SeptiBurnAlert: AI/ML tool integrated with qPCR assay to score the risk of burn sepsis from the first blood draw after ICU admission

Nabarun Chakraborty[1], Alexander Lawrence[2], Aarti Gautam[1], Stacy Ann-Miller[1], Lauren T. Moffatt[3], Jeffrey W. Shupp[3], and Rasha Hammamieh[1]

[1]Integrative Readiness Systems Biology, CMPN, WRAIR, Silver Spring, MD, [2]ORISE, Silver Spring, MD,

[3]The Burn Center, MedStar Washington Hospital Center; Department of Surgery, Georgetown University School of Medicine, Washington, DC

Abstract

Introduction: Nearly 30% of burn patients eventually develop sepsis. However, determining sepsis in burn patients is particularly challenging because the pathophysiology of burn sepsis differs significantly from typical sepsis in critically wounded patients. As a result, standard methods for detecting sepsis often fail in burn patients, increasing their risk of mortality.

Capability Description: The goal is to develop a molecular assay capable of predicting septic onset within the first day of ICU admission, providing a time advantage of approximately 70 hours compared to traditional diagnostic methods.

Methods: At MedStar Washington Hospital Center, blood samples were collected from burn patients starting at ICU admission (time 0) and continuing until discharge. Sixteen patients who developed sepsis at any point during their ICU stay were identified as "pre-septic" patients and compared with age-, gender-, and TBSA-matched "non-septic" controls. RNA profiles from whole blood were analyzed using a proprietary AI/ML tool to develop a biomarker panel capable of predicting sepsis onset within the first 24 hours of ICU admission. A risk score model ranging from 0 to 10 was developed, with "0" indicating the lowest and "10" the highest risk of sepsis onset.

Results: The resulting tool, named SeptiBurnAlert, is a 6-gene panel capable of predicting sepsis with >80% sensitivity and specificity within 2 hours of blood collection.

Applicability to Medical Roles of Care: The rapid turnaround time of SeptiBurnAlert, using standard qPCR tools, makes it a potentially valuable diagnostic tool for Role 2/3 facilities.

Significance: SeptiBurnAlert detects sepsis within a day of burn incidents, offering clinicians a critical 72h advantage to customize therapy and reduce unnecessary antibiotic use.

Developmental Status of Technology: A worldwide patent application has been submitted, and FDA pre-submission is complete. Recruitment of subjects for independent validation is ongoing, and a non-exclusive licensing process is underway.

Biomarker discovery: Differentially expressed genes were seeded to AI/ML tool, developed in house, named Biomarker discovery process at binomial decision point (2BDP). This data was processed to find best performing panels with 6-8 features that can answer the following binomial questions- "Will this subject ever be diagnosed with sepsis?" Logistic regression model was used compute the following equation, $logit(P) = a + b X_1 + c X_2 + \cdots + \alpha X_n$....., here "x" is the gene expression value and logit(P) is the score to assess the risk of mTBI or the chances to recover slowly.

Established tools and Gaps

Table 1.

Assay	Technique	Target (host vs. pathogen)	% sensitivity/ % specificity
Iridica Plex ID (Abott Molecular)	Multiplex broad-range PCR/ESI-MS	Pathogen, able to detect polymicrobial infection	45-83%/69-84%
SeptiFast (Roche Dx)	Multiplex target-specific qPCR/ in situ hybridization/ melt analysis	Pathogen, able to detect polymicrobial infection	63-83%/83-95%
SeptiTest (Molzyme)	Universal PCR/ sequencing	Pathogen, able to detect polymicrobial infection	11-87%/83-96%
MinION (Oxford Nanopore technology)	Sequencing	Pathogen, potentially able to detect polymicrobial infection	NA/97-100%
SeptiCyte Lab (Immunexpress)	4-gene qPCR	Host whole blood	NA/95%

Table 1. FDA-Approved Nucleic Acid Test Kits. The top four featured kits target pathogens. The primary challenges of this approach are the high background from the host genome and the low concentration of target amplicons at early stages, which can overwhelm the targets of interest. SeptiCyte is the only FDA-approved nucleic acid test kit that targets the host's whole blood. A major limitation of SeptiCyte is that the assay was optimized for critically ill surgical patients, whose background inflammation levels are typically lower than those of burn patients.

Box 1. Justifications to find burn sepsis-specific biomarkers

Major differences between sepsis in the general population and sepsis in burn patients:

- ➢Burn patients lose the first barrier to infection—their skin
- ➢Burn patient is continuously exposed to inflammatory mediators as long as the wound remains open
- ➢All burns >15–20% TBSA suffers persistent "SIRS" for months after the wound is closed
- ➢Diagnosis of sepsis in patients with severe burns (>20% of TBSA) is complicated due to
- ➢Overlapping of clinical signs of the post-burn hypermetabolic response with those of sepsis
- ➢A persistent a hyper-inflammatory state in burn patients

Results

Figure 1.

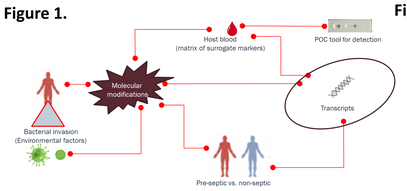

Figure 1. Outline of our approach: Our hypothesis is that the molecular landscape of the host is perturbed much earlier than the clinical manifestation of sepsis. Strategic curation of these molecular signatures can distinguish patients at risk of sepsis onset at an early time point, providing clinicians with a critical time advantage for intervention.

Figure 2.

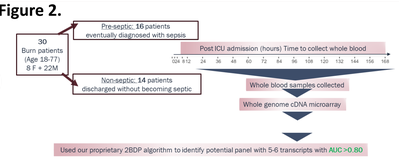

Figure 2. Study outline: Subjects were recruited at MedStar Washington Hospital Center. The PaxGene whole blood tubes were transferred to WRAIR, where the assay and analysis took place.

Box 2. Brief introduction of SeptiBurnAlert

SeptiBurnAlert

What?:
Blood gene transcript panel readily implementable on targeted rapid IVD platform, such as qPCR

Goal:
Predict the risk of sepsis onset in burn patients within the first 24 hours of ICU admission

Mode of Detection:
A risk (or likelihood) score of sepsis onset. A prognostic tool to be used in the hospital setting

Capability gap to be addressed by this tools:
Following are available capabilities with comparatively delayed turnover, and SeptiBurnAlert is poised to mitigate these limitations

- Quick Sepsis Related Organ Failure Assessment (qSOFA)
- Mann-Salinas criteria: Predictive, not diagnostic and aimed only at thermally injured patients.
- Systemic Inflammatory Response Syndrome (SIRS): limited specificity in burn patients due to their naturally high inflammatory
- Sepsis-3: Incorporate the use of the SOFA score, but as this was not validated in burn patients

Figure 3.

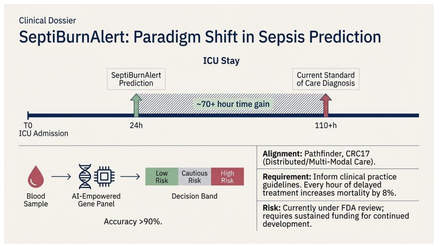

Figure 3. Project Outline. SeptiBurnAlert can predict the onset of sepsis within the first 24 hours of a patient's ICU stay, potentially providing clinicians with more than 72 hours of advance notice. We aim to achieve this by screening the blood gene landscape using an AI/ML-empowered algorithm.

Figure 4.

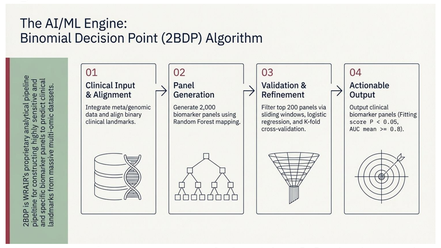

Figure 4. AL/ML-empowered algorithm [1]. The biomarker discovery process at the binomial decision point (2BDP) was used to identify panels with less than 10 features capable of predicting the onset of sepsis in burn patients with high efficacy. This algorithm first curates high-performing features using Random Forest, followed by feature classification with machine learning tools.

$$logit(P) = a + b X_1 + c X_2 + \cdots + \alpha X_n \ldots\ldots\ldots\ldots$$

a, b, c... n = constant values X1, X2, Xn : RT-PCR data

Equation 1. The deliverable of 2BDP-logistic regression-based classification

$$x' = to_{min} + \frac{(x - from_{min}) \cdot (to_{max} - to_{min})}{from_{max} - from_{min}}$$

X': Final value; X: Logit(p);
$to_{min} = 0$, $to_{max} = 10$,
$from_{max}$= highest value in the data, $from_{min}$: lowest value in the data

Equation 2. Conversion of logit(P) value to 0-10 score to facilitate easy reading

Diagnostic Panel

Table 2.

# of transcripts	AUC	Specificity	Sensitivity	McFadden's R^2	Adjusted R^2
8	0.96	0.88	0.93	0.40	0.37
9	0.96	0.85	0.93	0.40	0.37
10	0.96	0.85	0.97	0.45	0.43
9	0.96	0.85	0.95	0.45	0.43
9	0.96	0.85	0.95	0.43	0.40
7	0.95	0.85	0.93	0.38	0.36
8	0.95	0.85	0.93	0.38	0.35

Table 2 [2]. Top-performing biomarker panels for predicting sepsis using the first blood draw upon ICU admission

Figure 5.

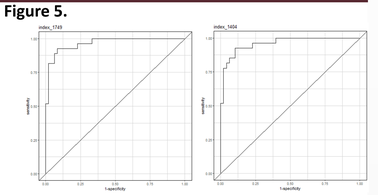

Figure 5. Representative AUC curves of some of the panels

Figure 6.

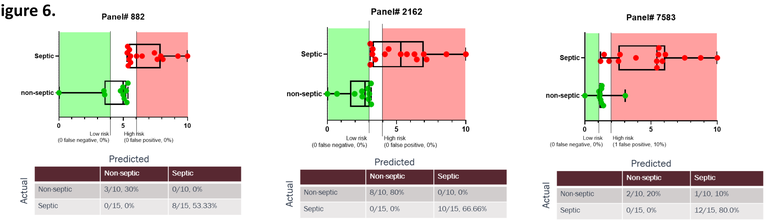

Figure 6. Representative bands with scores from 0 to 10 are shown: the green area indicates low-risk patients who do not require antibiotic administration, while the red area represents high-risk patients who require immediate antibiotics. The white section is the gray zone, where patients require standard monitoring. Confusion matrices are also presented. SeptiBurnAlert has the potential to reduce antibiotic misuse by approximately 80%

Figure 7..

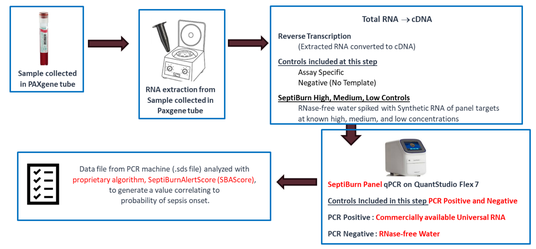

Figure 7. Assay flow chart to outline the current protocol. Ongoing efforts aim to reduce the hands-on time by incorporating SeptiBurnAlert to a handheld device.

Conclusions

Performance metrics of SeptiBurnAlert in comparison to the current protocols and the predicate, namely SeptiCyte :

	Rapid diagnosis (<24h since ICU admission)	Burn Specific diagnosis	Host blood-based detection
Standard method: ICU checklist (qSOFA, SIRS etc.)	×	√ / ×	√ / ×
SeptiCyte	√	×	√
SeptiBurnAlert	√	√	√

References:

1. Biomarker discovery process at binomial decision point (2BDP): Analytical pipeline to construct biomarker panel. Computational and Structural Biotechnology Journal. 2023 Jan 1;21:4729-42
2. Hammamieh R, Chakraborty N, Campbell R, LAWRENCE AB, Jett-Tilton M, Shupp J, Gautam A, MCLAWHORN M, inventors; United States Department of the Army, assignee. Method of managing clinical outcomes from specific biomarkers in burn patients. 2023 Mar 9.

Acknowledgment: CCC, as the funding source.

To learn more, visit: www.wrair.health.mil