Correlation of quantitative proton MR spectroscopy with local histology from stereotactic brain biopsy to evaluate heterogeneity of brain tumors

Steve H. Fung, MD¹, Edward F. Jackson, PhD², Samuel J. Hassenbusch III, MD, PhD³, Jeffrey S. Weinberg, MD³, Sanjay K. Singh, MD¹, Krista R. McAlee, RT², Brian K. Law, BS³, Ashok J. Kumar, MD¹

1. MD Anderson Cancer Center, Department of Diagnostic Imaging – Neuroradiology, Houston, TX
2. MD Anderson Cancer Center, Department of Imaging Physics, Houston, TX
3. MD Anderson Cancer Center, Department of Neurosurgery, Houston, TX

Certificate of Merit, 105th Annual Meeting of the American Roentgen Ray Society
May 15-20, 2005, New Orleans, Louisiana
Introduction

• Gliomas, especially high-grade gliomas such as anaplastic astrocytoma (AA) and glioblastoma multiforme (GBM), are spatially heterogeneous from loss of cell regulation, variation in metabolism and cell density.

• Therapeutic decisions are based on tumor grade, which is predictive of patient prognosis.

• Accurate tumor grading requires histological proof of area with highest anaplasia. Heterogeneity of gliomas presents a challenge for deciding where to biopsy.

• Number of biopsies taken is generally small, so sampling error can prevent accurate tumor grading.

• Need for image guidance to optimize sampling yield.
Grading for diffuse astrocytic neoplasm

- A - nuclear atypia
- M - mitoses
- E - capillary endothelial proliferation
- N - necrosis

WHO Grade = score + 1 (max 4)
Introduction (continued)

- T2-hyperintense regions correspond to areas of mixed tumor and vasogenic edema. Contrast-enhancing regions represent areas of blood-brain barrier breakdown.

- Region of contrast enhancement traditionally used for surgical target for active tumor, but tumor often infiltrate well beyond enhancing regions.
Introduction (continued)

- Proton MR spectroscopy ($\text{^1H-MRS}$) has been increasingly used for evaluating metabolic changes in brain tumors.
  - **Cho** (3.2 ppm) Increased from membrane synthesis in rapidly dividing tumor cells.
  - **Cr** (3.0 ppm) Metabolism marker; reactants in creatine kinase high-energy phosphate reaction. Decreased in gliomas.
  - **NAA** (2.0 ppm) Neuronal marker; synthesized in neuronal mitochondria from acetyl-CoA and aspartate. Decreased in gliomas.
  - **Lac-Lip** (1.1 to 1.6 ppm) Found in high-grade gliomas, lymphomas, metastases and radiation necroses.
• Potential of $^1$H-MRS to guide stereotactic biopsy only recently evaluated.

• Technical limitations of earlier studies, which used single-voxel techniques that provided a single aggregate spectrum representative of the entire tumor. Single-voxel techniques provide no information on local intratumoral variability of metabolic abnormality and extent of tumor infiltration.

• Earlier multi-voxel techniques have also been applied without direct reference to specific biopsy sites.

• Only recently have multi-voxel studies been attempted correlating localized spectral data to stereotactic biopsy locations:
Introduction (continued)
Retrospective study between 2002 and 2004 of 21 patients at MD Anderson with suspected or known primary brain tumors who had MR imaging and multi-voxel $^1$H-MRS as part of their preparation for image-guided stereotactic biopsy.

**Objective:**
Correlation of calculated local metabolite ratios from multi-voxel $^1$H-MRS and local histology obtained from stereotactic biopsy to

(1) determine the extent of heterogeneity present in brain tumors as reflected by variations in spectral data,

(2) ascertain if tumor type and grade can be accurately predicted, and

(3) explore the role of $^1$H-MRS in improving the yield of stereotactic brain biopsies by directing target areas toward regions of maximal spectral abnormality.
MR Imaging and Spectroscopy:

- MR imaging and multi-voxel $^1$H-MRS acquired within 24-hours prior to stereotactic biopsy using a 1.5-T whole-body clinical MR scanner (Signa, General Electric Medical Systems, Milwaukee, WI) with a standard head coil.

- Pre-contrast MR imaging with FLAIR FSE:
  TR 10002 ms, TE 157.5 ms, TI 2200 ms, 20 x 20 cm FOV, 256 x 160 matrix, 5 mm section thickness, 1.5 mm gap.
Methods

- Multi-voxel $^1$H-MRS using point-resolved spectroscopy (PRESS):
  TR 1000 ms, TE 144 ms, 20 x 20 cm FOV, 24 x 24 matrix, 0.83 cm x 0.83 cm x 1.5 cm voxel size, 1.0 cm³ resolution, 2 averages.
  Water and outer volume spatial suppression pulses applied to reduce spectral contamination.
• Post-contrast MR imaging with T1W SE with Gd-DTPA:
  TR 400 ms, TE 8 ms, 28 x 28 cm FOV, 256 x 192 matrix, 3 mm section thickness,
  1 mm gap.

• Followed by T1W 3D FSPGR with Gd-DTPA:
  TR 11.3 ms, TE 4.2 ms, flip angle 25°, 28 x 28 cm FOV, 256 x 192 matrix, 1.8 mm
  section thickness.
Methods (continued)

• Data from MRI and multi-voxel $^1$H-MRS then transferred off-line to a GE Advantage workstation (v4.1, General Electrical Medical Systems, Milwaukee, WI).

• At each $^1$H-MRS voxel, following metabolite peak areas integrated:
  • Choline (Cho, 3.2 ppm)
  • Creatine and phosphocreatine (Cr, 3.0 ppm)
  • $N$-acetylaspartate (NAA, 2.0 ppm)
  • Lactate and/or lipid containing compounds (Lac-Lip, 1.1 to 1.6 ppm)

• Following metabolite ratios calculated:
  • Cho/Cr
  • Cho/NAA
  • NAA/Cr

• Data then transferred to a frameless stereotactic image-guidance system (Stealth Station, Medtronic Sofamor Danek, Memphis, TN) for intraoperative visualization.
Methods (continued)

Image-guided Stereotactic Biopsy:

- Stereotactic biopsy performed using a Leksell stereotactic frame (Elekta, Stockholm) under local anesthesia and conscious sedation.
- Variable number (2-10) of sampling levels along each stereotactic biopsy tract, depending on tumor location and size, imaging and $^1$H-MRS characteristics, initial frozen section diagnosis and potential for obtaining higher grade material.
- At each sampling level, multiple biopsy specimens were taken at 12, 3, 6 and 9 o’clock positions; each specimen typically 3 mm x 2 mm x 2 mm.
- Parts of the specimens were used for frozen sectioning and the remainder fixed in neutral formalin and embedded in paraffin for further pathologic examination, including immunohistochemistry for cell-specific markers.
MR Spectroscopy and Histology Correlation:

- Software image reconstruction used to reconstruct stereotactic biopsy tract by aligning MR images to recorded snapshots from the screen of the frameless stereotactic image-guidance system.
- $^1$H-MRS dataset resampled to center a 0.83 cm x 0.83 cm x 1.5 cm voxel at each biopsy sampling level based on the coordinates obtained during surgery.
- Metabolite spectrum of each resampled voxel then analyzed, and ratios of Cho/Cr, Cho/NAA and NAA/Cr correlated to local histology from biopsy.
- Datasets were excluded when the stereotactic biopsy site was clearly out of the $^1$H-MRS volume plane, and when there was poor shimming and poor SNR.
Methods (continued)

Statistical Analysis:

- Analysis of variance (ANOVA) and pairwise Tukey-Kramer multiple comparisons used to test if metabolite ratios of biopsy samples of different tumor types and grades were statistically different.

- Spearman rank-order correlation used analyze correlation of metabolite ratios and corresponding local histology tumor grade in heterogeneous tumors.

- Differences resulting in $P$ values of less than 0.05 were considered statistically significant.
Results

19 of 21 patients had $^1$H-MRS data of sufficient quality to allow comparison of spectral characteristics with local histology:

- 1 WHO grade I glioma – pilocytic astrocytoma (JPA)
- 4 WHO grade II glioma –
  - 2 diffuse low-grade astrocytoma
  - 2 oligodendroglioma
- 5 WHO grade III glioma –
  - 2 anaplastic astrocytoma (AA)
  - 3 anaplastic oligodendroglioma (AO)
- 3 WHO grade IV glioma – glioblastoma multiforme (GBM)
- 4 Lymphoma
- 1 Gliosis
- 1 Radiation necrosis from treated GBM

Of the 19 patients, 56 biopsy locations with histology could be correlated to resampled $^1$H-MRS voxels centered at their respective biopsy sites.
Comparison of Local Histology to Cho/Cr Centered at Biopsy Locations

Grade of tumor from which biopsy was taken:
- gliosis
- lymphoma
- WHO I
- WHO II
- WHO III
- WHO IV
- necrosis

Local histology of biopsy:
- Gliosis
- Lymphoma
- JPA
- Astro
- Oligo
- AA
- AO
- GBM
- Necrosis
Comparison of Local Histology to Cho/NAA Centered at Biopsy Locations

Grade of tumor from which biopsy was taken:
- ○ gliosis
- △ lymphoma
- ◊ WHO I
- □ WHO II
- ▲ WHO III
- ▼ WHO IV
- △ necrosis

Local histology of biopsy:
- Gliosis
- Lymphoma
- JPA
- Astro
- Oligo
- AA
- AO
- GBM
- Necrosis
Comparison of Local Histology to NAA/Cr Centered at Biopsy Locations

Grade of tumor from which biopsy was taken:
- ○ gliosis
- ▲ lymphoma
- ◊ WHO I
- □ WHO II
- ● WHO III
- ▲ WHO IV
- ▲ necrosis

Local histology of biopsy:
- Gliosis
- Lymphoma
- JPA
- Astro
- Oligo
- AA
- AO
- GBM
- Necrosis
Results (continued)

Correlation of Metabolite Ratios with Histology

• ANOVA analysis demonstrated significant differences among the metabolite ratios of the different tumor types and grades with Cho/Cr ($P<0.0001$), Cho/NAA ($P=0.020$) and NAA/Cr ($P=0.002$).

• Tukey-Kramer pairwise comparisons of metabolite ratios of low-grade astrocytoma, oligodendroglioma, AA, AO and GBM failed to show any significant differences.

• Significant differences were found between
  - Cho/Cr of non-tumor (i.e. gliosis and radiation necrosis) and lymphoma ($P=0.038$)
  - Cho/Cr of lymphoma and WHO grade II glioma ($P=0.015$)
  - Cho/NAA of non-tumor and GBM ($P=0.036$)

• For NAA/Cr, no significant differences were found with pairwise comparisons.
Local histology of biopsy site:

- Gliosis
- Lymphoma
- JPA
- Astro
- Oligo
- AA
- AO
- GBM
- Necrosis

Cho/Cr and Cho/NAA Centered at Biopsy Locations
Conclusion

• Considerable heterogeneity of tumor was present from the biopsy specimens; 48% of patients had tumors that were histologically heterogeneous.

• Almost all biopsy locations yielding non-tumor, i.e. gliosis or radiation necrosis, had Cho/Cr and Cho/NAA < 2.

• Almost all biopsy locations yielding lymphoma had Cho/Cr > Cho/NAA.

• Except for some of the low-grade astrocytomas and oligodendrogliomas, biopsy locations yielding tumor including lymphoma had Cho/Cr or Cho/NAA > 1.5.

• General trend of increasing Cho/Cr and Cho/NAA was found with increasing local glioma grade. The exception was JPA, which had elevated Cho/Cr and Cho/NAA similar to high-grade gliomas despite its low-grade tumor classification.

• General trend of decreasing NAA/Cr was found with increasing local astrocytoma grade. Although trends in metabolite ratios were observed with increasing local glioma grade, the differences were not significant.
Conclusion (continued)

- Considerable intertumoral variability and overlap in metabolite ratios of astrocytoma, oligodendroglioma, AA, AO, GBM and lymphoma precluded accurate differentiation of tumor type and grade based on metabolite ratios alone.

- Presence of Lac-Lip elevation may be helpful in narrowing the diagnosis by suggesting the diagnosis of a high-grade glioma, especially GBM, lymphoma or radiation necrosis.

- Considerable amount of biopsy-confirmed tumor extended outside of contrast-enhancing regions within T2-hyperintense regions. In some cases, biopsy-confirmed tumor was found even at the outer margins of T2-hyperintense regions.

- $^1$H-MRS may be helpful in determining the full extent of tumor boundaries, which is important for optimizing margins for surgical resection and radiation therapy planning.
Conclusion (continued)

- Local intratumoral variations in Cho/Cr and Cho/NAA correlated with local histology grade in most instances, with biopsies obtained in regions of local maximum Cho/Cr or Cho/NAA generally yielding highest-grade histology.

Calculated probabilities for obtaining higher grade tumor if one biopsied only max Cho/Cr or max Cho/NAA:

- 100% for WHO grade II glioma
- 75% for WHO grade III glioma
- 50% for WHO grade IV glioma
- 100% for Lymphoma

Calculated probabilities for obtaining higher grade tumor if one biopsied only contrast-enhancing regions:

- 67% for WHO grade II glioma
- 78% for WHO grade III glioma
- 40% for WHO grade IV glioma
- 33% for Lymphoma

- All locations in this study where local maximum Cho/Cr or Cho/NAA coincided with region of contrast enhancement always yielded highest-grade histology.

- $^1$H-MRS in combination with contrast-enhanced MRI may improve stereotactic brain biopsy yield by providing metabolic information of possible higher grade histology imbedded within heterogeneous brain tumors.
Limitations of the Study

• Retrospective study with small number of patients in each series of tumor type.

• Study used 2D multi-voxel $^1$H-MRS technique. 3D multi-voxel technique permits better correlation of spectroscopy data with reconstructed stereotactic biopsy tract. Present MR technology also permits use of 3D multi-voxel $^1$H-MRS technique without significant compromise in scan time.

• Spectroscopy of contralateral brain parenchyma not used for normalization. Metabolite ratios used in other studies included Cho/nCr, NAA/nCr, Lac-Lip/nCr, and Cho/nCho.

• Study did not take into account degree of tumor infiltration. A tumor infiltration score can be used, i.e.
  0 – normal tissue
  1 – minimal or equivocal infiltration of tumor
  2 – mild infiltration of tumor
  3 – moderate infiltration of tumor
  4 – heavy infiltration of tumor
  5 – pure solid tumor