

**Diffusion tensor imaging of normal appearing white matter as a biomarker for radiation-induced late delayed cognitive decline**

Running title: Diffusion tensor imaging as a cognitive decline biomarker

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## **Conflicts of Interest Notification**

The authors declare that no actual or potential conflicts of interest exist.

## **Abstract**

### *Purpose*

To determine whether early assessment of cerebral white matter degradation can predict late delayed cognitive decline after radiation therapy (RT).

### *Methods and Materials*

Ten patients undergoing conformal fractionated brain RT participated in a prospective diffusion tensor magnetic resonance imaging (MRI) study. MRI was acquired prior to RT, 3 weeks and 6 weeks during RT, and 10, 30, and 78 weeks after starting RT. Diffusivity variables in the parahippocampal cingulum bundle and temporal lobe white matter were computed. Quality of life (QOL) survey and neurocognitive function tests were administered pre-RT and at post-RT MRI follow-ups.

### *Results*

In both structures, longitudinal diffusivity ( $\lambda_{||}$ ) decreased and perpendicular diffusivity ( $\lambda_{\perp}$ ) increased following RT, with early changes correlating to later changes ( $p < 0.05$ ). Radiation dose correlated with increase in cingulum  $\lambda_{\perp}$  at 3 weeks, and patients with >50% of cingula volume receiving >12 Gy had a higher increase in  $\lambda_{\perp}$  at 3 weeks and 6 weeks ( $p < 0.05$ ). Post-RT changes in verbal recall scores were linearly correlated with late changes in cingulum  $\lambda_{||}$  (30 weeks,  $p < 0.02$ ). By receiver operating characteristic curves, early cingulum  $\lambda_{||}$  changes predicted post-RT changes in verbal recall scores (3 weeks and 6 weeks,  $p < 0.05$ ). Neurocognitive test scores were significantly correlated with QOL survey results.

### *Conclusions*

The correlation between early diffusivity changes in the parahippocampal cingulum and the late decline in verbal recall suggests that diffusion tensor imaging may be useful as a biomarker for predicting late delayed cognitive decline.

### **Key Words**

diffusion tensor imaging; neurocognitive function; late-delayed effects; biomarker; quality of life

### **Ethical Guidelines**

This study was prospectively approved by the University of Michigan Medical School Institutional Review Board. Data and safety monitoring was performed by the University of Michigan Radiation Oncology Department and reported to the University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Board. Patient confidentiality was maintained during all phases of the study.

## Introduction

A limitation on radiation therapy (RT) for cranial tumors is toxicity to the central nervous system. Radiation induced brain injury is traditionally divided into three temporal phases: acute, early delayed, and late delayed. While acute and early delayed effects are transient, late delayed injury is usually permanent and is characterized by necrosis and histopathological abnormalities. Severe late delayed effects are typically seen 6 or more months after RT and at doses greater than 60 Gy.<sup>1</sup> However, permanent late delayed cognitive deterioration can occur at doses less than 60 Gy. This late delayed cognitive decline is observed without necrosis or lesions visible on T1- and T2-weighted magnetic resonance imaging (MRI).<sup>2</sup> It has been psychologically characterized as declines in short-term memory, attention, and executive function.<sup>3</sup> The effect of late delayed cognitive decline on quality of life has become increasingly important as survival times lengthen.<sup>4</sup> Chang et al. have demonstrated that the risk of neurocognitive decline in patients receiving whole-brain RT and stereotactic RT is greater than in those receiving stereotactic RT alone;<sup>5</sup> therefore early tissue biomarkers of neurocognitive decline would be beneficial for guiding radiation delivery and improving outcomes.

Our understanding of what causes late delayed cognitive decline is still limited. Traditional theories have considered radiation-induced encephalopathy to be exclusively a result of damage to cells of either oligodendrocyte or vascular endothelium lineage.<sup>1</sup> Accumulating evidence now indicates that a single target of radiation is unlikely, and that astrocytes, microglia, neurons, and neural stem cells are also involved in a long-term, multi-factorial, and dynamic process.<sup>1, 6</sup> However,

as a proximal cause of late neurocognitive dysfunction, white matter damage as manifested by demyelination and axonal degradation most likely plays a role. In 2001, Akiyama et al. observed demyelination in a rat model of radiation-induced late delayed neurocognitive decline.<sup>7</sup> In humans, numerous cross-sectional studies have correlated white matter degradation with cognitive decline in adult survivors of childhood cancers.<sup>8-10</sup> However, these studies were done retrospectively and years after treatment. A prospective longitudinal study can better address how white matter changes after brain irradiation and whether the changes correlate with late delayed cognitive decline.

One of the best methods for evaluating white matter degradation is diffusion tensor magnetic resonance imaging (DTI). DTI makes use of the fact that within the brain, water molecules preferentially move along the long axis of myelinated fiber bundles, leading to unequal diffusion (anisotropy) in that direction. By quantifying the magnetic resonance signal of water molecule motion in multiple directions, DTI produces a 3-dimensional image of the diffusivity characteristics of the brain. DTI has been shown to be more sensitive than T1- and T2-weighted MRI for detecting subtle white matter damage.<sup>11</sup> We anticipated that DTI might be able to detect white matter degradation associated with late delayed cognitive decline not visible by traditional MRI modalities.

Few studies have used DTI to investigate the effects of radiation on the human brain longitudinally. In 2008, Haris et al. published longitudinal data showing decreased anisotropy in higher dose areas of subcortical white matter.<sup>12</sup> Our previous study tracked DTI changes in the corpus callosum of 25 patients,

showing significant and dose dependent changes in diffusivity as early as 4 weeks after finishing radiotherapy.<sup>13</sup> However, these previous longitudinal studies did not examine the functional consequences of radiation-induced white matter damage. Both evaluated white matter structures chosen for ease of contouring, and neither measured neurocognitive function, leaving the link between white matter damage and functional impairment incomplete.

The purpose of the present work was to examine the connection between white matter degradation and radiation-induced late delayed cognitive decline. We hypothesized that early radiation-induced changes in white matter integrity would progress into more advanced white matter degradation and cause late delayed cognitive decline; thus early DTI changes could be used as a biomarker for late delayed neurotoxicity. To test this hypothesis, we performed serial DTI and neurocognitive tests on patients before and after radiotherapy treatment. We aimed to address three questions: (1) how the DTI characteristics of normal appearing white matter change after radiotherapy, (2) how these changes are related to radiation dose, and (3) whether these changes are correlated to neurocognitive function.

## **Materials and Methods**

### *Study design*

Ten patients with low-grade or benign tumors were enrolled in a prospective, IRB approved, clinical MRI study. All patients underwent a standard, 6-week course of daily fractionated conformal cranial RT with a median dose of 54 Gy



to 95% of planning target volume (Table 1). Overall function was assessed prior to RT with the Karnofsky Performance Status scale (KPS), the Folstein Mini-Mental State Exam (MMSE), and RTOG neurological function class. All patients had a KPS score of  $\geq 80$ , MMSE score  $\geq 27$ , and neurological function class of 1 or 2, indicating high overall function. KPS, MMSE, and neurologic function class were also assessed at each scan time point after RT.

#### *Diffusion tensor MRI*

Patients underwent an MRI scan at 6 time points: 1-2 weeks pre-RT, 3 weeks into RT, 6 weeks into RT (end of RT), and 10 weeks, 30 weeks, and 78 weeks after starting RT. All MRI scans were done on a 1.5T scanner (GE Healthcare, Milwaukee, WI). MRI series included T1- and T2-weighted images, DTI, and post-contrast T1-weighted images. DTI was acquired using a spin-echo echo-planar imaging sequence with a repetition time of 10,000 ms, echo time of 70 ms, 360 mm<sup>2</sup> field of view, 128 × 128 matrix, and 4 mm slice thickness, with a 0 mm gap. For each axial slice, diffusion-sensitizing gradient encoding with a diffusion weighting factor of  $b = 1,000$  s/mm<sup>2</sup> was applied in nine non-collinear directions, and one set of null images with  $b = 0$  s/mm<sup>2</sup> was acquired.

#### *Image registration and processing*

Diffusion tensor images were co-registered to the post-contrast T1-weighted image set acquired pre-RT. The diffusion-weighted images were registered using mutual information and affine transformation, which included 12 parameters (9 for 3 × 3 rotation and shearing matrix and 3 for translation). Similarly, the treatment

planning computed tomography scan and spatially distributed radiation dose maps were co-registered to the post-contrast T1-weighted image set acquired before RT.

### *DTI analysis*

DTI was used to assess the structural changes in the white matter fiber tracts. Several diffusion indices can be calculated from the diffusion tensor. Two commonly reported indices are the trace diffusivity (Dtr) and the fractional anisotropy of diffusion (FA). Dtr, the sum of the diffusivities in the three orthogonal directions, is an orientation-independent measure of the total displacement of water molecules. FA is a unitless index that measures the degree of anisotropic diffusion. FA values are between 0 and 1, with a greater value denoting greater anisotropy. Additional information can be provided by eigen-diffusivities ( $\lambda_1$ ,  $\lambda_2$ , and  $\lambda_3$ ) along the three principal directions.<sup>14</sup> In the brain, the largest eigen-diffusivity,  $\lambda_1$ , represents water diffusion along the direction parallel to the axonal fibers, hereafter labeled  $\lambda_{||}$ , which is sensitive to axonal injury and astrogliosis but not demyelination.<sup>15, 16</sup> The  $\lambda_2$  and  $\lambda_3$  components denote diffusivities in the two orthogonal directions perpendicular to the axonal fibers. The mean of  $\lambda_2$  and  $\lambda_3$  is termed  $\lambda_{\perp}$ , which is sensitive to demyelination.<sup>15, 17</sup> Maps of Dtr, FA,  $\lambda_{||}$ , and  $\lambda_{\perp}$  were calculated using FIAT, an imaging software package developed in house.<sup>18</sup>

### *Volumes of interest*

Normal-appearing white matter (i.e., tissue that appeared normal on T1-weighted and T2-weighted MRI) was studied in the parahippocampal cingulum bundle and the subcortical temporal lobe white matter. Diffusivity differences in these structures have previously been associated with cognitive impairment.<sup>19, 20</sup>

The volumes of interest were contoured on multiple axial levels using a combination of T1-weighted and FA images acquired prior to RT (Figure 1). Hippocampal gray matter was contoured first (not used for DTI indices). Parahippocampal cingulum bundle was defined as cingulum white matter at the same axial levels as the hippocampus with a pre-RT FA value  $\geq 0.250$ , a value chosen as a reasonable lower bound for white matter structures based on prior experience.<sup>13</sup> Temporal lobe white matter was contoured at the same axial levels with the assistance of auto-segmentation in FIAT. The volumes were copied onto pre-RT T2-weighted images and edited to exclude any tumor or peritumoral edematous tissue. Volumes were then transferred to co-registered images from later time points, and linearly translated to ensure good fit over the structures of interest and maximizing mean contour FA. Because FA was used for volume adjustment, it was not included in analysis.

Mean values of  $\lambda_{||}$ ,  $\lambda_{\perp}$ , and Dtr were obtained for each structure at each time point from the DTI maps. Primary outcomes evaluated were the percent changes in diffusivity indices from pre-RT to later time points.

Fraction of volume receiving  $\geq 12$  Gy ( $\%V_{12}$ ) and mean dose (volume-weighted average dose) to each structure was also obtained from the co-registered radiation dose map.  $V_{12}$  was chosen due to QUANTEC recommended use in normal tissue dose limits for radiosurgery.<sup>21</sup> The linear-quadratic model was applied on a voxel-by-voxel basis to find the biologically corrected dose using  $\alpha/\beta$  ratio of 2.50.<sup>22</sup> Hereafter, the biologically corrected doses will be referenced as “dose”.

*Neurocognitive function tests*

All patients underwent standardized neurocognitive function tests prior to RT and at the post-RT follow-ups. The tests included the Hopkins Verbal Learning Test (verbal recall), the Controlled Oral Word Association Test (verbal fluency), and Trail Making Tests A & B (executive function).<sup>3</sup>

Late delayed cognitive decline was defined as the difference between score at 78 weeks compared to post-RT maximum score (at 10 weeks or 30 weeks).

Previously published data has shown that the average intra-subject test-retest variability of these neurocognitive tests is approximately  $\pm 0.25$  times the age-matched standard deviation;<sup>23-25</sup> therefore, a decline in neurocognitive test score greater than 0.5 times the age-matched standard deviation was considered neurocognitive decline. This is similar to thresholds used in previous large clinical trials.<sup>5, 26</sup>

Along with the neurological function tests, two standardized quality of life questionnaires (EORTC QLQ-C30 and EORTC QLQ-BN20)<sup>27, 28</sup> were administered prior to RT and on the same days as the post-RT MRI scans. After the QOL results were correlated to neurocognitive test scores, 5 questions related to self-assessment of cognitive performance were chosen retrospectively for additional comparison to neurocognitive test scores (Table 5). Due to its post-hoc nature, the latter comparison was considered significant at  $p < 0.01$ .

#### *Statistical analysis*

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS Software Products, Chicago, IL). A pairwise two-tailed Student's *t* test was used to evaluate differences between volumes and across time points. Associations

between DTI indices, time of MRI scan, cognitive test scores, and radiation dose were evaluated using Pearson's product-moment correlation. Receiver operating characteristic analysis was used to evaluate the predictive value of DTI changes, age, and radiation dose on cognitive test scores. Results of statistical tests were considered statistically significant at  $p < 0.05$ .

## Results

### *Clinical and radiological findings*

None of the patients exhibited tumor progression by 30 weeks after the start of radiation. Patient number 8 exhibited progression after 30 weeks and did not contribute DTI data after that time. No patient showed radiation-induced lesions on T2-weighted or pre- and post-gadolinium T1-weighted images up to 78 weeks. There were no interval changes in tumors or tumor-related hyperintensities. All patients maintained a KPS score  $\geq 80$ , MMSE score  $\geq 26$ , and RTOG neurological function class of 1 or 2, indicating that major neurological function was preserved.

### *Dose delivery*

Mean  $\%V_{12}$  and average mean dose to contoured structures across all patients are reported in Table 2. There was no significant difference between left and right hemisphere doses among paired structures.

### *Temporal changes in DTI characteristics*

Decreases in longitudinal diffusivity ( $\lambda_{||}$ ) and increases in perpendicular diffusivity ( $\lambda_{\perp}$ ) from pre-RT values were observed in the cingula and temporal lobe white matter beginning 3 weeks after starting RT and remaining until 78 weeks

after starting RT, indicating white matter degradation (Table 3, Figure 2). Changes in  $D_{tr}$  from pre-RT were never more than  $\pm 2\%$  and not significant at any time point in any structure.

In the cingula, the increase in  $\lambda_{\perp}$  compared to pre-RT values was significant at the scans 6 weeks after the start of RT and later, and the decrease in  $\lambda_{\parallel}$  was significant at scans 10 weeks and later. By 78 weeks, mean  $\lambda_{\parallel}$  in the cingula had decreased 4.8% and mean  $\lambda_{\perp}$  had increased 6.6%. Additionally, the percent change in cingula  $\lambda_{\parallel}$  at 6 weeks after starting RT was positively correlated with the percent decrease in cingula  $\lambda_{\parallel}$  at 30 weeks after starting RT, indicating that early changes predicted late changes ( $R = 0.698, p < 0.05$ , Figure 3).

In the temporal lobe, the magnitudes of the changes were smaller than those in the cingula; by 78 weeks after the start of RT, mean  $\lambda_{\parallel}$  in the temporal lobe had decreased 2.4% and mean  $\lambda_{\perp}$  had increased 2.8%. Temporal lobe changes in  $\lambda_{\parallel}$  and  $\lambda_{\perp}$  were significant at 30 weeks and 78 weeks respectively after starting RT.

#### *Dose dependency*

We evaluated the effect of radiation dose to white matter structures on changes in  $\lambda_{\parallel}$  and  $\lambda_{\perp}$  after beginning RT. We found that the mean dose to cingula was positively correlated to the increase in  $\lambda_{\perp}$  at 3 weeks into RT, when approximately one-half of the prescribed radiation dose had been received ( $R = 0.493, p < 0.05$ , Figure 4). By 6 weeks after starting RT, the trend remained but the correlation was not significant ( $R = 0.376, p = 0.12$ ). At 3 weeks and 6 weeks into RT, patients with a cingula  $\%V_{12}$  greater than 50% had an average increase in  $\lambda_{\perp}$  of 5.3% and 6.0% respectively, while those with a cingula  $\%V_{12}$  less than 50% had little change in  $\lambda_{\perp}$  ( $p$

< 0.03, Figure 5). We found no significant correlation between mean dose and changes in cingulum  $\lambda_{||}$  or temporal lobe white matter diffusivity.

### *Neurocognitive function changes*

Mean post-RT Z-score changes on the neurocognitive function tests are displayed in Table 4. On average, 33% of patients exhibited neurocognitive decline, a proportion very similar to those found in previous large clinical trials of brain radiotherapy.<sup>5, 26</sup> Because of tumor progression, patient 8 was not included in neurocognitive function analyses. To check for confounding correlations, we confirmed that age was not significantly correlated to Z-score change after RT.

Diffusivity index changes were correlated to neurocognitive test results. By Pearson's product-moment correlation, changes in cingula  $\lambda_{||}$  at 30 weeks were significantly correlated with post-RT Z-score change in HVL T Total Recall ( $R = 0.730, p < 0.02$ , Figure 6). Receiver operating characteristic curves suggested that early changes in cingula  $\lambda_{||}$  predicted neurocognitive decline in HVL T Total Recall (Table 6). At 3 and 6 weeks after starting RT, changes in cingula  $\lambda_{||}$  significantly predicted decline in HVL T Total Recall, and changes in PCB  $\lambda_{||}$  at 30 weeks showed a trend towards prediction. However, wide confidence intervals indicate that a larger study is needed to determine the actual predictive value of this measurement. Scores on neurocognitive tests besides HVL T Total Recall did not correlate with DTI indices; however, all three patients with late delayed decline in Total Recall also declined in Controlled Oral Word Association, and one of three declined in Delayed Recall. Changes in cingula  $\lambda_{\perp}$  and temporal lobe DTI indices did not correlate with neurocognitive score changes.

We considered the relationship between radiation dose to the structures and neurocognitive function. Mean doses to cingula, temporal lobe white matter, or hippocampi were not significantly related to changes in neurocognitive test scores. %V<sub>12</sub> of structures was also not significantly related to changes in neurocognitive test scores. This indicates that change in cingula  $\lambda_{||}$  was a better predictor of late delayed cognitive decline than structure dose alone.

In order to determine the impact of cognitive decline on patient quality of life, we looked at the relationship between neurocognitive test scores and EORTC QLQ-C30 and EORTC QLQ-BN20 survey results. Scores on the Hopkins Verbal Learning Test Total Recall component and Trail Making Tests A&B were significantly correlated to the QOL survey scores ( $p < 0.05$ ; Table 5). Scores on the Hopkins Verbal Learning Test Total Recall component were also significantly correlated with responses on 5 questions chosen from the quality of life questionnaires to represent self-assessment of cognitive performance, at a higher significance threshold for post-hoc analysis ( $p < 0.01$ ; Table 5).

## Discussion

In this study, we compared  $\lambda_{||}$ , an imaging biomarker of white matter axonal integrity, with neurocognitive function following cranial RT. Changes in cingula  $\lambda_{||}$  at 30 weeks after starting RT were correlated with neurocognitive function changes, and decreases in cingula  $\lambda_{||}$  at 3 weeks and 6 weeks after starting RT were predictors for neurocognitive decline. Furthermore, changes in cingula  $\lambda_{||}$  at 6 weeks predicted further changes in cingula  $\lambda_{||}$  at 30 weeks. These findings are consistent



with our hypothesis that early white matter degradation eventually develops into injury substantial enough to cause late delayed cognitive decline. They also support our proposal that by identifying patients on a steeper early “trajectory” of white matter degradation, one may be able to predict which patients are at risk for radiation induced neurocognitive decline. However, while a correlation was observed between radiation dose and white matter diffusivity changes and between diffusivity changes and neurocognitive function, radiation dose to structures did not predict neurocognitive function changes, **which does not support the use of dose-sparing techniques to prevent neurocognitive decline.** This may be because individual dose tolerances due to genetic factors or other susceptibilities have a larger effect on neurocognitive decline risk, supported by the fact that in the general population only 30-60% of patients receiving cranial radiation experience neurocognitive sequelae<sup>2,3</sup> **Also, analysis of mean dose and %V<sub>12</sub> alone is an introductory approach, and a more complex study of normal tissue complication probability using a complete dose-volume histogram may reveal a dose dependent interaction in a larger, more homogeneous study population. Even if specific structures for dose sparing are not identified, this does not preclude the use of DTI as a biomarker for at-risk patients to receive prophylactic pharmaceutical or neuropsychological intervention; Gehring et al. review the current research in these fields.<sup>29</sup>** As none of our patients received chemotherapy, we are unable to say how that may affect white matter injury or neurocognitive decline.

One consideration in the validity of our study was the effect of tumor progression itself on neurocognitive function change. Meyers and Hess have

demonstrated that tumor progression is a risk factor for neurocognitive decline, and that cognitive deterioration precedes progression by 6 weeks on average.<sup>30</sup> However, continued clinical surveillance showed that no patient in our study had tumor progression less than one year after follow-up ended, implying that the observed cognitive decline was radiation-induced and not due to tumor progression.

The observed post-RT decreases in  $\lambda_{||}$  and increases in  $\lambda_{\perp}$  are similar to previous observations of RT-induced white matter changes.<sup>12, 16</sup> While increases in vascular permeability may increase overall diffusion and reduce anisotropy,<sup>11</sup> we believe that changes observed were primarily due to white matter damage, due to the lack of increase in  $D_{tr}$  that would be expected if overall diffusion was increased post-RT.

As a supplement to our major questions, we considered the clinical relevance of late delayed cognitive decline and found that scores on some neurocognitive function tests were correlated to quality of life survey results and to patients' self-assessment of cognition. This indicates that cognitive function changes as measured on standardized tests are substantial enough to cause appreciable detriment to quality of life. Quality of life has been increasingly recognized as an important outcome in the treatment of CNS tumors.<sup>3</sup> We propose that resources would therefore be well spent on understanding the causes, risk factors, and prevention of late delayed cognitive decline.

Future studies are intended to examine multiple white matter structures and to consider the effect of dose gradients within structures and the influence of radiation field design. The results of this study are encouraging for the development of DTI as

a biomarker for radiation damage and late delayed cognitive decline. Our ultimate goal is to create better prediction metrics and improve applicability to individual patients.

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## Figure Legends

*Figure 1.* Example of volume locations from patient 10. Left: Axial pre-RT T1-weighted image. Right: Axial pre-RT FA image (white is high anisotropy). Volumes outlined in black. Medial to lateral: Parahippocampal cingulum bundle, hippocampal gray matter, temporal lobe white matter.

*Figure 2.* A: Changes in mean  $\lambda_{||}$  over time. B: Changes in mean  $\lambda_{\perp}$  over time. Solid lines correspond to cingula, dashed lines to temporal lobe. Error bars are  $\pm$  standard error. \*Significant change from pre-RT values.

*Figure 3.* Linear regression plot of percent change in cingula  $\lambda_{||}$  at 6 weeks to percent change in cingula  $\lambda_{||}$  at 30 weeks. Each dot represents one patient's cingula.  $R = 0.698, p < 0.05$ .

*Figure 4.* Linear regression plots of percent change in cingulum  $\lambda_{\perp}$  to dose at 3 weeks after starting RT. Each dot represents right or left cingulum in one patient.  $R = 0.493, p < 0.05$ .

*Figure 5.* Box plot showing range of percent change in cingula  $\lambda_{\perp}$  at 3 weeks and 6 weeks in patients with  $\%V_{12}$  greater than and less than 50% of cingula volume.

Open box is  $\%V_{12} > 50\%$  ( $n = 6$ ), shaded box is  $\%V_{12} < 50\%$  ( $n = 4$ ). Box represents 25<sup>th</sup> to 75<sup>th</sup> percentile, band is median and whiskers are full range of values.

Difference at 3 weeks,  $p < 0.03$ , at 6 weeks,  $p < 0.01$ .



*Figure 6.* Linear regression plot of percent change in cingula  $\lambda_{||}$  at 30 weeks to HVL Total Recall Z-score drop. Each dot represents one patient's cingula.  $R = 0.730, p < 0.02$ .