Infection Treatment in High Risk Patients: Moving Pathogen Detection to the Point-of-Care

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Critical and Point-of-Care Testing Meeting
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Infections and Sepsis

• Sepsis remains the leading cause of death in non-coronary intensive care units\(^1\) and claims more lives than bowel and breast cancer combined globally\(^2\).

• Mortality ranges from 28 to 50% in cases of severe sepsis, but approaches 70% in cases of septic shock.\(^1\)

• Early recognition and appropriate treatment of sepsis is associated with improved outcomes.\(^3\)

• Delays in appropriate therapy decreases survival 17.6-fold during severe sepsis.\(^4\)

Culture-Based Pathogen Detection

PERFORMANCE OF BLOOD CULTURE

• Theoretical limit of detection (LoD) of 1 CFU/mL (Reality = 3.18 – 3,000 CFU/mL)

• Detects most culturable microorganisms

• Median time to qualitative results: 10.4 hours

• Median time to speciation results: 26.4 hours

• Median time to drug sensitivity results: 43.7 hours

• Affected by antimicrobial drugs and improper sample volumes

High Risk Sepsis Patients

• High risk patients include those that are more susceptible to sepsis and associated complications.

• Burns patients are an example of a high-risk sepsis population due to loss of their primary barrier to the environment.\textsuperscript{1,2}

• For example, 97% of burn patients with >20% total body surface area (TBSA) burns will acquire an infection over the course of their ICU stay.\textsuperscript{1}

• Up to 75% of burn related mortality is associated with wound infections.\textsuperscript{2}

• Burn sepsis definitions are not specific and may be masked by underlying conditions from chronic hypermetabolism and inflammation.\textsuperscript{1}

Pathogen Detection in Burn Patients

Patient is a 20 year old man status post motor vehicle accident with 90% TBSA 3rd and 4th degree burns and C1 pedicle and C4 foraminal fracture.

**Day 1**
- **RC1:** Collected
  - *H. influenzae*
- **WC1:** Collected
- **RC2:** Collected
  - Mold observed during dressing change.
- **WC2:** A. fumigatus, Rhizopus sp.
- **RC3:** Collected
  - Amphi B soaks
- **WC3:** MSSA, *E. faecalis*, Strep. *viridans*
  - Added Linezolid

**Day 2**
- **RCB:** Collected
  - *P. aeruginosa*

**Day 3**
- **WC3:** Collected
  - Discontinued Linezolid, Meropenem; Added Vancomycin,

**Day 4**
- **BCB:** Collected
  - Added Voriconazol, Meropenem

**Day 8**
- **BCC:** Negative

**Day 10**
- **BCC:** Negative

**Day 15**
- **RC1:** Collected

**Day 23**
- **RC3:** Collected
  - *P. aeruginosa*

**Day 27**
- **BCB:** Collected
  - *P. aeruginosa*

**Day 30**
- **BCC:** Negative

**Day 31**
- **BCC:** Negative

**Day 32**
- **BCC:** Negative

**Day 33**
- **BCC:** Negative

**Day 37**
- **Patient expired**

**Day 38**
- **Patient expired**

**Day 39**
- **Patient expired**
Patient is a 20 year old man status post motor vehicle accident with 90% TBSA 3rd and 4th degree burns and C1 pedicle and C4 foraminal fracture.

Pathogen Detection in Burn Patients

Molecular Pathogen Detection

Polymerase Chain Reaction

- Growth-Independent Method
- Amplifies nucleic acids: $N = N_0 \cdot 2^n$
- Detects conserved regions including resistance genes
- Analytical Turnaround Time: 1 – 6 hours
- Limits of Detection: $10^0$ to $10^2$ CFU/mL
PCR-Based Pathogen Detection in Trauma, Emergency, and Burn Surgery Patients

• **Hypothesis:** Multiplex PCR rapidly identifies pathogen nucleic acids from whole blood and detects the presence of occult infections when blood culture is inhibited.

• **Design:** Single-site, prospective observational trial

• **Population:** 50 adult trauma, emergency, and burn surgery (≥20% TBSA) with suspected sepsis.

• **Method:** 1.5mL of whole blood collected with blood culture samples and tested by Real-Time PCR (SeptiFast).

# SeptiFast Test Panel

<table>
<thead>
<tr>
<th>Gram Positive</th>
<th>Gram Negative</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoNS¹</td>
<td>Acinetobacter baumannii</td>
<td>Aspergillus fumigatus</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>Enterobacter aerogenes/ cloacae⁴</td>
<td>Candida albicans</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>E. coli</td>
<td>Candida glabrata</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>Klebsiella pneumoniae/ oxytoca⁴</td>
<td>Candida krusei</td>
</tr>
<tr>
<td>Strep. pneumoniae</td>
<td>Proteus mirabilis</td>
<td>Candida parapsilosis</td>
</tr>
<tr>
<td>Strep. sp.²</td>
<td>Pseudomonas aeruginosa</td>
<td>Candida tropicalis</td>
</tr>
<tr>
<td>MRSA (mec A gene)³</td>
<td>Serratia marcescens</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stentrophomonas maltophilia</td>
<td></td>
</tr>
</tbody>
</table>

1-Coagulase negative Staphylococcus aureus
2-Streptococcus agalactiae, pyogenes, viridans = Strep. Sp.
3-Separate test kit
4-No differentiation between these two subspecies
“SeptiFast” PCR Targets

Factor Essential for Methicillin-Resistance (Fem) A and B Regions

STAPHYLOCOCCUS AUREUS GENOME
NCTC 8325, Size: 2800 kb

16-23S ITS Primer (50):
GTTATTAWCTTGTAG (FP)
GTTATTAWCTTGTAGTGTT (RP)

CGAACACTAGCGATTATTTCCTTA (FP)
TTTCGAACACTAGCGATT (RP)

Probe Pairs (70):
CCGAGTGAATAAGAGTTTTAAA (anchor)
GCTTGAATTCTAAGAAATAATCG (sensor)

CTTGGTAAAAATCTTACTTACTTATCTAG (anchor)
AAGCAGAGTTTACTATGTAAATGAGCAT (sensor)

AGTGGTCAAAAGAACCACACAAGATTAATAA (anchor)
AGCTTATTAAAACACTCTTATTTAATCAGCGTTT (sensor)

Protein A Gene (spa)

16S - 23S Ribosomal Internal Transcribed Spacer Region

Chromosomal Cassette Recombinase (ccr)
S. aureus Open Reading Frame X (orfX) Region

Staphylococcal Chromosomal Cassette Region and Methicillin Resistance Gene (mecA)

PCR Testing in Burn Sepsis Patients

Patient is a 20 year old man status post motor vehicle accident with 90% TBSA 3rd and 4th degree burns and C1 pedicle and C4 foraminal fracture.

Day 1

Septic Shock
MAP: 40-50mmHg
Started 4 Vasopressors
Green exudate on wounds
Platelet: 88,000

BC_B: P. aeruginosa
PCRC: P. aeruginosa
BC_C: Negative

Added Posaconazole

PCR may have detected P. aeruginosa 38.7 hours earlier than culture, and could have identified an occult infection during antimicrobial therapy
ABA – MCTG
COMBAT CASUALTY GRANT:

“Rapid, Quantitative, PCR-Based Detection of Staphylococcus aureus in Burn Sepsis Patients”

PI: Nam K. Tran, PhD
NIH Clinical Trials Registration Number: NCT01140269
UCD IRB Approval Number: 200918586
USAMRMC HRPO Log Number: A-15774.0 (Core Protocol)
Study Model: Randomized Controlled Trial

**RECRUITMENT**

**Inclusion Criteria**
- Age $\geq$ 18 years
- $\geq$ 20% TBSA burns

**Exclusion Criteria**
- Age $<$ 18 years
- Unable to consent
- IV Antibiotic allergies
- Non-survivable injuries

**BLOCK RANDOMIZATION (240 Patients)**

**CONTROL (120)**
- Observational group
- No PCR testing
- Routine laboratory testing
- Standard of care treatment

**EXPERIMENTAL (120)**
- Treatment group
- PCR testing for *Staphylococcus aureus*
- Routine laboratory testing
- Standard of care treatment
- Quantitation of positive PCR results (blinded)
CURRENT PARTICIPATING SITES

- UCDMC
  Sacramento, CA
- Torrance Memorial
  Torrance, CA
- U. Cincinnati
  Cincinnati, OH
- U. Washington
  Seattle, WA
- Nathan Speare
  Philadelphia, PA
- U. Miami
  Miami, FL
Staphylococcus aureus

- Gram positive cocci found in groups.
- Coagulase and catalase positive
- Produces capsules (types 5 and 8 are common human pathogens)
- Expresses beta-lactamase to confer penicillin resistance
- Colonizes 10 to 20% of adults
- Methicillin resistant strains (MRSA) associated with higher mortality.

GeneXpert PCR Testing

Nasal

Wound

BC

Time (min) 2 60 - 70 RESULTS
GeneXpert *S. aureus* Gene Targets

- Factor Essential for Methicillin-Resistance (Fem) A and B Regions
- STAPHYLOCOCCUS AUREUS GENOME
  - NCTC 8325, Size: 2800 kb
  - OrfX Primer: GGATCAAAACGGCCTGCACA (FP)
    TTACTACGTGTTGAAGACGA (RP)
  - OrfX Probes: CCCGCGCGTAGTTACTGCGTTGTAAGACGTCGCCGCGGG
    CCCGCGCATAGTTACTGCGTTGTAAGACGTCGCCGCGGG
    CCCGCGCGTAGTTACTACGTGTTGAAGACGTCGCCGCGGG
- mecA Primers: GTCAAAAAATCATGAACCTCATTTACTATTATAG
  ATTTCAATATGTAAATTCTCCTCCACATCTC
  CAAATTTATCTCGTAATTACCTTCTGCC
  CTCTGCTTTATATTATAAAAATTCGCTG
  CACTTTATTTTTCACATGGAGTTTGAAC
- mecA Probes: AAACAAAGCAATAGAATCATCAGAT
  GAGATAGGCAATCGGTTCCTCAGAAGATGTA

PRELIMINARY DATA
2010-2012
Control vs. PCR Group Demographics

**Abbreviations:** BSI, bloodstream infections; ICU, intensive care unit; LOS, length of stay; MODS, multiple organ dysfunction score; TBSA, total body surface area.

- **TBSA (%)**
  - Control: 35%
  - PCR: 32%
  - $P = 0.747$

- **Age (years)**
  - Control: 42 years
  - PCR: 41 years
  - $P = 0.538$

- **ICU LOS (days)**
  - Control: 50 days
  - PCR: 52 days
  - $P = 0.506$

- **BSI (Frequency)**
  - Control: 1 case
  - PCR: 2 cases
  - $P = 0.418$

- **Admit MODS**
  - Control: 3 cases
  - PCR: 4 cases
  - $P = 0.575$

- **Vent Days**
  - Control: 15 days
  - PCR: 10 days
  - $P = 0.052$
Distribution of Men vs. Women Between Study Groups

![Bar graph showing the distribution of patients by gender and study group. The graph compares the number of male and female patients in the control and PCR groups. The x-axis represents gender (Male and Female), and the y-axis represents the number of patients. The control group has 21 patients, with 14 males and 7 females, and the PCR group has 18 patients, with 12 males and 6 females. The p-value is 0.555, indicating no significant difference in distribution.]
MRSA vs. MSSA Infections

Control (n = 21)  PCR (n = 18)

P = 0.212
MRSA/MSSA Antibiotic Days Between Control vs. PCR Groups

“Non-vancomycin” = nafcillin or cefazolin
PCR Turnaround Time vs. Culture

<table>
<thead>
<tr>
<th></th>
<th>SSTI</th>
<th>BSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>3.178258065</td>
<td>32.5144</td>
</tr>
<tr>
<td>Culture</td>
<td>83.30322581</td>
<td>53.3768</td>
</tr>
</tbody>
</table>

**Abbreviations:** BSI, bloodstream infection; PCR, polymerase chain reaction; SSTI, skin and soft tissue infection
**Proof of Concept: Serial Quantitative PCR Testing**

**History:** Patient is a 40 year old man with 20% total body surface area burns to the face, head, neck, left upper back, bilateral hands, and lower left extremity from a house fire. Blood cultures, respiratory cultures, and wound cultures were collected on day 5 for clinical suspicion of burn sepsis (*American Burn Association Sepsis Trial*).

<table>
<thead>
<tr>
<th>Time</th>
<th>Event Description</th>
<th>CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>0945</td>
<td>Vancomycin Started 750mg IV q8H</td>
<td></td>
</tr>
<tr>
<td>0934</td>
<td>WC report 2+ Gram Positive Cocci</td>
<td></td>
</tr>
<tr>
<td>1015</td>
<td>WC report <em>S. aureus</em> (MIC pending)</td>
<td></td>
</tr>
<tr>
<td>1205</td>
<td>WC report MSSA</td>
<td></td>
</tr>
<tr>
<td>0900</td>
<td>Wound culture (WC) Collected PCR swab sample collected</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>PCR detects 1,029 CFU of MSSA from wound swab</td>
<td></td>
</tr>
<tr>
<td>2030</td>
<td>Mupirocin started on wounds</td>
<td></td>
</tr>
<tr>
<td>1900</td>
<td>Wound culture (WC) Collected</td>
<td></td>
</tr>
<tr>
<td>2030</td>
<td>Mupirocin started on wounds</td>
<td></td>
</tr>
<tr>
<td>661</td>
<td>CFU of <em>S. aureus</em></td>
<td></td>
</tr>
<tr>
<td>1380</td>
<td>CFU of MSSA</td>
<td></td>
</tr>
<tr>
<td>792</td>
<td>CFU of MSSA</td>
<td></td>
</tr>
</tbody>
</table>

PCR testing provided definitive results 4 days faster than culture. Quantitative PCR correlated with treatment efficacy.
Moving Rapid Pathogen Detection to the Point-of-Care
Evolution of Rapid Pathogen Detection Devices
Evolution of Rapid Pathogen Detection Devices
Evolution of Rapid Pathogen Detection Devices

**CENTRAL LAB**
Processing: Manual or semi-automated processing
**Menu:** Culturable pathogens
**TAT:** ~2-3 days
**Cost:** $118/set
Evolution of Rapid Pathogen Detection Devices

- **CENTRAL LAB**
  - Processing: Manual
  - Menu: 25-plex
  - TAT: <24 hours
  - Cost: $500/batch

- **TAT**: 

- **PORTABILITY**: 

[Image of bottles and a device]
Evolution of Rapid Pathogen Detection Devices

NEAR PATIENT
Processing: Auto
Menu: Singleplex
TAT: ~1-2 hours
Cost: $50/test
Evolution of Rapid Pathogen Detection Devices

**BEDSIDE**
- Processing: Auto
- Menu: Multiplex(?)
- TAT: ~1-2 hours
- Cost: ???

**TAT**

**PORTABILITY**
Challenges for Point-of-Care Rapid Pathogen Detection

- Rapid (<1 hour) identification of both pathogen and resistance profile (antimicrobial susceptibility).

- Miniaturization and automation of complex pre-analytical processing for molecular assays → CLIA waived device!

- Adequate analytical sensitivity and specificity. Limits of detection of < 100 CFU/mL to detect early sepsis.

- Environmental robustness for tests performed in non-hospital settings.

- Pathogen detection from minimally or unprocessed samples (e.g., whole blood).
The Bright Future of POC Rapid Pathogen Detection

T2 Magnetic Resonance (T2MR)

- Direct detection of bacterial and fungal pathogens in 1 hour
- No sample processing required
- Limits of detection < 1 CFU/mL

T2 Biosystems
The Bright Future of POC Rapid Pathogen Detection

Surface Enhanced Raman Spectroscopy (SERS)

- Direct detection of pathogens in <1 hour
- Potential to determine antimicrobial susceptibility
- Handheld platform
Conclusions

• Sepsis remains a significant health care problem and exhibiting substantial mortality and morbidity.

• High risk patients have increased susceptibility for sepsis and often have underlying conditions that mask the presence of infection.

• Current microbiological culture-based pathogen detection is unable to rapidly identify the pathogen causing sepsis.

• Rapid molecular pathogen detection techniques have proven to improve patient outcomes by accelerated targeted antimicrobial therapy, however, these devices remain mostly located in the central laboratory.

• Challenges with moving rapid pathogen detection to the point of care includes acceptable TAT, automation, complexity, and analytical performance.

• Future POC pathogen detection technologies will favor direct identification using innovative methods, may involve antimicrobial susceptibility testing, and provide analytical performance that is at least comparable to current methods.
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QUESTIONS?