

Biomarker Assessment For Neurotrauma Diagnosis and Improved Triage System (BANDITS): Interim Analysis of Feasibility Study of Biomarkers of Severe TBI

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ABSTRACT

Biomarker Assessment for Neurotrauma Diagnosis and Improved Triage System (BANDITS) seeks to develop blood-based biomarkers useful for diagnosis, management and prognosis of traumatic brain injury (TBI) experienced by military personnel and ultimately useful in far-forward combat environments. Researchers estimate that more than 20% of combatants in Iraq and Afghanistan will sustain TBIs, a significant percentage of which will be severe. 101 severe TBI patients were recruited from 7 sites. Injury severity score was inferred by the Glasgow Coma Scale (GCS: Severe TBI GCS 3-8). Patients requiring placement of ventriculostomy to relieve intracranial pressure were enrolled. Appropriate control groups were included. We measured levels of ubiquitin C-terminal hydrolase-L1 (UCH-L1), GFAP and a breakdown product to alpha-spectrin (SBDP145) in samples of blood and cerebrospinal fluid (CSF) from severe TBI patients. CSF levels in UCH-L1, GFAP and SBDP145 are highly specific and sensitive biomarkers of severe TBI when assessed within the first 6 hrs after injury. Analyses demonstrated that biomarkers measured in CSF or serum were highly sensitive and specific predictors of severe brain injury and early mortality. Increased levels of biomarkers were associated with increased injury detected by CT as well as measures of secondary insults.

BACKGROUND

- Biomarker Assessment for Neurotrauma Diagnosis and Improved Triage System (BANDITS) seeks to develop blood-based biomarkers useful for diagnosis, management and prognosis of traumatic brain injury (TBI) experienced by military personnel and ultimately useful in far-forward combat environments. Researchers estimate that more than 20% of combatants in Iraq and Afghanistan will sustain TBIs, a significant percentage of which will be severe. Current methods to assess injury severity are based on CT scans and neurological examinations such as the Glasgow Coma Scale (GCS). These measures are not very sensitive or reliable and are not typically available on the battlefield. There are no rapid, definitive and cost effective blood-based diagnostic tests for TBI.
- This study measured levels of ubiquitin C-terminal hydrolase-L1 (UCH-L1), GFAP and a breakdown product to alpha-spectrin (SBDP145) in samples of blood and cerebrospinal fluid (CSF) from severe TBI patients. UCH-L1 is present in almost all neurons and averages 1-5% of total soluble brain protein. GFAP is an intermediate filament protein found in astrocytes within the CNS. Although highly enriched in CNS, GFAP has also been located in rat peripheral organs including kidney and testes. SBDP145 is a breakdown product to alpha-spectrin produced by the proteolytic activity of the calcium dependent protease, calpain into a 145 kDa fragment. Calpain activation is a prominent marker of oncotic-necrotic cell death. alpha-Spectrin is highly enriched in axons within the CNS, although located in peripheral organs as well, including testes. Previous studies in our own and other laboratories, however, have suggested the potential utility for all these biomarkers for acute brain injury.

METHODS

101 Severe TBI patients were recruited from 7 sites (Univ of Pecs-Hungary, Univ of Szeged-Hungary, Maryland Shock Trauma, Orlando Regional Medical Center, Univ of CA-Davis, Univ of Padua-Italy, Univ of Miami). Injury severity score was inferred by the Glasgow Coma Scale (GCS: Severe TBI GCS 3-8). Patients requiring placement of a ventriculostomy to relieve intracranial pressure were enrolled in the study. Control groups included subjects for cerebrospinal fluid collection (control A) who had not had a TBI but had a lumbar puncture or intraventricular placement for other medical reasons. Serum samples were also taken from healthy normal controls. All patients were 18 years or older. CSF samples were collected after first placement of ventriculostomy and every 6 hrs thereafter, if available. Blood samples were taken on admission and every 6 hrs thereafter. CSF and blood sampling continued for as long as the patient stayed in the ICU or for up to 10 days after injury.

Severe TBI	
n	101
Age, years Mean (SD)	48.78 ± 19.83
Min-Max	19-88
F/M, n	24/77
F/M, %	23.8/ 76.2 %
Ethnicity	Not Hispanic or Latino 99
Race	Hispanic or Latino 2 Caucasian 89 Asian 3 Black or African American 8 Other 1
GCS	3 30 4 15 5 15 6 15 7 16 8 10
Mechanism of Injury:	Motor Vehicle Accident 34 Moto Cycle Accident 4 Gun Shot Wound 2 Fall 43 Sport Injury 4 Assault 14 Other 34

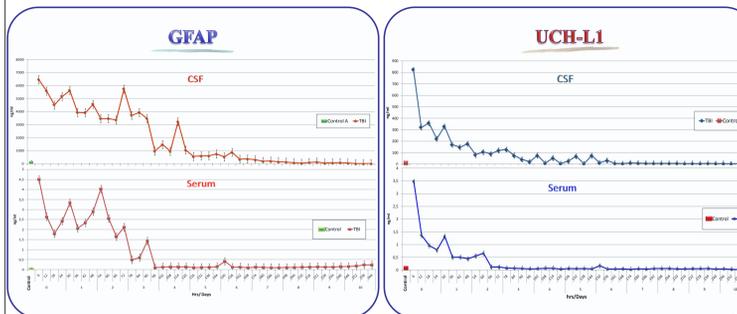
CSF and blood samples were stored at -70°C and shipped to Banyan Biomarkers, Inc. for further analyses. Levels of GFAP, UCH-L1 and SBDP145 were measured by sandwich ELISAs. UCH-L1 and SBDP145 assessments employed proprietary Banyan ELISAs. GFAP was measured by a commercially available ELISA kit (BioVendor USA).

Control Groups

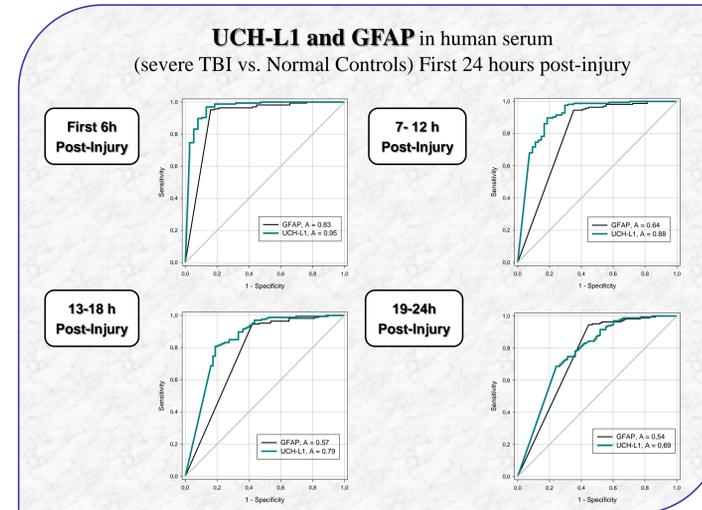
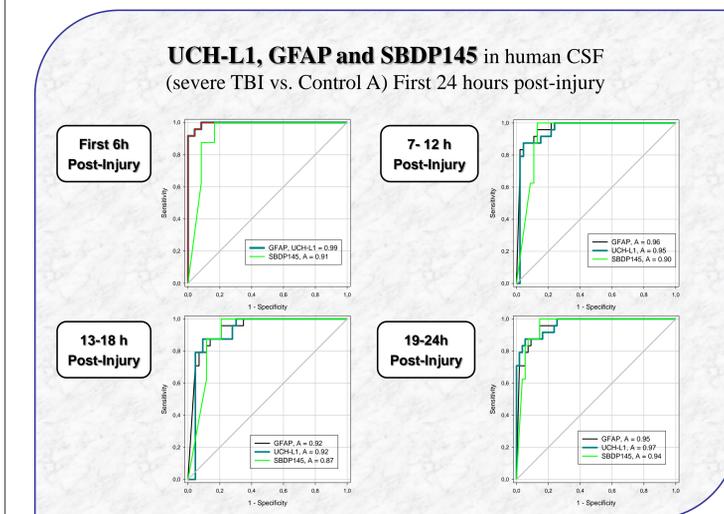
Control A (CSF)		Normal Controls (Serum)	
n	24	n	167
Age, years Mean (SD)	57.12 ± 16.63	Age, years Mean (SD)	36.91 ± 14.06
Min-Max	23-83	Min-Max	18-74
F/M, n	7/17	F/M, n	72/95
F/M, %	29.2/ 70.3 %	F/M, %	43.1/ 56.9 %
Ethnicity	Not Hispanic or Latino 24	Ethnicity	Not Hispanic or Latino 146 Hispanic or Latino 13 Caucasian 8 Asian 53 Black or African American 7 Other 4
Race	Hispanic or Latino 24	Race	Caucasian 53 Asian 7 Black or African American 4 Middle Eastern 1
GCS	15 24	Alcohol (Y/N)	114/47 (N/A 6)
Procedures:	Lumbar Puncture 11 Intraventricular Placement 13	Drug	19/ 147 (N/A 1)
Causes:	Hydrocephalus 20 Cyst (Colloid, Subarachnoidal) 2 SAH – a. comm. anterior aneurysm. 1 Lumbar liquor-drainage after ICH 1 Other 1	Smoke (Y/N)	54/112 (N/A 1)
		Health	Excellent 78 Good 74 Average 13 N/A 2

RESULTS

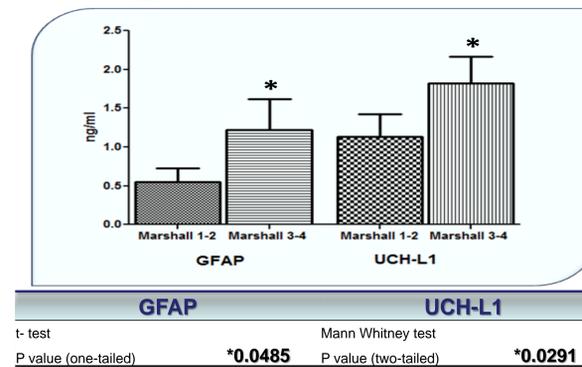
Temporal Profile



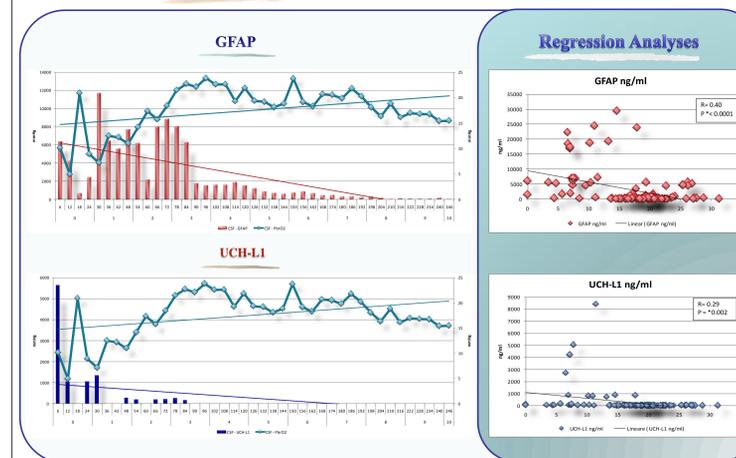
ROC Curve Analysis of Diagnostic Utility



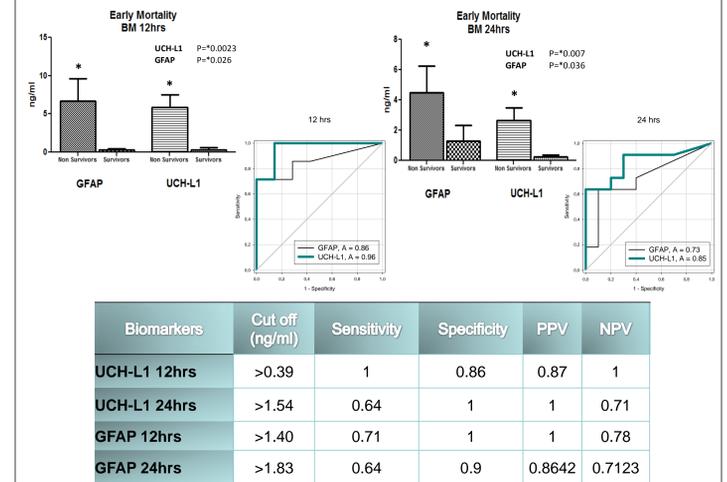
Biomarkers vs. CT Scan



Biomarkers vs. Tissue Oxygenation



Prediction of Mortality: Sensitivity and Specificity



CONCLUSIONS

- Serum levels of UCH-L1 and GFAP are highly specific and sensitive indicators of severe TBI within the first 6 hrs after injury.
- CSF levels in UCH-L1, GFAP and SBDP145 are highly specific and sensitive biomarkers of severe TBI when assessed within the first 6 hrs after injury.
- Analyses of CT employing a Marshall score classification indicated that serum levels of UCH-L1 and GFAP were significantly elevated in Marshall score 3-4 vs. 1-2, suggesting that increasing injury severity for those Marshall score ranges was associated with higher biomarker elevations.
- Initial analyses of biomarker levels associated with secondary insults detected by continuous physiological monitoring indicate increased levels can be associated with adverse physiological events following TBI.
- Levels of UCH-L1 and GFAP were significantly correlated with decreasing tissue oxygenation measured by LICOX probes, suggesting biomarker levels can be associated with secondary insults produced by inadequate oxygen substrate availability.
- Serum levels of UCH-L1 and GFAP were sensitive and specific predictors of early mortality prior to discharge even when measured within the first 12 hrs after injury.

Future Studies

- Continue accrual of severe TBI patients in multicenter trial.
- Complete more extensive analyses of data set from first 101 patients focusing on biokinetics of biomarkers, enhanced understanding of relationship between biomarkers and secondary insults, CT profiles and outcome including Glasgow outcome score.

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