Submission Guidelines:

- Up to 2 figures or tables
- Abstract limit 3000 characters, not including spaces

Title: PROGNOSTIC ABILITY OF CELLULAR HOST RESPONSE TEST IN DIAGNOSIS AND RISK STRATIFICATION OF PATIENTS WITH SUSPECTED INFECTION IN NEED OF ICU-LEVEL CARE

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Background:

Sepsis, a dysregulated host immune response to infection leading to life-threatening organ dysfunction[1], is a common, fast-moving condition, and the leading cause of in-hospital death. Most cases develop in the community and present to Emergency Departments (ED)[2], where urgent action is required to prevent resultant morbidity and mortality[3]. However, the presentation of sepsis is nonspecific, making the diagnosis difficult, especially in early stages when intervention is most efficacious. One readily available stratification method that has been gaining traction is ICU level of care (ICU LOC). In this study, we assessed the risk-stratification value of ICU LOC compared to a cellular host-response test (HR) in a population of suspected infection patients.

Methods:

A semi-quantitative in-vitro HR test uses deformability cytometry to assess leukocyte biophysical properties from whole blood in <10 min. The test generates an Index (0.1 - 10.0), stratified into 3 interpretation bands (Band 1, Band 2, Band 3) of increasing sepsis likelihood[4].

Adult patients presenting to the ED with signs or suspicion of infection were prospectively enrolled at multiple US sites (02/2016–10/2021). EDTA-anticoagulated blood was assayed within 5 hours from draw using the test, and patients were followed by retrospective chart review. Infection and sepsis status were determined via retrospective physician adjudication per Sepsis-3 criteria. Necessity for ICU care (ICU -/+) was determined through objective cardiovascular (MAP < 60 or SOFA-CV ≥2), respiratory (PF ratio < 200), or renal ([Creatinine > 5 and (HCO_3^- < 12 or K^+ > 6)] or BUN > 120) criteria within 3-days of presentation. Missing data was not imputed.

Results:

We analyzed a cohort of 730 patients (sepsis prevalence 19.2%) and found 605 patients (82.9% of total) did not meet necessity for ICU level of care (ICU-) and 125 (17.1%) met at least one criteria (ICU+, Table 1). The ICU- cohort consisted of 351 (58.0%) Band 1, 181 (23.0%) Band 2, and 115 (19.0%) Band 3 patients, while the ICU+ cohort consisted of 36(28.8%) Band 1, 38 (30.4%) Band 2, and 51 (40.8%) Band 3. Across all interpretation bands, the most common

criteria for ICU+ patients was respiratory (49.0%), followed by cardiovascular (45.3%) and renal (5.7%), with 19.5% of patients meeting multiple criteria.

The majority of patients retrospectively adjudicated septic were in the ICU- group (N=66, 57.4%). Furthermore, 30% of sepsis-associated mortalities were observed in this ICU- group (Table 1). Regardless of ICU care necessity, the HR test achieved appropriate diagnostic performance and risk stratification, with increasing rates of sepsis-3, and sepsis-associated mortality across increasing interpretation bands (Fig 1 A,B). Notably, there were no Band 1 patients with sepsis-associated mortality in either of the ICU- or ICU+ cohorts (Fig 1 B). Finally, the diagnostic performance of the HR test for risk of sepsis was observed to be similar irrespective of the need for ICU level of care (ICU- : Sensitivity – 95.5% [87.1-99.1%], Specificity: 88.5% [77.5-94.6%]; ICU+: Sensitivity – 91.8% [80.4-97.7%], Specificity: 73.7% [58.9-85.1%]).

Conclusions:

The results of this study suggest both that ICU level of care is an inappropriate metric for the identification of sepsis and it's severity and that a host response test for early sepsis detection and risk stratification in the ED may provide a more specific solution.

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Figures/ Tables:

Table 1. Rates of ICU-/ICU+ care levels across host response interpretation bands and sepsis status.

	ICU -		**ICU +**		
	Non-Septic	**Septic**	**Non-Septic**	**Septic**	**Total**
Band 1	348 (89.9%)	3 (0.8%)	33 (8.5%)	3 (0.8%)	387 (100.0%)
Band 2	122 (68.9%)	17 (9.6%)	23 (13.0%)	15 (8.5%)	177 (100.0%)
Band 3	69 (41.6%)	46 (27.7%)	20 (12.0%)	31 (18.7%)	166 (100.0%)

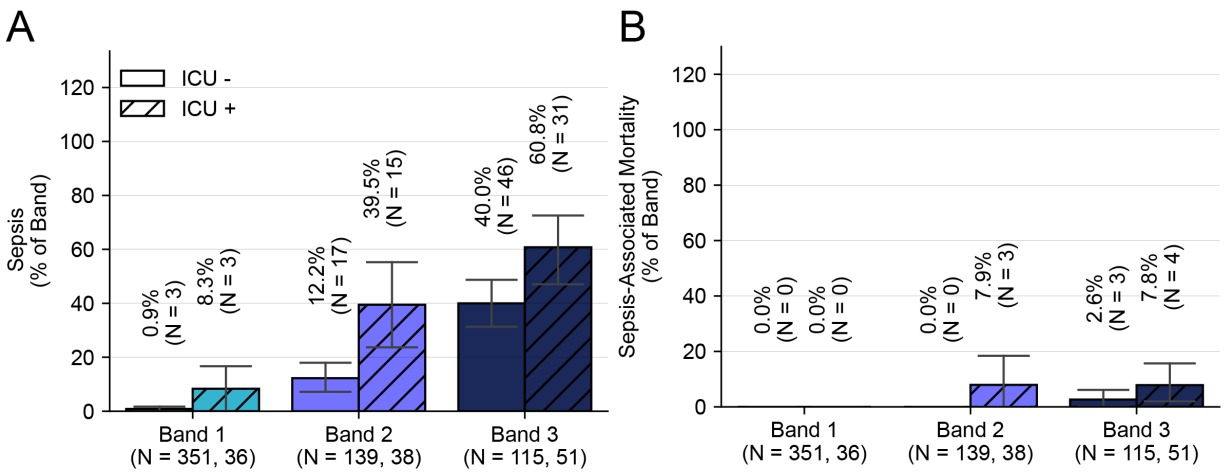

Figure 1 (A) and (B) rates of sepsis-associated moralities across ICU care need and interpretation bands.

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Introduction

Sepsis, a dysregulated host immune response to infection leading to life-threatening organ dysfunction[1], is a common, fast-moving condition, and the leading cause of in-hospital death. The majority of cases develop in the community and present to Emergency Departments (ED)[2], where urgent action is required to prevent resultant morbidity and mortality[3]. However, the presentation of sepsis is nonspecific, making the diagnosis difficult, especially in early stages when intervention is most efficacious. Discerning sepsis from other conditions is especially important and challenging in critically ill Emergency Department (ED) patients, in whom the initiation of therapy for sepsis may preclude or delay the treatment of alternative, life-threatening diagnoses.

One readily available stratification method that has been gaining traction is ICU level of care (ICU LOC). In this study, we assessed the risk-stratification value of ICU LOC compared to a cellular host-response test, the IntelliSep test, in a population of suspected infection patients.

Methods

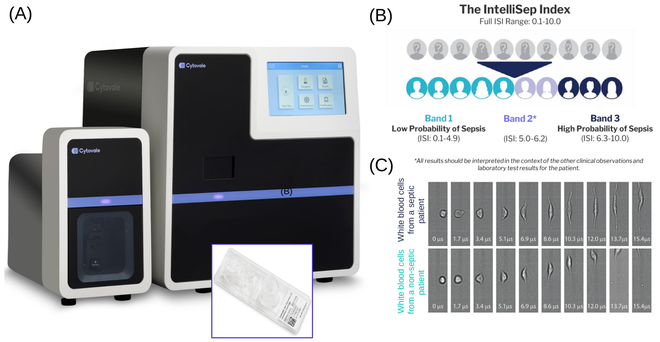

Figure 1: (A) Photograph of the Cytovale system, a benchtop instrument on which the IntelliSep test is performed (inset) the IntelliSep microfluidic cartridge; (B) The IntelliSep reported result: the IntelliSep Index and Interpretation Bands; (C) Time series of cell deformation for a representative leukocyte of a septic Band 3 patient (top) and a non-septic Band 1 patient (bottom).

The IntelliSep test

The Cytovale IntelliSep test is an FDA cleared, semi-quantitative test that assesses cellular host response via deformability cytometry of leukocyte biophysical properties and is intended for use in conjunction with clinical assessments and laboratory findings to aid in the early detection of sepsis with organ dysfunction manifesting within the first 3 days after testing. It is indicated for use in adult patients with signs and symptoms of infection who present to the ED. The test is performed on a K2 EDTA anticoagulated whole blood sample.

The test results in the IntelliSep Index (ISI), a single score between 0.1-10.0, in <10 minutes. The score is stratified into three discrete interpretation bands based on the probability of sepsis with organ dysfunction manifesting within the first three days after testing: Band 1 (low), Band 2, and Band 3 (high)[4-7] (Fig. 1-B).

Biophysical properties such as deformability, density, and size of neutrophils and monocytes are thought to shift with degranulation, neutrophil extracellular trap (NET) formation[8-10], or maturity that occurs during the dysregulated immune activation associated with sepsis[10,11]. As such, these properties differ in cells from septic patients when compared to quiescent white blood cell (Fig. 1-C).

Study Design & Setting

- Multiple prospective observational studies at medical centers across the USA enrolling 5 distinct but similar cohorts (Feb. 2016 – Oct. 2021).
- Adults presenting to the ED with signs (SIRS 2+ with at least one being aberration of temperature or WBC count) or suspicion (clinician order for culture of a body fluid) of infection were prospectively enrolled (n =1002)[12].
  - *Exclusions: hematologic malignancies, receipt of a cytotoxic chemotherapeutic agent (past 3 months), hematopoietic stem cell or solid organ transplants, or transfers from other acute care facilities.*
  - *Only patients with ICU level of care information were included – missing data not imputed (n=730 final population)*
- Necessity for ICU care (ICU -/+) was determined through objective cardiovascular (MAP < 60 or SOFA-CV ≥2), respiratory (PF ratio < 200), or renal ([Creatinine > 5 and (HCO3- < 12 or K+ > 6)] or BUN > 120) criteria within 3-days of presentation.
- EDTA-anticoagulated blood was assayed with the IntelliSep test within 5 hours of blood draw, and patients were followed by retrospective chart review for outcome information. Treating physicians were blinded to the ISI results and patients received standard of care.
- Infection and sepsis status was determined via retrospective physician adjudication (blinded to ISI).

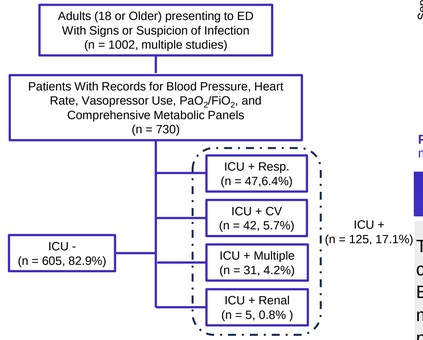

Figure 2: Flow chart for selection of evaluable subjects from multiple prospective observational studies.

Results & Discussion

- Final cohort of 730 patients (sepsis prevalence 15.7%) with 605 patients (82.9%) that did not meet necessity for ICU level of care (ICU-) and 125 (17.1%) met at least one criteria (ICU+, Table 1, Figure 2).
- ICU- cohort consisted of 351 (58.0%) Band 1, 139 (22.3%) Band 2, and 115 (19.0%) Band 3 patients, while the ICU+ cohort consisted of 36 (28.8%) Band 1, 38 (30.4%) Band 2, and 51 (40.8%) Band 3.
- Across all interpretation bands, the most common criteria for ICU+ patients was respiratory (n = 47, 6.4% of total population), followed by cardiovascular (n = 42, 5.7%), multiple criteria (n = 31, 4.2%), and renal (n = 5, 0.8%, Figure 2)
- The majority of patients retrospectively adjudicated septic were in the ICU- group (N=66, 57.4%, Table 1), with 30% of sepsis-associated mortalities were observed in this ICU- group (Figure 3B).
- Regardless of ICU care necessity, the IntellliSep test achieved appropriate diagnostic performance and risk stratification, with increasing rates of sepsis-3, and sepsis-associated mortality across increasing interpretation bands (Figure 3 A,B), with no Band 1 patients with sepsis-associated mortality in either of the ICU- or ICU+ cohorts (Figure 3B). (Sepsis 3 ROC Metrics: ICU- : Sensitivity – 95.5% [87.1-99.1%], Specificity: 88.5% [77.5-94.6%]; ICU+: Sensitivity – 91.8% [80.4-97.7%], Specificity: 73.7% [58.9-85.1%]).

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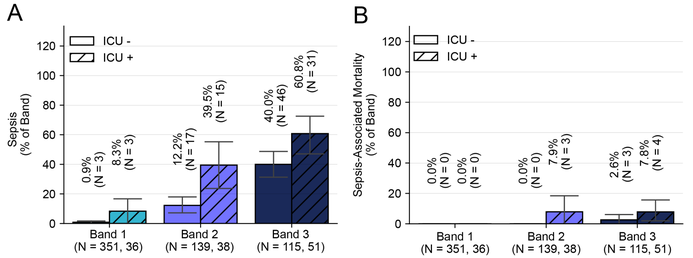

Figure 3. Fraction of septic patients across ICU -/+ and Interpretation Bands (A) and fraction of sepsis-associated mortalities across ICU -/+ and Interpretation Bands (B)

Table 1. Patient case report summaries showing discrepancies between ICU-level care and IntelliSep Interpretation Band / Sepsis-3 status

IntelliSep: Band 1 **ICU Care +** **Not Septic**	**Demographics:** Male, 40s **Medical Hx:** chronic pancreatitis, alcohol & tobacco abuse **Chief Complaint:** rapid weight loss, decreased appetite, nausea, and diarrhea. **Temp:** 98.2ºF, **HR:** 150 BPM, **RR:** 18 BPM **WBC:** 13.3 (×10³/µL); **Lactate:** 9.7 (mmol/L) **BldCx:** (1) returned a contaminant, (2) negative
Hospital Course/Clinical Assessment: Patient with refractory and persistent hypotension requiring Levophed, an elevated lactate (9.7) which decreased to (6.7) and liver dysfunction (INR 1.5) requiring hydrocortisone. CT imaging revealed a pancreatic head mass with biliary ductal dilation and diffuse wall thickening concerning for colitis. Biopsy was not consistent with an abscess. Ultimately, no evidence of colitis, or infection were found. MRCP confirmed presence of a mass with resulting biliary ductal dilation and refractory hypotension was secondary to profound volume depletion. **HLOS**: 6 (1 ICU – stepped down next day)	
IntelliSep: Band 3 **ICU Care -** **Septic**	**Demographics:** Male, 60s **Medical Hx:** atrial fibrillation, ESRD, sick sinus syndrome **Chief Complaint:** 3-day history of generalized weakness and malaise. **Temp:** 101.2ºF, **HR:** 85 BPM, **RR:** 17 BPM **WBC:** 16.0 (×10³/µL); **Lactate:** 1.2 (mmol/L) **BldCx:** positive for Enterococcus faecalis (x2)
Hospital Course/Clinical Assessment: Patient had thrombocytopenia and was hypotensive (MAP <70) which responded to volume resuscitation. Patient found to have enterococcal bacteremia and a vegetation on RV pacemaker lead which was removed. Blood cultures cleared and patient received definitive therapy with prolonged course of IV antibiotics of ampicillin and ceftriaxone. **HLOS**: 13 (0 ICU)	

Conclusions & Clinical Implications

The results of this study suggest that ICU level of care does not correlate with sepsis diagnosis or with severity of illness due to sepsis. Because ISI provides a metric of immune dysregulation, it may provide more actionable information for the management of potentially septic patients.

Acknowledgements

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