Biomarkers of Traumatic Brain Injury

Cameron Jeter, Ph.D.
Traumatic Brain Injury (TBI)

- Vehicle accidents, falls, violence
- Blunt, penetrating, blast
- Mild, moderate, severe
- Primary and secondary injuries

Each Year in the U.S.
- 1 million head-injured treated in emergency departments
- 50,000 die from head injury; 80,000 with life long disabilities
- Higher than incidence of Alzheimer’s and Parkinson’s combined

#1 killer of individuals under 45 years of age
Cost exceeds $76 billion
Famous Cases of TBI

Bob Woodruff: Blast Injury

Dave Duerson: Chronic Traumatic Encephalopathy

Gabrielle Giffords: Gun shot wound to head

Natasha Richardson: Epidural Hematoma
TBI Pathophysiology

Modified from Ottens et al/ Mass Spec Rev 2006
What is a Biomarker?

An indicator of a specific disease state.

**EXAMPLES**
- Body temperature
- Blood pressure
- Imaging
- Genetic variants
- Proteins, metabolites, inflammatory markers

**APPLICATIONS**
- Differentiation of disease subtypes
- Early detection of subclinical disease
- Pathophysiological underpinnings
- Prognostic assessment of disease course
- Selection of appropriate treatment
- Monitoring of response to therapy
An indicator of a specific disease state.

**Example Sources**

- Damaged tissue
- Cerebrospinal fluid
- Saliva
- Urine
- Blood

**Blood-Brain Barrier**

- Endothelial Cell
- Tight Junction
- Foot process of astrocyte
Contribution of Biomarkers to Clinical Care

- Can a biomarker be useful for patient care?
- Does the biomarker add new information?
- Will the biomarker help the clinician to manage patients?

Morrow & de Lemos *Circulation* 2007
Contribution of Biomarkers to Clinical Care

- Cancer antigen 125 (CA125) for Ovarian Cancer
- Prostate-specific antigen (PSA) for Prostate cancer
- Cardiac troponin T for Myocardial Infarction

Morrow & de Lemos Circulation 2007
TBI Pathophysiology

Modified from Ottens et al/ Mass Spec Rev 2006
Biomarker Discovery

**BIOMARKER DISCOVERY APPROACHES**

- **Top-down**
  - Glial fibrillary acidic protein (GFAP)
  - Neuron-specific enolase (NSE)
  - S100B
  - Cleaved-Tau

- **Bottom-up**
  - No prior assumptions (i.e., unbiased)
  - Would hate to miss an ideal, but unforeseen biomarker
Biomarker Discovery

Target selection
(e.g. mRNA, protein, peptide, metabolites, etc.)

Approach selection
(e.g. biased versus unbiased)

Sample collection

Sample processing
(e.g. extraction, fractionation, derivatization)

Sample analysis

Candidate selection and validation

Diagnostic accuracy determination

Biomarker signature
To determine if the blood plasma from brain-injured patients contains reproducible biomarkers that

• Are indicative of injury severity.

• Appear prior to elevations in intracranial pressure (ICP).
Collect serum samples from TBI patients and healthy volunteers

↓

Immunodepletion of high abundance serum proteins

↓

iTRAQ labeling of pooled serum samples from each group

↓

Validation of candidates using ELISA

↓

Test predictability of verified candidates using independent samples
Finding the Needles in the Haystack

12 of the most highly abundant serum proteins comprise ~95% of total protein content.
## Study Design

<table>
<thead>
<tr>
<th></th>
<th>HV n=20</th>
<th>Ortho Injury n=15</th>
<th>Mild TBI n=18</th>
<th>Severe TBI n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>25.15 ± 6.7</td>
<td>38.3 ± 15.5</td>
<td>36.5 ± 13.2</td>
<td>25.8 ± 15.5</td>
</tr>
<tr>
<td><strong>Gender (M:F)</strong></td>
<td>16:4</td>
<td>10:5</td>
<td>13:5</td>
<td>16:4</td>
</tr>
<tr>
<td><strong>Glasgow Coma Scale (GCS)</strong></td>
<td>NA</td>
<td>14.7 ± 1.0</td>
<td>14.7 ± 0.5</td>
<td>3.65 ± 1.2</td>
</tr>
<tr>
<td><strong>Injury Severity Score (ISS)</strong></td>
<td>NA</td>
<td>8.3 ± 4.0</td>
<td>5.4 ± 2.2</td>
<td>27.8 ± 7.8</td>
</tr>
</tbody>
</table>

Jeter et al., 2012
Arginine Metabolism

Jeter et al., 2012
Arginine after TBI

Jeter et al., 2012
Inflammation Signaling after TBI

Jeter et al., 2012
Urea Cycle after TBI

Jeter et al., 2012
Extracellular Remodeling after TBI

Jeter et al., 2012
Energy Metabolism after TBI

Jeter et al., 2012
Clinical case of elevated intracranial pressure
Elevated Intracranial Pressure (ICP)

- Develops within 72 hours of injury
- ICP elevated when > 25 mm Hg

**Consequences of Elevated ICP**
- Tissue herniation
- Cerebral hypoperfusion
- Ischemia
- Exacerbated brain damage
- Death
Rationale for markers of elevated ICP

- Early diagnosis prior to elevation of ICP
- Allow for appropriate clinical management
- Evacuation of “at risk” patients from far-forward battlefields and rural areas
Intracranial Pressure (ICP) after TBI

A. Peak ICP in severe TBI patients by study day

B. creatine

Jeter et al., 2012
Diagnostic Value of a Biomarker

**Sensitivity**
Percent true positive
Does not miss a diagnosis

**Specificity**
Percent true negative
Does not falsely diagnose

**Receiver Operating Characteristic (ROC) Curve**
Graph of sensitivity vs. 1-specificity
Diagnostic accuracy: area under the curve (AUC)

- AUC = 0.5 - 0.7  poor
- AUC = 0.7 - 0.8  fair
- AUC = 0.8 - 0.9  good
- AUC = 0.9 - 0.98 very good
- AUC = 0.98 - 1.0 excellent
Biomarkers of Elevated Intracranial Pressure

Jeter et al., 2012
Future Directions

VALIDATION
Verify biomarkers in new patient cohort

THERAPEUTIC INTERVENTIONS
Proline/hydroxyproline supplementation
Creatine supplementation
Other Identified Pathways of Interest

Tryptophan metabolism
- Kynurenine → Tryptophan → Serotonin
  - Indolepyruvate → Indole
  - Indoleacetic acid → Indoxyl
  - Indolepropionate (IPA) → Indoxyl sulfate

Branched chain amino acid catabolism
- Leucine
- 4-methyl-2-oxopentanoate
- Isovaleryl-CoA → Acetoacetate
- 3-methylcrotinonyl-CoA
- 3-hydroxy-3-methylglutaril-CoA

Aromatic amino acid metabolism
- Phenylalanine
  - Phenylpyruvate (PPA)
  - Phenylacetate (PAA)
  - Phenyllactate (PLA)
  - Phenylacetylglycine

Redox homeostasis
- Methionine
  - N,N-dimethyl glycine
  - Phosphatidyl-ethanolamine
  - Homocysteine
  - Choline
  - Cystathionine
  - δ-Ketobutyrate → 2-hydroxybutyrate

γ-glutamyl-amino acid
- 5-oxoproline → Cysteine
- Glutathione

Fatty Acid Synthesis
- Acetyl-CoA → Acetoacetate

Dopamine → Noradrenaline → Adrenaline
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