Evaluating potential of multi-parametric MRI using co-registered histology: Application to a mouse model of Glioblastoma.

H. Al-Mubarak\textsuperscript{a,b*}, A. Vallatos\textsuperscript{c}, L. Gallagher\textsuperscript{a}, J. Birch\textsuperscript{d}, A. J. Chalmers\textsuperscript{e}, W.M. Holmes\textsuperscript{a}

\textsuperscript{a}Glasgow Experimental MRI centre, Institute of Neuroscience and Psychology, University of Glasgow, G61 1QH, U.K.
\textsuperscript{b}Department of Physics, College of science, University of Misan, Iraq.
\textsuperscript{c}Centre for Clinical Brain Sciences, University of Edinburgh, EH16 4SB, UK.
\textsuperscript{d}Beatson Institute for Cancer Research, UK.
\textsuperscript{e}Wolfson Wohl Translational Cancer Research Centre, Institute of Cancer Sciences University of Glasgow, G61 1QH, UK.

*Haitham Al-Mubarak e-mail: haitham_f99@outlook.com or haitham_f99@yahoo.com
Antoine Vallatos e-mail: antoine.vallatos@ed.ac.uk.
Lindsay Gallagher e-mail: Lindsay.Gallagher@glasgow.ac.uk
Joanna Birch e-mail: j.birch@beatson.gla.ac.uk
Anthony Chalmers e-mail: Anthony.Chalmers@glasgow.ac.uk
William Holmes e-mail: william.Holmes@glasgow.ac.uk
**Key words:** Glioblastoma, Multi-parametric MRI, mpMRI, Linear regression, Infiltration of Tumor cells, co-registration.

1. Introduction

Magnetic Resonance Imaging has proven to be a powerful tool in clinical diagnosis. Clinical MRI is capable of generating a range of image contrasts (e.g. T1W, T2W, DWI, FLAIR and Gd-T1) which are commonly assessed individually by qualitative visual inspection [1, 2]. However, it has long been hypothesized that better diagnoses could be achieved by combining these multiple images, so called multi-parametric or multi-spectral MRI. However, this hypothesis has never been rigorously tested [3].

It is generally agreed that histopathology is the gold standard for characterising diseased tissue and for validating imaging biomarkers [4]. For human patients this is limited to biopsy (e.g. brain tumor) or post mortem specimens (e.g. carotid plaques [5]), which are cut into thin sections and stained to reveal complex differentiated structures at the cellular level [6]. Even then, the localization/registration of histology sections with MR images is very challenging.

In the absence of histopathology, there are currently two strategies to analyze mpMRI data. The first involves extracting relevant features such as volume, signal intensity and texture, then applying a model to those factors [7-9]. The second strategy is to perform a voxel-wise (voxel by voxel) analysis [10-12], transforming voxel values from each imaging modality to create a single image map by applying either a linear or non-linear function.

GBM brain tumors are heterogeneous in nature, with GBM cells infiltrating into adjacent normal tissue [13, 14]. For surgical resection and radiotherapy, planning is important to accurately identify the outer boundary of this infiltration [15]. It is widely acknowledged that conventional MRI (T1W, T2W, ADC, Gd-T1) cannot detect GBM infiltration beyond the contrast enhanced region of Gd-T1 images. It has been speculated that the combination of image data from various MRI modalities (morphological, functional and metabolic) has the potential to provide the radiologist with a single tumor map (Fig.1) by extracting more information from the individual images.
An example of the voxel-wise approach is the work of Kazerooni, Mohseni [3], who proposed that using a combination of MRI images (ADC, PWI and T2W), followed by a segmentation method, could identify the extent of GBM infiltration and help in its delineation before surgery. Further, several studies have recommended including additional imaging biomarkers adopted from diffusion and perfusion modalities [16-18]. However, these clinical studies lack direct verification with the commonly agreed gold standard of histology.

Even when studying animal models of disease, where the animal can be readily sacrificed, the comparison between MR images and histology sections has been mostly qualitative and largely limited by the difficulties of co-registration. However, in a recent publication we achieved high quality registration of histology with MRI, not by improving on current image registration algorithms but by focusing on improving the quality of the histology sections [19]. We applied this stacked in-plane histology (SIH) methodology to a mouse model of GBM, which resulted in a registered multi-dimensional datasets of MR images and histology. This allowed a direct voxel-by-voxel assessment of individual MRI modalities, which found that invasion of tumor cells can be detected beyond the edematous region by using a perfusion weighted image (ASL) [20]. In this paper, we describe how we further interrogated this unique dataset to quantitatively test the hypothesis that, by combining data from individual MRI modalities, multi-parametric MRI has the potential to improve tumor detection.

Figure 1: Reconstruction of a single tumor map from different MRI modalities when combined with linear regression analysis.
2. Methods and Materials

In this study we used a previously published multi-dimensional dataset consisting of registered MR images and histology from a mouse model of GBM [18, 19]. The methods used in acquiring this dataset are summarized below.

2.1 Animal Preparation

Ten immunocompromised CD1 nude mice (20-25g, Charles River Laboratories) were intracranially injected the G7 Glioblastoma model (10^5 cells per mouse) derived from a primary human tumor cell line. This cell line produces a tumor bulk with invasive edges in vivo that replicates the human disease. For the MRI session, the mouse was anaesthetized with 5% isoflurane for induction at 30:70 O_2/N_2O ratio. Next, the mouse was placed in the MRI cradle and, using isoflurane at 2%, maintained using a 40:60 ratio of O_2/N_2O (1 L min^-1). The head of the mouse was restrained with both ear and tooth bars to prevent movement during scanning. A rectal probe was used to monitor body temperature, which was maintained at 37±1 °C by an enclosed water circuit that surrounded the mouse. Experiments were carried out under license from the UK Home Office in accordance with the Animals (Scientific Procedures) Act, 1986, incorporating European Directive 2010/63/EU and approved by the University of Glasgow Ethical Review Panel.

2.2 MRI acquisitions

MRI experiments were performed on a Bruker Biospec Avance 7T imaging system (Bruker Biospin, Ettlingen, Germany). Radiofrequency excitation was achieved using a 72mm diameter birdcage volume resonator, with the signal detected using an actively decoupled 4-channel phased array receive-only head surface coil (Rapid Biomedical, Wurzburg, Germany). MRI was performed with a field-of-view 2×2 cm on five 1.5 mm thick coronal slices centered at 4 mm posterior from the rhinal fissure. T2 mapping and T2-weighted imaging were conducted using a Multi-Slice Multi-Echo (MSME) and RARE sequence (TE=30 ms, TR=4394 ms, matrix=176x176, slice thickness=1.5mm, 7min). Diffusion-weighted imaging (DWI) was conducted using a 4-shot spin-echo echo planar imaging DWI scan (TE=37.63 ms, TR=4,500 ms, matrix=128×128, 1.5 mm slice thickness, 6 directions, b-values = 0, 1000 s.mm^-2, 10 min). Contrast-enhanced T1 imaging (CE-T1) was conducted using a RARE acquisition (TE=12.3 ms, TR=800 ms, matrix=176×176, 8 min). Images were acquired before and 5 min after Gadolinium-DTPA injection. In the last scanning sessions,
T2W_{histology} experiments with 9 slices (slice thickness=0.5 mm) were also carried out with a relaxation enhancement (RARE) sequence (TE=46 ms, TR=5000 ms, matrix=176×176, 9 min, RARE factor =8). Following in vivo scanning, a doped water phantom was scanned to correct the sensitivity bias of the RF surface receiver coil.

Figure 2 shows the experimental protocol. At the conclusion of the MRI session, animals were euthanized immediately following the last MRI scan and brains were extracted and stored frozen at -20 °C to enable later ex vivo studies. Histology sections were then cut and stained using either hematoxylin and eosin (H&E) or Human Leukocyte Antigen (HLA) to identify the human tumor cells. For more details about the histology, see Al-Mubarek and et al. [19].

![Injecting mice with G7 cells](image1.png) ![MRI scan and mouse sacrificed](image2.png)

Figure 2: Experimental protocol. At week zero, 10 mice were orthotopically implanted with G7 glioblastoma cells. Mice were imaged using MRI in week 12. After the last MRI session, mice were euthanized and prepared for histology.

### 2.3 MRI and Histology image processing

Non-uniform detection sensitivity associated with the use of a surface receiver coil can adversely affect the registration processes. This was corrected using phantom images. T1W, T2W and DWI images were normalized using corresponding pre-acquired water phantom MRI images with the same parameters [21]. Apparent Diffusion Coefficient (ADC) maps were calculated by fitting the DWI data to the mono-exponential equation of the Stejskal and Tanner model [22]. All data were resized to match the T2W matrix (176×176). To separate the mouse brain from the background and to reduce processing time, the brain area was manually delineated and separated by applying an active contour method [23]. A non-linear diffusion filter was applied (No. of iterations=100, Lambda=0.2, and epsilon=1) to remove noise and preserve the sharpness of the edges in the image [24]. For more details
regarding the creation of SIH methodology and histology pre-processing, see [19]. The flow chart below (Fig.3) summarizes the image processing steps for both MRI and SIH. All datasets in this work were processed using in-house MATLAB script (MATLAB R2020a).

Figure 3: Pipeline shows MRI and histology image processing steps which allows for co-registration MR images and SIH to create IRM and IRMave.

### 2.4 Multiple Linear Regression

To identify the optimal combination of the different MRI modalities, we applied a multiple linear regression model containing interaction terms, which can be written as

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_1 X_2 + b_5 X_1 X_3 \ldots \ldots \ldots$$  

Equation 1
Where the dependent variable Y represents the normalized SIH value and the independent variables $X_1, X_2, X_3, X_4$ represent the normalized MRI parameters $T2W, ADC, FA, T2map$.

Multiple regression was performed using MATLAB R2020a with a built-in function (regrass). Initially, this Interaction regression method, (equation 1), was applied to the MRI/histology data of each individual mouse, producing interaction regression maps (IRM). For each mouse, the $b$ values were then used to construct a single tumor map, labelled IRM (Figure 5).

To test whether a common set of $b$ values could be applied to all mice, the average of the individual $b$ values was taken. For each mouse, these average $b$ values were again used to construct a single tumor map, labelled IRMave (Figure 5).

Figure 4a shows the relationship between normalized SIH values and the individual MRI modalities ($T2W, T2map ADC and FA$). Figure 4b shows that the scatter plot of individual voxels’ SIH values against the IRM value for an individual mouse has a positive correlation (0.62). Figure 4c, shows a scatter plot for the same animal, of SIH verse IRMave.

3. Statistical Analysis

The performance of the multiple linear regression analysis was evaluated using three statistical methods:
1. The Pearson coefficient, which is used to identify if two or more variables are related to each other. The correlation coefficient \( r \) is the numerical assessment of the strength of the relationship between the X and Y values in the data set consisting of (X, Y) pairs.

2. To test the accuracy of each modality of the MRI and IRM maps, tumour volumes of interest (VOI) were manually selected. These were compared to our gold standard VOI selected from the stacked in-plane histology.

3. Receiver Operating Characteristic (ROC) curve analysis was used to compare abnormal regions of interest probed by the MRI, IRM and histology [25]. A comparison of abnormal regions gives measurements of True Negative (TN), False Positive (FP), False Negative (FN) and True Positive (TP) used to calculate sensitivity, specificity, accuracy, and Dice.

\[
\text{Sensitivity} = \frac{TP}{TP + FN} \quad \text{Equation 2}
\]

\[
\text{Accuracy} = \frac{TP + TN}{TP + FN + FP + TN} \quad \text{Equation 3}
\]

\[
\text{Specificity} = \frac{TN}{TP + TN} \quad \text{Equation 4}
\]

\[
\text{Dice} = \frac{2 \times TP}{FP + FN + 2 \times TP} \quad \text{Equation 5}
\]

Using Graph Pad prism 6 (Ver.6.01, 2012), all data were analyzed using ANOVA and the \( t \)-test to compare abnormal tumour-related regions in both MRI and histology images. All values are reported as mean ± standard deviation (STD).

4. Results

Qualitative and quantitative assessments were used to evaluate the individual MRI modalities and the regression maps. One of the qualitative methods was a visual comparison. Figure 5 shows a comparison between the individual images (T2W, ADC, FA, and T2map) and the interaction regression maps (IRM and IRMave). This comparison shows a significant improvement in tumor region detection as well as the detection of the
infiltration of tumor cells beyond the edema when using IRM rather than individual MR images.

Figure 5: Comparison of original MR images (T2W, ADC, FA, and T2map), linear regression maps (IRM, IRMave) and SIH.

The stacked in-plane histology (SIH) provides a ‘ground truth’ for a quantitative assessment of the individual MRI modalities and the interaction regression maps (IRM and IRMave). As the MRI data has been co-registered with the SIH, each MRI voxel has a corresponding ‘ground-truth’ SIH voxel (example scatter plots are shown in figure 4). This allows correlation coefficient analysis between individual MRI modalities, IRM / IRMave and the SIH ‘ground truth’. Figure 6 illustrates that IRM (r>0.5), and IRMave (r>0.3) had higher correlation coefficient values than T2W, ADC, FA, and T2map. Statistically, the following clinically relevant r values were used: an r value less than 0.4 was considered poor, an r value of 0.4-0.59 was considered fair, an r value of 0.6-0.74 was considered good, and an r value greater than 0.74 was considered excellent.
Figure 6: The Pearson correlations between individual MRI (T2W, ADC, FA and T2map), IRM, IRMave against SIH in the brain after discarding the background.

Figure 7: Volumetric tumor comparison between interaction regression maps and SIH. All tumor volumes are represented by mean ± STD manually delineated from the individual MRI maps and the interaction regression maps at week 12. Also shown is the tumor volume manually delineated from histology (SIH). ANOVA and t-test were used and showed statistical significance (P<0.0125) between T2W, ADC, FA, and T2map and SIH and no statistical significance (P>0.025) between IRM and IRMave.

Volumetric analysis is also a powerful tool for studying tumors and the effects of cancer treatment. However, in this study, we compared tumor volumes identified using individual MRI modalities, interaction regression maps and histology (SIH) to ascertain the benefits of the interaction regression method. The tumor VOIs for nine mice (one did not develop a tumor) were obtained by manual delineation of both interaction regression maps and the
corresponding SIH map by two observers, each with more than 3 years’ experience. Figure 7 shows the average and standard deviation of tumor volumes for four MRI modalities with an Interaction regression map at week 12. The mean tumor volumes selected by IRM (15.73±3.9mm³) and IRM_{ave} (15.14±3.64mm³) exhibited no statistically significant difference when compared with SIH mean volume (15.3± 2.54mm³).

ROC analysis was employed to evaluate the accuracy of detection of the tumor region. This method included four scenarios: true positives (TP) and true negatives (TN), where the identification between MRI and histology regions was correct, and false positives (FP) and false negatives (FN), where differences existed between the MRI and histology regions.

Figure 8A shows the sensitivity of both individual MRI modalities and interaction regression maps. The high sensitivity of interaction regression maps indicates that they are more sensitive to detecting the tumor burden than individual MRIs. Figure 8B shows that specificity is indicative of accurate tumor detection. The slightly higher specificity values of individual MRIs when compared with those of interaction regression maps may be due to individual MRIs being totally inside the histology tumor region. There was no significant difference between individual MRIs and interaction regression maps with respect to accuracy and Dice values (Fig.8C-D).
Figure 8: ROC analysis between MRI, interaction regression maps and SIH which enables evaluation of the accuracy of detection of the tumor region compared with SIH (A) Sensitivity (B) Specificity (C) Accuracy (D) Dice.

5. Discussion

5.1 Glioblastoma and multi-parametric MRI

GBM is the most common and aggressive primary brain tumor, with a median survival time of 10.6 months after diagnosis [26]. GBM cells can progressively infiltrate neighboring normal brain regions [13, 14]. It is widely acknowledged that conventional MRI (T1W, T2W, ADC, Gd-T1) cannot detect GBM infiltration beyond the contrast enhanced region of Gd-T1 images. However, changes beyond the contrast enhanced region have been detected with MRS [27], MRI measurements of cerebral blood volume (CBV) [28] and cerebral blood flow (CBF) [29].

In recent years, the clinical research community has paid more attention to mpMRI. It is considered that the combination of image data from various MRI modalities (morphological, functional and metabolic) has the potential to provide the radiologist with a single tumour map by extracting more information from the individual images [3]. Although the selection of MRI modalities and how they are combined is much debated. Several studies have attempted to use mpMRI to detect the GBM tumor infiltrative region. Kazerooni, Mohseni [3] proposed that using a combination of MRI images (ADC, PWI and T2W) followed by a
segmentation method could find the extent of GBM and help in its delineation before surgery. Furthermore, several studies have recommended including additional imaging biomarkers adopted from diffusion and perfusion modalities as a means of achieving deeper insight into the physiological behavior of glial brain tumors [1, 16]. For example, Jensen and Schmainda [30] stated that, by combining several MRI modalities (morphological and functional) with a segmentation algorithm, they were able to distinguish between the invasion of the tumor and the normal tissue inside edema. Further, a combination of Dynamic Contrast Enhanced (DCE) and Dynamic Susceptibility Contrast (DSC) MRI with an unsupervised segmentation algorithm showed promising results for distinguishing between vasogenic edema and tumor-containing edema, which appear similar in conventional MRI [31]. However, all these clinical studies lacked direct verification with the commonly agreed gold standard of histology.

5.2 Quantitative assessment of the multi-parametric MRI hypothesis

MRI is capable of generating a range of individual image contrasts, giving morphological, functional and metabolic information [32, 33]. It has long been hypothesized that better diagnoses could be achieved by combining these multiple images (the so called multi-parametric (mpMRI) or multi-spectral MRI). Here, we have sought to quantitatively test this hypothesis for the first time, using a previously published dataset of co-registered MR images and histology, from a mouse model of glioblastoma [18, 19].

The immunocompromised mice were intracranially injected with G7 Glioblastoma cells, which were derived from a primary human tumor cell line. The histology sections were then stained for Human Leukocyte Antigen (HLA), which is very specific in the mouse model, as it stains only cells that originated from the implanted human tumor cells. Hence, the HLA stacked in-plane histology (SIH) provided a gold standard for characterizing the co-registered MRI voxels as either normal or containing tumor cells (see figure 9).

A comprehensive visual comparison between individual MRI, interaction regression maps, and SIH showed that the regression maps were more visually comparable with SIH. A voxel-wise correlation analysis (Figure 6) showed a poor correlation between the individual MRI modalities and normalized SIH, whereas the correlation was fair for the regression methods. In addition, a statistical difference was found between tumor volumes identified by the
individual MRI modalities and the “ground truth” SIH. However, there was no significant difference between tumor volumes determined from the regression maps (IRM and IRM_{ave}) and those determined from the “ground truth” SIH. Further, ROC analysis showed lower sensitivity of individual MRI modalities than for the regression maps. This provides quantitative evidence that mpMRI can better distinguish between the tumor region and normal tissue, allowing better delineation of the tumor boundary including infiltration of tumor cells.

It should be remembered that the current results were obtained at a high magnetic field (7Tesla) in a mouse model of human glioblastoma infiltration. Hence, these results (e.g. b values) cannot be directly translated to human patients in the clinic. For example, MRI relaxation times and image contrast will differ at different clinical MRI field strengths (1.5 and 3Tesla). However, they do provide a rigorous justification for mpMRI.

Figure 9: Voxel-wise scatter plots of normalized SIH and MRI data. A) SIH against T1W; B) SIH against T2W; C) SIH against ADC. The SIH maps are used to define each voxel as normal tissue or containing tumor cells; colors indicate whether MRI categorization was true positive (green), true negative (blue) or false negative (Red).

6. Conclusion

For the first time we are able to conclude that mpMRI, as hypothesized, provides more information than the individual images. An interactive linear regression model was better at identifying the whole tumor region than when using individual MR images alone. Development and translation of such techniques could allow improved brain tumor diagnosis, prognosis, and monitoring.

Acknowledgments

H. Al-Mubarak would like to thank the Ministry of Higher Education and Scientific Research of Iraq for financial support. Contract grant sponsor: The Brain Tumour Charity; Contract grant number: 26/160.nt.
References


