Proteomic Studies: Insights into MS

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Disclosures

Consultant: AbbVie, Accordant, Acorda, Bayer, Biogen, Genentech/Roche, Genzyme/Sanofi, Novartis, Serono, Teva

Research: Actelion, Novartis, Opexa

Magnetization Transfer Ratio in NAWM*

- Increasing evidence that demyelination/ neuronal damage occurs preferentially in cortical gray matter
  - ? due to pathology outside brain parenchyma (meningeal inflammation, CSF-mediated factors)
- WM lesions next to ventricles could be similar
- Investigated N=43 RRMS, N=28 SPMS, N=38 healthy controls using MTI to study concentric WM bands

*_Brain_ 2015; 138:1239

Magnetization Transfer Ratio in NAWM*

- MTR in NAWM was significantly ↓ in MS
- In controls MTR was highest in WM band adjacent to ventricles, declined with distance
- ↓ MTR greater in SPMS vs. RRMS, most marked in bands closest to ventricles
  - both RRMS and SPMS showed this periventricular gradient
- Conclusion: findings c/w CSF or ependymal mediated pathogenesis

*_Brain_ 2015; 138:1239

MS CSF Biomarkers*

- Biomarkers involve
  - predictive/diagnostic
  - disease activity
  - treatment response
- Biomarker development involves
  - discovery phase
  - validation process

*_Lancet Neurol_ 2014; 13:113

MS CSF Biomarkers*

- Molecular MS biomarkers
  - exploratory
  - validated
  - clinically useful

*_Lancet Neurol_ 2014; 13:113

Clinically Useful CSF Biomarkers*

- IgG oligoclonal bands
- IgG index
- Anti-aquaporin 4 antibodies

*_Lancet Neurol_ 2014; 13:113

Validated CSF Biomarkers*

Multiple studies, high agreement

- Kappa free light chains
- IgM oligoclonal bands
- Neural cell adhesion molecule-1 (NCAM1)
- Nitric oxide metabolites
- Matric metalloproteinase 9 (MMP-9)

*_Lancet Neurol_ 2014;13:113

Validated CSF Biomarkers*

Multiple studies, high agreement (cont)

- Myelin basic protein
- Osteopontin
- Chemokine ligand 13 (CXCL13)
- Glial fibrillary acidic protein (GFAP)

*_Lancet Neurol_ 2014;13:113

Validated CSF Biomarkers*

Multiple studies

- Measles, rubella, varicella zoster reaction
- Chitinase 3-like-1 (CHI3L1)

Others

- Neurofilaments
- IL17, TNF, IL12 and 23
- BAFF

*_Lancet Neurol_ 2014;13:113

MS Biomarker Challenges*

- Pre-analytical variability (sample collection, timing, storage, other factors)
  - accounts for up to 68% of total lab errors
- Poor study design (heterogeneity, small sample size, confirmation using different techniques)
- Statistical analysis
- Nonspecific markers
- Practicality
- Lack of established surrogate endpoints

*_Lancet Neurol_ 2014;13:113

Micro RNAs in MS*

- Endogenous small (~22 NT long) noncoding RNAs that regulate gene expression at post transcriptional level (typically ↓ encoded proteins)
- Dysregulated miRNA expression implicated in immune, neurologic, cardiovascular diseases; cancer
- Can be detected in body fluids

*_MSJ_ 2015; DOI 10.1177/1352458515578771; _Neurology_ 2012; 79:2160

Micro RNAs in MS*

- miRNA in CSF (N=53 MS, N=39 OND controls), global miRNA profiling
  - miR-922, miR-181c, miR-633 differentially regulated in MS vs. OND; miR-181c and miR-633 also differentiated RRMS from SPMS

*_MSJ_ 2015; DOI 10.1177/1352458515578771; _Neurology_ 2012; 79:2160

Proteomics*

- Term first coined in 1997; refers to large scale study of proteins in complex biological sample (protein purification and mass spectrometry)
- Other omics technologies include genomics (genomes), transcriptomics (all RNA), metabolomics (metabolites), lipidomics (lipids), pharmacogenomics (gene-drug effects)
- Proteome refers to entire complement of proteins, including modifications

*_Proteomics Clin Appl_ 2015; May 15

Proteomics*

- Advanced unbiased discovery technique
  - can measure large number of samples in short time period
- Mass spectrometry: technology detects/ quantifies proteins in complex biological matrix
  - can distinguish proteins that differ by single hydrogen atom
  - initial steps (affinity capture, organelle/protein fractionation) often enrich

**Proteomics Clin Appl* 2015; May 15

Proteomics*

- Proteomics may identify biomarkers, potential drug targets, new regulatory mechanisms
- Must go through discovery and validation stages

**Proteomics Clin Appl* 2015; May 15

CSF Proteomic Issues*

- Inconsistent results an issue
- Good sample collection
  - caudo-rostral gradient
  - circadian rhythm, gender and aging influences
  - potential impact of tobacco, alcohol, drug use
- Blood contamination
- BBB disruption

*_Proteomics Clin Appl_ 2007; 1:805

CSF Proteomic Issues*

- Sample processing
- Sample storage
- Processing CSF before proteomics
  - desalting
  - enriching low abundance proteins
  - pooling samples

*_Proteomics Clin Appl_ 2007; 1:805

Chitinase 3-Like-1*

- CHI3L1 (YKL-40, human cartilage glycoprotein 39, breast regression protein 39) is an enzymatically inactive protein, produced by Mϕ, neutrophils, astrocytes
  - member of chinitase/chitinase-like proteins
- Involved in tissue remodeling, inflammation, cell survival
- Proposed as both diagnostic and prognostic biomarker

*MSJ 2015; DOI 10.1177/1352458514561906; DOI10.1177/1352458515574148; *Brain* 2015; 138:918

Chitinase 3-Like-1*

- Study compared CSF proteomic of CIS patients who did/did not convert to MS
  - N=813 CSF samples from 15 European MS centers
  - mean F/U 5.4 years
- CHI3L1 measured by ELISA
  - CSF levels appear CNS derived
- CHI3L1 levels ↑ in those with second clinical attack
  - risk factor for conversion to MS (independent of MRI, CSF OCBs)
  - risk factor for disability (time to EDSS 3)

*MSJ 2015; DOI 10.1177/1352458514561906; DOI10.1177/1352458515574148; *Brain* 2015; 138:918

Chitinase 3-Like-1*

- In CSF proteomic study of control vs. RRMS, followed by ELISA verification in CSF/serum of CIS, RRMS, progressive MS
  - 22/527 proteins differed between RRMS vs. controls; included CHI3L1 and CHI3L2
  - CSF CHI3L1 levels ↑ with stage of MS (CIS, RR, progressive)
  - in contrast CSF CHI3L2 were only ↑ in RRMS, not progressive MS

*_MSJ_ 2015; DOI 10.1177/1352458514561906; DOI10.1177/1352458515574148; _Brain_ 2015; 138:918

CSF Proteomic Studies*

- Evaluated N=22 SPMS, N=7 SPMS on lamotrigine, N=12 NIND, N=10 HC for 26 preselected proteins
  - one protein (MY15B) significantly different in SPMS vs. HC, 3 proteins differed SPMS vs. NIND, 2 proteins differed NIND vs. HC, 11 significantly differed in untreated SPMS vs. treated SPMS

*_Clin Proteomics_ 2012; 9:9; _J Prot Res_ 2013; 12:1101; _PLoSONE_ 2014; 9:e103984; _PLoSONE_ 2010; 5: e12442

CSF Proteomic Studies*

- Evaluated effect of natalizumab on CSF proteome at baseline and 1 year (N=17 discovery set, N=20 validation set
  - 3 proteins significantly decreased: Ig mu-chain C region, haptoglobin, CHI3L1
- Evaluated RRMS (N=11), PPMS (N=10), C (N=10)
  - protein jagged-1 ↓↓ in PPMS vs. RRMS; vit D binding protein only detected in RRMS

*_Clin Proteomics_ 2012; 9:9; _J Prot Res_ 2013; 12:1101; _PLoSONE_ 2014; 9:e103984; _PLoSONE_ 2010; 5: e12442

CSF Proteomic Studies*

- Evaluated N=24 CIS, N=16 RRMS, N=11 progressive MS
  - secretogranin II and protein 7B2 ↑ RRMS vs. progressive MS ($p<0.05$)
  - fibrinogen and fibrinopeptide significantly ↓ in CIS vs. progressive MS ($p<0.04$)
  - tymosin β4 peak differentiated CIS and RRMS ($p=0.013$)

*_Clin Proteomics_ 2012; 9:9; _J Prot Res_ 2013; 12:1101; _PLoSONE_ 2014; 9:e103984; _PLoSONE_ 2015; May 5

CSF Proteomic Studies*

- Evaluated CIS (N=65), RR (N=72), progressive MS (N=42) vs. NIND (N=22), OND (N=20)
  - 151 differentially expressed proteins in MS
  - involved disease related pathways (aldosterone regulated sodium reabsorption, renin angiotensin system, notch signaling pathway, vitamin digestion and absorption system)

*_Clin Proteomics_ 2012; 9:9; _J Prot Res_ 2013; 12:1101; _PLoSONE_ 2014; 9:e103984; _PLoSONE_ 2015; May 5

MS CSF Proteome Review*

- Must consider expected effects
  - RRMS has pronounced inflammation, leakage of BBB
  - progressive MS has lower overt inflammation; age matched controls esp important
- This review evaluated 14 studies from 2010-2014
  - evaluated 8-84 MS, 0-36 controls in discovery phase; found 2-67 proposed biomarkers
  - 8 verification phase studies, involving 20-187 MS, 0-58 controls; 1-16 verified biomarkers

*_Biochm Biophys Acta_ 2015; 1845:746

MS CSF Proteome Review*

- ten verified biomarkers involved haptoglobin and Ig mu-chain C (pre vs. post natalizumab), α1 antichymotrypsin and α2 hsglycoprotein (↑ SPMS), vit D binding protein CHI3L1, protein jagged 1, contactin 1, kallikrein 6, apolipoprotein D,
- 6/10 linked to immune cells/inflammatory response
- all but 1 were highly abundant, not requiring depletion/fractionation

- Conclusion: large potential but much work still needed, esp for low abundance proteins

**Biochm Biophys Acta* 2015; 1845:746

MS CSF Proteomic Study*

- High resolution mass spec used to identify CSF proteins in first attack MS, relapsing MS, controls
- First attack patients showed distinct, shared profile
- First attack patients distinguished by gray matter (axon, synapse, neuron) pertinent antigens vs. myelin antigens

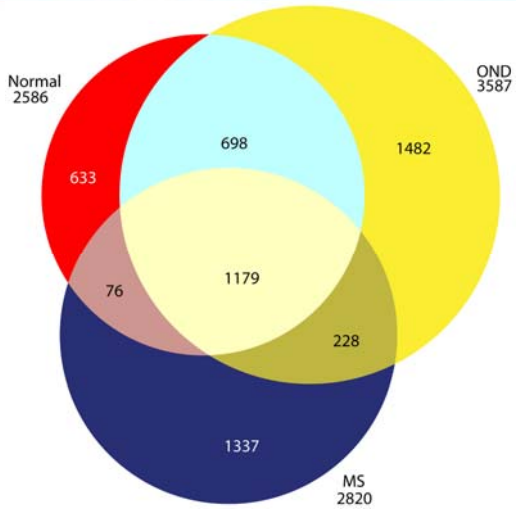
*PloS One 2013; 8:e66117

MS CSF Proteomic Study*

- Supports gray matter involvement in early MS; raises possibility that first attack is orchestrated not random
- Diagnostic and pathogenetic insights

*PloS One 2013; 8:e66117

CSF Proteome



Gray Matter Is Targeted in First-Attack Multiple Sclerosis
undefined, Thomas E. Angel, Tao Liu, Athena A. Schepmoes, Fang Xie, Jonas Bergquist, László Vécsei, Denes Zadori, David G. Camp II, Bart K. Holland, Richard D. Smith, Patricia K. Coyle

Table 1. Significant CSF Brain Protein Changes Increased in First Attack CIS-MS vs. Established RR-MS, Controls

	Fold change			
Protein name	First-attack CIS vs. RR	First-attack CIS vs. Control	Established RR vs. Control	Function
Nogo receptor	8.04	2.62	−3.07	Regulates axonal growth, regeneration, synaptic recovery; decreases amyloid beta levels
Kallikrein-6 (Neurosin)	2.79	1.06	−2.64	Serine protease, produced by activated macrophages, active against extracellular matrix, amyloid precursor protein, myelin basic protein, alpha synuclein.
Cerebellin-1	2.67	1.46	−1.83	Synapse integrity, plasticity, stimulates norepinephrine release
Ceruloplasmin	1.78	1.70	−1.05	Iron transport, binds copper
Dickkopf-3 (RIG-like 7–1)	1.78	1.12	−1.59	Affects synapse formation, signaling
Amyloid beta precursor-like protein 1	1.68	0.97	−1.73	Involved in synapse maturation, postsynaptic function, neurite outgrowth.
Activated leukocyte cell adhesion molecule (CD166)	1.45	1.16	−1.24	Neurite extension, controls MMP-2 activation,expressed on neurons, activated T and B cells, monocytes.
Neural cell adhesion molecule 2	2.34	1.05	−2.24	Type 1 membrane glycoprotein, implicated in interneuronal and glia-neuronal adhesion, reparative and remyelinating activity.
Neural epidermal growth factor like 2/cerebral protein-12	2.12	1.54	−1.38	Secreted glycoprotein involved in neural cell growth and differentiation.

doi:10.1371/journal.pone.0066117.t001

Schutzer SE, Angel TE, Liu T, Schepmoes AA, et al. (2013) Gray Matter Is Targeted in First-Attack Multiple Sclerosis. PLoS ONE 8(9): e66117. doi:10.1371/journal.pone.0066117
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0066117

Table 2. Decreased in first-attack CIS-MS vs established RR-MS and controls

	Fold change			
Protein name	First-attack CIS vs. RR	First-attack CIS vs. Control	Established RR vs. Control	Function
Clusterin (Apolipoprotein J, complement lysis inhibitor)	−1.07	−1.65	−1.54	Secreted chaperone, involved in protein folding/aggregation, clearance of misfolded proteins, protects against apoptosis and complement cytolysis.
Brevican	−1.14	−2.25	−1.98	Brain specific proteoglycan involved in cortical CNS development.
Neuronal cadherin	−1.60	−2.14	−1.34	Synapse adhesion, axon outgrowth and guidance, neuronal recognition, dendritic spine density, adhesion molecule.
Chitinase-3-like 1 protein	−2.81	−1.43	1.97	Secreted by activated macrophages; plays role in response to pathogens, ability of cell to respond to microenvironment.
Neogenin	−2.83	−1.94	1.46	Transmembrane receptor involved in neuronal differentiation, apoptosis, repulsive axon guidance, cell adhesion mechanisms.

doi:10.1371/journal.pone.0066117.t002

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http://www.plosone.org/article/info:doi/10.1371/journal.pone.0066117

Table 3. Increased in first-attack CIS-MS vs established RR-MS, but Decreased in first-attack CIS-MS vs Controls

	Fold change			
Protein name	First-attack CIS vs. RR	First-attack CIS vs. Control	Established RR vs. Control	Function
Multifunctional protein MFP (collagen alpha 1 18) chain, Endostatin)	3.11	−1.45	−4.51	Extracellular matrix protein, antiangiogenic
Dystroglycan 1	2.02	−1.06	−2.15	Laminin binding component,scaffolds axin to cytoskeleton,cell adhesion receptor.
Contactin 2	1.70	−1.17	−1.99	Neuronal membrane protein that functions as adhesion molecule,involved in axonal connections, expressed on axons and juxtaparanodal region of myelinating oligodendrocytes.
Ephrin type A receptor 4	1.40	−1.05	−1.46	Member of protein-tyrosine kinase family, involved in signal transduction,axon and dendritic development.
Neural cell adhesion molecule L1 like protein	1.30	−1.17	−1.52	Neural recognition molecule involved insignal transduction, synaptic plasticity, neuriote outgrowth, suppresses neuronal death.
Contactin 1	1.14	−1.45	−1.65	Neuronal membrane protein, axon-myelinating glial cell signaling, oligodendrocyte generation via NOTCH 1 ligand.

doi:10.1371/journal.pone.0066117.t003

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http://www.plosone.org/article/info:doi/10.1371/journal.pone.0066117

Summary

- Value of CSF as MS biomarker source is clear
- CSF proteomics still in early stage
- Important to standardize techniques
- CSF proteomic may ultimately provide critical insights into MS