Highly sensitive single molecule array immunoassay measurement of t-Tau, NF-L, GFAP, and UCH-L1 biomarkers in concussion/mild traumatic brain injury serum samples

I. Introduction

Central nervous system (CNS) proteins released into cerebral spinal fluid (CSF) and peripheral blood have been associated with brain injury, and are candidate prognostic biomarkers of concussion and mild to moderate traumatic brain injury (t-MTBIs). The accurate and reproducible measurement of these markers such as total Tau (t-Tau), neurofilament light chain (NFL), glial fibrillary acidic protein (GFAP) and terminal hydrolase L1 (UCH-L1), in blood requires highly sensitive detection technologies. Recently, single molecule array immunoassays (Simoa) have enabled the accurate quantitation of candidate concussion/t-MTBIs biomarkers in blood.

II. Methods

To quantify CNS protein markers of concussion/t-MTBIs in human serum, a single molecule array (Simoa) lab immunoassay platform utilizing microfluidic and paramagnetic bead digital technology (Quanterix) was performed in a multiplex format (4-plex) to generate quantitative measurements of t-Tau, NF-L, GFAP and UCH-L1. Two sample groups consisting of 30 concussion/t-MTBIs patients each, with blood collected within 1-4 hours (avg 1.8 ± 0.8 hr) and 8-16 hr (avg 12.5 ± 2.2 hr) post-injury, respectively, were compared with 30 healthy controls. Sample groups were aged matched: control group: range = 20-45 years, avg = 33.8 ± 7.5, median 33.5; t-MTBIs group: range = 20-50 years, avg = 33.0 ± 5.5, median = 33.5.

Quanterix Simoa digital immunoassay

• Digital immunoassay platform
• High sensitivity (picogram/mL)
• Wide dynamic range
• Full automation: high precision and reproducibility
• 300 samples/batch
• Homebrew assay capabilities
• Multiplexing capabilities
• RUG-Assays
• Potential for implementation in an CLIA/CAP-accredited platform

III. Results

T-Tau, NF-L, GFAP and UCH-L1 in serum from c/mTBIs patients, and healthy controls

• A significant elevation of median GFAP, UCH-L1 and NF-L levels were observed in both the 1-4 hr (p = 0.0002, p = 0.0001, p = 0.0001) and 8-16 hr (p = 0.0013, p = 0.0001, p = 0.0001) c/mTBIs groups relative to healthy controls (Figure 1, 2, 3).

• Smaller, but significant increases in median t-Tau levels were also observed for both c/mTBIs groups relative to healthy controls (p = 0.018 and p = 0.0003), respectively (Figure 4).

• Median levels of GFAP and NF-L trended toward higher levels in the 1-4 hr c/mTBIs sample group related to the 8-16 hr c/mTBIs group, while median t-Tau levels trended higher in the 8-16 hr c/mTBIs group (Figure 1-4).

• Moderate, but significant correlations were observed between t-Tau and UCH-L1, and NF-L and GFAP in the 1-4 hr c/mTBIs group ( Spearman’s r = 0.65, 0.56) and in the 8-16 hr c/mTBIs group (r = 0.59, 0.45), including a correlation between NF-L and UCH-L1 (r = 0.53) (Table 1).

GFAP & UCH-L1 in c/mTBIs patients (n=30) vs healthy controls (n=30) at 1-4 and 8-16 hr post-c/mTBIs

IV. Conclusion

• Highly sensitive immunoassays (Simoa, Quanterix) were used to evaluate CNS proteins (t-Tau, NF-L, UCH-L1 and GFAP) levels in post-c/mTBIs human serum.

• Significant elevations of median GFAP, UCH-L1, and NF-L levels were observed in both the 1-4 hr and 8-16 hr c/mTBIs groups relative to healthy controls. Smaller but significant increases in median t-Tau levels were observed for both c/mTBIs groups.

• Median levels of GFAP and NF-L trended toward higher levels in the 1-4 hr c/mTBIs group related to the 8-16 hr c/mTBIs group, while the median t-Tau level trended higher in the 8-16 hr c/mTBIs group. The evaluation of longitudinal sample collections will be required to further define these changes in biomarker levels.

• Overall correlations were observed between t-Tau vs UCH-L1, NF-L vs GFAP and t-Tau vs GFAP in the 1-4 hr and 8-16 hr c/mTBIs groups. The above observations are consistent with previous reports describing both GFAP and UCH-L1 proteins as acute biomarkers of mild to moderate TBI (Papa et al., JAMA Neurol. 2016; 72 (5):S51-560).

• This study demonstrates the feasibility of quantifying CNS proteins in serum within 1-4 and 8-16 hr of concussion/t-MTBIs using a sensitive, multiplex immunoassay platform.

Table 1

<table>
<thead>
<tr>
<th>Summary of biomarker correlations</th>
<th>1-4 hr c/mTBIs</th>
<th>8-16 hr c/mTBIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tau vs UCH-L1</td>
<td>0.562</td>
<td>0.0001</td>
</tr>
<tr>
<td>NF-L vs GFAP</td>
<td>0.9002</td>
<td>0.477</td>
</tr>
<tr>
<td>Tau vs NF-L</td>
<td>0.316</td>
<td>0.337</td>
</tr>
<tr>
<td>Tau vs GFAP</td>
<td>0.033</td>
<td>0.099</td>
</tr>
<tr>
<td>Tau vs UCH-L1</td>
<td>0.055</td>
<td>0.0027</td>
</tr>
<tr>
<td>GFAP vs UCH-L1</td>
<td>-0.195</td>
<td>-0.248</td>
</tr>
<tr>
<td>GFAP vs NF-L</td>
<td>-0.195</td>
<td>-0.248</td>
</tr>
</tbody>
</table>

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

*Non-Mann Whitney test

Group or comparison: Median value N
Healthy control | 7.38 | 28
UCH-L1 1-4 hr | 20.19 | 0.0001 | 30
UCH-L1 8-16 hr | 38.57 | 0.0001 | 30

Group or comparison: Median value N
Healthy control | 3.247 | 30
UCH-L1 1-4 hr | 20.19 | 0.0001 | 30
UCH-L1 8-16 hr | 38.57 | 0.0001 | 30

*UCL = upper control limit

For Table 1, correlations between biomarker levels and age were not observed with the exception of NF-L (consistent with the existing literature).

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