Sepsis Genomics

Gene Expression to Stratify Sepsis

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Canada Critical Care Forum
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Stratification of Sepsis

Why?

Stratification

Inform patient selection for clinical trials

Prognostic Enrichment

Predictive Enrichment
Enrichment

According to the FDA

• The prospective use of any patient characteristic to select a study population in which detection of a drug or intervention effect is more likely than it would be in an unselected population.
Prognostic Enrichment

According to the FDA

- Choosing patients with a greater likelihood of having a disease-related event.
- No effect on relative effect.
- But, increases absolute effect.
- Sample size $\rightarrow$ effect size and event rate.
- Prognostic enrichment $\rightarrow$ decrease the necessary sample size.
Predictive Enrichment

According to the FDA

• Choosing patients more likely to respond to an intervention or drug.
• Can lead to larger effect size, both absolute and relative.
• Sample size \(\rightarrow\) effect size and event rate.
• Predictive enrichment \(\rightarrow\) decrease the necessary sample size.
• *Right drug/intervention for the right patient.*
Transcriptomics

Discover biomarkers and gene expression signatures for **stratification**

- **Prognostic Enrichment Strategies**
- **Predictive Enrichment Strategies**
Transcriptomics

Discover biomarkers and gene expression signatures for **stratification**

- **Prognostic Enrichment Strategies**
- **Predictive Enrichment Strategies**
Developing a Clinically Feasible Personalized Medicine Approach to Pediatric Septic Shock

Hector R. Wong1, 2, Natalie Z. Cvijanovich3, Nick Anas4, Geoffrey L. Allen5, Neal J. Thomas6, Michael T. Bigham7, Scott L. Weiss8, Julie Fitzgerald9, Paul A. Checchia9, Keith Meyer10, Thomas P. Shanley11, Michael Quasney11, Mark Hall12, Rainer Gedeit13, Robert J. Freishtat14, Jeffrey Nowak15, Raj S. Shekhar16, Shira Gertz17, Emily Dawson18, Kelli Howard1, Kelli Harmon1, Eileen Beckman1, Erin Frank1, and Christopher J. Lindsell19

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Gene expression-based endotypes of septic shock
Gene expression based endotypes of septic shock

• 300 patients combined in derivation and test cohorts.
• Endotype A patients are younger, “sicker”, and have worse outcomes.
• Logistic regression adjusting for illness severity, age, and comorbidity: *allocation to endotype A is independently associated with mortality (O.R. = 2.8).*
Gene expression based endotypes of septic shock

- 100 genes corresponding to adaptive immunity and glucocorticoid receptor signaling.
- **Repressed** in endotype A.
- Adaptive immunity enhancing therapies are now being considered for sepsis.
- Predictive enrichment?
Gene expression based endotypes of septic shock

- 100 genes corresponding to adaptive immunity and glucocorticoid receptor signaling.
- **Repressed** in endotype A.
- The role of adjunctive corticosteroids remains a controversy in the field
- Predictive enrichment?
Gene expression based endotypes of septic shock

Prescription of corticosteroids independently associated with 4 times the risk of mortality
Preliminary Analysis

Temporal Endotype Changes

• All of the previous data involved “day 1”.
• Do patients change endotype over time and how do those changes associate with outcome?
• 192 patients assigned endotypes on “day 1” and “day 3”
• Preliminary results:
  – A → A (n = 41); 15% mortality
  – A → B (n = 33); 18% mortality
  – B → B (n = 86); 6% mortality
  – B → A (n = 32); 3% morality
• Adjunctive corticosteroids associated with 11 times the risk of mortality in the A → A group.
Gene expression based endotypes of septic shock

Endotype A

Endotype B

Feasible approach to predictive enrichment?
Transcriptomics

Discover biomarkers and gene expression signatures for *stratification*

Prognostic Enrichment Strategies

Predictive Enrichment Strategies
Identification of genes associated with mortality in pediatric septic shock

Prognostic Enrichment Genes

ANOVA; FDR 5%
Genes differentially regulated: survivors vs. non-survivors

Support Vector Machines: Top 5% mortality predictor genes

137

4,397
Identification of genes associated with mortality in pediatric septic shock

Prognostic Enrichment Genes

ANOVA; FDR 5%
Genes differentially regulated: survivors vs. non-survivors

Support Vector Machines: Top 5% mortality predictor genes

20

4,280

117

ANOVA; FDR 5%
Genes differentially regulated: survivors vs. non-survivors

Support Vector Machines: Top 5% mortality predictor genes
Identification of genes associated with mortality in pediatric septic shock

Prognostic Enrichment Genes

Working list of candidate prognostic enrichment (stratification) genes
Final Selection of Biomarkers

• Which genes are biologically plausible?
• For which genes can we feasibly measure the protein product in the serum compartment?
## Candidate sepsis stratification biomarkers

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL3</td>
<td>C-C chemokine ligand 3; a.k.a. MIP-1α</td>
</tr>
<tr>
<td>LCN2</td>
<td>Lipocalin 2; a.k.a. NGAL</td>
</tr>
<tr>
<td>MMP8</td>
<td>Matrix metallopeptidase 8; a.k.a. neutrophil collagenase</td>
</tr>
<tr>
<td>RETN</td>
<td>Resistin</td>
</tr>
<tr>
<td>THBS</td>
<td>Thrombospondin 1</td>
</tr>
<tr>
<td>GZMB</td>
<td>Granzyme B</td>
</tr>
<tr>
<td>HSPA1B</td>
<td>Heat shock protein 70kDa 1B</td>
</tr>
<tr>
<td>CCL4</td>
<td>C-C chemokine ligand 4; a.k.a. MIP-1β</td>
</tr>
<tr>
<td>IL8</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>LTF</td>
<td>Lactotransferrin</td>
</tr>
<tr>
<td>ELA2</td>
<td>Neutrophil elastase 1</td>
</tr>
<tr>
<td>IL1A</td>
<td>Interleukin 1α</td>
</tr>
</tbody>
</table>
PERSEVERE

• PEdiatRic SEpsis biomarker Er Risk modEl.
• Multi-biomarker-based risk model to assign a mortality probability for children with septic shock.
• CART methodology to derive a decision tree.
Root N = 335

CCL3 ≤ 160 N = 234

- HSPA1B ≤ 3.27E6 N = 207
  - Outcome Number Rate
    - Death 8 0.039
    - Survived 199 0.961

- HSPA1B > 3.27E6 N = 27
  - Outcome Number Rate
    - Death 14 0.060
    - Survived 220 0.940

CCL3 > 160 N = 121

- IL8 ≤ 507 N = 55
  - Outcome Number Rate
    - Death 6 0.222
    - Survived 21 0.778

- IL8 > 507 N = 66
  - Outcome Number Rate
    - Death 22 0.333
    - Survived 44 0.667

IL8 ≤ 829 N = 174

- IL8 > 829 N = 33
  - Outcome Number Rate
    - Death 6 0.182
    - Survived 27 0.818

GZMB ≤ 55 N = 30

- MMP8 ≤ 47513 N = 40
  - Outcome Number Rate
    - Death 1 0.025
    - Survived 39 0.975

- MMP8 > 47513 N = 15
  - Outcome Number Rate
    - Death 4 0.267
    - Survived 11 0.733

GZMB > 55 N = 36

Age ≤ 0.5 years N = 8

- Age > 0.5 years N = 22
  - Outcome Number Rate
    - Death 5 0.625
    - Survived 3 0.375
    - Death 0 0.000
    - Survived 22 1.000
### Outcome Analysis

#### Root Node
- **N = 335**
- **Death:** 41, **Rate:** 0.115
- **Survived:** 314, **Rate:** 0.885

#### CCL3 ≤ 160, N = 234
- **Death:** 14, **Rate:** 0.060
- **Survived:** 220, **Rate:** 0.940

#### CCL3 > 160, N = 121
- **Death:** 27, **Rate:** 0.223
- **Survived:** 94, **Rate:** 0.777

#### HSPA1B ≤ 3.27E6, N = 207
- **Death:** 8, **Rate:** 0.039
- **Survived:** 199, **Rate:** 0.961

#### HSPA1B > 3.27E6, N = 27
- **Death:** 6, **Rate:** 0.222
- **Survived:** 21, **Rate:** 0.778

#### IL8 ≤ 507, N = 55
- **Death:** 5, **Rate:** 0.091
- **Survived:** 50, **Rate:** 0.909

#### IL8 > 507, N = 66
- **Death:** 22, **Rate:** 0.333
- **Survived:** 44, **Rate:** 0.667

#### IL8 ≤ 829, N = 174
- **Death:** 2, **Rate:** 0.011
- **Survived:** 172, **Rate:** 0.989

#### IL8 > 829, N = 33
- **Death:** 6, **Rate:** 0.182
- **Survived:** 27, **Rate:** 0.818

#### GZMB ≤ 55, N = 30
- **Death:** 5, **Rate:** 0.167
- **Survived:** 25, **Rate:** 0.833

#### GZMB > 55, N = 36
- **Death:** 17, **Rate:** 0.472
- **Survived:** 19, **Rate:** 0.528

#### MMP8 ≤ 47513, N = 40
- **Death:** 1, **Rate:** 0.025
- **Survived:** 39, **Rate:** 0.975

#### MMP8 > 47513, N = 15
- **Death:** 4, **Rate:** 0.267
- **Survived:** 11, **Rate:** 0.733

#### Age ≤ 0.5 years, N = 8
- **Death:** 5, **Rate:** 0.625
- **Survived:** 3, **Rate:** 0.375

#### Age > 0.5 years, N = 22
- **Death:** 0, **Rate:** 0.000
- **Survived:** 22, **Rate:** 1.000

### TERMINAL NODE
Low risk terminal nodes
N = 236
Mortality risk: 0.0 to 2.5%
<table>
<thead>
<tr>
<th>Feature</th>
<th>Outcome</th>
<th>Number</th>
<th>Rate</th>
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<td>1.000</td>
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Intermediate risk terminal nodes
N = 75
Mortality risk: 18.2 to 26.7%
High risk terminal nodes
N = 44
Mortality risk: 47.2 to 62.5%
Test characteristics of PERSEVERE

<table>
<thead>
<tr>
<th></th>
<th>Non-survivor</th>
<th>Survivor</th>
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<tr>
<td>Predicted Non-Survivor</td>
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## Test characteristics of PERSEVERE

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<tr>
<td>Predicted Non-Survivor</td>
<td>38</td>
<td>81</td>
</tr>
<tr>
<td>Predicted Survivor</td>
<td>3</td>
<td>233</td>
</tr>
</tbody>
</table>

- PPV: 32% (CI 24% to 41%)
- +LR: 3.6 (CI 2.9 to 4.4)
- NPV: 99% (CI 96% to 100%)
- -LR: 0.1 (CI 0.0 to 0.3)

- Sensitivity: 93% (CI 79% to 98%)
- Specificity: 74% (CI 69% to 79%)

Excellent performance during prospective validation.

AUC = 0.883
Related Developments

• Have derived and validated an analogous model for adults with septic shock.
  – *More than 800 subjects representing 3 countries.*

• Have derived and validated a temporal version of the model that takes into account changes in biomarker concentrations over 3 days.
  – *Adjunct monitor for therapeutic effectiveness.*
Using PERSEVERE for Prognostic Enrichment

- Trial simulation.
- Population: Children with septic shock and “TAMOF”.
- Intervention: plasmapheresis.

ClinicalTrials.gov
A service of the U.S. National Institutes of Health

Patient Plasma Response and Outcome in Septic Shock With Thrombocytopenia Associated Multiple Organ Failure in Children (TAMOF)

The recruitment status of this study is unknown because the information has not been verified recently.

Verified May 2009 by Children’s Healthcare of Atlanta.
Recruitment status was Recruiting

Sponsor:
Children’s Healthcare of Atlanta

Information provided by (Responsible Party):
Children’s Healthcare of Atlanta

ClinicalTrials.gov Identifier:
NCT00118664

First received: July 1, 2005
Last updated: March 14, 2012
Last verified: May 2009
History of Changes
Using PERSEVERE for Prognostic Enrichment

108 patients with TAMOF + eligible for plasmapheresis
Overall mortality = 38%

PERSEVERE-based mortality risk

- 39 True Positives
- 35 False Positives
- 2 False Negatives
- 32 True Negatives
Using PERSEVERE for Prognostic Enrichment

108 patients with TAMOF + eligible for plasmapheresis

Overall mortality = 38%

PERSEVERE-based mortality risk

Low mortality risk group EXCLUDED from the trial

39 True Positives

35 False Positives

2 False Negatives

32 True Negatives
Using PERSEVERE for Prognostic Enrichment

108 patients with TAMOF + eligible for plasmapheresis
Overall mortality = 38%

Higher mortality risk group INCLUDED in the trial

Selects a population with an overall mortality of 53%

39 True Positives
35 False Positives
32 True Negatives
Using PERSEVERE for Prognostic Enrichment

108 patients with TAMOF + eligible for plasmapheresis
Overall mortality = 38%

Higher mortality risk group INCLUDED in the trial
Selects a population with an overall mortality of 53%
Increased event rate: “prognostic enrichment”

- 39 True Positives
- 35 False Positives
Using PERSEVERE for Prognostic Enrichment

<table>
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<th>Relative mortality reduction with plasmapheresis</th>
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</tr>
<tr>
<td>50%</td>
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Power = 0.8; alpha = 0.05
Using PERSEVERE for Prognostic Enrichment

<table>
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<th>Relative mortality reduction with plasmapheresis</th>
<th>No. of un-stratified patients required in each study arm</th>
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<tr>
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Using PERSEVERE for Prognostic Enrichment

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<th>No. of <strong>un-stratified</strong> patients required in each study arm 38% Mortality</th>
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<td>10%</td>
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Power = 0.8; alpha = 0.05
Using PERSEVERE for Prognostic Enrichment

**Prognostic Enrichment:**
- Select a population with a greater event rate.
- Increase the absolute effect size.
- Reduce the number of patients necessary for a trial

<table>
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<tr>
<td>30%</td>
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<td>2559</td>
<td>1434</td>
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Power = 0.8; alpha = 0.05
Identification of genes associated with mortality in pediatric septic shock

Prognostic Enrichment Genes

Working list of candidate prognostic enrichment (stratification) genes
The 12 PERSEVERE biomarkers were selected from this candidate list of 117 genes.

105 candidate genes were “left on the table”
Stratification of septic shock using previously unconsidered candidate genes

• Preliminary modeling
  – Six models having AUCs > 0.9
  – “Best” model: AUC 0.97, Sensitivity 100%, Specificity 89%

• Dilemma:
  – Best performing model with no biological information
  – Vs.
  – Excellent performing model with interesting biological information
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• Keith Meyer, MD: Miami Children’s Hospital, Miami, FL.
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• Mark Hall, MD: Nationwide Children’s Hospital, Columbus, OH.
• Rainer Gedeit, MD: Children’s Hospital of Wisconsin, Milwaukee, WI.
• Robert Freishtat, MD: Children’s National Medical Center, Washington, DC.
• Jeffery Nowak, MD: Children’s Hospital and Clinics of Minnesota, Minneapolis, MN.
• Raj Shekhar, MD: Riley Hospital for Children, Indianapolis, IN.
• Shira Gertz, MD: Joseph M. Sanzari Children’s Hospital, Hackensack, NJ.
• Emily Dawson, MD: The University of Chicago Comer Children’s Hospital, Chicago, IL.
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- Patrick Lahni
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- Will Hanna
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