Imaging Correlates of MS Pathology

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Outline:

1. MRI-tissue correlation: what we know and what we think we know
2. Clinical aspects: correlation of basic clinical sequences
3. What have we learned from the MSLP?
   1. Spectrum of tumefactive MS
   2. IP correlations
   3. The use of ADC map
4. Imaging the cortex: field strength vs pulse sequence vs both
5. Imaging of wet vs paraffin embedded samples
6. Conclusion

MRI-Pathology correlation

• Most of our knowledge re: MRI is empirical!
  – New pulse sequences are not typically developed with tissue correlational studies conducted “right away”
    • In vivo vs ex vivo studies: in vivo correlation w models is more commonly done during development
    • MRI-pathology studies are scarce
      – Over 9400 MRI-related MS papers on PubMed
      – Less than 80 MRI of ex vivo MS samples
  – Special problem related to MS studies:
    • Tissue samples: early “aggressive” disease (biopsy)
    • Burnt out chronic disease: autopsy
**MRI-Pathology correlation**

- MRI is NOT in vivo histology
  - It’s not because of “resolution” that MRI and histology look different!
  - Histology: stains provide sensitivity and specificity
  - MRI: pulse sequences scrutinize the physico-chemical environment of (mainly) water molecules, and this determine sensitivity and specificity

- $^1$H MRI is always a “proton map” of tissue, with images generated using a variety of contrast mechanisms

**MRI correlations of basic pulse sequences**

- “Correlation Between Pathological and MRI Findings in MS” Milan on June 10–11, 2011.

**“Every MS lesion is T2 hyperintense”**

- Most WML-s are
- GM/CL-s: typically not (not on FSE/TSE sequences)
- $T2^*$ may be more sensitive, but clearly more prone to artifacts
- Main problem: T2 weighted sequences are sensitive, but lack any specificity
  - demyelination, remyelination, focal inflammatory infiltration, gliosis, axonal loss, small vessel ischemic changes, etc.
- Lesion appearance, location, and signal behavior on additional MRI sequences
- **Location:** Barkhof DIS criteria
T2 weighted FSE  FLAIR  DIR

FSE T2  FLAIR
T1 hypointense lesions

- AKA “T1 “black holes” (T1BH)
- Consequence of “increased water signal”: marked oedema and demyelination with or without matrix destruction
- Can completely disappear as inflammation resolves: acute T1BH
- Chronic foci of T1 hypointensity—persistent (chronic) T1 black hole: severe tissue destruction, axonal loss, matrix destruction
**Gadolinium enhancement**

- Acute or active and chronic or non-active lesions by MRI: *NOT the same as what these mean in the pathological sense!*
- On MRI, these are based on evidence of **BBB** breakdown as indicated by contrast enhancement.
- HOWEVER: several factors affect enhancement:
  - Gadolinium dose
  - Delay and characteristics of image acquisition
  - Steroid treatment of acute attacks
  - Contrast enhancement persists for 2–6 weeks, but:
  - short lived “fluctuating” enhancement in studies with high temporal resolution!

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**Active vs inactive lesions**

- Is USPIO the answer?
- NOT FDA approved
- “In vivo monocyte/macrophage stain”
- USPIO enhancement:
  - Can be seen in areas without signal change on T2-weighted images
  - Can be seen in the absence of Gd enhancement
  - May suggest prelesional accumulation of monocytes preceding or independent of lesion formation
  - May extend for a long time beyond Gd enhancement, which is the present marker for active lesions

- USPIO derived data suggest that infiltration of macrophages into the brain occurs through different mechanisms from blood–brain barrier damage
Cross-sectional patterns of lesion enhancement: gadolinium vs USPIO

Vellinga M M et al. Brain 2008;131:800-807

USPIO enhancement of PPMS lesion

Radiology,
http://pubs.rsna.org/doi/abs/10.1148/radiol.12111416
What have we learned from the MSLP re: pathology-MRI correlation?
Basic pulse sequences

The Radiographic Spectrum of Biopsy Proven Inflammatory Demyelinating Disease (IDD)

- To describe radiographic features of a biopsied cohort with pathologically confirmed inflammatory demyelinating disease (IDD)
- To compare demographic, clinical course and disability profiles between tumefactive and non-tumefactive MS.
Brain; Lucchinetti et al. 2009

• Retrospective review of MR scans from 168 patients with pathologically confirmed IDD
  • 206 pre-biopsy MRI scans
  • 442 follow-up brain MRI scans
• Cases derived from a larger MS biopsy resource (700+ biopsies) collected from 3 centers (Mayo, Austria, Germany).
• Clinical data collection
  • Face-to-face interview/exam 98
  • Limited clinical data 29

METHODS

• MR scans were analyzed for:
  • Biopsy lesion location, size, enhancement pattern, T2W hypointense rim, T1W hypointensity, mass effect, edema
  • Multifocal lesions
  • Barkhof criteria
  • Tumefactive lesions
    • Defined strictly by lesion size > 2.0 cm
Clinical Characteristics of Biopsy/MRI Cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median</th>
<th>(range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>1.2F:1M</td>
<td></td>
</tr>
<tr>
<td>Age at biopsy (yrs)</td>
<td>39</td>
<td>(11-70)</td>
</tr>
<tr>
<td>Disease duration to 1st MRI (days)</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Disease duration at last F-U (yrs)</td>
<td>3.8</td>
<td>(0.27-33)</td>
</tr>
<tr>
<td>EDSS at last F-U</td>
<td>2.5</td>
<td>(1-3.5/-IQR)</td>
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### Clinical course prior to biopsy

<table>
<thead>
<tr>
<th>Category</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>First attack</td>
<td>59</td>
</tr>
<tr>
<td>Relapsing</td>
<td>32</td>
</tr>
<tr>
<td>S Progressive</td>
<td>2</td>
</tr>
<tr>
<td>P Relapsing</td>
<td>1</td>
</tr>
<tr>
<td>P Progressive</td>
<td>3</td>
</tr>
<tr>
<td>Uncertain</td>
<td>3</td>
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</table>

### Diagnosis last F-U

<table>
<thead>
<tr>
<th>Category</th>
<th>%</th>
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<tbody>
<tr>
<td>CDMS</td>
<td>79</td>
</tr>
<tr>
<td>Possible MS</td>
<td>7</td>
</tr>
<tr>
<td>Isolated syndrome</td>
<td>13</td>
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<tr>
<td>Uncertain</td>
<td>1</td>
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</table>

Index Attack Symptoms

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Patients (no.)</th>
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<tbody>
<tr>
<td>Cognitive</td>
<td>54</td>
</tr>
<tr>
<td>Brainstem/ cranial nerve</td>
<td>53</td>
</tr>
<tr>
<td>Motor</td>
<td>50</td>
</tr>
<tr>
<td>Sensory</td>
<td>47</td>
</tr>
<tr>
<td>Brainstem</td>
<td>36</td>
</tr>
<tr>
<td>Sphincter</td>
<td>6</td>
</tr>
<tr>
<td>Visual field cut</td>
<td>1</td>
</tr>
<tr>
<td>Seizure</td>
<td>4</td>
</tr>
<tr>
<td>Optic neuritis</td>
<td>3</td>
</tr>
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</table>
Treatments
<4 Weeks Prior To Pre-biopsy MRI

<table>
<thead>
<tr>
<th>Patients (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids</td>
</tr>
<tr>
<td>Plasma exchange</td>
</tr>
<tr>
<td>Interferon β</td>
</tr>
<tr>
<td>Glatiramer acetate</td>
</tr>
<tr>
<td>Mitoxantrone</td>
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</tbody>
</table>

Biopsy Lesion Location

- Frontal 32%
- Parietal 30%
- Sub/juxta cortical 9%
- Deep gray 1%
- Temporal 4%
- Infratentorial 4%
- Corpus Callosum 5%
- Occipital 7%
- Periventricular 8%
### Biopsied Lesion Characteristics

**Lesion size >2 cm in 67%**

<table>
<thead>
<tr>
<th>Edema</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>25</td>
</tr>
<tr>
<td>Mild (0.1-1 cm)</td>
<td>39</td>
</tr>
<tr>
<td>Moderate (1-3 cm)</td>
<td>21</td>
</tr>
<tr>
<td>Marked (&gt;3 cm)</td>
<td>1</td>
</tr>
<tr>
<td>Uncertain</td>
<td>14</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Mass effect</th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>52</td>
</tr>
<tr>
<td>Mild (sulcal effacement)</td>
<td>21</td>
</tr>
<tr>
<td>Moderate (&lt;1 cm subfalcine herniation)</td>
<td>14</td>
</tr>
<tr>
<td>Marked (&gt;1 cm subfalcine herniation)</td>
<td>1</td>
</tr>
<tr>
<td>Uncertain</td>
<td>12</td>
</tr>
</tbody>
</table>

### PRE-BIOPSY LESION T1W DEGREE OF HYPOINTENSITY RELATIVE TO NAWM

- **74% severe**
- **24% mild/mod**
- **3% isointense**

### Hypointensity Evolution

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>More</td>
<td>19%</td>
</tr>
<tr>
<td>Same</td>
<td>40%</td>
</tr>
<tr>
<td>Less</td>
<td>12%</td>
</tr>
<tr>
<td>Isointense</td>
<td>8%</td>
</tr>
<tr>
<td>Uncertain 2° Bx</td>
<td>21%</td>
</tr>
</tbody>
</table>
**Common Prebiopsy Lesion Enhancement Patterns**

- Ring, arc, open ring: 42.5%
- Heterogenous: 32%
- Open ring cortex: 8%
- Homogenous: 8%

**Uncommon Prebiopsy Lesion Enhancement Patterns**

- Diffuse/patchy: 4%
- Nodular >2mm: 0.5%
- Fluffy: 1%
- No enhancement: 15%
- OTHER
  - Punctate: 3%
  - Concentric: 1%
  - No enhancement: 15%
**Lesion Geography**

**Patterns I and II**
- Sharp MΦ border

**Patterns III**
- Fuzzy MΦ border

**Pattern I**
- T cell/macrophage associated

**Pattern II**
- Antibody/complement associated

**Pattern III**
- Distal oligodendro-gliopathy

**Pattern IV**
- Primary oligodendrocyte degeneration
Ring/Rims Correlate with Accumulation of Macrophages at ACTIVE PLAQUE edge

Correlation of Enhancement Pattern of Biopsied Lesion with Immunopatterns (n=127 pts)

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern I/II</td>
<td>70%</td>
</tr>
<tr>
<td>Pattern III</td>
<td>30%</td>
</tr>
</tbody>
</table>

*5 Balo concentric cases
Ring Enhancement and T2 hypointense Rims Colocalize

Correlation of T2 Rims with Immunopattern (n=127)

P = < 0.001
TUMEFACTIVE IDD SUBGROUP

<table>
<thead>
<tr>
<th></th>
<th>Pre-Biopsy</th>
<th>Last F/U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multifocal lesions</td>
<td>66/83 (80%)</td>
<td>74/92 (80%)</td>
</tr>
<tr>
<td>Barkhof criteria</td>
<td>38/83 (46%)</td>
<td>43/92 (47%)</td>
</tr>
</tbody>
</table>

- 80% had a single tumefactive episode
- 20% had recurrent tumefactive lesions
### TUMEFACTIVE vs NonTUMEFACTIVE MS

<table>
<thead>
<tr>
<th></th>
<th>Nontumefactive</th>
<th>Tumefactive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td>1M : 1.3F</td>
<td>1.1M : 1F</td>
</tr>
<tr>
<td><strong>Age at Bx (yrs)</strong></td>
<td>42 (11-69)</td>
<td>39 (19-70)</td>
</tr>
<tr>
<td><strong>Disease course</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite MS (%)</td>
<td>59</td>
<td>62</td>
</tr>
<tr>
<td>Probable (%)</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>CIS (%)</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td><strong>EDSS at last F/U</strong></td>
<td>2.0 (1.5-3.5)</td>
<td>2.5 (1-3.5)</td>
</tr>
<tr>
<td><strong>Barkhof (%)</strong></td>
<td>66</td>
<td>47</td>
</tr>
</tbody>
</table>

**LIMITATIONS**

- Retrospective
- Non-standardized scans
  - Variable Gad regimens
  - Multiple institutions
  - Older MR techniques
- Single observer
CONCLUSIONS:
PREBIOPSY LESION

- Frontal/parietal lesions predominate
- Mild-moderate edema in majority
- Mild-moderate mass effect in 35%
- Ring-enhancement most common
- T1W hypointensity severe in most

CONCLUSIONS:
COHORT

- Majority of tumefactive and non-tumefactive cases are multifocal at biopsy
- Many met Barkhof criteria at biopsy, most by F/U
- Tumefactive IDD represents a large proportion of our biopsied cohort
CONCLUSIONS:
TUMEFACTIVE vs NONTUMEFACTIVE IDD

• Tumefactive MS is often multifocal
• 20% develop recurrent tumefactive lesions
• Tumefactive MS typically follows a clinical and radiographic course similar to prototypic MS
• Tumefactive MS does not portend a poorer prognosis


Funded by NMSS RG 3185-A-2 (CFL); 3185-B-3 (CFL) “The MS Lesion Project”
What have we learned from the MSLP re: pathology-MRI correlation? The role of in vivo ADC maps

The use of ADC maps in biopsy proven CNS IDD

- **Goal**: to analyze and describe DWI characteristics in a cohort of biopsy-proven CNS IDD cases

- Compare diffusion characteristics of ring-enhancing lesions vs. abscesses and tumors
The use of ADC maps in biopsy proven CNS IDD

• **40** prebiopsy ADC maps from **30** CNS IDD patients

• **Studied parameters**: size, T2-weighted (T2W) hypointense rim, enhancement and ADC pattern

• ADC patterns of CNS IDD ring-enhancing lesions were compared to a cohort of 35 patients with ring-enhancing tumors or abscesses

(A) Dark ring/arc pattern with bright facilitated center; (B) Isointense ring pattern with a bright, facilitated center; (C) Homogeneously dark pattern

(D–F) Baló’s concentric sclerosis: (D) Dark arc of restricted diffusion (E) ADC and T2W dark ring/arc colocalization. (F) concentric arc enhancement pattern

*Abou Zeid N et al. Neurology 2012;78:1655-1662*
Results:

- IDD lesions displayed **a spectrum of peripheral ADC patterns** at the lesion edge:
  - restricted diffusion (low ADC), 33%
  - increased diffusion (high ADC), 60%
  - normal diffusion (homogeneously isointense), 7%.
- 93% of lesions showed **gadolinium enhancement**
  - ring, 52%; heterogeneous, 34%; homogeneous, 7%
- **Hypointense T2W rim** was observed in 53%
- A ring pattern on ADC (isointense or dark) was **associated with T2W hypointense rims** (*p = 0.02*) but **not with ring enhancement**

![Evolution of ADC pattern on serial MRI scans over a 12-day interval](image)

Evolution of ADC pattern on serial MRI scans over a 12-day interval (A and D) Boxed region demonstrates change in ADC pattern (B and E) Regions of restricted diffusion observed in A are not initially associated with T2-weighted signal abnormalities (B) until the subsequent MRI (E, arrows). (C and F) Similarly, gadolinium enhancement develops in the previous areas of restricted diffusion 12 days later (F, arrows).
Results:

• On serial imaging, 4 of 7 (57%) CNS IDD patients demonstrated changes in ADC patterns

• Peripheral restriction was more common in IDD ($p = 0.006$) than in tumors or abscesses, whereas central restriction was only observed in abscesses

• Restricted lesions in the same stage were more common in the non-IDD cohort (42% vs 20%), with a uniform restricted pattern seen only in abscesses

Cortical Demyelination in Early MS
Lucchinetti, Popescu, et al. NEJM 365:23; Dec 8th, 2011

• We studied cortex obtained “en passant” during diagnostic biopsy of white matter lesions

• Represents random sampling of cortex
Hypothesis:

- MS lesions will form in WM areas directly connected with CL-s
  - Vascular connection
  - Direct connection via WM pathways

- If so, why not study this with MRI??
GM-WM
MS Lesion Connectivity

Future lessons from the MSLP: upcoming reassessment
**MSLP reassessment 2014-2017**

- Reassessment of a subcohort of biopsy proven cases
- MRI, detailed clinical descriptors, neurocognitive assessment
- MRI: all 3D scans
  - 3D FLAIR, T2
  - 60-direction DTI; DWI and ADC
  - Conventional and proprietary 3D DIR
  - 3D PSIR
  - 3D SWI
  - Customized FSE-IR
  - Field map

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**Imaging the cortex: high field strengths and its benefits**
Cortical Demyelination in Chronic MS

(Kutzelnigg/Lucchinetti et al. Brain 2005)

Pathological Evidence of MS Cortical Onset

(Popescu et al. Neurology 2011)

- 33 year old Caucasian woman
- Incidentally found Gad-enhancing cortical lesion
- No other white matter lesions
- Cortical resection → inflammatory demyelinating disease
Types of cortical lesions detected by 7-T MRI

- Type I: leukocortical
- Type II: intracortical
- Types III and IV: subpial lesions extending partly or all the way through cortex but not to subcortical WM

Fast low-angle shot (FLASH)-T2* and T2w-turbo spin-echo (TSE) images

MS#1: RR-MS with type IV lesion
MS#3 and MS#16: SP-MS, all CL types

Cortical plaques: greater contrast on FLASH- T2* scans than on T2-TSE images

Reproducibility of 7-T MRI in detecting cortical multiple sclerosis pathology

Example of reproducibility in cortical lesion types detection (subpial and leukocortical) in a SP-MS case
Appearance of CL-s on phase and magnitude images

(A) WM (red square) and leukocortical (blue square) lesions in SPMS;
(B) leukocortical lesion (blue square) in RRMS;
(C) subpial lesions (green squares) on magnitude (a) and phase (b) images in SPMS

The high-susceptibility ring, clearly visible in the leukocortical lesion of the RRMS case, was not seen in any of the other lesions in the 2 SPMS cases. This ring, hypothesized to be due to the expression of iron rich macrophages, may reflect the varying extent of inflammation that characterizes different lesions.
Ex Vivo Imaging:
How to gain sensitivity?
Formalin fixed brains are useful for magnetic resonance imaging (MRI) study.

Abstract

We carried out magnetic resonance imaging (MRI) studies on human brains which had been fixed in formalin solution for over 2 years and had been proven neuropathologically to be cases of multiple sclerosis (MS), progressive multifocal leukoencephalopathy (PML), and Balo’s concentric sclerosis (Balo). Using spin echo (SE) and inversion recovery (IR) pulse sequences to detect demyelinated lesions in a living person with MS, the demyelinated lesions of the fixed brains in cases of MS, PML and Balo definitely reappeared, although T1 and T2 in the grey and white matter were reduced following fixation. High signal areas on SE images corresponded not only to the characteristic distribution of demyelinated lesions in the white matter but also to sparse myelin, gliosis and mild perivascular cuffing in the white matter around the demyelinated foci in cases of the fixed MS, PML and Balo brains. On the IR images, only MS plaques were evident. This MRI study of fixed brains proved useful to elucidate clinicopathological correlations.

Stereotactic co-registration of magnetic resonance imaging and histopathology in post-mortem multiple sclerosis brain

Neuropathology and Applied Neurobiology

Stereotactic co-registration of magnetic resonance imaging and histopathology in post-mortem multiple sclerosis brain

Effects of formalin fixation on magnetic resonance indices in multiple sclerosis cortical gray matter
Quantitative magnetic resonance of postmortem multiple sclerosis brain before and after fixation

Magnetic resonance imaging as a tool to examine the neuropathology of multiple sclerosis
40% axonal density

MTR: 0.22
T1-CR: 0.66

50%

MTR: 0.27
T1-CR: 0.77

90%

MTR: 0.34
T1-CR: 0.96

Cylindrical home-made tissue containers. The base ring of the container was manufactured from 140 mm inner diameter clear PVC sewer pipe of 28 (A) or 22 mm (B) height

Comparison between magnetic resonance imaging (MRI)-visible (red) and invisible (blue) cortical lesions

PD-weighted MRI vs PLP stain

Left column, 3a–7a: histological sections of the MRI visible lesion shown in 2a.

Right column, 3b–7b: MRI-invisible lesion shown in 2b

3: Nissl stain
7: Bodian silver
4: HLA-DR
5: GFAP
6: fibrinogen

No difference whether MRI visible or not!!
• MRI-visible CLs did not seem to differ from MRI-invisible CLs in terms of histopathology or qMRI measures

• However: visible ones were significantly larger!
  • mean 13.3 ± 1.7 mm(2) versus 6.9 ± 1.3 mm(2); p = 0.001

• The number of MRI-visible lesions correlated with:
  – overall CL load in the brain slice (r = 0.96, p < 0.01)
  – overall percentage of demyelination (r = 0.78, p < 0.01)
    per hemispheric brain slice
DIR-pathology comparison

- 3D DIR sensitivity to detect CLs: 18%
  - 1.6-fold increase compared to 3D FLAIR!
  - At 1.5 Tesla
  - Improves to 37% with retrospective scoring; 2.0-fold higher than 3D FLAIR
- Leukocortical lesions: 83% using 3D DIR
  - (65% sensitivity for 3D FLAIR)
  - improved to 96% with retrospective scoring (91% for 3D FLAIR)
- Intracortical lesions: 3D DIR detected more than 2-fold more than 3D FLAIR
  - improved to >3-fold with retrospective scoring
- Specificity of 3D DIR: ~90%
Ex vivo sample imaging at Mayo Clinic: wet specimen

Formalin fixed CNS samples imaged in formalin or Fomblin

#130 – normal WM suppression
#190 – intracortical

#200 - intracortical
# 210 – intra/subcortical
Ex vivo sample imaging at Mayo Clinic: paraffin embedded specimen

MRI at 16.4 Tesla          H&E grayscale          H&E
Conclusions

• While pathology remains the gold standard for tissue studies, correlative MRI studies are gaining grounds (ex vivo MRI-pathology correlation, ex vivo-in vivo correlation)
• Most of our clinical knowledge is empirical, with ongoing efforts re: tissue-MRI correlation
• Lessons from the MSLP can be utilized in every day clinical practice using commonly available pulse sequences
• Clear need to define the role of newer pulse sequences in pathology-MRI correlations: new extension to MSLP studies
• Cortical lesion detection is improving, but is incomplete even with ex vivo modalities
• Advanced ex vivo imaging of wet and embedded specimen opens new doors to pathology-MRI correlations

THANK YOU!

Questions?
pirko@mayo.edu
Sporadic non-winter day in Rochester, Minnesota

Mayo Clinic Locations