Bridging Proteomics to the Clinic – A Multivariate Blood Test for Disease Activity in Multiple Sclerosis

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OCTAVE

HUPO BUSAN 2023 SEP. 17-21

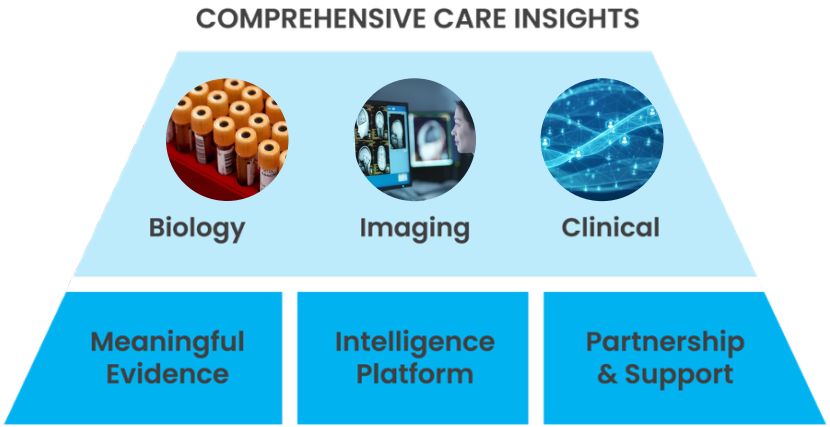
Disclosures

Ferhan Qureshi is an employee of Octave Bioscience.

The Octave Precision Care Solution for Neurodegenerative Diseases

Neurodegenerative Diseases represent a vast emerging field of innovation. Includes Multiple Sclerosis, Parkinson's and Alzheimer's.

Complex, high cost, with devastating outcomes. Poorly characterized, lack of metrics and tools



MSDA TEST
Objective measurement of MS disease activity

MRI Insights
MS expert neurorads
Structured reports
AI/ML algorithms

Clinical Insights
Continuous member monitoring with Octave's technology and MS nurse care partners

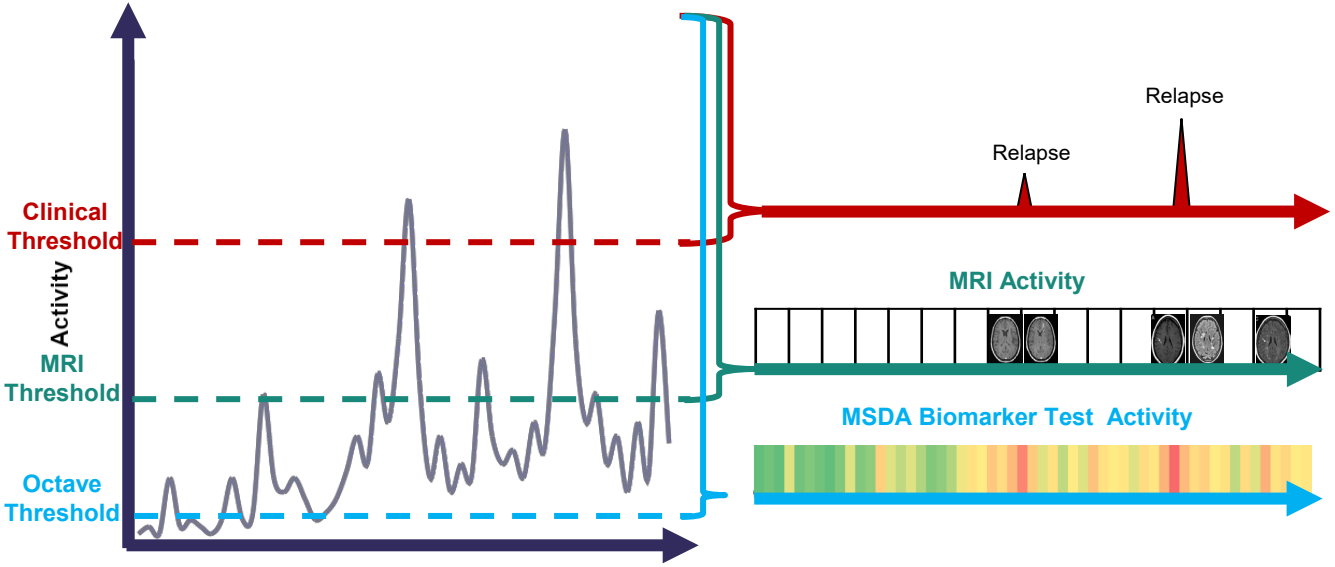
Mission to improve patient lives & outcomes

Address significant challenges, issues, unmet needs in the MS ecosystem

Creating new objective measurement tools to improve and expand clinical insights & decisions

Octave Platform integrates all findings for each patient and at the population level

Biomarker Data Streams for Multiple Sclerosis



- Routine MRI interpretation provides a lagging indicator of neurodegeneration, retrospective, poor correlation with clinical presentation and future activity
- Clinical assessments are limited to retrospective analysis of perceived symptoms, typically qualitative and subject to pronounced recall bias
- **Biology is closest to the truth**

Octave developing novel serum biomarker tests using the Olink platform, to add disease insights

Real-time assessment of underlying disease pathways, mechanisms

Dynamic measure of both **inflammation and degeneration processes**

Addresses issues of **Rx selection, Rx response, flares,** smoldering disease

Disease Activity test validated and clinically launched, Disease progression test development underway

MSDA Test Development Process

Feasibility	Discovery	Development	Validation
82 Samples Serum Pools Matched Normals	**305 Samples** ACP Cohort CLIMB Cohort #1	**753 Samples** F4: CLIMB Cohort #2 F5: EPIC Cohort F6: Basel Cohort PROMOTE Cohort	**>2000 Samples** SUMMIT Cohort RMMSC Cohort SMSC Cohort SUNY Buffalo Cohort Bern Cohort
• Market Research to refine product profiles • Identified candidate biomarkers and analytical platforms • Proof of Concept Studies • Initiated biology modeling project to investigate causation	• Added Olink Platform • Evaluated endpoints of disease activity and progression • Established CLIA/CAP accredited Lab • Prepared for Development (reagents, samples, team, tech stack for data science)	• Finalized selection of Top 21 biomarkers • Optimized analytical performance of individual assays • Manufactured 2 Custom Assay Reagent Lots • Developed pilot algorithms for classification and regression	• Analytical Validation completed • Clinical Validation completed • Beta program completed • Routine Clinical Use launch • Additional collaborations with pharma, academic centers and COEs ongoing to expand claims and clinical utility

220 biomarkers → 1416 biomarkers → 21 biomarkers → 18 Biomarker Validation MSDA Test

1400 -> 800 -> 200 -> 21

Iterative ranking process: Univariate associations across independent samples, Dimensionality reduction via regularization, Stochastic accuracy-weighted multivariate feature importance, biology modeling to ensure comprehensive coverage of MS pathophysiology, Analytical performance specifications

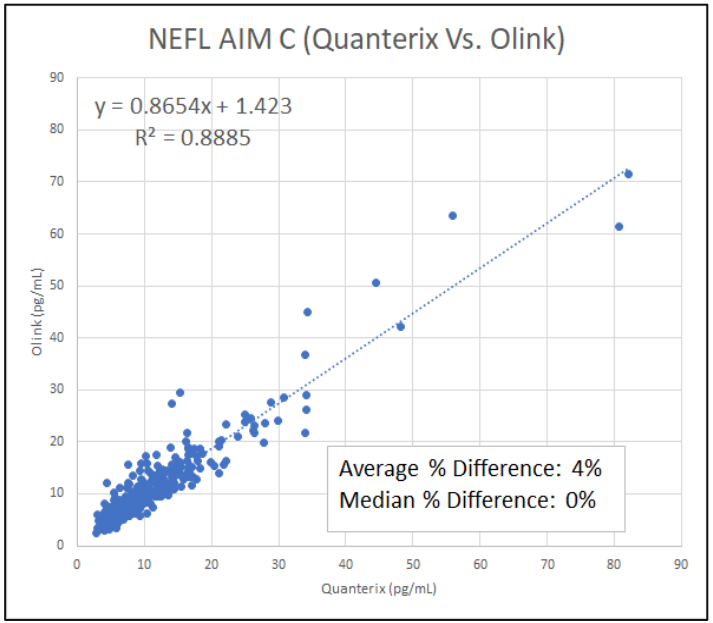
Olink Proteomic Technology

Extensive library with key content of interest broadly representing relevant pathways

PEA, dual recognition - excellent specificity and high sensitivity

High-quality service offerings and certification to perform assays in-house

Custom panel capability with novel targets, optimization, and fit-for-purpose validation



- Partnered with Olink to develop & manufacture a 21-plex MS specific custom assay panel.
- Optimized performance, absolute quantification, calibrated to ‘gold standard’ assays.
- Completed extensive analytical characterization and validation (biomarkers and scores).
- 3 reagent kit lots manufactured and bridged to date. 4th lot in-process
- MSDA Test is an LDT performed in Octave’s CLIA certified and CAP accredited laboratory.
- >40 clinics in the USA are now using MSDA, ongoing retrospective and prospective trials with pharma

Octave Custom Assay Panel Protein Analytes

Count	Marker	Name (Alias)	Associated Pathways, Cell Types
1	NEFL	Neurofilament Light	Neurodegeneration
2	MOG	Myelin-oligodendrocyte glycoprotein	oligodendrocyte, immune-mediated demyelination
3	CD6	Cluster of Differentiation 6	T cell, Th1, Th17
4	CXCL13	C-X-C Motif Chemokine Ligand 13, BLC	immune activation, B cell homing
5	CXCL9	CXCL9, Monokine Induced by Gamma Interferon, MIG	Immune Response, Inflammation
6	CDCP1	CUB domain-containing protein 1	T cell migration
7	CCL20	MIP-3 alpha	immunoregulatory and inflammatory processes
8	OPG	Osteoprotegerin, TNFRSF11B	inflammation, T cell activation, IFN-B treatment
9	IL-12B	Interleukin 12B	innate & adaptive immunity, Th1, overexpression observed in CNS in MS
10	APLP1	Amyloid Beta Precursor Like Protein 1	synaptic maturation during cortical development, regulation of neurite outgrowth
11	GH*	somatotropin, Growth Hormone	growth, cell reproduction and regeneration
12	VCAN*	Versican, versican proteoglycan	cell motility, cell adhesion, proliferation, proliferation, migration and angiogenesis
13	TNFRSF10A	TRAILR1, DR5 - Death Receptor 5	Cell Signaling and Apoptosis
14	COL4A1*	Collagen alpha-1(IV) chain	cell proliferation, migration, ECM
15	SERPINA9	serpin family A member 9	B cell
16	PRTG	Protogenin	neurogenesis, demyelinating
17	FLRT2	Leucine-rich repeat transmembrane protein	cell-cell adhesion, cell migration and axon guidance
18	TNFSF13B	BAFF	B cell, Inflammation
19	OPN	Osteopontin	Immune modulation
20	CNTN2	Contactin 2	cell adhesion, proliferation, migration, and axon guidance of neurons
21	GFAP	Glial Fibrillary Acidic Protein	Astrocytes, demyelination and neuro-axonal injury

*Biomarker not utilized in final MSDA Test algorithm

Biological Corroboration of Selected Features

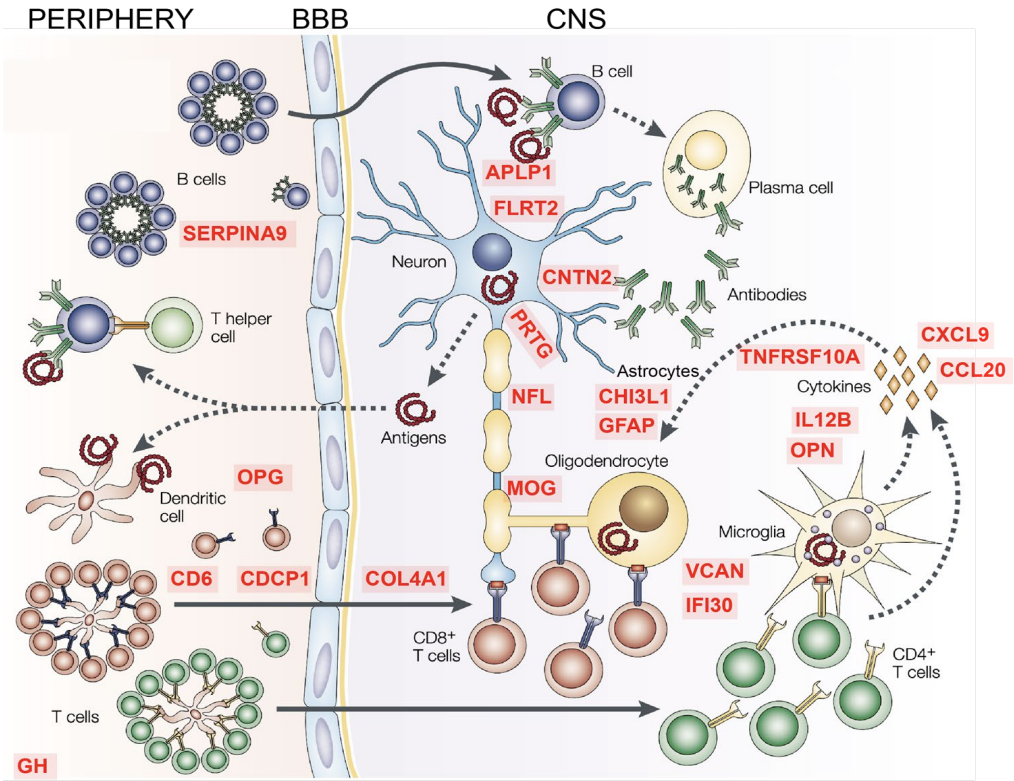
Statistically significant features were investigated for relevance to MS neurophysiology

- Computational Biology models
- Automated/manual curation of open-source ontologies (Uniprot, Pubmed, GO, etc.)
- Probabilistic Graph Network for literature aggregation
- Protein-Protein Interaction modeling (STRING, Cytoscape)
- Gene Set Enrichment (Enrichr for functional annotations)
- Spatial Expression Profiling (Human Brain Atlas, Allen Brain Atlas)

Key Pathways and Processes:

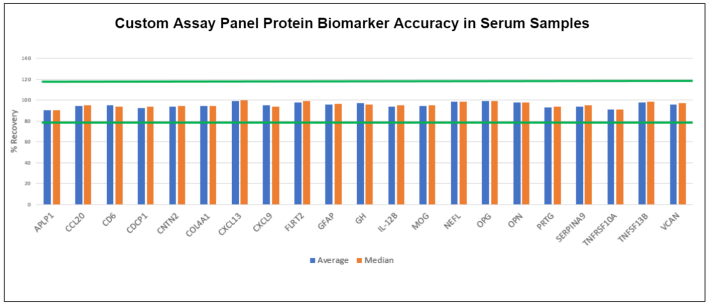
- Leukocyte Differentiation
- Microglial / Astrocytic Activation
- BBB Disruption
- Neuroinflammation
- Neurodegeneration
- Axon Guidance and Regeneration
- Iron Clearance and Recycling
- Cholesterol Clearance and Recycling
- Neuroprotection
- Demyelination

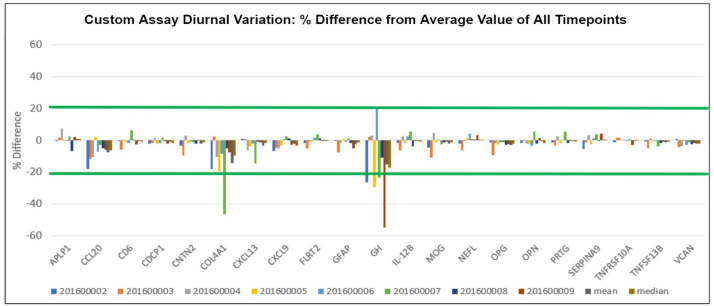
Mechanistic Understanding of MS - Octave Biomarkers



Analytical Validation of Octave's MS Custom Assay Panel

Dual Lot Approach: Two Development Lots of Reagents + Kits manufactured and evaluated
Dual Site Approach: (1) Characterization at Olink and (2) Validation at Octave
Accuracy: Sample mixing (endogenous protein). Correlation to previous runs using R&D assays.
Precision: Intra and Inter-Assay CV for individual Biomarkers using serum pools included in every run to date and forthcoming runs. Includes assay drift assessment.
Sensitivity: LOB, LOD, LLOQ and ULOQ
Interference: RF/HAMA, Endogenous substances, Common drugs, MS therapeutics
Cross Reactivity: Intra-panel (cross talk) and homologous proteins (AA sequence coverage ≥90% and sequence identity ≥50% according to Protein BLAST)
Stability: Accelerated + Real Time for Reagents and Samples including Freeze/Thaw cycles
Robustness: Equipment, Personnel , Olink vs Octave R&D vs Octave CLIA
Diurnal Variation: First Study 6 timepoints - Day 1, Day 2, Day 3, Day 4, Day 5 and Day 12
Reference Ranges: Established for MS Population
Kit Qualification and Release: Three stage process (1) Olink (2) Octave Manufacturing (3) Octave Clinical: Includes Correlation, Accuracy, Sensitivity and Precision
QC Process: Assay protocol designed with 3 levels of internal controls for each sample and 4 Process Controls per plate. Specifications established for accuracy and precision of calibrators, controls, & samples.





Analytical Validation Completed For Both Individual Proteins and MSDA Algorithm Scores
Manuscript Published March 2023 - Proteomics Clinical Applications

MSDA Test Algorithm and Clinical Validation

September 17-21, 2023
Busan I BEXCO, Korea
2023.hupo.org
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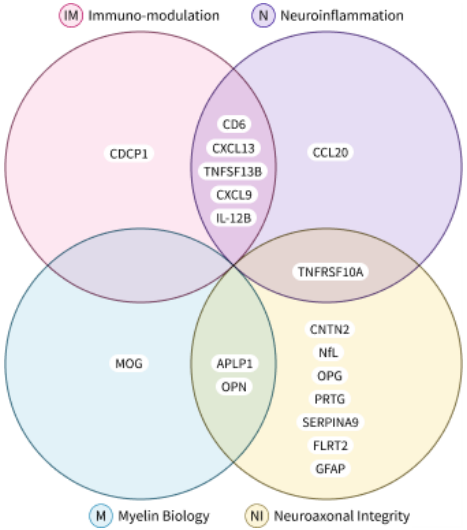
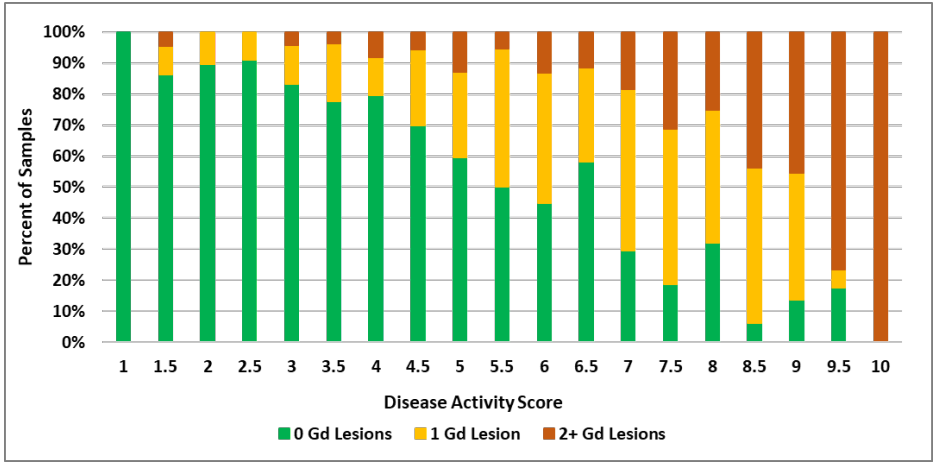
MSDA algorithm leverages 18 protein biomarkers and their biological categorizations to determine **4 Disease Pathway Scores** and an **Overall Disease Activity Score**. Model was trained versus count of Gd+ lesions on an associated MRI (reflecting active inflammation) and provided a quantitative endpoint for Disease Activity level categorization. Algorithm also associated significantly with New/Enlarging T2 lesions and Active/Stable status.



4 Disease Pathway Scores and Disease Activity Score are Scaled From 1.0 to 10.0 with 0.5 intervals. Thresholds established corresponding to Low, Moderate and High Disease Activity

Plot of MSDA Score Distribution for Samples from Clinical Validation Study (n=188) and Post Validation Focal Inflammation Study (n=126)



MSDA Score Reflects Both Likelihood and Severity of Disease Activity

KEY RESULTS →

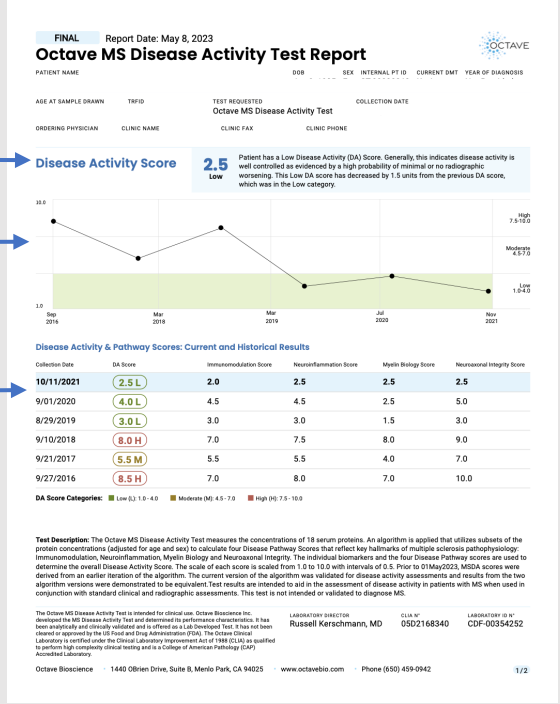
M/H score is 4.5 times more likely to have one or more Gd lesions than a patient with a L score.
H score is 21 times more likely to have two or more Gd lesions than a patient with a L/M score.

MSDA Test Report: Comprehensive, Yet Easy to Interpret

Overall Disease Activity Score

Longitudinal Journey

DA & Pathway Scores



FINAL Report Date: May 8, 2023

Octave MS Disease Activity Test Report

PATIENT NAME DOB SEX INTERNAL PT ID CURRENT DMT YEAR OF DIAGNOSIS

AGE AT SAMPLE DRAWN TRFID TEST REQUESTED Octave MS Disease Activity Test COLLECTION DATE

ORDERING PHYSICIAN CLINIC NAME CLINIC FAX CLINIC PHONE

Disease Activity Score

2.5 Low

Patient has a Low Disease Activity (DA) Score. Generally, this indicates disease activity is well controlled as evidenced by a high probability of minimal or no radiographic worsening. This Low DA score has decreased by 1.5 units from the previous DA score, which was in the Low category.

Disease Activity & Pathway Scores: Current and Historical Results

Collection Date	DA Score	Immunomodulation Score	Neuroinflammation Score	Myelin Biology Score	Neuroaxonal Integrity Score
10/11/2021	2.5 L	**2.0**	**2.5**	**2.5**	**2.5**
9/01/2020	4.0 L	4.5	4.5	2.5	5.0
8/29/2019	3.0 L	3.0	3.0	1.5	3.0
9/10/2018	8.0 H	7.0	7.5	8.0	9.0
9/21/2017	5.5 M	5.5	5.5	4.0	7.0
9/27/2016	8.5 H	7.0	8.0	7.0	10.0

DA Score Categories: Low (L): 1.0 - 4.0 Moderate (M): 4.5 - 7.0 High (H): 7.5 - 10.0

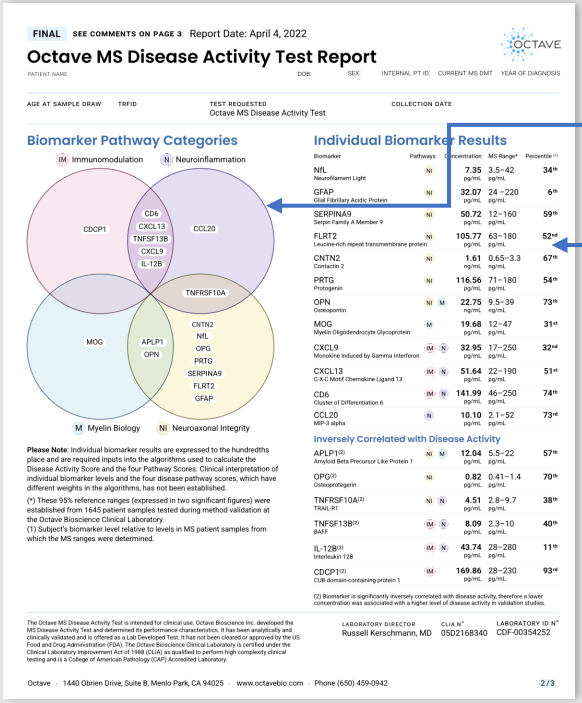
Test Description: The Octave MS Disease Activity Test measures the concentrations of 18 serum proteins. An algorithm is applied that utilizes subsets of the protein concentrations (adjusted for age and sex) to calculate four Disease Pathway Scores that reflect key hallmarks of multiple sclerosis pathophysiology: Immunomodulation, Neuroinflammation, Myelin Biology and Neuroaxonal Integrity. The individual biomarkers and the four Disease Pathway scores are used to determine the overall Disease Activity Score. The scale of each score is scaled from 1.0 to 10.0 with intervals of 0.5. Prior to 01May2023, MSDA scores were derived from an earlier iteration of the algorithm. The current version of the algorithm was validated for disease activity assessments and results from the two algorithm versions were demonstrated to be equivalent.Test results are intended to aid in the assessment of disease activity in patients with MS when used in conjunction with standard clinical and radiographic assessments. This test is not intended or validated to diagnose MS.

The Octave MS Disease Activity Test is intended for clinical use. Octave Bioscience Inc. developed the MS Disease Activity Test and determined its performance characteristics. It has been analytically and clinically validated and is offered as a Lab Developed Test. It has not been cleared or approved by the US Food and Drug Administration (FDA). The Octave Clinical Laboratory is certified under the Clinical Laboratory Improvement Act of 1988 (CLIA) as qualified to perform high complexity clinical testing and is a College of American Pathology (CAP) Accredited Laboratory.

LABORATORY DIRECTOR Russell Kerschmann, MD CLIA № 05D2168340 LABORATORY ID № CDF-00354252

MS-specific Pathways

Individual Biomarker Concentrations and Percentiles



FINAL SEE COMMENTS ON PAGE 3 Report Date: April 4, 2022

Octave MS Disease Activity Test Report

PATIENT NAME DOB SEX INTERNAL PT ID CURRENT MS DMT YEAR OF DIAGNOSIS

AGE AT SAMPLE DRAW TRFID TEST REQUESTED Octave MS Disease Activity Test COLLECTION DATE

Biomarker Pathway Categories

Please Note: Individual biomarker results are expressed to the hundredths place and are required inputs into the algorithms used to calculate the Disease Activity Score and the four Pathway Scores. Clinical interpretation of individual biomarker levels and the four disease pathway scores, which have different weights in the algorithms, has not been established.

(*) These 95% reference ranges (expressed in two significant figures) were established from 1645 patient samples tested during method validation at the Octave Bioscience Clinical Laboratory.
(1) Subject's biomarker level relative to levels in MS patient samples from which the MS ranges were determined.

Individual Biomarker Results

Biomarker	Pathways	Concentration	MS Range*	Percentile (1)
NfL Neurofilament Light	NI	7.35 pg/mL	3.5–42 pg/mL	34th
GFAP Glial Fibrillary Acidic Protein	NI	32.07 pg/mL	24 –220 pg/mL	6th
SERPINA9 Serpin Family A Member 9	NI	50.72 pg/mL	12–160 pg/mL	59th
FLRT2 Leucine-rich repeat transmembrane protein	NI	105.77 pg/mL	63–180 pg/mL	52nd
CNTN2 Contactin 2	NI	1.61 ng/mL	0.65–3.3 ng/mL	67th
PRTG Protogenin	NI	116.56 pg/mL	71–180 pg/mL	54th
OPN Osteopontin	NI M	22.75 ng/mL	9.5–39 ng/mL	73rd
MOG Myelin Oligodendrocyte Glycoprotein	M	19.68 pg/mL	12–47 pg/mL	31st
CXCL9 Monokine Induced by Gamma Interferon	IM N	32.95 pg/mL	17–250 pg/mL	32nd
CXCL13 C-X-C Motif Chemokine Ligand 13	IM N	51.64 pg/mL	22–190 pg/mL	51st
CD6 Cluster of Differentiation 6	IM N	141.99 pg/mL	46–250 pg/mL	74th
CCL20 MIP-3 alpha	N	10.10 pg/mL	2.1–52 pg/mL	73rd

Inversely Correlated with Disease Activity

APLP1 (2) Amyloid Beta Precursor Like Protein 1	NI M	12.04 pg/mL	5.5–22 pg/mL	57th
OPG (2) Osteoprotegerin	NI	0.82 pg/mL	0.41–1.4 pg/mL	70th
TNFRSF10A (2) TRAIL-R1	NI N	4.51 pg/mL	2.8–9.7 pg/mL	38th
TNFSF13B (2) BAFF	IM N	8.09 pg/mL	2.3–10 pg/mL	40th
IL-12B (2) Interleukin 12B	IM N	43.74 pg/mL	28–280 pg/mL	11th
CDCP1 (2) CUB domain-containing protein 1	IM	169.86 pg/mL	28–230 pg/mL	93rd

(2) Biomarker is significantly inversely correlated with disease activity, therefore a lower concentration was associated with a higher level of disease activity in validation studies.

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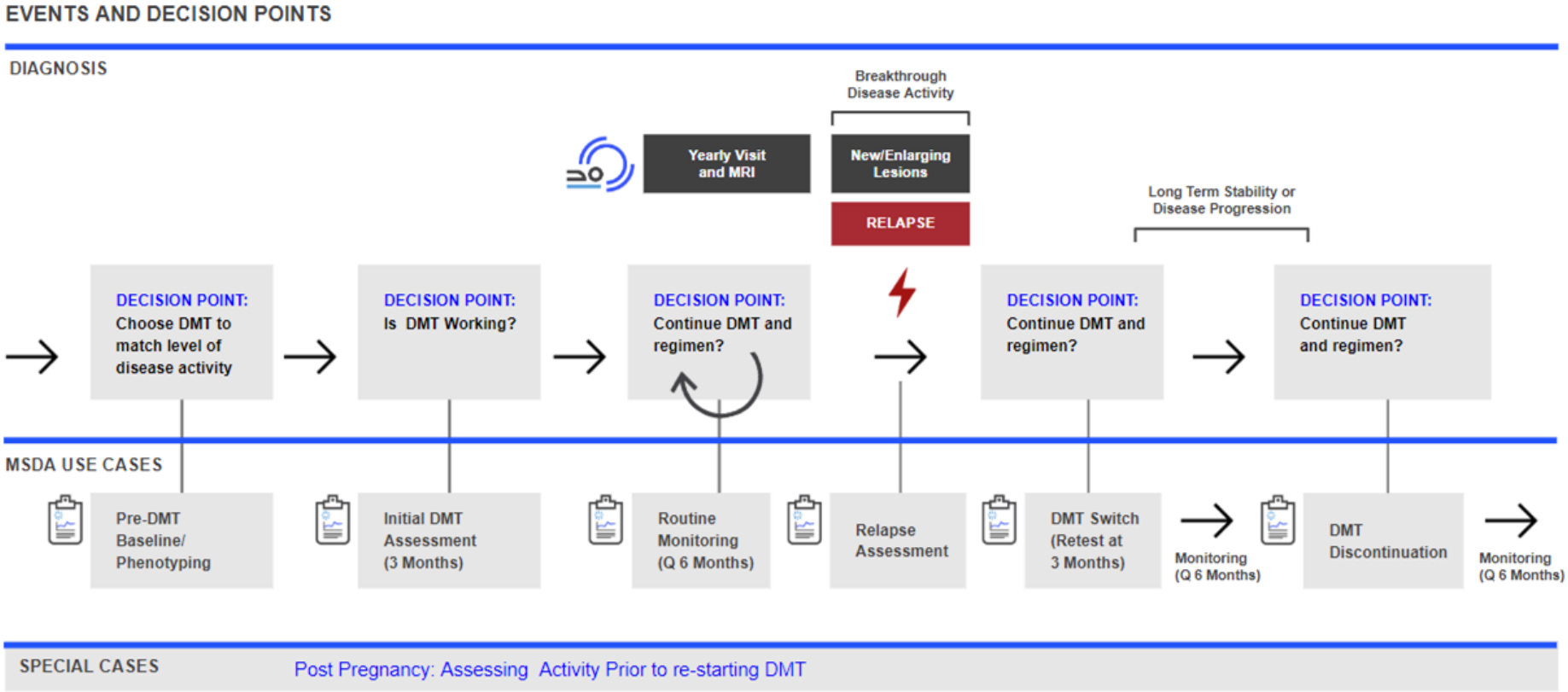
MSDA Test Patient Journey – Actionable Insights



MSDA Case Study: Longitudinal Pre-Post DMT Switch

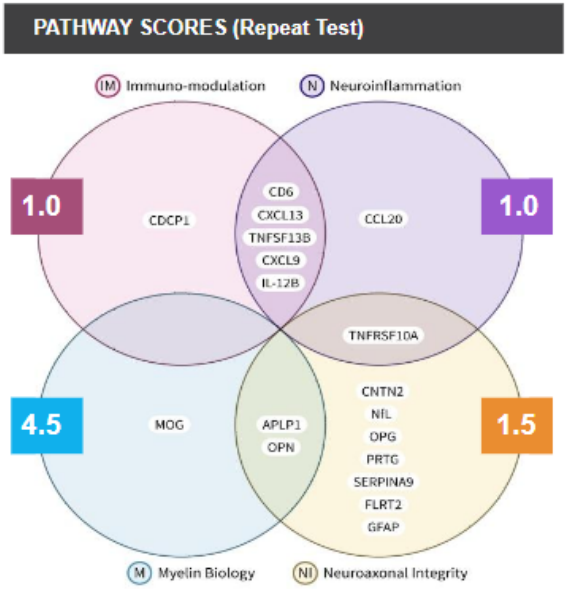
From an early adoption clinic (now getting longitudinal results). Patient was on HE DMT but had a High MSDA score + relapse. Switched to alternate HE DMT and Low MSDA score confirmed disease stability.

Patient History

35 year old female diagnosed at age 29. Was on Natalizumab (High Efficacy) but had a relapse. Was determined to have neutralizing abs and was switched to Ocrelizumab. Longitudinal MSDA result was obtained 8 months later.

Actions Considered with MSDA Test

High Score of 8.0 in Aug 2022 (baseline) corroborated relapse symptoms despite patient being on HE DMT. Switched to alternate HE DMT. Longitudinal Low MSDA score from Apr 2023 post DMT switch confirms patient is now clinically stable.



DISEASE ACTIVITY SCORE (Baseline Aug 2022)

8.0 HIGH

DISEASE ACTIVITY SCORE (Apr 2023)

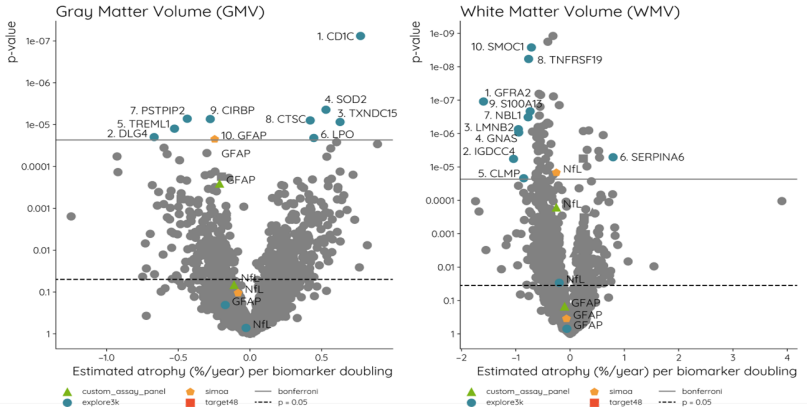
1.5 Low

Summary and Future Directions

- Octave leveraged Olink's cutting-edge, proteomic platform to develop and manufacture a custom assay panel for MS
- MSDA Test was validated and launched in 2021, now being used in over 40 clinics across USA and growing
- MSDA is also being utilized by pharma for both retrospective and prospective studies
- Octave custom assay panel is also being utilized for additional assessments in MS (differential diagnosis, disease progression)
- Proteomic deep-scan for Octave MS studies first expanded to 3K analytes in 2022, now >5K analytes in 2023 using Olink Explore HT as part of Disease Progression test development project
- Now expanding and applying our Precision Care Solution to other neurodegenerative diseases: Parkinson's, Alzheimer's, and beyond

Disease Progression Phenotypes

Serum Biomarkers (Explore 3K) at Baselline Relative to Radiographic Endpoints of Progression: GMV and WMV)



Thank you for your attention!

Octave

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Rocky Choi
Helene Rönnevall
Lena Zettergren

MS patients who contributed biospecimens and the research teams that provided data from the following cohorts:

ACP – Accelerated Cure Project

CLIMB - Brigham & Women's Health

EPIC - UCSF

SMSC – University Hospital Basel

PROMOTE – University of Pittsburgh

SUMMIT Consortium

RMMSC – Rocky Mountain Multiple Sclerosis Center

CEG – SUNY Buffalo BNAC

Together Through Proteomics

HUPO BUSAN 2023 SEP 17-21

22nd Human Proteome Organization World Congress

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SEPTEMBER 17-21, 2023

BEXCO, Busan, Korea

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