SENSITIVE AND SPECIFIC DIAGNOSIS OF SEPSIS IN CRITICALLY ILL CHILDREN UTILIZING HOST GENE EXPRESSION

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Immunexpress
the signature diagnostics for sepsis

INTRODUCTION

Sepsis, generalized inflammation due to infection, is one of the most common causes of childhood mortality worldwide. Additionally, children who survive sepsis are at higher risk for subsequent infection, hospital readmission, late mortality, and likely prolonged, impaired functional status and health-related quality of life. Early recognition, hemodynamic restoration, and antimicrobial administration improve sepsis survival. Early sepsis recognition represents the foundation for sepsis goal-directed therapy. A test that could rapidly and definitively discriminate between infection negative systemic inflammation (INSI) and sepsis, early in the disease process when therapeutic intervention could be most effective, would have significant diagnostic and clinical utility for critically ill children.

Culture-based tests for bloodstream infections produce false negatives and false positives in 30-50% of sepsis cases, and are too slow (>24 hours) to provide real-time diagnoses in cases of suspected sepsis. Therefore, attention has turned toward identifying host-response biomarkers that might be useful for discriminating sepsis from phenotypically similar conditions such as INSI.

Immunexpress has developed and submitted to the FDA a molecular diagnostic test, SeptiCyte® LAB, that discriminates INSI and clinical severe sepsis syndrome (CSSS) in adult critical care populations, with an AUC 0.82-0.90.[3]

An ideal sepsis diagnostic test should provide clinical utility when applied at the first suspicion of sepsis, even before organ dysfunction becomes evident. In adults, the ability of SeptiCyte® LAB to discriminate sepsis from INSI appears to be independent of the severity of organ dysfunction caused by sepsis, as measured by APACHE IV or SOFA scores.

The present study examined whether SeptiCyte® LAB can also be used to differentiate sepsis from INSI among critically ill children. We quantitated mRNA transcript levels in peripheral blood using both next-generation sequencing (NGS) and RT-qPCR. Here in we report the first evidence that SeptiCyte® LAB can discriminate CSSS from INSI with high sensitivity and specificity. In addition, we show that there is no significant correlation between SeptiCyte® LAB scores and commonly used indices of illness severity and organ dysfunction, indicating that the assay is not simply measuring inflammation intensity and organ dysfunction.

PRIMARY HYPOTHESIS

SeptiCyte®LAB will differentiate children with infection negative systemic inflammation (INSI) from children with clinical severe sepsis syndrome (CSSS) with high sensitivity and specificity.

METHODS

• This research was funded through a collaborative agreement between Seattle Children's Hospital Research Institute and Immunexpress.

• Genotypes And Phenotypes in Pediatric Severe Sepsis was an IRB-approved, prospective, proof-of-concept, observational cohort study (Seattle Children's Hospital IRB, #14761). Written informed permission was documented by subjects' parents before any study procedures were undertaken.

• Children in the INSI cohort underwent congenital cardiac defect corrective surgery requiring cardiopulmonary bypass, that is known to induce an INSI response for ~24 hours thereafter. All children in this cohort were > 4 kg and were culture negative. Children in the CSSS cohort had confirmed/highly suspected infection (microbial culture orders, prescription of antimicrobials), exhibited SIRS criteria, and demonstrated at least cardiovascular or pulmonary organ dysfunction. Both immune-competent and immune-compromised patients were included in the CSSS cohort.

• Demographic, infection, illness severity and organ dysfunction information were collected during subject transit through the PICU. Illness severity was quantified utilizing Pediatric Risk of Mortality, version III (PRISM III). Composite organ dysfunction was quantified utilizing Pediatric Logistic Organ Dysfunction, version II (PELOD II). Cardiovascular, pulmonary, and renal organ dysfunctions were individually quantified utilizing the vasoactive-inotropic score, oxygenation index, and serum creatinine respectively.

• Biosample collection and processing: At PICU admission, 2.5 mL blood samples were collected into PAXgene blood RNA tubes for RNA expression analysis. For patients suspected of sepsis, blood was also collected for culture, with culture-positive samples reflexed to MALDI. RNA was isolated and analyzed for yield, concentration, purity, and integrity (Agilent Bioanalyzer RIN score).

• Gene expression data generation: Next-generation sequencing (NGS) employing the Illumina Mi-Seq was used to analyze the transcriptome from peripheral blood samples. A standard MiSeq protocol was used (globin mRNA reduction, ribosomal RNA depletion, library preparation, 2x50 bp paired-end sequencing, minimum 25 million reads per library). NGS gene expression results were confirmed by RT-qPCR.

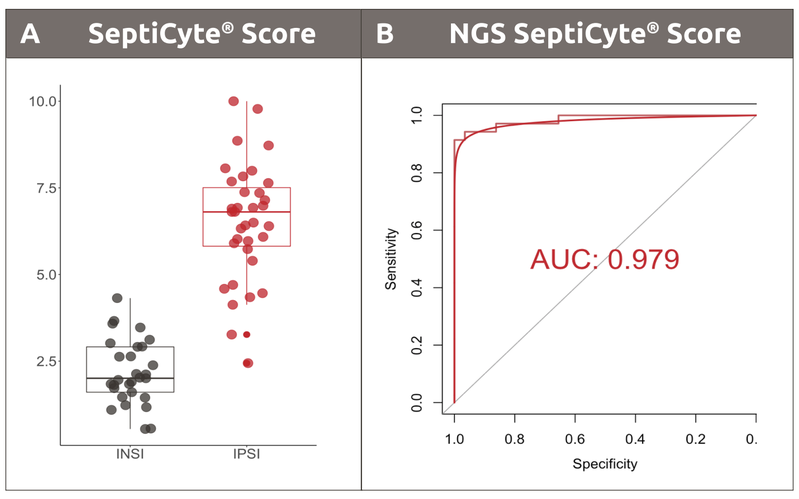
• Data analysis: SeptiCyte® LAB, an adult-derived RNA expression signature for discriminating CSSS from INSI, is defined as follows:

• SeptiCyte® LAB score = log (I [PLAC8]) – log (I [PLA2G7]) + log (I [LAMP1]) – log (I [CEACAM4]) where each "I [ ]" represents the intensity of expression of the RNA transcript within the brackets.

• Performance of SeptiCyte® LAB was evaluated using ROC curve analysis and checked for potential confounders including age, immune status, and blood culture results. Correlation analyses were performed between the SeptiCyte® LAB score measured at day 1 after PICU admission, and various measures of illness severity.

RESULTS

Variable	INSI	CSSS
Age (Years)	7.3 ± 5.6 5.3 [3.1, 13.4]	9 ± 6.7 13.5 [1.7, 14.9]
% Female Gender	41.4	48.6
% Immune Competent	96.6	62.9
% Culture Positive	0	71.4
PRISM III Score	7 ± 4.6 7 [5.0, 8.0]	8.7 ± 6.4 7 [3.5, 11.5]
PELOD Score (Day 1)	5.1 ± 2.2 5 [5.0, 6.0]	4.8 ± 2.8 5 [2.5, 6.5]
Vasoactive-Inotropic Score (Day 1)	12.6 ± 15.3 7 [5.0, 10.0]	20 ± 23.5 15 [5.5, 25.0]
Oxygenation Index (Day 1)	4.9 ± 5.1 3.2 [2.5, 3.7]	7.8 ± 8.4 5 [2.1, 7.9]
Serum Creatinine (Day 1)	0.5 ± 0.4 0.5 [0.4, 0.5]	0.7 ± 0.6 0.6 [0.4, 0.8]



• Characteristics of the study cohort, consisting of 29 INSI subjects and 35 CSSS subjects, are displayed in the table to the left with data summarized as mean ± SD, or median [interquartile range]. Performance of SeptiCyte® LAB was confirmed by retesting 29 CSSS and 12 INSI patient samples using RT-qPCR, producing a ROC AUC of 0.954.

• A Kolmogorov Smirnov test (p=0.11) and F-test for equality of variance about two Gaussian distributions (F=0.4262, numerator df=9, denominator df=17, p=0.1943) revealed no significant differences in SeptiCyte® LAB scores for immune-competent and immune-compromised subjects within the CSSS cohort.

• Similarly no significant differences in SeptiCyte® LAB score ROC AUC for INSI vs. culture-negative CSSS and INSI vs. culture-positive CSSS subjects were observed.

• No relationships between the SeptiCyte® LAB score and various indicators of subject illness severity including PRISM score, day 1 PELOD score, vasoactive-inotropic score, oxygenation index, and serum creatinine were noted.

• Since the upper bound of the 95% CI AUC for SeptiCyte® LAB is 1, no attempts to demonstrate enhancement of performance through addition of other clinical parameters was pursued. On the contrary, it is more likely that either noise would be added, or else overfitting would occur since, in the present study, patient samples were limited in number.

Discrimination of CSSS From INSI, Based on NGS Data

Transcript counts were estimated from RNA sequencing on the Illumina HiSeq platform. Panel A: SeptiCyte® LAB score for CSSS versus INSI, box-and-whisker plot. Whisker boundaries are defined by Q1-1.5 IQR and Q3+1.5 IQR, where IQR = interquartile range. Panel B: corresponding ROC curve.

CONCLUSIONS

1. **In a preliminary fashion we evaluated the performance of SeptiCyte® LAB, a 4-gene transcript classifier, comparing children with CSSS versus INSI, by ROC analysis.**
2. **An AUC >0.9 was obtained for discriminating these two groups, using transcript frequency data generated by either NGS or RT-qPCR.**
3. **Because the SeptiCyte® LAB gene signature was discovered and validated in an entirely independent adult cohort, this pilot investigation constitutes a preliminary validation of the SeptiCyte® LAB signature among critically ill children.**
4. **There was no significant correlation between the SeptiCyte® LAB score and PRISM, PELOD and a variety of other markers of specific organ dysfunction, indicating that information provided by SeptiCyte® LAB is independent of illness severity. Instead, the magnitude of SeptiCyte® LAB score correlates directly with the probability of sepsis.**
5. **SeptiCyte® LAB may have clinical utility for discriminating sepsis versus infection-negative causes of systemic inflammation among critically ill children.**
6. **A broader investigation of the performance of SeptiCyte® LAB among children in more heterogeneous care settings and with a variety of infection diagnoses is warranted.**

References

[1] Seattle Children's Hospital, University of Washington [2] Immunexpress, Seattle, WA [3] McHugh L, Seldon TA, Brandon RA, et al. A molecular host response assay to discriminate between sepsis and infection-negative systemic inflammation in critically ill patients: Discovery and validation in independent cohorts. PLoS Med 2015; 12 (12): e1001916. doi: 10.1371/journal.pmed.1001916. PMID: 26645559

Abstract 1024. Crit Care Med 2015; 43 (12, Suppl): A 1024.