Multiple Serum Biomarker Panels Identify Brain Injured Patients in CT-negative Populations.

Introduction

Head injury nearly 5 million patients into emergency departments per year in the US.[1] Only a small percentage of these patients have a positive CT scan, showing evidence of intracranial hemorrhage or skull fracture. In the 90% of patients with a negative CT result, routine structural MRI scanning reveals structural abnormalities in up to one third of patients, and advanced neuroimaging methods, such as diffusion tensor imaging (DTI), detects abnormalities in an even larger fraction. Thus, there is still a need to identify TBI pathology in CT negative patients, as they are fractions of those are at risk for persistent post-concussive symptoms that may affect their overall quality of life, and are candidates for clinical trials of targeted therapies. Blood based diagnostic tests measuring changes in physiological levels of circulating biomarkers may aid in identifying CT negative patients at risk for long-term consequences.

Several protein biomarkers discovered in serum and CSF have been described in TBI, including our own work showing alteration of Neuronogranin and other proteins in neuroblastoma.[1] A major test to be done is to see if such proteins are useful as single biomarkers to help confirm a diagnosis of brain injury in CT negative patients. The current study was designed to evaluate the utility of 8 brain-specific protein biomarkers alone, and in combination, to diagnose brain injury in CT negative patients.

Methods

Samples:

Biomarker assays were performed on 254 brain-injured patients from 2 independent studies and compared with 250 healthy control serum samples. All studies were conducted under IRB-approved study protocols at each respective institution. HeadSMART, a prospective study being conducted at Johns Hopkins University, was the main TBI cohort used in the study. Average baseline blood draw was 4.2 hours from injury. Subsequently patients were evaluated at 7 additional time points out to 6 months post injury. Additional samples were obtained from the COBRIT study (5 CT negative patients).[1] Healthy controls (n= 250) were recruited from Baylor College of Medicine (patients not presenting for health assessment of any kind, recruited from companions of ED patients). For TBI patients clinical data was compiled, including detailed neurocognitive and neuroimaging results, consistent with NIH Common Data Elements (CDE). Patient demographics are described in Tables 1 and 2. All TBI patients used in the study met either ACRM criteria for TBI diagnosis (129/192 patients), or ACP criteria for CT (64/192 patients).

Biomarker Assays:

Serum biomarker concentrations for Intercellular Adhesion Molecule-5 (ICAM5), brain-derived Neurotrophic Factor (BDNF), Metallothionein 3 (MT3), Neurotrophic (NTRG and citrullinated NTRGN), Neuro-specific Enolase (NSE), Glial Fibrillary Acidic Protein (GFAP), Beta-Synuclein (SNCB) were assessed in duplicate using high sensitivity sandwich ELISA tests across replicate assays. Detection technologies were either Mass Scale Discovery (MSD) electrochemiluminescence or peroxidase-mediated calorimetric detection with 3,3',5,5'-tetramethylbenzidine (TMB). The values obtained were subjected to selected statistical (logistic regression, performed in STATA v14) and machine learning (LogitBoost) analyses with 10-fold cross validation (Weka Version 3.7.12).

Sensitivity

Univariate analysis of single analytes did not demonstrate sufficient combined sensitivity and specificity for clinical utility (both >0.9), with the possible exception of SNCB (See Table 3, below). However, significant improvements in the combined sensitivity and specificity for the classification of CT negative patients as brain-injured are observed with three-analyte panels (sensitivity and specificity >0.95), using classical logistic regression analysis (performed in STATA, see Table 5, below) or machine learning algorithms such as LogitBoost (performed in Weka, see Table 4 and with example ROC analysis of test performance in Figure 5). Cross validation of study results in both logistic regression and LogitBoost, yielded similar results.

Table 3: Single markers were analyzed using LogitBoost. The algorithm calculated the optimal values for combined sensitivity and specificity. The algorithm was further challenged to optimize for sensitivity only and subsequently calculate the resulting specificity.

Table 4: Selected panels of 3 marker combinations were analyzed using LogitBoost. The algorithm calculates the optimal values for combined sensitivity and specificity of the 3-marker panels. The algorithm was further challenged to optimize for sensitivity only and subsequently calculate the resulting specificity.

Table 5: Analysis of 3 marker panels was also performed using Logistic Regression in order to compare the performance of LR to LogitBoost.

Discussion

An objective biomarker-based test for diagnosis of brain injury in the absence of a positive CT requires a combination of both high sensitivity and specificity for the successful identification of CT negative patients who are at risk following suspected brain injury. None of the individual 8 markers that we tested was sufficiently sensitive and specific to accurately diagnose brain injury positive / CT negative patients. Three-marker panels were able to classify patients as brain injured in the CT negative population with high sensitivity and specificity (> 0.95).

Further studies need to address the brain specific nature of each of these markers, including the contribution of some proteins from the hematopoietic compartment to the detected serum levels and normal ranges (e.g., BDNF, Synuclein B, NSE). Furthermore, additional cohorts and additional time points post-injury should be studied to ascertain the optimal sampling time post-injury. These studies are ongoing in our laboratories in multiple patient cohorts, and are part of the design of HeadSMART for those purposes.

Table 1: HeadSMART Study Patient Demographics and Clinical Data.

Table 2: Healthy Control Patient Demographics.

Figure 1. Comparison of trial designs and time of blood sampling from TBI cohorts used in the study.

Figure 2. Conceptual View of the TBI Continuum of Care. Following brain injury, short term damage may be observed in the first few days following initial injury. Patient samples from the first blood draw post injury (average 4.2 hours post injury; range of 1.5–24 hours post injury) were analyzed by ELISA for independent detection of 8 biomarkers.

Figure 3. Comparison of Biomarker Assays for CT negative patients and Healthy Controls. Box plots analyses show data distribution for individual serum biomarkers in CT Negative patients (CTNeg) and healthy controls (HC) in the study, compiled in standard statistical software. Wilcoxon Rank Sum tests were used to compare the medians between CT negative TBI and healthy controls. Significant differences in median protein concentration were found for GFAP, ICAM5, MT3, citrullinated Neurogranin, and SNCB (p<0.0001), and for NSE (p=0.0057), but not for BDNF (p=0.0058) or Neurogranin (p=0.1545). Results for 4 selected antigens are shown. Outliers were removed from the analysis (defined as values >150% of the interquartile distance), as indicated on each plot.

Figure 4: ROC Analysis of 3 biomarker panels. An example ROC curve from which AUC, sensitivity and specificity were calculated. Examples shown are from analysis with the LogitBoost algorithm (Weka v3.7.12), evaluating the combination SNCB, GFAP and MT3 panel.

Table 6: Multiple Serum Biomarker Panels Identify Brain Injured Patients in CT-negative Populations.

References Cited