Multivariate proteomic analysis and the relationship with axonal pathology in multiple sclerosis: a longitudinal 5-year diffusion tensor imaging study

Dejan Jakimovski[1], Ferhan Qureshi[2], Murali Ramanathan[3], Victor Gehman[2], Michael G Dwyer[1], Niels Bergsland[1,4], Anisha Keshavan[2], Kelly Leyden[2], Bianca Weinstock-Guttman[5], Robert Zivadinov[1,6]

[1]Buffalo Neuroimaging Analysis Center, Department of Neurology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, State University of New York, Buffalo, NY, USA; [2]Octave Bioscience, Menlo Park, CA, USA; [3]Department of Pharmaceutical Sciences, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, State University of New York, Buffalo, NY, USA [4]IRCCS, Fondazione Don Carlo Gnocchi, Milan, Italy; Jacobs Comprehensive MS Treatment and Research Center, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, State University of New York, Buffalo, NY, USA; [6]Center for Biomedical Imaging at the Clinical Translational Science Institute, University at Buffalo, State University of New York, Buffalo, NY, USA;

Background

- There is an increased interest in development and use of serum-derived biomarkers for monitoring of multiple sclerosis (MS).
- The ability to concurrently measure several biomarkers which represent multiple MS-specific pathophysiologic pathways using a multivariate proteomic panel could further improve the sensitivity and specificity relative to any of the biomarkers individually.

Objective

- To determine the predictivity of multivariate proteomic assay for concurrent and future microstructural axonal brain pathology in a heterogeneous group of persons with multiple sclerosis (pwMS).

Methods

- A proteomic analysis was obtained on serum samples from 202 persons with MS (148 relapsing-remitting pwMS and 54 progressive pwMS) at the baseline and 5-year follow-up.
- The concentration of 21 proteins related to multiple pathways of MS pathophysiology were derived using a custom assay panel validated for MS disease assessments. (Table 1)
- The severity of microstructural axonal brain pathology was quantified by a 3T MRI-based diffusion tensor imaging (DTI). Fractional anisotropy (FA) and mean diffusivity (MD) of normal-appearing brain tissue (NABT), normal-appearing white matter (NAWM), gray matter (GM), and T2 and T1 lesions were calculated.
- Age, sex and body -mass index-adjusted step-wise regression models were used.

Table 1. List of proteomic biomarkers analyzed using the multivariate assay.

Marker	Name (Alias)
NfL	Neurofilament Light
MOG	Myelin-oligodendrocyte glycoprotein
CD6	Cluster of Differentiation 6
CXCL13	C-X-C Motif Chemokine Ligand 13, BLC
CXCL9	CXCL9, Monokine Induced by Gamma Interferon, MIG
CDCP1	CUB domain-containing protein 1
CCL20	MIP-3 alpha
OPG	Osteoprotegerin, TNFRSF11B
IL-12B	Interleukin 12B
APLP1	Amyloid Beta Precursor Like Protein 1
GH	Somatotropin, Growth Hormone
VCAN	Versican, versican proteoglycan
TNFRSF10A	TRAILR1, DR5 - Death Receptor 5
COL4A1	Collagen alpha-1 (IV) chain
SERPINA9	Serpin family A member 9
PRTG	Protogenin
FLRT2	Fibronectin leucine-rich repeat transmembrane protein
TNFSF13B	BAFF
OPN	Osteopontin
CNTN2	Contactin 2
GFAP	Glial Fibrillary Acidic Protein

Results

- Glial fibrilary acidic protein (GFAP) was the most common and highest ranked proteomic biomarker associated with greater concurrent microstructural CNS damage ($p<0.001$).
- Higher baseline GFAP levels were significant predictors of future wide-spread microstructural damage as measured by NABT FA and MD (standardized β=-0.397/0.327, $p<0.001$), NAWM FA (standardized β=-0.466, $p<0.0012$), GM MD (standardized β=0.346, $p<0.011$) and T2 lesions MD (standardized β=0.416,$p<0.001$) at the 5-year follow-up. (Table 2)
- Serum levels of MOG, NfL, contactin-2 and osteopontin proteins were additionally and independently associated with worse concomitant and future axonal pathology.
- Higher GFAP was associated with future disability progression (Exp(B)=8.647, p=0.004).

Figure 1. Cross-sectional correlation matrix (heatmap) between follow-up proteomic data and follow-up diffusion tensor imaging outcomes in persons with multiple sclerosis.

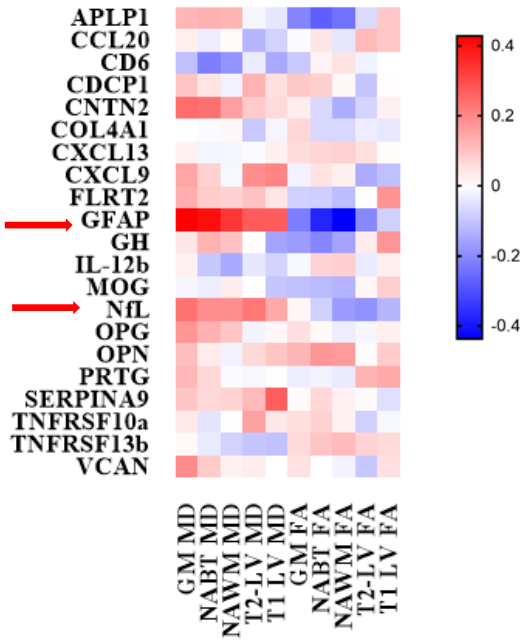

Figure 2. Longitudinal correlation matrix (heatmap) between baseline proteomic data and follow-up diffusion tensor imaging outcomes in persons with multiple sclerosis.

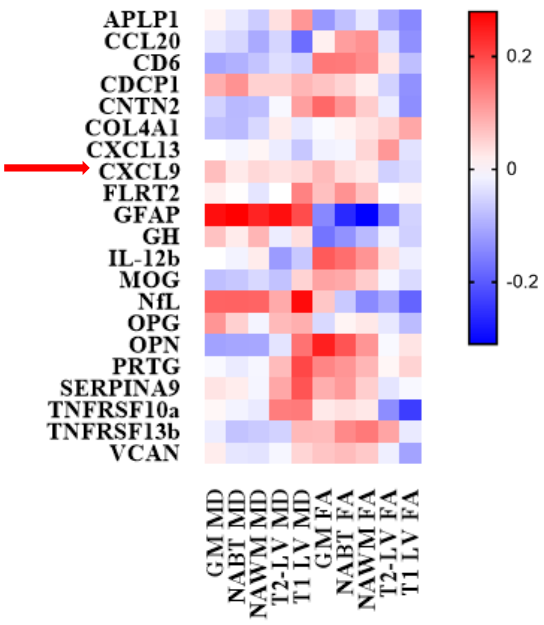

Table 2. Linear step-wise regression determining associations between baseline proteomics and future microstructural DTI-based outcomes (at follow-up)

Fractional anisotropy (FA)	Predictors	R^2	Std β	p-value
NABT FA	GFAP	0.113	-0.397	<0.001
	BMI	0.156	0.233	0.005
	MOG	0.191	0.253	0.01
	Sex	0.231	0.203	0.013
NAWM FA	GFAP	0.146	-0.466	<0.001
	MOG	0.178	0.221	0.025
	BMI	0.205	0.168	0.044
GM FA	BMI	0.053	0.198	0.021
	Sex	0.103	0.154	0.07
	OPN	0.133	0.205	0.021
	APLP1	0.161	-0.245	0.011
	CD6	0.193	0.192	0.029
T2-LV FA	Age	0.082	-0.287	0.001
T1-LV FA	Sex	0.079	0.283	0.003

Mean diffusivity (MD)	Predictors	R^2	Std β	p-value
NABT MD	GFAP	0.041	0.327	0.001
	MOG	0.076	-0.224	0.027
NAWM MD	GFAP	0.046	0.214	0.012
GM MD	GFAP	0.041	0.346	0.001
	MOG	0.087	-0.256	0.011
T2-LV MD	GFAP	0.072	0.416	<0.001
	MOG	0.118	-0.332	0.001
	TNFRSF10a	0.152	0.196	0.025
T1-LV MD	NfL	0.09	0.299	0.003

Legend: GFAP – glial fibrillary acidic protein, MOG - myelin oligodendrocyte glycoprotein, IL-12B – interleukin-12 subunit B, FLRT2 - fibronectin leucine rich transmembrane protein 2, TNFRSF10a - tumor necrotic factor receptor superfamily member 10a, NfL – neurofilament light chain, DTI – diffusion tensor imaging, MD – mean diffusivity, FA – fractional anisotropy, GM – gray matter, NABT – normal-appearing brain tissue, NAWM – normal-appearing white matter, LV – lesion volume.

Conclusion

- Multiple proteomic biomarkers are independently associated with greater severity of axonal brain pathology as measured by diffusion tensor imaging.
- Baseline serum GFAP levels can predict future disability progression.

Disclosures

- Dejan Jakimovski and Niels Bergsland have nothing to disclose.
- Ferhan Qureshi, Anisha Keshavan and Kelly Leyden are employees of Octave Bioscience. Victor Gehman was an employee of Octave Bioscience at the time the study was performed
- Murali Ramanathan received research funding from the National Multiple Sclerosis Society, Department of Defense and National Institute of Neurological Diseases and Stroke.
- Michael G. Dwyer received compensation from Keystone Heart for consultant fees. He received financial support for research activities from Bristol Myers Squibb, Mapi Pharma, Keystone Heart, Protembis and V-WAVE Medical.
- Bianca Weinstock-Guttman received honoraria for serving in advisory boards and educational programs from Biogen Idec, Novartis, Genentech, Genzyme and Sanofi, Janssen, Abbvie and Bayer. She also received support for research activities from the National Institutes of Health, National Multiple Sclerosis Society, Department of Defense, and Biogen Idec, Novartis, Genentech, Genzyme and Sanofi.
- Robert Zivadinov has received personal compensation from Bristol Myers Squibb, EMD Serono, Sanofi, Keystone Heart, Protembis and Novartis for speaking and consultant fees. He received financial support for research activities from Sanofi, Novartis, Bristol Myers Squibb, Octave, Mapi Pharma, Keystone Heart, Protembis and V-WAVE Medical.

University at Buffalo The State University of New York
BNAC Buffalo Neuroimaging Analysis Center
OCTAVE
Buffalo Neuroimaging Analysis Center (BNAC)
Department of Neurology,
School of Medicine and Biomedical Sciences,
University at Buffalo