Biomarkers of Traumatic Brain Injury
Implications for the Next Generation of Clinical Trials

Ramon Diaz-Arrastia, MD, PhD
Department of Neurology
University of Pennsylvania

Outline of Presentation

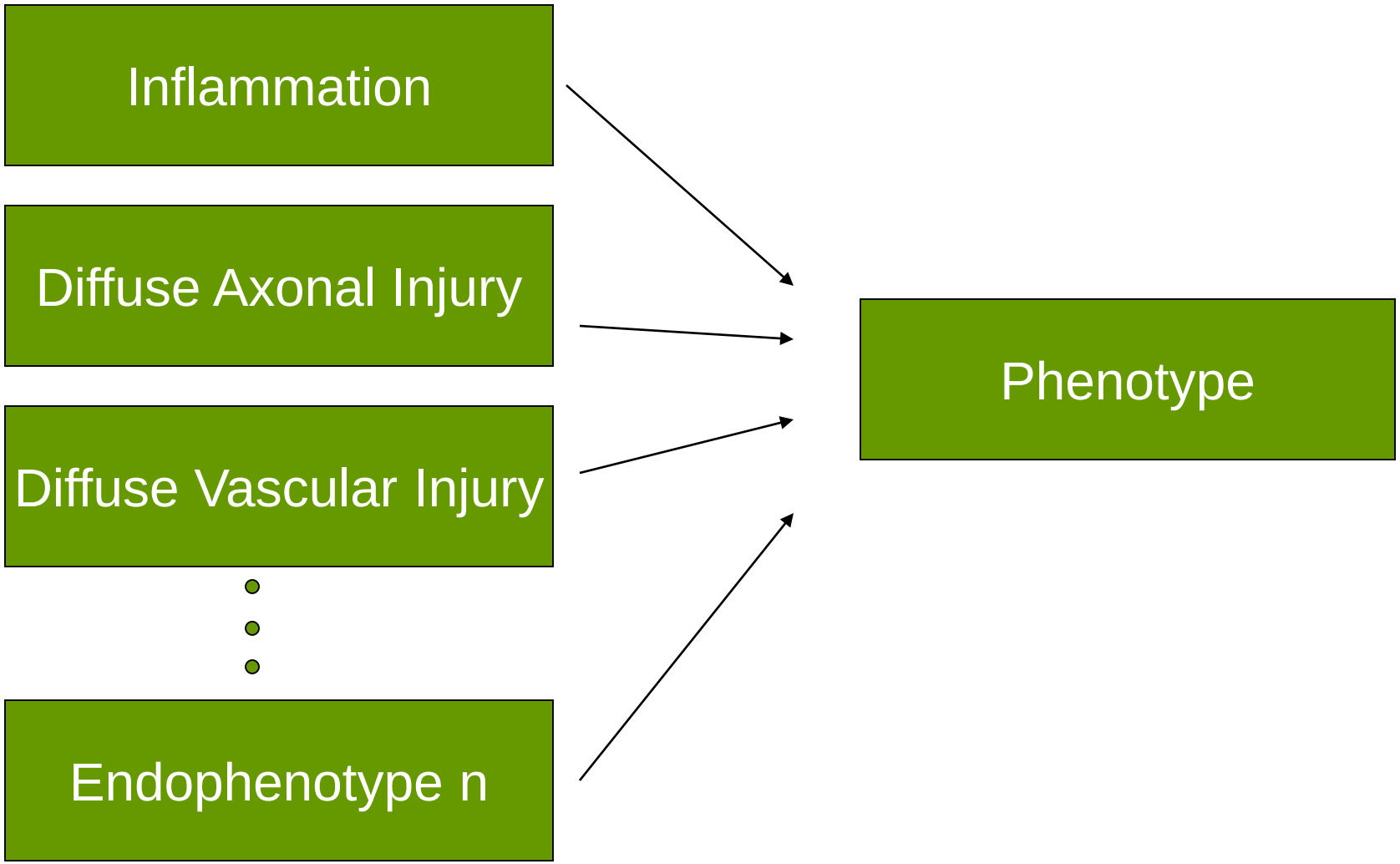
- Need for Endophenotype-directed biomarkers
- Types of Biomarkers and Context of Use
- Example: Glial Fibrillary Acidic Protein (GFAP)
- Example: C-Reactive Protein (CRP)
- Example: Neurofilament Light Chain (NfL)

Endophenotype

- An internal or intermediate phenotype that is closer to the underlying pathophysiology of disease (whether genetic or environmental)
- A continuous, quantitative variable (as opposed to phenotype which is usually a categorical variable)
- Measured quantitatively through physiologic, biochemical, or imaging technique.
- Synonyms: Endotype, subphenotype

Almasy & Blangero (2001) *Am J. Hum Genet* 105:42-44

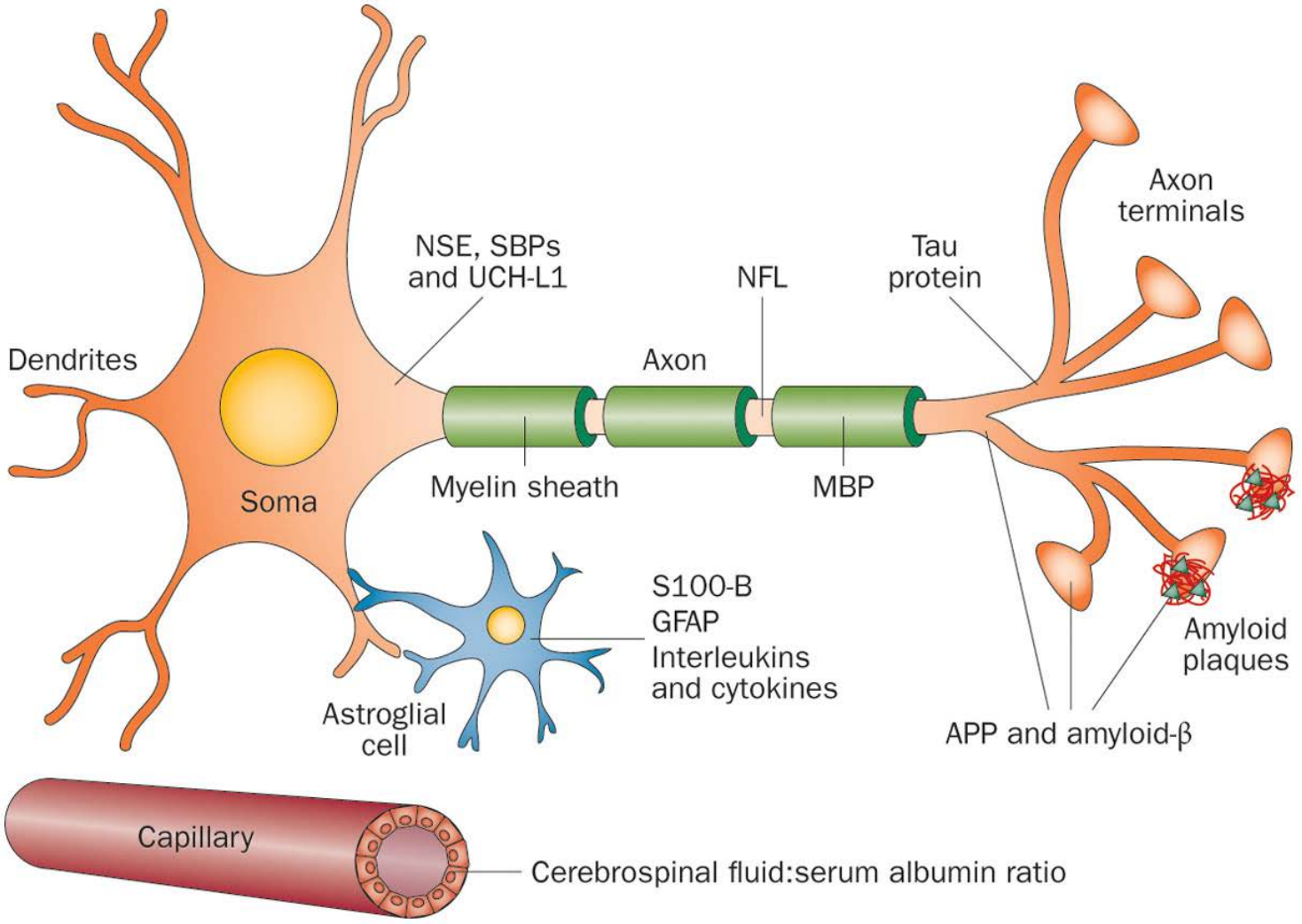
Endophenotypes of TBI



What do we need to know for the next generation of clinical trials?

- Biomarkers to measure endophenotypes should be developed iteratively between clinical and preclinical studies
  - *Observational studies humans*—Natural history of endophenotype in humans with TBI. Identify subset of patients likely to merit therapy
  - *Preclinical studies*—Confirm mechanistic benefit of therapy and establish pharmacodynamic relevance of biomarker
  - *Biomarker-driven Phase II clinical trials*—To establish optimal dose, timing, and duration of therapy

Candidate biomarkers for TBI



Zetterberg et al, *Nat. Rev. Neurol*. 2013

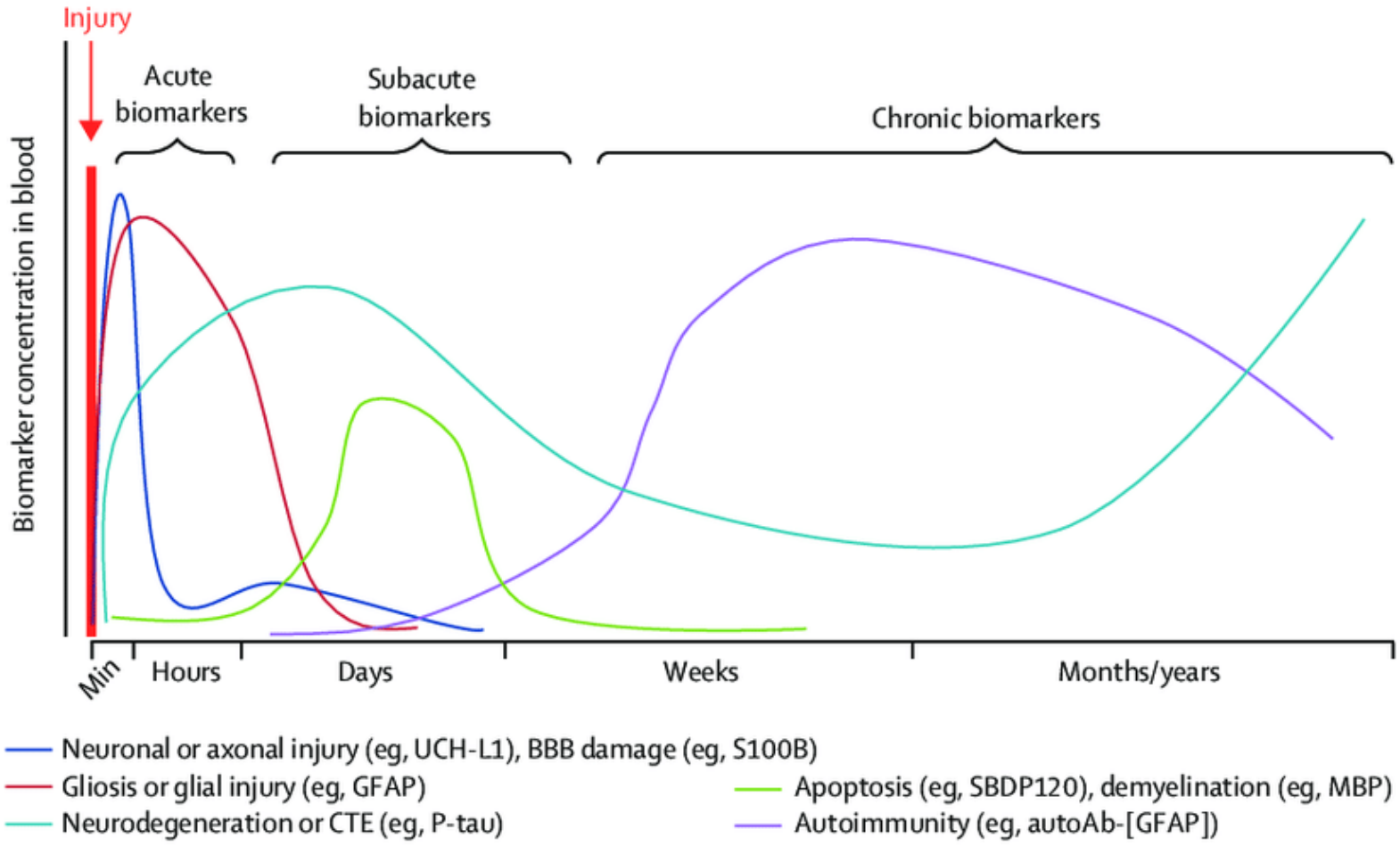
TRACK-TBI Precision Medicine Initiative

Candidate Endophenotype-directed Biomarkers		
Measuring neuroinflammation	*Measuring diffuse axonal injury*	*Measuring diffuse vascular injury*
• Free water fraction • DCE-MRI • IL-1-β, IL-6, IL-10, TNF-α	• Diffusion Tensor Imaging • Regional brain volumes • NfL, Tau, SNTF	• Cerebral Blood Flow (CBF) • Cerebrovascular Reactivity (CVR) • vWF, cFN, PDGFR-β

TRACK-TBI Clinical Trials Network (TRACK-TBI NET)

Candidate Phase 2 acute TBI drug candidates		
Targeting neuroinflammation	*Targeting diffuse axonal injury*	*Targeting diffuse vascular injury*
• IL-1 receptor antagonist • Minocycline/NAC • Imatinib	• Cyclosporine A • Omega-3 FA • Dronabinol	• Simvastatin • Glyburide • Candesartan • CN-105 (ApoE mimetic)

Longitudinal Evolution of Brain Injury Biomarkers



Maas et al, *Lancet Neurology* 2017

Types of Biomarkers

- Biomarker:
  - *A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention*
- Diagnostic biomarker:
  - *Measure used to identify individuals with disease or condition of interest, or to define a subset of the disease*
- Prognostic biomarkers:
  - *Baseline measurements which categorize patients by degree of risk for disease progression, and informs about the natural history*
  - Used to select patients likely to have a problem that warrants therapy
- Predictive biomarkers:
  - *Baseline characteristics that categorize patients by their likelihood of response to a particular treatment.*
  - Used to measure the presence in the patient of the mechanism targeted by therapy
- Pharmacodynamic biomarkers:
  - *Dynamic measurements which show that biologic response has occurred in a patient after a therapeutic intervention.*
  - Used to demonstrate target engagement by therapy, and fine tune issues of dose, duration, timing of therapy

Robb et al, *JAMA* March 15, 2016 (315) :11

Clinical Needs: Pre-hospital

- Scene of accident; sidelines of sports event; combat setting
  - Inform decision to transfer to ED for medical evaluation
  - Inform decision to bypass nearest ED in favor of a neurosurgical specialty facility
- Need high sensitivity / moderate specificity
- Must be detectable in blood or other biologic fluid within minutes
- Impact:
  - Improve utilization of ED services
  - Accelerate transfer to specialized neurosurgical centers when such care needed

Clinical Needs: Emergency Department

- Identify patients in need of cranial CT
  - Excessive number of normal cranial CTs performed
- Identify subset of patients who may benefit from cranial MRI
- Inform counseling at ED discharge
  - Identify patients likely to develop PCS
- Select patients for clinical trials of neuroprotective/neurorestorative therapies

Clinical Needs: Intensive Care Unit

- Identify patients at risk for secondary neural injury
  - Ischemia
  - Intracranial hypertension
  - Inflammation
- Select patients for clinical trials of neuroprotective/neurorestorative therapies
- Inform decisions regarding intensity of care and benefit of rehabilitation services

Clinical Needs: Rehabilitation Unit and Chronic Care

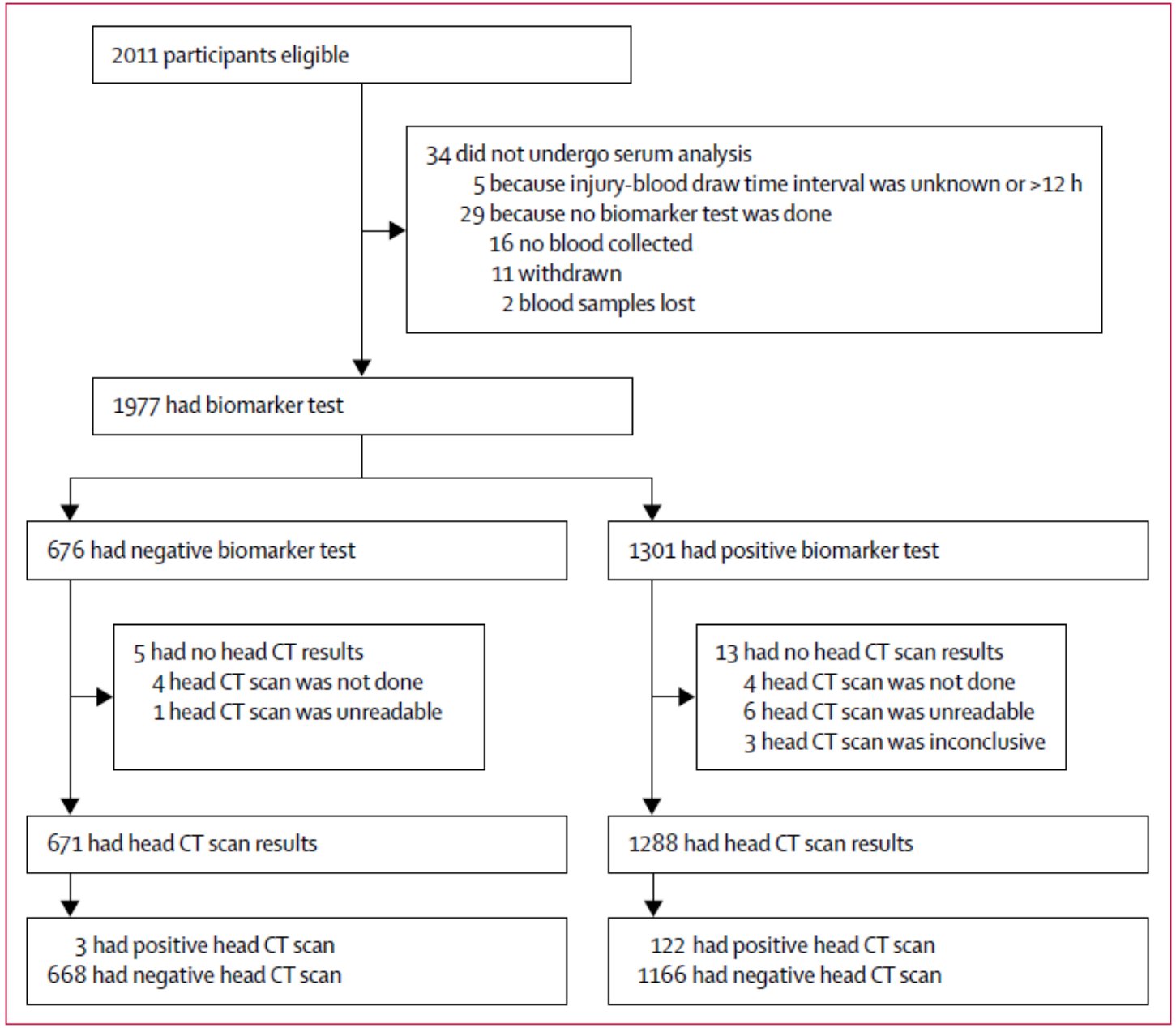
- Identify patients at risk for late complications of TBI
  - Post-traumatic epilepsy
  - Post-traumatic dementia / Chronic Traumatic Encephalopathy
- Identify mechanisms of post-TBI comorbidities
  - Post-traumatic headaches
  - Post-traumatic neuropsychiatric disorders
- Select patients for clinical trials of therapies designed to prevent late complications

Glial Fibrillary Acidic Protein (GFAP)

- Intermediate filament protein
- Highly specific to astrocytes
  - Induced by neural injury
  - Released upon disintegration of cytoskeleton
- Elevated in CSF and serum after severe TBI
- Elevated in mild TBI

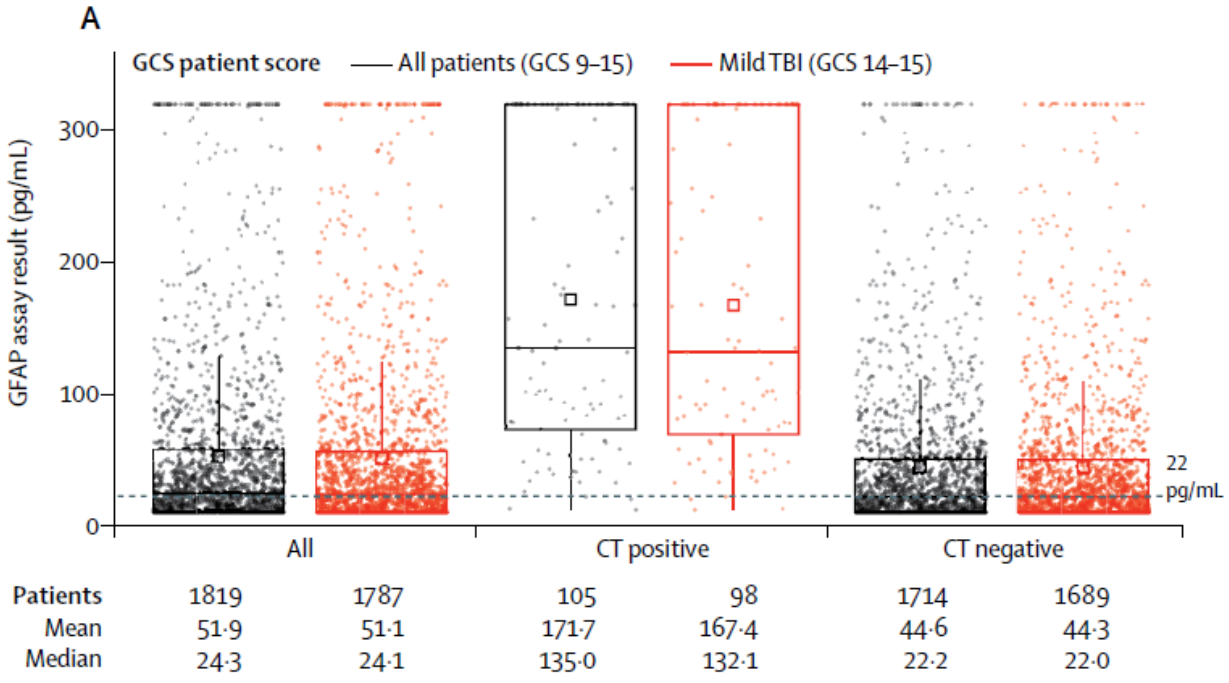
GFAP and UCHL-1 in ED:

The ALERT Study

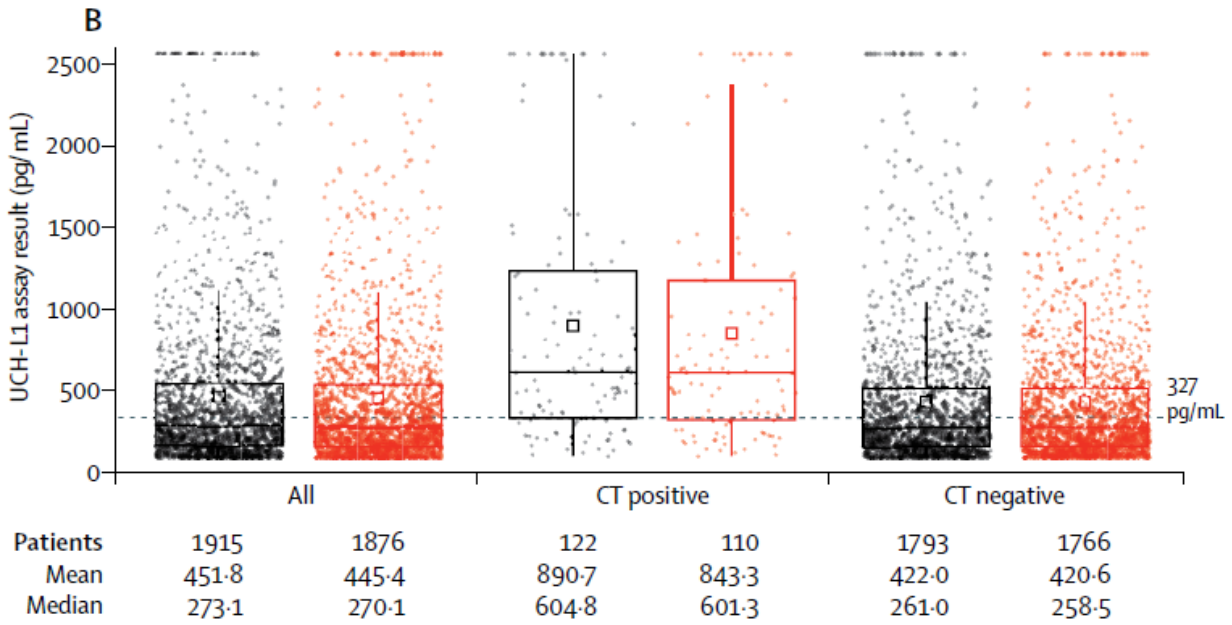


Bazarian et al, *Lancet Neurol*. 2018

GFAP and UCHL-1 in ED: The ALERT Study



GFAP



UCH-L1

Bazarian et al, *Lancet Neurol*. 2018

GFAP and UCHL-1 in ED: The ALERT Study

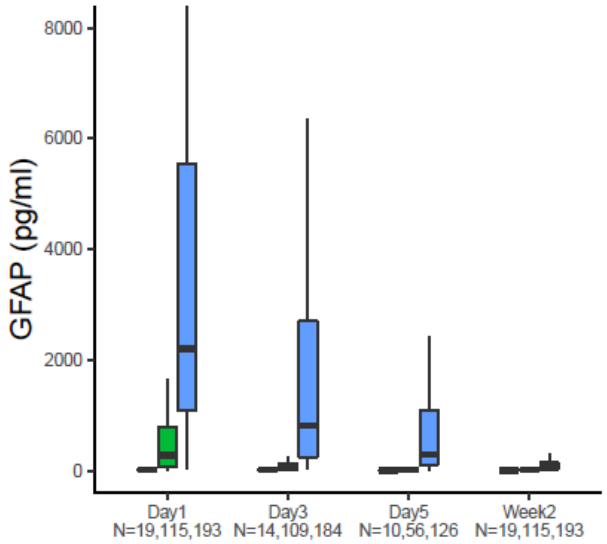
	Sensitivity	Specificity	PPV	NPV	LRP	LRN
GCS 9–15 (n=1959)	0·976 (0·931–0·995)	0·364 (0·342–0·387)	0·095 (0·079–0·112)	0·996 (0·987–0·999)	1·5 (1·455–1·616)	0·07 (0·00–0·153)
GCS 14–15 (n=1920)	0·973 (0·924–0·994)	0·367 (0·345–0·390)	0·088 (0·073–0·105)	0·995 (0·987–0·999)	1·5 (1·457–1·618)	0·07 (0·00–0·159)
Neurosurgically manageable lesions (n=8)	1·00 (0·631–1·00)	0·344 (0·323–0·365)	0·006 (0·003–0·012)	1·00 (0·995–1·00)	1·5 (1·447–1·602)	0·0 (0·00–0·093)

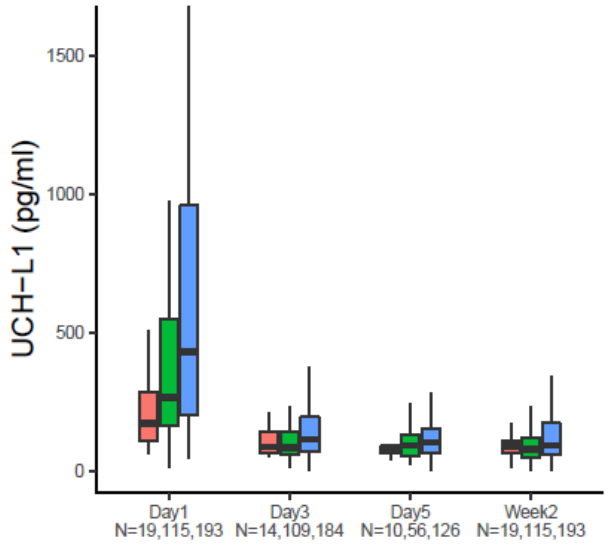
Data in parentheses are 95% CIs. PPV=positive predictive value. NPV=negative predictive value. LRP=likelihood ratio positive. LRN=likelihood ratio negative.

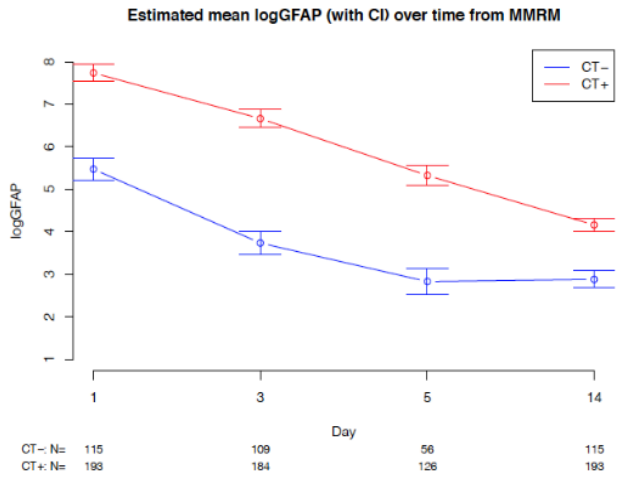
- In combination, GFAP and UCH-L1 have excellent sensitivity and decent specificity for identifying trauma-related CT lesions
- If implemented, could reduce cranial CT use in the ED by 34-35%
- Number of neurosurgically manageable lesions small (n=8).
  - Confidence intervals large for this critical subset
- ALERT did not compare performance of biomarkers vs. available clinical guidelines

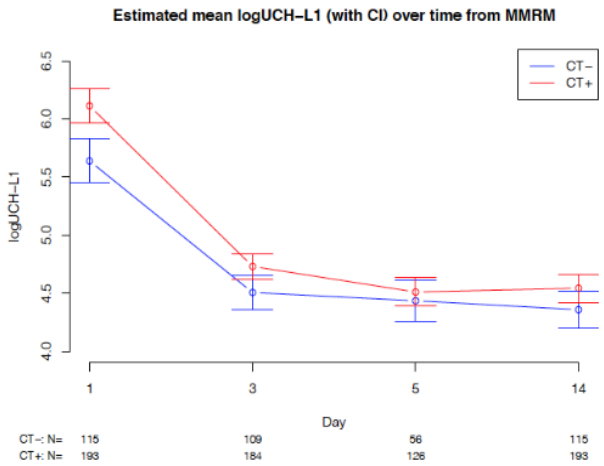
Bazarian et al, *Lancet Neurol*. 2018

GFAP remains elevated for several days after mTBI



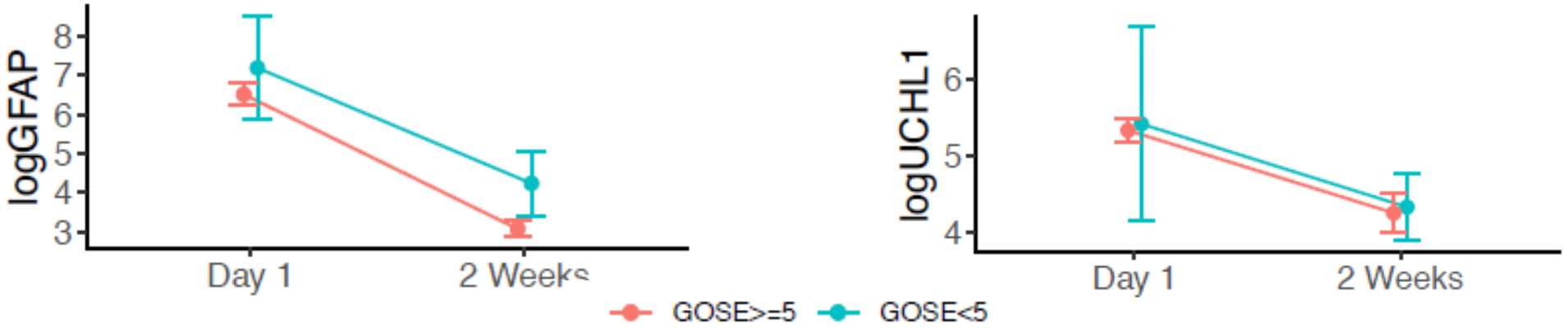






Puccio, TRACK-TBI Investigators [submitted]

GFAP remains elevated for several days after mTBI

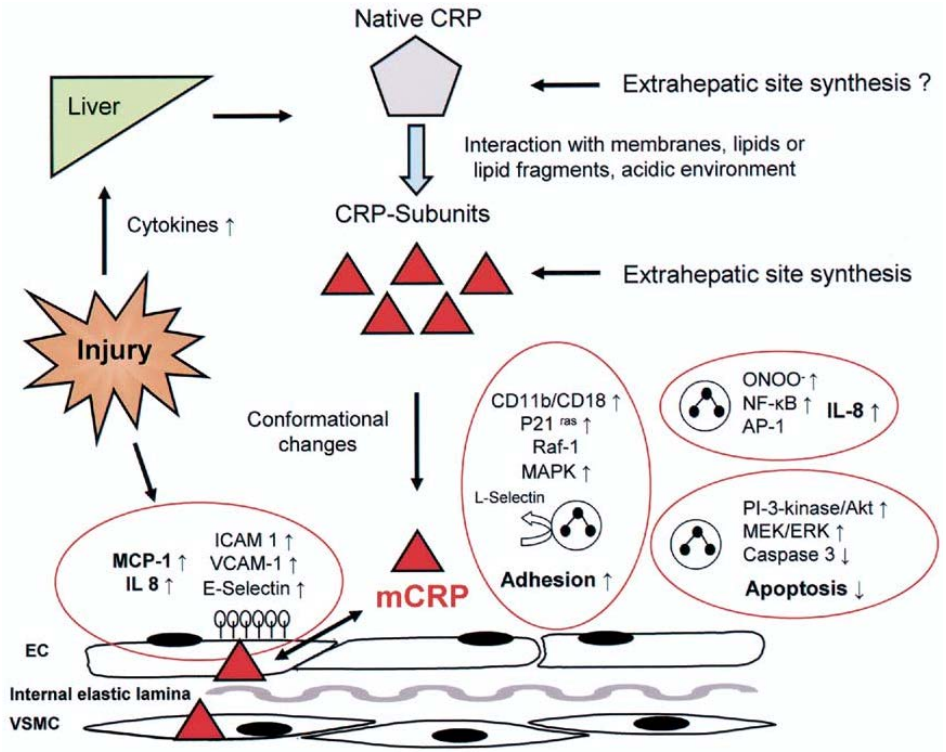


GFAP	3m GOSE 1 4 v. 5 8 AUC	95% CI Lower	95% CI Upper	6m GOSE 1 4 v. 5 8 AUC	95% CI Lower	95% CI Upper
Log D1	0.609	0.304	0.915	0.400	0.082	0.718
Log D14	0.805	0.559	1.000	0.828	0.623	1.000

Puccio, TRACK-TBI Investigators [submitted]

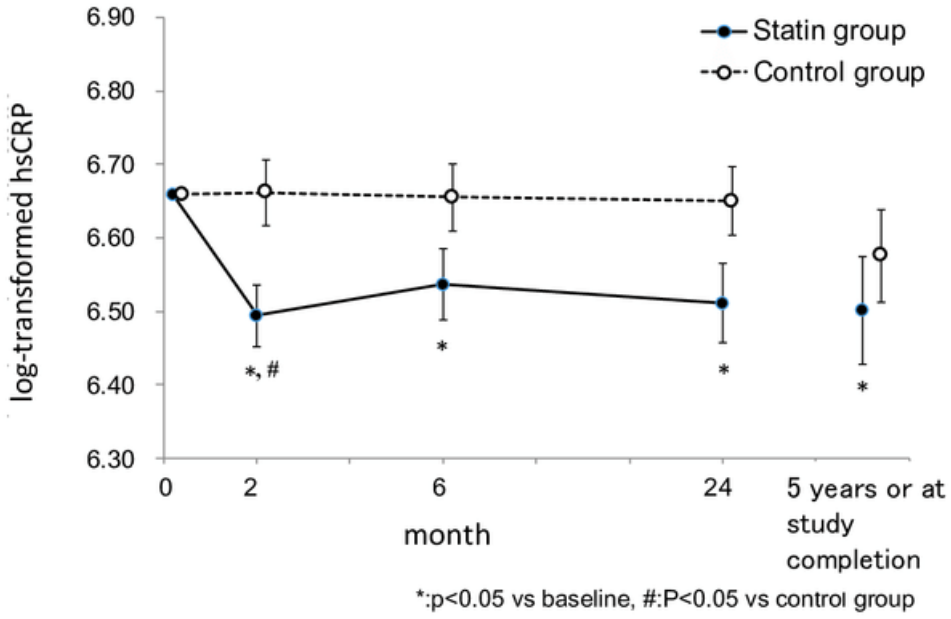
C-Reactive Protein

CRP is an Acute Phase Reactant and Inflammatory Mediator



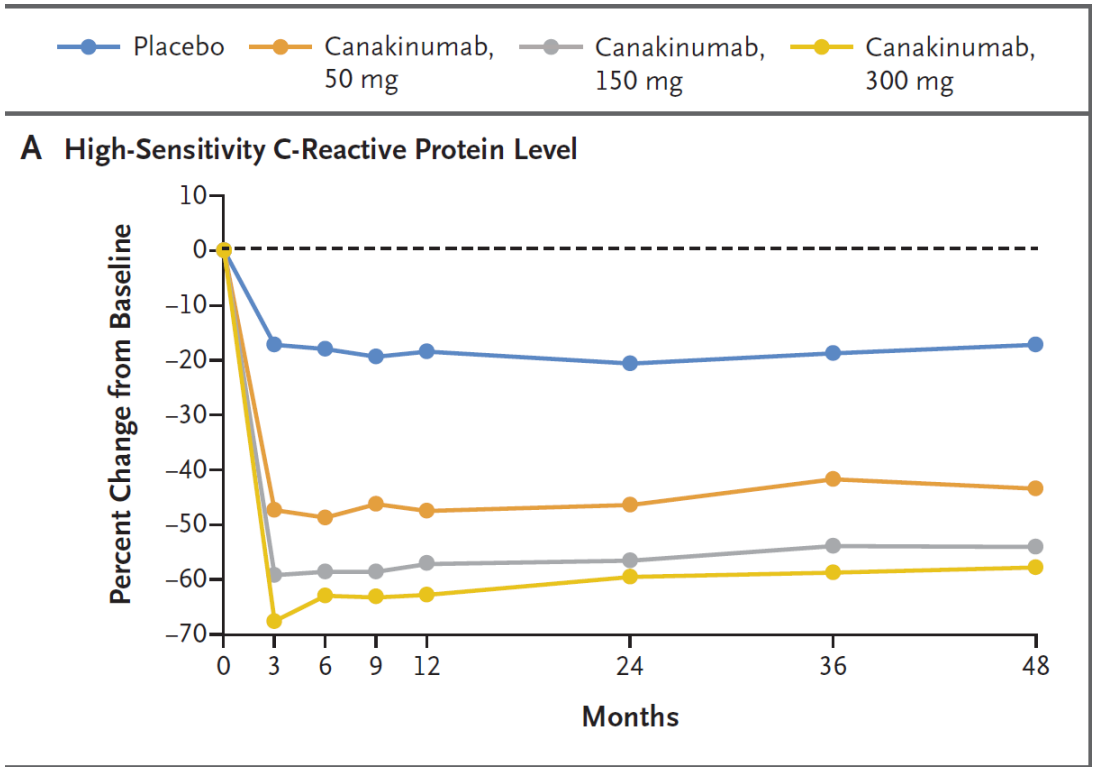
Schwedler et al, *Am J Kid Dis* 2006

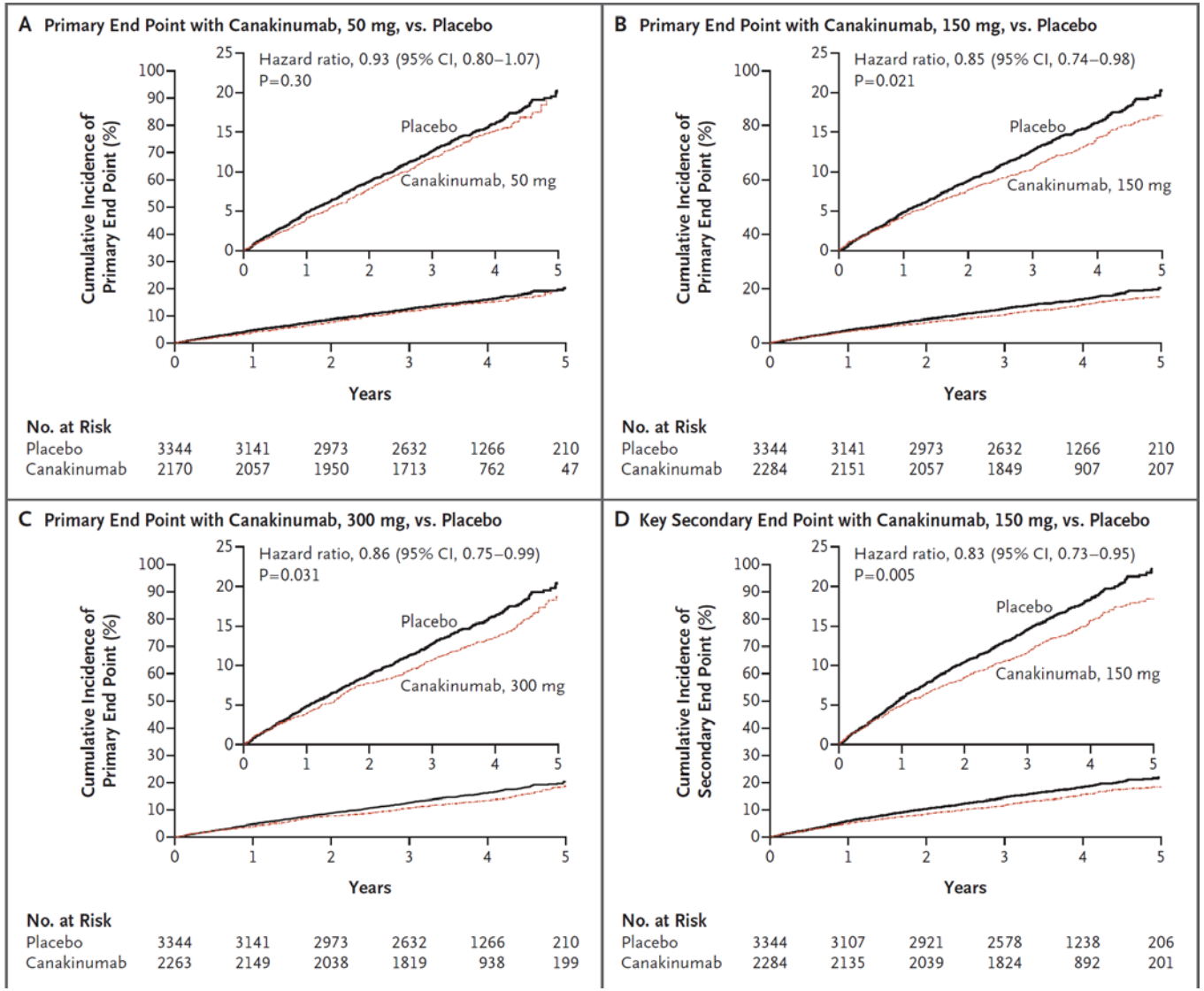
Statin therapy reduces hsCRP after acute ischemic stroke



Kitagawa et al, *J Atheroscl Thromb* 2017

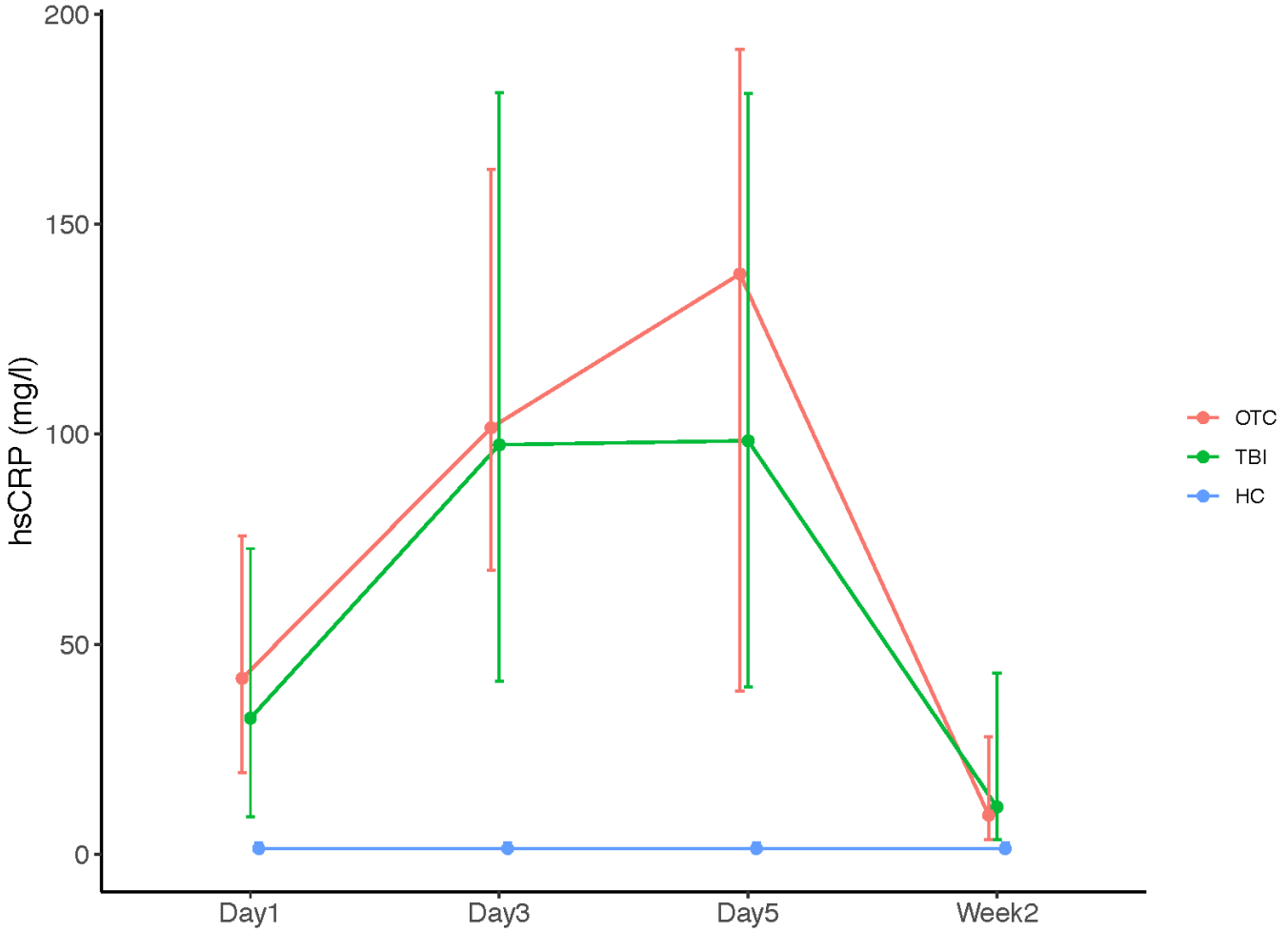
hsCRP as a Pharmacodynamic Biomarker of anti-IL1b Therapy





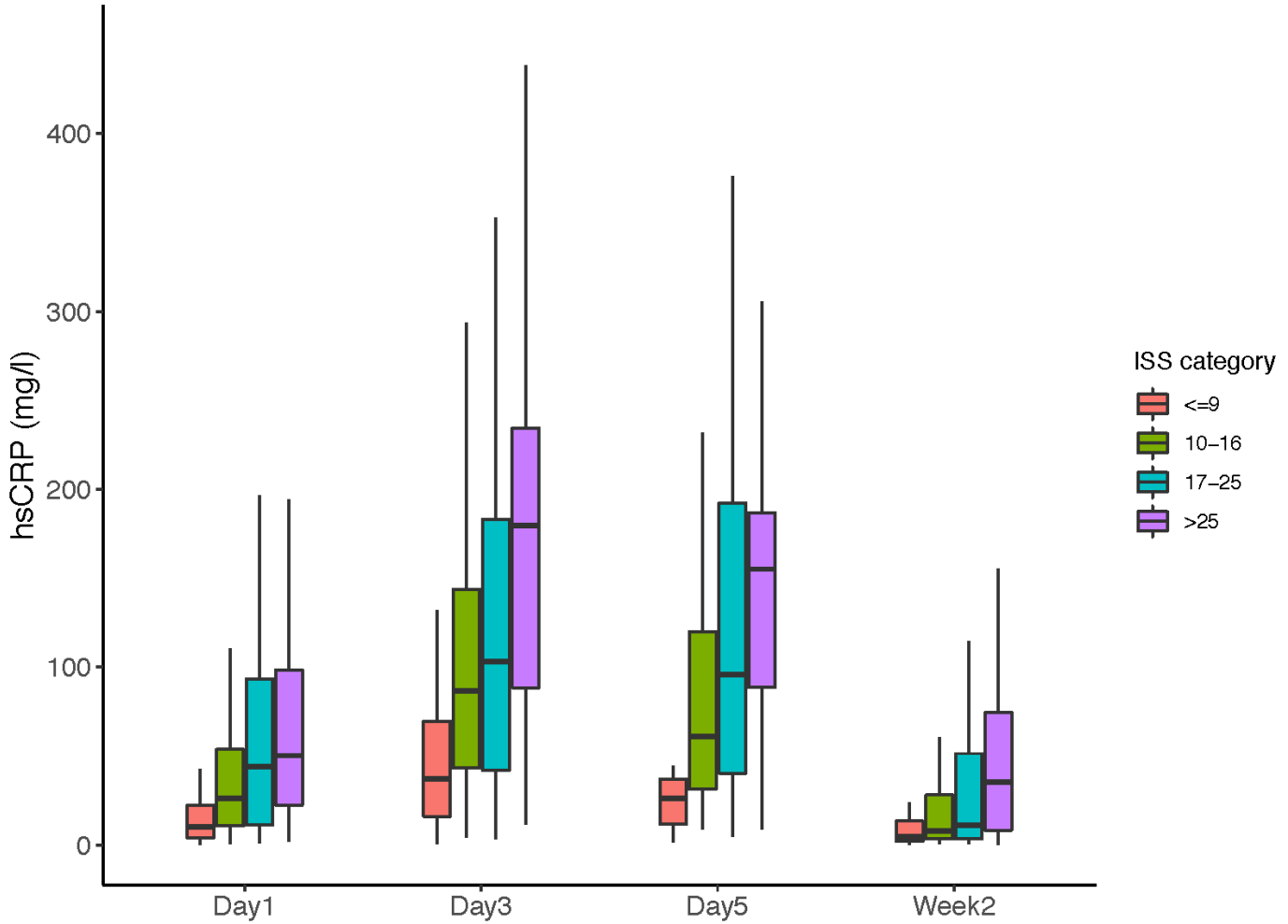
Ridker et al *NEJM* 2017

TRACK-TBI Phase I Cohort
hsCRP is elevated in TBI and Orthopedic Controls



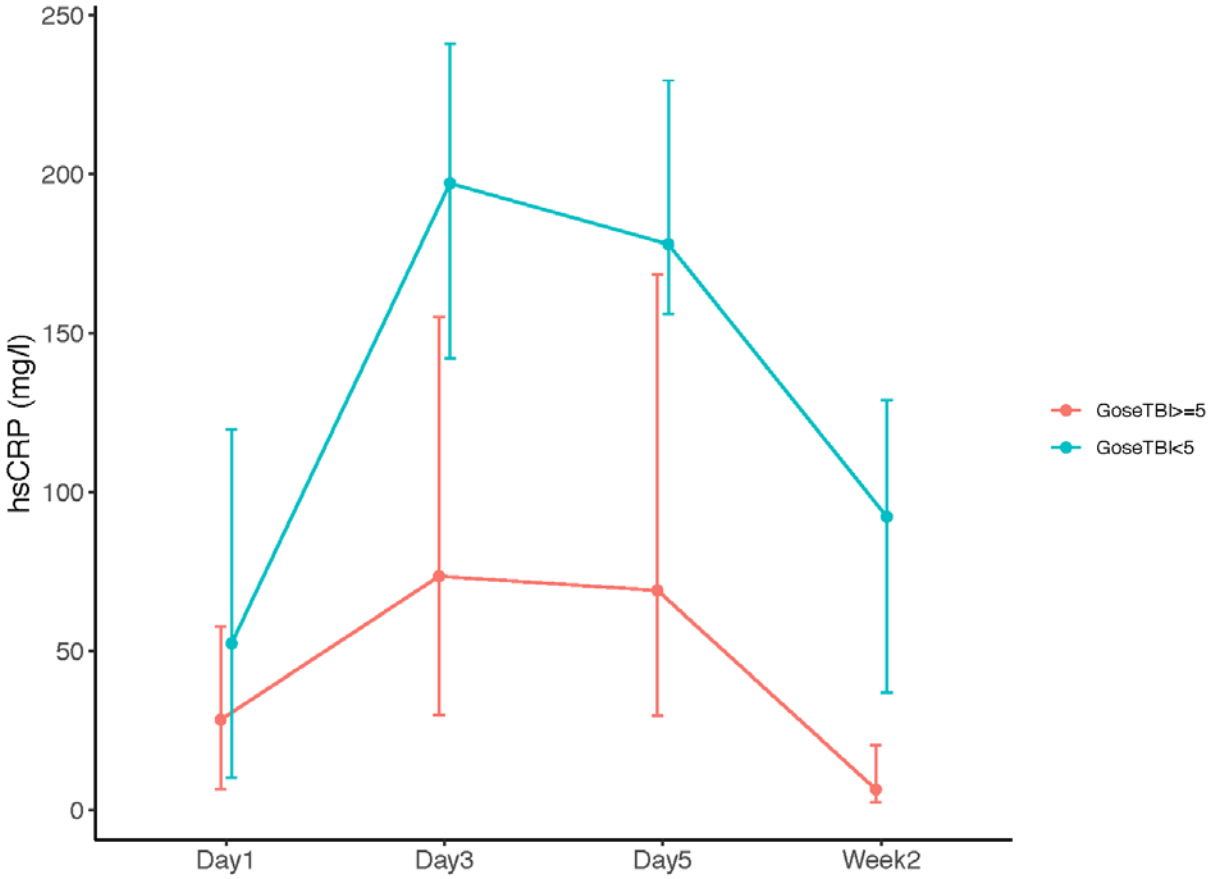
Xu et al *J Neurotrauma* 2020

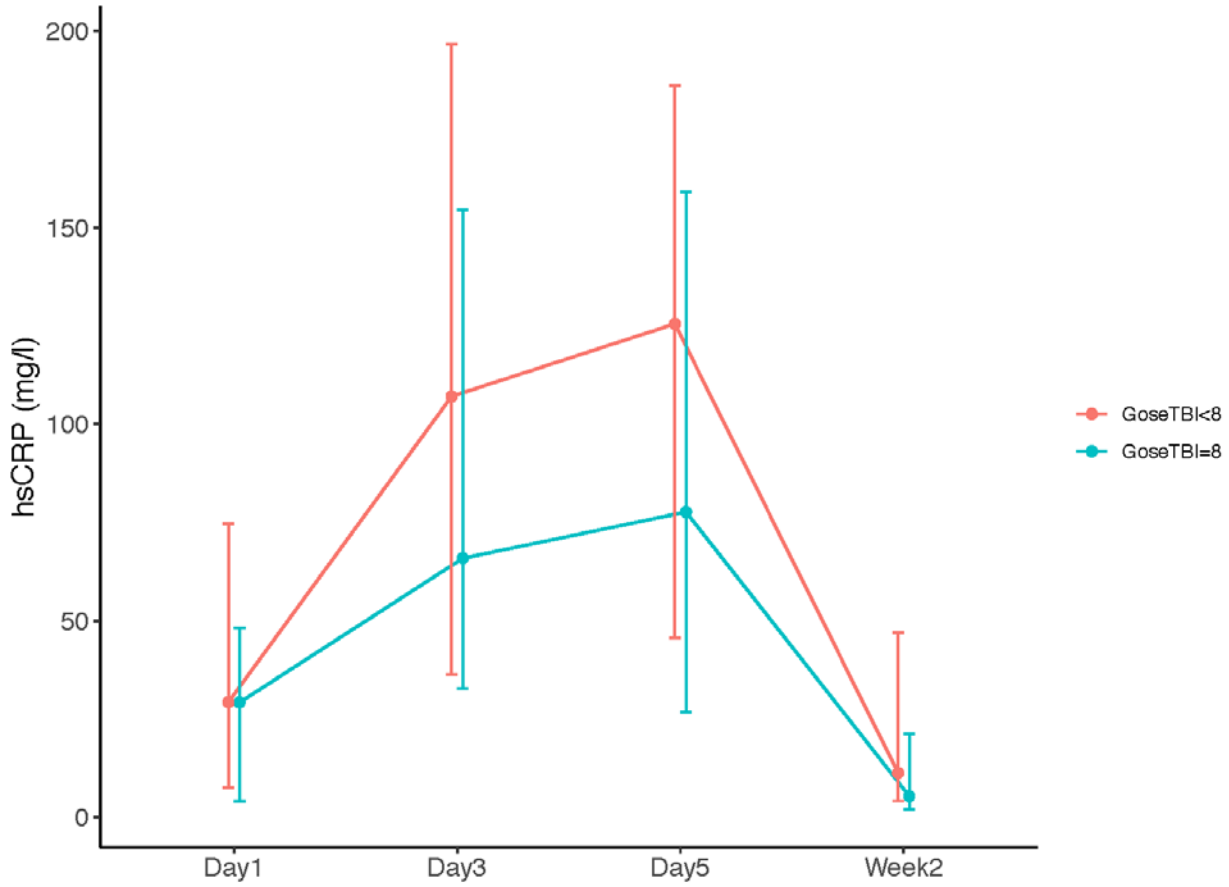
TRACK-TBI Phase I Cohort
hsCRP is Associated with Systemic Injury



Xu et al *J Neurotrauma* 2020

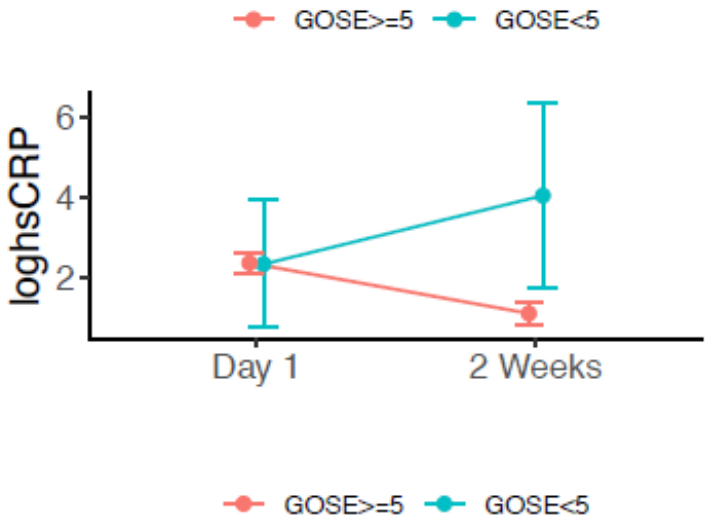
TRACK-TBI Phase I Cohort
hsCRP is Prognostic of Unfavorable Neurologic Outcome





Xu et al *J Neurotrauma* 2020

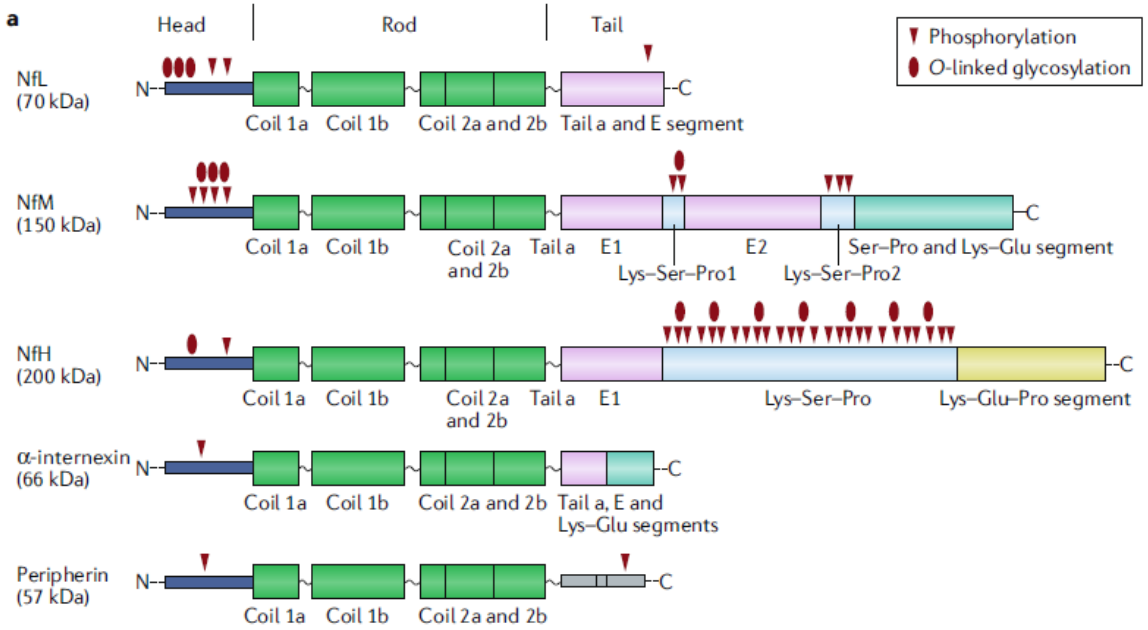
TRACK-TBI Phase I Cohort
hsCRP and GFAP are Prognostic of Unfavorable Neurologic Outcome

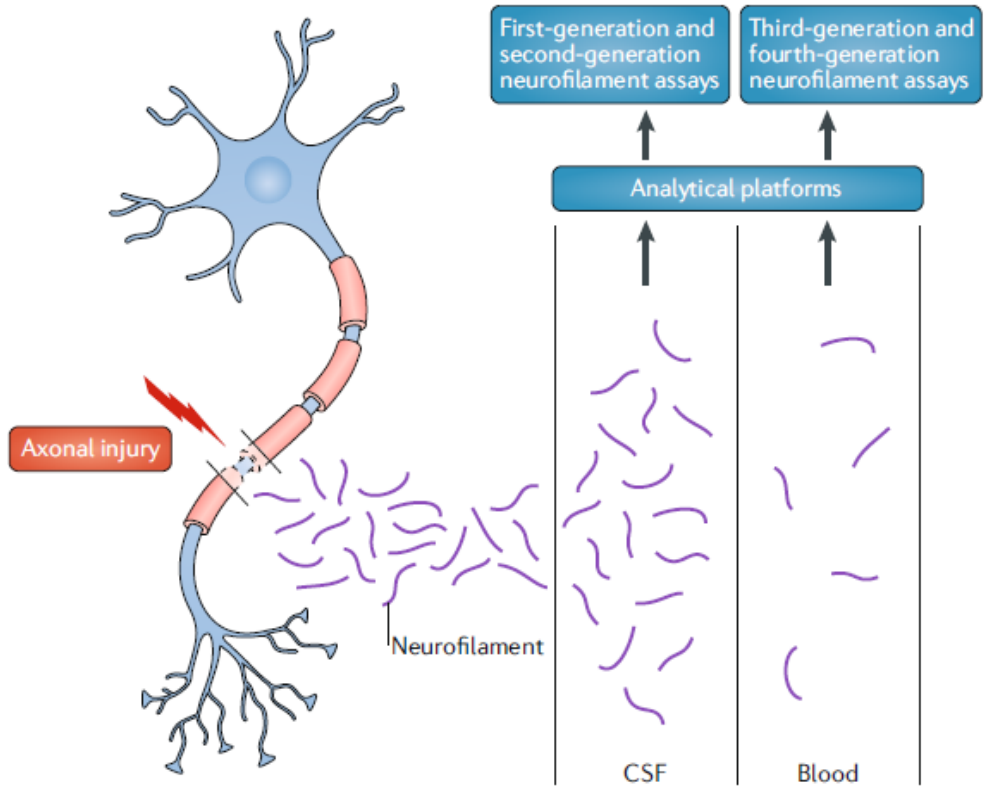


	AUC (95% CI)
Day 1	
GFAP	0.768 (0.662-0.875)
hsCRP	0.640 (0.521-0.760)
GFAP + hsCRP	0.765 (0.655-0.875)
Day 3	
GFAP	0.873 (0.798-0.949)
hsCRP	0.800 (0.729-0.871)
GFAP + hsCRP	0.902 (0.846-0.959)
Day 5	
GFAP	0.900 (0.827-0.972)
hsCRP	0.777 (0.691-0.862)
GFAP + hsCRP	0.911 (0.850-0.971)
2-Week	
GFAP	0.890 (0.823-0.956)
hsCRP	0.892 (0.839-0.944)
GFAP + hsCRP	0.939 (0.900-0.978)

Xu et al *J Neurotrauma* 2020

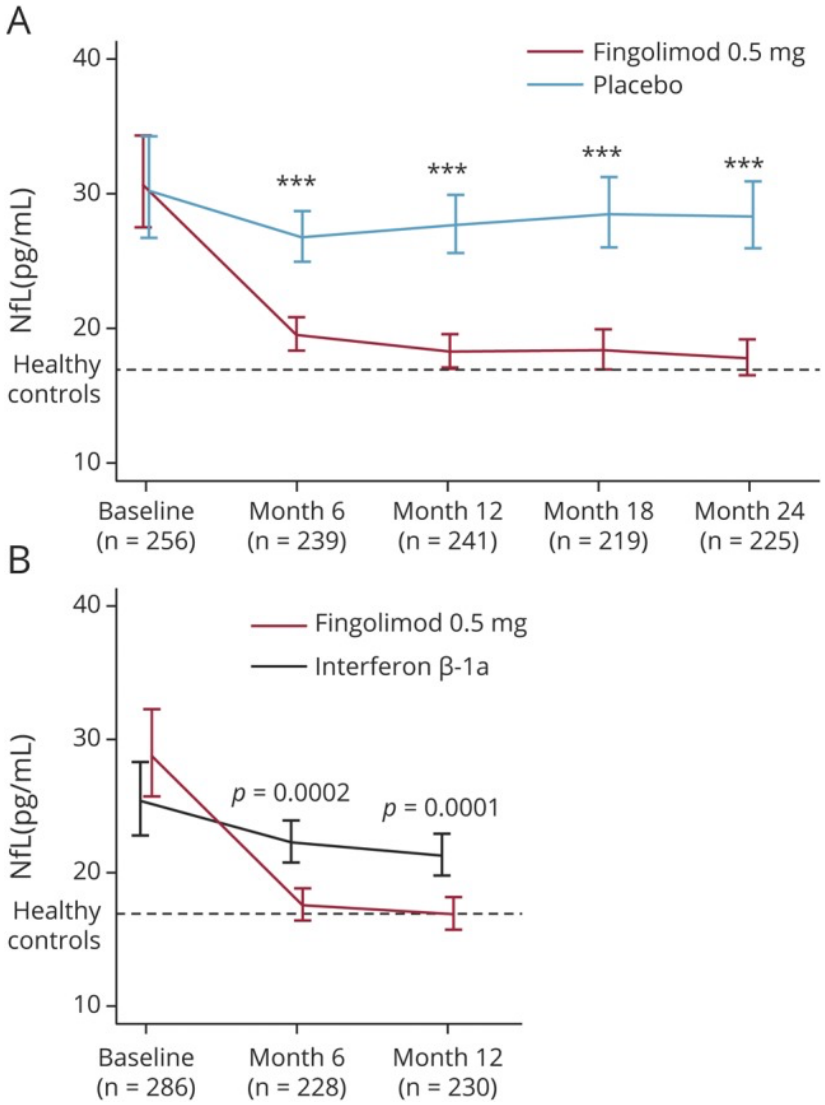
Neurofilament Light Chain





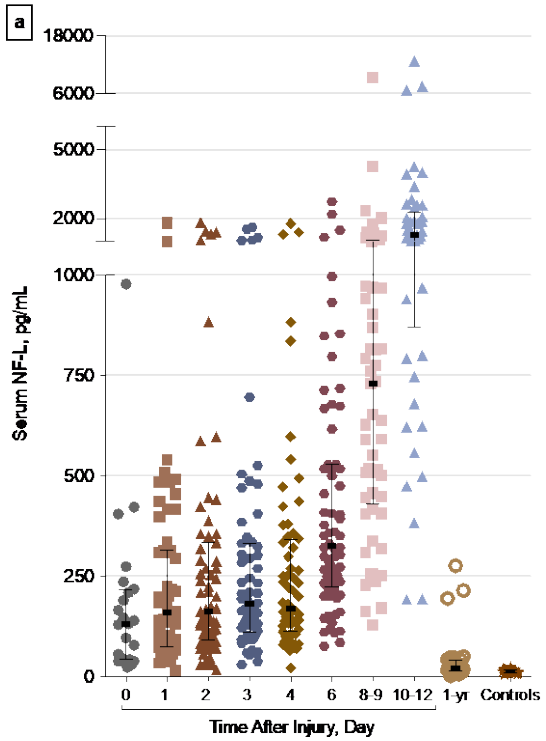
Khalil et al *Nature Review Neurol* 2018

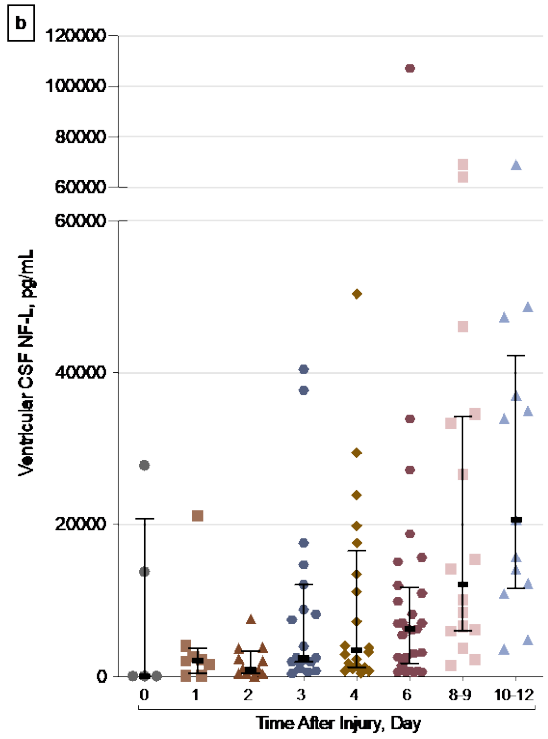
Serum NfL is a Pharmacodynamic Biomarker in Multiple Sclerosis

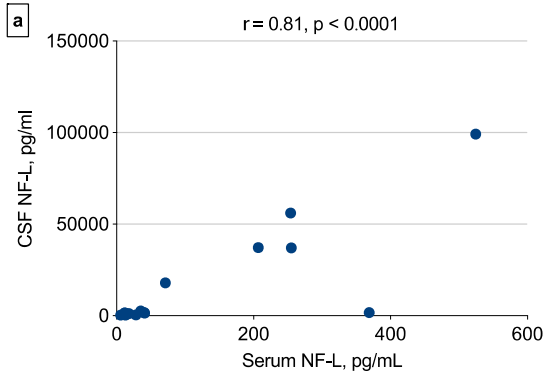


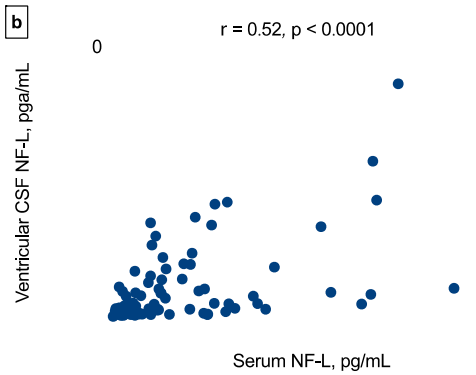
Kuhle et al *Neurology* 2017

NfL in Moderate to Severe TBI—Gothenburg ICU Study



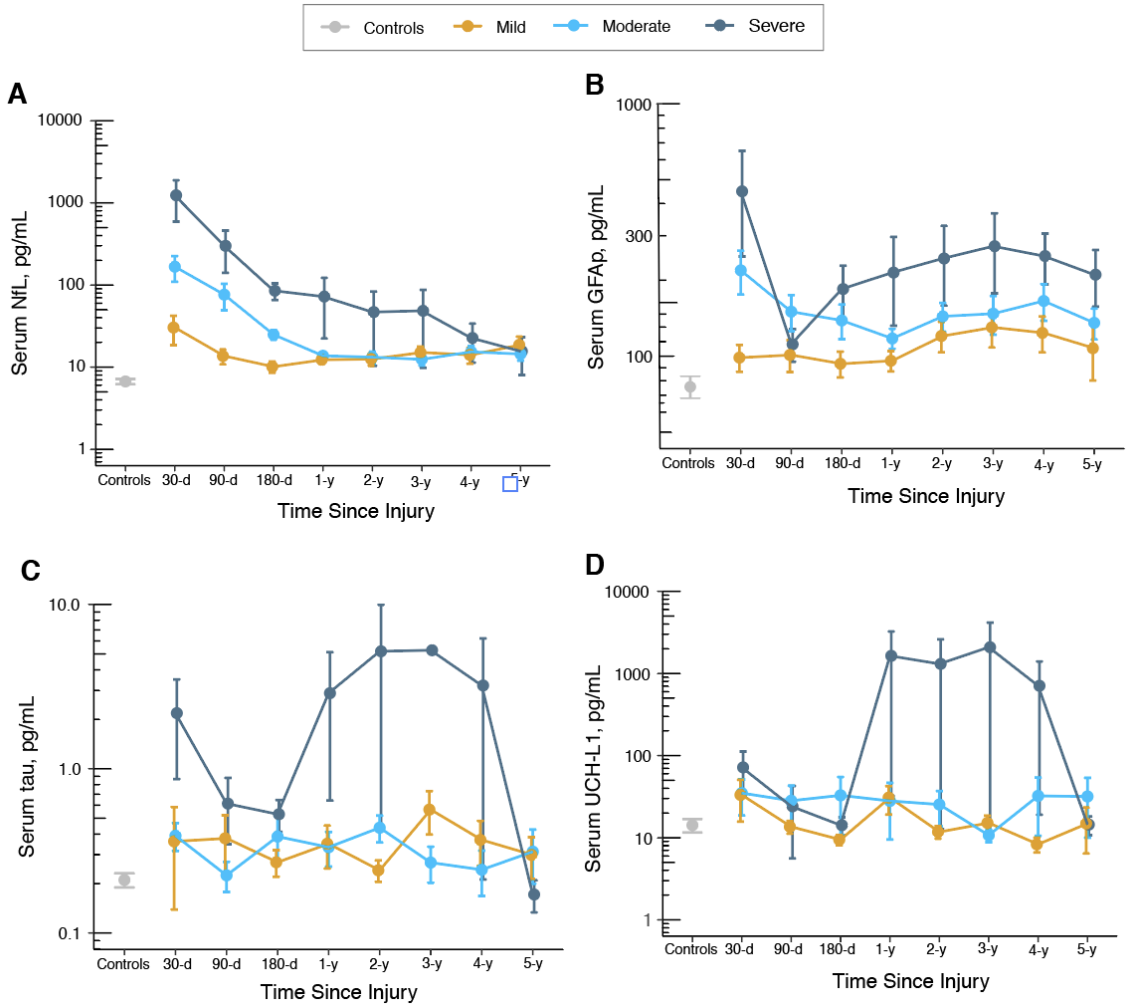






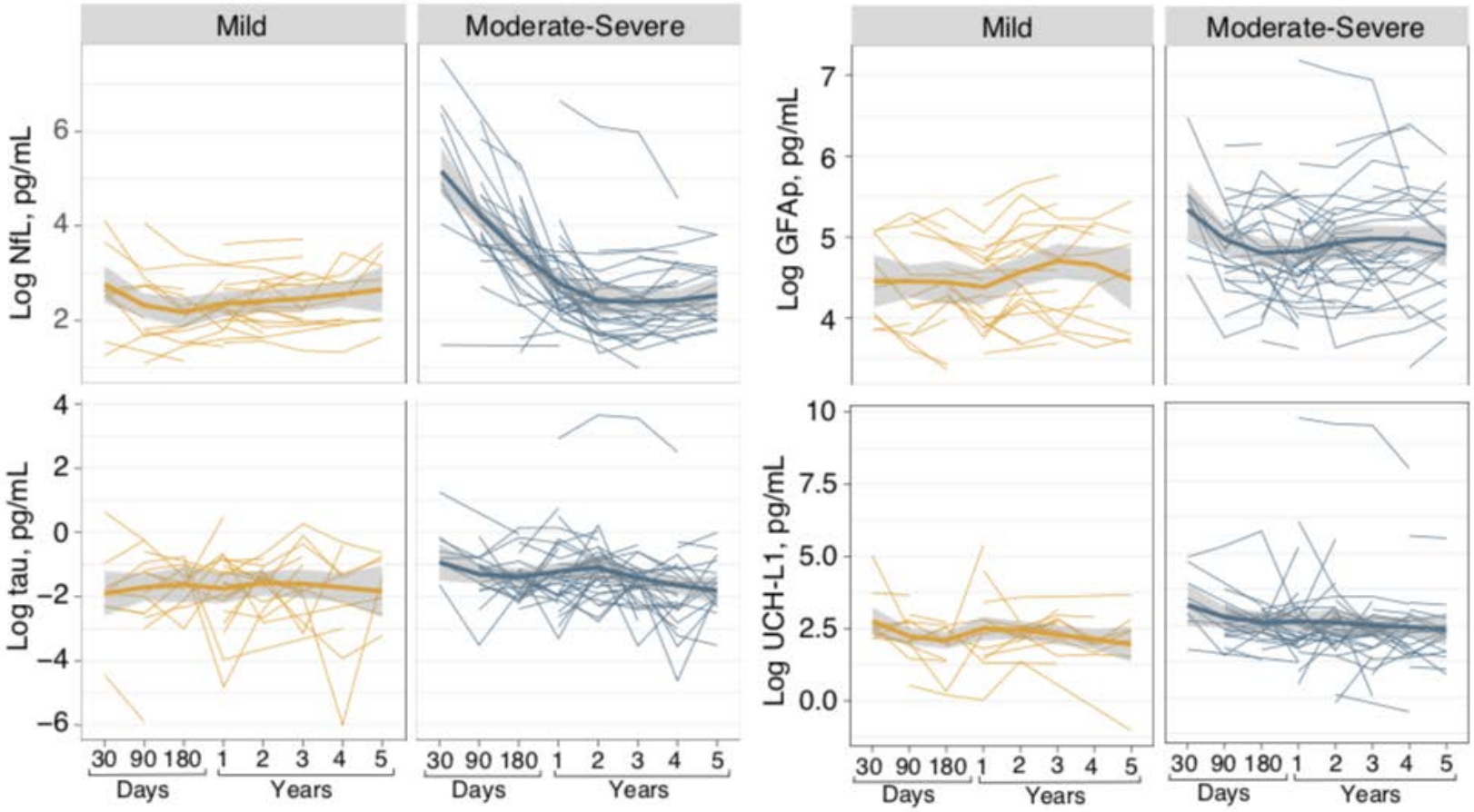
Modified from Shahim et al *Sci Rep* 2016

NfL in Chronic TBI—CNRM Study



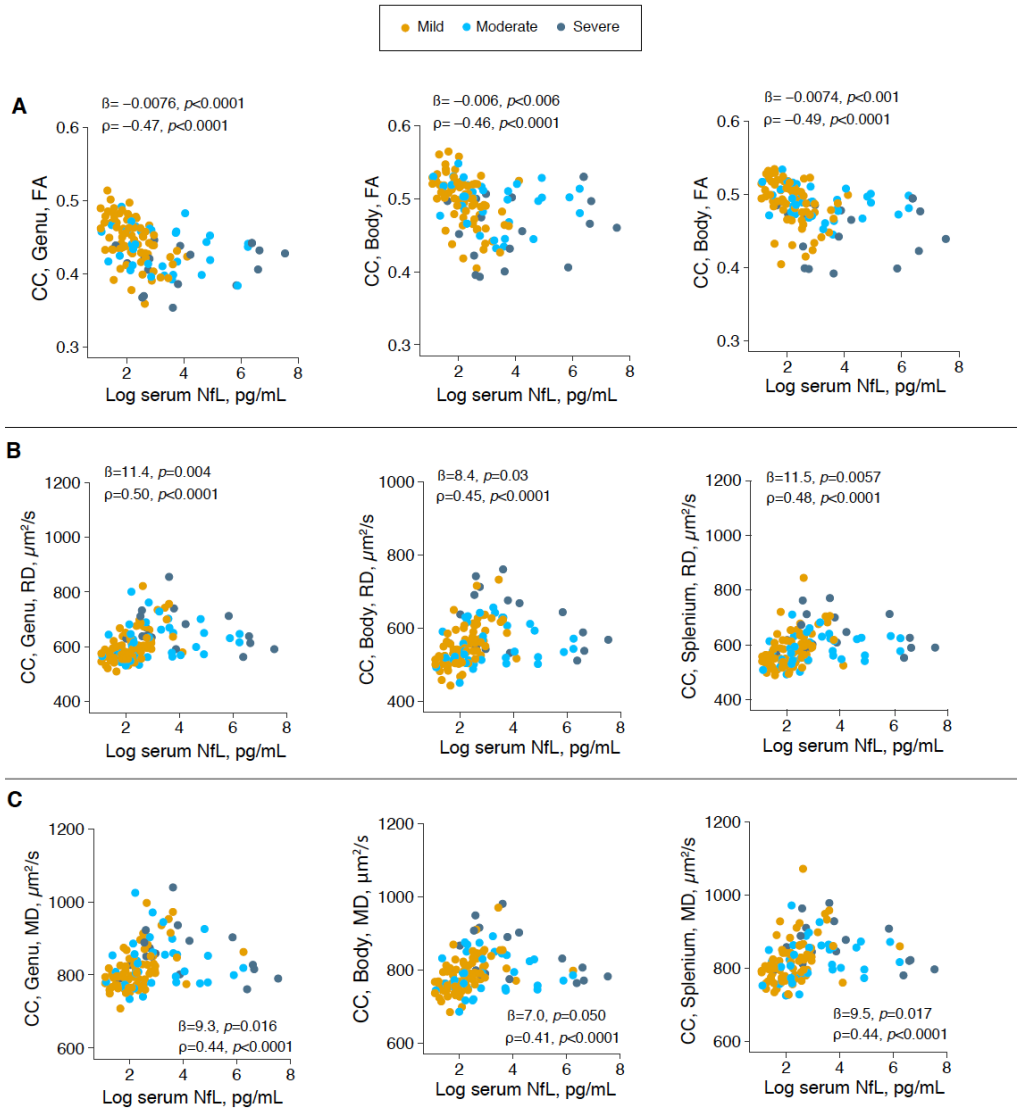
Shahim et al, in press

NfL in Chronic TBI—CNRM Study



Shahim et al, in press

NfL in Chronic TBI—CNRM Study



Shahim, et al in press

NfL in Chronic TBI—CNRM Study

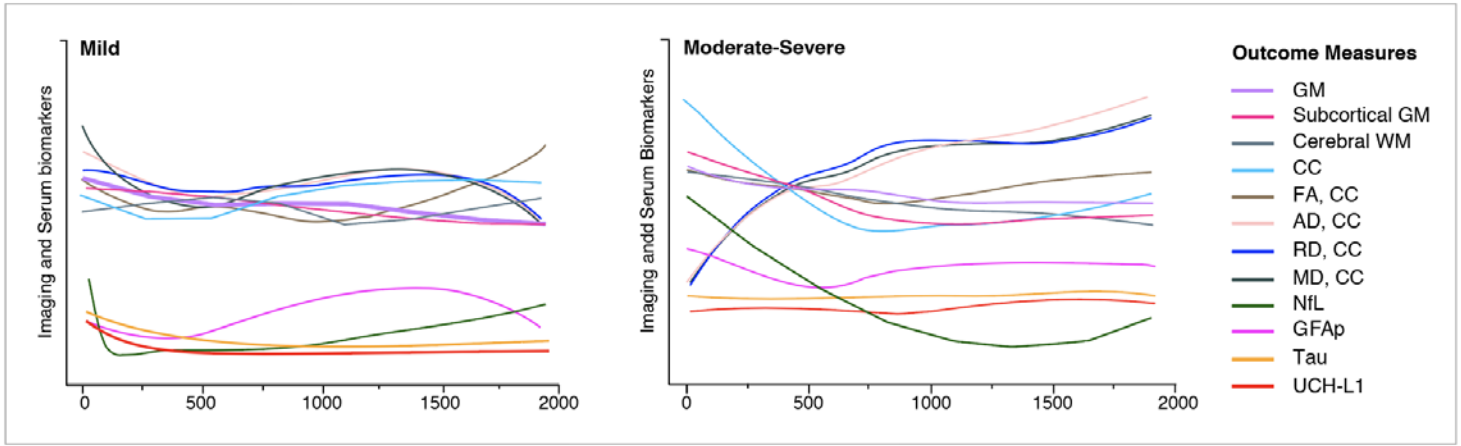


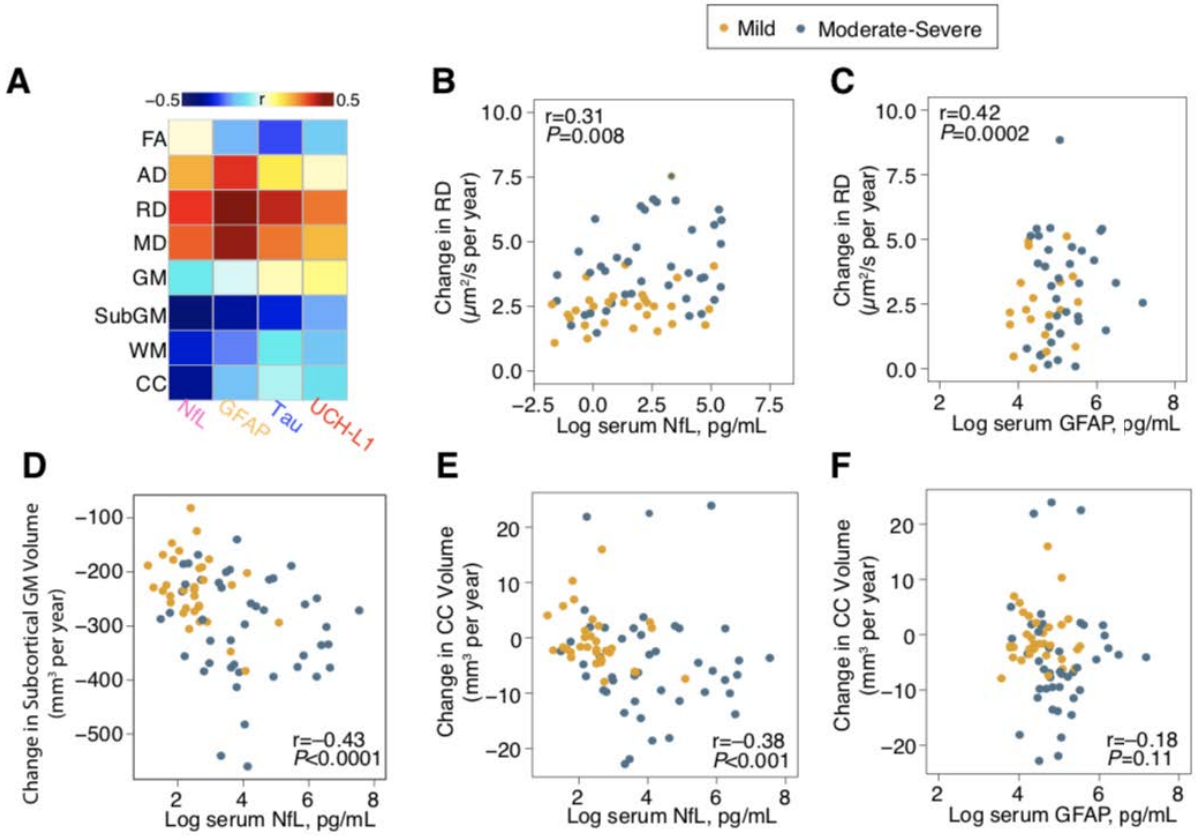
Table 2. Assessment of traumatic axonal injury following mild and moderate-severe head trauma

Variables	Mild		Moderate-Severe		Moderate-Severe *vs* Mild		Time*Moderate-Severe	
	Slope	*P* value	Slope	*P* value	Slope	*P* value	Slope	*P* value
DTI, CC								
FA	–0.0014	0.16	–0.0005	0.43	–0.01	0.27	0.0009	0.47
AD, µm²/s	–0.89	0.59	3.34	**0.0036**	20.3	0.07	4.2	**0.033**
RD, µm²/s	0.18	0.87	1.66	**0.039**	29.0	**0.010**	2.7	**0.021**
MD, µm²/s	–0.074	0.95	2.25	**0.009**	29.0	**0.010**	2.3	0.11
MRI, volume, mm³								
CC	4.2	0.15	–5.0	**0.013**	–8.9	0.72	–9.2	**0.0087**
Cerebral WM	1179	0.07	–1550	**0.001**	–626	0.94	–2729	**<0.001**
Subcortical GM	–110	0.17	–327	**<0.0001**	–2814	**0.004**	–217	**0.022**
Total GM	–1783	**0.004**	–1737	**<0.0001**	–1783	**0.020**	46	0.94
Serum biomarkers								
NfL, log pg/mL	0.002	0.96	–0.33	**<0.0001**	1.28	**<0.0001**	–0.33	**<0.0001**
GFAP, log pg/mL	0.003	0.88	–0.022	0.10	0.52	**<0.0001**	–0.026	0.30
Tau, log pg/mL	0.076	0.35	–0.11	**0.034**	0.78	**0.004**	–0.18	0.052
UCH-L1, log pg/mL	–0.27	0.0018	–0.06	0.29	0.22	0.61	0.21	**0.041**

Abbreviations: FA = fractional anisotropy; AD = axial diffusivity; RD = radial diffusivity; MD = mean diffusivity; CC = corpus callosum; GM = gray matter; WM = white matter; NfL = neurofilament light; GFAP = glial fibrillary acidic protein; UCH-L1 = ubiquitin carboxy-terminal hydrolase-L1. The slope estimates and *P* values are from mixed models adjusted for age, sex, and education.

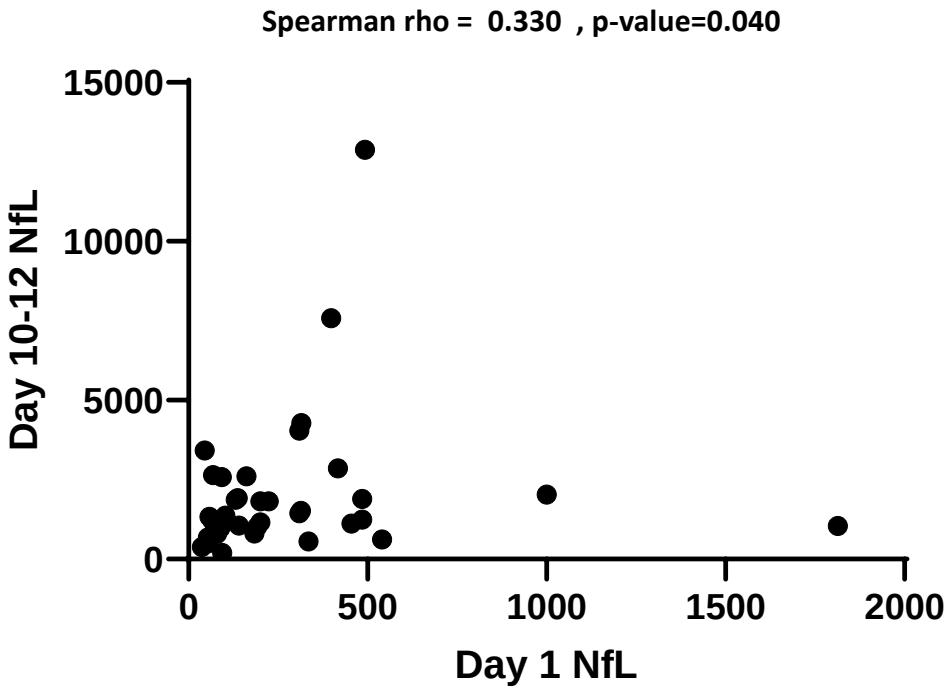
Shahim, Gill, Chan et al unpublished data

NfL in Chronic TBI—CNRM Study
Relationship of Moledular and Imaging Biomarkers



Shahim, Gill, Chan et al unpublished data

Relationship of Acute with Subacute Bimarker Levels

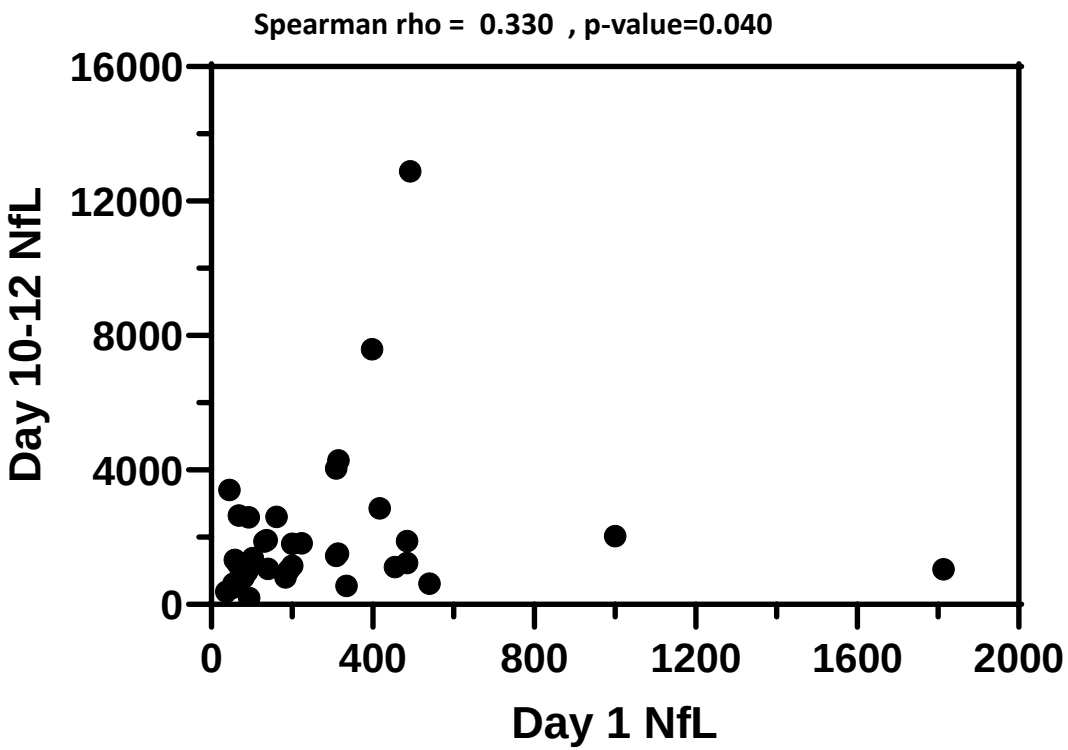


Δ Log NfL (Day 14-Day 1)	S.D.	Cohen's d	n
0.045	0.25	0.18	486
0.045	0.21	0.21	343
0.09	0.25	0.36	123
0.09	0.21	0.43	87
0.135	0.25	0.54	55
0.135	0.21	0.64	39

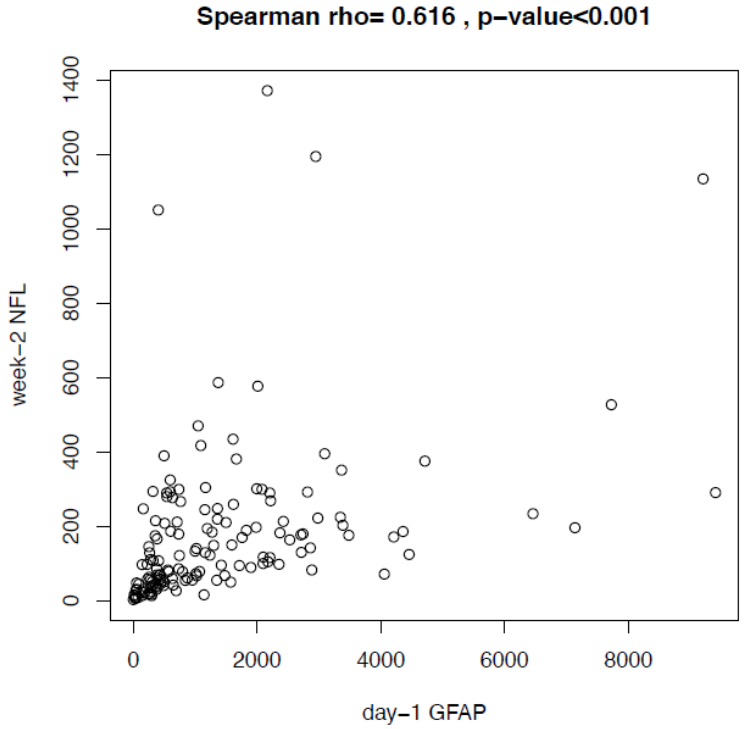
Modified from Shahim et al *Sci Rep* 2016

Courtesy of S. Jain, UCSD

Relationship of Acute with Subacute Bimarker Levels

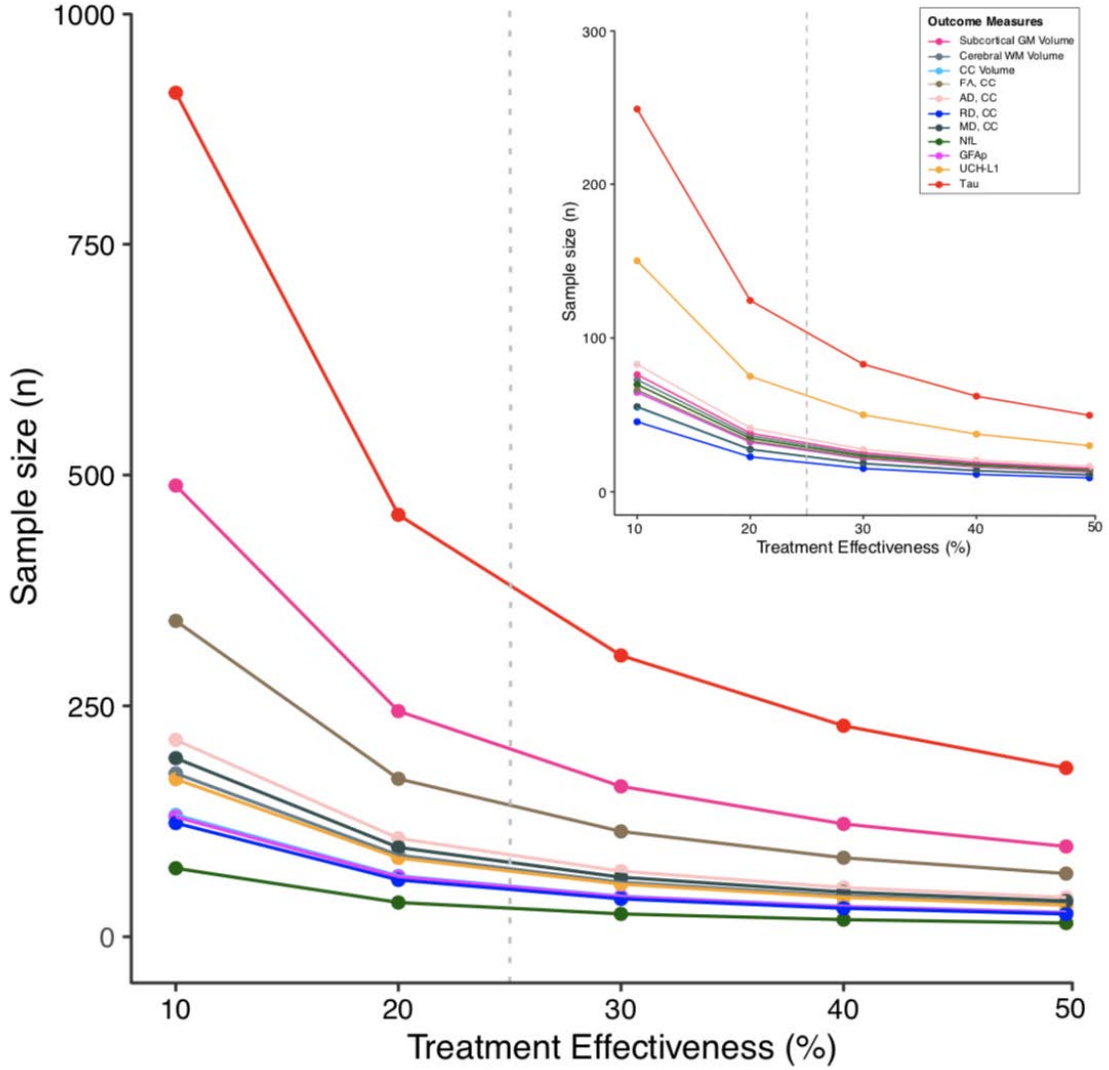


Modified from Shahim et al *Sci Rep* 2016



Courtesy of S. Jain, UCSD

NfL in Chronic TBI—CNRM Study
Relationship of Moledular and Imaging Biomarkers



Shahim, Gill, Chan et al unpublished data

Conclusions

- Endophenotype-specific biomarkers will be needed to inform the next generation of TBI clinical trials
  - Prognostic and Pharmacodynamic Biomarkers
  - Multiple time points, beyond the acute period
- hsCRP shows promise as a biomarker of systemic inflammation, which impacts TBI recovery
- Neurofilament Light Chain shows promise as a biomarker of Traumatic Axonal Injury
  - Sample size of studies using NfL appropriate for Phase 2 studies
- Much work remains to be done

Penn TBI Clinical Research Initiative

University of Pennsylvania Clinical TBI Initiative

- Danielle Sandsmark
- Andrea Schneider
- Megan Moore
- Cillian Lynch
- Alexa Walter
- Erika Silverman
- Cian Dabrowski
- Brigid Magdamo
- My Duyen Le
- Leroy Wesley
- Justin Morrison
- Hannah Zamore

NIH / USUHS (CNRM)

- Pashtun Shahim
- Jessica Gill
- Kimbra Kenney
- Carol Moore
- Leighton Chan

NIH/NINDS

- U01 NS099046
- U01 NS086090
- U24 NS107199
- U01 NS114140
- R01 NS061860

TRACK-TBI

- Geoff Manley
- Sonia Jain
- Ava Puccio

DoD/USAMRMC

- CNRM
- TED
- MTEC
- W81XWH1920002
- DM180187

Penn Dept Health