Neurology 4-Plex A (Tau, NF-Light, GFAP, UCH-L1) A Multiple Sclerosis Study

Introduction

The Quanterix Simoa HD-1 Analyzer uses digital ELISA to achieve unprecedented sensitivity when measuring cerebrospinal fluid (CSF) and blood biomarkers associated with neurodegenerative diseases such as Multiple Sclerosis (MS), an autoimmune disease characterized by demyelination of the central nervous system that eventually results in permanent disability[1]. There are four types of MS: relapsing-remitting MS (RRMS), secondary-progressive MS (SPMS), primary-progressive MS (PPMS) and progressive-relapsing MS (PRMS). The expanded disability status scale (EDSS) uses impairment in eight categories (including vision, cerebellar, and motor function) to evaluate the severity of MS[2]. EDSS scores range from zero to ten in increments of 0.5, with zero representing no impairment and scores above five representing moderate to severe impairment. EDSS score and physician diagnosis are the current method for categorizing the severity of the disease in patients.

This study explored the correlation of disease severity in patients with RRMS, which accounts for more than 85% of the types of MS, with ultrasensitive measurements of human Tau protein, neurofilament light protein (NF-Light), glial fibrillary acidic protein (GFAP), and ubiquitin carboxyl terminal hydrolase L1 (UCH-L1). The following Simoa Assays were chosen because MS demyelination is accompanied by axonal damage, which releases Tau and NF-Light into extracellular fluid and elevates CSF levels[3]. Studies have shown NF-Light and Tau levels can be associated with rate of brain atrophy[4], MS onset and progression[5], and MS severity[6]. UCH-L1 is the most abundant protein in the brain and maintains neural ubiquitin levels[7]. A decline in UCH-L1 function has been associated with neurodegenerative disease, and the resulting ubiquitinated protein aggregates are characteristic of diseases including Alzheimer's and Parkinsons[7]. Lastly, elevated GFAP levels are indicative of astrogliosis and chronic lesion formation, both hallmarks of MS[8]. Like Tau and NF-Light, recent studies have shown that baseline levels of GFAP are correlated with MS severity and can be used to predict future rate of neurological decline[8].

Experimental design

Human samples (plasma EDTA) were obtained from Bioreclamation from 12 apparently healthy donors and 16 with RRMS. RRMS patients were categorized as mild, moderate, or severe based on a physician's diagnosis

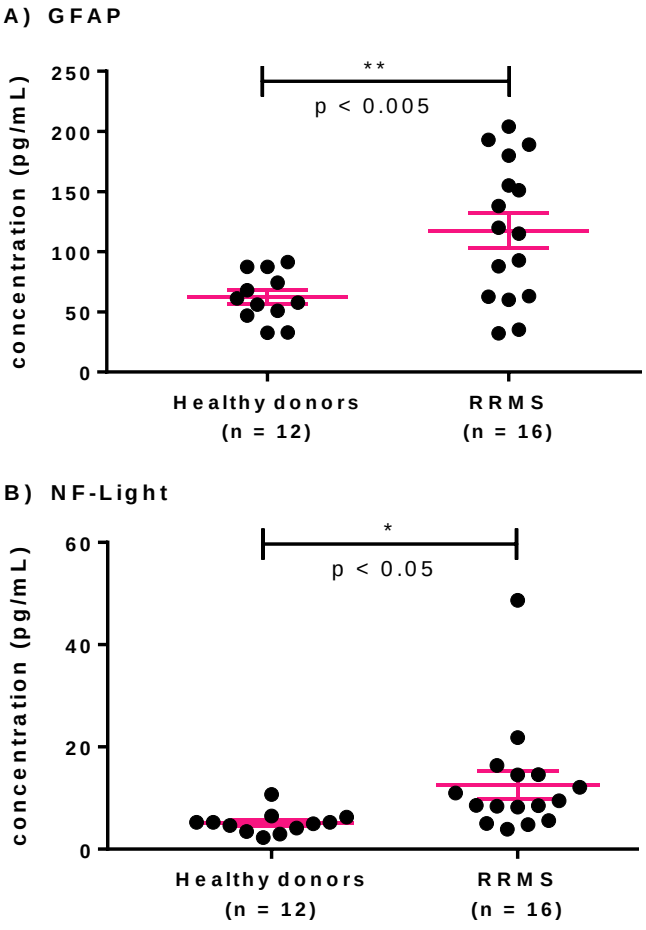

Figure 1. Concentration in healthy and RRMS donors for a) GFAP and b) NF-Light. Error bars depict mean and SEM.

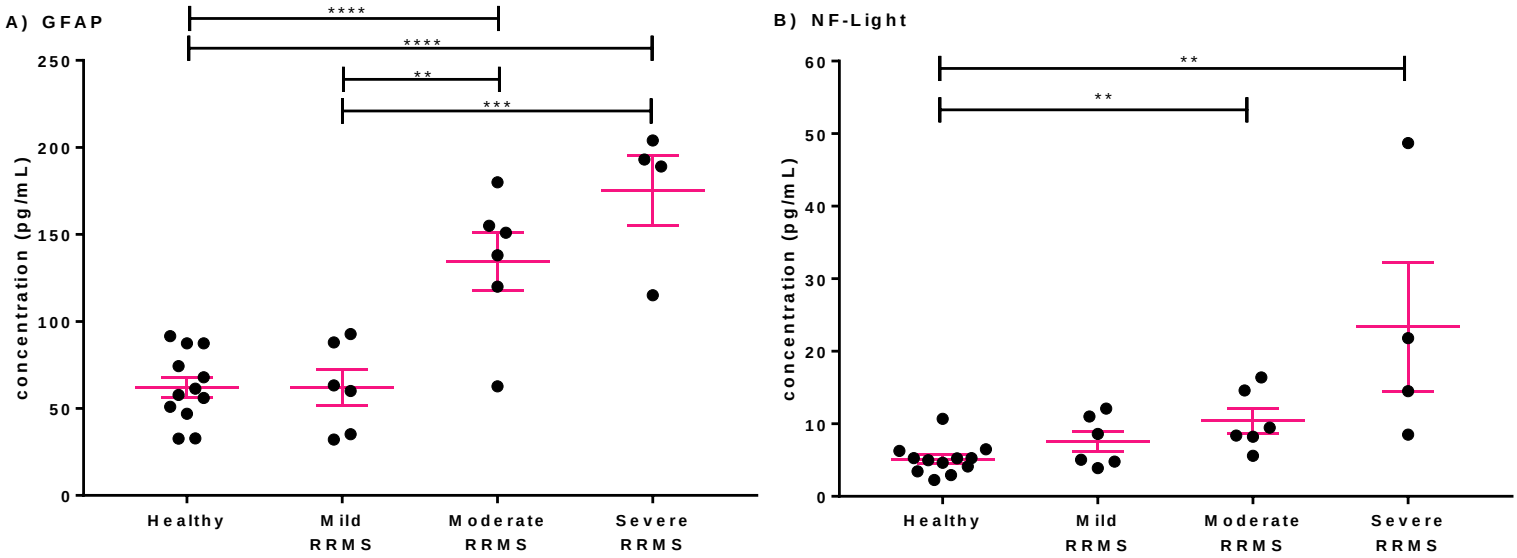

Figure 2. Concentration in healthy vs. MS stratified by severity according to EDSS score for a) GFAP and b) NF-Light. Error bars depict mean and SEM.

with EDSS scores typically less than 3 for mild, greater than 5 for severe, and 3-5 for moderate RRMS. All samples were tested on Human Neurology 4-Plex "A" (GFAP, Tau, NF-Light and UCH-L1), item 102153, and were prepared according to the package insert for automated analysis by the HD-1 Analyzer. After approximately 2.5 hours, concentration results were generated for all four biomarkers.

Results

Mean concentrations for healthy and RRMS donors for Tau (2.02±0.277 vs. 1.31±0.288) and UCH-L1 (8.96±1.93 vs. 20.3±8.57) did not show a significant increase in RRMS as compared to healthy donors ($p > 0.05$). UCH-L1 in a single donor showed a large increase in concentration (147 pg/mL) as compared with other healthy and RRMS donors. Mean concentrations for GFAP and NF-Light show a significant increase in RRMS as compared with healthy donors, with $p < 0.005$ for GFAP (62.2±5.82 vs. 117±14.4, Figure 1A) and $p < 0.05$ for NF-Light (5.14±0.623 vs. 12.6±2.89, Figure 1B).

Stratification of RRMS severity showed additional significance for GFAP and NF-Light (Table 1 and Figure 2), and to a lesser extent UCHL1 (Table 1). A significant increase above healthy control was observed in both moderate RRMS (5.31±1.47 above healthy, $p < 0.005$) and severe RRMS (18.2±4.87 above healthy, $p < 0.005$) for NF-Light. NF-Light also showed a slight increase in mild RRMS (2.43±1.33 above healthy), although not statistically significant ($p = 0.09$). Furthermore, NF-Light showed an increase in mean concentration for moderate vs. mild RRMS (2.88±2.22) and severe vs. moderate RRMS (12.9±8.87).

Similarly, GFAP also showed a significant increase in measured concentration in moderate RRMS (72.2±14.0 above healthy, $p < 0.001$) and severe RRMS (113±15.0 above healthy, $p < 0.001$). Unlike NF-Light, no difference between healthy control and mild RRMS was observed, but an increase in severe vs. moderate RRMS was noted (40.8±26.1), although not significant.

Conclusions

In this small population of RRMS samples stratified by severity of the disease, two of the four biomarkers, NF-Light and GFAP, in the Neurology 4-Plex A Simoa kit showed significant increase in measured analyte as the severity of the disease progressed. UCH-L1 showed promise in further quantifying measurable differences in disease severity, but would require a larger population of samples to determine if this noted increase for a single patient is relevant to the disease progression or correlated with a flare-up.

Due to the inherently subjective nature of the EDSS score, a quantitative biomarker test is ideal for more accurate initial diagnoses as well as monitoring treatment for individual patients with MS. The 4-plex offers more information than the individual assays alone can provide to better determine this information.

Analyte	Healthy	Mild RRMS	Moderate RRMS	Severe RRMS
GFAP	62.2 ±5.82	61.9 ±10.4	135 ±16.5	175 ±20.3
NF-Light	5.14 ±0.623	7.57 ±1.43	10.4 ±1.7	23.4 ±8.87
Tau	2.02 ±0.277	1.0 ±0.459	1.09 ±0.218	2.08 ±0.864
UCH-L1	8.96 ±1.93	10.5 ±1.09	12.9 ±3.75	46.0 ±33.7

Table 1. Mean concentrations (pg/mL) measured in healthy donors and patients with MS, stratified by severity according to EDSS score.
*Individual results for severe RRMS in UCH-L1: 7.95, 13.4, 15.5 and 147 pg/mL.

Note that samples from patients diagnosed with RRMS in this study were actively receiving medical treatment, which may affect results.

References

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