Blood biomarkers for assessment of mild traumatic brain injury and chronic traumatic encephalopathy

Matthew Hiskens and Andrew Fenning PhD.

Clinical Pharmacology Laboratory, Central Queensland University

Email: m.hiskens@cqu.edu.au

Manpreet Romana/AFP/Getty https://www.theguardian.com/world/2009/jul/15/first-photograph-ied-afghanistan-roadside-bomb

Outline

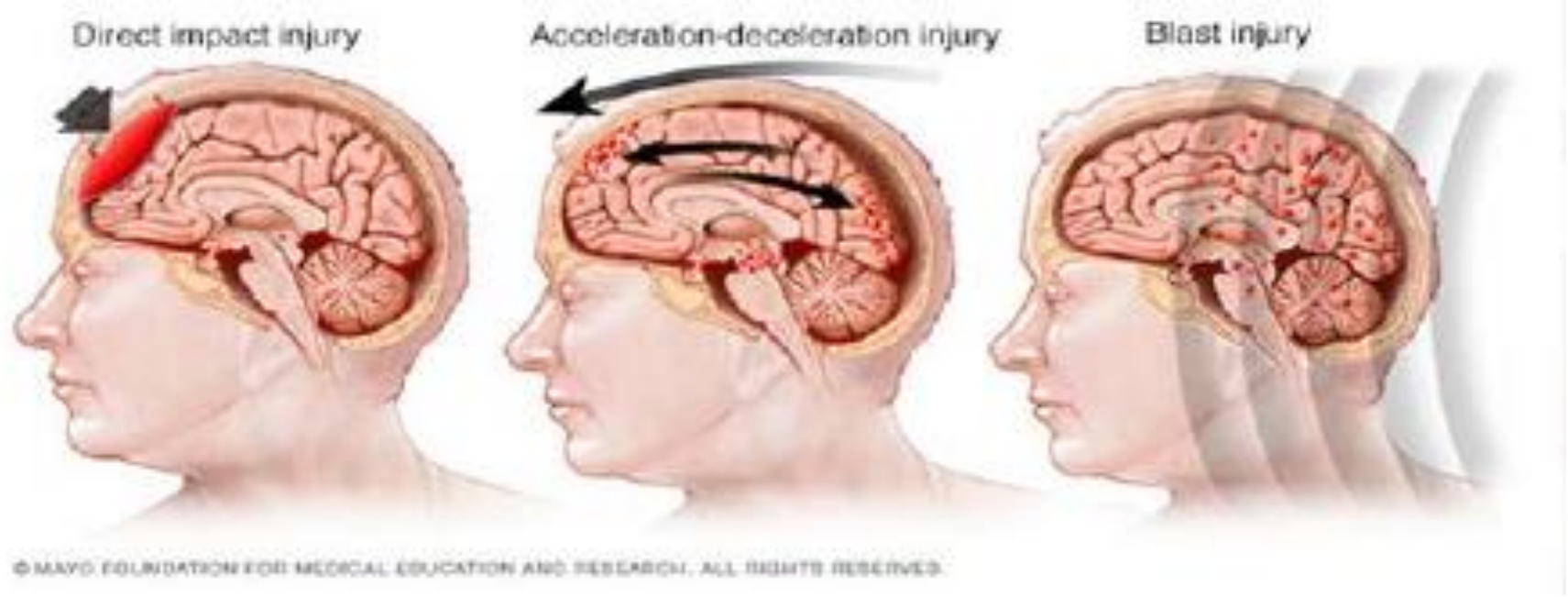
- mTBI prevalence and consequences
- mTBI aetiology and pathophysiology
- Clinical limitations in diagnosis
- Access and limitations of biomarkers
- Markers of neuronal damage
- Markers of astrocyte damage
- Markers of inflammation
- miRNAs
- Ongoing challenges

Poll Question #1

- What is your area of involvement in health services?
  - Clinical research
  - Basic research
  - Rehab medicine
  - Other clinician

Mechanisms of mTBI

- Direct impact (blunt force)
- Acceleration/deceleration
- Blast



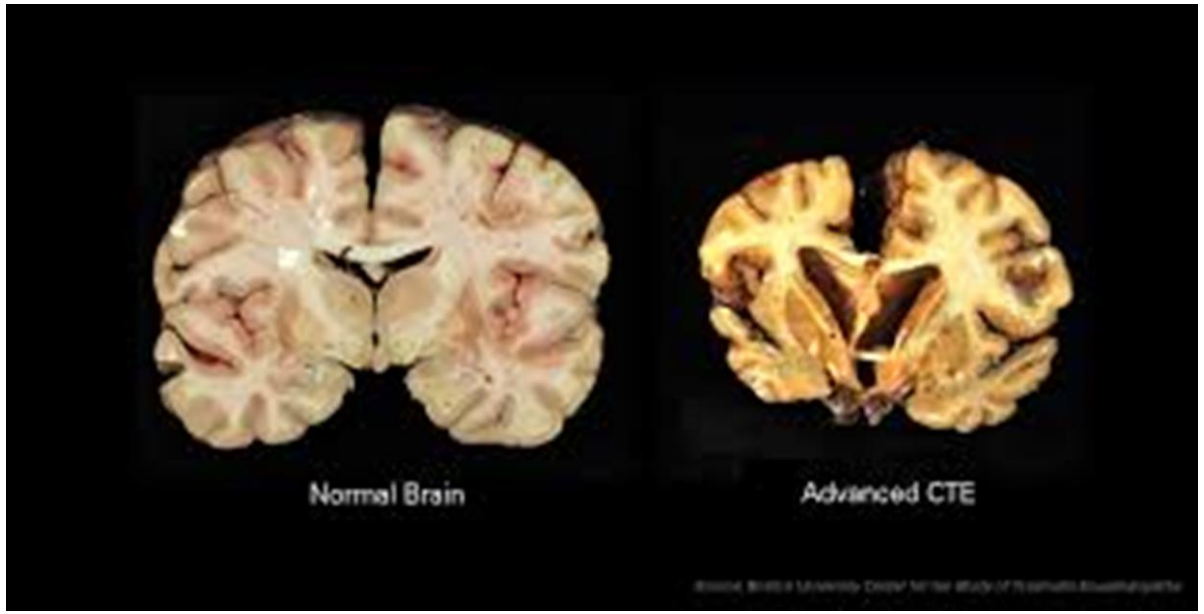
mTBI in the military

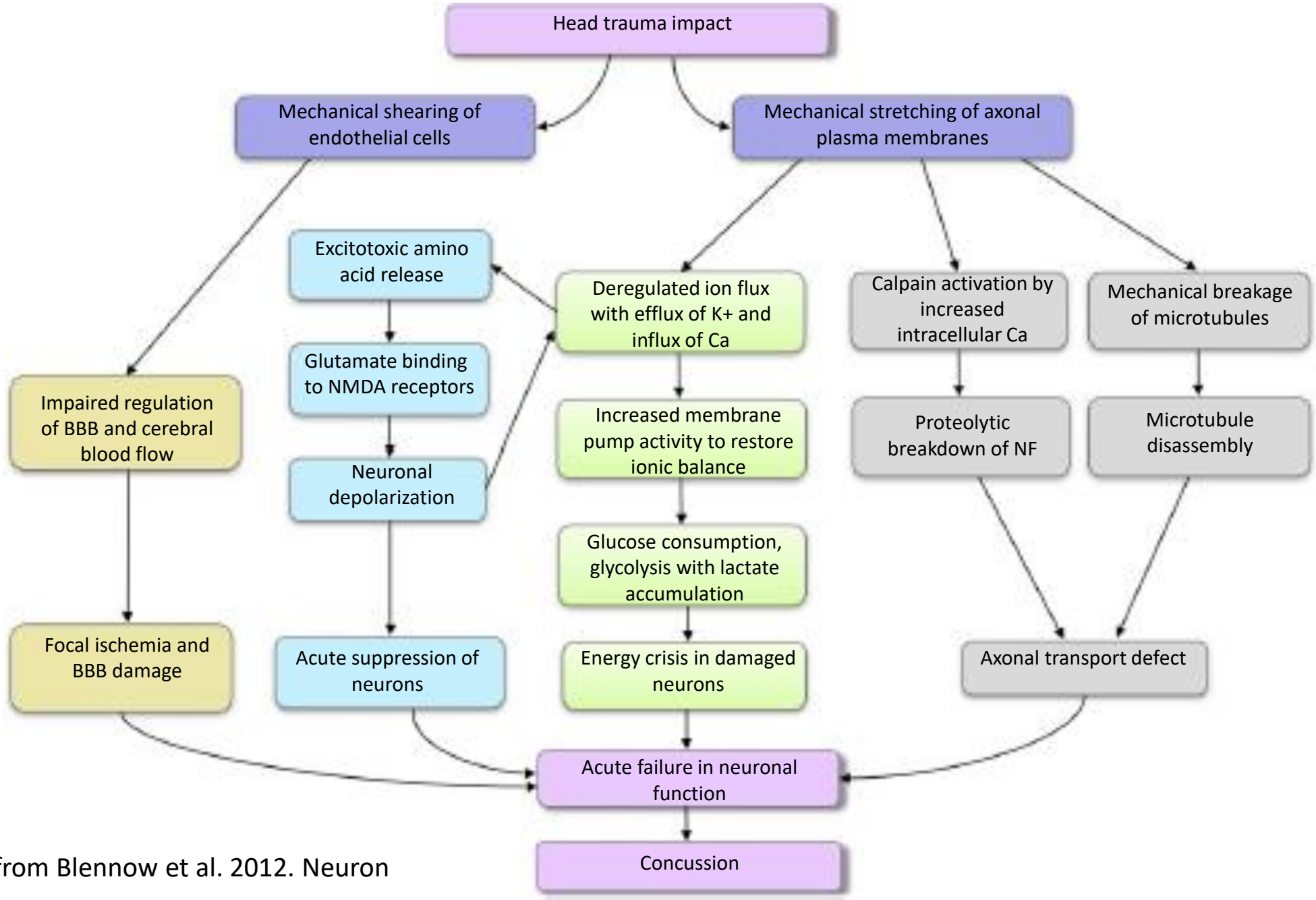
- Iraq & Afghanistan wars led to a considerable number of TBIs, most commonly from IED blast
- ~20% of returning veterans suffered from TBI between 2000 and 2019
- ~82% of TBI was classified as mild

Defence and Veterans Brain Injury Center. DoD Worldwide Numbers for TBI. http://dvbic.dcoe.mil/dod-worldwide-numbers-tbi.

Aetiology and pathophysiology

- Mitochondrial dysfunction, proliferation of free radicals, inflammation mediated by microgliosis, glial cell injury, neurotransmitter reuptake, microvasculature stenosis, calcium overload, atrophy of white matter





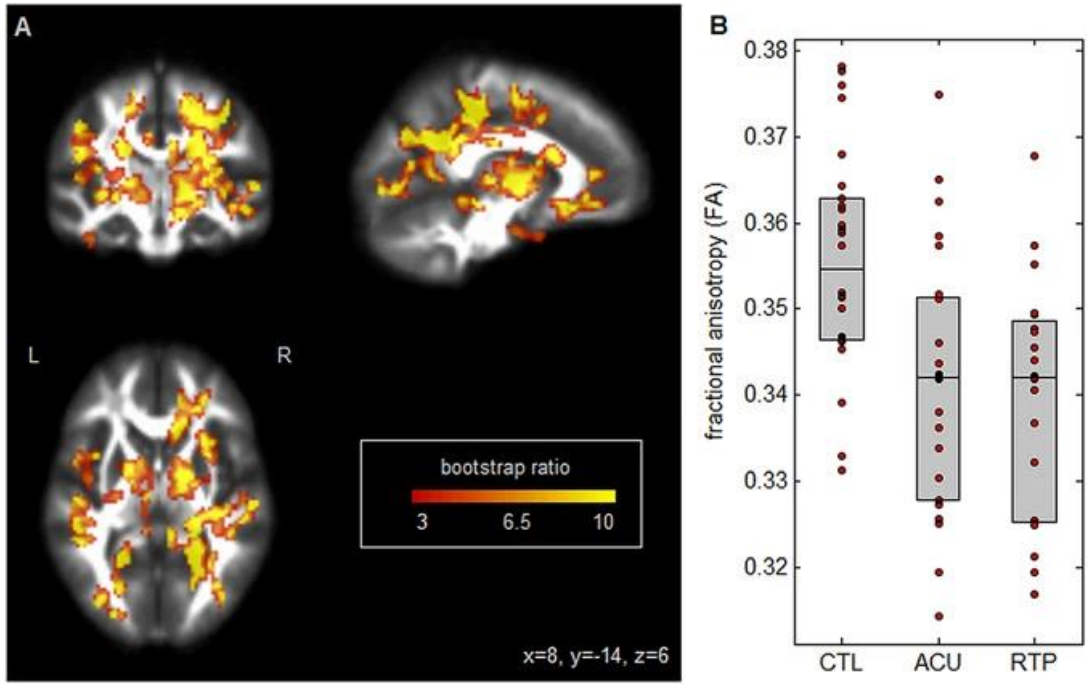
Adapted from Blennow et al. 2012. Neuron

Consequences

- Acute symptoms
  - Fatigue, dizziness, headache, irritability, memory impairment, photosensitivity, sleep disturbances
- Long term consequences have been overlooked
  - Undetectable in standard CT and MRI
  - Repetitive mTBI or significant single blast injury linked with psychiatric, cognitive, and motor impairment
  - Neuropathological change involving p-tau aggregation, TDP-43 proteinopathy in neurons, irregular astrocyte and cell process distribution at the depths of the cortical sulci

Screening and diagnosis limitations

- Imaging – MRS, fMRI, DTI
- Clinical tools – GCS, SCAT



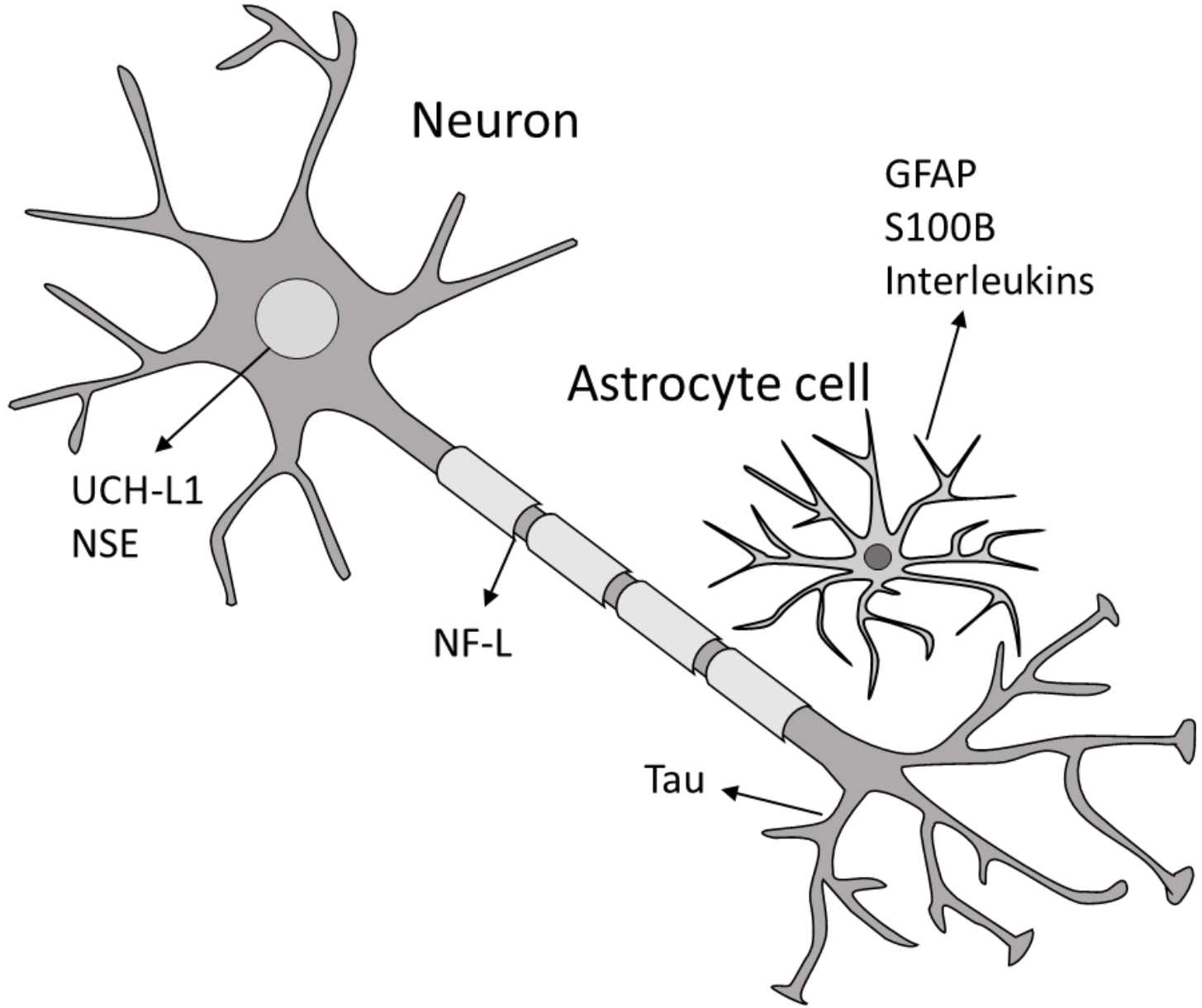
Churchill et al., 2017. Scientific Reports

Poll question #2

- As a clinician, what is your familiarity with blood biomarkers in mTBI?
  - Current use in diagnosis and prognosis
  - Some awareness of current status of development
  - Limited background

Access to biomarkers

- Proteins in peripheral blood or CSF
  - Limited in saliva, urine, tears
- Limitations in blood
  - Blood brain barrier permeability
  - Proteolytic degradation
  - Liver or kidney clearance
  - Carrier protein binding
  - Serum vs plasma assays
  - Contamination with erythrocytes or platelets


Neuron
GFAP
S100B
Interleukins
Astrocyte cell
UCH-L1
NSE
NF-L
Tau

Markers of neuronal damage

- Neuron cytoskeleton formed by actin filaments, tubulin microtubules, and neurofilaments
- These structures may be damaged by mechanical and chemical injury following mTBI
- Axonal transport of proteins and organelles may be impaired
  - Formation of NFTs and neuronal death

Tau

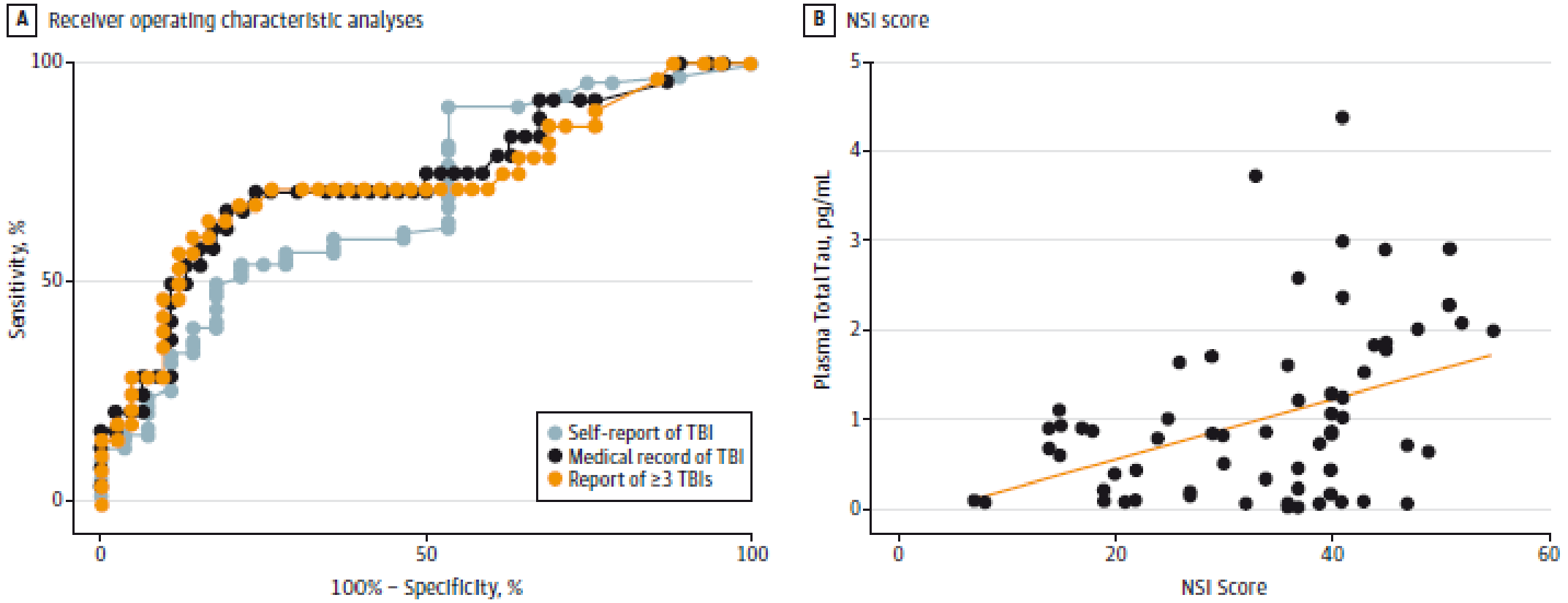
- Structural MT protein
- Hyperphosphorylated tau (p-tau) results in NFT aggregation in the cortex as seen in CTE
- Military personnel – plasma total tau (t-tau) increased up to 18 months compared to healthy controls (Olivera et al. 2015). Cumulative effect, levels higher in those with three mTBIs than with one mTBI. Relationship with t-tau and PCS severity.
- T-tau may not have the required sensitivity for sub-concussive diagnosis
- Lack of correlation between blood and CSF tau concentration

Original Investigation

Peripheral Total Tau in Military Personnel Who Sustain Traumatic Brain Injuries During Deployment

Anlys Olivera, PhD; Natasha Lejbman, BS; Andreas Jeromin, PhD; Louis M. French, PsyD; Hyung-Suk Kim, PhD; Ann Cashion, PhD; Vincent Mysliwiec, MD; Ramon Diaz-Arrastia, MD, PhD; Jessica Gill, RN, PhD

Figure 2. Specificity of Tau in Traumatic Brain Injuries (TBIs) and Associated Chronic Postconcussive Disorder Symptoms



Neurofilament light (NfL)

- Prominent in large myelinated axons that project into the subcortical white matter – these are particularly vulnerable to mTBI
- Following trauma, NFs are released into CSF and serum
- Serum NfL shows greater diagnostic and prognostic utility than GFAp, tau, and UCH-L1 for subacute and chronic TBI (Shahim et al. 2020)

UCH-L1

- Found in the cytoplasm of neurons of the brain and peripheral nervous system
- Peak at 8 hr, decrease over following 48 hrs
- Has been approved as part of a composite panel for predicting presence of intercranial lesions to rule out unnecessary CT scans
- Failed to differentiate mTBI and orthopaedic patients in acute care setting (Posti 2017)
- Lack of correlation with head impacts or imaging in college football players (Puvenna 2014)

Neuron-specific enolase

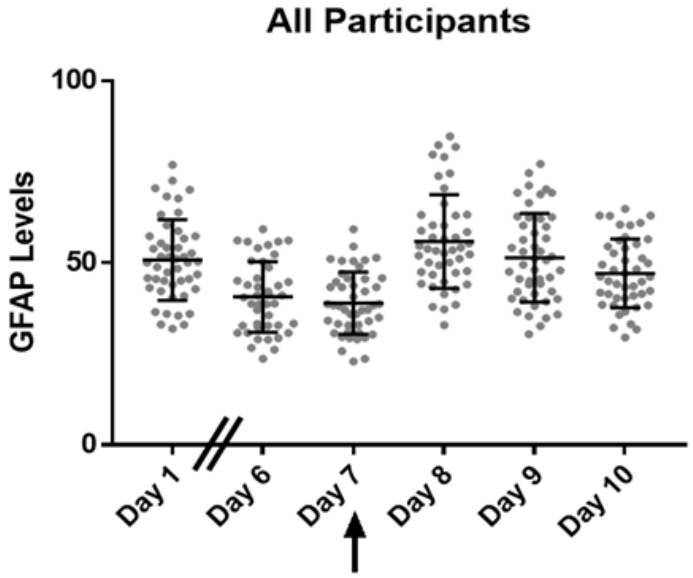
- Glycolytic enzyme elevated in the blood following neuron damage or death
- Expressed by other cells such as erythrocytes
- Demonstrated utility in moderate and severe TBI, but does not appear to be sensitive enough in blood for mTBI

Markers of astrocyte damage

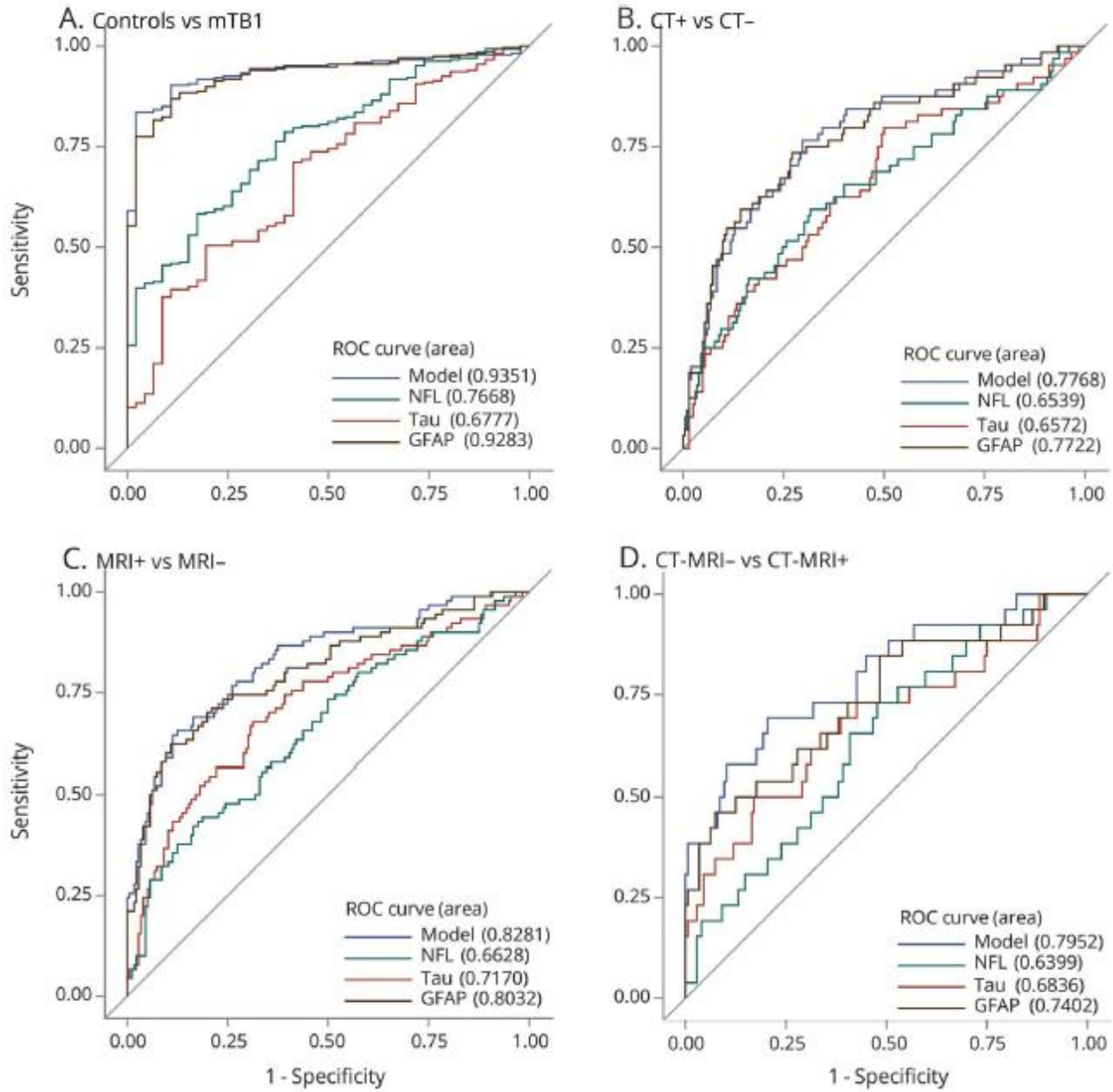
- Support structures that respond to the metabolic demand of neurons
- Contribute to BBB
- Transformation following mTBI trauma for structure and defence
- Severe or persistent insults cause permanent change to structure and function, inhibiting CNS regeneration

GFAP

- Structural protein of the astrocyte cytoskeleton
- Moderate increase may aid neuroregeneration in recovery, excessive elevation indicates neuroinflammation and gliosis
- Approved to differentiate patients with abnormal CT scans with controls in conjunction with UCH-L1
- Some inconsistencies with utility need to be clarified



Tschiffley et al. 2019. J Neurotrauma



Gill et al. 2018. Neurology

S100B

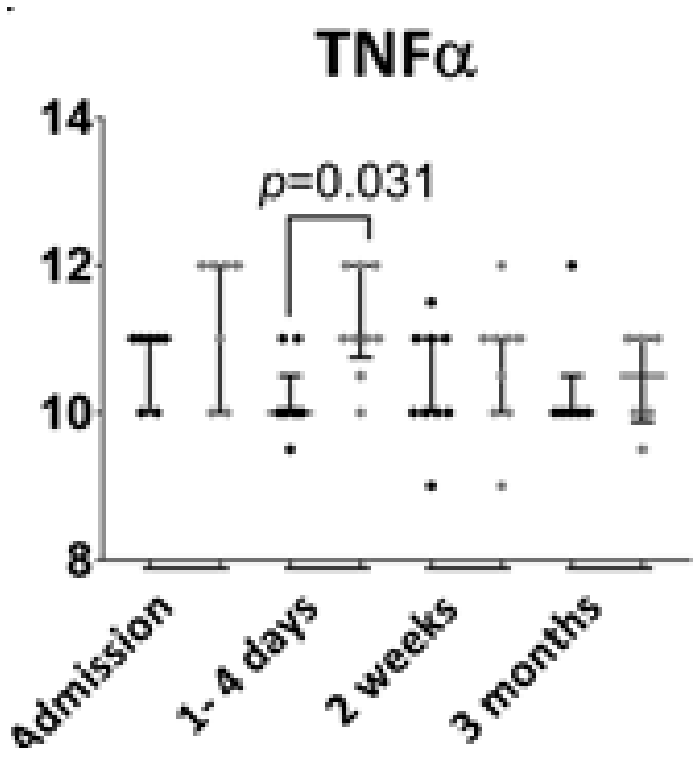
- Calcium binding protein with roles in signal transduction
- Following mTBI S100B is released by astrocytes to protect against secondary inflammation
- At high levels contributes to neuron death and tau hyperphosphorylation
- Also a marker of other cellular diseases and elevated following physical activity
- Traditionally been measured in more severe forms of TBI

Inflammation

- Driven by microglia
  - Release of pro and anti-inflammatory cytokines, chemokines, interferons, reactive oxygen and nitrogen species, prostaglandins, excitotoxins
- Provide mechanism of defence, but greater damage results in neurons, dendrites, and synaptic connections if release is too great

TNF

- Major signalling interleukin, but not specific to neuroinflammation
- Most commonly investigated in moderate and severe TBI
- In paediatric concussion, increased plasma TNF in the days following injury for those with symptoms, while no difference was seen in IL-6, IL-10, tau, NSE, S100B, and GFAP (Parkin et al. 2019)



IL-6

- Pro and anti-inflammatory properties
- Promotes nerve growth factor synthesis and counteracts NMDA toxicity
- Elevated in high school and collegiate football players who were concussed within 6 hours of injury, and correlated with symptom duration (Nitta et al. 2019)
- Serum concentrations are also increased in orthopaedic injury, burns and exercise

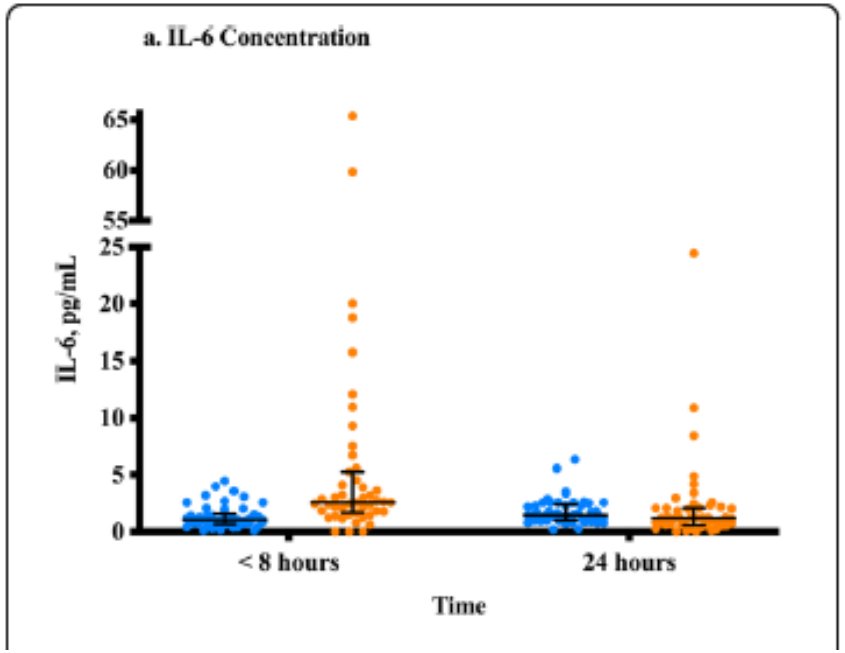
IL-10

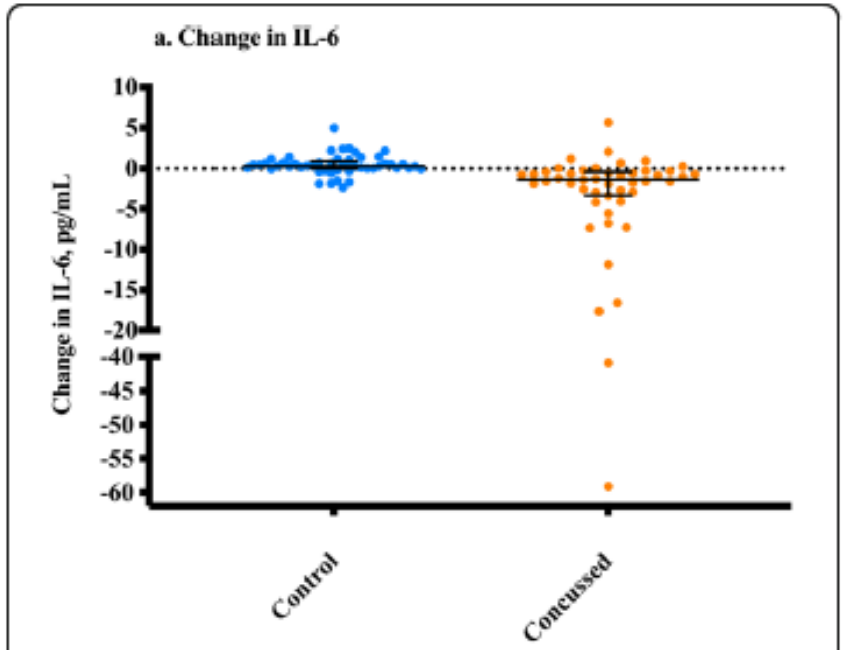
- Able to differentiate between CT positive and CT negative patients more successfully than S100B (Lagerstedt et al. 2018)
- Associated with the presence of behavioural symptoms such as PTSD in military subjects with and without TBI (Bersani et al. 2016; Devota et al. 2017)
- Compared to controls, military personnel who had experienced mTBI in the previous 18 months had elevated concentrations with levels related to PTSD symptoms (Gill et al. 2018)
- Serum levels are higher than CSF, indicating potential peripheral expression

RESEARCH ARTICLE Open Access

Interleukin-6 is associated with acute concussion in military combat personnel

Katie A. Edwards[1,2*], Jessica M. Gill[1,3], Cassandra L. Pattinson[1], Chen Lai[1], Misha Brière[4], Nicholas J. Rogers[5], Denise Milhorn[5], Jonathan Elliot[6] and Walter Carr[7,8]





Change in IL-6 across time was greater for the concussed group than healthy control, with no differences between groups in the change of IL-10 or TNFα.

CCL11

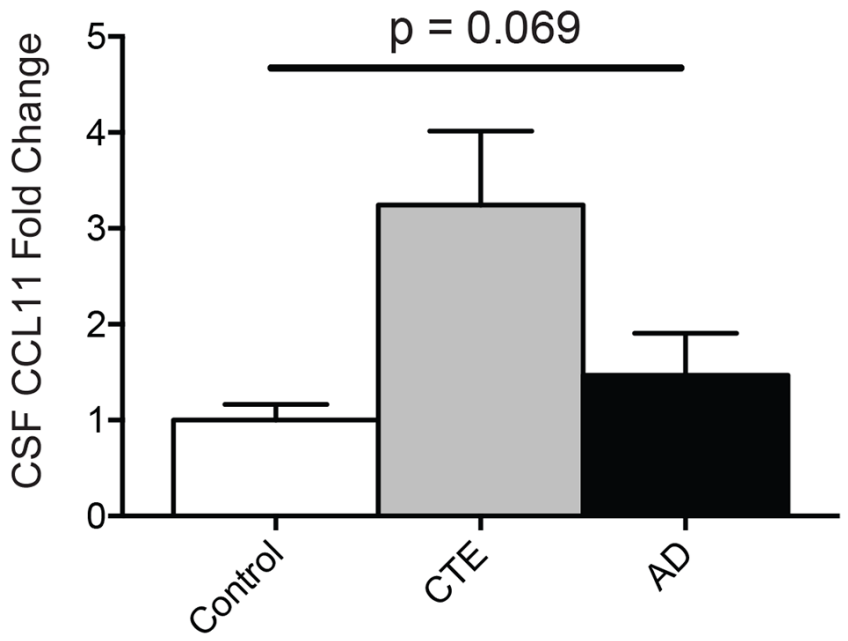
- Chemokine generated in the choroid plexus, also contributed by glial cells following CNS trauma
- Excess levels trigger ROS and excitotoxicity leading to tau pathology
- Significantly elevated in CSF of former American football players with CTE diagnosed at autopsy. A correlation between CCL11 levels and tau pathology, and between levels and number of years playing football (Cherry et al. 2017).
- Differentiate between diagnosis of AD

RESEARCH ARTICLE

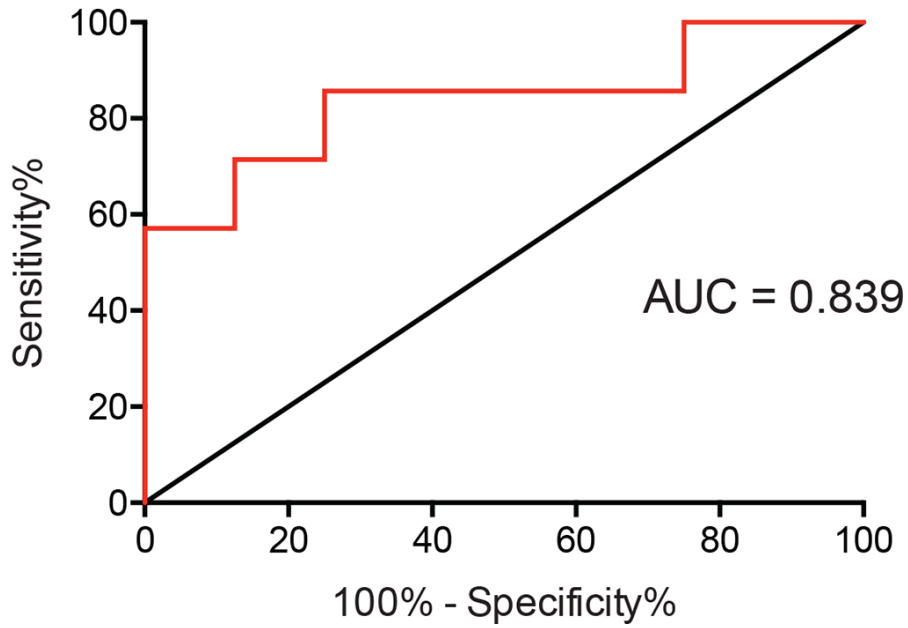
CCL11 is increased in the CNS in chronic traumatic encephalopathy but not in Alzheimer's disease

Jonathan D. Cherry[1,2,3]*, Thor D. Stein[1,2,4], Yorghos Tripodis[5], Victor E. Alvarez[1,2,3,4], Bertrand R. Huber[1,2,3], Rhoda Au[2,6], Patrick T. Kiernan[1], Daniel H. Daneshvar[1], Jesse Mez[1,2], Todd M. Solomon[1], Michael L. Alosco[1,2], Ann C. McKee[1,2,3,4,7]

A



B



miRNA markers

- Non-coding nucleotide sequences that regulate gene expression
- May have benefits over protein biomarkers
  - Cross the BBB more readily
  - Protected by microvesicles, exosomes, or carrier proteins
  - Detection in fluids such as saliva
- Diagnosis - serum differences seen in military veterans with and without mTBI in miR-671-5p (Pasinetti et al. 2012)
- Preliminary results from the Chronic Effects of Neurotrauma Consortium Biomarker Discovery Project have identified exosomal MicroRNAs in Military Personnel with mTBI (Devoto et al. 2020)

Ongoing challenges

- Consistency with sample preparation techniques and use of assays
- Time-dependent expression changes
- Differentiating the mechanisms of biomarker expression – BBB vs glymphatic system vs expression change

Poll question #3

- After viewing this talk, how relevant do you think blood biomarkers are in diagnosis/prognosis for blast injury and mTBI?
  - Very
  - Somewhat
  - Not at all
  - Other, please comment

Questions?