How to use biomarkers of traumatic and ischemic brain injury

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Outline

• Definition of biomarkers
• Potential utility of biomarkers
• Steps necessary to bring biomarkers to the bedside
• Systematic review of the literature in Ped TBI
• Some specific studies - TBI
• Global cerebral ischemia and cardiac arrest
Traumatic Brain Injury (TBI)

- Most common cause of death and acquired disability in children and young adults

**Causes**
- Falls
- MVA
- Bicycle
- Sports
- Assault

Definitions and Examples

- Analytes in biological samples
- Any measure that can predict a disease state or a response to a drug
- Examples
  - Physiological – Blood Pressure, Heart Rate
  - Laboratory – Troponin
  - Highly complex imaging modalities
  - Multi-marker genomic/proteomic panels
  - Proteins/lipids or metabolites in blood, CSF, saliva or urine
• Biomarkers have potential utility as diagnostic, prognostic, and therapeutic adjuncts in the setting of traumatic brain injury.

• They could be used to help determine which patients should receive which treatments.

• Two approaches are being used, namely, assessing markers of structural damage and quantifying mediators of the cellular, biochemical, or molecular cascades in secondary injury or repair.
Utility of biomarkers in disease

• Many biotechnology/pharmaceutical companies are interested in discovering and validating biomarkers as surrogate endpoints
  – Goal: Cost cutting
• $$$ millions have been spent on failed drug trials in critical care
• Many companies hope that ...If a new drug fails to improve the (surrogate) biomarker levels of disease then this drug should not be tested further and they should move on to the next drug
Problems with biomarker research

- Discovery must be done in one dataset and validation in another dataset
  - Often this sequence is not followed
- Sample sizes are often too low
- Retrospective datasets are used
- Sensitivity and specificity of tests vary from manufacturer to manufacturer
- Cross reactivity of the tests varies
- Quality of assays varies across laboratories
- Often there is a lack of knowledge of the biology ie. A lack of understanding of how the biomarkers reflect the mechanisms of traumatic brain injury. Fingerprints of injury (eg. metabolomics) are difficult to interpret.
Rationale for studying biomarkers to for prognosis

• TBI can have devastating effects on the long-term function of children and adults.
• We remain unable to predict long-term function and neuropsychological outcome in the acute period (i.e., in the ICU) post-injury.
• If we were able to accurately predict outcome early during hospitalization, we could stratify patients for rehabilitation interventions, to improve outcomes.
• End-of-life decisions are being made without accurate prognosis.
"My body is still in the intensive care unit"
History of biomarkers as tests of prognosis in TBI

• Single markers of structural damage to different brain cells (eg. S100B, neuron specific enolase) or small groups of molecules from the same family in serum and cerebrospinal fluid.
  – Good correlation with outcome but not highly sensitive or specific

• Evolved to more complex approaches:
  – Proteomics, lipidomics, metabolomics, multiplex immunoassays
The Molecular Fingerprint of the Neurovascular Unit

MOLECULAR FINGERPRINT → BLOOD → MOLECULAR FINGERPRINT

Endothelial cell

MOLECULAR FINGERPRINT → BRAIN → MOLECULAR FINGERPRINT

Astrocyte

MOLECULAR FINGERPRINT → CSF → MOLECULAR FINGERPRINT

Leukocyte

Glycoproteins

Basement membrane

Neuron

Oligodendrocyte
Protein and amino acid mediators of injury

Tsz-Yan (Milly) Lo

Cytokines, Adhesion molecules

Apoptotic proteins

Brain specific proteins

Excitatory amino acids

hsp

IEG

Time

Brain Trauma
Systematic review: Biomarkers which are associated with outcome in children with severe TBI?
Shibata ARO, LoT-Y, Hutchison JS, Guerguerian A-M

• Brain specific
  – Myelin Basic Protein (MBP), S100B, Glial Fibrillary Acid Protein (GFAP), Nerve Growth Factor (NGF), neuron specific enolase (NSE)

• Inflammatory
  – L-selectin, Interleukin (IL)-1β, IL-6, IL-8 and IL-10

• Apoptotic
  – Bcl-2

• Other
  – Heme oxygenase 1 (HO-1), Quinolinic acid, fibrin degradation products (FDP) and doublecortin (DCX).

• Amino acids
  – aspartate, glutamate and glycine
Biomarkers associated with age

• IL-10
• Caspase-1
• PARP
• Glutamate
• S-100b
• IL-6
• Trend: P-selectin, HO-1
Serum GFAP

GFAP Concentration (ng/ml)

ICU Day

1 2 3 4 5 6 7 8 9 10 11

Reference

Fraser D et al. PCCM 2010
Multi-variable receiver operating characteristic curve for predicting Glasgow outcome scale at 6 months following injury (N=28 children with TBI)

AUC 0.98
S100B + L-selectin
S100B + IL-6
Sensitivity 100%
Specificity 96%

Lo TY et al. J Neurotrauma 2009
More complex approaches to biomarker discovery research

- Proteomics
- Lipidomics
- Metabolomics
Hierarchical clustering of 95 differentially expressed proteins in comparison to admission GCS and S100B in children with TBI

# Brain-selective proteins identified in sera (8 hours post-TBI) of 6 paediatric patients

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Tissue specificity</th>
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</thead>
<tbody>
<tr>
<td>Spectrin alpha chain, brain</td>
<td>Non-erythroid; cleaved by calpain and caspase-3; detected in CSF after TBI</td>
</tr>
<tr>
<td><strong>Neuron-specific</strong> enolase (NSE)</td>
<td>The alpha/gamma heterodimer and the gamma/gamma homodimer found in neurons</td>
</tr>
<tr>
<td>Neurofilament triplet H protein (200 kDa neurofilament protein)</td>
<td>Neuron-specific cytoskeletal protein</td>
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<tr>
<td>Amyloid beta A4 protein precursor (APP) (ABPP)</td>
<td>Highly enriched in the brain</td>
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<tr>
<td>Prostaglandin-H2 D-isomerase precursor (PDG2 synthase)</td>
<td>Abundant in the brain and CNS where it is expressed in the blood-brain barrier and secreted into the CSF</td>
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<tr>
<td>Microtubule-associated protein tau (Neurofibrillary tangle protein)</td>
<td>Expressed in neurons. Isoform PNS-tau – in peripheral nervous system; others are expressed in the CNS</td>
</tr>
<tr>
<td>Contactin 3 precursor (Brain derived immunoglobulin superfamily protein- BIG-1)</td>
<td>Expressed in the brain – frontal and occipital lobe, cerebellum and amygdala</td>
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Objective: To determine if eicosanoids and leukotrienes are elevated in the serum of children with severe TBI compared to healthy controls

• Methods:
  – Measured 22 lipids in serum using mass spectrometry
  – Compared 6 severe TBI to 8 healthy controls

• Results:
  – 8/22 (36%) lipids were elevated in the serum of children with TBI compared to controls
Novel post-traumatic outcome prediction using acute serum levels of amino and organic acids Lo T et al. PCCM 2011 A112

• Objective: Determine if serum amino and organic acids at day 1 post-injury are associated with outcome at 6 months post-TBI in children

• Methods:
  – Measured 36 different amino and organic acids using mass spectrometry
  – Compared 4 children with bad outcome to 14 children with good outcome

• Results:
  – 6 amino acids ↓ in those with bad outcome
  – 3 – methyl-histidine ROC AUC 1.0
Multimodal approach to prognosis

• Clinical parameters
  – Eg. Severity of injury
• Serum and CSF biomarkers
• Novel MRI techniques
• Neuro electrophysiology
Perfusion CT

Sham

forebrain

hindbrain

no perfusion

2VO

MICe – John Sled
Systemic (serum) inflammatory response

<table>
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<tr>
<th></th>
<th>Sham</th>
<th>24h</th>
<th>72h</th>
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<tbody>
<tr>
<td><strong>IL-1beta</strong></td>
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<tr>
<td>pg/mg of total protein</td>
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<td><strong>sICAM-1</strong></td>
<td></td>
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<td>ng/mg of total protein</td>
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IL-1β expression in cerebral cortex

Sham

Ischemia
Neurologic Prognosis after Cardiac Arrest

G. Bryan Young, M.D.
Figure 2. Decision Algorithm for Use in Outcome Prediction for Comatose Survivors of Cardiac Arrest.

Major confounders include the use of neuromuscular blocking agents, large doses of sedative drugs, the use of hypothermia, the presence of organ failure, and shock. FPR denotes false positive rate, NSE neuron-specific enolase, and SSEP somatosensory evoked potential. Numbers in parentheses are 95% confidence intervals. Data are from Wijdicks et al. 22
Thank-you
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