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UTILITY OF PLASMA BIOMARKERS IN THE VALIDATION OF REPORTED LOC & PTA FOR GCS 13-15 PATIENTS: A TRACK-TBI PILOT STUDY

JEFFREY BRENNAN

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Jeffrey Brennan, BS, MS
2020

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PTA FOR GCS 13-15 PATIENTS: A TRACK-TBI PILOT STUDY

by

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Presented to the Faculty of The University of Texas

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UTILITY OF PLASMA BIOMARKERS IN THE VALIDATION OF REPORTED LOC &
PTA FOR GCS 13-15 PATIENTS: A TRACK-TBI PILOT STUDY

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School of Public Health, 2020

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Depending on a brain injury patient to accurately report their duration of unconsciousness and amnesia is clearly flawed. We constructed biomarker-based models to validate these self-reported measurements using a GCS 13-15 subset of participants from TRACK-TBI, a multi-institutional study on traumatic brain injury. Potential covariates were assessed for significant interactions with biomarker level. We report that the predictions of LOC and PTA failed. GFAP exhibited the most consistent serum level increase between categories of both LOC and PTA. Given a number of issues with the study sample, we recommend continued investigation on the prediction of LOC and PTA using a larger database.

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BACKGROUND

Epidemiology of TBI

Traumatic brain injury (TBI), a condition commonly associated with contact sports and military combat, disproportionately affects the elderly. Patients older than 74 have the highest rate of TBI related hospital visits out of any age group with an incidence of 2.2 per 100 people.¹ This doubles the next highest adult rate of 1.1 per 100 people between the ages of 15 and 24.¹ Every year, 69 million people suffer TBI, with mild TBI (mTBI) – a classification characterized by shorter durations of unconsciousness and reduced impairment of cognitive function – comprising 81% of those cases.² Falls are the leading cause of injury among all age groups, with an incidence of 0.4 per 100 people.¹ Moreover, the risk of adverse outcomes like dementia from a single mTBI event increases with age. Compared to trauma patients without TBI, hazard ratios of dementia increase from 1.08 (95% Confidence Interval: 0.77 – 1.49) among mTBI patients aged 55-64 to 1.25 (95% Confidence Interval: 1.09 – 1.44) for mTBI patients older than 85.³

The elderly simultaneously face the highest incidence of TBI visits, the most common cause of injury, and the most severe consequences from a single mTBI event. Despite this overwhelming burden, diagnostic guidelines remain inconsistent and depend on self-reported measures. These measures are subject to recall bias, especially for injuries where no witness was present. This is particularly problematic in elderly populations, since approximately one in four people older than 65 live

alone.⁴ To address this limitation, it is crucial to improve current diagnostic methods and explore objective measures that minimize reporting bias.

Clinical Approach to TBI

When a patient seeks care for their head injury, the receiving physician's diagnosis is critical. Current guidelines suggest a diagnosis of mTBI when one of the following conditions are present: 1) a loss of consciousness (LOC) period – where the patient was unaware of themselves and their surroundings – lasting less than 30 minutes; 2) post-traumatic amnesia (PTA) – where the patient is unable to remember events after the injury – lasting less than 24 hours; 3) a Glasgow Coma Scale (GCS) score – a brief battery to assess verbal, motor, and visual function – between 13 and 15; 4) presence of physiological symptoms such as vomiting, headaches, and disorientation.^{5,6}

If the physician diagnoses a patient with TBI when they don't have it, their patient may have to pay for an unneeded treatment. For example, the physician may request a computed tomography (CT) scan to check for skull damage,⁷ a magnetic resonance image (MRI) to check for brain bleeds,⁸ and may even suggest that the patient goes to regular physical therapy to regain motor function.⁹

On the other hand, if the physician fails to diagnose TBI when it is present, the opportunity for time-sensitive intervention may be missed. In the short term, the patient's balance will be impaired,⁹ which could lead to another fall if they are sent

home too early. mTBI is associated with increased risk for subsequent neurological disease, including Parkinson's Disease, Alzheimer's Disease, and cognitive impairment.¹⁰ In the long term, the patient may miss the opportunity for preventative intervention. For example, anti-inflammatory drugs like ibuprofen can protect against the onset of Parkinson's¹⁰ and Alzheimer's Disease.¹¹ A misdiagnosis means that the patient will be unprepared for their increased risk.

GCS scores primarily drive TBI diagnosis, however there is criticism regarding the limitations of this method.^{12–15} TBI defined by GCS score includes only three classifications – mild (GCS 13-15), moderate (GCS 9-12), and severe (GCS 3-8).¹⁷ GCS 13-15 patients are at increased risk of depression, post-concussion symptoms, and attention deficits even a year after their injury.¹⁷ Yet, these patients are classified as having “mild” TBI, which is confusing to both the patient and their loved ones. Moreover, GCS results are impacted by patient intoxication, which often presents together with head trauma.^{18,19} Diagnostic limitations stemming from the simplicity of the GCS have prompted the call for a nuanced classification scheme. Brain trauma is complex, and classification should consider multiple clinical features of brain injury²⁰ including demographic factors,²¹ radiologic findings,²² and the durations of LOC and PTA.²⁰

To inform a refined classification of TBI and ensure that care is appropriately directed, reported durations of LOC and PTA must be accurate. Recall bias plagues the clinical utility of self-reported LOC and PTA, especially when the head injury was

not observed by a witness. Objective biomarkers that are associated with brain trauma can be expected to validate these subjective estimates, and improve the resulting patient plan for care.

Plasma Biomarkers

In response to the inherent limitations of subjective measures, neurotrauma researchers have investigated biomarkers associated with TBI. Currently identified blood-based biomarkers include Glial Fibrillary Acidic Protein (GFAP), Ubiquitin C-Terminal Hydrolase L1 (UCH-L1), phosphorylated tau (P-Tau), and total tau. After a brain injury, proteins associated with neural function are released into the bloodstream.²³ GFAP is a protein that regulates the repair of astrocytes, which transmit electrical signals in the brain. Elevated plasma levels of GFAP have been observed to correlate with brain injuries where astrocyte cells are damaged.²³ UCH-L1 is an enzyme found in neuron cells. After a TBI, UCH-L1 is cleaved from damaged neurons and can be found elevated in blood.²³ Tau serves as a binding protein that absorbs the shock from a rapid head movement, such as the whiplash from a car accident.²³ Under stress, tau proteins are excessively phosphorylated. Total tau count alone is not informative for TBI. However, phosphorylated tau (P-Tau) (AUC = 0.711; $p < 0.001$) and the ratio of P-Tau to total tau (AUC = 0.748; $p < 0.001$) are able to differentiate TBI severity.²⁴ These four biomarkers are well-

studied in their relation to TBI and represent different characteristics of brain damage.

Gap in Knowledge

Although LOC and PTA are key factors informing TBI diagnosis, literature regarding biomarker methods to improve their reliability are lacking. Studies in this field typically consider the prognostic value of biomarkers, such as correlations with functional outcome and recovery at 3 and 6 months.^{26,27,29–32} Recent studies have extended these findings by predicting outcomes with multivariate biomarker panels.^{32–34}

In order to develop an accurate TBI classification scheme, biomarkers need to validate subjective indicators of brain damage. Several studies have identified significant associations between LOC/PTA and radiologic findings (CT^{7,33,34}, MRI^{35,36}). However, these imaging tests are expensive and MRI testing is uncommon for GCS 13-15 patients.³⁷ Plasma biomarkers offer a cheaper alternative to quickly validate these self-reported measures at the time of injury.

Research Question

To determine if a panel of plasma biomarkers (GFAP, UCH-L1, P-Tau, total tau), adjusted for potential covariates (age, sex, time between injury and blood

draw), can predict the presence of LOC, and separately, the presence of PTA, in a population of GCS 13-15 hospital patients with potential brain injuries.

Study Aims

1. To determine the efficacy of a four-item plasma biomarker panel (GFAP, UCH-L1, P-Tau, total tau) adjusted for potential confounders (i.e. age, sex, time between injury and blood draw) in correctly classifying the presence of LOC in a population of 136 hospital patients from three sites (San Francisco, Pittsburgh, Austin) with diagnosed potential mild TBI and a recorded duration of LOC. The efficacy of plasma biomarkers were quantified using the area under the curve (AUC) from a receiver operating characteristic (ROC) curve.
2. To determine the efficacy of a four-item plasma biomarker panel (GFAP, UCH-L1, P-Tau, total tau) adjusted for potential confounders (i.e. age, sex, time between injury and blood draw) in correctly classifying the presence of PTA in a population of 114 hospital patients from three sites (San Francisco, Pittsburgh, Austin) with diagnosed potential mild TBI and a recorded duration of PTA. The efficacy of plasma biomarkers were quantified using AUC.

Hypotheses

1. We expect that the LOC biomarker panel will be able to predict the presence of LOC in a test subset of TRACK-TBI Pilot patients with a higher AUC than the PTA panel for predicting the PTA model.

Public Health Significance

The expected outcomes from the proposed project are a validated prediction of LOC and PTA using plasma biomarkers. We expect that findings from this study will promote additional research on validating the clinical indicators of TBI. Currently, discrepancies in TBI diagnosis between medical professionals are unfortunately common.^{38,39} These insufficient classifications result in missed cases and limit opportunities for early intervention. With a revised classification scheme, at-risk patients will have a better chance of receiving the care they need, and the severity of long-term neurological impacts can be limited with preventative measures.

METHODS

Parent Study Design & Population

TRACK-TBI is currently the largest study of American TBI patients.⁴⁰ A pilot study was conducted to assess the feasibility of the ongoing U01 and LONG studies. Members of the pilot study were patients ascertained from one of three level one trauma centers (University of California – San Francisco; University of Pittsburgh

Medical Center; University Medical Center Brackenridge). between April 1st, 2010 and September 30th, 2010. This population includes 300 suspected TBI patients.⁴⁰

Study participants were approached by study personnel during peak hospital hours, contingent on their medical records matching the eligibility criteria for TRACK-TBI. Ability to consent was determined using the Galveston Orientation and Amnesia Test or the presence of a legally authorized representative.⁴⁰ Participants were compensated for their time.

Patients were provided consent forms and self-selected into different subsets of the study. All patients who consented provided their medical history and CT scan results as part of standard emergency department care. A subset of these patients additionally consented to a standard blood draw for proteomic markers, including GFAP, UCH-L1, P-Tau, and total tau. Inclusion and exclusion criteria varied by the study subset (Appendices A & B). Since all data was collected within 24 hours of injury, TRACK-TBI provides a unique opportunity to assess the relationship of biomarkers and TBI diagnostic criteria.

Study Population

In this analysis, we will include participants with suspected TBI. Participants must have recorded levels for at least 3 of 4 biomarkers (GFAP, UCH-L1, P-Tau, total tau) collected within 24 hours of their injury. Suspected TBI patients must have

a recorded duration of either LOC or PTA (including missing and unknown durations), and a GCS score between 13 and 15.

Study Design

The proposed study is a cross-sectional study on the diagnostic utility of plasma biomarker levels for identifying the presence of LOC and PTA using a subset of TRACK-TBI Pilot participants.

Exposures

The main exposures are biomarker levels of GFAP, UCH-L1, P-Tau, and total tau. Biomarker levels were obtained within 24 hours of patient injury. 8 ml of blood was drawn from the patient's vein, unless an arterial line was already installed as part of standard care. Blood samples were centrifuged for 7 minutes at 4000 RPM, separated into plasma and serum, then frozen at -80°C.⁴⁰ Samples were sent to a biorepository at the UCSF DNA Bank at Mission Bay for storage and analysis.⁴⁰

Outcomes

The outcomes for this study are presence of LOC and PTA. Both measures were obtained by an interview of either the participant or a witness. To assess LOC, participants were asked "Did you have a period of time after the event when you were completely unconscious. That means you had no ability to think, speak or

move and were completely unaware of the world around you.”⁴⁰ Witness reports were utilized to determine LOC when available. To assess PTA, participants were asked “Was there a period of time after the injury for which you have no memory? If so, how long did it take for your memory to return to normal or become consistent.”

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Covariate Assessment

Following head trauma, the levels of particular biomarkers are expressed depending on the age of the patient, as well as the time between trauma and when their blood was drawn. These factors, along with other potential covariates (Appendix C) were assessed for their association with biomarkers in the prediction of LOC & PTA. The values for all covariates were obtained from patient medical records and interviews at the study sites. If these covariates are not considered, then the biomarker-based logistic regression model will have a higher chance of incorrectly classifying the LOC and PTA of patients who are outliers for these factors.

Data Analysis

Demographics and Outcome Relationships

All biomarker levels were assessed for normality using Shapiro-Wilk tests, boxplots, and normal probability plots. If a biomarker exhibits normality as indicated

by the normal probability plot and a Shapiro-Wilk p-value > 0.05, then parametric statistical tests of association were used to analyze their relationship with LOC and PTA. Otherwise, we will use nonparametric tests to obtain these associations. Wilcoxon Rank Sum non-parametric tests were ultimately chosen to analyze the relationship between biomarkers and the presence of LOC and PTA.

Covariates & LASSO

A least absolute shrinkage and selection operator (LASSO)⁴² identified covariates (Appendix C) in the data. LASSO is a shrinkage method that aids in model selection by identifying predictors which are most likely to be important in the prediction of LOC/PTA. While fitted values are typically computed using least squares, LASSO uses an additional lambda parameter to minimize the potential variance of model predictions. Predictors that exhibit a large degree of variation (and therefore not predictive of the outcome), have their beta coefficients shrunk to 0, excluding them from the model.⁴³

LASSO was implemented using the `cv.glmnet()` function from the `glmnet` package in R. A matrix of all predictors was cross-validated using the default parameters of 10 folds and no variable weighting. The resulting lambda values were used to compute beta coefficients of the model. If the coefficient was shrunk to 0, then the predictor was not included in the model.

Model Fitting

Biomarker models were fit using logistic regression based on potential covariates (Appendix C) for dichotomized groups of LOC and PTA. LOC and PTA models were trained using two-thirds of the GCS 13-15 sample. The remaining one-third of the sample was used to report the findings to account for potential overfitting of the training data.⁴⁴ The training process is described as follows: a) LASSO identifies predictors for the LOC and PTA models. b) Model diagnostics (linearity, multicollinearity, outliers) are reviewed (detailed below). If model assumptions are violated, remedial measures (transformations, investigation of outliers and extraneous variables) were taken (detailed below).

Diagnostics

Linearity

For a logistic regression models, linearity was assessed between continuous predictors and the log odds of LOC/PTA.⁴⁵ A scatter plot matrix of all continuous predictors was constructed with the log odds of LOC/PTA, and a restricted cubic splines curve was applied to each continuous variable and visualized in the scatter plot with the `geom_smooth()` r function to identify deviations from normality. For predictors which visually appear to be nonlinear, this relationship was quantified by conducting a likelihood ratio test that compares a model with the linear term with a

restricted cubic spline transformed predictor. If the resulting p-value was > 0.05 , then the predictor was transformed with the spline terms.

Multicollinearity

Multicollinearity occurs when two predictors are strongly correlated with each other. Since the interpretation of coefficients from a multivariable model assumes that all other predictors are held constant, multicollinearity will inhibit model interpretation.⁴⁴ A variance inflation factor (VIF) was used to identify multicollinearity using the `vif()` function from the `car` package in R. If the VIF value between two predictors was greater than 10, multicollinearity was addressed.⁴⁴

Outliers

Outliers are defined as extreme observations that strongly influence either the fitted values or other regression coefficients in a statistical model.⁴⁴ Outliers were assessed visually by comparing the fitted model's estimated probability against the leverage, change in Pearson chi-square, and Cook's distance.⁴⁶ Leverage is computed as the diagonal elements of the model's hat matrix, which equals $x_j(X'VX)^{-1}x_j$ and serves as an indicator of distance from the computed mean value of the full model data.⁴⁶ The change in Pearson chi-square is computed as the model's squared residuals divided by $1 -$ the diagonal elements from the model's hat matrix.⁴⁶ Cook's distance is calculated as the squared model residuals multiplied by

the diagonal elements of the hat matrix divided by $1 - \text{the diagonal elements of the hat matrix}$.⁴⁶ Observations exhibiting large values (leverage values > 0.2 , change in Pearson chi-squared values > 3 , Cook's Distance values greater than > 0.2 with > 0.8 estimated probability) were declared as potential outliers and investigated further.

Remedial Measures

If linearity fails, then the predictor was transformed using restricted cubic splines.⁴⁴ If multicollinearity is present, then the violating predictors were investigated.⁴⁴ If an outlier exceeds one of the three cutpoints, then it was investigated and removed from the model if determined to be an error in the data by a subject matter expert.⁴⁴ After these remedial measures were taken, model selection and diagnostics were conducted again as described above.

Interaction

Interaction is defined as when one predictor alters the magnitude of the relationship between a predictor and the outcome (LOC or PTA).⁴⁴ To identify interaction terms, pairwise interactions were computed on the main effects from the LASSO selected model. Next, a series of likelihood ratio tests were used to compare the model with one of the interaction terms to the LASSO selected model with the main effects but no interaction term. If the test has a p-value $< 0.05 / n$, where n is

the number of computed pairwise interactions, then the interaction term was included in the model.

Model Analysis

The sensitivity and specificity for all models were visualized using receiver operating characteristic (ROC) curves. LOC and PTA were predicted using the fitted models from the training set on the remaining third of the GCS 13-15 patients. Once the LOC and PTA models met all regression assumptions, two finalized model were reported with a log-odds cutpoint that favored higher sensitivity in the respective ROC curve.

RESULTS

Demographics and Outcome Relationships

Table 1. Demographics of TRACK Pilot GCS 13-15 Patients (n=178)

Overall (N=178)	
Age	
Mean (SD)	42.6 (17.9)
Range	16.0 - 93.0
Sex	
Male	124 (69.7%)
Female	54 (30.3%)
Race*	
White	146 (82.0%)
Black	16 (9.0%)

Overall (N=178)	
Asian	7 (3.9%)
Unknown	7 (3.9%)
American Indian	1 (0.6%)
Pacific Islander	1 (0.6%)
Study Site	
San Francisco	70 (39.3%)
Pittsburgh	60 (33.7%)
Austin	48 (27.0%)
Glasgow Coma Scale Score	
15	146 (82.0%)
14	29 (16.3%)
13	3 (1.7%)
Mechanism of Injury*	
Fall	63 (35.4%)
Motor Vehicle	35 (19.7%)
Assault	24 (13.5%)
Striking	23 (12.9%)
Self-Inflicted	17 (9.6%)
Firearm	10 (5.6%)
Piercing	5 (2.8%)
Other	1 (0.6%)
Time Between Injury and Blood Draw (min)	
Mean (SD)	648.9 (400.7)
Range	30.0 - 1433.0

*Race was not included as a variable in the LOC and PTA models. Mechanism of injury was collapsed into “Fall” and “Other” to avoid overweighting injury mechanisms with infrequent counts.

Patients from the TRACK Pilot were 42.6 years old on average (± 17.9 years), with the youngest enrolled patient at 16 and the oldest at 93 in this sample. The majority of patients were male (69.7%) and white (82.0%). Of the three study sites, San Francisco enrolled the most patients (70 which constituted 39.3% of the GCS 13-15 sample). Most patients had a GCS score of 15 (82.0%), indicating that they did not exhibit any noticeable signs of impaired consciousness at the time of assessment. Most patients were admitted to the hospital after a fall (35.4%). Other more severe injuries comprised a sizeable portion of the sample: motor vehicle accidents (19.7%), assaults (13.5%), self-inflicted injuries (9.6%), and injuries from a firearm (5.6%). Patients were seen enrolled and evaluated soon after their injury, with a mean blood draw time of 10 hours, and a maximum delay of less than 24 hours.

Table 2. Comparison of LOC Model Predictors by LOC Status (n = 178)

	LOC+ (N=101)	LOC- (N=35)	Unknown (N=42)	p value
Age				0.130 ¹
Mean (SD)	39.9 (15.9)	47.1 (21.3)	45.2 (18.8)	
Range	16.0 - 76.0	18.0 - 93.0	16.0 - 77.0	
Sex				0.977 ²
Male	71 (70.3%)	24 (68.6%)	29 (69.0%)	
Female	30 (29.7%)	11 (31.4%)	13 (31.0%)	
Study Site				0.056 ³
San Francisco	40 (39.6%)	19 (54.3%)	11 (26.2%)	
Pittsburgh	31 (30.7%)	12 (34.3%)	17 (40.5%)	

	LOC+ (N=101)	LOC- (N=35)	Unknown (N=42)	p value
Austin	30 (29.7%)	4 (11.4%)	14 (33.3%)	
Glasgow Coma Scale Score				0.150 ³
15	81 (80.2%)	33 (94.3%)	32 (76.2%)	
14	17 (16.8%)	2 (5.7%)	10 (23.8%)	
13	3 (3.0%)	0 (0.0%)	0 (0.0%)	
Mechanism of Injury				0.988 ²
Fall	36 (35.6%)	12 (34.3%)	15 (35.7%)	
Other	23 (65.7%)	65 (64.4%)	27 (64.3%)	
Time Between Injury and Blood Draw (min)				0.338 ¹
Mean (SD)	609.1 (389.6)	706.46 (436.7)	696.62 (394.7)	
Range	30.0 - 1410.0	50.0 - 1412.0	58.0 - 1433.0	

LOC+ indicates a loss of consciousness duration greater than 0 minutes. LOC- indicates a duration of 0 minutes. P-values < 0.05 declared significant.

1. Kruskal-Wallis Test
2. Chi-Square Test
3. Fisher Exact Test

After stratification of patient demographics by LOC, study site exhibited some difference by LOC strata, but were not statistically significant at the selected alpha value of 0.05. Those with a reported loss of consciousness were more likely to be seen at the San Francisco location, while those with an unknown LOC status were most likely to be seen at the Pittsburgh clinic. Patients with unknown LOC were more likely to have been in a motor vehicle accident than those with reported LOC. Moreover, patients with LOC were more likely to have suffered blunt force trauma (striking) than either the LOC- or LOC unknown patients. Mechanism of injury, GCS,

and time between injury and blood draw were roughly homogenous across LOC strata.

Table 3 - Comparison of PTA Model Predictors by PTA Status (n = 178)

	PTA+ (N=72)	PTA- (N=60)	Unknown (N=46)	p value
Age				0.130 ¹
Mean (SD)	39.6 (16.7)	43.3 (19.5)	46.4 (17.2)	
Range	16.0 - 74.0	16.0 - 93.0	18.0 - 77.0	
Sex				0.538 ²
Male	49 (68.1%)	40 (66.7%)	35 (76.1%)	
Female	23 (31.9%)	20 (33.3%)	11 (23.9%)	
Study Site				< 0.001 ²
San Francisco	24 (33.3%)	35 (58.3%)	11 (23.9%)	
Pittsburgh	18 (25.0%)	14 (23.3%)	28 (60.9%)	
Austin	30 (41.7%)	11 (18.3%)	7 (15.2%)	
Glasgow Coma Scale Score				0.518 ³
15	59 (81.9%)	51 (85.0%)	36 (78.3%)	
14	12 (16.7%)	7 (11.7%)	10 (21.7%)	
13	1 (1.4%)	2 (3.3%)	0 (0.0%)	
Mechanism of Injury				0.705 ²
Fall	23 (31.9%)	22 (36.7%)	18 (39.1%)	
Other	49 (68.1%)	38 (63.3%)	28 (60.9%)	
Time Between Injury and Blood Draw (min)				0.088 ¹
Mean (SD)	587.9 (407.5)	650.9 (410.3)	741.6 (366.2)	
Range	126.0 - 1433.0	50.0 - 1412.0	30.0 - 1380.0	

PTA+ indicates a loss of consciousness duration greater than 0 minutes. PTA- indicates a duration of 0 minutes. P-values <0.05 declared significant.

1. Kruskal-Wallis
2. Chi-Square Test
3. Fisher Exact Test

Patients stratified by PTA exhibited a significant difference in study site that is more pronounced than when stratified by LOC. Patients without PTA were primarily seen in San Francisco, while patients without a reported PTA were primarily seen in Pittsburgh. There were more unknown PTA durations (28) in the Pittsburgh clinic than PTA+ (18) or PTA- (14). While the San Francisco site had primarily PTA- patients, the Austin site had primarily PTA+ patients. Time between injury and blood draw was, on average higher among the unknown group. Other demographics did not exhibit considerable differences when stratified by PTA. Sex, GCS, and mechanism of injury did not differ between PTA strata.

Fig 1a: Outcomes and Numeric Predictors

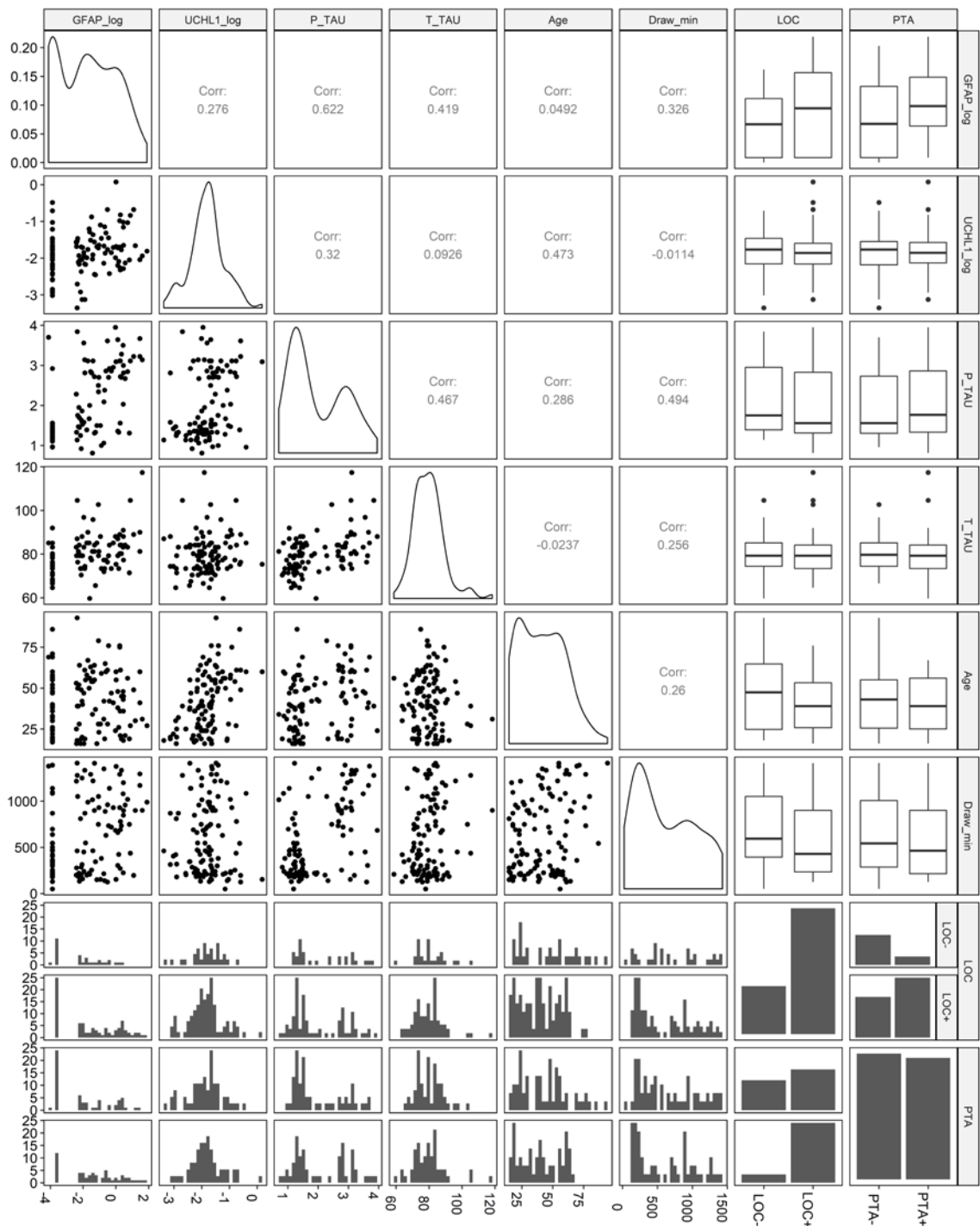
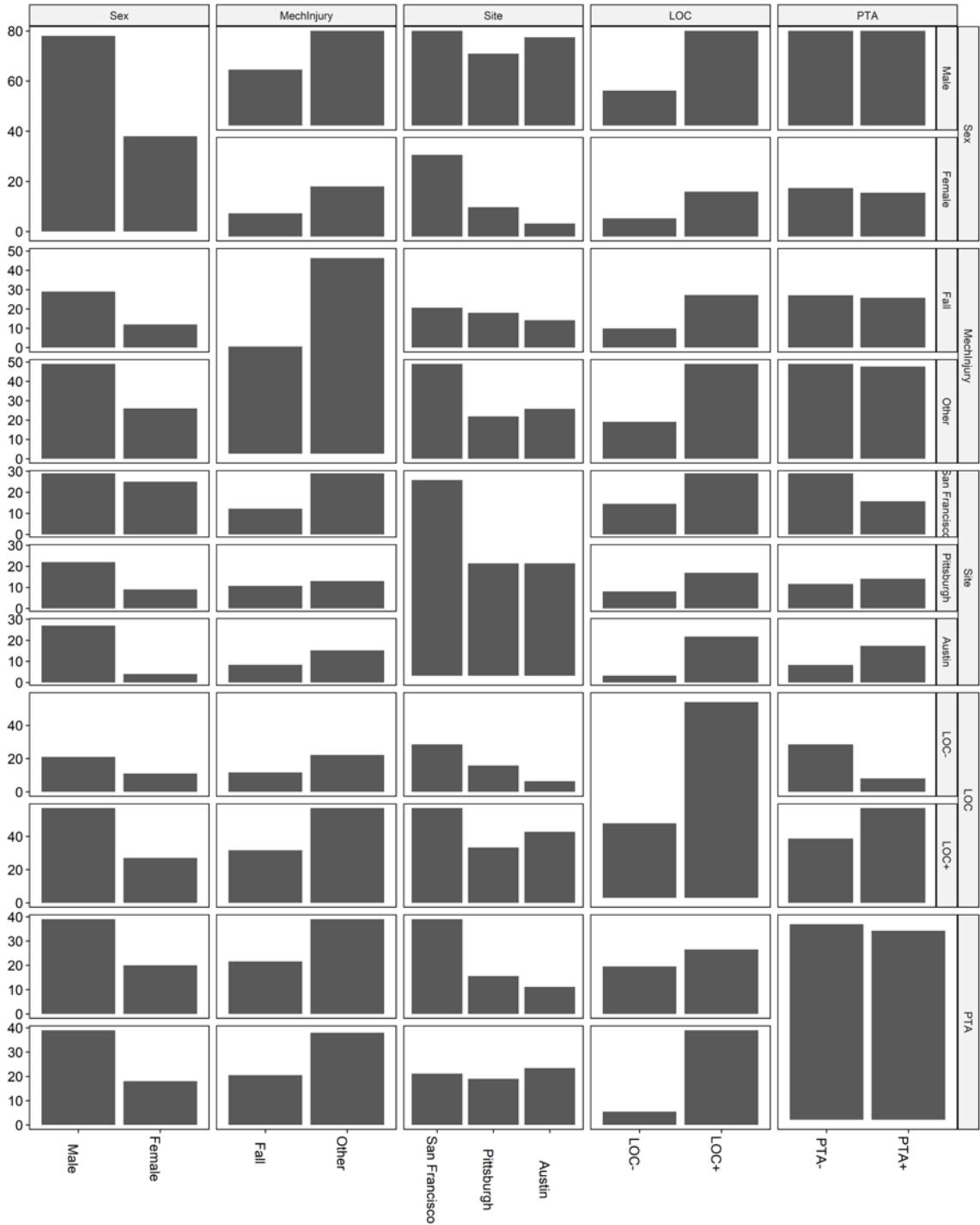
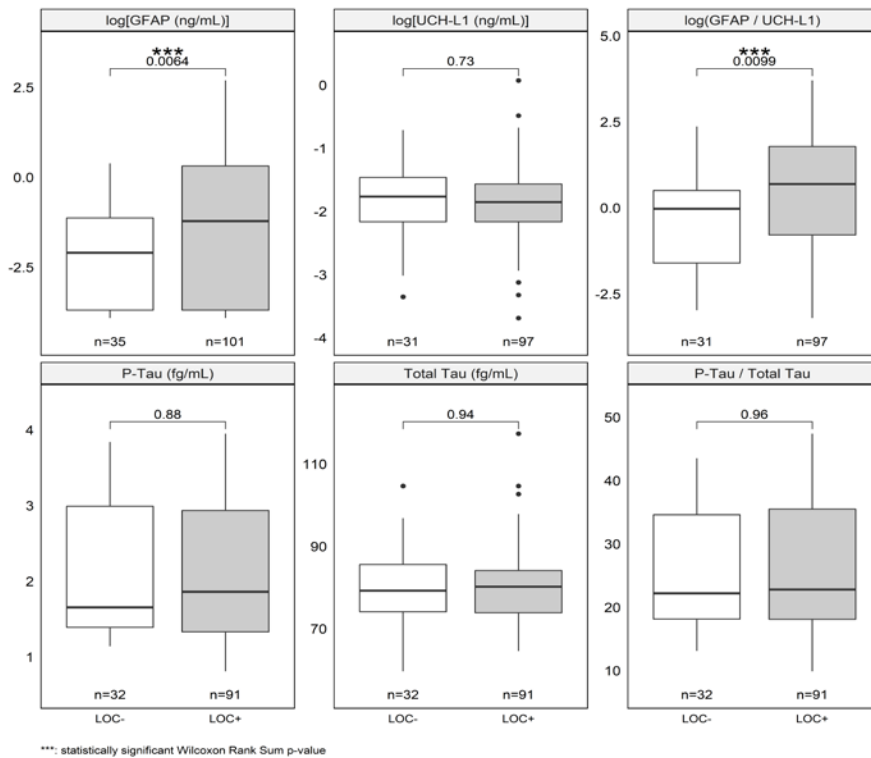


Fig 1b: Outcomes and Categorical Predictors



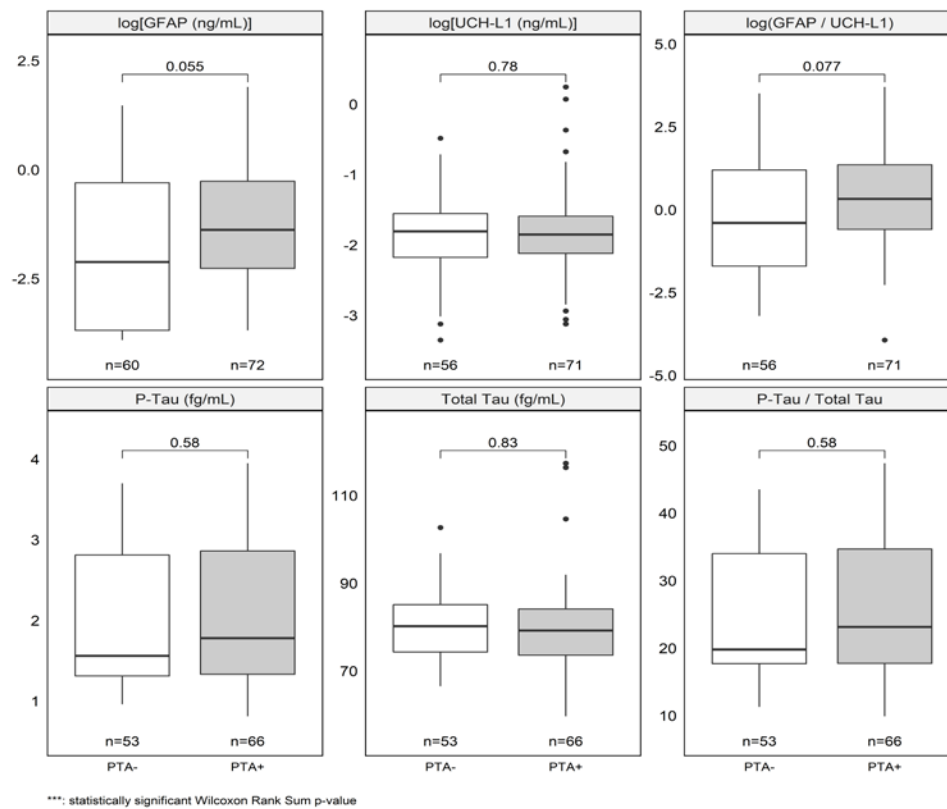
The univariate relationships of all biomarkers, covariates, and outcomes that comprise the LOC and PTA logistic regression models were visualized for a preliminary understanding of their potential association. Moderately strong correlations are observed for log(GFAP) and P-Tau (0.622), age and log(UCH-L1) (0.473), log(GFAP) and T-Tau (0.419), P-Tau and draw duration (0.494). Numeric predictors appear to vary by study site, with patients treated in Pittsburgh contributing to higher values of log(GFAP), P-Tau, and time between injury and blood draw than either San Francisco or Austin. Biomarker levels do not appear to strongly differ by presence of LOC or PTA. There is a notable repetition of log transformed GFAP levels at the lower end of the distribution, which represent the lower limit of detection for the GFAP blood test. Although these measurements are valid, the repeated values may have exerted influence over the final model prediction.

Fig 2: LOC & Biomarker Univariate Significance



Biomarker values are the key predictor of interest for this analysis. We conducted Wilcoxon Rank Sum tests for each biomarker with LOC. Log(GFAP) and log(GFAP/UCH-L1) exhibited statistically significant associations (0.0064 and 0.0099), respectively. For both biomarkers, the median level was elevated in LOC+ patients as compared to LOC- patients. However, the variance for both of these biomarkers is large across LOC categories. There is not a consistent or distinct increase in levels of log(GFAP) or log(GFAP / UCH-L1) between LOC- to LOC+.

Fig 3: PTA & Biomarker Univariate Significance



There were no statistically significant associations observed for biomarker levels and the presence of PTA. Similar to the LOC findings. Again, these relationships are not consistent and do not provide a clear distinction between PTA status. From these findings, biomarker levels alone do not appear to differentiate between LOC or PTA.

LOC Model Fitting

Table 4: LOC Initial Main Effects Model

<i>Predictors</i>	LOC		
	<i>Odds Ratios</i>	<i>Confidence Interval</i>	<i>p</i>
(Intercept)	5248.325	1.873 – 35700834.619	0.042
GFAP_log	1.477	0.888 – 2.620	0.149
UCHL1_log	1.276	0.375 – 4.406	0.694
P_TAU	1.462	0.380 – 6.014	0.583
T_TAU	0.921	0.835 – 1.008	0.080
Age	0.961	0.909 – 1.008	0.130
Draw_min	1.001	0.999 – 1.003	0.467
Sex [Female]	1.297	0.308 – 5.970	0.728
Site [Pittsburgh]	1.151	0.125 – 9.182	0.896
Site [Austin]	9.221	1.661 – 79.323	0.020
MechInjury [Other]	1.657	0.430 – 6.477	0.458

Observations 76

LOC: Loss of Consciousness

Draw_min: Time between injury and blood draw

Site: Study Site (“San Francisco” as the reference category)

Sex: Male used as reference category

MechInjury: Mechanism of Injury (“Fall” used as reference category)

A preliminary LOC model containing all potential main effects was fit for the LASSO variable selection. The confidence interval for the intercept of 1.87 –

35700834.62 indicates that this model is unstable and affected by the large parameter odds ratios. The Austin study site was the only statistically significant predictor ($p = 0.020$), with patients treated there having 9.22 the odds of being LOC+ as compared to San Francisco patients, holding all other predictors constant. The primary biomarkers of interest do not exhibit statistically significant coefficients. Given the extreme values present in the confidence intervals for this model, additional remediation was required

Table 5: LOC Model with LASSO Selected Predictors

LOC			
<i>Predictors</i>	<i>Odds Ratio</i>	<i>Confidence Interval</i>	<i>p</i>
(Intercept)	1793.580	1.734 – 3411754.818	0.039
GFAP_log	1.575	0.995 – 2.675	0.067
T_TAU	0.934	0.856 – 1.012	0.101
Age	0.970	0.931 – 1.007	0.131
Draw_min	1.001	0.999 – 1.003	0.449
Site [Pittsburgh]	1.343	0.178 – 9.559	0.767
Site [Austin]	8.670	1.711 – 69.467	0.018
MechInjury [Other]	1.583	0.426 – 5.884	0.486
Observations	76		
LOC – Loss of Consciousness			
Draw_min – Time between injury and blood draw			
T_TAU – Total-Tau			

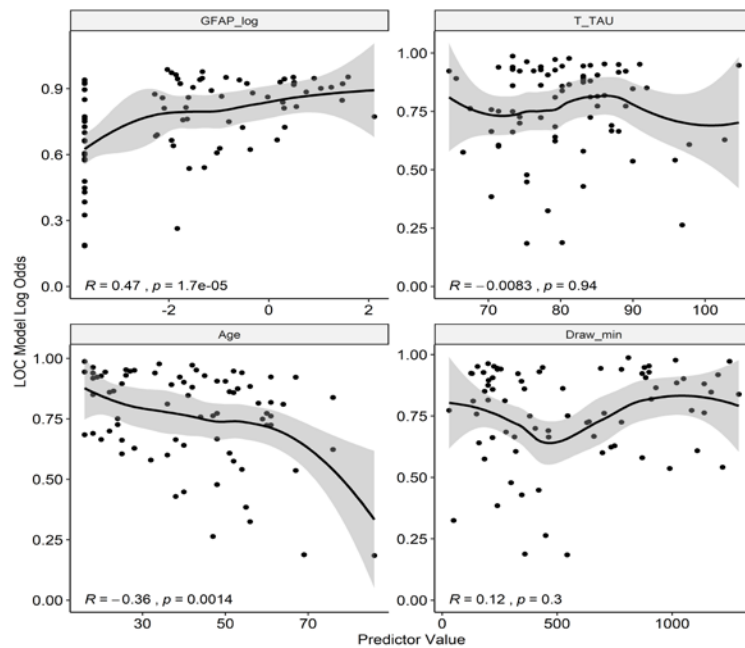
After LASSO variable selection, several predictors were removed from the preliminary model. The coefficients of log(UCH-L1), P-Tau, Sex, and Injury were shrunk to 0, suggesting that these predictors were not predictive of LOC status. Similar to the main effects model, the Austin site remains the only statistically significant coefficient in this model, with patients treated in Austin having 8.67 times the odds of being LOC+ as compared to patients from San Francisco, holding all other predictors constant.

Pairwise interactions were fit on the LASSO selected main effects. When compared to the full LASSO-selected model, no combination produced a likelihood ratio test p-value less than $0.05 / n$, where n was the number of pairwise comparisons. Since the inclusion of an interaction term did not produce a significantly different model, the simpler main effects model was chosen for diagnostics and ROC analysis.

Diagnostics

Linearity

Fig 4: LOC LASSO Model: Numeric Predictor Linearity Investigation



Numeric predictors from the LASSO model were visualized to assess the underlying linearity assumption of the LOC model. There were four numeric predictors in the LASSO selected LOC model: log(GFAP), T-Tau, Age, and time between injury and blood draw. The values for each predictor was visualized against the log-odds produced from the LOC model using a scatter plot and restricted cubic splines. Age and time between injury and blood draw exhibited potentially non-linear relationships to the log-odds of the model. Likelihood ratio tests comparing the LOC

model to a model with a restricted cubic spline term replacing the predictor were used to determine non-linearity.

Table 6: LOC LASSO Model: Time Between Injury and Blood Draw Non-Linearity Investigation

Model 1: LOC ~ GFAP_log + T_TAU + Age + Draw_min + Site + MechInjury

Model 2: LOC ~ GFAP_log + T_TAU + Age + splines::bs(Draw_min) + Site + MechInjury

Model	Resid. Df	Resid.	Dev Df	Deviance	Pr(>Chi)
1	68	67.813			
2	66	65.883	2	1.9302	0.3809

Resid. Df – Residuals degree of freedom

Dev Df – Deviance degrees of freedom

Pr(>Chi): Chi-Squared p-value

With a p-value of 0.3809, there is not enough evidence to suggest that the model containing a restricted cubic spline transformed time between injury and blood draw significantly differs from the initial LASSO selected model. Time between injury and blood draw was retained as a linear predictor for the remainder of the LOC analysis.

Table 7: LOC LASSO Model: Age Non-linearity Investigation

Model 1: LOC ~ GFAP_log + T_TAU + Age + Draw_min + Site + MechInjury

Model 2: LOC ~ GFAP_log + T_TAU + Age + splines::bs(Draw_min) + Site + MechInjury

Model	Resid. Df	Resid.	Dev Df	Deviance	Pr(>Chi)
1	68	67.813			

2	66	62.614	2	5.1989	0.07431
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Resid. Df – Residuals degree of freedom

Dev Df – Deviance degrees of freedom

Pr(>Chi): Chi-Squared p-value

With a p-value of 0.0743, there is not enough evidence to suggest that the model containing a restricted cubic spline transformed age significantly differs from the initial LASSO selected model at an alpha level of 0.05. Age was retained as a linear predictor for the remainder of the LOC analysis in an effort to produce an interpretable and stable final model.

Multicollinearity

Table 8 – Variance Inflation Factors of LASSO Selected Predictors – LOC Model

	VIF	Df
GFAP_log	1.897653	1
T_TAU	1.438008	1
Age	1.213917	1
Draw_min	1.503269	1
Site	2.280941	2
MechInjury	1.131081	1

VIF – Variance Inflation Factor

Df – Degrees of Freedom

Since the coefficients of a logistic regression model are interpreted with the other predictors held constant, the LASSO model was assessed for multicollinearity to ensure that the selected predictors were not strongly related with each other. All predictors exhibited a VIF value lower than the cutpoint of 10 for multicollinearity.

The largest VIF observed was 2.28 for the study site predictor. These values are within normal ranges and indicate that the model's

Outliers

Fig 5: LOC LASSO Model: Leverage

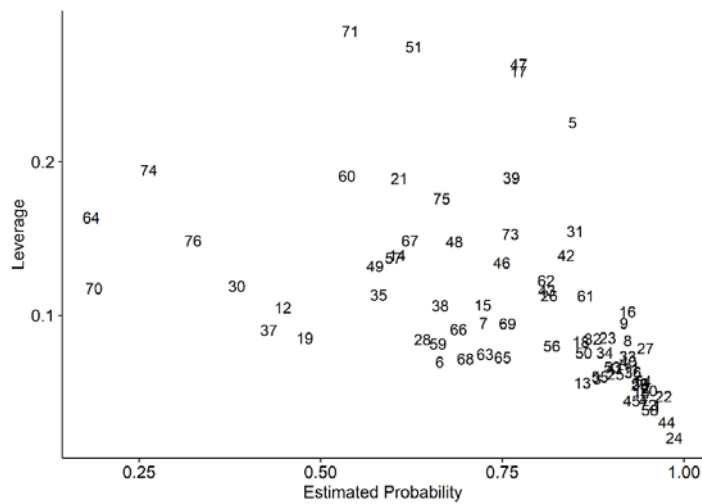


Fig 6: LOC LASSO Model: Pearson's Chi-Squared

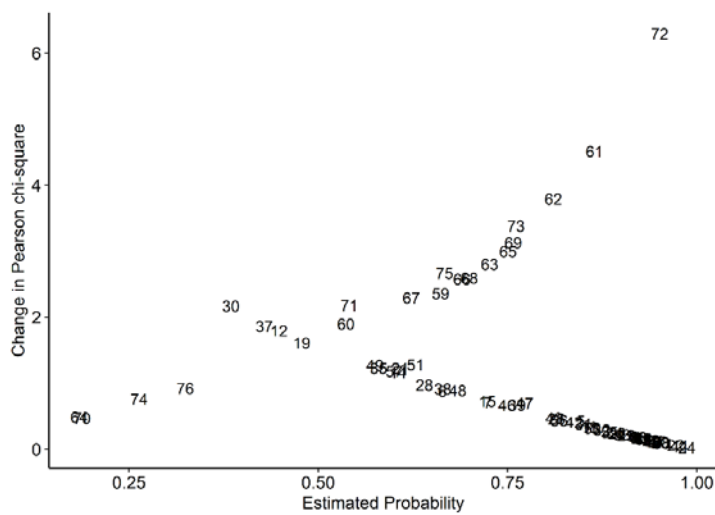


Fig 7: LOC LASSO Model: Cook's Distance

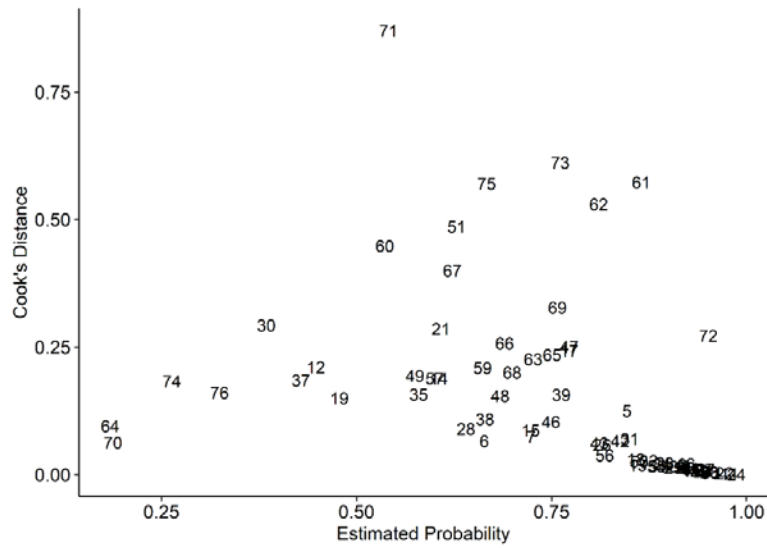
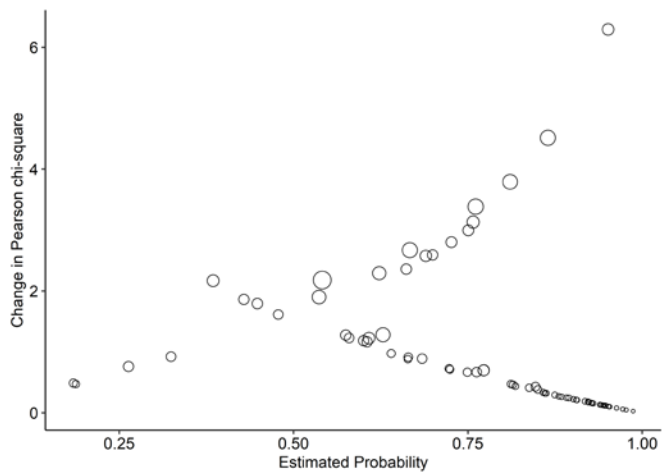


Fig 8: LOC LASSO Model: Pearson's Chi-Squared and Cook's Distance



There are five observations that exceed the leverage cutpoint of 0.2: 71, 51, 4, 17, and 5. (Figure 5). Observations 62, 61, and especially 72 exhibit a large amount of influence on the probability from the model, and warrant further investigation (Figure 6). The Cook's distance plot indicates that observations 62 and 71 (Figure 7). When visualizing the change in Pearson chi-square using the magnitude of leverage as the size of the points, the previously identified outliers all exhibit relatively similar levels of leverage. Given this list of potential predictors, observations 61, 62, and 72 were chosen for further investigation.

Table 9: Summary Statistics of Selected Outliers

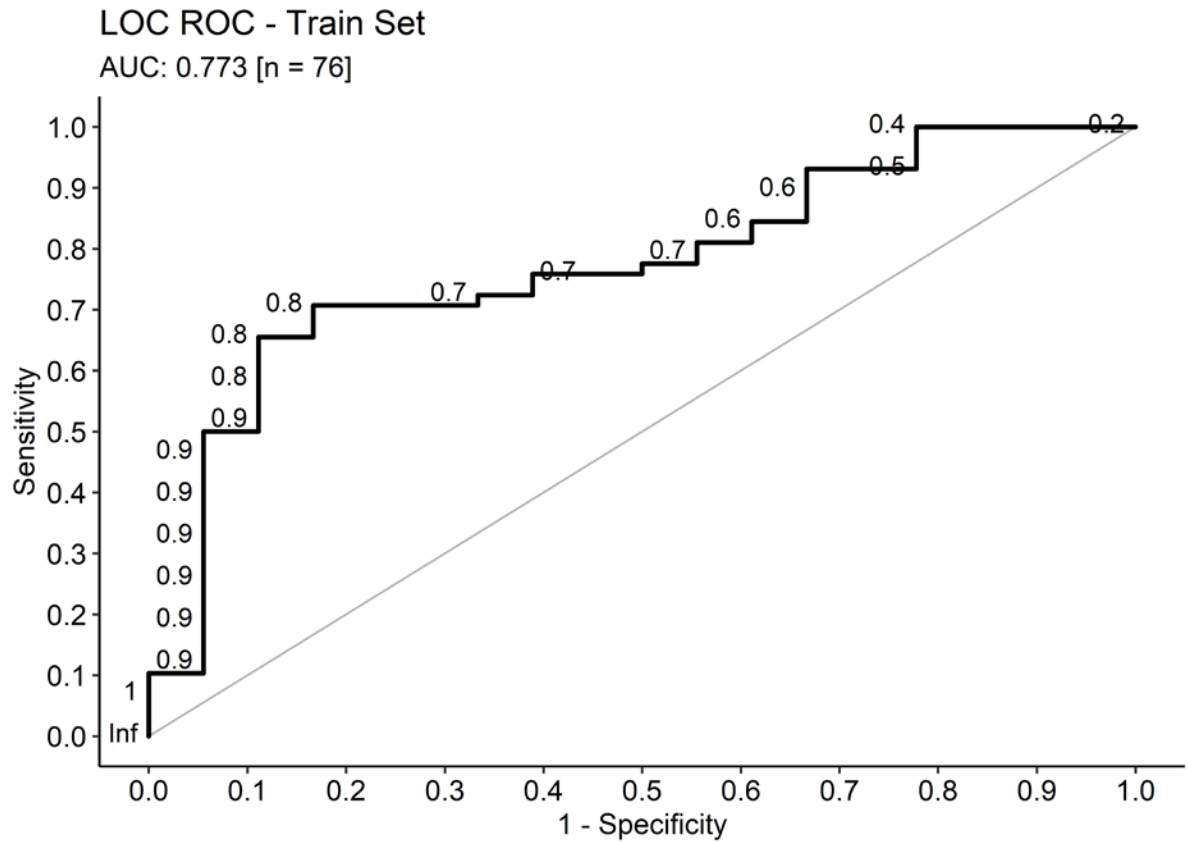
LOC		GFAP_log		UCHL1_log			
Min.	0	Min.	-2.0956	Min.	-2.071		
1st	0	1st	-1.5199	1st	-1.858		
Median	0	Median	-0.9442	Median	-1.645		
Mean	0	Mean	-1.2985	Mean	-1.604		
3rd	0	3rd	-0.8999	3rd	-1.371		
Max.	0	Max.	-0.8557	Max.	-1.097		
P_TAU		T_TAU		Age		Sex	
Min.	1.45	Min.	79.22	Min.	23	Male	2
1st	1.475	1st	80.19	1st	25.5	Female	1
Median	1.5	Median	81.17	Median	28		
Mean	1.883	Mean	82.8	Mean	38.33		
3rd	2.1	3rd	84.59	3rd	46		
Max.	2.7	Max.	88.02	Max.	64		
Draw_min		Site		MechInjury			
Min.	135	San Francisco	0	Fall	2		
1st	144.5	Pittsburgh	1	Other	1		
Median	154	Austin	2				

Mean	406.3					
3rd	542					
Max.	930					

These outliers all reported no duration of LOC despite having above average levels of GFAP, and Total-Tau. The outliers were younger than the base population (mean 38.33 compared to mean 42.59) and their blood was drawn earlier (mean 406.3 compared to mean 648.87). While these differences likely account for their influence on the model predictions, the predictor values for these patients are not extreme enough to justify removing them from the model. Moreover, their removal did not produce notable improvements in model AUC. The suspected outliers were retained in the final model.

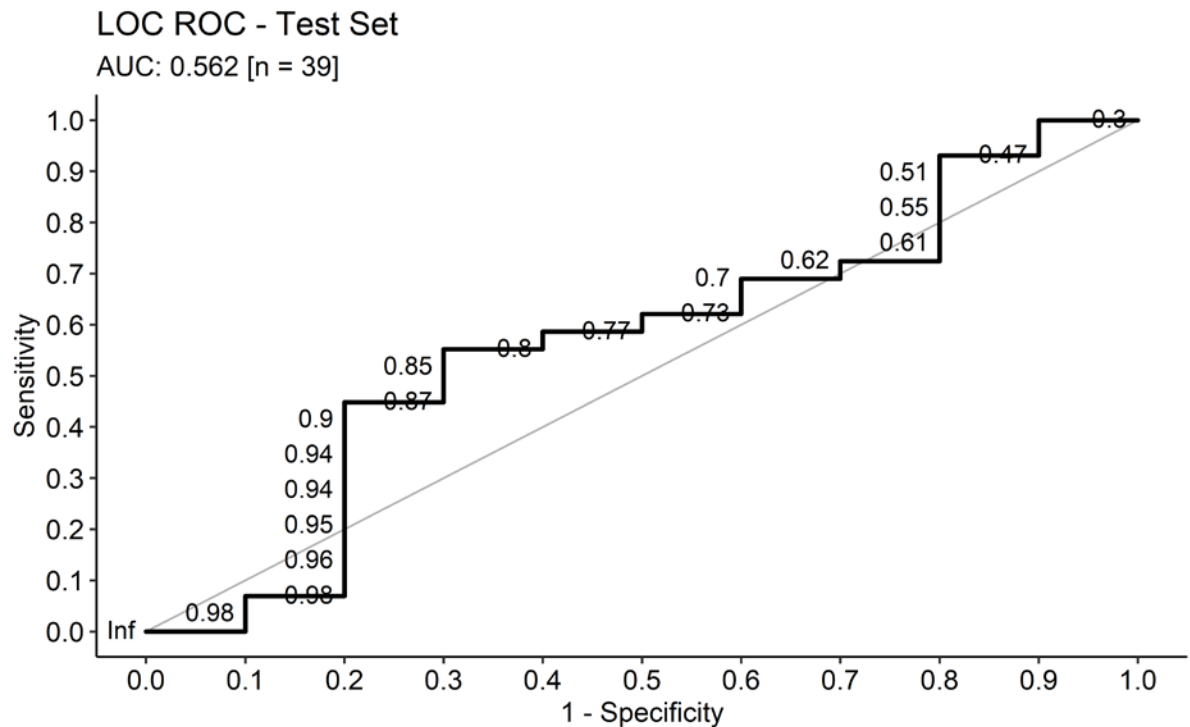
Model Performance

Fig 9: LOC LASSO Model: ROC Curve on Training Data



The LOC model achieved an area under the curve (AUC) of 0.773 on the training set (n = 76). This model performance is acceptable, but unsurprising given the lack of associations observed from the univariate analysis of model predictors. The large values for decision cutpoints indicate that the model primarily predicted a LOC+ outcome for patients in the training set.

Fig 10: LOC LASSO Model: ROC Curve on Test Data



When the model fit on the training data was applied to the test data (n = 39), the resulting ROC curve produced an AUC of 0.562 – which is marginally more reliable than a random guess. The poor AUC indicates that the selected predictors cannot reliably predict LOC in practice. The observed difference between the training and test AUC suggests that the training model was based on unique predictor-outcome relationships in the training set that were not generalizable to new data.

Table 10: LOC LASSO Model: Confusion Matrix

Predicted	Reported (n=39)	
	LOC+	LOC-
LOC+	2	8
LOC-	2	27

LOC: Loss of Consciousness

The following logistic regression model was fit on 76 patients from the training set: $\text{LOC} = 7.492 + 0.454_{\text{GFAP}} - 0.069_{\text{T-Tau}} - 0.069_{\text{Age}} + 8\text{e-}4_{\text{Draw_min}} + 0.295_{\text{Site:Pittsburgh}} + 2.160_{\text{Site:Austin}} + 0.459_{\text{Injury:Other}}$. Sensitivity was valued higher than specificity, and a cutpoint of 0.47 was chosen to classify the fitted values from the model. With this selection, the model produced a sensitivity of 0.5 and a specificity of 0.771 for an overall accuracy of 0.7436 (Table 10).

PTA Model Fitting

Table 11: PTA Initial Main Effects Model

<i>Predictors</i>	PTA		
	<i>Odds Ratio</i>	<i>Confidence Interval</i>	<i>p</i>
(Intercept)	4247.471	2.897 – 16362410.646	0.033
GFAP_log	1.431	0.940 – 2.271	0.106
UCLH1_log	2.775	0.908 – 9.590	0.087
P_TAU	1.008	0.369 – 2.948	0.988
T_TAU	0.942	0.870 – 1.013	0.116

Age	0.967	0.923 – 1.008	0.123
Draw_min	1.000	0.998 – 1.002	0.774
Sex [Female]	2.485	0.704 – 9.772	0.169
Site [Pittsburgh]	1.092	0.163 – 7.285	0.926
Site [Austin]	2.730	0.751 – 10.813	0.135
MechInjury [Other]	1.066	0.324 – 3.458	0.915
Observations	75		

PTA: Post Traumatic Amnesia

Draw_min: Time between injury and blood draw

Site: Study Site (“San Francisco” as the reference category)

Sex: Male used as reference category

MechInjury: Mechanism of Injury (“Fall” used as reference category)

A preliminary PTA model containing all potential main effects was fit for the LASSO variable selection. Similar to the first LOC model, the confidence interval for the intercept of 3.77 – 14533149.24 indicates that this model is unstable and overfit to the training data. There are no predictors that exhibit statistically significant coefficients at an alpha value of 0.05.

Table 12: PTA Model with LASSO Selected Predictors

<i>Predictors</i>	PTA		
	<i>Odds Ratio</i>	<i>Confidence Interval</i>	<i>p</i>
(Intercept)	3.905	0.455 – 40.073	0.227
GFAP_log	1.221	0.881 – 1.717	0.236

UCHL1_log	1.719	0.672 – 4.884	0.277
Site [Pittsburgh]	0.792	0.187 – 3.257	0.746
Site [Austin]	2.960	0.900 – 10.554	0.080
Observations	75		

PTA – Post Traumatic Amnesia

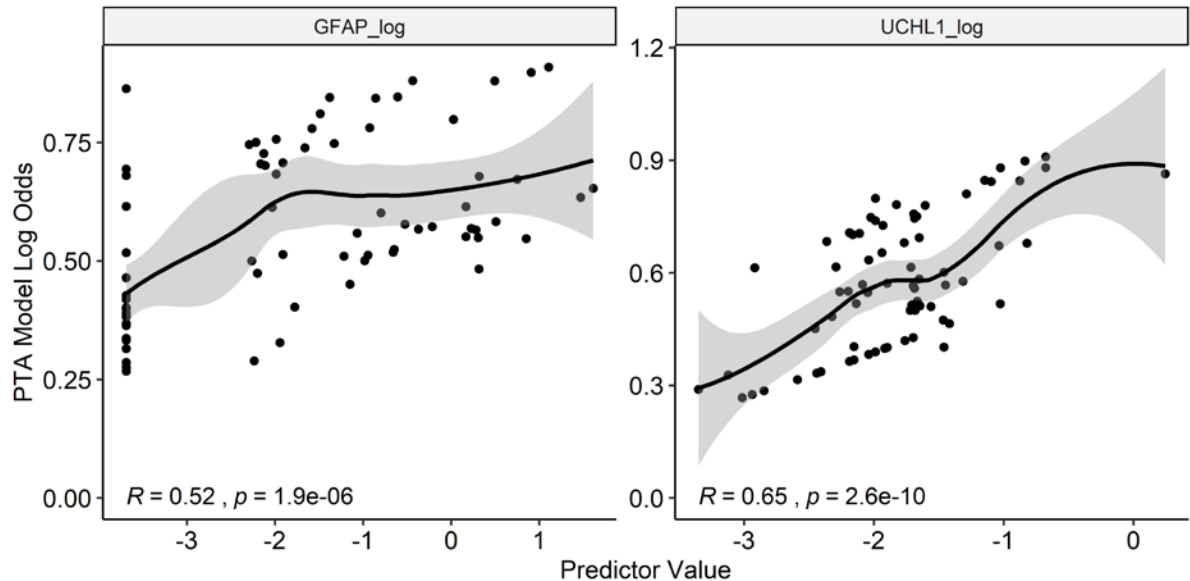
Site: Study site (San Francisco as the reference category)

Many predictors were removed from the preliminary model after LASSO variable selection. The coefficients of, P-Tau, Total tau, Sex, Injury, and time between injury and blood draw were shrunk to 0, suggesting that these predictors have no impact in the prediction of PTA. There are no statistically significant coefficients in this restricted model. Pairwise interactions were fit on the LASSO selected main effects. When compared to the full LASSO-selected model, no combination produced a likelihood ratio test p-value less than $0.05 / n$, where n was the number of pairwise comparisons. Since the inclusion of an interaction term did not produce a significantly different model, the simpler main effects model was chosen for diagnostics and ROC analysis.

Diagnostics

Linearity

Fig 11: PTA LASSO Model: Numeric Predictor Linearity Investigation



There were two numeric predictors in the LASSO selected PTA model: log(GFAP) and log(UCH-L1). Both biomarkers appear to have approximately linear relationships with the log-odds of the PTA model, and both likelihood ratio tests did not produce a significant p-value. Both log(GFAP) and log(UCH-L1) were retained as linear predictors in the PTA model for the remainder of the analysis.

Multicollinearity

Table 13: PTA LASSO Model: Variance Inflation Factors of Predictors

	VIF	Df
GFAP_log	1.203613	1
UCHL1_log	1.193526	1
Site	1.341334	2

VIF – Variance Inflation Factor

Df – Degrees of Freedom

All three exhibited a VIF value lower than the cutpoint of 10 for multicollinearity. The largest VIF observed was 1.34 for the study site predictor. These values are within normal ranges and do not indicate a violation of the no multicollinearity assumption for logistic regression models.

Outliers

Fig 12: PTA LASSO Model: Leverage

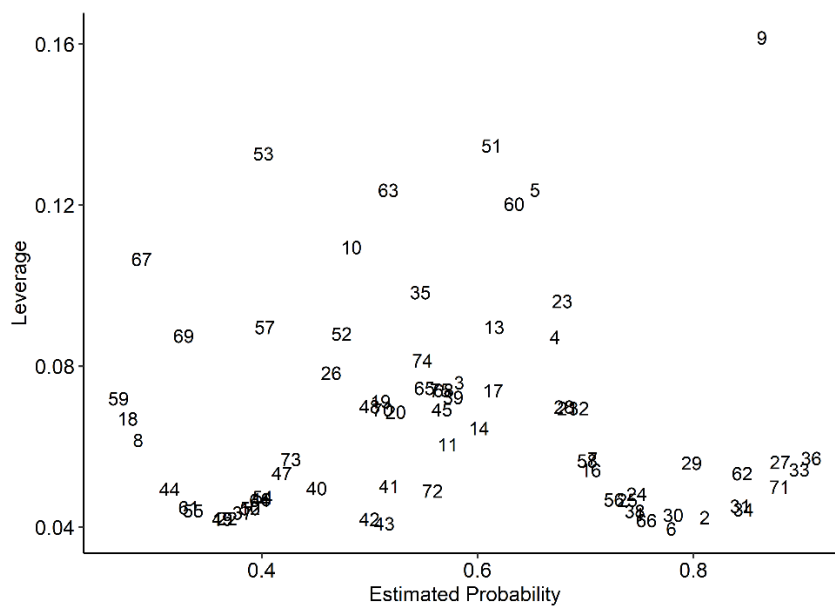


Fig 13: PTA LASSO Model: Pearson's Chi-Squared

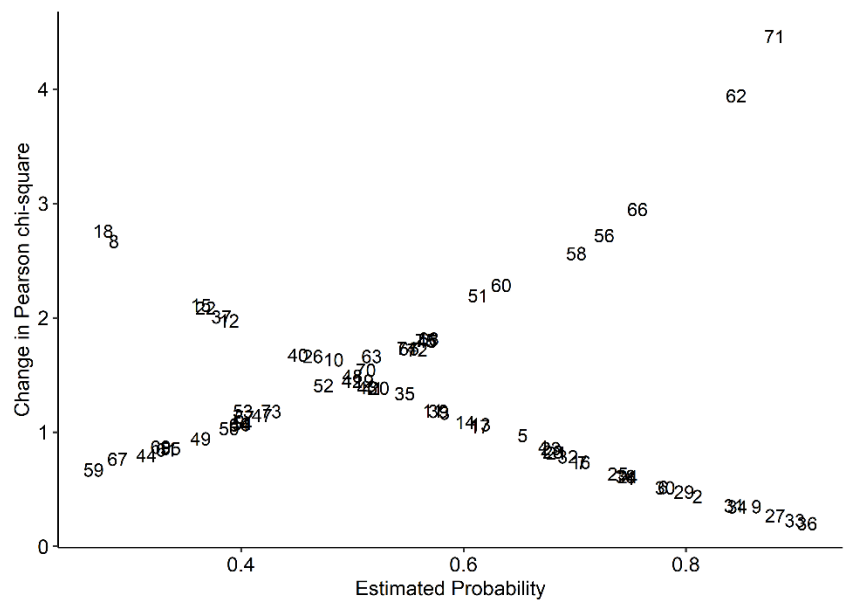


Fig 14: PTA LASSO Model: Cook's Distance

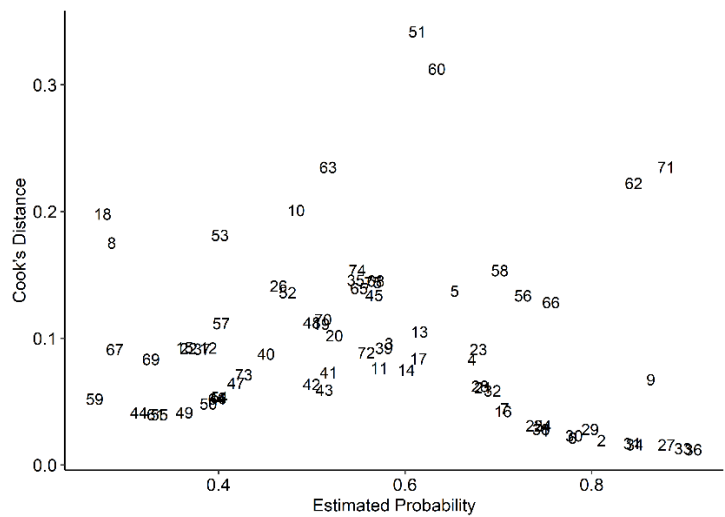
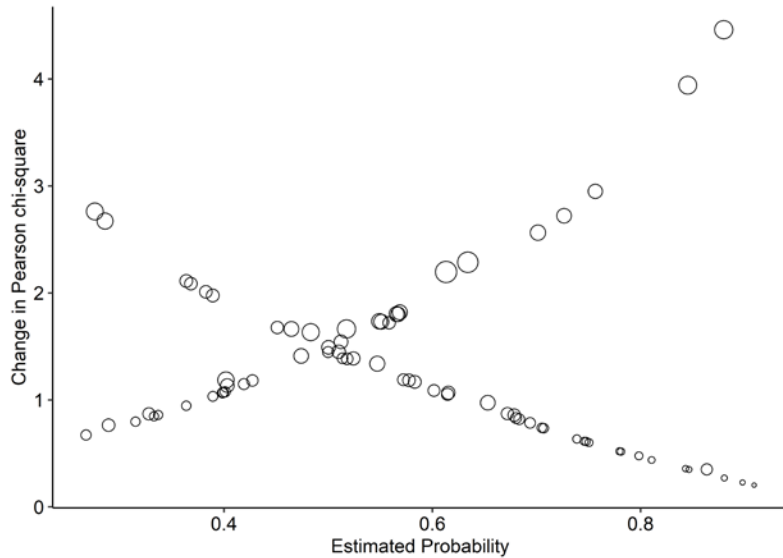


Fig 15: PTA LASSO Model: Pearson's Chi-Squared and Cook's Distance



There are no observations that exceed the leverage cutpoint of 0.2. (Figure 12). Observations 62, 71 exhibit a large amount of influence on the probability from the model, with a change in Pearson's chi-square larger than 3, and warrant further investigation (Figure 13). The Cook's distance plot indicates that observations 62 and 71 (Figure 14). When visualizing the change in Pearson chi-square using the magnitude of leverage as the size of the points, the previously identified outliers all exhibit relatively similar levels of leverage (Figure 15). Given this list of potential predictors, observations 62 and 71 were chosen for further investigation.

Table 14: Summary Statistics of Selected Outliers

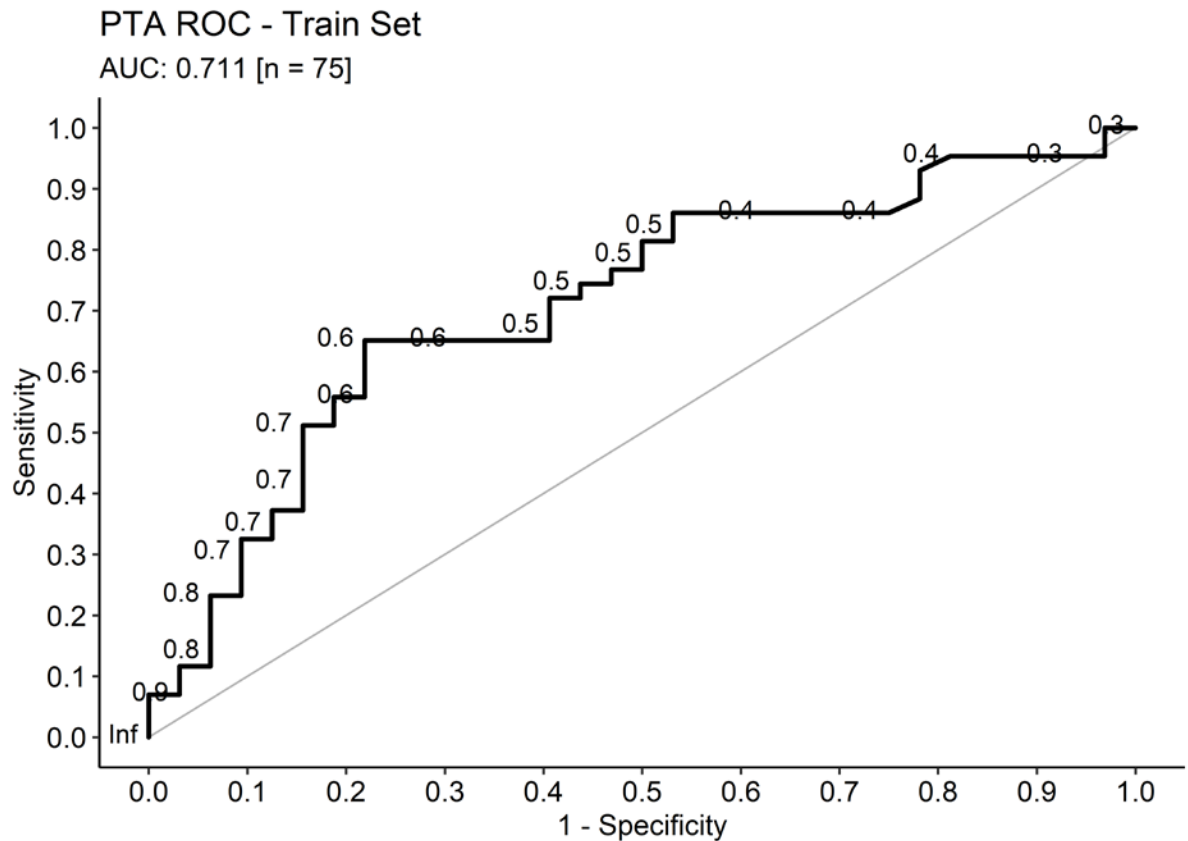
PTA		GFAP_log		UCHL1_log		
Min.	0	Min.	-1.37833	Min.	-1.0244	

1st	0	1st	-0.91038	1st	-0.9876		
Median	0	Median	-0.44243	Median	-0.9508		
Mean	0	Mean	-0.44243	Mean	-0.9508		
3rd	0	3rd	0.02553	3rd	-0.9139		
Max.	0	Max.	0.49348	Max.	-0.8771		
P_TAU		T_TAU		Age		Sex	
Min.	2.03	Min.	77.26	Min.	43	Male	2
1st	2.225	1st	80.68	1st	45.75	Female	0
Median	2.42	Median	84.11	Median	48.5		
Mean	2.42	Mean	84.11	Mean	48.5		
3rd	2.615	3rd	87.53	3rd	51.25		
Max.	2.81	Max.	90.95	Max.	54		
Draw_min		Site		MechInjury			
Min.	225	San Francisco	0	Fall	0		
1st	232.5	Pittsburgh	0	Other	2		
Median	240	Austin	2				
Mean	240						
3rd	247.5						
Max.	255						

Both outliers reported no duration of PTA despite having above average levels of GFAP, UCH-L1. Both outliers were also enrolled at the Austin site. Their blood was drawn earlier (mean 240 compared to mean 648.87), and neither suffered a fall. While these differences likely account for their influence on the model predictions, the predictor values for these patients are not extreme enough to justify removing them from the model. Moreover, their removal did not produce notable improvements in model AUC. The suspected outliers were retained in the final model.

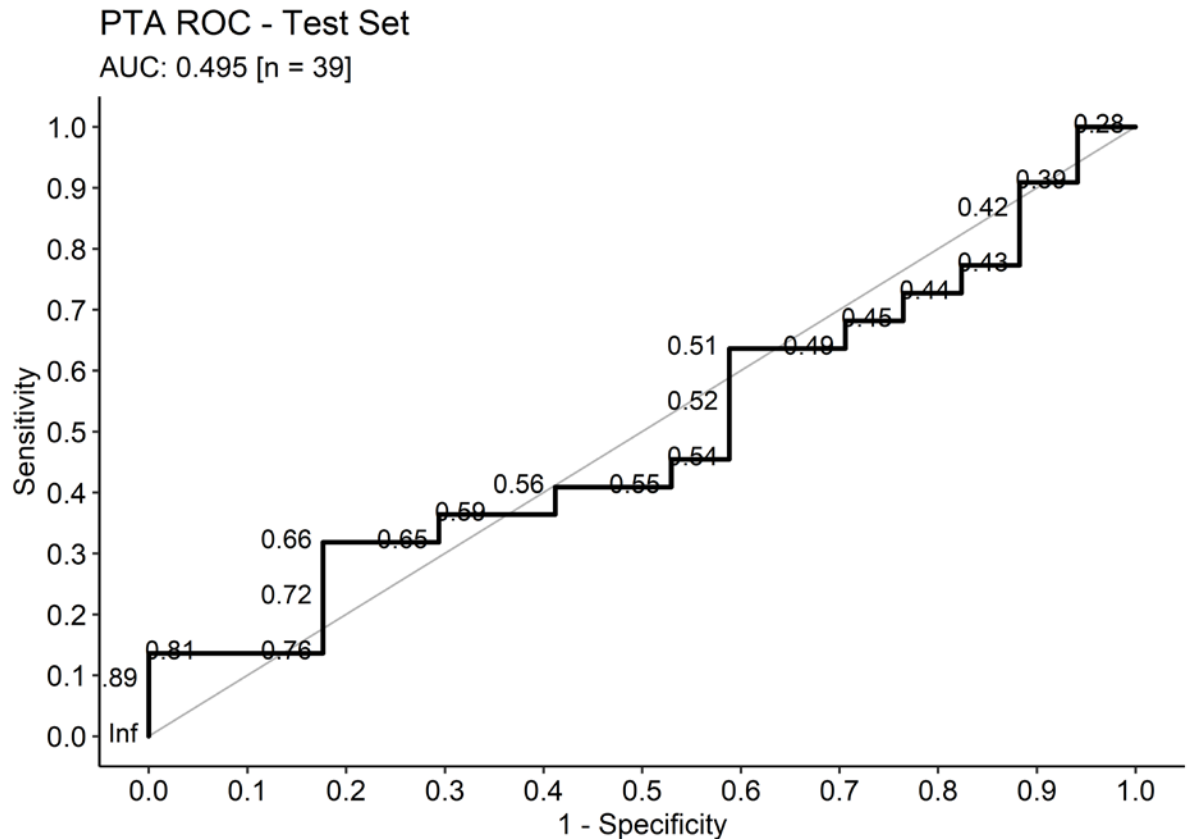
Model Performance

Fig 16: PTA LASSO Model: ROC Curve on Training Data



The PTA model achieved an AUC of 0.711 on the training set, which is acceptable but not excellent.⁴⁷ Since no significant relationships were observed in univariate analysis of PTA and the model predictors, this is expected. With a cutpoint of 0.5 corresponding to a specificity of 0.5, the PTA produced fewer false positives than the LOC model.

Fig 17: PTA LASSO Model: ROC Curve on Testing Data



When the model fit on the training data was applied to the test data (n = 39), the resulting ROC curve produced an area under the curve of 0.495 – which is worse than a random guess. Similar to the LOC model, the low AUC observed for the PTA model indicates that the selected predictors cannot reliably predict PTA using the TRACK Pilot sample. The decrease in AUC between the training and test ROC curves suggests that the training model performance was based on unique predictor-outcome relationships in the training set that were not generalizable to new

data. There were only three predictors selected for the model, including an indicator of where the patient was treated. While there may have been a pattern of PTA status by study site, this does not have biological relevance and would naturally make incorrect predictions given a different set of data.

Table 15: Classification matrix – dichotomized PTA model

Predicted	Reported	
	PTA+	PTA-
PTA+	1	16
PTA-	2	20

PTA: Post Traumatic Amnesia

The following logistic regression model was fit: $PTA = 1.3622 + 0.199_{\log(GFAP)} + 0.542_{\log(UCHL1)} - 0.234_{\text{Site:Pittsburgh}} + 1.085_{\text{Site:Austin}}$. Sensitivity was valued higher than specificity, and a cutpoint of 0.39 was chosen to classify the fitted values from the model. With this selection, the model produced a sensitivity of 0.333 and a specificity of 0.557 for an overall accuracy of 0.5385 (Table 15).

DISCUSSION

In this cross-sectional study, we examined the utility of a plasma biomarker panel adjusted for demographic covariates in the prediction of LOC and PTA. To our knowledge, this is the first effort to do so. Our logistic regression models did not reliably predict the presence of both LOC and PTA. Despite a modest classification of each outcome in the training sets, these predictions were no better than random guesses when applied to the test sets. $\log(\text{GFAP})$ and $\log(\text{GFAP} / \text{UCHL1})$ exhibited univariate significance with LOC (Figure 2), however this association was weak and disappeared when adjusted for other covariates in the LOC model. No significant associations were identified in univariate comparisons of biomarker levels with PTA (Figure 3).

Mechanism of injury was selected by LASSO as a predictor for the LOC model. Falls were compared against “Other”, a collapsed category which included more severe mechanisms of injury including gunshots, striking blunt force trauma, and car accidents. There is evidence which suggests that both focal injuries (like gunshots and piercing wounds) contribute to LOC as well as diffuse axonal injuries (car accidents and blunt force trauma).^{48–51} However, our ability to utilize injury as an outcome differentiator was limited by this grouping. Even with the initial injury categories (Table 1), many key descriptors of the injury were absent. The severity of injury from car accidents varies by collision type. Head-on collisions and high-speed collisions with stationary objects typically produce the greatest alteration in

consciousness.⁵² Lateral collisions also contribute to a disproportionate duration of unconsciousness since seatbelts primarily limit forward movement.⁵² Further, focal injury types did not describe which portion of the brain was affected, which strongly contributes to the effects on consciousness and amnesia.⁵¹ Patients entering the ER for a fall are typically older and exhibit more mild alterations in consciousness as compared to car accidents, which makes age an important covariate for the LOC model.⁵³

GFAP and UCH-L1 were the two biomarkers selected in the LOC model. GFAP was also selected in the PTA model. Additionally, GFAP exhibited a significant univariate relationship with the presence of LOC. The GFAP finding is consistent with other studies that have identified this biomarker as a key predictor of TBI.^{54–59} Of note, elevated levels of GFAP are associated with focal injuries, while elevated levels of UCH-L1 are associated with diffuse injuries on CT.⁶⁰ This relationship with injury type warrants further investigation. Although UCH-L1 is associated with memory formation, LASSO did not select it as a predictor for PTA.⁶¹

For both outcomes, LASSO selected the study site as a key predictor. While this improved the AUC for the training set, the relationship between LOC/PTA outcome and where the patient was treated has no biological basis. All clinical sites followed the same TRACK-TBI protocol, indicating that these differences were likely due to random chance. The model was making outcome predictions based on where the patient was treated, rather than more plausible associations like biomarker levels

and mechanism of injury. The selection of this variable was expected after our exploration in figure 1. Study site was a primary contributor to the variation in the predictors of interest. More than sex or either outcome, study site was associated with the largest difference in biomarker values, with higher levels consistently observed for the Pittsburgh site. Study site also exhibited variation in the outcomes. LOC+ and PTA+ cases were disproportionately treated in Austin.

Previous studies interested in prediction using TBI variables have focused on CT findings, mortality, and neuropsychological test performance.^{20,62–65} The common relationship with these studies is that they focused on concretely separate and easily measurable outcomes. Since LOC and PTA are both self-reported and unreliable, rigorous data collection and a large sample size are likely required to mitigate the effects of misreported durations. In this analysis, we compared durations of 0 minutes for LOC and PTA against any duration greater than 0 minutes. The lack of univariate biomarker associations observed for these collapsed levels may indicate that the chosen split is not informative. A more discrete split in outcome duration (eg. 0 minutes compared to 30 minutes of LOC or PTA) may exhibit better prediction performance. The 30-minute mark is classically used to differentiate mild from moderate TBI⁵, and it would be useful to identify patients exhibiting alterations of consciousness lasting this duration.

CONCLUSION

Strengths

Previous efforts incorporating biomarker models in TBI studies have primarily focused on 1) predictions of outcome^{32,66,67} (cognition, motor skills etc. as measured by neuropsychological assessments) and 2) identification of TBI associated biomarkers.^{68–71} Studies focused on the diagnostic criteria of TBI (LOC/PTA duration) use costly approaches, such as handheld EEG devices.⁷² Our study directly assesses the relationship of four distinct biomarkers with LOC and PTA using a universal blood draw. Moreover, this blood draw was achieved with 24 hours for all patients, which mitigates time-dependent level differences that occur in biomarkers after injury.

TRACK-TBI is joined by other multi-institutional efforts to understand TBI. CREACTIVE (n=7000)⁷³ focuses on severe TBI, and CENTER-TBI (n=5400)⁷⁴ focuses on TBI cases between ER, ICU, and hospital cohorts in Europe. TRACK extends these efforts in America with a substantial collection of TBI-associated genomic and proteomic markers. These markers, initially explored in the TRACK Pilot, have been utilized in the larger TRACK U01 and LONG studies to identify additional outcome associations. Since LOC and PTA are routinely collected as part of standard TBI care, this study could be replicated in a larger patient cohort to determine if the prediction inaccuracy stems from the small sample size or a true lack of association between biomarkers and LOC/PTA. Through the stronger

understanding of this relationship, we can improve the efficiency and targeting of patient care.

Limitations

Study limitations originate from the small TRACK Pilot dataset and the inherent limitations of self-reported measures. For binary logistic regression, 10 events per predictor (EPV) or more is recommended to produce a stable model.⁷⁵ Others have argued that an EPV of 10 is too conservative and that an EPV of 5 - 9 observations is more realistic when considering many covariates.⁷⁶ Our training sample for LOC had 76 patients, and our training sample for PTA had 75 patients. There were nine main effects (four biomarkers and five demographic covariates) considered for each model. With only the main effects, there were 8.333 observations per predictor in the training models. This restricted our ability to include pairwise interaction and polynomial terms. The test set for both models only had 35 observations and could ideally support 3 predictors, with a maximum of 7 using a relaxed EPV of 5.

Together with a small sample size, predictors of the model were unbalanced. There were very few female patients and patients with a GCS of 13 or 14. Most importantly, the mechanism of injury – which is strongly linked with the pathology of trauma – lacked sufficient observations for every category that was recorded. Levels of the outcome were also unbalanced. There were 101 LOC+ patients, but only 35

LOC- patients. We implemented a stratified train/test split to ensure that both sets had an equal proportion of LOC+ and LOC- cases. However, the frequency of LOC+ cases meant that the model was assigning a log-odds of > 0.5 for the majority of patients, resulting in many false positives when applied to the test set. This was also observed for PTA model to a lesser degree. There were 72 PTA+ cases and 60 PTA- cases. In turn, a cutpoint of 0.5 corresponded to a sensitivity of 0.7 and a specificity of 0.55, indicating that the distribution of PTA log-odds value was more balanced.

Participants were incentivized to join the study and there was a lack of patients older than 60. Male patients and injuries from car accidents were more common than would be expected from the general population.⁷⁷ Blood draws were centrifuged and later analyzed at a separate facility. To validate LOC & PTA duration in clinic, biomarker levels need to be measured much sooner. These limitations could be improved in a larger study with LOC/PTA durations recorded in minutes and biomarkers measured on-site with a rapid screening tool.

The selected biomarkers and covariates were not predictive of either LOC or PTA, however an expanded study that utilizes a similar intake protocol with more patients, study sites, and biomarkers (such as TRACK U01) may mitigate the limitations identified in this pilot data.

Conflicts of Interest

None.

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APPENDICES

A. TRACK-TBI Pilot Inclusion Criteria⁴⁰

Component 1: Enrollment
1. Age 0-100
2. Documented/verified TBI (ACRM Criteria)
3. Injury occurred < 24 hours ago
4. Acute brain CT for clinical care
5. Ability to provide informed consent
Component 2: Blood Draw
1. Informed consent for the blood draw
2. Blood draw within 24 hours of presentation

B. TRACK-TBI Pilot Exclusion Criteria⁴²

Component 1: Enrollment
1. Patients presenting later than 24 hours after their injury
2. Patients presenting who do not need a CT scan
3. Patients in custody or incarcerated
4. Patients who are a potential danger to themselves or others
Component 2: Blood Draw
1. Patients who do not consent to a blood draw
2. Two unsuccessful venipuncture attempts

C. TRACK-TBI Covariates of Interest⁴⁰

Covariate	Values

Age	Years
Time between injury and blood draw	Minutes
Sex	<ul style="list-style-type: none"> • Male • Female
Study site	1 – 3 (UCSF; UPMC; UMCB)
Mechanism of Injury	<ul style="list-style-type: none"> • Assault • Direct impact: blow to head (striking) • Fall • Piercing fragment • Gunshot • Motor Vehicle • Self-inflicted