Median SOFA (3-day max)	3

Septic Patients in Band 3 (N = 79)	
Admitted to Hospital	77
Admitted to ICU	20
In-Hospital Mortality	7
Length of Stay (prior to passing, days)	8
Median Length of Stay (for survivors, days)	7
Median SOFA (3-day max)	4

Figure 2. The distribution and relationship of infection, organ dysfunction, and sepsis within each interpretation band and selected outcomes of patients with sepsis. **A**, 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. **B**, Selected outcomes in patients adjudicated as septic within each interpretation band. SOFA = Sequential Organ Failure Assessment.

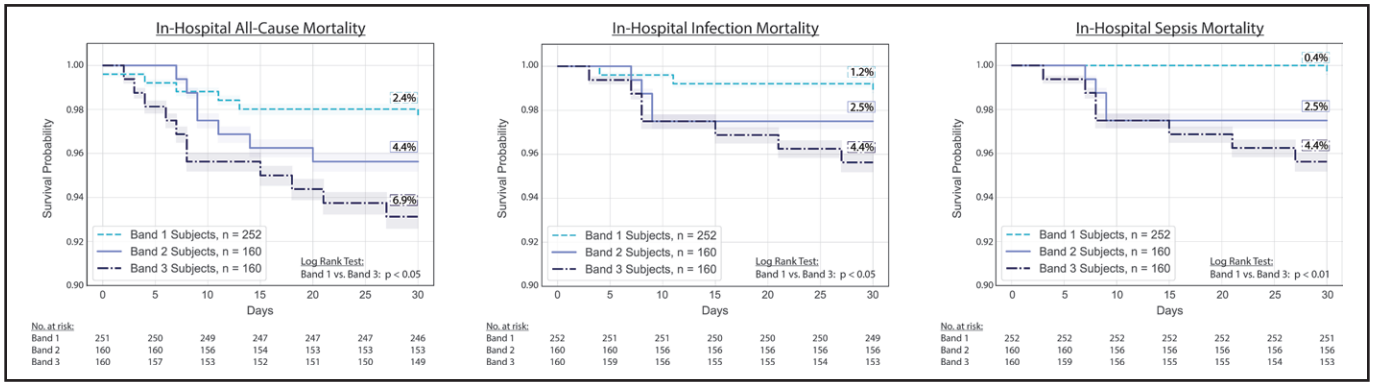


Figure 3. Probability of survival during the first 30 days of hospitalization for all patients enrolled, (**A**) all-cause in-hospital mortality, (**B**) in-hospital mortality with adjudication of infection, and (**C**) in-hospital mortality with adjudication of sepsis, stratified by IntelliSep Index (ISI) interpretation band. Plots were created with right-censoring, with the assumption that patients discharged from the emergency department (ED) or hospital survived ≥ 30 days in the absence of evidence to the contrary (e.g., return to the ED, discharged to hospice, or other indication in the electronic health record, which was reviewed after 30 d, that the patient had deceased). *Shading* depicts 95% CIs. At each timepoint, the number at risk, per ISI interpretation band, is noted below the figure panel. Presumed cause of death for all patients who suffered in-hospital mortality during the first 30 days of hospitalization is provided in Supplementary Material (http://links.lww.com/CCX/B289).

DISCUSSION

This study confirms preliminary findings (21) that the IntelliSep test rapidly stratifies patients presenting to the ED with signs of suspicion of infection from low (band 1) to high (band 3) probability of sepsis. The ISI also provides risk stratification for adverse outcomes, including the development or worsening of organ dysfunction, increased resource utilization, and in-hospital mortality. The results are broadly applicable, as they include a racially and physiologically diverse patient population and a variety of pathogens. This study also illustrates the difficulty facing ED providers in diagnosing and managing sepsis, as many patients without sepsis received appropriate sepsis care while many patients with sepsis did not. These findings support the need for a rapid, easily-interpreted diagnostic and risk-stratification tool for sepsis to help guide appropriate therapy.

A semiquantitative assessment of the host response, the ISI assesses the state of immune activation (27), not the presence or absence of infection, and these processes can occur independently. In the study population, the overall prevalence of infection was 50%. Although the prevalence of infection increased across ISI interpretation bands, more than 25% of patients with adjudicated infection fell within band 1, suggesting that these patients have infection without systemic activation and dysregulation of the immune response. Because a dysregulated host response is a major factor contributing to the adverse outcomes of sepsis, it follows that patients in band 1 would be at lower risk for the expected adverse outcomes associated with sepsis. Although infected patients in band 1 had fewer hospital-free days than noninfected patients in the same band, they had similar severity of illness, organ dysfunction, and need for ICU admission (**S1-Fig. 2**, http://links.lww.com/CCX/B289).

As noted in **Figure 2*A***, the prevalence of infection increases across interpretation bands, and the proportion of those with infection who were adjudicated to have sepsis also increases across interpretation bands. In total, 73.1% of patients in band 3 were adjudicated to have infection, with nearly two-thirds of these (49.4% of the total) adjudicated to have sepsis. As expected, infection-associated mortality increased across interpretation bands, with patients in band 2 having outcomes and clinical courses intermediate to those of bands 1 and 3, which may indicate a continuum of immune dysregulation across bands. Furthermore, a number of patients in band 1 had organ dysfunction, suggesting that an alternative etiology, rather than a dysregulated host response, is the cause of organ dysfunction in these patients. Importantly, the deaths in band 1 occurred in patients without sepsis, further supporting the possibility that an alternative etiology (as noted in S1-Table 4, http://links.lww.com/CCX/B289) is the mechanism of deterioration in these patients, even in those with infection. This finding underscores the potential importance of evaluating an alternative diagnosis for patients in band 1.

The ISI is pathogen agnostic test, providing rapid risk stratification independent of the pathogen, as suggested by the ISI's ability to risk stratify patients early in the COVID-19 pandemic (25). A list of potential pathogens identified through various diagnostic methods is presented in S1-Table 6 (http://links.lww.com/CCX/B289). If integrated into a process for decision support, the ISI may help identify a large population of patients—those with band 1 results—in whom pathogen identification (by serology, molecular diagnostics (28), or traditional cultures) may not be necessary, and in whom symptomatic or guideline-based therapy for specific infection (29–31) is sufficient. Such an approach may potentially reduce the laboratory and personnel costs associated with pathogen detection (32) as well as the costs associated with false positives and contaminated specimens.

On the other hand, because of the high rate of infection in band 3, the ISI also identifies a group of patients who warrant a rigorous investigation for infection. The correlation of the ISI with clinical outcomes, especially in those with infection, suggests that it may aid in defining the pathophysiology of the sepsis syndrome and in managing patients with signs or suspicion of infection. Clinicians fear that failure to act promptly on high-risk patients may lead to adverse, infection-associated outcomes; however, discerning high-risk from low-risk patients is difficult. Furthermore, excessive intervention consumes resources, exposes patients to the harm of treatment and admission, delays alternative diagnoses, and complicates antimicrobial stewardship. Patients in band 3 have high rates of blood culture positivity (40%); however, 25% did not have cultures ordered, suggesting that some patients in band 3 may have had undiagnosed bacteremia. The ISI has the potential to play an important role in aiding

clinicians in such difficult decisions as when to collect or defer blood cultures (33–35), admit patients, or administer broad-spectrum antibiotics independently or as part of bundled care. This study illustrates the potential impact of the ISI on clinical care, as many patients without sepsis who fell within the low-risk band 1 result received resource-intensive treatment necessary for the effective treatment of sepsis. For example, 23% (95 of 420) patients without sepsis received antibiotics within 3 hours, whereas 57% (86 of 152) patients with sepsis did not. These findings illustrate the potential of an ISI-based process to conserve resources or redirect them to high-risk patients who are more likely to benefit.

This study has several limitations. The purpose of the study was to assess the diagnostic and prognostic performance of the ISI, not to compare it to other available biomarkers or diagnostics. Clinicians were blinded to the result of the ISI, so we could not assess the influence that the ISI may have on sepsis management. Also, there is no reference standard for the diagnosis of sepsis and, despite a rigorous process for adjudication, the result is dependent upon subjective interpretation, which may lead to misreporting of the true performance of the ISI as a sepsis diagnostic. The coincidence of the enrollment period and the COVID-19 Delta surge may have impacted the adjudication process as well as influenced clinical outcomes such as mortality, which was lower than expected. Although we report several clinical outcomes, the study was not powered for their detection. Finally, there was no standardized treatment for enrolled patients, and variability among sites and clinicians in medical management complicated the adjudication process and assessment of clinical outcomes.

CONCLUSIONS

The ISI rapidly identifies patients with the underlying biology of sepsis, and it can serve as a tool for both diagnostic aid and risk stratification. This study identifies several potential opportunities for IntelliSep to improve the process of care for patients presenting to the ED with signs of suspicion of infection, including the timely delivery of care and improved resource utilization. Further studies are needed to assess the impact the ISI may have on sepsis management through rapid identification and informing decisions on care delivery.

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