Defining ARDS with Molecules or The Value of Combining Biological and Clinical Measurements in ARDS

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Disclosures

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GlaxoSmithKline grant - pathogenesis of ARDS from sepsis
Objectives

* Evidence for the value of biology in characterizing a more comprehensive phenotype of ARDS, including patients with early acute lung injury

* Treatment Implications for integrating both biologic and clinical variables in ARDS
Risk of Mortality in ARDS Depends in Part on the Clinical Risk Factors

The magnitude of injury to the lung endothelium and epithelium and the responses of inflammatory cells are not the same among different patient groups with ARDS.

Ware & Matthay; NEJM, 2000
Biological Heterogeneity in ARDS: Trauma vs. Non-trauma

Table 5. Bivariate and multivariate analysis of baseline biomarker levels in patients with acute lung injury

<table>
<thead>
<tr>
<th>Biomarker, Day 0</th>
<th>n</th>
<th>Median Value, Trauma</th>
<th>Median Value, Nontrauma</th>
<th>Unadjusted p Value</th>
<th>Multivariable p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1, ng/mL</td>
<td>1307</td>
<td>410 (272–738)</td>
<td>765 (480–1223)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SP-D, ng/mL</td>
<td>1075</td>
<td>58 (37–94)</td>
<td>95 (45–211)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Protein C, % of control</td>
<td>1308</td>
<td>58 (45–78)</td>
<td>53 (35–84)</td>
<td>&lt;.014</td>
<td>.07</td>
</tr>
<tr>
<td>vWF, % of control</td>
<td>1088</td>
<td>226 (142–331)</td>
<td>355 (215–551)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PAI-1, ng/mL</td>
<td>1304</td>
<td>56 (36–104)</td>
<td>70 (37–157)</td>
<td>.045</td>
<td>.95</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>1302</td>
<td>300 (128–674)</td>
<td>259 (100–850)</td>
<td>.44</td>
<td>.047</td>
</tr>
<tr>
<td>IL-8, pg/mL</td>
<td>1309</td>
<td>21 (20–59)</td>
<td>44 (20–103)</td>
<td>&lt;.002</td>
<td>.30</td>
</tr>
<tr>
<td>sTNFr-1, pg/mL</td>
<td>1091</td>
<td>2535 (1945–3332)</td>
<td>3748 (2340–7661)</td>
<td>&lt;.001</td>
<td>&lt;.004</td>
</tr>
</tbody>
</table>
How can biomarkers help us test new treatments for ARDS?

- Cardiologists have troponin & BNP
- Oncologists use biologic markers for classification and treatment decisions
- Asthma research is using Th1 and Th2 and other biomarkers
- In Sepsis we use lactate -- what can we use in ARDS?

- How can biomarkers help us in ARDS?
  - New insights into pathogenesis, heterogeneity and the responses to treatment
  - Identify patients earlier (prediction)
  - Identify patients at highest risk (risk stratification) and thus provide more focus to clinical therapeutics
How can biomarkers help us in ARDS?
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Impact of Lung Protective Ventilation on Biological Markers of Lung Injury in ARDS Network Trial

Biological Impact of Lung Protective Ventilation in ARDS Net Trial

- Lower levels of IL-6
- Lower levels of IL-8
- Lower levels of sTNR1
- Reduced levels of SP-D
- Lower levels of RAGE

NEJM, 2000
RAGE and Mortality in ARDS Network Trial of Lung Protective Ventilation

Calfee CS et al, Thorax, 2008
Patients with High Baseline Plasma RAGE levels Had the Most Benefit from Low Tidal Volume

Calfee CS et al, Thorax, 2008
Angiopoietins 1/2: The Basics

* Ang-2 is a context-dependent ligand for Tie-2 receptor
* Released upon endothelial cell activation
* Promotes vessel destabilization and inflammation, increases permeability

David S et al, J Pharm Exp Ther 2013
Elevated Ang-2 in Plasma and Edema Fluid in ARDS

**Ang-2: Mediator of Endothelial Injury**

- Serum from septic patients with high Ang-2 levels disrupts endothelial architecture
- Enhances permeability to albumin in Transwell monolayer system

Parikh SM et al, PLOS Med 2007
Ang-2: Mediator of Organ Injury

- Mice injected with recombinant Ang-2 had significantly increased vascular permeability in multiple organs
- Increased extra-vascular lung water
- Increased histologic severity of lung injury

Parikh SM et al, PLOS Med 2007
Ang-2: Clinical Studies

* Multiple studies demonstrating elevated plasma levels in septic patients
* Frequently correlated with organ injury, mortality
* Dynamic marker

David S et al, CCM 2012
“Fluid Conservative” vs. “Fluid Liberal” Management of ALI/ARDS
Ventilator free days to day 28

Fluid Conservative: 14.55
Fluid Liberal: 12.09

P = 0.0002
Plasma angiopoietin-2 in clinical acute lung injury: Prognostic and pathogenetic significance

Carolyn S. Calfee, MD, MAS; Diana Gallagher, MD, MMSc; Jason Abbott, BS; B. Taylor Thompson, MD; Michael A. Matthay, MD; the NHLBI ARDS Network

* 1000 subjects enrolled in FACTT trial (NEJM 2006)

* Plasma Ang-2 at baseline significantly higher in nonsurvivors than survivors:
  * 10,674 pg/ml vs. 7854 pg/ml, p<0.0001
Modifying the Endothelium Via Intravascular Pressure

- Mechanically stretched endothelial cells promote inflammation
  - Cytokine secretion
  - Calcium mobilization

- P-selectin expression increases with increases in lung venular pressure

Kuebler et al, JCI 1999
Ang-2 is a Dynamic Marker in ARDS

* Rising Ang-2 levels from Day 0 to 3 were particularly poor prognostic sign
* Ang-2 levels were preferentially decreased by fluid conservative therapy (13.2% greater than fluid liberal group)
Two cohorts:

- Single center cohort: VALID Study (Vanderbilt)
  - 100 patients with ARDS and severe sepsis
  - 44 direct (pneumonia, aspiration); 56 indirect (non-pulmonary sepsis)
- Multicenter trial population: FACTT trial, NHBLI ARDS Network
  - 853 subjects
  - 620 with direct ARDS; 233 with indirect ARDS
Ang-2: Higher in Indirect ARDS in Single and Multicenter Studies

Ware et al, submitted, 2014
How can biomarkers help us in ARDS?

- New insights into pathogenesis, heterogeneity and the responses to treatment
- Identify patients earlier (prediction)
- Identify patients at highest risk (risk stratification) and thus provide more focus to clinical therapeutics
Most experimental lung injury therapies that are successful in animal models are administered EARLY

Low incidence of ARDS based on clinical risk factor alone

Prediction scores developed to increase detection of patients at highest risk
  - Most notably, the Lung Injury Prediction Score (LIPS)
  - Low positive predictive value: 18%

Few studies of predictive biomarkers, most in small subgroups

Major focus of the new NHLBI PETAL Network
Testing a Panel of Biomarkers: The EARLI Cohort

* Early Assessment of Renal and Lung Injury cohort at UCSF
* Critically ill patients enrolled in ER upon triage to ICU (n=230)
  * Excluded patients with ALI at baseline or in subsequent 6 hours (n=49) or no P/F ratio (n=14)
* Early biospecimen collection
* Measured 5 biomarkers in plasma:
  * sRAGE: Epithelial injury, inflammation
  * Angiopoietins 1 and 2, vWF: Endothelial injury/permeability
  * Interleukin-8: Inflammation

Agrawal et al, AJRCCM 2013
Plasma Ang-2 and IL-8 were significantly higher among those who went on to develop ALI than those who did not.

Association of Ang-2 with future development of ALI was robust to adjustment for sepsis, severity of illness.

IL-8 was somewhat less robust.
Combination of LIPS > 4 and Ang-2 ≥ 12,100 pg/ml:
--PPV 40%
--NPV 100%

Figure 2: Receiver Operating Characteristic (ROC) curves. Ang-2 (Area under ROC[AUROC]: 0.74, 95%CI: 0.62-0.84) and the Lung Injury Prediction Score (LIPS, AUROC: 0.74, 95%CI: 0.65-0.84) each show good discrimination between those who will develop ALI and those who will not, but the combination of both LIPS and Ang-2 is superior to either alone (AUROC: 0.84, p=0.05 for test of equality of AUROC compared to LIPS alone).
Additive Value of Biomarkers

* How can biomarkers help us in ARDS?
  * New insights into pathogenesis, heterogeneity and the responses to treatment
  * Identify patients earlier (prediction)
  * Identify patients at highest risk (risk stratification) and thus provide more focus to clinical therapeutics
Molecular Phenotyping in ARDS

* Hypothesis: ARDS encompasses more than one distinct subphenotype, as defined by natural history, clinical characteristics, biomarkers, +/- response to therapy

* Study population: Two ARDSnet clinical trials
  * First cohort: ARMA (low tidal volume only; n=479)
  * Second cohort: ALVEOLI (low vs. high PEEP; n=549)

Calfee CS et al, Lancet Resp Med 2014
Latent Class Models

* Based on hypothesis that there are a number of identifiable classes within a broader group of patients
  * Underlying approaches developed > 100 years ago in the study of crab evolution
  * Computationally intensive
  * Does data distribution better fit with 1 class or 2 (or with 3, 4, …)?
Latent Class Modeling Procedures

- Clinical and biomarker data from baseline in each study as inputs that “identify” class
  - Analysis conducted independently in each cohort
- Then tested association of class with clinical outcomes
- Finally, tested whether response to PEEP in ALVEOLI differs based on class assignment

### Latent Class Model, ARMA Cohort

<table>
<thead>
<tr>
<th># classes</th>
<th>BIC*</th>
<th>Entropy**</th>
<th>N₁</th>
<th>N₂</th>
<th>N₃</th>
<th>N₄</th>
<th>N₅</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>39947.9</td>
<td>.78</td>
<td>318</td>
<td>155</td>
<td></td>
<td></td>
<td></td>
<td>.036</td>
</tr>
<tr>
<td>3</td>
<td>39760.2</td>
<td>.88</td>
<td>308</td>
<td>119</td>
<td>46</td>
<td></td>
<td></td>
<td>.59</td>
</tr>
<tr>
<td>4</td>
<td>39656.7</td>
<td>.86</td>
<td>212</td>
<td>126</td>
<td>43</td>
<td>92</td>
<td></td>
<td>.28</td>
</tr>
<tr>
<td>5</td>
<td>39583.8</td>
<td>.86</td>
<td>150</td>
<td>120</td>
<td>36</td>
<td>36</td>
<td>131</td>
<td>.64</td>
</tr>
</tbody>
</table>

* Bayesian Information Criterion: Is additional class worth added complexity? Decrease suggests yes.
** Entropy is an index of how well the classes are separated. It ranges from zero to one and values around .8 and up are generally considered a sign of a useful model.

ARMA Cohort, Continuous Variables
ARMA Cohort, Categorical Variables

p=0.007 for gender, p<0.0001 for others

Calfee CS et al, Lancet Resp Med, in press
ALVEOLI Cohort, Continuous Variables

[Graph showing two lines labeled Class 1 and Class 2, with variable values decreasing over time.]
ALVEOLI Cohort, Categorical Variables

p=NS for female, 0.004 for race, <0.0001 for others

Clinical Outcomes Differ By Class

<table>
<thead>
<tr>
<th>Clinical Outcome</th>
<th>ARMA Cohort</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Class 1</td>
</tr>
<tr>
<td>90d Mortality</td>
<td>23%</td>
</tr>
<tr>
<td>Ventilator Free Days</td>
<td>17.8</td>
</tr>
<tr>
<td>Organ Failure Free Days</td>
<td>14.5</td>
</tr>
</tbody>
</table>

*Analyses adjusted for uncertainty re: class assignment; unadjusted analyses were similar*
Clinical Outcomes Differ By Class

**Clinical Outcomes**

<table>
<thead>
<tr>
<th>Clinical Outcome</th>
<th>ARMA Cohort</th>
<th>ALVEOLI Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Class 1</td>
<td>Class 2</td>
</tr>
<tr>
<td><strong>90d Mortality</strong></td>
<td>23%</td>
<td>44%</td>
</tr>
<tr>
<td><strong>Ventilator Free Days</strong></td>
<td>17.8</td>
<td>7.7</td>
</tr>
<tr>
<td><strong>Organ Failure Free Days</strong></td>
<td>14.5</td>
<td>8.0</td>
</tr>
</tbody>
</table>

*Analyses adjusted for uncertainty re: class assignment; unadjusted analyses were similar.*
* ALVEOLI was considered a “negative” study
* We asked whether there was evidence of response to randomly assigned PEEP within either class
* Significant interaction for mortality (p=0.049)

<table>
<thead>
<tr>
<th></th>
<th>Mortality in Class 1 (n=404)</th>
<th>Mortality in Class 2 (n=145)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low PEEP</td>
<td>16%</td>
<td>51%</td>
</tr>
<tr>
<td>High PEEP</td>
<td>24%</td>
<td>40%</td>
</tr>
</tbody>
</table>

Similarly, significant interactions between PEEP and class for ventilator-free days (p=0.018) and organ failure free days (p=0.003)

Is class simply a surrogate for severity of disease? No.
  * No significant interactions with APACHE (p=0.58-0.99)
Identification of Class Using 3 Markers

* Exploratory analysis: Three variables with greatest difference in mean between the classes in ARMA as predictors in ROC analysis
  * IL-6, sTNFr-1, vasopressor use
  * Area under the curve in ALVEOLI: 0.93
  * With addition of IL-8, bicarbonate: 0.97
Summary of Results

* Latent class analysis carried out independently on two separate cohorts reveals two subphenotypes within ARDS
  * Very similar findings between the two cohorts, despite considerable differences in clinical and biomarker data
* Class 2 is characterized by much higher levels of inflammatory biomarkers, more shock, more acidosis, less trauma, more sepsis
* Class 2 has much worse clinical outcomes than Class 1
* Response to treatment may differ based on class

“Hyper-inflammatory” Class 2 is clinically recognizable

- High levels of inflammatory biomarkers, severe shock and acidosis
- Yet class cannot be identified on basis of any single variable alone, including ARDS risk factor, vasopressors

- Not simply a reflection of organ failure severity
  - Severity of ARDS, renal failure, hepatic failure were not cardinal features of either phenotype

- Plasma protein biomarkers contributed prominently to phenotype
  - Development of rapid assay will be key for translating to trials
The addition of biologic markers to clinical variables in ARDS provides

* More insight into pathogenesis
* Enhanced ability to determine prognosis
* Enhanced capacity to predict development of ARDS
* Probably an improved ability to identify patients for clinical therapeutic interventions, including ventilator and pharmacologic interventions
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