Gene expression profiling in pediatric septic shock: biomarker and therapeutic target discovery

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CCTST Grand Rounds
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Gene expression profiling in pediatric septic shock

- NIH-sponsored.
- Multiple centers submitting biological samples and clinical data.
- Whole blood-derived RNA.
- Microarray-based measurements of mRNA expression at the level of the entire genome.
- Parallel serum samples for validation studies and biomarker development.
Goals of expression profiling

• Biomarker and gene expression-based stratification.
• Discovery of novel targets and pathways.
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Rationale for stratification in septic shock

• Septic shock is more of a syndrome than a distinct “disease.”
• As a syndrome, it is likely that multiple “disease subclasses” and “disease strata” exist.
• The multiple failures of septic shock clinical trials perhaps reflect our misguided approach to septic shock as a single “disease” entity.
• Effective stratification or staging may allow for more specifically targeted therapies and for more effective clinical trials.
Current state of the art for septic shock sub-classification……..

• Physiologic: “warm” shock vs. “cold” shock.

• Microbiologic: gram negative, gram positive, or fungal.
Genome-level expression profiles in pediatric septic shock indicate a role for altered zinc homeostasis in poor outcome

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Physiol Genomics 30:146-155, 2007
Potential genes of interest selectively upregulated in nonsurvivors

• CC chemokine ligand 4 (a.k.a. MIP-1β)
• Granzyme B
• Interleukin-8
• Metallothionein 1E
• Metallothionein 1K
• Solute carrier family 39, member 8 (zinc transporter)
• Suppressor of cytokine signaling 1
• Transferrin
• Thrombospondin
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Interleukin-8 as a Stratification Tool for Interventional Trials Involving Pediatric Septic Shock

Hector R. Wong1, Natalie Cvijanovich2, Derek S. Wheeler1, Michael T. Bigham1, Marie Monaco1, Kelli Odoms1, William L. Macias3, and Mark D. Williams3

1Division of Critical Care Medicine, Cincinnati Children’s Hospital Medical Center and Cincinnati Children’s Research Foundation, and Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio; 2Division of Critical Care Medicine, Children’s Hospital and Research Center Oakland, Oakland, California; and 3Eli Lilly Research Laboratories, Indianapolis, Indiana

Am J Respir Crit Care Med. 178:276, 2008

IL-8 serum level < 220 pg/ml, obtained within 24 hours of ICU admission.

95% probability of survival with standard care (C.I. 90 – 98%).

Prospectively validated in an independent database (n > 300).

Proposal: use IL-8 as an **exclusion** biomarker in future pediatric septic shock clinical trials as a means of optimizing the risk to benefit ratio.
Multi-biomarker-based stratification for septic shock: *rationale*

- The IL-8 strategy is appealing.
  - High negative predictive value
  - Simplicity
- But, sensitivity, specificity, and positive predictive values not very robust.
- Can we develop a biomarker-based stratification tool that can meet a broader range of clinical and research needs?
- Can a multi-biomarker based approach meet these needs?
Multi-biomarker sepsis risk model

• Used microarray data from 100 patients to objectively derive a panel of 15 candidate outcome biomarkers for sepsis.
<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Description</th>
<th>Fold Induction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL3</td>
<td>C-C chemokine ligand 3; a.k.a. MIP-1α</td>
<td>2.8</td>
</tr>
<tr>
<td>LCN2</td>
<td>Lipocalin 2; a.k.a. NGAL</td>
<td>2.7</td>
</tr>
<tr>
<td>MMP8</td>
<td>Matrix metallopeptidase 8; a.k.a. neutrophil collagenase</td>
<td>2.6</td>
</tr>
<tr>
<td>RETN</td>
<td>Resistin</td>
<td>2.4</td>
</tr>
<tr>
<td>THBS</td>
<td>Thrombospondin 1</td>
<td>2.2</td>
</tr>
<tr>
<td>GZMB</td>
<td>Granzyme B</td>
<td>2.2</td>
</tr>
<tr>
<td>HSPA1B</td>
<td>Heat shock protein 70kDa 1B</td>
<td>2.1</td>
</tr>
<tr>
<td>ORM1</td>
<td>Orosomucoid 1, acute phase protein with unknown function</td>
<td>2.0</td>
</tr>
<tr>
<td>CCL4</td>
<td>C-C chemokine ligand 4; a.k.a. MIP-1β</td>
<td>1.9</td>
</tr>
<tr>
<td>IL8</td>
<td>Interleukin-8</td>
<td>1.8</td>
</tr>
<tr>
<td>LTF</td>
<td>Lactotransferrin</td>
<td>1.8</td>
</tr>
<tr>
<td>ELA2</td>
<td>Neutrophil elastase 1</td>
<td>1.8</td>
</tr>
<tr>
<td>IL1A</td>
<td>Interleukin 1α</td>
<td>0.5</td>
</tr>
<tr>
<td>SULF2</td>
<td>Sulfatase 2; extracellular modulator of heparan sulfate proteoglycans</td>
<td>0.5</td>
</tr>
<tr>
<td>FGL2</td>
<td>Fibrinogen-like 2; acute phase protein similar to fibrinogen</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Nonsurvivors relative to survivors*
Plan

- Assay 15 serum biomarkers in a derivation cohort of patients (n = 220) using a multi-plex platform.
- Multi-variable logistic regression to derive a risk model: *individual patient outcome and illness severity*.
- “PERSEVERE” (PEdiatRic SEpsis biomarkEr Risk modEl)
- Validate PERSEVERE in a validation cohort: *200 prospectively enrolled patients*.
Septic shock as a syndrome....

Implies the existence of septic shock “subclasses”

Distinct gene expression patterns and biological processes

Distinct clinical phenotypes

Can genome-wide expression profiling identify subclasses of children with septic shock beyond the dichotomy of “alive” vs. “dead”? 
Identified 3 subclasses of children with septic shock based exclusively on differential gene expression patterns.
Identification of expression-based subclasses

SUBCLASS A

SUBCLASS B

SUBCLASS C
**Post-hoc** phenotype analysis of expression-based subclasses

- Subclass A patients had significantly higher:
  - *Illness severity*
  - *Rates of organ failure*
  - *Mortality (36% vs. 11*)
Identification of expression-based subclasses
Can we get this type of classification closer to the bedside?

• Identified top 100 class-defining genes based on leave-one-out cross validation procedures (Support Vector Machine).
• Depict expression of these 100 genes using “GEDI” mosaics.
http://www.childrenshospital.org/research/ingber/GEDI/gedihome.htm

“Sample-oriented” rather than “gene-oriented”

Graphical output: mosaics / engrams that give a "face" to microarray data (SOM).

Intuitive pattern recognition.
Group A  Group B  Group C

REFERENCE MOSAICS

ALL TRUE GROUP A PATIENTS

INDIVIDUAL PATIENT MOSAICS
REFERENCE MOSAICS

ALL TRUE GROUP B PATIENTS

INDIVIDUAL PATIENT MOSAICS
Toward a clinically feasible gene expression-based subclassification strategy for septic shock: Proof of concept

Hector R. Wong, MD; Derek S. Wheeler, MD; Ken Tegtmeier, MD; Sue E. Poynter, MD; Jennifer M. Kaplan, MD, MS; Ranjit S. Chima, MD; Erika Stalets, MD; Rajit K. Basu, MD; Lesley A. Doughty, MD

Objective: To develop a clinically feasible stratification strategy for pediatric septic shock, using gene expression mosaics and a 100-gene signature representing the first 24 hrs of admission to the pediatric intensive care unit.

Design: Prospective, observational study involving microarray-based bioinformatics.

Setting: Multiple pediatric intensive care units in the United States.

Patients: Ninety-eight children with septic shock.

Interventions: None other than standard care.

Measurements and Main Results: Patients were classified into three previously published, genome-wide, expression-based subclasses (subclasses A, B, and C) having clinically relevant phenotypic differences. The class-defining 100-gene signature was depicted for each individual patient, using mosaics generated by the Gene Expression Dynamics Inspector (GEDI). Composite mosaics were generated representing the average expression patterns for each of the three subclasses. Nine individual clinicians served as blinded evaluators. Each evaluator was shown the 98 individual patient mosaics and asked to classify each patient into one of the three subclasses, using the composite mosaics as the reference point. The respective sensitivities, specificities, positive predictive values, and negative predictive values of the subclassification strategy were ≥84% across the three subclasses. The classification strategy also generated positive likelihood ratios of ≥16.8 and negative likelihood ratios of ≤0.2 across the three subclasses. The κ coefficient across all possible interevaluator comparisons was 0.81.

Conclusions: We have provided initial evidence (proof of concept) for a clinically feasible and robust stratification strategy for pediatric septic shock based on a 100-gene signature and gene expression mosaics. (Crit Care Med 2010; 38:1955–1961)

Key Words: microarray; gene expression; stratification; staging; septic shock; pediatrics
Expression based subclasses

• Gene expression-based subclasses of patients with septic shock exist.
• The subclasses can be identified by clinicians using gene expression mosaics.
• The subclasses can be identified in the first 24 hours of admission.
• The subclasses have clinically relevant phenotypes.
• Recently validated in a different patient cohort.
• Subclass identification has the potential to direct therapy (i.e. “theragnostics”).
100 subclass-defining genes

Correspond to adaptive immunity and glucocorticoid receptor signaling

These genes are \textit{repressed} in the subclass A patients, relative to subclasses B and C
Other biomarker work in progress…

• Discovery of biomarkers to predict severe, persistent, septic shock-associated kidney injury (SSAKI).
• Meeting criteria renal failure at 7 days post ICU admission.
• “Resuscitation unresponsive” renal failure.
• Have identified 21 genes that predict SSAKI, within the first 24 hours of ICU admission, with 98% sensitivity and 80% specificity.
Goals of expression profiling

• Biomarker and gene expression-based stratification.

• Discovery of novel targets and pathways.
Potential Targets and Strategies

- Zinc
- Matrix metallopeptidase 8
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Genome-level expression profiling in children with septic shock

Large number of genes that directly depend on zinc homeostasis or play a direct role in zinc homeostasis.

Functional validation: nonsurvivors have abnormally low serum zinc concentrations.
Decreased serum zinc levels in nonsurvivors of septic shock

<table>
<thead>
<tr>
<th>Serum Zinc (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivors</td>
</tr>
<tr>
<td>Nonsurvivors</td>
</tr>
</tbody>
</table>

*Significant difference between groups.
Genome level expression profiling in children with septic shock

Large number of genes that directly depend on zinc homeostasis or play a direct role in zinc homeostasis.

**Functional validation:** nonsurvivors have abnormally low serum zinc concentrations.

Large number of genes involved in **T cell function** and **antigen presentation**.

These repression patterns are evident within 24 hours of admission, and persist at least into 72 hours of illness.
NORMAL ZINC HOMEOSTASIS IS ABSOLUTELY CRITICAL FOR NORMAL FUNCTIONING OF THE IMMUNE SYSTEM.
Zinc supplementation in sepsis?

- Animal models demonstrate efficacy.
- Just completed phase 1 trial of intravenous zinc supplementation in critically ill children.
- Adult phase 2 studies commencing.
Potential Targets and Strategies

• Zinc
• Matrix metallopeptidase 8
MMP-8

• Matrix metalloproteinase-8 (a.k.a. neutrophil collagenase).
• Primarily involved in degradation of extracellular matrix (collagen type 1).
• Also involved in chemokine processing.
• MMP-8 null animals are viable and are resistant to TNF-mediated acute hepatitis.
MMP-8 is consistently the highest expressed gene in patients with sepsis or septic shock in all of our microarray studies thus far.
MMP-8 mRNA expression increases with illness severity in sepsis and septic shock.

# $p < 0.05$ vs. control; * $p < 0.05$ sepsis vs. septic shock.
MMP-8 plasma activity increases with illness severity in sepsis and septic shock.

# p < 0.05 vs. control and sepsis
MMP-8 mRNA expression is higher in septic shock nonsurvivors vs. survivors

* p < 0.05 vs. survivors
Higher MMP-8 mRNA expression correlates with more organ failure (n = 180)

# $p < 0.05$ vs. 1$\text{st}$ and 2$\text{nd}$ quartiles
Maybe MMP-8 plays an important role in sepsis...
Survival study: CLP in wild-type vs. MMP null mice

N = 20 per group
Other observations regarding MMP-8 in sepsis

• MMP-8 null mice have less inflammation after CLP.
  – Tissue neutrophil infiltration.
  – Systemic cytokines and NF-κB activation.
  – But clear bacteria effectively.

• We can fully replicate the MMP-8 null phenotype in wild-type animals treated with 2 different classes of MMP-8 inhibitors after CLP.
Phosphonic acid-based MMP-8 inhibitor is beneficial in murine sepsis

Survival Proportion

Time (Hours)

0.0 0.2 0.4 0.6 0.8 1.0

0 20 40 60 80 100 120 140 160

Vehicle

Inhibitor
MMP-8 as a novel therapeutic target in septic shock?

• Inhibition of MMPs was major focus in the cancer field about 10 years ago.
• Failed.
• Many compounds on the shelves of pharmaceutical companies .........
A case for development-based therapeutic strategies

- Identified genes differentially regulated across 4 developmental age groups (n = 180 patients with septic shock):
  - *Neonate*: 0 to 28 days.
  - *Infant*: 1 month through 1 year.
  - *Toddler*: 2 through 5 years.
  - *School age*: 6 through 10 years.
TREM-1: Triggering receptor expressed on myeloid cells; critical for amplifying inflammation during infection.

Genes corresponding to TREM-1 signaling are repressed in the “neonate” group.
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Inhibition of TREM-1 is being considered as a therapeutic strategy for septic shock.

Inhibition of TREM-1 may not be biologically indicated in the neonate group.
Funding Acknowledgement

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Thank You