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A Novel Quantitative, Multianalyte Immunoassay to Detect Neuroinflammation following Traumatic Brain Injury

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Introduction

Traumatic brain injury (TBI) affects up to 10 million people worldwide.[1] Mild traumatic brain injury (mTBI) accounts for between 70–90% of all TBI cases. An estimated 10–20% of all veterans of recent U.S military conflicts have sustained mTBI.[2] The primary goal of TBI biomarker research is to identify molecular changes in the brain that could help determine if the brain was injured and help monitor the recovery process.[3]

Proinflammatory biomarkers are released following brain injury and induce a neuroinflammatory response. The prolonged presence of these biomarkers can affect neurons and brain functioning. Inflammation can also alter cell phenotypes and cause increased cytokine production and neural damage. A single blood inflammatory biomarker may not indicate brain pathology; however, evaluating multiple biomarkers may be more informative and allow for a more accurate diagnosis. We evaluated multiple neuroinflammatory markers using Simple Plex™, a novel quantitative, multianalyte immunoassay platform that delivers high precision and accuracy with ≤ 25 µL of sample. Our results identify a potential inflammatory profile for TBI.

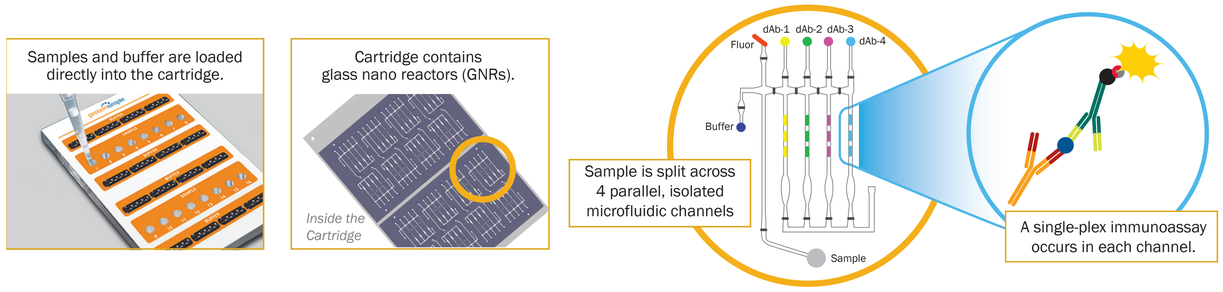
Methods

Serum and plasma samples from individuals diagnosed with varying symptoms of TBI were purchased from Discovery Life Sciences. TBI severity, amount of time from diagnosis, course of treatment, age, gender, and race varied between samples. Control serum and plasma samples were obtained in-house from apparently healthy donors. No medical information was available on these individuals.

The levels of 14 analytes, BAFF/BLyS/TNFSF13B, CCL2/MCP-1, Chitinase 3-like 1 (CHI3L1), CXCL8/IL-8, CXCL10/IP-10, EGF, HGF, IL-1β/IL-1F2, IL-6, IL-10, IL-15, Proprotein Convertase 9/PCSK9, TNF-α, and VEGF, were analyzed in the serum and plasma samples using the Simple Plex™ multianalyte immunoassay platform (ProteinSimple). TBI and control samples were diluted either 1:2 or 1:10 with Sample Diluent and vortexed prior to assaying. All values outside the dynamic range of the standard curve (Lower Limit of Quantification [LLOQ] and Upper Limit of Quantification [ULOQ]) were excluded from data analysis. The concentration of each biomarker in each sample was quantified by comparison to the standard curves that are preloaded onto the cartridge bar code.

The concentrations of Enolase 2/Neuro-Specific Enolase and ICAM-1/CD54 were measured in the serum and plasma samples using the Human Enolase 2/Neuron-Specific Enolase Quantikine® ELISA (R&D Systems, Catalog # DENL20) and the Human ICAM-1/CD54 Allele-Specific Quantikine® ELISA (R&D Systems, Catalog # DCD540), respectively. The assays were performed per individual kit protocols.

Assay Principle



Analytical Performance Characteristics for the Simple Plex™ Platform

Analyte	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)	Average Intra-assay	Average Inter-assay	Average Linearity
BAFF/ BLyS/TNFSF13B	0.31	1.0	8990	3.9%	8.3%	92%
CCL2/MCP-1	0.27	0.619	2560	4.7%	10.2%	89%
CHI3L1	3.79	4.92	63750	4.0%	10.0%	90%
CXCL8/IL-8	0.06	1.031	3180	6.6%	6.9%	109%
CXCL10/IP-10	0.12	0.323	920	5.7%	3.9%	95%
EGF	0.11	0.287	2370	4.9%	7.2%	105%
Enolase 2/Neuron-Specific Enolase	38	313	20000	2.8%	6.7%	100%
HGF	3.77	5.49	15160	7.2%	5.4%	105%
ICAM-1/CD54	53	630	40000	4.9%	8.6%	109%
IL-1β/IL-1F2	0.04	0.241	890	2.2%	4.9%	97%
IL-6	0.10	0.619	2560	3.9%	7.7%	116%
IL-10	0.16	0.354	5530	6.0%	7.1%	111%
IL-15	0.20	0.396	1950	7.2%	5.6%	87%
PCSK9	3.36	9.79	56970	5.4%	6.3%	109%
TNF-α	0.27	1.90	5000	4.1%	4.7%	111%
VEGF	2.37	2.762	2220	5.5%	6.7%	103%

With the exception of Enolase 2/Neuron-Specific Enolase and ICAM-1/CD54, these assays are correlated to their respective Quantikine® ELISA with a slope of 0.9 - 1.1 and $R^2 > 0.9$.

indicates values are from the respective Quantikine® ELISA **LOD**= Limit of Detection **LLOQ**= Lower Limit of Quantification **ULOQ**= Upper Limit of Quantification

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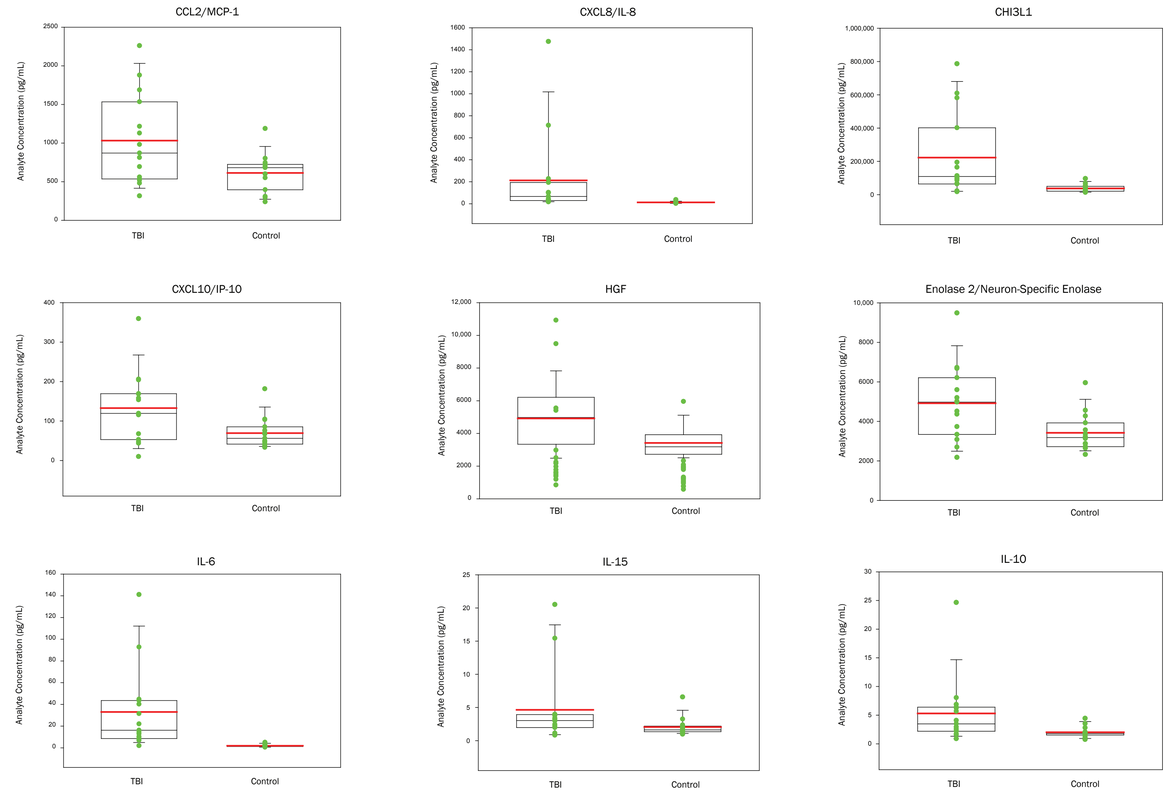
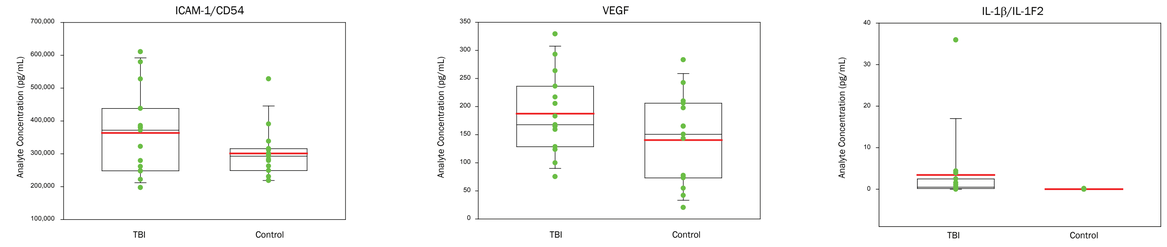
Results and Discussion

Analytes with Increased Concentrations in TBI Samples Compared to Control Samples



- Several of the analytes, including CHI3L1, HGF, and IL-10, whose levels were increased in the serum of TBI subjects have been previously reported to be putative indicators of neuroinflammation.[4-7] In addition, IL-10 has been shown to be secreted intrathecally after severe TBI and is believed to down-regulate subsequent inflammatory events.[7]
- The chemokines CCL2/MCP-1, CXCL8/IL-8, and CXCL10/IP-10 were increased in the samples from TBI subjects. Changes in the expression levels of chemokines are increasingly being implicated in the pathogenesis of several central nervous system (CNS) disorders.[8] In general, these chemo-attractants are involved in the recruitment of inflammatory cells. The varying concentrations of the chemokines may reflect the immune cell type and current level of cell recruitment that is occurring during TBI.
- Enolase 2/Neuron-Specific Enolase levels were increased in TBI samples. This analyte is thought to be a marker of neural injury as it is released into the cerebrospinal fluid (CSF) and systemic circulation from injured neurons. Studies have shown that elevated serum levels of Enolase 2/Neuron-Specific Enolase can be detected in a variety of conditions associated with CNS damage such as stroke, TBI, multiple sclerosis, and Alzheimer's disease.[9-12] It has also been shown that malignant neuroendocrine tumors produce increased levels of Enolase 2/Neuron-Specific Enolase.[13]

Analytes with Concentrations That Were Not Different between TBI and Control Samples



- The levels of ICAM-1/CD54 were not significantly different between TBI and control samples. This is surprising as this analyte has been associated with impaired vasodilation and microvascular damage, and has been shown to be upregulated in response to inflammation.[14, 15]
- IL-1β/IL-1F2 levels were also not different between TBI and control samples; however, this analyte was detected in 10 of the 15 TBI samples while none of the control samples scored above the limits of detection. IL-1β/IL-1F2 is the protypic proinflammatory cytokine and was the first cytokine shown to have actions in the brain.[16]
- There were no perceived differences in the levels of BAFF/BLyS/TNFSF13B, PCSK9,TNF-α (data not shown), and VEGF between TBI and control samples.

Summary

In an effort to improve the diagnosis and prognosis of TBI, researchers are attempting to identify proteins in the peripheral blood that could act as markers of TBI. The purpose of our study was to determine if the Simple Plex™ multianalyte immunoassay platform could serve as a highly efficient technique that could help researchers identify possible TBI blood biomarkers.

We compared the levels of proteins in serum samples from control individuals and individuals diagnosed with TBI using the Simple Plex™ platform and Quantikine® ELISAs. We found that the concentrations of several proteins that are used as markers of inflammation and neural damage were higher in serum from TBI patients compared to control serum. Our results also demonstrate the broad utility of the Simple Plex™ platform as a tool for discovery by allowing the comparison of multiple biomarkers simultaneously from a single small sample volume. This study is just a small part of a growing portfolio of research that is attempting to identify a potential inflammatory profile for TBI in order to help assess the risk, diagnosis, and severity of the injury.