Association of early chronic systemic inflammation with depression at 12 months post-traumatic brain injury and a comparison of prediction models

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Background: Post-traumatic depression (PTD) is a common condition after traumatic brain injury (TBI), which is believed to be potentiated by systemic inflammation. The objective of this study was to study the role of early chronic (1-3 months post-TBI) systemic neuroinflammation on 12 months PTD following moderate-to-severe TBI and build prediction models.

Methods: Data from participants (n=149) recruited from inpatient rehabilitation centers at the University of Pittsburgh Medical Center (UPMC) was used. Distributions 33 different neuroinflammatory markers, derived from blood samples collected 1-3 months post-injury, were graphed. Descriptive statistics for selected covariates (age, sex, injury severity, 1-6 months antidepressant use history, premorbid depression) were summarized using mean, median, interquartile range (IQR), standard deviations (SD), and percentages (%). Simple logistic regressions were used to identify several biomarkers associated with PTD (p-value <0.10). Principal components analysis (PCA) and ridge regression were then employed to create an overall inflammatory load score (ILS). PTD prediction model performance was compared using a logistic regression and a random forest modeling and their variations (up-sampling) using both internal and external validations.

Results: 1-3 months MIP-1α, RANTES, ITAC, MIP-3α, IL-1b, TNFα, sIL-6R, IL-21, GM-CSF, MIP-1b, IL-7, IL-10, and Fractalkine were associated (p-value < 0.10) with 12 months PTD in the univariate logistic regressions. The ridge regression-based ILS outperformed the first three PCA-based ILS [area under the curve, AUC=84.52% (ridge) vs. 83.62% (3-PCA) and 81.62% (1-PCA)].

An internal validation approach using 100 bootstrapped datasets identified random forest model with up-sampling procedure as the best performing model (92.4% average accuracy, 69.9% average sensitivity, and 96.2% average specificity). PTD significantly mediated the ILS-functional outcomes relationships.

Conclusion: Early chronic systemic inflammation specific to different areas of immune function can help predict PTD with considerable accuracy. A random forest model with an up-sampling procedure performed better than logistic regression in all prediction metrics using a robust internal (bootstrapping) validation.

Public health significance: Depression is treatable, and biomarkers associated with depression have utility as a screening tool for PTD prevention and early treatment, minimizing negative consequences like suicidality. It may have additional benefits for daily functioning, including cognition, behavior, and community reintegration.

Keywords: *depression, neuroinflammation, traumatic brain injury*.

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Preface

First, I begin by expressing my gratitude to Allah for giving me the strength to finish this dissertation amid different crises. It would never be possible to stay strong and work towards this thesis without his kind blessings.

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1.0 INTRODUCTION

Background

More than 2.8 million individuals are diagnosed each year with traumatic brain injury (TBI) (Taylor et al., 2017). The lifetime costs of TBI in USA is estimated at $60 billion annually (Langlois et al., 2006). The pathophysiology of TBI includes the primary trauma and secondary neuro-metabolic crisis potentiated by inflammation, excitotoxicity, ischemia, and edema. Depression is one common secondary condition after TBI (Jorge et al., 2004). The economic burden of depression is estimated at $210 billion annually in the general population (Greenberg et al., 2015). Approximately one-third of general people diagnosed with depression fail standard treatment (The Council on Scientific Affairs, American Medical Association et al., 1999). Because depression is both prevalent and treatable, prevention and early detection are of great importance for clinicians. Previous research showed patients with TBI are roughly 7.9 times more likely to develop depression compared to the general population (Juengst et al., 2015). Correctly predicting depression status among individuals with TBI may help reduce risky behaviors like suicidal endorsement and attempts. The identification of individualized biomarkers associated with TBI and depression may help predict risk of depression after TBI.

Neuroinflammation and post-traumatic depression

Neuroinflammation is known as a secondary injury mechanism following TBI and a major contributor to chronic outcomes (Donat et al., 2017; Kim et al., 2015). The role of neuroinflammation post-TBI is multifaceted (Chio et al., 2015; Finnie, 2013; A. Kumar & Loane,

2012; Xu et al., 2017). Importantly, neuroendocrine-immune cross-talk, governed by the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes coordinate key signaling of cellular immunity and chemokine signaling in the periphery that impacts neuroinflammation (A. K. Wagner & Kumar, 2019). Systemic inflammatory mediators have been shown in multiple studies to both influence and reflect neuroinflammatory pathology associated with major depressive disorder (MDD) (R. Dantzer, 2008; D'Mello & Swain, 2016; Moriarity et al., 2020) and secondary depressive syndromes including that recently observed among those with the novel coronavirus (COVID-19) (Steardo & Verkhratsky, 2020). Systemic inflammation has been associated with anti-depressant non-responsiveness (Bombardier, 2010). It is also known to be associated with post-traumatic stress disorder (PTSD) (von Känel et al., 2010), which is commonly known to co-occur with depression (Gros et al., 2012). The role of acute inflammation on post-traumatic depression (PTD) was studied in the TBI space and sVCAM-1, sICAM-1, and sFAS, which are generally related to death and damage of cells and platelets causing inflammation, were found to be associated with 6-month PTD (Juengst et al., 2015). The role of chronic inflammation on depression has long been identified (Michael Maes, 1995; Michael Maes et al., 2012), but early chronic inflammation in relation to PTD has remained under-studied in the TBI space, which if associated with PTD, can be very informative for clinicians to detect and treat depression early.

The Department of Physical Medicine and Rehabilitation of the University of Pittsburgh collects data, including blood samples on TBI patients from the level 1 inpatient rehabilitation center at the University of Pittsburgh Medical Center (UPMC) facilities including Presbyterian and Mercy Hospitals through IRB approved study protocols. The investigators measured 34

inflammatory responses in serum samples, believed to be related to different patient outcomes post injury, using a Luminex® bead array assay. These multiplex assays used microsphere technology where assay beads were tagged with various fluorescent-labeled markers. The binding for each protein onto the multiplex bead was analyzed with a fluorescence detection laser optic system. The Human High Sensitivity T cell Magnetic Bead Panel included interleukin (IL)-10, IL-12p70, IL-13, IL17A, IL-1β, IL-2, IL-21, IL-4, IL-23, IL-5, IL-6, IL-7, IL-8, Macrophage Inflammatory Protein (MIP)-1α, MIP-1β, Tumor Necrosis Factor (TNF)-α, Fractalkine, Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Interferon-inducible T-cell alpha chemoattractant (ITAC) and Interferon (IFN)-γ. The Human Neurodegenerative Disease Magnetic Bead included soluble Intracellular Adhesion Molecule (sICAM)-1, Regulated upon Activation, Normal T-cell Expressed and Secreted (RANTES), Neural Cell Adhesion Molecule (NCAM) and soluble Vascular Adhesion Molecule (sVCAM)-1. The Human Soluble Cytokine Receptor Magnetic Bead Panel included soluble (s)CD30, soluble glycoprotein (sgp)130, soluble IL-1 receptor (sIL-1R)-I, sIL-1RII, sIL-2α, sIL-4R, sIL-6R, sTNFRI, and sTNFRII. Assay specifics for these data have been described in detail and published elsewhere (Vijapur et al., 2020).

Often individual interpretations of these biomarkers are not of direct importance because biologically they work together as a complex signaling network to influence PTD. An overall composite score representing a patient's inflammatory profile or inflammation burden, created by the biomarkers that influence PTD, could be more relevant in understanding the biodiversity of the immune system in its relationship to PTD. Such an overall composite score can also be created as a linear combination of discriminant inflammatory biomarkers and be called an inflammatory load score (ILS). There is a gap in the literature wherein there are no known attempts to develop

an inflammatory load score for PTD prediction. The main articles on such an score formulation for other outcomes were mostly based on an unweighted approach (R. G. Kumar et al., 2015; Raj G. Kumar et al., 2015; Santarsieri et al., 2015). Since inflammatory biomarker cascades are usually correlated, stable weights corresponding to the biomarkers in the linear combination can be obtained using methods such as principal component analysis (PCA) or ridge regression. Weights obtained by PCA do not depend on the outcome of interest, while weights obtained by ridge regression are predicated on the inflammatory relationship to outcome.

Role of other covariates on post-traumatic depression

Patient characteristics such as age, sex, and injury severity can influence inflammatory response and hence may have an impact on PTD. Individuals with preinjury psychological disorder or diagnosis of depression can also be related to post-injury depressive symptoms (Alway et al., 2016; Bombardier et al., 2016; Rogers & Read, 2007). Information on medications, especially antidepressants that individuals were taking prior to injury can help inform and predict later depression (Price et al., 2011), although antidepressants may be less effective among patients with TBI (Neurobehavioral Guidelines Working Group et al., 2006). Lesions identified on computed tomography (CT) scan data during acute hospitalization may also reflect differences in long-term health outcomes, such as depression (Hamani et al., 2011; Hudak et al., 2011; Koolschijn et al., 2009; Maller et al., 2010; Mayberg, 2003; Mettenburg et al., 2012; Sheline et al., 2003). Concurrent employment and substance abuse can also be related to depression (Awan, DiSanto, Juengst, Kumar, Bertisch, Niemeier, Fann, Kesinger, et al., 2020). For predictive models the concurrent variables are not relevant, but our previous research shows preinjury employment and substance abuse status predict post-injury employment and substance abuse (Awan, DiSanto,

Juengst, Kumar, Bertisch, Niemeier, Fann, Sperry, et al., 2020), so these variables can be brought in to see how well they help predict 12 months depression.

Self-reported MDD through Patient Health Questionnaire-9 (PHQ-9) is often categorized as a binary (depressed/non-depressed) outcome (Fann, Berry, et al., 2009). Of note, PHQ-9 is a validated self-administered battery for screening symptom endorsement and symptom severity associated with MDD; this instrument scores each of the 9 Diagnostic and Statistical Manual-IV (DSM-IV) criteria for MDD as "0" (not at all) to "3" (nearly every day). Logistic regression is an old and widely used method for binary classification (Cox & Snell, 1969). There are more recent tree-based algorithms such as random forest that can also perform binary classification. Logistic regression describes the relationship between one dependent binary variable and one or more nominal, ordinal, interval or ratio-level independent variables. Random forests, also known as random decision forests, are a popular ensemble method that can be used to build predictive models for both classification and regression problems. Ensemble methods use multiple learning models to gain better predictive results. For the random forest, the model creates an entire collection of random uncorrelated decision trees to arrive at the best possible prediction. The ideas of `bagging' (selecting subsets of features and growing the full trees) and ensembles (combination of decision trees to increase the classification accuracy) were popularized by an extension of the very first algorithm of random forest (Ho, 1995) and the algorithm developed by Leo Breiman (Breiman, 2001).

There have been several studies that have compared the predictive performance of a logistic regression and a random forest with different datasets. One such study compared the prediction performance of the onset of a civil war (Muchlinski et al., 2016). However, different datasets may

show superior prediction accuracy of one approach over another under different conditions. For example, logistic regression can work equally well when signal-to-noise is low, and the sample size is comparatively small, but random forest will be superior with more data on the same problem. Logistic regression is still used even when less predictive because it is more interpretable and faster. However, model performance should be evaluated through some kind of cross-validation before deciding which approach has better predictive accuracy. Tuning any parameter for improved model performance should be based on the out-of-sample model performance measures (average over the hold-out folds in a cross-validation, for example) and order of sampling should be maintained during cross-validation while comparing multiple models.

Objectives of the study

The primary objective of this study was to investigate the influence of early measures of chronic systemic inflammation on 12 months depression after moderate-to-severe TBI adjusting for premorbid depression, injury severity, demographic characteristics, and other features specified above. The secondary objective was to compare PCA-based and ridge regression-based ILS calculations. The third objective was to compare the predictive performance of a logistic regression and a random forest model in predicting PTD. This study can support early detection and proactive treatment of "at risk" individuals in order to prevent or reduce the functional devastation associated with depression post TBI.

2.0 METHODS

In this section, I discuss the data source and variables in subsection 2.1, the description of the statistical methodology used in subsection 2.2, and statistical analyses in the order they were performed in subsection 2.3. I used PCA to derive the PCA-based ILS, logistic regression with ridge penalty (including only selected biomarkers) to derive the ridge-based ILS, and then compared PCA-based and ridge-based ILS to find which one performed better in a logistic regression with other covariates. Finally, I selected the ridge-based ILS (based on area under the curve (AUC)) for further analysis and compared the prediction performance of logistic regression and random forest. Logistic regression was used twice: first, to derive the ridge-based ILS (using only the biomarkers) and then while comparing the predictive models (using ILS and all other covariates).

2.1 Data

2.1.1 Data source and participants

Data from a prospective cohort study of individuals (N=149) with moderate-to-severe TBI, recruited from the inpatient rehabilitation centers through Mercy (MER) facilities at the University of Pittsburgh Medical Center (UPMC), were collected and analyzed. Moderate-to-severe TBI status was based on admission total Glasgow coma scale (GCS) score <13, positive findings on

head CT scan, loss of consciousness >30 minutes, and/or post-traumatic amnesia >24 hours (Carlson et al., 2009). Patients were followed up to 15 months post injury in accordance with site-specific institutional review board approved protocols and provided informed consent. In order to be included in the analysis, participants must have had an indicator of post-traumatic depression (PTD) at 12 months, as defined below.

2.1.2 Outcome

The main outcome of interest was post-traumatic depression (PTD) at 12 months following moderate-to-severe TBI. PTD status was calculated by using the Patient Health Questionnaire-9 (PHQ-9), which consists of items where subjects are asked if they have been bothered by the following problems in the past two weeks: 1) little pleasure or interest in doing things (anhedonia), 2) feeling down, depressed, or hopeless (depressed mood), 3) sleeping too little or too much, 4) feeling tired or having little energy, 5) poor appetite or overeating, 6) feelings of worthlessness or guilt, 7) concentration problems, 8) psychomotor retardation or agitation, and 9) thoughts of suicide ("Thoughts that you would be better off dead or of hurting yourself in some way"). The PHQ-9 has demonstrated appropriate validity to be used as a screening tool for MDD. In accordance with Diagnostic and Statistical Manual-IV (DSM-IV) criteria for diagnosing MDD, participants were characterized as having PTD if they reported at least five symptoms, including at least one of the cardinal symptoms (depressed mood or anhedonia).

2.1.3 Covariates

We initially included 34 different inflammatory responses (pro- and anti-inflammatory cytokines) that were measured using a Luminex® bead array assay: IL-2, IL-1b, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IFN-gamma, GM-CSF, TNFα, ITAC, Fractalkine, MIP-3a, IL-17A, IL-21, IL-23, MIP-1a, MIP-1b, sTNFRI, sCD30, sgp130, sIL-1RI, sIL-1RII, sIL-2Ra, sIL-4R, sIL-6R, sTNFRII, sICAM-1, RANTES, NCAM, sVCAM-1. We excluded sIL-1RI as it was poorly assayed and was highly missing (data unavailable at all time points for about 70% of individuals in the study).We also included characteristics such as age, sex, injury severity, pre-existing psychological disorder (Yes/No), premorbid employment (Yes/No), premorbid substance abuse (Yes/No), and use of antidepressant during first 6 months post-injury (Ever/Never) as covariates. The list of antidepressants used to extract the information on antidepressant use status is provided below in Table 1.

Table 1: List of antidepressants used to identify 1-6 months antidepressant use status

Tricyclics: Anafranil (clomipramine), Asendin (amoxapine), Elavil (amitriptyline), Norpramin (desipramine), Pamelor (nortriptyline), Sinequan (doxepin), Surmontil (trimipramine), Tofranil (imipramine), Vivactil (protiptyline)
Selective serotonin reuptake inhibitors (SSRIs): Celexa (citalopram), Lexapro (escitalopram), Luvox (fluvoxamine), Paxil (paroxetine), Prozac (fluoxetine), Zoloft (sertraline)
Monoamine oxidase inhibitors (MAOIs): Nardil (phenelzine), Parnate (tranylcypromine)

Others: Desyrel (trazadone)

The Glasgow coma scale (GCS) was used to measure the severity of the neurological injury. This tool rates a patient's level of injury on 4-6-item scales based on assessing eye opening, verbal, and motor response. GCS ranges from 3-15 and lower scores mean more severe neurological injury (*The Glasgow Structured Approach to Assessment of the Glasgow Coma Scale*, n.d.). Computed tomography (CT) scan data were available from individual medical records obtained at various time point during acute hospitalization on subdural hemorrhage (SDH), subarachnoid hemorrhage (SAH), extradural hematoma (EDH), intraventricular hemorrhage (IVH), intraparenchymal hemorrhage (IPH), intracerebral hematomas (ICerH), diffuse axonal injury (DAI), and contusion. Based on other CT subtypes, evidence of intracranial hemorrhage (ICH), and extra- and intra-axial lesions were also created.

Aside from the inflammatory biomarkers and CT variables, we also considered other covariates to use as adjustments, regardless of their significance in bivariate analyses. For example, premorbid depression was associated with 12-months PTD status in one study (Ouellet et al., 2018). Also, we assumed that first 6 months antidepressant use status post-TBI could also inform 12-months PTD. Age and sex are well known risk factors to be adjusted for in most epidemiological studies. We adjusted for neurological injury severity, since differing levels of injury severity can result in different levels of recovery. We also included preinjury psychological disorder as a covariate, as PTD can develop directly or indirectly through pre-existing psychological and psychosocial factors (Juengst et al., 2017).

2.2 Statistical methods

The statistical methods used in this dissertation are described below in the order they were used.

2.2.1 Principal components analysis (PCA)

Principal component analysis (PCA) is a popular dimension-reduction technique that explains the variance-covariance structure of a set of variables through a few linear combinations of these variables. We have used PCA to explain the variation in the correlated biomarkers using only the first few dimensions. If $X_1, X_2, \dots, X_p$ are p random variables with variance-covariance matrix Σ and correlation matrix ρ, which has eigenvalues $\lambda_1 \geq \lambda_2 \geq \cdots \geq \lambda_p \geq 0$, and corresponding eigenvectors $e_1 = [e_{11}, e_{12}, \dots, e_{1p}]$, $e_2 = [e_{21}, e_{22}, \dots, e_{2p}]$, $\dots$, $e_1 = [e_{p1}, e_{p2}, \dots, e_{pp}]$, then the linear combinations

$$Y_1 = e_{11}X_1 + e_{12}X_2 + \cdots + e_{1p}X_p$$

$$Y_2 = e_{21}X_1 + e_{22}X_2 + \cdots + e_{2p}X_p$$

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$$Y_p = e_{p1}X_1 + e_{p2}X_2 + \cdots + e_{pp}X_p$$

are called the principal components, where $Var(Y_i) = e_i \Sigma e_i^T = \lambda_i$ and $Cov(Y_i, Y_k) = e_i \Sigma e_k^T$ for $i, k = 1, 2, \ldots, p$. Hence, the variance explained by the first principal component (Y_1) is the maximum and can often be taken to represent the index for variables $X_1, X_2, \ldots, X_p$.

2.2.2 Logistic regression

For binary (Bernoulli) response variable Y, where $Y = 0, 1$, such as in our case where the outcome is PTD ($Y = 1$) or no PTD ($Y = 0$), if $P(Y = 1) = p$, then for covariates $X_1, X_2, \ldots, X_k$, a logistic regression model can be written as,

$$log \frac{p}{1-p} = \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_k X_k.$$

This regression models the log odds of $Y = 1$ and the predicted probability is given by,

$$\hat{p} = \frac{e^{\widehat{\beta}_1 X_1 + \widehat{\beta}_2 X_2 + \cdots + \widehat{\beta}_k X_k}}{1 + e^{\widehat{\beta}_1 X_1 + \widehat{\beta}_2 X_2 + \cdots + \widehat{\beta}_k X_k}}.$$

2.2.3 Logistic regression with ridge penalty

Ridge penalty stabilizes the coefficients and their standard errors in the presence of correlated data. The biomarkers considered in this study are correlated because of their biological function. We used the ridge-based penalty to stabilize the β coefficients and used the predicted $X\beta$ as the linear combination of the correlated biomarkers, where X is the matrix of the selected biomarkers. If x_i is the *i*-th row of a matrix of *n* observations

with *p* predictors and a column of ones to accommodate the intercept, and β is the column vector of the regression coefficients, then the constrained maximization for penalty parameter is given by the following (Duffy & Santner, 1989; Le Cessie & Van Houwelingen, 1992):

$$l_\lambda(\beta) = \sum_{i=1}^{n} [y_i x_i \beta - \log(1 + e^{x_i \beta})] - \lambda \sum_{j=1}^{p} \beta_j^2.$$

The coefficients obtained by maximizing this equation are more stable when the predictors are correlated because adding some bias reduces the variance of the parameter estimates. The predicted log odds using these coefficients can be used as an outcome-dependent index of the correlated predictors.

2.2.4 Random forest for binary classification

Random forest is a process of combining many decision trees with bootstrapped data producing different leaf nodes. For binary classification problem (e.g. PTD/no PTD), it merges the classification of all decision trees and counts the maximum vote for classification. How nodes on a decision tree branch depends on the Gini index, which is given by,

$$Gini = 1 - \sum_{i=1}^{c} (p_i)^2,$$

where p_i represents the relative frequency of the class we are observing in the dataset and c represents the number of classes (c=2 in our case). This index ranges from 0 (homogeneous) to 1 (heterogeneous) and is a measure of how each variable contributes to the homogeneity of the nodes and leaves in the resulting random forest. Each time a variable is used to split a node, the Gini coefficient for the leaf nodes are calculated and compared to that of the original node. The root of each split is chosen based on the variable split with the lowest Gini index.

2.2.5 Up-sampling

When data are imbalanced in terms of outcome cases and non-cases, the most used classification algorithms do not work well because the focus of these algorithms is minimizing the error rate rather than identifying positive cases correctly. While this effect can be minimized by moving along the threshold for classification, over-sampling the minority class can sometimes produce better sensitivity (Kuhn & Johnson, 2013; Ling & Li, 1998). The process involves resampling the minority class to increase the corresponding frequencies or weights by replication, without increasing information (Chen et al., 2004). Both logistic regression and random forest were performed using up-sampling of PTD cases and the predictive performance metrics were compared with the ones where the threshold was chosen based on fixing sensitivity (at 80%) using the training data.

2.2.6 Diagnostic and prediction accuracy metrics

To compare the diagnostic ability of different logistic regression models, as their discrimination threshold is varied, the receiver operating characteristic (ROC) curve is a widely used graphical tool. The concordance statistic (c-statistic) or area under the curve (AUC) is the probability that a classifier will rank a randomly chosen positive instance higher than a randomly chosen negative one (assuming 'positive' ranks higher than 'negative'). AUC ranges between 0 and 1 and higher values mean greater discriminative capability. For a 2-class prediction problem, prediction accuracy measures are based on the confusion matrix, which can be defined as follows.

Confusion Matrix	**Reference/True category**	
Predicted category	Event	No Event
Event	A	B
No Event	C	D

The accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), detection rate, and detection prevalence are calculated as follows based on this confusion matrix.

$$Accuracy = \frac{A + D}{A + B + C + D},$$

$$Sensitivity = \frac{A}{A + C},$$

$$Specificity = \frac{D}{B + D},$$

$$Positive\ Predictive\ Value = \frac{A}{A + B},$$

$$Negative\ Predictive\ Value = \frac{D}{C + D},$$

$$Detection\ Rate = \frac{A}{A + B + C + D},$$

$$Detection\ Prevalence = \frac{A + B}{A + B + C + D},$$

$$Balanced\ Accuracy = \frac{Sensitivity + Specificity}{2},$$

$$Yuden's\ Index = Sensitivity + Specificity - 1.$$

We have evaluated these metrics using an external validation with a 70-30 split of the original data into training and test sets. Because our sample size was small with complete data on all biomarkers and covariates, we also performed a 'strong internal validation' which is often recommended instead of a split or *k*-fold cross validation when the sample size is small (Harrell Jr & Slaughter, 2001). This validation process involves taking a bootstrap sample from the original data and using it as a training set. The model built on the training set is tested on the original sample. Hence the original sample now serves as a test set. The bootstrapping is repeated *B* times and the average of the performance metrics on the test set (here, the original sample) are reported over the *B* iterations.

2.3 Statistical analysis

2.3.1 Descriptive analysis

Medians of monthly values of 1-3 months biomarkers were used to represent a measure of *early chronic* biomarkers. A median is not affected by outliers; hence this also took care of any possible outliers present in the biomarker data. We calculated the descriptive statistics of the covariates by PTD status using summary measures mean, median, interquartile range (IQR), and standard deviation (SD) for continuous variables, and percent (%) for categorical variables. The Wilcoxon rank-sum (Mann-Whitney) test, Chi-square test, and logistic regression were used to test for bivariate associations with PTD.

2.3.2 Inflammatory load score (ILS)

Since inflammatory responses are typically highly correlated to each other, it is often of interest to look at the burden of the inflammatory responses rather than the biomarkers individually. To do this, clinicians can create an ILS using inflammatory biomarkers to represent an individual's inflammatory burden by weighting each inflammatory marker based on its importance. This composite score can provide an overall idea about a individual inflammatory burden, which could be more relevant to the clinicians in making treatment-related decisions.

The first step of creating the ILS for our study was to assess which biomarkers were statistically important (p-value < 0.10) to PTD using simple logistic regressions. Variable selection

techniques require complete data on all biomarkers, therefore using a variable selection technique requiring complete data on all 33 biomarkers would significantly reduce the analytic sample size. Our biomarker data had high missingness due to random assay failures or research participant loss to follow up, and all biomarker values were not available for the study participants. Therefore, selecting only a subset of these 33 biomarkers would result in less missingness in the ILS creation procedures described below, maximizing our analytic sample size. The variables were standardized (mean-centered and scaled by the standard deviation) to show the change in odds ratio (OR) for one standard deviation change in the standardized biomarker to make the ORs comparable (Agresti, 2003).

Two potential methods of creating this ILS are to use: 1) principal component analysis (PCA) or 2) ridge regression. In PCA, the components are the linear combinations of original biomarkers with different weights that represent the correlation of the biomarkers and the component scores. Ridge regression introduces a small amount of bias to reduce the variance of the estimates and weight the biomarkers based on their association with the outcome. The predicted log odds from this ridge regression can be taken as a weighted inflammatory load score (wILS) that is equivalent to a weighted sum of the biomarker levels. Both methods deal with high dimensionality of the inflammatory markers. PCA is a dimension-reduction technique that produces principal components only based on the biomarkers that maximize the variation in the biomarker space. It does not weight the biomarkers based on any outcome associated with the biomarkers. Ridge regression is a modelling approach which uses information regarding the association of the outcome with the biomarkers. However, this may or may not be an advantage in

the predictive model, as the weights derived from the training set will be applied to the validation set, wherein the association betwen PTD and the candidate inflammatory biomarkers could differ.

We created two versions of the ILS based on PCA and ridge regression. The composite score created by using a ridge regression model will be called a weighted ridge-based inflammatory load score and the composite score by using a PCA will be called a weighted PCA-based inflammatory load score. We used ridge regression to create the ILS because 1) the biomarkers are usually correlated among themselves to some degree and 2) we weighted the biomarkers depending on their relationship to PTD, adjusting for other covariates and all other biomarkers. Ridge regression improves the feasibility of generating more reliable estimates (with reduced standard error) by adding a penalty term in the presence of multicollinearity (Hoerl & Kennard, 1970). We performed a 5-fold cross validation (CV) 1000 times to choose the most stable ridge penalty parameter using the R package *glmnet* (Friedman et al., 2010). We multiplied these coefficients with the log scaled biomarkers to get a weighted ILS. For PCA, we used the base function *princomp* in R and multiplied the biomarker values with the loadings of the first component to get the PCA-based ILS.

2.3.3 Predictive models: logistic regression and random forest

We used the ILS along with age, sex, GCS, antidepressant use, preexisting psychological disorder and premorbid depression to find the association of the ILS with PTD. To compare the discrimination capability of the PCA-based and ridge-based ILS, we compared the area under the

receiver operating characteristic (ROC) curve. From this we selected the ILS that had greater discriminatory power.

We also compared the predictive performance of logistic regression model to random forest for our problem. To assess model performance in the study, we performed an external validation by dividing the data into training and test sets with a 70-30 ratio and a 'strong internal validation' by taking 100 bootstrap samples. When comparing the predictive ability of the logistic regression and random forest, we used ROC, confusion matrix, sensitivity, specificity, accuracy, PPV, NPV, detection rate, and detection prevalence. We compared both models through a 5-fold cross-validation repeated 10 times. The logistic regression and random forest models and their corresponding accuracy measured were obtained using the *caret* package in R. All analyses were performed using R, version 3.6.2 (R Core Team, 2019) in RStudio, version 1.3.959 (RStudio Team, 2019).

3.0 RESULTS

3.1 Descriptive statistics

The sample characteristics by PTD status are presented in Table 2. Among the study participants, 76.5% were men and 23.5% were women with an average age of approximately 39 years. Among them, 18.1% had premorbid depression, 31.5% had pre-existing psychological disorder and 28.9% had record of antidepressant use in the first 6 months after their injury. The percentages of CT lesions are also recorded. The Fisher's exact test showed that participants with premorbid depression had a higher prevalence of depression at 12 months (33.3%) compared to those who did not have premorbid depression (14.1%). None of the other variables were significantly different between PTD and no PTD status.

Table 2: Descriptive statistics of the covariates by PTD status at 12 months (row percentages presented in the 3rd and 4th columns)

Covariates	Total (N=149)	Present (N=31)	Absent (N=118)	p-value
Age at injury				0.792*
Mean (SD)	38.9 (17.6)	38.3 (15.2)	39.1 (18.3)	
Median [Min, Max]	34.0 [17.0, 78.0]	35.0 [18.0, 69.0]	32.5 [17.0, 78.0]	
Sex				0.126†
Male	114 (76.5%)	20 (17.5%)	94 (82.5%)	
Female	35 (23.5%)	11 (31.4%)	24 (68.6%)	
GCS				0.847*
Mean (SD)	8.43 (3.48)	8.48 (3.35)	8.42 (3.53)	
Median [Min, Max]	8.00 [3.00, 15.0]	8.00 [3.00, 15.0]	8.00 [3.00, 15.0]	

Covariates	Total (N=149)	Present (N=31)	Absent (N=118)	p-value
Missing	4	0	4	
Premorbid depression				0.045††
Present	27 (22.7%)	9 (33.3%)	18 (66.7%)	
Absent	92 (77.3%)	13 (14.1%)	79 (85.9%)	
Missing	30	9	21	
Pre-existing psychological disorder				0.550†
Present	47 (32.6%)	12 (25.5%)	35 (74.5%)	
Absent	97 (67.4%)	19 (19.6%)	78 (80.4%)	
Missing	5	0	5	
Antidepressant use (first 6m)				0.236†
Yes	43 (31.9%)	8 (18.6%)	35 (81.4%)	
No	92 (68.1%)	19 (20.65%)	73 (79.35%)	
Missing	14	4	10	
CT SDH				0.465†
Present	96 (69.6%)	23 (24.0%)	73 (76.0%)	
Absent	42 (30.4%)	7 (17.7%)	35 (83.3%)	
Missing	11	1	10	
CT SAH				0.523†
Present	97 (70.3%)	23 (23.7%)	74 (76.3%)	
Absent	41 (29.7%)	7 (17.1%)	34 (82.9%)	
Missing	11	1	10	
CT EDH				0.784††
Present	22 (15.9%)	4 (18.2%)	18 (81.8%)	
Absent	116 (84.1%)	26 (22.4%)	90 (77.6%)	
Missing	11	1	10	
CT IVH				0.288†
Present	42 (30.4%)	12 (28.6%)	30 (71.4%)	
Absent	96 (69.6%)	18 (18.8%)	78 (81.3%)	
Missing	11	1	10	

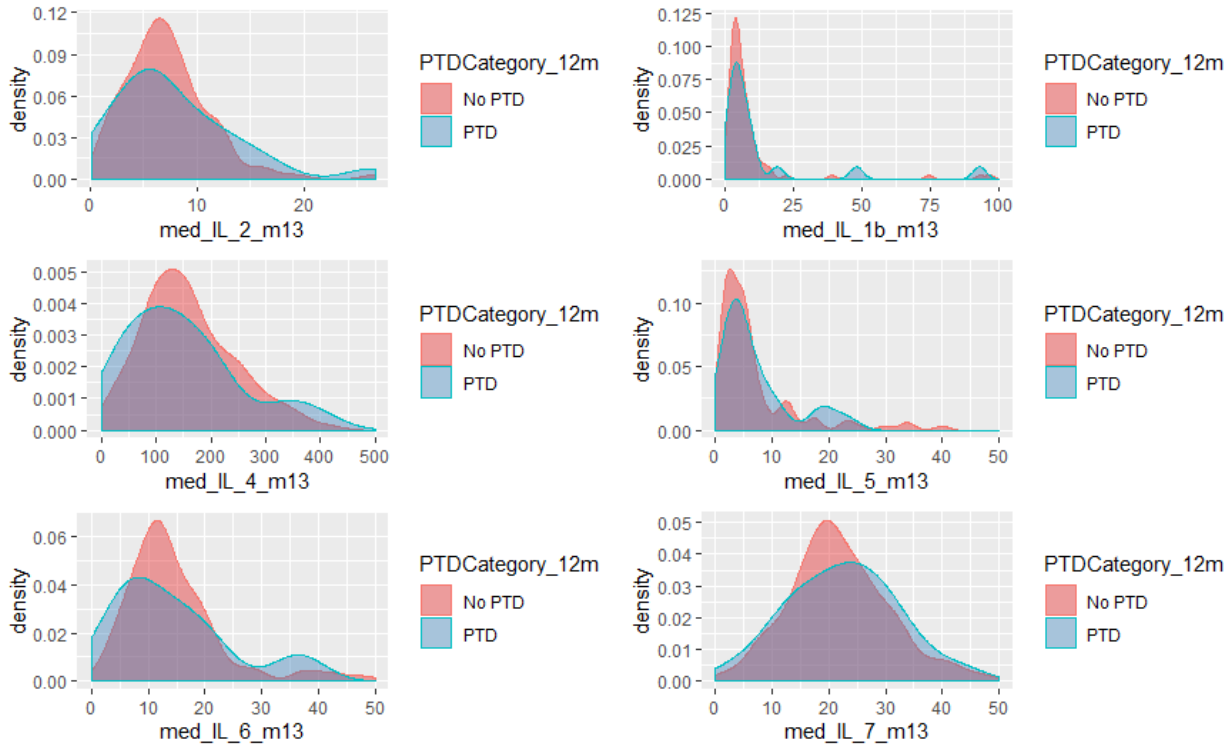
Covariates	Total (N=149)	Present (N=31)	Absent (N=118)	p-value
CT IPH				0.597†
Present	68 (49.3%)	13 (19.1%)	55 (80.9%)	
Absent	70 (50.7%)	17 (24.3%)	53 (75.7%)	
Missing	11	1	10	
CT ICerH				0.524††
Present	3 (2.2%)	1 (33.3%)	2 (66.7%)	
Absent	135 (97.8%)	29 (21.5%)	106 (78.5%)	
Missing	11	1	10	
CT DAI				0.364††
Present	17 (12.3%)	2 (11.8%)	15 (88.2%)	
Absent	121 (87.7%)	28 (23.1%)	93 (76.9%)	
Missing	11	1	10	
CT Contusion				0.964†
Present	80 (58.0%)	18 (22.5%)	62 (77.5%)	
Absent	58 (42.0%)	12 (20.7%)	46 (79.3%)	
Missing	11	1	10	
CT ICH				0.999††
Present	131 (94.9%)	29 (22.1%)	102 (77.9%)	
Absent	7 (5.1%)	1 (14.3%)	6 (85.7%)	
Missing	11	1	10	
CT extra-axial				0.999††
Present	126 (91.3%)	28 (22.2%)	98 (77.8%)	
Absent	12 (8.7%)	2 (16.7%)	10 (83.3%)	
Missing	11	1	10	
CT intra-axial				0.540†
Present	107 (77.5%)	25 (23.4%)	82 (76.6%)	
Absent	31 (22.5%)	5 (16.1%)	26 (83.9%)	
Missing	11	1	10	

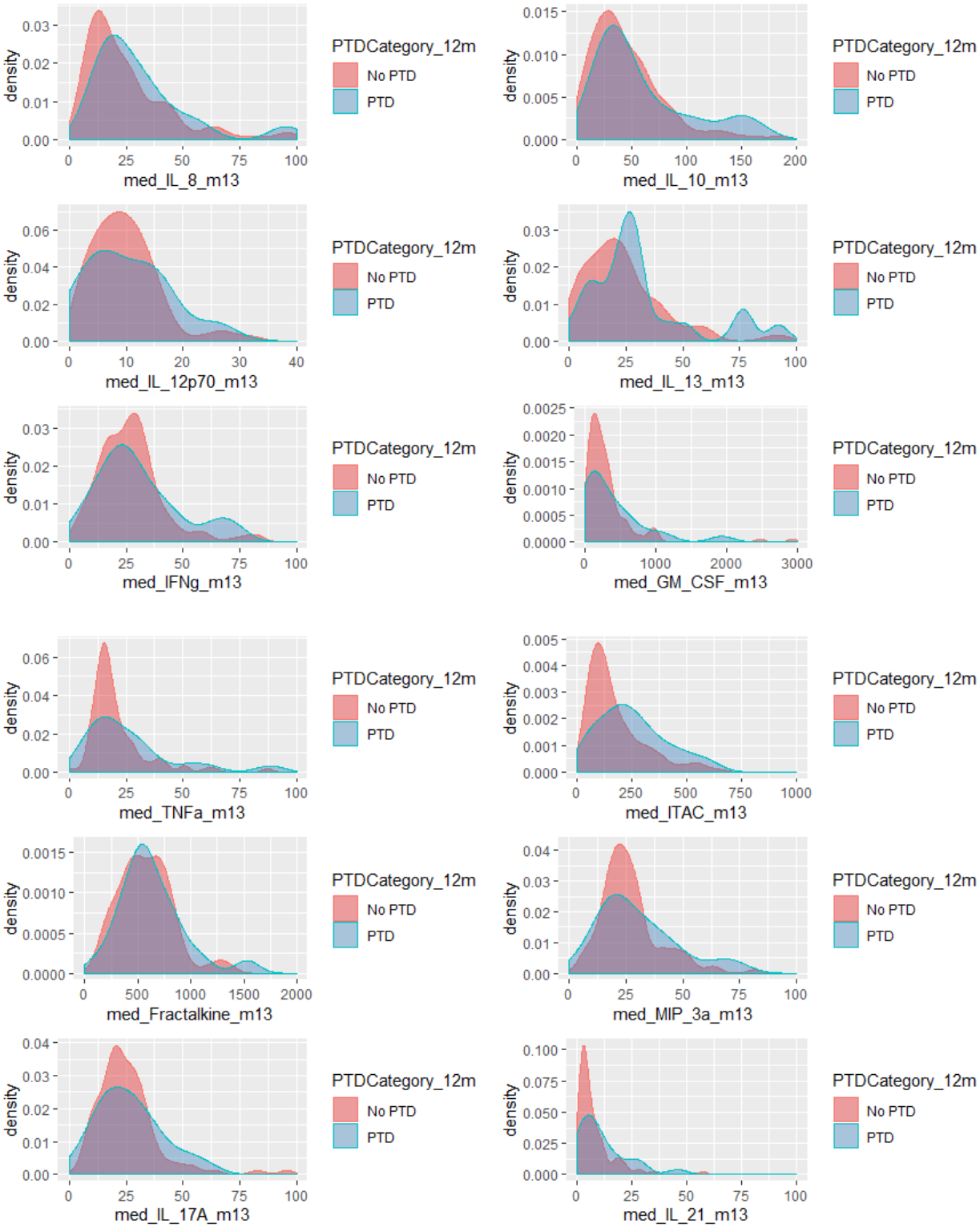
∗ Mann-Whitney test
† Chi-square test
†† Fisher's exact test

3.2 Bivariate analysis

The medians of the 1-3 months standardized biomarker levels are presented in Figure 1. There were some visible differences in the distribution of the biomarkers between PTD and no PTD status, with participants who had PTD having higher values in all biomarkers, except for sIL-6R.

Figure 1: Distributions of the medians of the 1-3 months standardized biomarkers by PTD status




density
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med_IL_10_m13
med_IL_12p70_m13
med_IL_13_m13
med_IFNg_m13
med_GM_CSF_m13
med_TNFa_m13
med_ITAC_m13
med_Fractalkine_m13
med_MIP_3a_m13
med_IL_17A_m13
med_IL_21_m13
PTDCategory_12m
No PTD
PTD


density
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4e-04
2e-04
0e+00
0 1000 2000 3000 4000 5000
med_IL_23_m13
PTDCategory_12m
No PTD
PTD
density
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0.015
0.010
0.005
0.000
0 100 200 300 400 500
med_MIP_1a_m13
PTDCategory_12m
No PTD
PTD
density
0.020
0.015
0.010
0.005
0.000
0 50 100 150 200
med_MIP_1b_m13
PTDCategory_12m
No PTD
PTD
density
0.00075
0.00050
0.00025
0.00000
0 1000 2000 3000 4000 5000
med_sTNFRI_m13
PTDCategory_12m
No PTD
PTD
density
0.015
0.010
0.005
0.000
0 50 100 150 200
med_sCD30_m13
PTDCategory_12m
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PTD
density
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1.0e-05
5.0e-06
0.0e+00
100000 150000 200000 250000
med_sgp130_m13
PTDCategory_12m
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PTD
density
0.03
0.02
0.01
0.00
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PTDCategory_12m
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PTD
density
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0.00010
0.00005
0.00000
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PTDCategory_12m
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PTD
density
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0.0010
0.0005
0.0000
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PTDCategory_12m
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PTD
density
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0.00025
0.00000
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PTDCategory_12m
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PTD
density
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2e-05
0e+00
10000 20000 30000 40000
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PTDCategory_12m
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PTD
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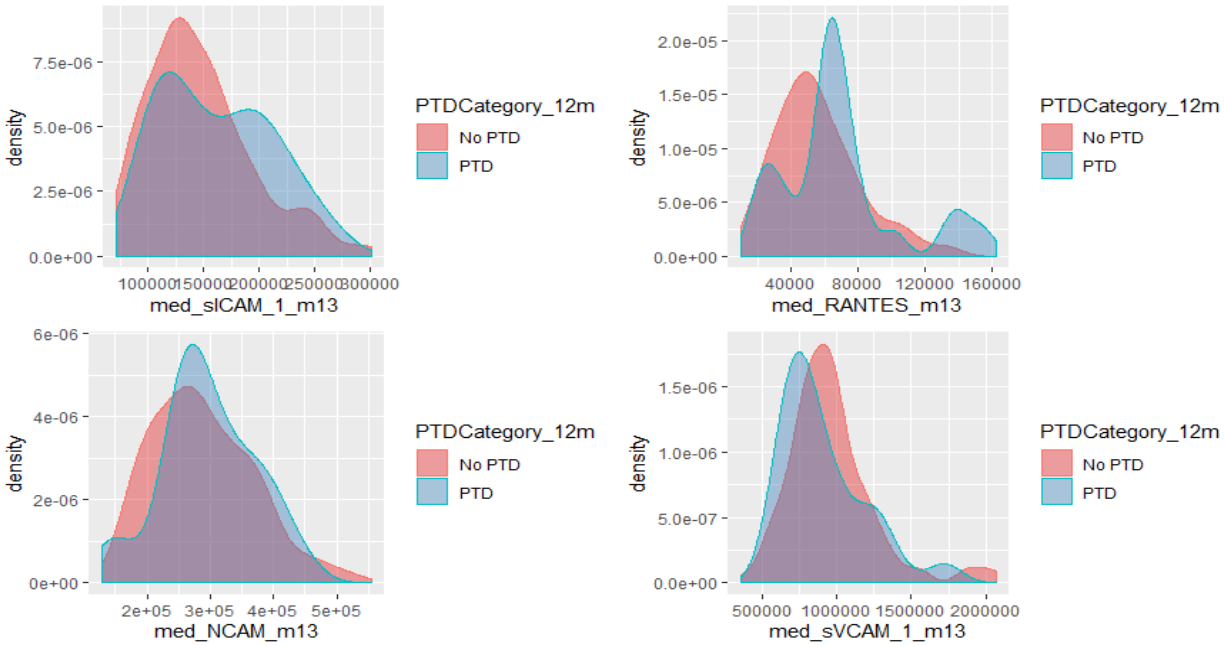
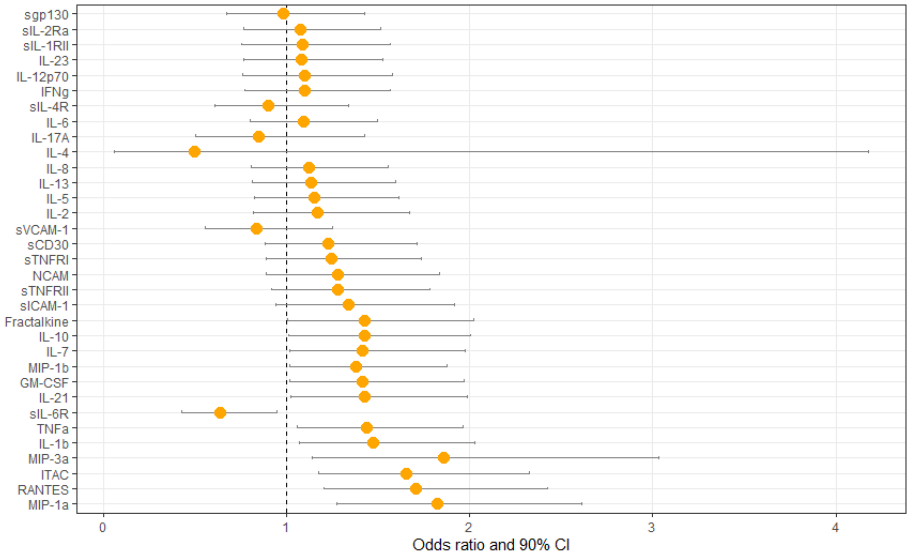
No PTD
PTD



We summarized all other odds ratios and their 90% confidence intervals in Figure 2. The figure shows evidence that there is variation in the effects of the markers on odds of PTD.

Figure 2: Odds ratios with 90% CI from univariate logistic regressions of 12 months PTD with standardized medians of 1-3 months biomarkers, sorted by the descending order of p-values



Among all the 33 biomarkers considered, 13 (IL-1b, IL-7, IL-10, GM-CSF, TNFα, ITAC, Fractalkine, MIP-3a, IL-21, MIP-1a, MIP-1b, sIL-6R, and RANTES) were significant at the 10% level. Higher levels of each these inflammatory markers were significantly associated with increased odds of PTD, except for sIL-6R, higher levels of which were associated with decreased odds of PTD. We presented the statistically significant results in Table 3 ($\alpha = 0.10$).

Table 3: Bivariate logistic regression of PTD status with standardized 1-3 months biomarker medians

Biomarker	β	OR	P-value
MIP-1a	0.60	1.82	0.006
RANTES	0.53	1.71	0.012
ITAC	0.50	1.65	0.016
MIP-3a	0.62	1.86	0.037
IL-1b	0.39	1.47	0.046
TNFα	0.36	1.44	0.054
sIL-6R	-0.45	0.64	0.062
IL-21	0.35	1.42	0.081
GM-CSF	0.35	1.41	0.083
MIP-1b	0.32	1.38	0.087
IL-7	0.35	1.41	0.088
IL-10	0.35	1.42	0.091
Fractalkine	0.36	1.43	0.095

3.3 Creating inflammatory load score using ridge regression and PCA

To choose the best value for the tuning parameter of ridge regression, λ, we performed a 5-fold cross-validation 1000 times; the resulting distribution of λ is presented in Figure 3a. The mode of this empirical distribution was $\lambda = 0.7414409$. The inflammatory load score (ILS) was created using a ridge regression based on this λ. The coefficient paths for different values of the tuning

parameter (λ) from the ridge regression are presented in Figure 3b. It shows that the parameter estimates become stable very quickly as we increase the values of the penalty parameter λ.

Figure 3: (a) Distribution of best lambda from repeated CV and (b) plot of coefficient from the ridge regression for creating ILS

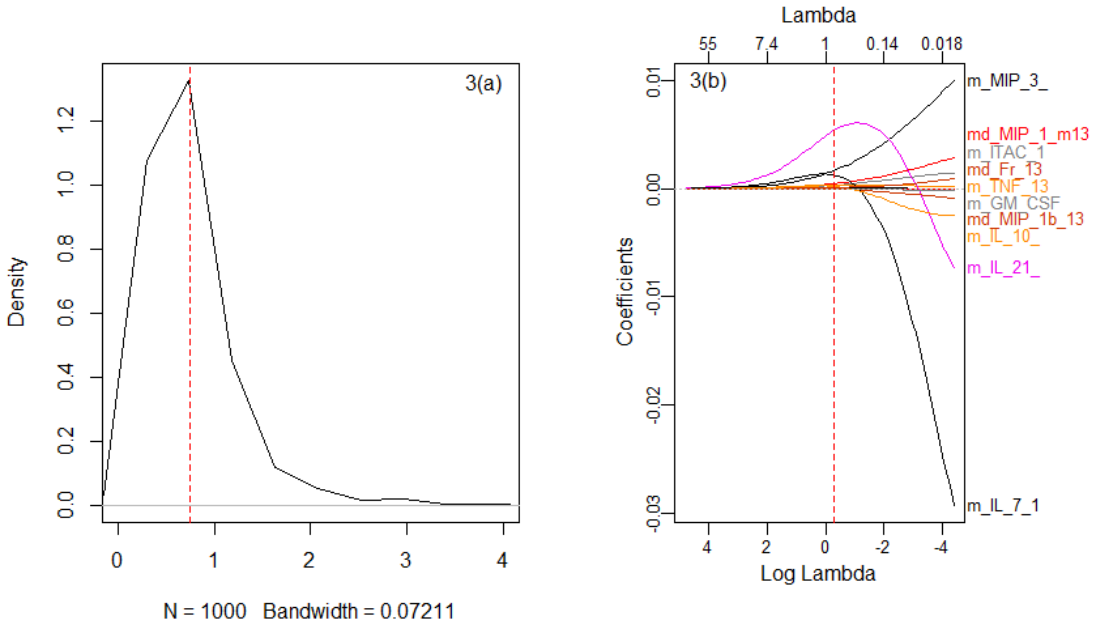


Table 4 shows the ridge-penalized coefficients for the standardized variables.

Table 4: Coefficients of the standardized biomarkers in the ridge regression

Biomarker	Coefficient
MIP-1a	0.096
RANTES	0.062
ITAC	0.050
MIP-3a	0.089
IL-1b	0.02
TNFα	0.028
sIL-6R	-0.053
IL-21	0.041
GM-CSF	0.010
MIP-1b	0.003

IL-7	0.020
IL-10	0.011
Fractalkine	0.039

The PCA-based ILS was created using the first component score, which explained about 45.65% variation in the selected biomarkers. The 2nd and 3rd PCs explain 13.90% and 10.56% of the total variation. The remaining PCs do not represent the overall inflammatory burden well and would not explain much additional variation, hence they were discarded from the analysis. A scree plot of the principal components with the bars representing percent variation explained is shown in Figure 4. The coefficients of the biomarkers in the linear combinations in PC1, PC2, and PC3 are given in Table 5. In the section below, we compare the PCA-based ILS with the ridge regression-based ILS.

Figure 4: Scree plot of the principal components with percent variation explained

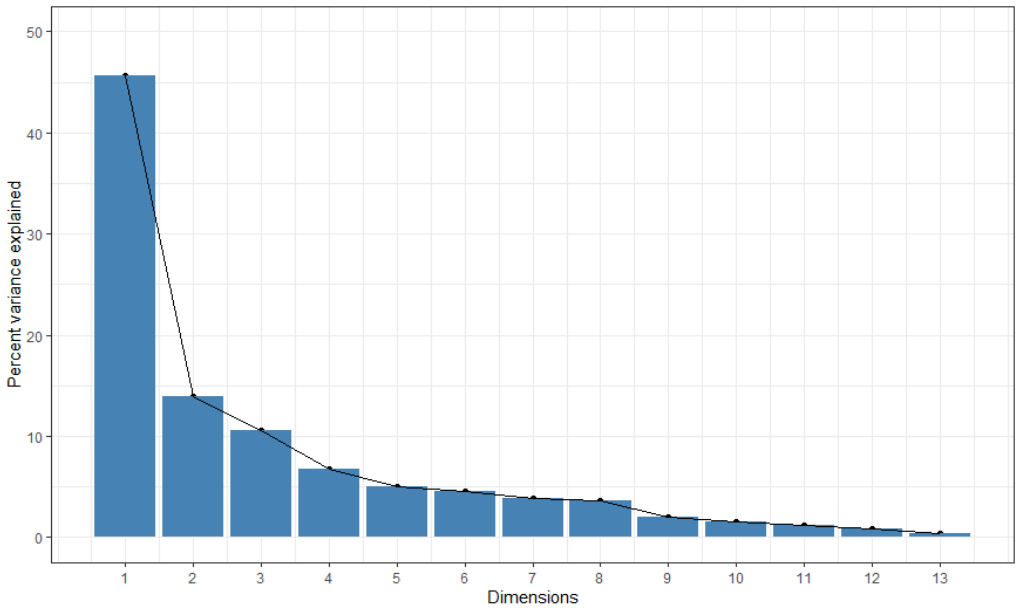


Table 5: Coefficient of the biomarkers in the first three principal components

Biomarker	PC1	PC2	PC3
MIP-1a	0.327	0.110	0.072
RANTES	0.162	0.200	0.522
ITAC	0.220	0.008	0.534
MIP-3a	0.305	-0.234	-0.079
IL-1b	0.320	-0.408	-0.086
TNFα	0.316	-0.386	-0.052
sIL-6R	-0.030	-0.137	0.542
IL-21	0.359	0.218	0.040
GM-CSF	0.245	0.145	-0.308
MIP-1b	0.297	-0.372	-0.051
IL-7	0.349	0.148	-0.034
IL-10	0.259	0.307	-0.062
Fractalkine	0.237	0.486	-0.155

3.4 Comparison of ridge-based and PCA-based ILS

We compared the PCA-based ILS with the ridge regression-based ILS in terms their discriminative ability of PTD and no PTD cases. We included age, sex, GCS, premorbid depression status, and antidepressant use status in the first 6 months as covariates and presented the adjusted odds ratios (AORs) from logistic regressions using ridge-based ILS, first PC-based ILS, and first three PC-based ILS in Tables 6a, 6b and 6c, respectively. The adjusted odds of PTD are estimated to increase by 32% (AOR=1.32, p-value=0.002) with each ten-point increase in the ridge-based ILS (i.e., one-unit increase in the 10× scaled ILS) (Table 6).

Table 6: Full logistic regression model with ridge-based ILS (n=96)

Variables	Estimate	Adjusted OR	Std. Error	P-value
(Intercept)	1.56	4.76	1.69	0.356
Age	-0.02	0.98	0.02	0.386
Sex: Men	0.2	1.22	0.87	0.814
GCS	0.13	1.14	0.1	0.196
Premorbid depression: Yes	1.11	3.03	0.79	0.162
Antidepressant in first 6m: Yes	-0.56	0.57	0.85	0.509
ILS (ridge)×10	0.28	1.32	0.09	0.002

The odds of PTD are estimated to increase by 38% (AOR=1.38, p-value=0.002) with each one-point increase in the first PC-based ILS (Table 7).

Table 7: Full logistic regression model with PCA-based ILS with the first component (n=96)

Variables	Estimate	Adjusted OR	Std. Error	P-value
(Intercept)	-2.55	0.08	1.19	0.032
Age	-0.02	0.98	0.02	0.396
Sex: Men	-0.03	0.97	0.81	0.97
GCS	0.13	1.14	0.1	0.203
Premorbid depression: Yes	1.02	2.77	0.78	0.192
Antidepressant in first 6m: Yes	-0.46	0.63	0.83	0.579
ILS (PC1)	0.32	1.38	0.1	0.002

The odds of PTD are estimated to increase by 43% (AOR=1.43, p-value=0.002) with each one-point increase in the first PC-based ILS (Table 8). The 2nd and 3rd PC-based ILS were not significantly associated with PTD.

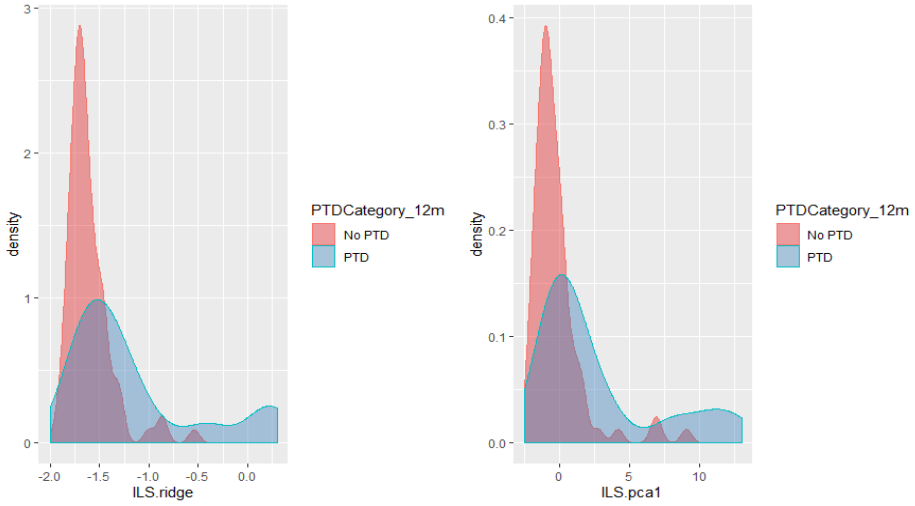
Table 8: Full logistic regression model with PCA-based ILS with the first three components (n=96)

Variables	Estimate	Adjusted OR	Std. Error	P-value
(Intercept)	-2.56	0.08	1.22	0.035
Age	-0.02	0.98	0.02	0.411
Sex: Men	0.06	1.06	0.87	0.944
GCS	0.12	1.13	0.1	0.26

Premorbid depression:				
Yes	1.1	3	0.79	0.163
Antidepressant in first 6m:				
Yes	-0.56	0.57	0.83	0.503
ILS (PC1)	0.36	1.43	0.12	0.002
ILS (PC2)	0.24	1.27	0.2	0.246
ILS (PC3)	0.07	1.07	0.26	0.796

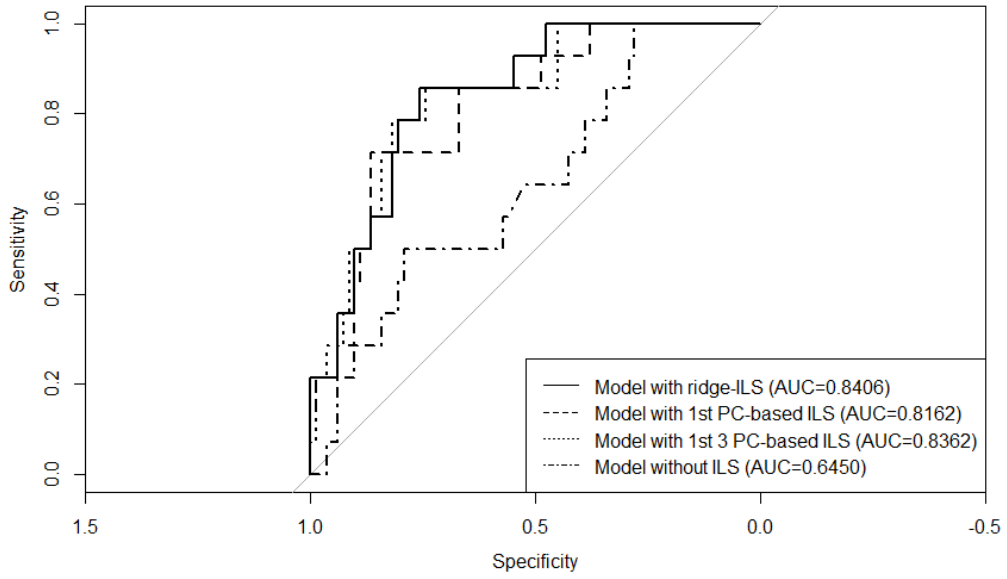
The distribution of ridge-based and 1st PC-based ILS values between PTD and no PTD categories are presented in Figure 5. The distributional difference between the PTD and no PTD categories look very similar in shape for both ILS. Note that the ranges of X-axis (i.e., ILS values) are different because of the arbitrariness of the different methods being used to create them. To aid the interpretation, the ILS variables were transformed into percentile ranks. A one percentile change in the ridge-based ILS was associated with 4.40% higher odds of PTD (OR=1.04, p-value=0.003) while a one percentile change in the first PC-based ILS was associated with 4.35% higher odds of PTD (OR=1.04, p-value=0.003). Hence, there was very little difference between the two ILS.

Figure 5: Distribution of ridge and PC1-based ILS by PTD status



We calculated the predicted probabilities of PTD from the models with PCA-based ILS, ridge-based ILS, and without ILS and compared the area under the receiver operating characteristics curves in Figure 6. The model without any ILS had an AUC of 64.5%, whereas the ROC for the model with 1st PC-based ILS was 81.6%. The model with ridge-based ILS outperformed the first three PC-based ILS (AUC=83.6%) only marginally and had an AUC of 84.1%. The DeLong's test for two correlated ROC curves did not show any statistically significant difference between the two curves (Z = 1.60, p-value = 0.110).

Figure 6: ROC comparison on the full model with and without ILS



3.5 Prediction performance of logistic regression and random forest model

The results of the prediction performance of logistic regression and random forest model on the test data are presented in Table 9. The sensitivity was set to 80% using the training data to find the probability threshold for classification. The test set consisted of 28 observations with 4 PTD cases. The overall accuracy of the logistic regression model was 85.71% with a sensitivity of 75% and a specificity of 87.5%. The positive predictive value was only 50%. The prevalence of PTD was about 14% in the sample, but the detection prevalence was 21.43%. The Yuden's index was 62.5% which meant the model was useful in predicting PTD to some extent. The random forest model failed to detect any of the 4 PTD cases in the test data. Hence, the other metrics were not reported.

Table 9: Accuracy measures on the test data using a 70-30 split with sensitivity set at 80% for training data

Estimate	Logistic regression	Random forest
Accuracy [95% CI]	0.8571 [0.6733, 0.9597]	0.8571 [0.6733, 0.9597]
Sensitivity	0.7500	0
Specificity	0.8750	1
Positive predictive value	0.5000	-
Negative predictive value	0.9545	-
Detection rate	0.1071	-
Detection prevalence	0.2143	-
Balanced accuracy	0.8125	-
Yuden's index	0.6250	-

The reason for the poor performance of the random forest model was the small size of the test set and the high class-imbalance. Therefore, we up-sampled PTD cases in the test data to evaluate the models (Table 10). With up-sampling the random forest model had higher accuracy compared to the logistic regression model (89.29% vs. 78.57%), but the sensitivity was still only

50% for the random forest model while it was 75% for the logistic regression model. However, there were only 4 PTD cases in the test data and the sensitivity metric can be arbitrary. The Yuden's indexes do not suggest that either of these models was very useful in predicting PTD, with values close to 50%.

Table 10: Accuracy measures on the test data using a 70-30 split and up-sampling of PTD cases

Estimate	Logistic regression	Random forest
Accuracy [95% CI]	0.7857 [0.5905, 0.917]	0.8929 [0.7177, 0.9773]
Sensitivity	0.7500	0.50000
Specificity	0.7917	0.95833
Positive predictive value	0.3750	0.66667
Negative predictive value	0.9500	0.92000
Detection rate	0.1071	0.07143
Detection prevalence	0.2857	0.10714
Balanced accuracy	0.7708	0.72917
Yuden's index	0.5417	0.45833

The sample size was small enough to not provide us with a moderately sized test set. Moreover, there was high class-imbalance present in our sample. Hence, we performed a 'strong internal validation'. The process involved taking B=100 bootstrap samples and using them each time to train the model and using the original sample as the test set. The averages of the prediction performance metrics were reported in Table 11 for classifications with thresholds chosen by setting sensitivity at 80% using the training data. The detection prevalence was very high (30.85%) for the logistic regression model while it was quite conservative for the random forest model (7.31%). As a result, the sensitivity of the random forest model was also poor (48.21%). The specificity of the random forest model was, however, almost perfect (99.67%). The positive predictive value of the random forest model (96.69%) was significantly higher than that of the logistic regression

model (35.10%). Overall, the Yuden's indexes were not convincing as a measure of overall predictive performance for either of these models (expected to be > 0.5).

Table 11: Strong internal validation by bootstrapping with sensitivity set at 80% for training data

Estimate	Logistic regression	Random forest
Accuracy [95% CI]	0.7442 [0.647, 0.8257]	0.9217 [0.8489, 0.9664]
Sensitivity	0.6807	0.4821
Specificity	0.755	0.9967
Positive predictive value	0.351	0.9669
Negative predictive value	0.9346	0.9187
Detection rate	0.0993	0.0703
Detection prevalence	0.3085	0.0731
Balanced accuracy	0.7179	0.7394
Yuden's index	0.4357	0.4789

Lastly, we performed the strong internal validation using the up-sampling technique with both logistic regression and random forest (Table 12). The random forest model with up-sampling outperformed all other models in almost all metrics. The accuracy was 92.4%, with a sensitivity of 69.9% and a specificity of 96.2%. The performance of the logistic regression model was also improved (accuracy: 77.81%, sensitivity: 63.93%, specificity: 80.18%). However, it suffered from poor positive predictive value (36.17%). The detection prevalence was still high (26.25%). The detection prevalence of the random forest model (13.4%) was almost close to the PTD prevalence in the sample. The Yuden's index was 66.1% for the random forest model with up-sampling, which was the best among the models we tried.

Table 12: Strong internal validation by bootstrapping with up-sampling of PTD cases

Estimate	Logistic regression	Random forest
Accuracy [95% CI]	0.7781 [0.6822, 0.8562]	0.924 [0.852, 0.967]
Sensitivity	0.6393	0.699
Specificity	0.8018	0.962
Positive predictive value	0.3617	0.778
Negative predictive value	0.9294	0.95
Detection rate	0.0932	0.102
Detection prevalence	0.2625	0.134
Balanced accuracy	0.7206	0.831
Yuden's index	0.4411	0.661

4.0 DISCUSSION

Diagnostic and Prediction Performance of the Models

We used ridge regression and PCA to obtain patient-specific ILS, which are novel approaches in the TBI space. The diagnostic performance of ILS created using ridge regression and PCA was tested using the whole cohort. The model with ridge-based ILS marginally succeeded to surpass the PCA derived model using a three PC-based ILS. We believe both methods can be useful in creating an ILS and are comparable in terms of their diagnostic performance based on AUC. We then proceeded with the ridge-based ILS to compare the predictive performance of logistic regression and random forest with our data. The predictive accuracy metrics were better for the random forest model when using an up-sampling technique and when tested using a robust internal validation methodology. The independent test set with a 70-30 split was very small to assess which model performed better. When using our robust internal validation methodology, the logistic regression continued to have a relatively poor positive predictive value. However, the sensitivity and specificity were moderate while using the threshold set by fixing training set sensitivity at 80%. The advantage of using a logistic regression for classification is its ease of use for clinicians. Thresholds can be defined, possibly by averaging over the ones identified with the bootstrapping and can be readily used. The best performing model, the random forest model with up-sampling technique, requires setting up an app or a calculation system that can classify a new patient into PTD or no PTD categories. Logistic regression was also more open to classifying cases as PTD, while the random forest models were conservative. The random forest models (both with

and without up-sampling) achieved very high specificity. However, in our case, early detection of a person with PTD is more important than correctly detecting a patient with no PTD. Hence, sensitivity holds significant weight when choosing between model complexity and convenience.

Inflammatory Hypothesis of Depression & Sickness Syndrome

Looking at cytokine or inflammatory load has potential relevance in the context of antidepressant treatment (Köhler et al., 2018). What has been deemed the "inflammatory hypothesis of depression" suggests a dynamic interplay between domains of the immune system, neurotransmitters, and neuro-circuitry influence behavioral changes, including the onset of depressive symptoms (Michael Maes, 1995; Miller & Raison, 2015). The development of depression is thought to be relevant to a pathogen host defense process. That is, an inflammatory response is mounted in response to environmental or pathogenic exposure or stressor.

Typically, a pro-inflammatory, anti-pathogenic response is intended to eliminate pathogen exposure (Raison & Miller, 2013). Exposure to pro-inflammatory cytokines, particularly chronically, can result in moods and behaviors related to "sickness syndrome" which overlap with depression symptoms (Raison et al., 2010; Slavich & Irwin, 2014). These symptoms include lack of energy and interest, decreased appetite, and fatigue, all of which are common in depression (Anisman et al., 2005; R. Dantzer, 2008; Robert Dantzer, 2006; Michael Maes et al., 2011; Myers, 2008; Reichenberg et al., 2001). While sickness behavior is adaptive and helps the body respond to acute injury or infection, it becomes maladaptive if it persists beyond 3-6 weeks after injury (Robert Dantzer, 2006; Michael Maes et al., 2011). This marks a transition from acute, adaptive behavior to a chronic process that can lead to depression (Charlton, 2000).

Other diseases with a prominent systemic pro-inflammatory state have also been associated with increased depression risk (Raison et al., 2010). For example, systemic and neuroinflammation have an impact on classic disease models such as multiple sclerosis (Christensen et al., 2013; Jadidi-Niaragh & Mirshafiey, 2011). Autoimmune diseases, including multiple sclerosis, have high depression rates wherein systemic inflammation is considered to be a central disease mechanism (Morris et al., 2015; Pryce & Fontana, 2016). Inflammatory mechanistic frameworks that drive depression also impact specific symptoms associated with depression such as fatigue and sleep dysregulation (Alekseeva et al., 2019). Following TBI, which induces a pro-inflammatory response to circulating brain-derived antigen, this susceptibly to depression is likely exacerbated. Systemic inflammatory signaling is also known to activate the HPA and the sympathetic nervous system (Elenkov, 2008; Elenkov et al., 2005), both of which are also persistently activated in the setting of major trauma, including TBI (Wagner Humoral triad) and impact neurotrophin signaling, which is also implicated with PTD (Failla et al., 2016) as well as MDD (Hing et al., 2018; Kraus et al., 2019; Mondal & Fatima, 2019). To our knowledge this is the first TBI study published directly implicating chronic inflammatory burden with PTD. To that end we have rigorously applied quantitative methods to identify inflammatory markers associated with PTD in our population as well as generate and validate a weighted ILS, based on inflammatory levels over the first three months post-injury that has significant predictive capacity. Markers used for ILS formulation implicate multiple arms of the immune system as increasing PTD risk at 12 months post injury. Below we outline the unique immune domain-specific profiles associated depression post-TBI.

Innate Immunity & Chemokines

The most relevant PTD markers in the ILS formation were among the innate, proinflammatory molecules (IL-1β, MIP-3a, MIP-1a) and chemo-attractant molecules (ITAC and RANTES). Additionally, GM-CSF, TNFα, and MIP-1b were associated with depression status albeit to a lesser degree. Historically, depressed patients, even in the absence of trauma, express increased serum pro-inflammatory biomarkers including IL-1β, IL-6, TNFα, and IFNγ and likewise, show compensatory increases in anti-inflammatory molecules IL-4 and IL-10 (R. Dantzer, 2008; Littrell, 2012; Michael Maes, 2011). Neurotransmitter depletion including serotonin, norepinephrine, and dopamine have all been implicated in the "monoamine hypothesis" of imbalanced brain chemistry related to depression (Bruno et al., 2020; Perez-Caballero et al., 2019; Spellman & Liston, 2020). After TBI, the hypothalamic pituitary adrenal (HPA)-axis exhibits a stress-like response to the neurologic insult that fails to normalize (Ranganathan et al., 2016; Martina Santarsieri et al., 2014; Amy K. Wagner et al., 2011) and a proinflammatory environment (M. Santarsieri et al., 2015; Schuster et al., 2017) similar to that observed with other disease associated sickness behavior inflammatory profiles and contributes to depressive symptoms post-TBI. These innate proinflammatory molecules also activate the HPA axis, and, in turn, affect serotonin precursor levels (Dunn et al., 1999), and serotonin signaling is widely implicated in depression (Krishnan & Nestler, 2008; M. Maes et al., 2011).

Likewise, chemokines are key in orchestrating the recruitment and activation of effector molecules to the sites of injury; however, they have also been implicated in HPA-axis and neuroendocrine dysregulation (Callewaere et al., 2007). With persistent chemo-attractant elevations into the chronic phase of recovery post-TBI, the neurogenesis reduction associated with

HPA-axis dysfunction may increase depression pathophysiology (Pariante & Lightman, 2008). The neuroendocrine dysfunction that accompanies depression, particularly after trauma, is a compelling area for further exploration.

Adaptive Immunity

Adaptive immunity related markers including IL-7, IL-21 and Fractalkine were implicated in depression at the $p<0.1$ threshold. While the cell-mediated innate immune relationship to depression was more dominant, there is still compelling evidence for humoral signaling and the immune response following TBI due to the interrelationships between innate and adaptive immune systems. In particular, T cell subsets may be imbalanced due to the dysregulated cytokine signaling in favor of a pathogenic Th1 phenotype and a down-regulation of Treg cells which would typically reduce chronic inflammation (Miller, 2010). In particular, IL-7 has been implicated in generating a sustained and effective immune response after TBI (Katzman et al., 2011). From a chronic TBI perspective, elevated IL-7 may have some maladaptive functions, as it is linked to numerous autoimmune disorders; further, an autoimmune response has recently been demonstrated after TBI (Zhang et al., 2014). Immunological memory associated with adaptive immunity may relate the experience of stress exposure (or elevated inflammatory profile) with the onset of depressive and mood disorders (Miller, 2010).

Soluble Molecules

sIL-6R was the only resulting molecule reduced in PTD cases. Membrane-bound IL-6R is the target receptor on the surface of white blood cells, including neutrophils, for IL-6-mediated

immune activation deemed “classical activation” (Baran et al., 2018; Rose-John, 2006). Upon activation, IL-6R is shed via proteolytic cleavage and released into circulation. The circulating sIL-6R has affinity for IL-6 and, when bound, the IL-6/sIL-6R complex acts via “trans-signaling” communicating pro-inflammatory signals far from the site of initial injury when bound to the ubiquitously expressed membrane-bound gp130 (Garbers et al., 2011). Noting the multifaceted nature of IL-6 and its spectrum of roles with respect to its receptors, the inverse finding between sIL-6R and PTD presence in this instance is complex. In that case, sIL-6R in isolation would be reduced when trans-signaling mechanisms are dominant and driving pathologic effects of IL-6 (Campbell et al., 2014). The depletion of serum sIL-6R in PTD may also be a result of increased blood brain barrier (BBB) crossing and increased IL-6 trans-signaling in the brain, further perpetuating neurological behavioral deficits (Patel et al., 2012). This relationship should be further explored by utilizing IL-6 family marker ratios to determine the balance of signaling mechanisms occurring.

Limitations and Future Directions

While the findings here are compelling there are some study limitations to consider. One limitation is the relatively low sample size for this study and the need for further validation of the ILS in an independent population. Larger study numbers may also allow for assessing if/how inflammatory load interacts with medication use to impact anti-depressant effectiveness. Also, there were no direct measurements of CNS inflammation. However future work should follow previously published methodologies (Mondello et al., 2020; Osier et al., 2018) to include blood extraction of CNS exosomes for measurement of inflammatory profiles in our TBI population.

There may also be additional inflammatory markers not measured here that may inform PTD risk. Exploring inflammatory marker associations with other secondary conditions such as post-traumatic epilepsy, headache, neuroendocrine dysfunction, cognition, and behavior may identify common inflammatory patterns associated with pathology and poor outcome. The inflammatory differences by PTD status suggest common immune-related pathophysiology underlying depression and other survivor-based outcomes after TBI from other of our work, such as headaches, cognition, and even functional deficits (DRS score) and global recovery (GOS score).

The ILS formulated here may have utility as a screening tool, that when paired with a clinical decision algorithm, as an effective early identifier of those at risk for PTD and potential responder to anti-inflammatory strategies and immunotherapy approaches that can be paired with other non-pharmacological strategies to curb depressive symptoms (e.g. exercise, cognitive behavioral therapy). Targeted immunotherapy approaches for likely responders may curb pathophysiological mechanisms after TBI that exacerbate PTD.

Some preliminary evidence suggests antidepressant medication and cognitive behavioral therapy may be efficacious for treating PTD (Fann, Hart, et al., 2009; Soo & Tate, 1996), however, previous systematic reviews have revealed that there are currently no psychotherapeutic or rehabilitation interventions that prospectively target depression or anxiety disorders after TBI (Hart et al., 2012; Ownsworth & Oei, 1998) Based on our findings, we hypothesize that the relative ineffectiveness of antidepressants, including selective serotonin re-uptake inhibitors (SSRIs), in the setting of TBI may, in part, be due to the inflammatory burden observed in the context of PTD. In fact, some studies support this hypothesis by showing increased SSRI efficacy with elevated IL-1β in the setting of MDD (Pineda et al., 2012). Future work should consider if/how our ILS

formulation informs likely respondership to SSRI treatment, both with/without co-treatment with an anti-inflammatory and/or immunotherapy strategy. Our previous work suggesting increased depression risk due to genetic variation in the *SLC6A4* gene (Failla et al., 2013) also provides an opportunity for future work to consider if/how serotonin system genetics might interact with inflammatory pathways to impact both PTD risk and treatment response.

The random forest model with up-sampling technique was the best performer in our internal validation process. But this model is not readily usable by someone who do not have the expertise to code for this model. To make it usable by the clinicians, we plan to build an R Shiny app in the future that can be used to assess new patient data without the need to know the coding. This will also help us understand better the effects and utility of ILS on model prediction.

Conclusions

These findings support a systemic inflammatory hypothesis for PTD. It is probable that inflammation is a common link between personal biology and recovery course that underlies multidimensional outcomes post-TBI. This study demonstrates great promise for early detection and/or risk stratification of PTD which can help clinicians explore the treatment options for depression before it becomes severe. Efforts in improving the prediction accuracy, especially the sensitivity, can be continued using other machine learning techniques. This study may also encourage research funding for obtaining a larger sample size, so that the relationships among the biomarkers and their association with PTD can be made clearer

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