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# General discussion





In this thesis I investigated the value of several specific Magnetic Resonance Imaging (MRI) techniques in patients with recurrent glioblastoma treated with bevacizumab. In these patients, a phenomenon known as pseudo-response complicates treatment response assessment. I investigated a variety of methods for measuring treatment response in this patient group. Additionally, I investigated growth patterns in newly diagnosed non-enhancing low-grade glioma using Diffusion Tensor Imaging (DTI).

## MAIN FINDINGS

### Conventional MRI techniques

The appearance of new lesions in recurrent glioblastoma patients on T1-weighted post-contrast images or T2-weighted/FLuid Attenuated Inversion Recovery (FLAIR) images (i.e. enhancing or non-enhancing lesions) early after the start of treatment clearly is associated with poor overall survival (OS) with a mean OS of just 3.2 months for those with a new lesion compared to 11.2 months in those without a new lesion at 6 weeks follow-up. We can therefore conclude that the appearance of a new lesion is a strong predictor for OS. This effect is independent of treatment.

We compared the 2D Response Assessment in Neuro-Oncology (RANO) criteria with various volumetric methods: contrast-enhancing volume only (measured on either T1w post-contrast images or subtraction images) and contrast-enhancing volume plus non-enhancing volume (the latter measured on FLAIR images). The RANO criteria are the current method of choice for assessing treatment response in glioblastoma. According to the RANO criteria, progressive disease (PD) is defined as any of the following:  $\geq 25\%$  increase in the sum of perpendicular diameters of enhancing lesions or a significant increase in T2w/FLAIR non-enhancing lesions compared to baseline or best response scan after start of treatment, the appearance of new lesions, clear progression of non-measurable lesions, or clinical deterioration. Steroid dosage is also taken into account<sup>1</sup>. While the enhancing lesions are measured in 2D, the T2w/FLAIR non-enhancing lesions are not quantified. By measuring not only contrast-enhancing volume, but also non-enhancing volumes we hypothesized that survival prediction based on change in tumor size would improve.

Especially in patients treated with bevacizumab we hoped to find an improvement in survival prediction using volumetric measurements of non-enhancing lesions. Bevacizumab directly influences tumor vasculature (i.e. abnormal vasculature) by decreasing the permeability of the vessel wall, which may lead to a decrease in contrast-enhancement on T1-weighted post-contrast images. Since tumor activity has not decreased but is only masked, this effect is known as pseudo-response. Bevacizumab also alleviates edema and by doing so, decreases non-enhancing abnormalities on T2w/FLAIR images. At progression, an increase in non-enhancing tumor has been described (gliomatosis cerebri), but whether this pattern of progression occurs more often in patients treated with bevacizumab has been questioned<sup>2-5</sup>.

We assessed both lomustine-only treated and bevacizumab-treated (with and without lomustine) patients and found that determining PD with volumetric methods did not significantly improve survival prediction at 6 and 12 weeks follow-up in any of the treatment groups and nor did subtraction techniques improve survival

prediction. Since volumetric assessment is more time-consuming, these findings support the use of 2D evaluation.

However, the threshold set for volumetric PD was  $\geq 40\%$  for enhancing tumor volume<sup>6-8</sup> and  $\geq 25\%$  for non-enhancing volume<sup>9</sup>. To see whether different thresholds would lead to different results, we tested lower thresholds at both 6 and 12 weeks follow-up. We found that lowering the thresholds improved prediction and that this result persisted after removing patients with high percentages of increase in tumor size from the analysis. Despite the better survival prediction found with these lower thresholds, the number of additional patients categorized as progressors was quite low. This was especially the case in the bevacizumab-treated group in which only a small number of patients had increasing enhancing and/or non-enhancing tumor volume.

### Diffusion Weighted Imaging

We also explored whether Apparent Diffusion Coefficient (ADC) histogram analysis would allow better outcome assessment. Some promising results for treatment response assessment were observed in patients treated with both bevacizumab and lomustine. In this group, a decrease in histogram derived minimum ADC values ( $ADC_{\min}$ ) from the enhancing tumor volume from baseline to first follow-up significantly improved survival. Those with a decrease in  $ADC_{\min}$  of  $>27.5\%$  had a mean OS of 15.2 months versus 6.8 months in those without this decrease. This effect was however not found in the bevacizumab-only and lomustine-only treatment arms, nor were significant results found when analyzing histograms from non-enhancing volumes.

### Diffusion Tensor Imaging

A different diffusion technique is diffusion tensor imaging (DTI), which can be used to determine directionality of diffusion of water molecules. Where in DWI only the presence of diffusion restriction is measured (by measuring diffusion in 3 directions), many different directions of diffusion are measured with DTI. Subsequently, the general direction of diffusion within a voxel can be calculated. Directionality in the brain is strongest in white matter where diffusion occurs along white matter tracts (high anisotropic values) and lowest in the ventricles (high isotropic values). Disturbances in white matter architecture by a tumor are made apparent using DTI. A tumor can grow by infiltrating white matter tracts by pushing into them or growing alongside the fiber bundles.

DTI derived isotropic ( $p$ ) and anisotropic ( $q$ ) maps have previously been used by Price et al.<sup>10</sup> to discern different molecular subtypes of glioblastoma (i.e. *IDH*-mutated versus *IDH* wild-type) based on different patterns of growth into/along white matter tracts. The authors reconfirmed that Isocitrate dehydrogenase (*IDH*) mutated glioblastomas grow less invasively than *IDH* wild-type tumors and that this is associated with patient prognosis. We used the same technique in pretreated non-

enhancing (presumed low-grade) gliomas to discern *IDH*-mutated and *IDH* wild-type tumors as well. We also looked at 1p19q codeleted versus non-codeleted tumors (i.e. molecular oligodendrogliomas versus astrocytomas) to see if there were differences in growth patterns between these molecular subtypes. Only 4 patients with an *IDH*-WT tumor were available for this analysis, but these all showed different growth patterns. These same 4 growth patterns were also found in the *IDH*-mutated group. 1p19q codeleted and non-codeleted tumors were more evenly distributed, but no significant differences in growth patterns were found here either.

## METHODOLOGICAL CONSIDERATIONS

All four chapters on the BELOB-trial have their focus on finding a better method for treatment response assessment in bevacizumab-treated recurrent glioblastoma. Effectiveness of treatment can be determined by correlating this with the golden endpoint for oncology trials: overall survival (OS). To predict OS, a cox regression analysis can be performed in which hazard ratios (HRs) are calculated for those with and without progressive disease (PD) as determined per method. The evaluation method leading to the highest HR at a given time-point is the best method for predicting OS and most likely to reflect benefit in a phase III trial with OS as the primary endpoint. When interpreting these results, it is important to consider the number of patients that are categorized as progressors with a particular method and the overlap in confidence intervals between methods. This places results in a broader perspective (shown in **chapter 3.2**).

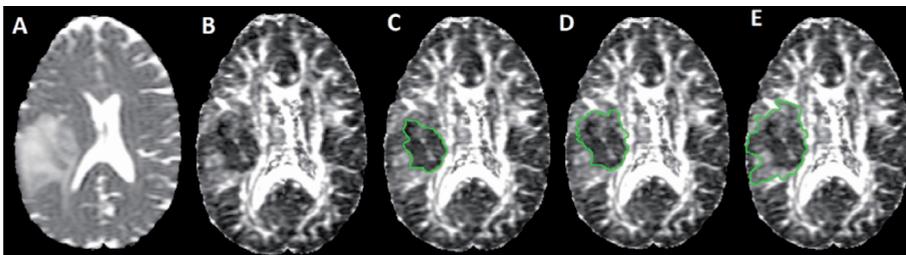
Different methods, such as DWI and perfusion imaging, can potentially provide other markers for treatment response in bevacizumab-treated patients. Regarding the results we found, it is important to note that our research took place within the context of a clinical trial and thus results are not directly generalizable to all patients with recurrent glioblastoma treated with other agents. This means that aside from validation in a different and larger cohort of patients, positive findings in our analysis would need to be confirmed in independent datasets and preferably in 'real life' clinical setting as well.

We also assessed non-enhancing glioma growth patterns with DTI. We reproduced a *p/q* mapping technique that was previously found successful for discerning molecular subtypes of glioblastoma<sup>10,11</sup>. The methodology included the drawing of volumes of interest (VOIs) on both the *p* and *q* maps and subsequently overlaying the two in order to detect mismatch. Mismatch was determined if the *p*-VOI exceeded the *q*-VOI by >0.5cm, although in the 2017 publication by Price, a cut-off of 1.0cm was

used. Three different patterns of mismatch were described: a minimally invasive pattern, a localized pattern, and a diffuse pattern.

Reproducing the exact methodology proved difficult: drawing a volume of interest (VOI) on the  $q$ -map was unreliable as many variations were possible within a single patient (**Figure 1**). Instead we overlaid the  $p$ -VOI on the  $q$ -map and scored where the  $p$ -VOI overlapped high intensity areas on the  $q$ -map (i.e. white matter tracts), basically measuring the invasiveness of the tumor into white matter tracts. However, the three categories described by Price et al. did not adequately define the patterns we encountered and we instead used 5 different categories. One of the reasons for doing so, is that we found that some tumors would expand into white matter tracts, while others would follow the tracts in a more infiltrative fashion.

Because of the many possible variations in delineation of the  $q$ -VOI, as well as the different patterns of infiltrative growth encountered, we were unable to reproduce the exact method used by Price. Possibly, our different patient characteristics, i.e. glioblastoma versus non-enhancing gliomas, are to blame. However, when evaluating the specific problems we encountered, we find that the technique itself is not reliable and/or robust enough for application. The lack of positive results and in particular the four different patterns found in the *IDH*-WT patients was unexpected given the highly positive results found by Price in glioblastomas. The difficulties we encountered reproducing the method were reflected in the low interrater agreement of 62.7%. In fact, the troubles we encountered using this technique could have obscured possible actual differences in growth patterns between the molecular subtypes of gliomas in our cohort.



**Figure 1.** DTI-derived isotropic map (A), anisotropic map (B), and three possible volume of interest (VOI) drawings (C, D, E).

### Clinical Implications and future directions

The association between the appearance of new lesions and survival has direct clinical implications. The association found was independent of treatment. We found that the negative impact of a new lesion on overall survival is stronger than that of increasing tumor volume. We scored and measured both enhancing and

non-enhancing new lesions of any size that persisted or increased in volume at the subsequent follow-up scan (i.e. 6 weeks later). While the appearance of a new lesion is already included in the definition of ‘progressive disease’ as described in the RANO criteria, we find that more emphasis on the appearance of new lesions is needed.

(Semi)-automated volumetric tumor measures have lower intra- and interrater variability than manual measures, including 2D measures<sup>7,12</sup>. Heterogeneous tumors with lots of necrosis, as is the case in glioblastoma, are more difficult to measure in an axial plane and the axial plane itself is influenced by head position and slice alignment. These are strong arguments for using volumetric instead of 2D measurements. However, volumetric methods are time-consuming for various reasons: I) it takes time to send the MRI-sequence to a dedicated work-station with software for volumetric segmentation and then it takes time to send the information back to the main system, and II) an segmentation by (semi)-automated techniques is based on algorithms that often include non-tumorous areas into the volume of interest (VOI). Examples are the inclusion of blood vessels and dura into an enhancing VOI and the inclusion of the cortical ribbon into a non-enhancing VOI. This means that manual work is needed to check the VOIs and to adjust them where necessary, which is time-consuming.

Only if (semi)-automated techniques became more readily available and faster than is currently the case, would we advise volumetric methods over 2D measures. At present there is no reason to switch to volumetric methods.

When it comes to the thresholds currently in use for determining PD, we see no reason to lower them at this time as the number of patients additionally categorized as progressors is quite low in our study cohort.

In recurrent glioblastoma patients treated with bevacizumab, only a few have increasing enhancing (and non-enhancing) tumor volumes at first follow-up, despite the absence of true anti-tumor activity of this agent. More advanced methods for measuring treatment outcome should therefore be considered, unless bevacizumab is only considered as a steroid-like drug. For instance, change in  $ADC_{min}$  in enhancing tumor in recurrent glioblastoma treated with both bevacizumab and lomustine is a very promising predictor for overall survival. There was a clear difference in survival between those with a decrease in  $ADC_{min}$  and those without. As mentioned before, these results will need to be validated in a different and larger cohort.

Since we were unable to discern different molecular subtypes of non-enhancing gliomas using  $p/q$ -mapping based on different growth patterns and because of the difficulties we encountered when using this technique, we currently do not recommend using this technique in non-enhancing gliomas.

Other techniques that could be used to look at white matter changes due to tumor infiltration are tractography, FA-skeletons, and fiber density mapping. In

tractography, the orientation of diffusion is determined for every voxel individually, after which the voxels are linked together to visualize diffusion occurring along white matter tracts<sup>13,14</sup>. White matter tracts can also be made visible by setting a threshold above which diffusion is considered anisotropic (generally FA is used) for the whole brain. A 'skeleton' of white matter tracts appears, commonly referred to as a FA-skeleton<sup>15</sup>. In both tractography and FA-skeleton techniques, a threshold is needed to prevent tracts from being 'drawn' outside the white matter. The chosen threshold affects the results. Fiber density mapping is a postprocessing method that includes the reconstruction of all fiber paths in the brain and calculations of fiber density values per voxel while also using measures from adjacent voxels. It is an additional measure to FA-skeleton maps and tractography and can help quantify the extent of destruction of white matter<sup>16</sup>. While the extent of destruction and the type of white matter damage can be determined using these techniques, it is unclear if they can be used to determine growth patterns and if results can provide information on the molecular subtype of the tumor. Instead of looking at affected white matter, one could also look at a variety of parameters to see which correlate with molecular tumor subtypes. This method is known as radiogenomics and it has the ability to look at very large datasets and numerous parameters from different MRI sequences (and other imaging methods) from which a predictive model can be calculated.

## CONCLUSIONS

There is currently no reason to change the 2D treatment response measures to volumetric measures in recurrent glioblastoma, nor is there a reason to adjust thresholds for measuring progressive disease. More attention to early appearing new lesions is justified to optimize survival prediction and deciding on the continuation of treatment within the currently used 2D RANO criteria.

Measuring change in minimum ADC measured in enhancing tumor from baseline to first follow-up is a promising tool for determining treatment response in recurrent glioblastoma treated with both bevacizumab and lomustine.

Possible differences in growth-patterns between different molecular subtypes of non-enhancing glioma cannot be discerned using DTI-derived isotropic and anisotropic maps as described by Price et al.

## REFERENCES

1. Wen PY, MacDonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *Journal of Clinical Oncology* 2010;28(11):1963-1972.
2. Iwamoto FM, Abrey LE, Beal K, et al. Patterns of relapse and prognosis after bevacizumab failure in recurrent glioblastoma. *Neurology* 2009;73(15):1200-1206.
3. Norden AD, Young GS, Setayesh K, et al. Bevacizumab for recurrent malignant gliomas: efficacy, toxicity, and patterns of recurrence. *Neurology* 2008;70(10):779-787.
4. Radbruch A, Lutz K, Wiestler B, et al. Relevance of T2 signal changes in the assessment of progression of glioblastoma according to the Response Assessment in Neurooncology criteria. *Neuro Oncol* 2012;14(2):222-229.
5. Wick W, Wick A, Weiler M, Weller M. Patterns of progression in malignant glioma following anti-VEGF therapy: perceptions and evidence. *Curr Neurol Neurosci Rep* 2011;11(3):305-312.
6. Pichler J, Pachinger C, Pelz M, et al. MRI assessment of relapsed glioblastoma during treatment with bevacizumab: volumetric measurement of enhanced and FLAIR lesions for evaluation of response and progression – a pilot study. *Eur J Radiol.* 2013;82(5):240-245.
7. Chow DS, Qi J, Miloushev VZ, et al. Semiautomated volumetric measurement on postcontrast MR imaging for analysis of recurrent and residual disease in glioblastoma multiforme. *AJNR Am J Neuroradiol.* 2014;35(3):498-503.
8. Wang MY, Cheng JL, Han YH, et al. Measurement of tumor size in adult glioblastoma: classical cross-sectional criteria on 2D MRI or volumetric criteria on high resolution 3D MRI? *Eur J Radiol.* 2012;81(9):2370-2374.
9. Gerstner ER, Chen PJ, Wen PY, et al. Infiltrative patterns of glioblastoma spread detected via diffusion MRI after treatment with cediranib. *Neuro Oncol.* 2010;12(5):466-472.
10. Price SJ, Allinson K, Liu H, et al. Less invasive phenotype found in isocitrate dehydrogenase-mutated glioblastomas than in isocitrate dehydrogenase wild-type glioblastomas: a diffusion-tensor imaging study. *Radiology* 2017;283(1):215-221.
11. Price SJ, Jena R, Burnet NG, et al. Improved delineation of glioma margins and regions of infiltration with the use of diffusion tensor imaging: an image-guided biopsy study. *AJNR Am J Neuroradiol* 2006;27(9):1969-1974.
12. Sorensen AG, Patel S, Harmath C, et al. Comparison of diameter and perimeter methods for tumor volume calculation. *J Clin Oncol* 2001;19(2):551-557.
13. Stadlbauer A, Nimsky C, Buslei R, et al. Diffusion tensor imaging and optimized fiber tracking in glioma patients: histopathologic evaluation of tumor-invaded white matter structures. *Neuroimage* 2007;34(3):949-956.
14. Bucci M, Madelli ML, Berman JJ, et al. Quantifying diffusion MRI tractography of the corticospinal tract in brain tumors with deterministic and probabilistic methods. *Neuroimage Clin* 2013;3:p.361-368.
15. Miller P, Coope D, Thompson G, Jackson A, Herholz K. Quantitative evaluation of white matter tract DTI parameter changes in gliomas using nonlinear registration. *Neuroimage* 2012;60(4):2309-2315.
16. Stadlbauer A, Buchfelder M, Salomonowitz E, Ganslandt O. Fiber density mapping of gliomas: histopathologic evaluation of a diffusion-tensor imaging data processing method. *Radiology* 2010;257(3):846-853.