A View of Brain Injury through the Eyes of the Clinical Pathology Laboratory

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Pathology Grand Rounds
This is the brain:
It is in your head.
I want to study it.
Disclosures - FStrathmann

- Collaboration for POC device development in TBI:
  - 3PDx
  - PLC Diagnostics Inc.
Objectives

• Describe the clinical workflow for TBI assessment

• State the underlying primary and secondary pathology in TBI

• Discuss the benefits and limitations of existing TBI biomarkers
Outline

Part 1 (Petron)
• Clinical Workflow for TBI assessment
• Presentation of cases

Part 2 (Strathmann)
• TBI Pathology
• Current biomarkers for TBI
• Direction of TBI biomarker research
Concussions
Feb 15, 2013

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What is a Concussion?

- Japanese Proverb:
  
- A problem clearly identified is half the solution
Closed Head Trauma without Structural Defects: Concussions

• Definition: “A trauma-induced alteration in mental status that may or may not be accompanied by loss of consciousness”

• No structural defects present on neuro-imaging

• Defects may be present on psychological exam
Why this matters...

- House Bill 204 “Protection of Athletes with Head Injuries Act”
  - Requires removal of a child from a sporting event when the child is suspected of sustaining a concussion or head injury
  - Prohibits a child from returning to play until they have received medical clearance from a “qualified healthcare provider trained in the evaluation and management of concussion”
Closed Head Injury with Structural Defect

- Structural defects are lesions that can be seen on neuro-imaging
- Structural defects are synonymous with brain damage
- Structural defects = no return to play
- Not “concussions”
Mechanisms of injury

- **Coup injuries**: a direct blow to a stationary skull that compresses/injures underlying brain at the point of impact.

- **Contracoup injuries**: injury to brain tissue on the opposite side of a blow or force as a result of a moving brain rebounding against the skull or from tearing of fragile subarachniod vessels.

- **Rotational injuries**: injury to brain tissue as a result of shearing forces between the skull and brain tissue.
High School Football Player Dies

By MICHAEL S. SCHMIDT and DAVE CALDWELL
Published: October 16, 2009

A Montclair High School football player who died Wednesday told a teammate that he still had postconcussion symptoms after he was cleared by doctors to return to play, the school’s interim principal said Thursday.

Ryne Dougherty, a junior linebacker, died Wednesday night. He was 16. He had a brain hemorrhage Monday while making a tackle during a junior varsity game. Doctors cleared Dougherty to return Oct. 6, roughly three weeks after he sustained a concussion in a practice Sept. 18. He first returned to action Friday, participating in one play for the varsity team....

....Dougherty is the third teenager to die in New Jersey in the last three months after participating in football activities; he is the second to die because of a brain injury. Dougherty is also at least the fourth high school player in the United States to die this year because of a head injury....
60% agree….How quickly a person recovers from a head injury depends primarily on how hard they work at recovering.

50% agree….Sometimes a second blow to the head can help a person remember things they had forgotten.

Invisible Injury…The person looks the same with no medical evidence of injury.

Expectation from self and others to ‘get over it’ and ‘get back into the game’.
Scenario:

“Sprained Ankle:”

“Sprained Brain”
Overview

— **Features:**

- 1. Can be caused by direct blow to head, neck, or anywhere on body (forces transmitted to head)

- 2. Typically results in rapid onset of neurologic impairment that resolves spontaneously and quickly, though may be prolonged
Overview

- 3. Acute clinical symptoms *typically* reflect a functional, rather than structural, disturbance (?)

- 4. Resolution typically follows a sequential course

- 5. Typically associated with normal neuroimaging (CT/MRI)
Macro to Micro

• Cellular Events
  – “A metabolic injury”
  – “Metabolic Mismatch”
  – “An energy crisis in the brain”

• Axonal Events
  – Spectrum of Diffuse Axonal Injury
Pathophysiology – Cellular Events

• Axonal stretching may lead to both changes in ionic permeability and injury to the cytoskeleton
• Release of excitatory neurotransmitters and uncontrolled ionic fluxes
• Energy dependent ion pumps activated (Na/K ATPase) in attempt to restore homeostasis
• Glucose demand/utilization increases acutely and then is diminished for up to 2-4 weeks
• Concurrently, local/regional decreases in cerebral blood flow
• Calcium influx and mitochondrial sequestration leads to impairment of oxidative metabolism and subsequent increase in Lactate

Barkhoudarian, Hovda et al. 2011  Giza and Hovda 2001
How the Brain’s Messaging System Works

Diagram showing the normal neuron function with dendrites, axon, synapse, and nerve cell body. The signal arrives at the neuron and travels down the axon to another cell. Neurotransmitters are released in an organized manner, triggering the next cell with a specific coded message.
Communication Breakdown

Neuron During Injury

During injury, potassium ions ($K^+$) rush out of the cell...

Neuron During Injury

...and toxic calcium ions ($Ca^{2+}$) rush into the cell, leading to metabolic dysfunction.

Neuron Following Concussion

Metabolic dysfunction results in energy crisis.

Neurometabolic Cascade Following Cerebral Concussion/MTBI

Neuron is extremely vulnerable in this condition, and further injury or stress may cause cell death or serious cell damage.
Post Concussion Symptoms

Cognitive
Mental fogginess
Poor attention
Poor memory

Mood
Irritability
Anxiety
Mood Swings

Physical
Dizziness
Headaches
Balance problems

Sleep
Can’t fall asleep
Sleep too much
Common Physical Symptoms

- Headache
- Dizziness and lightheadness
- Balance problems
- Blurry vision and light sensitivity
- Tinnitus and noise sensitivity
- Nausea
- Numbness
Common Cognitive Symptoms

• Feeling “dazed” or “foggy”
• Easily distracted
• Trouble doing more than one thing at a time
• Poor learning and memory
• Poor reading comprehension
• Poor mental stamina
• Problems with expression
Common Mood Symptoms

- Irritability
- Anxiety
- Mood swings
- Easily overwhelmed
- Emotional and behavioral outburst
- Lack of motivation
Exertional Effects

• Symptoms are worsened by:
  - Mental effort
  - Environmental stimulation
  - Emotional stress
  - Physical activity
At a football game...

- 16yo HS FB player presents to your office 3 days after sustaining frontal, helmet to helmet blow that produced 10 sec LOC, 10 min period of retrograde amnesia, 30 min period of post-traumatic amnesia. Evaluated at ER; CT neg. Now c/o fatigue, mod to severe bifrontal HA, bilat blurred vision, nausea, fogginess & difficulties with short-term memory.
According to recent published data, which acute or sub-acute symptom is most predictive of experiencing a protracted recovery (less than 3 wks)?

1. Acute Loss of consciousness
2. Presentation of retrograde amnesia
3. Subacute symptom of bilateral blurred vision
4. Subacute symptom of fogginess
5. None of the above, there are no defined prognostic indicators of outcome
Clinical Management Issues

ACUTE MANAGEMENT

- Rule out more serious intracranial pathology
  - CT, MRI, neurologic examination primary diagnostic tests

POST INJURY MANAGEMENT

- Prevent Second Impact Syndrome
- Prevent Cumulative Effects of Injury
- Prevent Post Concussion Syndrome

Determination of asymptomatic status is essential for reducing repetitive and chronic morbidity of injury.

Heads UP Booklet/CDC Report, 2007
How Long is Long Enough?

Preliminary animal and human studies suggest:

- There is **significant benefit from complete rest for several days** to reduce the secondary negative pathophysiological effects on recovery.

- Begin a program of **gradual exertion** before starting back to work/school/playing full time.

- Symptom free waiting period.
What Should We Do During Early Recovery

• **Sleep**: It is common for people to need more sleep in recovering from a concussion. 12-15 hours a day is not unusual.

• **Follow a routine**: get up and do a low demand activity during the day. Go to bed at the same time each night; take a nap during the day if needed.

• **Listen to your body**: If you feel tired or your symptoms worsen take a break. If you “push through” your symptom recovery will be prolonged. Reduce demand to match your available energy.
What Should We Avoid During Early Recovery

- **Avoid high risk activities**: Because of reduced reaction time the potential for further injury increases dramatically so, sports, skiing, snow or long boarding, cycling etc. should be avoided until cleared.

- **Avoid high stimulus environments**: Going to a busy shopping mall, noisy restaurant, rock concert, ballgame, party, full day of school and other high stimulus events will slow down recovery and increase symptoms. If you must do something demanding arrange an exit point if you become fatigued.
# Real Life Application of Recovery

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<th>Clinical and Physiological Recovery</th>
<th>Patient Experience</th>
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| **Super Acute (~first 5 days)** | Symptoms, cognitive dysfunction can be severe, disrupt daily activities.   
Brain in neurometabolic crisis-  
Inability to recruit resources | Symptoms, cognitive dysfunction render person unable to perform daily functions, return to work, etc. Exertion may negatively impact recovery |
| **Acute (~6-30 days)**   | Gradual improvement in symptoms, cognitive functions; full recovery in ~90% of cases.  
Brain on course back to normal metabolic state. Over recruitment of resources. | Gradual return to full function at work/school/home that requires more effort than customary to meet normal demands; fatigue present. |
| **Chronic (>30 days)**   | Small %age with persistent symptoms (PCS).  
Brain returned to normal state; co-morbidities influence recovery | Resume all normal activities without restriction, complications or accommodations. |

McCrea, 2008
Individual Recovery From Sports MTBI: How Long Does it Take?

N=134 High School Male Football Athletes

Collins et al., 2008, Neurosurgery
• Athletes with on-field **retrograde amnesia** were **10x** more likely to have a “poor outcome” at three days post concussion.

• Athletes with on-field **anterograde (PTA) amnesia** were **4.2x** more likely to have a “poor outcome” at three days post concussion.

• Brief LOC is **not predictive** of outcome.

On Field Symptom Summary

• **Brief LOC** is not predictive of subacute or protracted outcomes following concussion.

• **Amnesia** is important for acute presentation, but **may not be predictive** of a protracted recovery.

• Post-injury **dizziness and mental fogginess** is the **best predictor** of protracted recovery and “post-concussion syndrome.”

• Etiology of dizziness?
  • Migraine variant?
  • Central Vestibular Dysfunction?
  • Peripheral Vestibular Dysfunction?
  • Cervicogenic?
  • Psychologic?

• There is a need for better clinical tools to assess dizziness construct

*Collins, et al. AMSSM Conference, 2011*
Impact of Multiple Concussions

- 3+ concussions increase the risk of re-injury by 400%
- 3-4 concussions increase the risk of depression/mood disorders by 100%
- 5+ concussion increase the risk of permanent memory problems by 300%
- 75% of repeat concussions happen within 7 days
- 92% of repeat concussions happen within 10 days
- Lesser impact will produce concussion symptoms

McCrea, 2009
Risk Factors for a Complicated Recovery: Exceptions to the Rule?

- History of headaches
- History of multiple concussions
- History of LD/ADD
- Re-injury before symptom free period
- Psychiatric history
- Psychosocial stressors at the time of injury, lower psychological resilience
- Substance abuse
- Litigation/blaming others
- Limited social support

McCrea, 2009
Concussion Management

• Every concussion should be managed individually
  – No two concussions are the same, even in the same patient!!!

• Guidelines, not hard fast rules

• Trend is to more conservative

• Medico legal issues
ALWAYS

• Perform an *exercise challenge* before returning a player to competition

• Arrange for adequate *follow up* before leaving the locker room

• Always *document* your recommendations
A gymnast fell off the uneven bars during a competition and hit her head on the ground. She had a 2-minute loss of consciousness and had posttraumatic amnesia for 2 hours, which completely resolved. She was sent to the emergency room and had a CT scan of the head, which was negative for any intracranial bleeding. She was discharged to home. She now comes 2 days later to your office complaining of headache and the inability to concentrate in class.
This patient has signs and symptoms of:

1. postconcussive syndrome
2. continuing symptoms of a concussion
3. second-impact syndrome
4. epidural hematoma
5. malingering
CONCUSSION

Look on the bright side. For one brief, glorious moment, you forgot you were on the Cubs.
TBI: Pathophysiology and Key Biomarkers

Frederick G. Strathmann, PhD
Brain Injury Statistics

- 1.7 million people (US) require TBI-related medical treatment annually
- 16% of TBI cases require hospitalization
- 3% of TBI cases result in death
- Estimated 25% of TBI cases never seek medical care
Leading Causes of TBI

- Accidental injury
  - Automobile accidents
  - Sports
  - Falls
  - Violence/firearms
- Shaken Baby Syndrome
- Estimated $50 billion in economic costs (US) annually

- Military
  - Signature injury of Iraq and Afghanistan wars
- 20% of deployed personnel suffer TBI
  - 212,000 (2000 to 2011)
- $192 million on care in 2010
Pathophysiology of TBI

Primary Injury
• Direct tissue damage
• Contusions
• Lacerations
• Shearing damage

Secondary Injury
• Biochemical, cellular and physiological components
• Minutes to years
• Considerable symptom overlap
  – Parkinson’s, Alzheimer’s, Huntington’s, ALS

Long Term Consequences of TBI

- Unprovoked seizures
- Premature death
- Unemployment
- Aggressive Behavior
- Depression
- Alzheimer’s disease-type dementia
- Neurodegenerative hallmarks of dementia

Penetrating TBI

Moderate to Mild TBI

Secondary Injury: A Closer Look

Vasculature
- Perfusion ↓
  - hypoxia/ischemia
- BBB breakdown
  - Damage vs. response

Metabolism
- Oxygen availability ↓
  - Metabolic crisis
  - Neurometabolic cascade
- Brain Temperature ↓↑
- Toxic accumulation

TBI Injury: Key Characteristics for Current Biomarkers

- Blood-brain barrier disruption
- Vasculature disruption
- Intermixing of peripheral blood with CNS
- Diffuse axonal injury (DAI)
TBI Biomarker “Wish List”

Characteristics
• Sensitive
• Specific
• Present in blood
• Appropriate detection window

Comparison
• Liver
  – AST/ALT
  – Bilirubin, Coag, TP
• Heart
  – Cardiac Troponins
  – BNP
Prominent Biomarkers of TBI

Common Features

- Venipuncture
- CSF
- Markers of Necrosis
- BBB disruption necessary (?)

Primary

Secondary

s100B
NSE
GFAP
Imaging
Cell Types in the CNS

Neurons
• Transmission of electrical and chemical signals
  – GABAergic
  – Dopaminergic
  – Etc.

Glia
• Microglia
  – MØ of the CNS
• Oligodendrocytes
  – Myelination
• Astrocytes
  – Everything else
TBI Biomarkers by Cell Type

Neurons

• Neuron specific enolase
  – Glycolysis
• Tau (p)
  – Filament
• Neurofilament light protein

Neuronal Markers of TBI: NSE

- Dimeric enolase
  - Glycolysis
- Present within hours
- $t_{1/2}$ of 20 – 40 hours
- Released with cell necrosis

- Measured by Immunoassay
- Increases detection window
- Reports of low sensitivity & specificity
NSE Utility in TBI

Mild

Moderate - Severe


TBI Biomarkers by Cell Type

Macroglial
- Astrocyte
  - S100B
  - GFAP
- Oligodendrocyte
  - Myelin Basic Protein

Astrocyte Populations

Phenotype

• Distribution in the brain
  – White matter astrocytes
  – Gray matter astrocytes
  – Others

• Developmental differences

• Lineage differences
Early Events in Astrocyte Activation

• Transient
• Occurs distal to primary injury site where minimal tissue disruption is found
• Increase in size
• Nuclear hypertrophy
• Morphology changes to a stellate shape

• Increase production of:
  – Cytosolic enzymes
  – AO
  – Structural proteins
  – Organelles
  – Tropic and growth factors
  – Enhance adjacent cell survival
Late Events in Astrocyte Activation: Reactive Gliosis

- Cell hypertrophy
- *IF* upregulation
  - GFAP & S100B
- Extension, interdigitation
- Glial scar
- Inhibit neurite outgrowth

- “Reactive Astrocytes”
  - TNF-alpha
  - AA
  - NO
  - ROS

Trends in Neuroscience 2009;32(12):638-647
Astrocytic Markers of TBI: S100B

- Intracellular Ca binding
- Detected within 6 hours post-injury
- $t_{1/2} \approx 2$ hours
- Measured by immunoassay

- Concerns
  - Present in many non-CNS cell types
  - Widely used biomarker for malignant melanoma
  - Specificity diminished with multiple traumas
The Role of S100B in Injury

Low Concentration
- Promote neurite extension
- Promote neuron survival
- Regulate muscle development and regeneration

High Concentration
- Upregulates NOS, COX-2
- NO release and cell death
- Increase ROS production
- Perturbs Lipid homeostasis and cell cycle arrest

S100B and BBB Disruption

S100B Performance: Time Course

(A) Sensitivity vs. 1 - Specificity for TBI 12hr
- AUC = 0.80 (95% CI 0.51-1.09)

(B) Sensitivity vs. 1 - Specificity for TBI 24hr
- AUC = 0.74 (95% CI 0.45-1.03)

(C) Sensitivity vs. 1 - Specificity for TBI 48hr
- AUC = 0.67 (95% CI 0.37-0.96)

S100B Performance: Time Course

A. AUC = 0.80 (95% CI 0.51-1.09)

B. AUC = 0.80 (95% CI 0.51-1.09)

C. AUC = 0.67 (95% CI 0.37-0.96)

D. TBI Combined

Astrocytic Markers of TBI: GFAP

- Intermediate filament
- Time of detection thought to indicate type and severity of injury
- $t_{1/2}$ not well defined
- Released after cell necrosis

- Measured by WB or immunoassay (clinical?)
- Exclusive to CNS
- Isoforms exist
  - Consequence?
GFAP Performance

Box plot showing plasma GFAP levels for Ischemic stroke, ICH, and Stroke mimic.

Graphs A, B, and C illustrate non-survival, survival A, and survival B respectively, with correlation coefficients and p-values.


Do TBI Biomarkers Help?

"...and it’s at this point when we began to be unsure where it all began and where it’s all going to end."
What else can biomarkers do?

• **S100B**
  – Window of opportunity for drug delivery to the brain

• **Combinations**
  – Determine injured cell types
  – Possibly indicate severity

• **Tie-breaker for clinically ambiguous cases**
TBI Injury with the University of Utah Football Team

• Can the use of S100B and NSE add to the diagnostic accuracy or efficacy in sports related TBI?

• Dr. David Petron
• Dr. Bradley Hayes
• Stefanie Schulte
• UofU Sports Medicine
New Trends in TBI-related Biomarker Research

- ApoE
- UCH-L1
- miRNA
- POC devices
- Imaging

Modified from G. Siuzdak MSACL 2013
## Biomarker Discoveries in TBI

<table>
<thead>
<tr>
<th>Classification</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Neuron Injury</td>
<td>NSE, tau, APP</td>
</tr>
<tr>
<td>Glial cell injury</td>
<td>s100B, GFAP, MBP</td>
</tr>
<tr>
<td>Cell death or Degeneration</td>
<td>NR2A/2B autoantibodies</td>
</tr>
<tr>
<td>Metabolic Alterations</td>
<td>Lactate, pyruvate, etc.</td>
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<tr>
<td>Physiological Monitoring</td>
<td>CBF, Brain temperature</td>
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<tr>
<td>Genomic, Proteomic</td>
<td>ApoE, miRNAs</td>
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</tbody>
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