Blood Biomarkers for Brain Injury

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TOPICS COVERED

What are we measuring?

How do markers get in the blood?

Examples of commonly used markers

Data from repetitive subconcussive impacts

Future of Blood Biomarkers
1.7 million TBIs per year in U.S.
1.4 million are classified as mild.
(Concussion/Subconcussion)

Concussion

Without radiological correlates
Most common form closed head injury
Proposed critical velocity at 25 feet/second
Headache
Depression
Loss of memory
Poor concentration
Change in personality
Cognitive Impairment

Effects of Concussion are cumulative.
Consequences of Mild Repetitive Trauma
Acceleration/Deceleration
Rotation /Torque
I nterneuron
Arteriole
Pyramidal neuron
Capillary
Pericyte
Astrocyte
To better understand potential implications for increased Expression OR Detection, need to consider the normal function of protein marker
A New Panel of Blood Biomarkers for the Diagnosis of Mild Traumatic Brain Injury/Concussion in Adults.

Shan R, Szmydynger-Chodobska J, Warren OM, Mohammad F, Zink BJ, Chodobski A.

A Biomarker for Concussion?

Pauline Anderson

December 02, 2015

March 25, 2015  Contact: David Gorenstein  401-863-1862

A panel of four readily detectable blood proteins can accurately indicate concussion, even helping distinguishing it from other injuries, according to a new study. Researchers at Brown University and Lifespan found the panel by employing the unusual strategy of looking at the body’s inflammatory response to trauma, which might also be a therapeutic target.

PROVIDENCE, R.I. [Brown University] — By looking at the molecular aftermath of concussion in an unusual way, a team of researchers at Brown University and the Lifespan health system has developed a candidate panel of blood biomarkers that can accurately
What? Normal Function?

Where? Origin of the protein: CNS or other?

Why? Increased/expressed in response to damage?

How? Blood brain barrier damage or other?
Blood Brain Barrier

Detected in blood post TBI and Orthopaedic injuries

10 nm pores

1st extracellular loop
Electrical barrier

Controls flow resistance
Charge discrimination of small solutes

Kawata et al., 2015
BBB proteins in blood

4.5 fold increase in OCCLUDIN post-TBI

Increased in Ortho injury

NO difference from concussed participants

<table>
<thead>
<tr>
<th>MARKER</th>
<th>Process/Structure</th>
<th>CSF</th>
<th>BLOOD</th>
<th>peak TBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-100-B</td>
<td>Astrocytes</td>
<td>less sensitive than Tau</td>
<td>Originates from outside brain increased after exercise</td>
<td>1-3h</td>
</tr>
<tr>
<td>GFAP</td>
<td>Astrocytes</td>
<td>&lt; sensitive than Tau &amp; NF</td>
<td>correlates with changes in imaging</td>
<td>24h</td>
</tr>
<tr>
<td>Total Tau</td>
<td>Axon</td>
<td>Peak 4-10 dpi</td>
<td>up in hypoxia (mTBI n/a)</td>
<td>1h</td>
</tr>
<tr>
<td>gamma Enolase</td>
<td>Neurons</td>
<td>in RBC and confounded by lysis of RBC</td>
<td></td>
<td>12h</td>
</tr>
<tr>
<td>SPECTRIN</td>
<td>Neurons (axonal damage)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF light</td>
<td>Axon</td>
<td>Peak 4-10 dpi</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

### Table 1 | Potential fluid biomarkers of mild TBI

<table>
<thead>
<tr>
<th>Marker</th>
<th>Process or structure affected</th>
<th>Research findings in mild TBI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cerebrospinal fluid</td>
<td>Blood</td>
</tr>
<tr>
<td>Cerebrospinal fluid:serum albumin ratio</td>
<td>Blood–brain barrier</td>
<td>Detection method not sensitive enough to changes in concentration&lt;sup&gt;53,54&lt;/sup&gt;</td>
</tr>
<tr>
<td>Interleukins and other acute-phase inflammatory response proteins</td>
<td>Neuroinflammation</td>
<td>NA</td>
</tr>
<tr>
<td>Total tau protein</td>
<td>Axon</td>
<td>Levels peak 4–10 days after injury&lt;sup&gt;64,71&lt;/sup&gt;</td>
</tr>
<tr>
<td>Myelin basic protein</td>
<td>Axon</td>
<td>NA</td>
</tr>
<tr>
<td>Neurofilament light polypeptide</td>
<td>Axon</td>
<td>Levels peak 4–10 days after injury&lt;sup&gt;64,71&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neurofilament heavy polypeptide</td>
<td>Axon</td>
<td>NA</td>
</tr>
<tr>
<td>γ-Enolase</td>
<td>Neuron</td>
<td>Levels confounded by lysis of red blood cells in blood-contaminated cerebrospinal fluid&lt;sup&gt;82&lt;/sup&gt;</td>
</tr>
<tr>
<td>S100-B</td>
<td>Astroglial cells</td>
<td>Levels are elevated, but a less sensitive marker than tau protein and neurofilaments&lt;sup&gt;71&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glial fibrillary acidic protein</td>
<td>Astroglial cells</td>
<td>Elevated levels, but a less sensitive marker than tau and neurofilament proteins&lt;sup&gt;54,71&lt;/sup&gt;</td>
</tr>
<tr>
<td>Secreted APP-α and APP-β</td>
<td>Axon</td>
<td>NA</td>
</tr>
<tr>
<td>Amyloid-β&lt;sub&gt;40&lt;/sub&gt; and amyloid-β&lt;sub&gt;42&lt;/sub&gt;</td>
<td>Plaque pathology</td>
<td>No change in levels&lt;sup&gt;57,74&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spectrin breakdown products</td>
<td>Neuron</td>
<td>NA</td>
</tr>
<tr>
<td>Ubiquitin carboxyl-terminal hydrolase isoenzyme L1</td>
<td>Neuron</td>
<td>NA</td>
</tr>
<tr>
<td>Peripheral blood mononuclear small noncoding RNA molecules</td>
<td>Unknown</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: APP, amyloid precursor protein; NA, not available; TBI, traumatic brain injury.

What? Normal Function?

Where? Origin of the protein: CNS or other?

Why? Increased/expressed in response to damage?

How? Blood brain barrier damage or other?
What is the Normal Function?

Calcium, copper, zinc binding protein to regulate intracellular calcium levels

Protective at low levels, toxic at high
Glial activation, neuroinflammation


Normal concentrations S100B engages RAGE and exerts trophic effects on neurons and decreases microglial activation.

Normal blood levels: 0.05 ng/ml

After Injury blood levels: up to 5 ng/ml
S100-β

Where?
Enriched in brain: astrocytes
pulmonary alveolar cells, cardiomyocytes, chondrocytes, adipocytes


S100-β in neuropathology

Most extensively used biomarkers for TBI:

- Astrocytes and Oligodendrocytes
- Originates outside the brain
- Peaks in blood 1-3h after TBI
- Increases after exercise
**S100-β**

**NORMAL**
- **S100-β** binds Zn+
- Tau mediated neurite outgrowth

**Pathological**
- Increased intracellular Ca+ binds **S100-β**
- Induces aberrant Tau phosphorylation

Kawata et al., 2015
S-100-B

Why are levels increased?

Traumatic Brain Injury

Fluctuates after musculoskeletal injury

Cardiac arrest

Obesity

Bone fracture

Increases after exercise


GFAP

What is the Normal Function?

Intermediate filament protein in astrocytic cytoskeleton

Maintain resistance to mechanical stress to stress

Where?

GFAP α is most abundant type and in Astrocytes

GFAP-β in non-myelinating Schwann cells

GFAP-γ bone marrow and spleen

When and How is it released?

Intracellular GFAP can translocate to extracellular space and correlates with severity of injury.

Release from brain into blood requires loss of astrocytic integrity and increased BBB permeability.


GFAP

Diffuse Injury: 0.75 ng/ml
Focal Injury: 2.95 ng/ml

Normal blood levels: 0.03-0.07 ng/ml
Diffuse brain injury

Normal levels of GFAP

*
GFAP

No significant differences in serum GFAP in patients with multiple traumas and TBI versus TBI alone.

GFAP levels in blood remain normal in patients with multiple traumas with out brain injury.

Levels in TBI correlate with CT abnormalities.

C-Tau caspase 3: higher post concussion than pre season
A-Tau ADAM10: correlates with post-concussion symptom duration

Figure 1 Possible biomarkers of traumatic brain injury

Zetterberg, H. et al. (2013) Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood
Nat. Rev. Neurol. doi:10.1038/nrneurol.2013.9
Researchers find promising new biomarkers for concussion

March 25, 2015  Contact: David Cronstein  401-863-1862

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Tauopathies

Epilepsy

Alzheimer’s Disease

HIV-associated ageing

Frontotemporal dementia

Chronic traumatic encephalopathy
Tauopathy in CTE

A-C) Coronal sections through superior frontal cortex

McKee et al (J Neuropathol Exp Neurol, V 68, July 2009, Fig. 2)
Tauopathy
(abnormal hyperphosphorylation)
Dendrites
Axons
Enrolled Temple University Football Players

Conducted a series of measures before and after summer football practices
Assessments
Head impact kinematic measurements using mouth guard and sideline monitoring system

A, triaxial accelerometer senses linear acceleration and triaxial gyroscope senses angular acceleration (i1 Biometrics. Inc., Kirkland, WA, USA). In-mouth sensor deactivates signal transmission when not placed in the mouth. B, sideline antenna receives impact data by radio transmission. Impact kinematics can be monitored real-time with the sideline laptop.
Example of Head Impact Kinematics

Peak Linear Acceleration

- Linear Accel X (g)
- Linear Accel Y (g)
- Linear Accel Z (g)
- PLA

Legend:
- Front/back
- Side/Side
- Top/Bottom
- Peak Linear
Measures taken before and after practice

Scat3 (Sport Concussion Assessment Tool)

Near Point of Convergence

Blood Draw
Amount of Change in blood levels of S100β per hit

F. Predictive change in S100β per hit

J. Neurotrauma under review
Described some common biomarkers

How markers get in the blood

Example of data from repetitive subconcussive impacts
Future of the Blood Biomarkers for Head Injury
TEMPLE
Kinesiology
Ryan Tierney

Neuroscience
Keisuke Kawata
Leroy Wesley
Jong Lee
Tommy Sim
Kim Ferrero

Athletics
Masahiro Takahagi
Victor Szwanki
Al Bellamy

Engineering
Kurosh Darvish
Suroush Assari

Ophthalmology
Jeff Henderer