OBSERVATIONAL STUDY

OPEN

Assessment of a Cellular Host Response Test as a Sepsis Diagnostic for Those With Suspected Infection in the Emergency Department

OBJECTIVES: Sepsis is a common cause of morbidity and mortality. A reliable, rapid, and early indicator can help improve efficiency of care and outcomes. To assess the IntelliSep test, a novel in vitro diagnostic that quantifies the state of immune activation by measuring the biophysical properties of leukocytes, as a rapid diagnostic for sepsis and a measure of severity of illness, as defined by Sequential Organ Failure Assessment and Acute Physiology and Chronic Health Evaluation-II scores and the need for hospitalization.

DESIGN, SETTING, SUBJECTS: Adult patients presenting to two emergency departments in Baton Rouge, LA, with signs of infection (two of four systemic inflammatory response syndrome criteria, with at least one being aberration of temperature or WBC count) or suspicion of infection (a clinician order for culture of a body fluid), were prospectively enrolled. Sepsis status, per Sepsis-3 criteria, was determined through a 3-tiered retrospective and blinded adjudication process consisting of objective review, site-level clinician review, and final determination by independent physician adjudicators.

MEASUREMENTS AND MAIN RESULTS: Of 266 patients in the final analysis, those with sepsis had higher IntelliSep Index (median = 6.9; interquartile range, 6.1–7.6) than those adjudicated as not septic (median = 4.7; interquartile range, 3.7–5.9; $p < 0.001$), with an area under the receiver operating characteristic curve of 0.89 and 0.83 when compared with unanimous and forced adjudication standards, respectively. Patients with higher IntelliSep Index had higher Sequential Organ Failure Assessment (3 [interquartile range, 1–5] vs 1 [interquartile range, 0–2]; $p < 0.001$) and Acute Physiology and Chronic Health Evaluation-II (7 [interquartile range, 3.5–11.5] vs 5 [interquartile range, 2–9]; $p < 0.05$) and were more likely to be admitted to the hospital (83.6% vs 48.3%; $p < 0.001$) compared with those with lower IntelliSep Index.

CONCLUSIONS: In patients presenting to the emergency department with signs or suspicion of infection, the IntelliSep Index is a promising tool for the rapid diagnosis and risk stratification for sepsis.

KEY WORDS: cellular viscoelastic properties; emergency department; immune dysregulation; leukocyte biomechanical properties; microfluidics; sepsis diagnosis

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Sepsis is a common emergency medical condition (1, 2), and delays in treatment are detrimental to patients and costly to healthcare systems (3–5). Most cases of sepsis arise in the community (6), so the initial diagnosis and treatment frequently occurs in the emergency department (ED) where time is short and information is limited. In addition, the definition of sepsis is dynamic, evolving from the 1992 consensus definition (7), to the current Sepsis-3 definition of "life-threatening organ dysfunction caused by a dysregulated host response to infection" (8). Despite the evolving definition, sepsis

remains a clinical syndrome, identified by a constellation of signs and symptoms, without a gold-standard test for diagnosis.

Infection by a pathogen and activation of the host innate immunity are fundamental processes of sepsis pathogenesis. During activation, the cells of innate immunity—monocytes and neutrophils—undergo biochemical and biophysical changes that allow them to fulfill their function (9, 10). Although progress has been made in the rapid identification of pathogens (11), there are limited options available for the quantification of the biophysical changes characteristic of the host response (12). Historically, the quantification of this response required laborious and time-consuming assays, which have only been assessed in the critically ill (13, 14). Our early work demonstrated that deformability cytometry that quantifies innate immune activation may be used to distinguish septic from nonseptic patients (15). More recently, we have expanded this work to develop the IntelliSep Index (ISI), a quantitative measure of the changes in cellular structure exhibited during the activation of the host immunity, which distinguishes sepsis from nonsepsis with an area under the curve of greater than or equal to 0.9 (16).

This study evaluates the utility of the ISI for diagnosing sepsis in the ED. We performed a prospective, observational study on a cohort of patients presenting to the ED with signs or suspicion of infection for assessment of the ISI as a diagnostic and prognostic marker for sepsis. We hypothesized that patients with a higher ISI would be more likely to have sepsis and associated increases in severity of illness.

METHODS

Study Population

Between April 22, 2019 and September 13, 2019, we enrolled 289 adult patients in a prospective, observational study at two hospitals in Baton Rouge, LA. Inclusion criteria were signs or suspicion of infection, with signs of infection defined as two or more modified (systemic inflammatory response syndrome [SIRS]) criteria (with at least one being aberration of temperature or WBC count) and suspicion of infection defined as a clinician order for culture of a body fluid (e.g., blood, urine, sputum). We excluded patients with an expected palliative course; history

of hematologic disorders, receipt of cytotoxic chemotherapy within 3 months of the ED encounter, prisoners, patients transferred from other acute care facilities, and patients unwilling or unable to consent. We included 266 subjects in the final analysis (**Fig. S1**, Supplemental Digital Content 1, http://links.lww.com/CCX/A678). The study protocol was approved by the Louisiana State University Health Sciences Center Institutional Review Board (IRB; LSUHSC-NO number 19-019) as well as by local, hospital-specific IRBs (Franciscan Missionaries of Our Lady University IRB: number 2019-012 and Baton Rouge General IRB: number 2018-017). The study team obtained written informed consent from all subjects.

ISI Performance and Results

Upon consent, coordinators obtained EDTA-anticoagulated peripheral blood samples for the IntelliSep test. A subset of patients consented for optional storage of a specimen for later measurement of alternative biomarkers, including procalcitonin. Clinical management proceeded via standard care. The IntelliSep test (Cytovale, San Francisco, CA) requires 100 microliters of whole blood (**Fig. S2**, Supplemental Digital Content 1, http://links.lww.com/CCX/A678). It assesses intracellular and nuclear changes that occur during leukocyte activation and provides a single score, the ISI, within 10 minutes. The ISI ranges from 0.1 to 10.0 (inclusive) and is stratified into three discrete interpretation bands of risk for sepsis: green (low risk, 0.1–5.4), yellow (intermediate risk, 5.5–6.7), and red (high risk, 6.8–10.0). The algorithm for calculating the ISI and limits for interpretation bands were derived from prior investigation (16).

Data Collection

Coordinators collected radiographic, historical, physical, and laboratory data for assignment of the presence of infection based on predefined criteria derived from the Centers for Disease Control and Prevention's National Healthcare Safety Network criteria (17) with minor modifications. As the purpose of these definitions is to define infections for surveillance, the definitions were modified to remove surveillance-specific language and be applicable to the ED setting. Coordinators also calculated baseline and daily

Sequential Organ Failure Assessment (SOFA) scores (18), for each of the first 3 days of hospitalization.

Confirmation of the Diagnosis

Because there is no reference standard for the diagnosis of sepsis (19), we relied on a structured adjudication process for determining the presence or absence of the diagnosis by Sepsis-3 criteria. Because of known clinician variability in the diagnosis of sepsis (20), each case underwent a rigorous, three-tiered process of adjudication: objective review (predetermined criteria for infection and SOFA calculations) and site-level clinical review were performed by on-site investigators. These data were compiled and electronically and transmitted to an external, independent adjudication committee for final determination using the Research Electronic Data Capture electronic data capturing tool (21). Study personnel in every tier of the process were blinded to the ISI results. The adjudication committee determined each case to be "Sepsis" or "Not sepsis" with "unanimous," "consensus," or "forced" determinations. Analyses of the adjudicated endpoint included each population. Details of the patients requiring a forced adjudication are in **Figure S3** (Supplemental Digital Content 1, http://links.lww.com/CCX/A678).

Statistical Analysis

Unless otherwise stated, p values are derived from an unpaired two-sample Welch's t test, where the null hypothesis is that the mean of the two samples is equal. Descriptive statistics are presented as means, SDs, medians, and interquartile ranges (Q1–Q3) for the 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. Finally, we constructed receiver operating characteristic (ROC) curves to illustrate the performance of the ISI for classification of patients as septic or not septic.

RESULTS

Table 1 contains the baseline characteristics of all 266 patients analyzed. The median age was 57 years (Q1–Q3, 40–72 yr), with 115 male (43.2%) and 151 female (56.8%). Of the total, 144 were White (54.1%), 115 were Black (43.2%), and seven were members of other races (2.6%). Regarding inclusion criteria, 109 (41%) were enrolled with the SIRS criteria, 110 (41.4%) with culture criteria, and 47 (17.6%) with both.

Adjudication of Sepsis

Adjudicators determined that 112 of the subjects (42.1%) had an infection, 119 (44.7%) had or developed at least one organ failure, and 55 (20.7%) had both infection and organ dysfunction. Of these 55 patients, adjudicators determined 48 (18.0%) met the Sepsis-3 standard of dysregulated immune response to infection as the cause of the organ dysfunction. Adjudicators reached unanimous conclusion in 215 patients (80.8%) and consensus conclusion in 40 of the patients for a total of 255 patients (95.9%) with either unanimous or consensus determination. The remaining 11 required a forced determination.

Characteristics of Patients with Sepsis

Characteristics of patients by adjudicated outcome are presented in **Table 2**. When compared with patients adjudicated as not septic, those with sepsis were older. There was no difference in gender or race. Patients with sepsis were more likely to have hypertension and/or autoimmune disease; however, there was no difference in the presence of diabetes, obesity, malignancy, or chronic kidney disease between those with sepsis and those without. In septic patients, the most common sources of infection, by objective criteria, were respiratory (25% of cases) and urinary (27.1% of cases).

ISI Values

Table 1 includes baseline data of patients by ISI interpretation band. The median ISI for all patients was 5.2. Using the prespecified cut offs (16), 143 (53.8%) were in the green band, 66 (24.8%) in the yellow, and 55 (20.7%) in the red. There were no significant differences in baseline demographics (age, sex, and race) or comorbidities across interpretation bands, except for hypertension, which was more common in the red band patients (40 [72.7%]) than in green band patients (72 [50.3%]; $p < 0.01$).

TABLE 1.

Baseline Characteristics of All Patients in Total as Well as All Patients Stratified by Interpretation Band

Characteristics		Total, $N = 266$	Green Band, $N = 143$	Yellow Band, $N = 68$	Red Band, $N = 55$	p
Age, median (Q1–Q3)		57 (40–72)	55 (38–72)	59 (41–69)	61 (47–77)	NS
Age (≥ 65), n (%)		103 (38.7)	51 (35.7)	25 (36.8)	27 (49.1)	NS
Gender (female), n (%)		151 (56.8)	86 (60.1)	38 (55.9)	27 (49.1)	NS
Race, n (%)	White	144 (54.1)	80 (55.9)	31 (45.6)	33 (60.0)	NS
	African American	115 (43.2)	60 (42.0)	33 (48.5)	22 (40.0)	
	Other	7 (2.6)	3 (2.1)	4 (5.9)	0 (0.0)	
Comorbidities, n (%)	Hypertension	155 (58.3)	72 (50.3)[a]	43 (63.2)	40 (72.7)[a]	< 0.01[a]
	Diabetes	72 (27.1)	36 (25.2)	20 (29.4)	16 (29.1)	NS
	Obesity (body mass index ≥ 40)	39 (14.7)	19 (13.3)	13 (19.1)	7 (12.7)	NS
	Cancer	37 (13.9)	18 (12.6)	11 (16.2)	8 (14.5)	NS
	Chronic kidney disease	31 (11.7)	15 (10.5)	9 (13.2)	7 (12.7)	NS
	Autoimmune disease	13 (4.9)	5 (3.5)	4 (5.9)	4 (7.3)	NS
Infected—meeting objective criteria by organ system, n (%)	Respiratory	30 (11.3)	9 (6.3)[a]	9 (13.2)	12 (21.8)[a]	< 0.05[a]
	Gastrointestinal	16 (6.0)	4 (2.8)	7 (10.3)	5 (9.1)	NS
	Urinary	34 (12.8)	18 (12.6)	5 (7.4)[a]	11 (20.0)[a]	< 0.05[a]
	Skin	24 (9.0)	8 (5.6)	10 (14.7)	6 (10.9)	NS
	Other	12 (4.5)	1 (0.7)[a,b]	5 (7.3)[b]	6 (10.9)[a]	< 0.05[a]; < 0.05[b]
	Not Infected	161 (60.5)	105 (73.4)[a,b]	34 (50.0)[a]	22 (40.0)[b]	< 0.01[a]; < 0.001[b]
Infected by adjudication, n (%)		112 (42.1)	38 (26.6)[a,b]	37 (54.4)[a]	37 (67.3)[b]	< 0.001[a]; < 0.001[b]
Organ dysfunction by adjudication, n (%)		119 (44.7)	53 (37.1)[a]	32 (47.1)	34 (61.8)[a]	< 0.01[a]
Infected with organ dysfunction by adjudication, n (%)		55 (20.7)	11 (7.7)[a,b]	19 (27.9)[a,b]	25 (45.5)[b,c]	< 0.001[a]; < 0.001[b]; < 0.05[c]
Septic by Sepsis-3 definition, n (%)		48 (18.0)	6 (4.2)[a,b]	17 (25.0)[a,c]	25 (45.5)[b,c]	< 0.001[a]; < 0.001[b]; < 0.05[c]
Adjudication, n (%)	Unanimous	215 (80.8)	121 (84.6)	50 (73.5)	44 (80.0)	NS
	Consensus	40 (15.0)	16 (11.1)	16 (23.5)	8 (14.5)	NS
	Forced	11 (4.1)	6 (4.2)	2 (2.9)	3 (5.5)	NS
Blood culture, n (%)	Number tested	113 (42.5)	37 (25.9)[a,b]	33 (48.5)[a,c]	43 (78.2)[b,c]	< 0.01[a]; < 0.001[b]; < 0.001[c]
	Number positive (of tested)	24 (21.2)	4 (10.8)[a,b]	8 (24.2)[a]	12 (27.9)[b]	< 0.05[a]; < 0.01[b]
Lactate measured, n (%)		126 (47.4)	37 (25.9)[b,c]	44 (64.7)[a,b]	45 (81.8)[a,c]	< 0.05[a]; < 0.001[b]; < 0.001[c]
Lactate, median (Q1–Q3)		1.5 (1.1–2.4)	1.3 (1.0–1.9)[a]	1.5 (1.1–2.5)	1.9 (1.3–3.0)[a]	< 0.05[a]
Admitted to hospital, n (%)		163 (61.3)	69 (48.3)[b,c]	48 (70.6)[b]	46 (83.6)[c]	< 0.01[b]; < 0.001[c]

(*Continued*)

TABLE 1. (*Continued*).
Baseline Characteristics of All Patients in Total as Well as All Patients Stratified by Interpretation Band

Characteristics		Total, $N = 266$	Green Band, $N = 143$	Yellow Band, $N = 68$	Red Band, $N = 55$	p
Admitted to ICU, n (%)		14 (5.3)	2 (1.4)[a]	5 (7.4)	7 (12.7)[a]	< 0.05[a]
Hospital-free days (admitted subjects), median (Q1−Q3)		25 (22−26)	25 (22−26)[a]	25 (23−26)	24 (21−25)[a]	< 0.05[a]
In-hospital mortality, n (%)		6 (2.3)	1 (0.7)	1 (1.5)	4 (7.3)	NS
Sequential Organ Failure Assessment, 3 d maximum, median (Q1−Q3)		2 (0−3)	1 (0−2)[a,c]	2 (1−3)[a,b]	3 (1−5)[b,c]	< 0.05[a]; < 0.01[b]; < 0.001[c]
Antibiotics Administered in the emergency department, n (%)	Antipseudomonal (aztreonam, cefepime, levofloxacin, meropenem, piperacillin/tazobactam)	54 (20.3)	13 (9.1)[a,b]	19 (27.9)[a]	22 (40.0)[b]	< 0.01[a]; < 0.001[b]
	Anti-MRSA (daptomycin, vancomycin, linezolid)	40 (15.0)	7 (4.9)[a,b]	18 (26.5)[a]	15 (27.3)[b]	< 0.001[a]; < 0.001[b]
	Other (excluding antipseudomonal and anti-MRSA)	59 (22.2)	31 (21.7)	14 (20.6)	14 (25.5)	NS
Acute Physiology and Chronic Health Evaluation-II, median (Q1−Q3)		5 (3−10)	5 (2−9)[a]	6.5 (3−10)	7 (3.5−11.5)[a]	< 0.05[a]
WBC (10^3 cells/μL), median (Q1−Q3)		12.1 (7.5−15.2)	9.0 (6.2−12.9)[b,c]	13.3 (9.0−17.2)[b]	15.3 (11.2−18.8)[b,c]	< 0.001[b]; < 0.001[c]
Platelets (10^3 cells/μL), median (Q1−Q3)		249 (192−313)	251 (196−311)[a]	275 (211−354)[b]	215 (155−270)[a,b]	< 0.05[a]; < 0.01[b]
Creatinine (mg/dL), median (Q1−Q3)		0.97 (0.8−1.5)	0.92 (0.8−1.3)	1.00 (0.8−1.4)	1.16 (0.8−1.9)	NS
Triage temperature (F), median (Q1−Q3)		98.3 (97.9−98.8)	98.1 (97.8−98.4)[a,b]	98.5 (98.0−99.6)[a]	98.9 (98.0−100.1)[b]	< 0.001[a]; < 0.001[b]
IntelliSep index, median (Q1−Q3)		5.2 (4.0−6.5)	4.1 (3.1−4.7)[a,b]	6.0 (5.7−6.4)[a,c]	7.4 (7.2−8.1)[b,c]	< 0.05[a]; < 0.01[b]; < 0.001[c]

MRSA = methicillin-resistant *Staphylococcus aureus*, NS = not significant, Q1−Q3 = interquartile range.

p values were obtained from an unpaired two-sample Welch's *t* test (except for hospital-free days, where the Mann-–Whitney *U* was due to the nonnormal distribution), with the null hypothesis that the mean of the two samples are equal.

Sepsis-3 Diagnosis

Patients adjudicated to meet Sepsis-3 criteria had significantly higher ISI values (median = 6.9; 95% CI, 6.5–7.3) than those adjudicated as not septic (median = 4.7; 95% CI, 4.5–4.9; *p* < 0.001) (Table 2). We assessed the capacity of the ISI to differentiate patients with signs or suspicion of infection who met Sepsis-3 criteria from those who did not through ROC curve analysis. Test performance was impacted by confidence in the adjudicated Sepsis-3 label: when adjudication was unanimous, the area under the ROC curve (AUC) for the ISI was 0.89, whereas inclusion of consensus and forced cases resulted in an AUC for the ISI was 0.84 and 0.83, respectively (**Fig. 1A**). Of the entire 266 patients, 48 patients met Sepsis-3 criteria with six of these (12.5%) in the green band, 17 (35.4%) in yellow, and 25 (52.1%) in red (Table 1). These findings result in a 4.2% probability of Sepsis-3 in the green band, 25% probability of Sepsis-3 in the yellow band, and 45.5% probability of Sepsis-3 in the red band (**Fig. 1B**). Patients in the red band, in addition to having a higher

TABLE 2.
Characteristics of Patients by Adjudication Result

Characteristics		Sepsis-3 Definition		
		Sepsis, N = 48	No Sepsis, N = 218	p
Age, median (Q1–Q3)		67 (54–77)	55 (39–71)	< 0.001
Age (≥ 65), n (%)		27 (56.2)	76 (34.9)	0.01
Gender (female), n (%)		26 (54.2)	125 (57.3)	NS
Race, n (%)	White	29 (60.4)	115 (52.8)	N
	African American	19 (39.6)	96 (44.0)	
	Other	0 (0.0)	7 (3.2)	
Comorbidities, n (%)	Hypertension	36 (75.0)	119 (54.6)	0.01
	Diabetes	15 (31.3)	57 (26.1)	NS
	Obesity (body mass index ≥ 40)	8 (16.7)	31 (14.2)	NS
	Cancer	8 (16.7)	29 (13.3)	NS
	Chronic kidney disease	9 (18.8)	22 (10.1)	NS
	Autoimmune disease	7 (14.6)	6 (2.8)	0.05
Infected–meeting objective criteria by organ system, n (%)	Respiratory	12 (25.0)	18 (8.3)	0.05
	Gastrointestinal	8 (16.7)	8 (3.7)	0.05
	Urinary	13 (27.1)	21 (9.6)	0.05
	Skin	8 (16.7)	16 (7.3)	NS
	Other	7 (14.6)	5 (2.3)	0.05
	Not infected	7 (14.6)	154 (70.6)	0.001
Infected by adjudication, n (%)		48 (100.0)	64 (29.4)	0.001
Organ dysfunction by adjudication, n (%)		48 (100.0)	71 (32.6)	0.001
Infected with organ dysfunction by adjudication, n (%)		48 (100.0)	7 (3.2)	0.001
Adjudication, n (%)	Unanimous	31 (64.6)	184 (84.4)	0.01
	Consensus	12 (25.0)	28 (12.8)	NS
	Forced	5 (10.4)	6 (2.8)	NS
Blood culture, n (%)	Number tested	39 (81.3)	74 (33.9)	0.001
	Number positive (of tested)	16 (33.3)	8 (3.7)	0.001
Lactate measured, n (%)		41 (85.4)	85 (39.0)	0.001
Lactate, median (Q1–Q3)		1.90 (1.25–3.05)	1.50 (1.00–2.25)	NS
Admitted to hospital, n (%)		46 (95.8)	117 (53.7)	0.001
Admitted to ICU, n (%)		8 (16.7)	6 (2.8)	0.05
Hospital-free days (admitted subjects), median (Q1–Q3)		22 (20–25)	25 (23–26)	0.001
In-hospital mortality, n (%)		3 (6.3)	3 (1.4)	NS
SOFA, 3 d maximum, median (Q1–Q3)		3 (2–5)	1 (0–2)	0.001

(*Continued*)

TABLE 2. (*Continued*).
Characteristics of Patients by Adjudication Result

Characteristics		Sepsis-3 Definition		
		Sepsis, N = 48	No Sepsis, N = 218	p
Maximum SOFA change from enrollment for those admitted, median (Q1−Q3)		0 (−1.0 to 1.0)	0 (−2.0 to 1.0)	NS
Antibiotics Administered in the emergency department, n (%)	Antipseudomonal (aztreonam, cefepime, levofloxacin, meropenem, piperacillin/tazobactam)	25 (52.1)	29 (13.3)	0.001
	Anti-MRSA (daptomycin, vancomycin, linezolid)	17 (35.4)	23 (10.6)	0.01
	Other (excluding antipseudomonal and anti-MRSA)	15 (13.6)	44 (20.2)	NS
Acute Physiology and Chronic Health Evaluation-II, median (Q−Q3)		9 (6−12)	5 (2−9)	0.001
WBC (10^3 cells/μL), median (Q1−Q3)		15.3 (8.5−17.6)	11.2 (7.4−14.1)	0.01
Platelets (10^3 cells/μL), median (Q1−Q3)		213 (150−280)	255 (196−319)	0.05
Creatinine (mg/dL), median (Q1−Q3)		1.42 (0.86−2.10)	0.92 (0.75−1.26)	NS
Triage temperature (F), median (Q1−Q3)		98.6 (97.8−100.4)	98.3 (97.9−98.7)	0.05
IntelliSep index, median (Q1−Q3)		6.9 (6.1−7.6)	4.7 (3.7−5.9)	0.001

MRSA = methicillin-resistant *Staphylococcus aureus*, NS = not significant, Q1−Q3 = interquartile range, SOFA = Sequential Organ Failure Assessment.

p values were obtained from an unpaired two-sample Welch's *t* test (except for hospital-free days, where the Mann-Whitney *U* was due to the nonnormal distribution), with the null hypothesis that the mean of the two samples are equal.

probability of sepsis, had higher probabilities of both infection and organ dysfunction (**Fig. 2***A*). **Figure 2***B* delineates clinical outcomes of patients adjudicated as septic in each interpretation band.

Severity of Illness Prognostication

Independent of adjudicated endpoints, red band patients were more likely to have higher severity of illness scores, to be admitted to the hospital, and to have adverse outcomes (Table 1). As depicted in **Figure S4***A* (Supplemental Digital Content 1, http://links.lww.com/CCX/A678), the 3-day maximum SOFA score increased with interpretation bands: 1 (Q1−Q3, 0−2) for green band patients, 2 (Q1−Q3, 1−3) for yellow band patients, and 3 (Q1−Q3, 1−5) for red band patients. Also, patients in the red band were more likely to have an increase in 3-day SOFA scores as compared to those in green (**Fig. S4***B*, Supplemental Digital Content 1, http://links.lww.com/CCX/A678). Acute Physiology and Chronic Health Evaluation-II scores had similar increases across interpretation bands, with a median of 5 (Q1−Q3, 2−9) for greens band patients, 6.5 (Q1−Q3, 3−10) for yellow, and 7 (Q1−Q3, 3.5−11.5) for red

(Table 1) (**Fig. S4***C*, Supplemental Digital Content 1, http://links.lww.com/CCX/A678).

Hospitalization and Antibiotic Use

Sixty-nine green band patients (48.3%) required hospital admission, whereas 48 yellow band patients (70.6%) and 46 red band patients (83.6%) required admission (Table 1) (**Fig. S4***E*, Supplemental Digital Content 1, http://links.lww.com/CCX/A678). Red band patients were more likely to require ICU admission (7; 12.7%), as opposed to patients in the green band (2; 1.4%; *p* < 0.05) (Table 1) (**Fig. S4***F*, Supplemental Digital Content 1, http://links.lww.com/CCX/A678). Once admitted, green band patients were more likely to have a higher number of hospital-free days than those in the red band (Table 1) (**Fig. S4***G*, Supplemental Digital Content 1, http://links.lww.com/CCX/A678). Red band patients were more likely to have blood cultures ordered and returned positive and more likely to receive antibiotics (Fig. S4, Supplemental Digital Content 1, http://links.lww.com/CCX/A678). Furthermore, the ISI effectively risk stratified patients adjudicated as having infection, independent of being adjudicated as having sepsis.
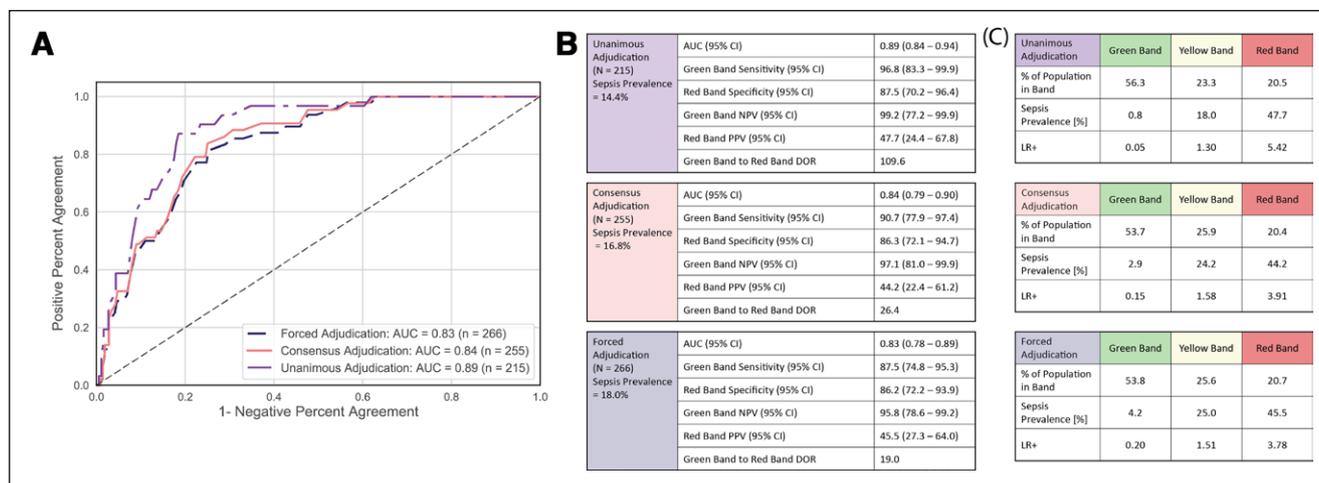
Figure 1. Diagnostic performance of the IntelliSep Index (ISI) in the differentiating of patients with sepsis as defined by the Sepsis-3 standard. **A**, Receiving operating characteristic curve for the ISI for various levels of confidence in the adjudicated endpoint. **B**, Diagnostic performance, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each level of adjudication (unanimous, consensus, and forced). **C**, Distribution of patients and prevalence of sepsis, as well as likelihood ratio for sepsis, within each band, stratified by level of adjudication. AUC = area under the curve, DOR = diagnostic odds ratio.

As noted above, patients in the red band were more likely to have infection, independent of being adjudicated as having sepsis. In total, 112 patients were adjudicated as having an infection, with 38 in the green band (26.6% of green band patients), 37 in the yellow band (54.4% of yellow band patients), and 37 in the red band (67.3% of red band patients). Among those adjudicated as infected, those in the red band had higher SOFA scores, were more likely to be admitted to the hospital and the ICU, have longer hospital length of stay, and were more likely to have positive blood cultures (**Fig. S5**, Supplemental Digital Content 1, http://links.lww.com/CCX/A678).

Comparison With Procalcitonin and Common Assessments of Organ Dysfunction

Of the 266 total patients enrolled, 186 (71.4%) consented for specimen storage for comparison of the ISI to alternative biomarkers including procalcitonin. In this subgroup, 33 (17.4%) were adjudicated as septic. **Figure S6** (Supplemental Digital Content 1, http://links.lww.com/CCX/A678) includes a comparison of the ISI and procalcitonin performance as depicted by ROC curves. Because there are no widely accepted cut off values for procalcitonin in the diagnosis of sepsis in the ED, in this analysis, both the ISI and procalcitonin were considered as continuous variables. The procalcitonin AUC was 0.79 as opposed to the ISI AUC of 0.84, for the diagnosis

of sepsis by Sepsis-3 criteria. **Table S1** (Supplemental Digital Content 1, http://links.lww.com/CCX/A678) includes a comparison of the ISI with a selection of other commonly assessed indicators of sepsis (22.

DISCUSSION

In this study of patients presenting to the ED with signs or suspicion of infection, we found the ISI, a quantification of innate immune activation, to be a reliable diagnostic indicator for Sepsis-3 (8, 23). Also, the ISI can assist in the risk stratification of these patients, as it correlates with severity of illness scores, the need for hospitalization, and ICU care. The ISI's rapid turnaround time (< 10 min) and collection in a standard, "purple-top" tube make it a potentially valuable tool for assisting ED clinicians in the efficient and effective identification and risk stratification of sepsis.

Achieving best outcomes in critical sepsis patients requires rapid identification and intervention with both appropriate antibiotic therapy (3, 5, 24) and effective cardiopulmonary support. Unfortunately, recognizing sepsis can be challenging in the ED (25), where these undifferentiated patients often present with a paucity of data. The Surviving Sepsis Campaign (26) and Centers for Medicare & Medicaid Services sepsis core measure were designed to help guide and improve sepsis care; however, this guidance may encourage clinicians to use broad-spectrum antibiotics and resource-intensive
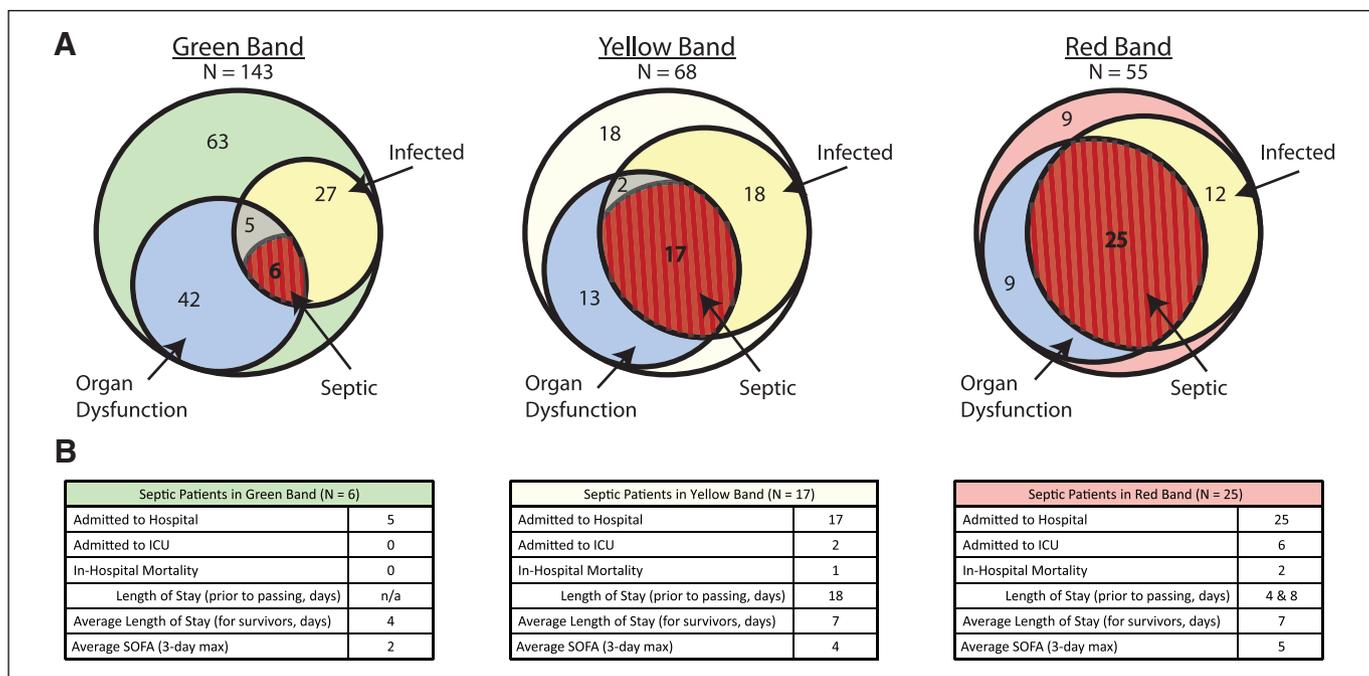
A

Figure 2. Clinical characteristics of patients within each interpretation band. **A**, Graphical representation of patients within each interpretation band for each component of sepsis: infection, organ dysfunction, and causation by dysregulated immunity (i.e., sepsis). **B**, Clinical metrics for severity of illness for those adjudicated as septic, including admission status, mortality, length of stay, and 3 d maximum (max) Sequential Organ Failure Assessment (SOFA) score.

interventions in patients with low acuity, a population in whom these measures are unlikely beneficial and may be potentially harmful (27–32). The identification of a low-risk population that clinicians can safely treat more judiciously is equally as important as the timely identification of high-risk patients who require the recommended, aggressive interventions (29).

With negative predictive values of 97% for Sepsis-3 in green band patients, the ISI can identify, early in the course of an ED visit, a subset of patients with signs or suspicion of infection in whom sepsis is unlikely, and the risk of adverse outcomes due to infection is low. This information can facilitate antibiotic stewardship and reduce the consumption of valuable resources. For example, green band patients had a low probability of positive blood cultures; however, one in three of these patients who received antibiotics received an antipseudomonal and/or anti–methicillin-resistant *Staphylococcus aureus* agent (**Fig. S4D**, Supplemental Digital Content 1, http://links.lww.com/CCX/A678 and **Fig. S4H**, Supplemental Digital Content 1, http://links.lww.com/CCX/A678). Green band may signal clinicians to approach these patients more deliberately and conservatively, encouraging evaluation for alternative etiologies prior to the initiation of sepsis-specific

therapy and supporting the use of narrower-spectrum antimicrobials. Furthermore, once admitted, the length of stay was lower for those in the green band (Fig. S4 *E–G*, Supplemental Digital Content 1, http://links.lww.com/CCX/A678), further indicating a lower severity of illness for this population, a finding which may support clinicians in the decision to discharge low-risk patients when the clinical course is uncertain.

In addition to identifying a low-risk subset of patients, the ISI also identifies a subset of patients with a markedly higher probability of sepsis, elevated severity of illness, and the associated risk of adverse outcomes. Over two thirds of patients in the red band were infected, as opposed to nearly one in four of green band patients. As opposed to those in the green band who often had localized infection, infected patients in the red band were more often systemically ill, as they had substantially higher severity of illness scores, received more antibiotics, and required higher rates of admission to the hospital and ICU (Fig. S5, Supplemental Digital Content 1, http://links.lww.com/CCX/A678). These findings indicate that red band patients, independent of adjudicated outcome, are more likely to have a severe infection that may benefit from aggressive, early interventions as defined by

current guidelines (26). Red band patients who are infected but not adjudicated as septic may indicate patients with systemic activation or dysregulation of the immune system who have not yet developed manifestations of organ failure, because many clinical and laboratory indicators lag behind the causative biologic processes (33–35). Red band patients who are not infected may have alternate causes of dysregulated immunity, such as severe exacerbations of autoimmune or other inflammatory disease. In patients with infection, a leading indicator of organ dysfunction caused by dysregulated immunity, such as the ISI, may allow clinicians to act on the basis of the dysregulated immunity itself, prior to clinically evident organ damage, potentially reducing sepsis-related morbidity for these patients.

In this study, our preliminary work also shows that the ISI compares favorably with procalcitonin as well as other biomarkers and more complex scoring systems for the risk stratification of patients with infection. The AUC for the ISI is at least as good as that of procalcitonin; however, the small sample size makes definitive comparison difficult. Additionally, there are no accepted cut off values for procalcitonin in the diagnosis of sepsis in the ED, which makes a direct comparison of positive and negative percent agreement with the adjudicated standard difficult. Despite the small sample size of our study, our assessment of the performance of procalcitonin is similar to other studies evaluating its performance for similar application (36, 37), and the performance of the ISI is also similar to our previous findings (16).

Our study has significant limitations. Although performed in two hospitals with different ED and inpatient medical staffs, both are in the same city. Also, despite enrolling a significant number of White and African-American patients, other races were not well represented. Furthermore, the prevalence of the primary endpoint (18%) of Sepsis-3 was relatively low in the study population, as was the acuity; however, we feel this reflects the daily reality of the ED setting and the current state of sepsis management in an undifferentiated patient population.

Finally, no reference standard exists for the diagnosis of sepsis, so we relied rigorous and structured adjudication processes with the flexibility of determining sepsis by either of the currently accepted definitions. Despite these efforts, the diagnosis of sepsis remains subjective (38). Similar to previous observations (14), confidence in the adjudicated endpoint impacts the perceived performance of the diagnostic test and may define upper (unanimous) and lower (forced) performance boundaries for the assay given our current understanding of the condition.

CONCLUSIONS

The ISI provides a sensitive indicator of host immune activation, a fundamental process in the development of sepsis. In this study of a rapid diagnostic for sepsis on a specimen collected within hours of ED presentation, the green, yellow, and red bands of the ISI correlate with retrospective adjudication of sepsis, Severity Of Illness scores, and clinical metrics. This tool may enable ED physicians to focus resources upon the most critical patients while treating less severe patients more conservatively. In addition, the test may help to define populations of patients with innate immune activation for future research and the administration of therapeutics. Further investigation will evaluate the role that quantification of host response and the ISI will play in clinical practice.

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