

ONLINE APPENDIX: ADDITIONAL IMAGE ACQUISITION DETAILS

The broad imaging study included sequences common to routine brain MR imaging studies, conventional and diffusion-weighted, in addition to advanced techniques like DSC for perfusion and DCE imaging for quantitating permeability. The specific acquisitions were chosen on the basis of hypothesized correlation with local pathology like cell density and were made purposefully broad to directly compare with the existing literature.

Routine anatomic sequences provide strong visualization of tumor and surrounding edema with general demarcation between healthy and diseased tissues. Because gliomas have generally increased cellularity, this contrast should also be indicative of cellular density.

Diffusion-weighted images were acquired using 4 b-values between 0 and 2000 and processed to generate ADC and exponential ADC maps.

Diffusion tensor imaging was similarly acquired with $b = 1200 \text{ s/mm}^2$ and 27 encoding directions and processed to map fractional anisotropy. The average diffusion coefficient from DTI was not used because it is redundant with ADC.

As an advanced imaging technique, DCE and DSC data have shown strong correlation with glioma pathology, including overall grade.¹ Because high-grade tumors tend to be more cellular, we expect derived parameters from DCE/DSC to correlate with cell density. DCE image series were recorded at TR = 1500 ms intervals following a 0.1-mmol/kg bolus of gadolinium contrast injected at 5 mL/s followed by saline. DSC was acquired with 60 phases at TR = 500 ms. DCE and DSC were processed using nordicICE with arterial deconvolution. An arterial input function was selected semiautomatically in the middle cerebral artery or anterior cerebral artery ipsilateral to the lesion. For DSC data, the arterial input function was measured in the superior sagittal sinus.

To compute the average tissue intensities for normalization, we drew a small ROI in homogeneous regions of 3 reference tissues: white matter, deep gray matter, and CSF. One set of ROIs was drawn for each patient and was used to scale all the images, and the position was verified by a neuroradiologist.

Normal White Matter Cell Density

A recent work by Roetzer et al² measured cell density using a postmortem histologic analysis of representative coronal brain slabs from adult patients with gliomas. They measured mean normal white matter cell density at 2581 [SD, 828] nuclei/mm². However, the histologic sections analyzed in their study were cut at 6- μm thickness. To correct to a value comparable with our measurements (with 4- μm sections), we used the Abercrombie method.^{3,4} The correction for the measured cell density is given by the ratio of section thickness plus nuclear diameter (H, in the equation below).

$$CD_{4 \text{ } \mu\text{m}} = CD_{6 \text{ } \mu\text{m}} \left(\frac{4 \text{ } \mu\text{m} + H}{6 \text{ } \mu\text{m} + H} \right).$$

We chose a nuclear diameter of 4.7 μm , the approximate size of an oligodendrocyte nucleus.⁵ This is a reasonable assumption because oligodendrocytes make up about 80% of the white matter cell population.⁶

Another method to estimate normal cell density would be to use estimates of total white matter cell count and white matter volume. Such estimates of cell number have been collected via the isotropic fractionator method,^{7,8} and estimates of white matter volume are available from analysis of imaging studies.⁹ From the ratio or number of cells per unit volume, there are formulas to estimate the density of cells that would be observed on a histologic section.^{3,4} From our calculations, this method yields only 877 nuclei/mm², considerably smaller than the values measured by Roetzer et al² as well as our own samples. Because of the methodologic similarities between the work of Roetzer et al and our own, we elected to work with normal values from their study, which we have reproduced in Table 2.

REFERENCES

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Online Table 1: Acquisition parameters for conventional imaging sequences^a

| | T1 | T1C ^b | T2 ^c | T2* | FLAIR ^d | SWAN ^e |
|-----------------------------|---------------------|---------------------|---------------------|------------------------|--------------------|---------------------|
| Section orientation | Axial | Sagittal | Axial | Axial | Sagittal | Axial |
| Pulse seq. name | MEMP | MEMP | FSE | 2D GRE (Gradient Echo) | 3D FSE | 3D FGRE |
| TR (ms) | 700 | 700 | 5800 | 700 | 7000 | 46 |
| TE (ms) | 10 | 11 | 76.512/80.832 | 15 | 124.923/131.379 | 23.06/23.5 |
| TI (ms) | NA | NA | NA | NA | 2071/2060 | NA |
| FA | 90° | 90° | 90°/125° | 20° | 90° | 15° |
| FOV (cm) | 16.5/22.0 | 16.5/24.0 | 19.6/23.8 | 16.5/22.0 | 25.6 | 20 |
| Matrix | 256 × 192 | 256 × 192 | 352 × 224 | 256 × 192 | 256 × 256 | 320 × 224 |
| BW (kHz) | 244.141 | 244.141 | 162.773 | 244.141 | 122.07 | 244.141 |
| Voxel size (mm) | 0.8594 × 0.8594 × 5 | 0.9375 × 0.9375 × 5 | 0.5469 × 0.5469 × 2 | 0.8594 × 0.8594 × 5 | 0.5 × 0.5 × 1.0 | 0.3906 × 0.3906 × 2 |
| Spacing between slices (mm) | 6.5 | 6.5 | 2 | 6.5 | 1/0.5 | 1 |
| No. of slices/volume | 23/24 | 20 | 85/95 | 23/24 | 154/166 | 68 |
| Total No. of slices | 23/24 | 20 | 85/95 | 23/24 | 158/308 | 68 |
| ETL | 1 | 1 | 8 | 1 | 140/211 | 6 |
| % Phase FOV | 75/100 | 75/100 | 70/85 | 75/100 | 100 | 100 |
| % Sampling | 100 | 100 | 100 | 100 | 100 | 69.1964 |
| Acquisition time (min:sec) | 1:51 | 2:47 | 4:50 | 1:45 | 8:55 | 3:48 |

Note:—SWAN indicates T2*-weighted angiography; FGRE, fast gradient recalled-echo; MEMP, multiecho multiplanar; FA, flip angle; BW, bandwidth; ETL, echo-train length; NA, not applicable; seq, sequence.

^a Two MR imaging scanners were used to acquire imaging data: a Signa HDxt ($n = 16$ patients in the final analysis) and a Discovery MR750 ($n = 7$). Parameters listed as #/# represent values for patients scanned with the Signa/Discovery, respectively.

^b A substitute TIC sequence from the same scanning session was used for analysis when the desired TIC sequence was lost due to a PACS malfunction. The replacement parameters for that 1 patient were the following FGRE 3D sequence: TR/TE, 8.124/1.756 ms; FA, 20°; matrix, 352 × 224; bandwidth, 195.312 kHz; voxel size, 0.5469 × 0.5469 × 1.8 mm; section spacing, 1.8 mm; 124 slices.

^c One patient had the following parameters for the T2-weighted image: TE, 86.076 ms; matrix, 256 × 192; bandwidth, 244.141 kHz; voxel size, 0.9766 × 0.9766 × 2 mm; % FOV, 80; ETL, 20.

^d Two patients scanned on the Discovery scanner had FLAIR sequences with substitute parameters: TE, 91.064 ms; FOV, 28 cm; voxel size, 0.5469 × 0.5469 × 1 mm.

^e One patient scanned on the Signa scanner had a SWAN acquisition with TR = 40.7 ms.

Online Table 2: Detailed list of physiologic (diffusion, dynamic susceptibility, dynamic contrast) MR imaging sequence parameters^a

| | DWI ^b | DTI | DCE ^c | DSC ^d |
|--------------------------------------|------------------------------------|---------------------------------------|-----------------------|-----------------------|
| Section orientation | Axial | Axial | Axial | Axial |
| Pulse seq. name | SE-EPI | SE-EPI | SPGR | GR-EPI |
| TR (ms) | 8000 | 10,175/10,000 | 3.1 | 1500 |
| TE (ms) | 99.7 | 90 | 1.1 | 25 |
| FA | 90° | 90° | 30° | 60° |
| FOV (cm) | 22 | 22 | 18 | 24 |
| Matrix | 128 × 128 | 128 × 128 | 256 × 160 | 128 × 160 |
| BW (kHz) | 1953.12 | 1953.12 | 488.281 | 1953.12/3906.25 |
| Voxel size (mm) | 0.8594 × 0.8594 × 3.5 | 0.8594 × 0.8594 × 3.5 | 0.9375 × 0.9375 × 3.5 | 0.9375 × 0.9375 × 3.5 |
| Spacing between slices (mm) | 3.5 | 2.5/3.5 | 3.5 | 3.5 |
| No. of slices/volume | 24 | 36 | 24 | 24 |
| Total No. of slices | 96 | 1008 | 1200 | 1440 |
| ETL | 1 | 1 | 1 | 1 |
| % Phase FOV | 100 | 100 | 75 | 100 |
| % Sampling | 100 | 100 | 100 | 100 |
| NEX | See below | 1 | 1 | 1 |
| B-values (\$/mm ²) (NEX) | 0 (1), 150 (1), 1000 (1), 2000 (2) | 1200 (1) (n = 27 encoding directions) | NA | NA |
| No. of phases | NA | NA | 50 | 60 |
| Acquisition time (min/sec) | 2:48 | 4:50 | 5:08 | 1:30 |

Note:—SE-EPI indicates spin-echo echo-planar imaging; GR-EPI, gradient recalled-echo echo-planar imaging; SPGR, echo-spoiled gradient echo; NEX, number of excitations averaged together to create a dataset; FA, flip angle; BW, bandwidth; ETL, echo-train length; NA, not applicable; seq., sequence.

^a Two MR imaging scanners were used to acquire imaging data: a Signa HDxt (n = 16 patients in final analysis) and a Discovery MR750 (n = 7). Parameters listed as #/# represent values for patients scanned the Signa/Discovery, respectively, together to create the dataset.

^b For DWI, 1 patient used a slightly different protocol with TR/TE = 8000/88 ms with 2 volumes acquired at 5-mm thickness. One patient had 45 slices per volume.

^c For DCE, 2 patients had a 15° flip angle with 5-mm section width and 16 slices per volume for 36 phases. One patient had a 220-mm FOV with 5-mm section thickness and 20 slices per volume for 60 phases.

^d For DSC, 3 patients had a modified flip angle of 90°, 5-mm section width, and 16 slices per volume. One patient also had a 12 × 128 matrix with 220-mm FOV.

Online Table 3: Model parameters used for predicting cell density^a

| Model | Specific Parameters | Description |
|-------------------|---|--|
| Decision tree | Complexity: tuned | Single decision tree |
| Random forest | No. of trees: 500 No. of variables (mtry): tuned Maximum terminal nodes: 44 | Breiman random forest algorithm |
| Neural network | Hidden layer nodes: tuned Decay: tuned Maximum iterations: 100 | Neural network with linear output for regression |
| Linear regression | Least-squares fitting | Linear model for regression and logistic regression model for classification |

^a Some specified hyperparameters were tuned during cross-validation by grid search, and others were fixed beforehand. A simple description of each algorithm is also included.

Online Table 4: Top performing variables for each imaging family within each fold of cross-validation as selected by the random forest variable importance listing^a

| Fold | Anatomic | Diffusion | Perfusion (DSC) | Permeability (DCE) |
|------------------------|------------|------------|-----------------|--------------------|
| 1 | T2 | FA | CBF | k_{ep} |
| 2 | T2 | FA | CBF | AUC |
| 3 | T2 | FA | CBF | AUC |
| 4 | T2 | FA | K2 | AUC |
| 5 | T2 | FA | K2 | k_{ep} |
| Final (% Increase MSE) | T2 (27.0%) | FA (18.0%) | CBF (11.3%) | AUC (16.1%) |

Note:—MSE indicates mean squared error; Inc, increase; k_{ep} , reverse transfer constant from DCE imaging; FA, fractional anisotropy.

^a The final 4-variable set was selected by most vote. Variable importance is measured as an increase in MSE when the variable is randomly permuted. A larger value indicates a larger importance in predicting CD.

Online Table 5: Top performing conventional variables from each fold of cross-validation listed in order of importance with each fold^a

| Fold | Variable 1 | Variable 2 | Variable 3 | Variable 4 |
|-------------------|------------|-------------|------------|--------------|
| 1 | T2 | T1C | T1 | FLAIR |
| 2 | T2 | T1C | T1 | FLAIR |
| 3 | T2 | T1 | T1C | FLAIR |
| 4 | T2 | T1C | T1 | FLAIR |
| 5 | T2 | T1C | T1 | FLAIR |
| Final (% Inc MSE) | T2 (26.7%) | T1C (18.9%) | T1 (10.3%) | FLAIR (9.4%) |

^a Four of the 6 conventional variables were chosen to maintain the same number as in conventional-plus-advanced imaging. For the final fixed variables, the variable importance is listed and a larger number indicates that the variable is more useful to the random forest algorithm.

Online Table 6: Average root-mean-square error for predicted-versus-observed cell density in cross-validation^a

| | All Variables (23 Inputs) | Variables Selected by RF Importance (4 Inputs) | All Conventional Variables (6 Inputs) | Variables Selected by RF, Conventional Only (4 Inputs) |
|----------------|------------------------------|---|--|---|
| Random forest | 1925 | 1975 | 2143 | 2099 |
| Linear | 2113 | 1957 | 2444 | 2386 |
| Neural network | 2672 | 2478 | 2470 | 2318 |
| Decision tree | 2555 | 2589 | 2377 | 2377 |

^a The variables used (columns) to train each predictive model are the same as in Table 4. “All Variables” is simply using all 23 imaging parameters of all 6 conventional sequences, whereas “RF Importance” and “RF Importance, Conventional” use the final 4 variable sets shown in Online Tables 4 and 5.