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Defining the True Extent of Glioblastoma Based on Probabilistic Tractography and FLAIR Images

PhD Dissertation Booklet

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List of publications relevant to the candidate's Dissertation:

I.David Kis, Laszlo Szivos, Mark Rekecki, Bayan Shukir, Adrienn Mate, Katalin Hideghety and Pal Barzo. Predicting the True Extent of Glioblastoma Based on Probabilistic Tractography. *Front. Neurosci.* 2022:16:886465. https://doi.org/10.3389/fnins.2022.886465. **IF: 3.2**

II.Bayan Shukir, Laszlo Szivos, Pal Barzo, and David Kis. 2025. Preoperative FLAIR Images for Identifying Glioblastoma Boundaries. https://doi.org/10.1186/s12880-025-01839-2. **IF: 3.2**

Objectives

- The aim of both studies were identifying the true extent of glioblastoma!
- Provide new insights into a critical aspect by evaluating the reliability of two distinct approaches: 1, probabilistic tractography and 2, preoperative FLAIR imaging
- Probabilistic tractography applied to map tumor invasion pathways, while examining preoperative FLAIR MRI as a predictor of glioblastoma extent

Background

- Glioblastoma is the most aggressive and fast-growing tumor of the CNS. Classified as G4 (WHO 2021).
- Majority of patients die within 16–20 months after the diagnosis
- Maximal safe resection (>90%) followed by the Stupp protocol considered as standard treatment procedure.
- Although surgery provides the best survival rate, the recurrence is almost inevitable.
- Theoretically, if the residual tumor volume could be minimized better therapeutic effects and longer survival would be possible.
- Therefore, accurately identifying the true extent of glioblastoma provides:

1, improving survival outcomes, and 2, minimizing the risk of severe, permanent

neurological deficits associated with surgery.

- Previous studies shown that the tumor mass identifiable on the MRI images does not correspond to the true extent of the tumor!
- Although the tumor mass and infiltrative part form one structural unit, only the tumor mass can be visualized easily on standard MRI images (fig 1, A)
- The surgical resection aiming at removing the tumor mass, visible on the MRI.

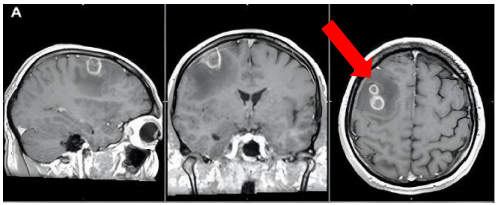

Figure 1: A. The preoperative CE-T1 image. Irregular ring-shaped contrast enhancement with a hypointense necrotic core, surrounded by prominent peritumoral edema and mass effect (red arrow)

- Due to glioblastoma's infiltrative nature, tumor cells can be present anywhere in the brain, e.g. (5-10) cm from the CE border.

- The recurrence usually originates from these infiltrative tumor cells (fig 1, B)

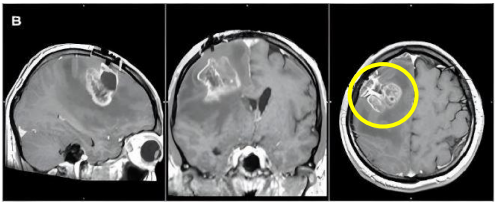

Figure 1: B. The size and the location of the tumor recurrence on CE-T1 (yellow circle). The main part of the recurrence is far away from the original tumor location

Clinical approaches

- Better survival can be accomplished if the resection margins exceeds the CE border.

- Tractography is an advanced application of diffusion MRI. The direction of the diffusion

movement of water molecules in the brain can be reconstructed.

- The major drawback of tractography is that the diffusion direction can be uncertain in several important brain regions (e.g., white and gray matter border, gray matter, basal ganglia, crossing fiber tracts, tumor, peritumoral edema) and, therefore, the result is unreliable
- To overcome this limitation Probabilistic tractography algorithm was used.
- In case of tumor, 1, it can potentially identify white matter fiber tracts that are infiltrated by the tumor (representing the connectivity distribution of the seed region). As well as, 2, the results can be thresholded to exclude the highest number of false positive and false negative results.

On the other hand,

- FLAIR imaging is believed to be more sensitive than CE-T1 for detecting infiltrated tumor cells; (i.e. FLAIR

hyperintensity often exceeds beyond CE lesions).

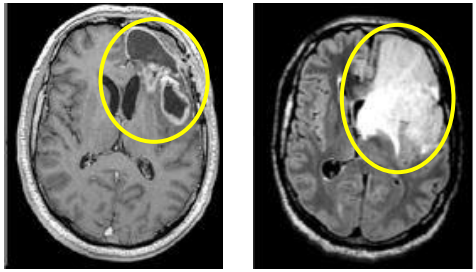

Figure 2. Radiographic features of glioblastoma on MRI. Left: post-gadolinium contrast with dense rim enhancement; Right: axial flair showing extensive vasogenic edema causing mass effect on the left lateral ventricle.

- However, studies demonstrated that the hyperintense region observed on FLAIR imaging does not primarily indicate tumor presence, but rather than reflects a composite signal of both microscopic tumor cells and edema
- Despite the effectiveness of FLAIR as imaging tool to localize glioblastoma, its prognostic value remains unclear!

- Despite advancements in imaging, no objective measurement for predicting the true extent of glioblastoma has been established yet!
- Our studies aimed to establish a clinically reliable and applicable imaging protocol for pre-surgical planning by evaluating the sensitivity and specificity of probabilistic tractography and FLAIR preoperatively.

Methodology

- The methodology of both studies followed the same pattern. There were minor adjustments in patient population, data processing techniques, and imaging protocols in FLAIR study.
- A total of 20 adults (>18 years) patients were screened and enrolled to the both studies.
- Inclusion criteria: 1, all patients diagnosed with glioblastoma or grade 3 anaplastic oligodendroglioma. 2, underwent either subtotal or total tumor resection during the primary surgery. 3, preoperative DTI and

FLAIR scans in addition to the routine head MRI protocol. 4, Follow-up MRI scans acquired every 3 months.

- Exclusion criteria: 1, loss to follow-up or 2, partial surgical resection.
- The following diagram summaries the preprocessing steps and data analysis calculations followed in both studies.

MRI Acquisition:
Total of 20 high-grade glioma patients. Pre-and post-CE high-resolution 3D axial FSPGR-T1 weighted, DTI, and preoperative FLAIR images
Mask Definition: Primary tumor masks and cortical and subcortical white matter masks
CORTICAL AND CUBCORTICAL masks:
2 standard atlases; JHU ICBM–DTI–white matter–81 (white matter into 54 regions) and AAL3 (cortex into 84 regions). Fig 1 A, B
TUMOR MASKS: pre- and postoperative CE-T1, and preoperative FLAIR (manually)
Image Preprocessing: Raw MRI files converted to NIfTI format using Chris Rorden's MRICron software. For tractography study: preprocessing steps, e.g. Eddy current correction, skull stripping, tensor reconstruction applied to DTI
For each patient sensitivity and specificity were determined at 13 different threshold levels of the tractography output. This step was wasn't necessary for FLAIR study
Sensitivity = A / (A + C)
Specificity = D / (D + B)
PPV= A / (A+B)
NPV= D /(D+C)
Probabilistic tractography and FLAIR study
Data analysis
True Positives (A): Brain regions overlapping with both the TC (or FLAIR mask) and the tumor recurrence mask.
False Positives (B): Brain regions overlapping only with the TC (or FLAIR mask), but not with the tumor recurrence mask.
False Negatives (C): Brain regions overlapping only with the tumor recurrence mask, but not with the TC (or FLAIR mask).
True Negatives (D): Brain regions not overlapping with either the TC (or FLAIR mask) or the tumor recurrence mask.
Tractography: DTI images used as seed region enabling the identification of white matter pathways infiltrated by the tumor.
Thresholding: To minimize low-probability connections (potential false positives) connectivity thresholds applied at 1-90% levels to define TC maps. Fig 2-4
Registration: each patients' DTI, FLAIR, and pre- and post-CE-T1 image aligned to the MNI152 1mm standard T1 brain template using FLIRT algorithm
FLAIR: Preoperative FLAIR tumor masks overlapped onto tumor recurrence tumor masks it was then how many brain regions covered by both tumor masks. Subsequently defining sensitivity and specificity Figs. 5 and 6

Results

- There were 2 neurological deficits observed within the two studies: 1, hemiparesis (35%) and, 2, speech disturbances (25%).
- Preoperatively, 40% did not exhibit severe neurological symptoms. The average Avg. KPS = 73% preoperatively, improving to 80% at 2 months postoperatively.

Probabilistic Tractography study

- Patient Characteristics (n=20)
- Data analyzed: 18 patients
- Sex ratio (F:M): 1:3
- Median age: 51.5 yrs
- Age 45–60 yrs: 50%
- Tumor location: Left hemisphere (75%), Frontal lobe (45%)
- Histology: 4 patients = Grade 3 anaplastic oligodendroglioma (20%)

Table 1: The group average sensitivity and specificity values (with standard error of the mean) are listed in this table at each threshold level.

*As the threshold increases the sensitivity decreases and the specificity increases. The highest values can be seen at 1 and 5% threshold levels.

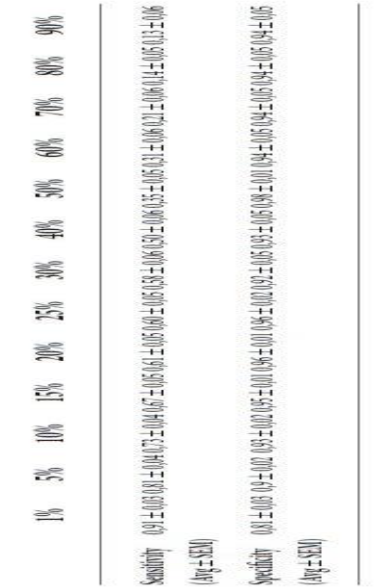

Table 1:The sensitivity and specificity values are listed in this table at each threshold level of the three example cases.

A: The results of Patient 14 are in concordance with the group average sensitivity and specificity values. B: The sensitivity and specificity of Patient 11. Please note that the specificity is high but the sensitivity is low even at 1% threshold level. This is because there is a smaller part of the recurrent tumor at the original site, which is covered by the TCs, therefore the ratio of the false positive results is low. The main part of the recurrence is far away from the original site, and this will result in a high false negative result. C: The results of Patient 13 are summarized in this table. The specificity is 100% at all threshold level while the sensitivity is very low even at 1%. This is due to the extensive local tumor recurrence which overlaps totally with all TCs It also exceeds its borders and affects several other brain regions resulting in a high rate of false negative results.

There were two outliers in the study group. These 2 patients showed remarkably lower sensitivity than the rest of the patients.

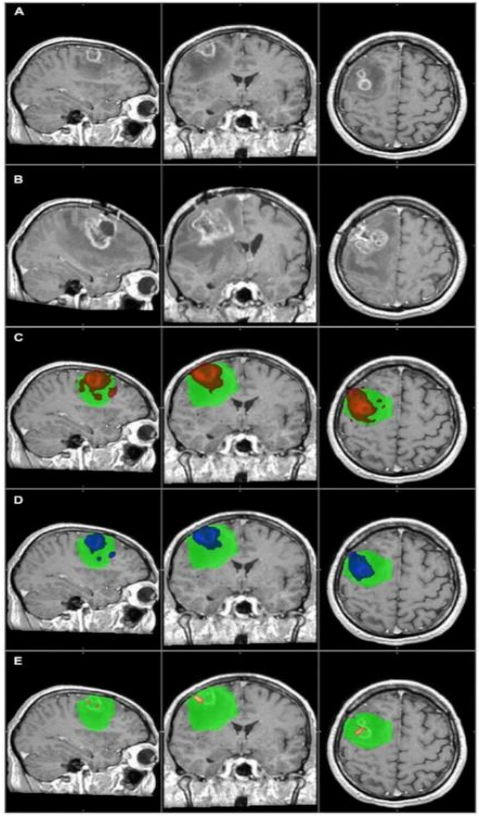

Figure 3: Case 1. Tumor Recurrence and TC Overlap. All images are in MNI152 1mm space. (A) Pre-op contrast-enhanced T1: non-enhancing tumor in left temporo-parietal region, histology confirmed glioblastoma (B) Tumor recurrence size and location. (C–E) Pre-op T1 with TC overlaps (1% red, 5% blue, 40% red-yellow) on recurrence mask (green). → Recurrence occurred distant from original tumor site, resulting in very low sensitivity at all thresholds (even 1%).

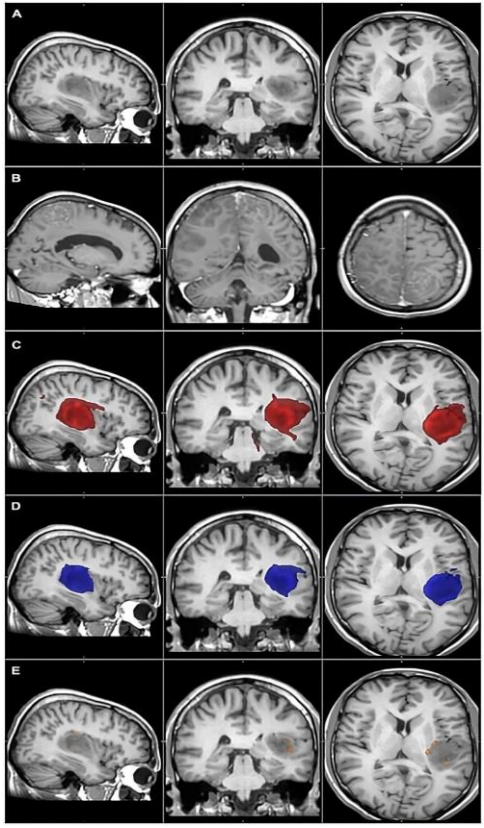

Figure 4: case 2. Tumor Recurrence and TC Overlap. All images are in MNI152 1mm space. (A) Pre-op contrast-enhanced T1: two right frontal tumors with ring enhancement (B) Tumor recurrence size and location. (C–E) Pre-op T1 with TC overlaps (1% red, 5% blue, 40% red-yellow) on recurrence mask (green). → TCs fully overlap recurrence mask (100% specificity) but recurrence extends beyond TCs (low sensitivity, even at 1%). Recurrence direction corresponds to TC distribution, reflecting fast tumor progression.

- Our results suggest that the presented method is a reliable and clinically feasible way to predict the true extent of glioblastomas.
- According to the literature 90–95% of the patients belongs to that group. Our study group is in concordance with that, as 90% of the recurrence was around the resection cavity.

FLAIR study

- Patient Characteristics (n=20)
- Data analyzed: 16 patients
- Sex ratio (F:M): 5:11
- Median age: 52 yrs
- Tumor location: Predominantly left frontal lobe
- Histology: 13 glioblastomas (81%), 3 oligodendrogliomas grade 3 (19%)
- Median survival: 17.5 months (range 2–72)

- Diagnostic performance: sensitivity 82.6%, specificity 84.7%.
- Although our method demonstrated high sensitivity and specificity, it did not provide a clear estimation of the true false-negative-to-true false-positive ratio within FLAIR imaging.
- i.e. hyperintensity on FLAIR imaging does not accurately distinguish between tumoral and non-tumoral areas such as edema.
- PPVs and NPVs defined to reflect the proportions of true positive and negative results.
- Group-level results showed high NPV (95.8%), indicating FLAIR reliably ruled out non-tumoral tissue, but moderate PPV (50.2%) due to false positives that overestimated tumor extent.

- Sensitivity 82.6%, specificity 84.7%, PPV was 50%, and NPV was 95.8%
- FLAIR detects abnormal regions but misses microscopic tumor infiltration
- Undetected cells near resection sites can cause recurrence and affect survival.
- Results limited by manual tumor mask delineation, modality registration errors, and small sample size.
- Future studies with larger samples and improved methods may yield more reliable results.

Two representative cases in which contradicting results are obtained should be considered.

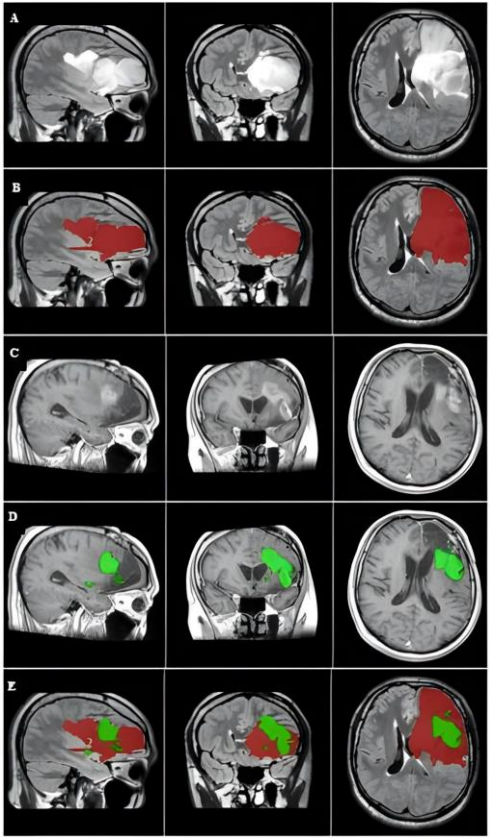

Figure 5: Case 1. Tumor Recurrence and FLAIR Overlap. All images are in MNI152 1mm space.
-High sensitivity vs low specificity most regions in primary tumor mask are true cancerous tissue
-Supports FLAIR imaging as effective for assessing tumor proliferation
-FLAIR hyperintensity may overestimate tumor boundaries
-Overes-timation can result from factors like edema
-Potential impact on diagnosis and treatment planning

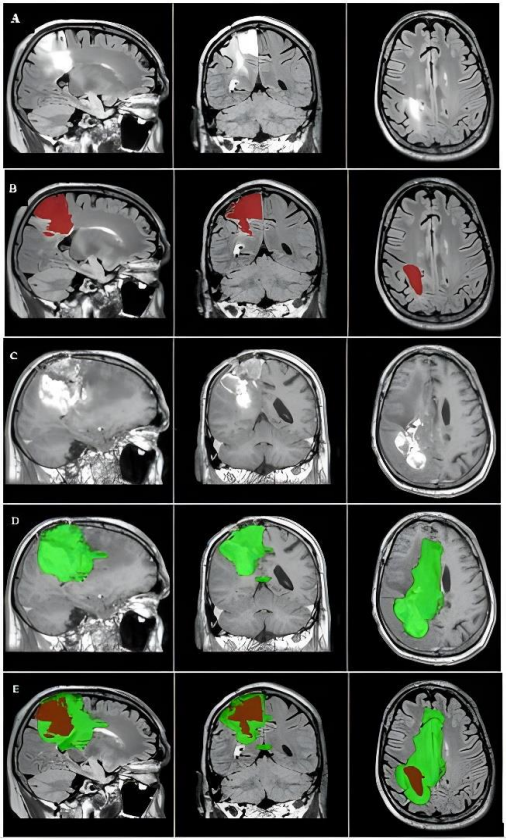

Figure 6: case 2. Tumor Recurrence and FLAIR Overlap. All images are in MNI152 1mm space.
-Higher specificity than sensitivity observed
-Tumor recurrence mask larger than original tumor mass
-Undetected regions by preoperative FLAIR were infiltrated by cancer cells
-Led to rapid tumor progression and poor overall survival

Conclusion

- Probabilistic tractography: visualizes infiltrated areas with high sensitivity and specificity → may support radical resection & personalized radiation
- FLAIR integration can enhance surgical precision and reduce overtreatment/recurrence risks
- According to our results we recommend incorporating DTI based probabilistic tractography and FLAIR into the MRI imaging protocol of glioblastoma to provide individual treatment options and better survival for the patients.

Table 2: Patients clinical data

*PFS, progression free survival; OS, overall survival.

*Patient (1-16): included in FLAIR study, Patient (1-20): included in Tractography study

Patients	Age (years) and Sex	Histology	Localization	Side	Time of Diagnosis	Date of Recurrence	Date of last Follow-up/Death	PFS (months)	OS (months)
1	26 Male	Glioblastoma	Frontalis	Right	2010.03	2012.08	2014.01	28	46
2	54 Female	Glioblastoma	Frontalis	Left	2010.04	2013.02	2013.03	34	35
3	39 Male	Glioblastoma	Parito-occipitalis	Right	2010.07	2012.05	2014.06	22	47
4	47 Male	Glioblastoma	Frontalis	Left	2010.09	2011.01	2012.05	4	20
5	34 Male	Glioblastoma	Frontalis	Left	2010.12	2011.05	2011.10	6	11
6	53 Male	Glioblastoma	Tempro-parietalis	Left	2011.02	2011.06	2012.02	4	12
7	51 Female	Oligodendroglioma-G3	Parietalis	Left	2011.02	2016.03	2017.02	61	72
8	67 Male	Glioblastoma	Temporalis	Left	2010.02	2012.07	2013.02	5	12
9	29 Male	Oligodendroglioma-G3	Fronto-parietalis	left	2012.05	2016.04	2017.09	47	64
10	67 Female	Oligodendroglioma-G3	Frontalis	Right	2012.06	2015.07	2015.07	37	37
11	52 Male	Glioblastoma	Temporalis	Left	2012.09	2013.02	2014.11	5	26
12	68 Male	Glioblastoma	Temporalis	Left	2013.01	2014.02	2014.09	13	20
13	46 Male	Glioblastoma	Frontalis	Right	2019.11	2020.03	2021.02	4	15
14	77 Female	Glioblastoma	Tempro-parietalis	Left	2019.10	2020.06	2020.08	8	10
15	56 Male	Glioblastoma	Parietalis	Left	2019.11	2020.01	2022.03	2	28
16	68 Male	Oligodendroglioma G3	Frontalis	Left	2020.04	2021.012	2022.02	8	10
17	36 Male	Glioblastoma	Parietalis	Left	2020.10	2021.01	2021.04	3	6
18	46 Female	Glioblastoma	Parietalis	Left	2021.01	2022.01	2022.02	1	2
19	55 Male	Glioblastoma	Tempro-parito-occipitalis	Right	2021.02	2021.04	2021.06	2	4
20	59 Male	Glioblastoma	Frontalis	Left	2021.10	2021.12	2022.02	2	3