CSF in MS: Background

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Disclosures

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

Research: Actelion, Novartis, Opexa
Background

CSF Analysis in MS

- Considered part of diagnostic assessment
- Can help to support MS diagnosis, and rule out other disorders
- Being done less often (several factors)
  - not required in 2010 diagnostic criteria
- Yet CSF pertinence is emphasized now more than ever before
- CSF is probably high yield biomarker source
**Background***

Entire human CSF about 140-150 ml
500 to 600 ml produced daily
- 20 ml replaced each hour
- 350 mcl per minute

Dual source
- 66%-80% from choroid plexus
- rest from blood-CSF barrier structures and CNS interstitial fluid (drainage); 280 ml total in brain

Reported surface area of BBB 20m² vs. 0.21 m² for choroid plexus (0.1%)


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**Background***

Secretory capacity of choroid plexus epithelium 0.4 ml/min/gram

Four choroid plexuses, in each ventricle
- major part of blood-CSF barrier (along with arachnoid membrane, circumventricular organs)

CSF secreted from ventricles, via foramen of Luschka and Magendie, into subarachnoid space

CSF returns to venous blood in brain sinuses (via arachnoid granulations, arachnoid villi)
- small fraction returns via perineural cranial nerve sheaths, dorsal nerve roots

*Physiol Rev 2013; 93:1847; J Neurosci 2013; 33:17553
**Background**

- CSF is 99% water, but contains proteins, lipids, hormones, microRNAs, and many other molecules/metabolites
- CSF serves multiple functions
  - protection, communication/transport, waste disposal, microenvironmental control
- Choroid plexus-CSF system coordinates development and health of the entire CNS

*Physiol Rev 2013; 93:1847; J Neurosci 2013; 33:17553

**CNS Immunology**

- CNS lacks conventional lymphatic system
- However both CSF and CNS interstitial fluid drain to regional lymph nodes
  - CSF also drains to blood
- CSF and APCs drain from subarachnoid space through cribiform plate via nasal mucosa
- Interstitial fluid drains via 100-150 nm wide basement membranes within walls of cerebral capillaries and arteries

*J Neuroimmune Pharmacol 2013; 8:840
Inflammatory cells readily enter CSF (vs. parenchyma)

Relationship between CSF and interstitial fluid differs for WM vs. GM

Gut microbiome, cervical lymph nodes, influence EAE

*J Neuroimmune Pharmacol 2013; 8:840

Role in Diagnosis
**MS Diagnosis**

- Diagnostic urgency, to institute early therapy
- Typical delay about 2 years
- No single diagnostic test (including MRI)
- CSF can play important role

**2010 Revised McDonald Diagnostic Criteria***

- Abnormal CSF involves either \( \geq 2 \) OCBs, or \( \uparrow \) IgG index
- CSF can be important to support inflammatory/demyelinating process, evaluate other diagnoses, predict MS
- Dropped using CSF to \( \downarrow \) MRI requirements (due to lack of data)
- CSF only noted for PPMS diagnosis

*Ann Neurol 2011; 69:292*
McDonald Criteria For Progressive From Onset*

One year of disease progression
(prospective/retrospective)

Two additional criteria

- brain DIS (≥1 T2 lesion in periventricular, juxtacortical, or infratentorial region)
- spinal cord DIS (≥2 T2 lesions)
- positive CSF

*Ann Neurol 2011; 69:292

CSF Evaluation Recommended

- Primary progressive MS
- Atypical MRI
- Atypical clinical presentation (age of onset, etc)
Evaluation of CSF biomarkers of intrathecal inflammation (sCD27, sCD21, sCD14) showed equal expression of activated T and B cells in progressive (PP, SP) MS as relapsing MS
  - about 10% in each MS subgroup lack intrathecal inflammation
  - activated T and B cells are preferentially imbedded in CNS tissue in progressive vs. relapsing MS
  - DMT efficacy dependent on CNS penetration

Oligoclonal IgM bands identified PPMS cohort with more aggressive clinical course, ↑ CSF B cells, ↑ contrast enhancing lesions

*Ann Neurol 2015; 2014; 76:231
Disclosures

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

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CSF: Lessons From Other Diseases

- Alzheimer’s
- Progressive multifocal leukoencephalopathy
- Parkinson’s and other dementias
Alzheimer’s Disease (AD)

- Most common form of dementia
  - accounts for 60% to >80% of cases
- Progressive mental, behavioral, functional decline and loss of ability to learn
- Characteristic neuropathology

AD

- Plaques consistent with aggregated amyloid β peptides (outside of neurons and in blood vessels, cerebral amyloid angiopathy)
  - mutations in genes encoding amyloid precursor protein (APP), and APP-processing enzymes, cause familial forms of Alzheimer’s
- Neurofibrillary tangles with hyperphosphorylated tau (intra-axonal protein)
  - no associated mutations in tau-encoding gene MAPT associated with Alzheimer’s

*Pharm Rep 2015; 67:195, Alzheimer’s Assoc Disease Facts
Healthy brain has 100 billion neurons, 100 trillion synapses
- information transfer at synapses fails, synapses and neurons lost
- Dramatic brain volume loss
- Recognizes preclinical, MCI, dementia stages
- In the US younger onset <65 years 4% (200,000); typically ≥ 65 years (5 million)
- By 2050, 13.8 million Alzheimer cases projected

Brain changes may begin ≥20 years before symptoms
- part of Alzheimer continuum
- amyloid can appear 20 to 30 years before dementia symptoms; as early as age 30
- amyloid ↓ in Alzheimer’s with age
Clinical phenotype heterogeneous
Up to 10% lack Aβ pathology
Clear correlation between NFT pathology and cognitive state (Braak and Braak)

Risk factors
- age
- family history
- APOE 4 gene (in general population 20% to 30% have one copy, 2% have two copies; 40%-65% of Alzheimer’s have one or two copies)
- mild cognitive impairment (MCI)
- cardiovascular risk factors
- social and cognitive engagement
- education
- TBI

*Pharm Rep 2015; 67:195, Alzheimer’s Assoc Disease Facts
Normally synthesized by neuronal cells to stabilize microtubules for proper functioning of neurons

Tau and its hyperphosphorylated version form main constituent of intracellular NFTs

Specific genetic variants of tau associated with familial forms of frontotemporal dementia

* Trends Mol Med 2015

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Tau pathology appears in distinct pattern (entorhinal cortex, hippocampus, cortical areas)

Tau pathology correlates with cognitive status

Tau may act as endopathogen

* Trends Mol Med 2015
**Aβ Hypothesis**

- Neurodegeneration in AD caused by deposition of Aβ in plaques
- Accumulation of Aβ in brain is main driver of AD pathogenesis
- Smaller Aβ aggregates/oligomers are key drivers
- Neurodegeneration, development of NFTs, follow from imbalance between Aβ production and clearance
- Strongly supported by genetic link

**AD Pathophysiology**

- **Amyloid hypothesis**
  - APP normally cleaved by α secretase; aberrantly processed by β and δ secretases
  - Aβ peptides aggregate into soluble oligomers, coalesce to fibrils, insoluble beta-sheet conformation
  - Aβ42 oligomers produced by neuron-astrocyte interactions; induce oxidative damage, tau hyperphosphorylation, toxic to synapses, mitochondria
  - Aβ plaques attract microglia with activation, proinflammatory cytokines stimulate more oligomers
**AD Pathophysiology**

- Oligos esp vulnerable to oxidative stress (↓ glutathione, high iron content)
- Aβ oligomers damage cholesterol-enriched membranes
- Oligomers removed by proteolytic degradation, uptake by astrocytes/microglia, passive flow into CSF, sequestration into vascular compartment

**Tau hypothesis**
- Altered, aggregated forms of tau act as toxic stimulus for neurodegeneration

**Inflammation hypothesis**

**AD Therapy**

- Anti-amyloid approach (targets Aβ protein)
  - Target amyloid transport
  - Modulate secretase enzymes
  - Target amyloid aggregation
  - Target amyloid clearance
  - Amyloid based vaccination therapy

*Pharm Rep 205, 67:195*
**AD Therapy**

- Target tau protein
  - inhibit phosphorylation
  - target microtubule stabilization
  - block tau oligomerization
  - enhance tau degradation
  - tau based vaccination therapy

- Target intracellular signaling cascades
  - eg., phosphodiesterase inhibitors, phospholipase A2 inhibitors

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**AD Therapy**

- Modulate neurotransmitter levels
  - acetylcholinesterase inhibitors
  - modulating gabaergic neurons
  - NMDA receptor antagonism
  - modulating serotonin receptor
  - histaminergic modulators
  - modulating adenosine receptor

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*Pharm Rep 205; 67:195
AD Therapy*

Target mitochondrial dysfunction
- to combat ↓ complex IV activity, ↑ ROS production
- lipoic acid/omega 3 FA, idebenone, CoQ10, methylene blue, SS-31

*Pharm Rep 205; 67:195

AD Therapy*

Target oxidative stress
- vitamins E and C, carotenoids, flavonoids, melatonin

Anti-inflammatory therapy
- NSAIDs, Cox-1 selective inhibitor

Miscellaneous (cholesterol lowering drugs, neuroprotective gonadotrophin hormones, neurotrophic factors, epigenesis, caspase inhibitors, NOS modulators, nucleic acid drugs, multi-target directed ligands)

*Pharm Rep 205; 67:195
Plaque pathology associated with low CSF levels of aggregation prone 42 AA form of Aβ (Aβ42)
- appears years before first clinical symptoms
- good predictive value for MCI
Tangle pathology associated with increased CSF phosphorylated tau (p-tau)
Neurodegeneration (injury to neurons, axons) associated with increased CSF total tau (t-tau)

**CSF Pattern*\**

↓ Aβ42, ↑ tau, ↑ p-tau detects MCI/incipient AD with 95% sensitivity, 87% specificity
Dementia progression more rapid with more marked CSF level changes

*JNNP 2014; 85:1426; J Clin Neurol 2015; 11:132*
CSF Biomarker Changes*

- N=265 cognitively normal middle aged cohort
- Longitudinal cognitive and CSF assessments
- Baseline CSF (Aβ42, p-tau, and ratios including t-tau) predicted MCI (mean time 5.41 years)

*Neurology 2013; 81:1753

CSF Biomarkers Standardization*

- CSF is most useful biologic fluid reflecting molecular events in the brain
- CSF biomarkers in clinical trials
  - surrogate endpoints
  - used diagnostically for inclusion/exclusion criteria
  - used to enrich study subjects (more rapid progression)
- Revised Alzheimer’s diagnostic criteria include biomarkers
Stage 1
Asymptomatic amyloidosis
- High PET amyloid tracer retention
- Low CSF Aβ1-42

Stage 2
Amyloidosis + Neurodegeneration
- Neuronal dysfunction on FDG-PET/fMRI
- High CSF tau/p-tau
- Cortical thinning/Hippocampal atrophy on sMRI

Stage 3
Amyloidosis + Neurodegeneration + Subtle Cognitive Decline
- Evidence of subtle change from baseline level of cognition
- Poor performance on more challenging cognitive tests
- Does not yet meet criteria for MCI

MCI ➔ AD dementia
CSF: Lessons from Alzheimer’s

- CSF diagnosis profile
  - early, more reliable diagnosis
- Combination better than single biomarker
- Proposed as screening for clinical trials
- Sheds light on pathophysiology
- Supports therapeutic approaches

Korean Consensus to Reduce Variability*

- Neuroimaging prior to LP
  - imaging in past year
  - repeat if new headaches, focal signs in interim
  - generally contraindicated if bleeding risk, low platelets, on anticoagulation
  - continuing antiplatelet therapy permissible
  - should be performed by experienced physicians

*J Clin Neurol 2015; 11:132
**Korean Consensus to Reduce Variability**

- Fasting for minimum of 6 hours recommended (or record last meal)
- Diurnal fluctuations of Aβ42 up to 4 fold (not confirmed in elderly); optimal time 8 AM-noon
- Needle (22-24g sprotte)
- Routine CSF analysis (cells, protein, glucose)
- Traumatic CSF should be discarded
- Volume 10-12 ml

*J Clin Neurol 2015; 11:132

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**Korean Consensus to Reduce Variability**

- Polypropylene tubes (vendor differences)
- Direct CSF transfer to freezing tubes
- Aβ42, t-tau, p-tau stable at RT for 5-14 days; delay ≤4 hours prior to deep freeze
- Centrifuge at 2000g for 10 min at RM
- Aliquot 400 mcl in 500 mcl tubes
- Freeze at -80°C
- One freeze thaw cycle

*J Clin Neurol 2015; 11:132
CSF and PML

- CSF studies diagnostically useful
- They have prognostic utility
- May reveal new helpful assays

PML Diagnosis

- Supportive clinical and MRI picture, with positive CSF JC virus PCR (or biopsy confirmation)
- CSF PCR very specific (92-99%) and sensitive (74-93%)
  - ultrasensitive quantitative PCR (≤50 copies/ml) preferred
  - TaqMan real time PCR detects ≥10 DNA copies/ml
- False negatives can occur
Natalizumab Associated PML Functional Outcomes and Predictors*

- Analysis of N=336 MS patients
- Survival 76%; mean F/U 16.1 months
  - mean time to death 4.7 months
- Survivors had
  - lower CSF JC viral load
  - younger, less disability
  - less extensive MRI disease

*J Neurovirol 2015

Natalizumab Associated PML Functional Outcomes and Predictors*

- Independent study of mefloquine for PML found ↓ in CSF JC virus DNA load from baseline to 4 weeks associated with better clinical outcome at 16 weeks

*J Neurovirol 2013; 19:351
**CSF Lipid Specific IgM Bands***

- Evaluated N=24 MS with PML, N=343 MS without, treated with natalizumab
- IgM bands ↓ PML risk (p<0.0001); associated with ↑ CSF CD4, CD8, B cells
- Protective effect even in JC virus antibody positive
- Higher PML risk in IgM band-, JC virus antibody positive (19% of cohort)
- IgM lipid bands are marker for highly inflammatory MS
  - ↑ relapse rate, lesion volumes

*Ann Neurol 2015; 77:447

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**CSF JC Virus Antibody Index***

- CSF JC virus AI assessed in N=37 PML, N=89 non-PML MS patients
- 26/37 (70%) of PML cases had AI >1.5, vs. 0% of controls (p<0.0001)
  - CSF antibodies detected in 44% of controls
- At time of first + PCR, 11/20 (55%) had AI >1.5; DNA levels <100 copies/ml in 14/20 (70%) of these cases; 8 (57%) had AI >1.5
- Conclusion: CSF JC virus antibody index (intrathecal production) is helpful diagnostic tool

*Ann Neurol 2014; 76:792
Natalizumab Effects on CSF*

- Evaluated N=59 MS treated with natalizumab, N=17 control MS; also studied N=37 PML MS patients
- Natalizumab ↓ CSF CD19 + B cells; ↓ CSF CD4/CD8 ratio; ↓ CSF IgG/IgM levels and IgG index
- Conclusion: natalizumab impacts CSF cell and immunoglobulin parameters

*MSJ 2014; Warnke et al.

Young Onset Dementia*

- Involves dementia onset before age 65
- Causes include Alzheimer’s (34%), vascular (18%), frontotemporal lobar degeneration (12%), alcoholic (10%), dementia with Lewy body (7%), other (19%)
- Lumbar puncture recommended to identify inflammatory causes (MS, vasculitides, infections)
  - SSPE, Whipple’s HHV-6, cryptococcosis, TB, prion disease (↑ 14-3-3 protein, ↑ tau)

*Lancet Neurology 2010; 9:793
**CSF Biomarkers for Dementia**

- Traditionally used to exclude infection, malignancy, neuroinflammation
- Used for young onset cognitive impairment, rapid disease course, unusual dementia syndromes, immunocompromised
- Prion disease suspected; CSF positive predictive value
  - ↑ 14-3-3 protein
  - ↑ S100B
  - ↑ t-tau/p-tau ratio
  - real-time quaking induced conversion technology

*Prion 2011; 5:150; JNHP 2014; 85:1420

**CSF Prion Protein Levels**

- Retrospective autopsy-confirmed study of N=30 AD vs. N=52 CJD; second cohort (N=104) of probable AD (N=55), probable sporadic CJD (N=26), controls (N=23)
- Evaluated commercial BetaPrion human EIA test kit
- CSF prion protein lowest in CJD (? Sequestration); ↑ in AD
- Conclusion: PRP is neuronal injury marker; low levels may be diagnostic biomarker for prion disease (along with ↑ 14-3-3, ↑ t-tau)

*JAMA Neurol 2015; 72:261, 267*
MSA is a rare neurodegenerative disorder that combines striatonigral degeneration, acquired olivopontocerebellar degeneration, and Shy-Drager syndrome

α synucleinopathy (along with Parkinson’s, dementia with Lewy body)

in glial cytoplasmic inclusions; to a lesser extent neurons

Candidate CSF biomarker studies suggest combinations will be more successful for diagnosis

Most promising are ↑ neurofilament light chain, catecholamine metabolites, and proteins such as α synuclein/DJ-1/t-tau
**CSF Biomarkers in Parkinson’s**

- CSF α synuclein level results inconsistent
  - blood cells can contaminate
- CSF AD markers predict cognitive decline
- CSF neurofilament light chains not elevated in Parkinson’s (vs. MSA, PSP)

*J Neurol Sci 2015; 352:84; Exp Neurol Biol 2014; 352; JNIP 2015; Jan 14; Park Rel Dis 2015*

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**Panel of nine CSF biomarkers identified atypical Parkinsonian syndrome vs. Parkinson’s**

- t-tau, p-tau, Aβ42
- α synuclein, neurofilament light chain
- α and β sAPP
- 2 inflammatory markers (MCP-1, YKL-40)

*J Neurol Sci 2015; 352:84; Exp Neurol Biol 2014; 352; JNIP 2015; Jan 14; Park Rel Dis 2015*
Summary

- A number of disorders are focused on studying CSF
- Goal is diagnostic, prognostic, pathophysiologic insights
- Combination of CSF biomarkers often favored
- CSF offers valuable body fluid for study