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


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 20–30% 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 function. Information 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, multiplex 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, CD54/ICAM-1, Enolase 2/Neuron-Specific Enolase, CXCL10/IP-10, EGF, HGF, IL-1α/IL-1β, IL-10, IL-12/IL-12p70, IL-15, IL-18, MIP-1α, MIP-1β, and VEGF, were evaluated in the serum and plasma samples using the SimplePlex™ multiplex 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 curve that is preloaded onto the cartridge barcode.

The concentrations of Enolase 2/Neuron-Specific Enolase and KIM-1/CDS4 were measured in the serum and plasma samples using the Human Enolase 2/Neuron-Specific Enolase Quantikine® ELISA (R&D Systems, Catalog # DEnol40) and the Human KIM-1/CDS4 Human-Specific Quantikine® ELISA (R&D Systems, Catalog # D20765), respectively. The assays were performed per individual lot protocols.

Analytical Performance Characteristics for the SimplePlex™ Platform

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LLOQ (pg/mL)</th>
<th>ULOQ (pg/mL)</th>
<th>% CV at LLOQ</th>
<th>% CV at ULOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1/CD54</td>
<td>0.11</td>
<td>7.2</td>
<td>6.7%</td>
<td>7.2%</td>
</tr>
<tr>
<td>BAFF/BLyS/TNFSF13B</td>
<td>0.12</td>
<td>5.5%</td>
<td>4.7%</td>
<td>6.7%</td>
</tr>
<tr>
<td>CXCL10/IP-10</td>
<td>0.16</td>
<td>6.7%</td>
<td>5.7%</td>
<td>8.7%</td>
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<tr>
<td>CHI3L1</td>
<td>0.287</td>
<td>5.7%</td>
<td>4.7%</td>
<td>8.3%</td>
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<tr>
<td>HGF</td>
<td>0.323</td>
<td>5.5%</td>
<td>4.7%</td>
<td>8.3%</td>
</tr>
<tr>
<td>IL-1α</td>
<td>0.354</td>
<td>7.7%</td>
<td>10.0%</td>
<td>109%</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.553</td>
<td>7.7%</td>
<td>10.0%</td>
<td>109%</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.553</td>
<td>7.7%</td>
<td>10.0%</td>
<td>109%</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>0.553</td>
<td>7.7%</td>
<td>10.0%</td>
<td>109%</td>
</tr>
<tr>
<td>IL-15</td>
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<td>7.7%</td>
<td>10.0%</td>
<td>109%</td>
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<tr>
<td>IL-18</td>
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<td>7.7%</td>
<td>10.0%</td>
<td>109%</td>
</tr>
<tr>
<td>IL-18p70</td>
<td>0.553</td>
<td>7.7%</td>
<td>10.0%</td>
<td>109%</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>0.553</td>
<td>7.7%</td>
<td>10.0%</td>
<td>109%</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>0.553</td>
<td>7.7%</td>
<td>10.0%</td>
<td>109%</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.553</td>
<td>7.7%</td>
<td>10.0%</td>
<td>109%</td>
</tr>
</tbody>
</table>

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.1,2 In addition, IL-10 has been shown to be secreted intrathecally after severe TBIs and is believed to be a marker of subsequent inflammatory events.3

• The chemokines CXCL2/ MCP-1, CXCL8/IL-8, and CXCL10/I-P10 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.4 In general, these chemokine-antagonists 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 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 observed 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.

• 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 increased vascular permeability and microvascular damage, and has been shown to be upregulated in response to neuroinflammation.1, 3

• Increased levels of IL-12p70 were also not different between TBI and control samples; however, this analyte was detected in 10 of the 35 TBI samples while none of the control samples scored above the limits of detection. IL-12p70 is the prototypic proinflammatory cytokine and was the first cytokine shown to have actions in the brain.5

• There were no perceived differences in the levels of BAFF/BLyS/TNFSF13B, PCSK9, TNF-α, and VEGF between TBI and control samples.

Analytes with Decreased Concentrations in TBI Samples Compared to Control Samples

• EGF was the only analyte whose levels were lower in TBI samples compared to 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 SimplePlex™ multiplex immunoassay could serve as a highly efficient technique that could help researchers identify possible TBI blood biomarkers.

We compared the levels of 10 proteins in serum samples from control individuals and individuals diagnosed with TBI using the SimplePlex™ 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 SimplePlex™ 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.

References