

ORIGINAL ARTICLE

The effect of region of interest strategies on apparent diffusion coefficient assessment in patients treated with palliative radiation therapy to brain metastases

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ABSTRACT

Background. Although diffusion-weighted magnetic resonance imaging (DW-MRI) is widely used in radiation therapy (RT) response studies, no standard of delineating the region of interest (ROI) exists. In this retrospective study, we evaluate the effect of four ROI strategies on the apparent diffusion coefficients (ADC) in patients receiving palliative RT to brain metastases.

Material and methods. Twenty-two metastases from nine patients, treated with whole-brain irradiation (30 Gy in 10 fractions) were analyzed. Patients were scanned with a 1T MR system to acquire DW- (eight b-values), T2*W-, T2W- and T1W scans, before start of RT (pre-RT) and at the 9th/10th fraction (end-RT). The following ROI strategies were applied. ROI_{b800} and ROI_{b0}: Entire tumor volume visible on DW(b = 800 s/mm²) and DW(b = 0 s/mm²) images, respectively. ROI_{b800vi}: Viable tumor volume based on DW(b = 800 s/mm²). ROI_{b800rep}: ROI_{b800} from pre-RT scan replicated to end-RT scan. Delineations were aided by co-registered T1W, T2W and T2*W images. ADC was estimated with two mono-exponential fits and one bi-exponential fit.

Results. Differences in absolute ADC values were non-significant across ROI strategy independent of fitting method, while significantly different between fitting methods. Evaluation of individual metastases showed that ROI strategies disagreed on the relative ADC change (from pre-RT to end-RT) in 13 of the 22 metastases when all fitting methods were added up.

Conclusion. The ROI strategies have an effect on the relative ADC change, which may be important for the assessment of individual patient's response to RT and the interpretation of the current literature.

Imaging biomarkers are becoming increasingly important in radiation therapy (RT) for detection and characterization of cancers, and for monitoring response to treatment. Diffusion-weighted magnetic resonance imaging (DW-MRI) is one such imaging modality that has become widely popular for treatment response in RT [1]. Unfortunately, DW-MRI is susceptible to great variability in terms of both technique (sequence, scanner system etc.) and strategies for defining the tumor region, the so-called region of interest (ROI).

DW-MRI has the ability to measure the random motion of water molecules. In biological tissue, molecular diffusion is confined within different compartments (e.g. extracellular and intracellular space), and the term apparent diffusion coefficient (ADC) is therefore used, representing the diffusion averaged over different compartments. The ADC is derived from the acquired DW-MRI images [2] when at least two *b-values* are implemented. The *b-value* defines the sensitivity of the DW-MRI sequence to diffusion, and usually the signal of the DW image is modeled

as a mono- or bi-exponential decay with increasing b-value [2,3].

The diffusivity of water within a tumor may depend on, e.g. the histology and cellularity of the tumor [4,5]. In large tumors, necrosis (water and protein rich fluid) may also be present, and in the course of RT the cellular response of tumors may include swelling, shrinkage, necrosis etc. [6,7]. This implies that the derived ADC may change during treatment, and that the ROI strategy may have an influence on the ADC change.

In this retrospective study we compared the derived ADC values from four ROI strategies in patients with brain metastases treated with palliative RT. The ADC was estimated with three fitting methods to assess possible interaction between ROI strategy and fitting method.

Material and methods

Patients

Twenty-two (N = 22) brain metastases from nine patients were analyzed. All patients were treated with palliative intent RT with a total dose of 30 Gy in 10 fractions (5 fractions/week), delivered as whole brain irradiation with 6 or 15 MV photons. Concomitantly, 150 mg of anti-inflammatory steroid (Prednisolone) was given daily during the course of RT. In eight of nine patients the Prednisolone course was started prior to the pre-RT scan, in one patient the day after pre-RT scan. The study was approved by the Danish Scientific Ethical Committee and the Danish data protection authorities. Additional patient characteristics in Supplementary Table I (available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2015.1061211>).

Magnetic resonance imaging

Patients underwent an imaging protocol consisting of DW-, T2W/T2*W- and T1W-MRI with gadolinium contrast (DOTAREM, 279.3 mg/ml, Guerbet, France), 0–3 day(s) before start of the RT course (pre-RT). At the end of the treatment course (fraction 9 or 10), patients were scanned using a shorter protocol, comprising T2W/T2*W and DW sequences (end-RT). For three patients the T2*W scan was not acquired. In all patients a 1T Philips Panorama MR system (Philips Healthcare, The Netherlands) was used with an eight-channel head coil. RT fixation mask was not used to avoid patient discomfort. Co-registration of DW images at different b-values (post-processing) secured limited motion effects. Total scan time was about 35 minutes when all images were acquired.

DW scans were implemented using a single-shot EPI, fat-saturated (DWIBS) SE sequence with eight b-values (0, 50, 100, 150, 400, 500, 600, 800 s/mm²) in all three gradient directions subsequently modulus averaged with TR/TE 7411 ms/110 ms, FA 90, matrix 116 × 90, resolution 1.8 mm × 1.8 mm, sl 4 mm, no gap. Additional sequence details in Appendix (available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2015.1061211>).

Region of interest strategies

All delineations were performed by a radiologist (HHJ) with more than seven years of experience in DW imaging, using a freehand manually contouring tool (Eclipse v.10.0, Varian Medical Systems, Inc., Palo Alto, CA, USA). The T2W and DW images were co-registered and ROIs were drawn individually on pre-RT and end-RT DW images, guided by T2W and T1W image overlays to detect areas with edema and necrosis for further tumor characterization. Necrosis is typically observed as hyper intense regions in the T2W image inside the tumor outline. Metastases with visible blood and regions with melanin in patients with malignant melanoma were defined with T2*W images and avoided in the ROIs. Four ROI strategies were applied (Figure 1) on pre-RT and end-RT scans:

ROI_{b800}: Delineation of the entire tumor suspect region based on the DW image with b = 800 s/mm² (b800).

ROI_{b800vi}: This ROI is equal to ROI_{b800} but excludes necrotic areas, hence only the *viable* tumor is delineated based on the b800 image.

ROI_{b800rep}: The pre-RT ROI_{b800} were replicated onto the co-registered end-RT DW b800 image. Image registration and ROI replication was done using the registration and contouring tool (Eclipse v.10.0), respectively.

ROI_{b0}: Delineation of the entire tumor suspect regions based on the DW image with b = 0 s/mm² (b0).

Apparent diffusion coefficient

The ADC values were derived by fitting DW data to two mono-exponential [2] and one bi-exponential [3] models, respectively, with in-house developed Matlab R2010a scripts (The Mathworks Inc., Natick, MA, USA). A non-linear least squares fitting algorithm was used in all cases.

Mono exponential fit – all b-values: DW data from eight b-values (0–800 s/mm²) were fitted to the mono exponential model $S(b) = S(0) \cdot \exp(-b \cdot \text{ADC})$, where $S(b)$ is the (arithmetic) mean signal of the ROI as a function of the b-value, and $S(0)$ is the mean signal at b = 0 s/mm².

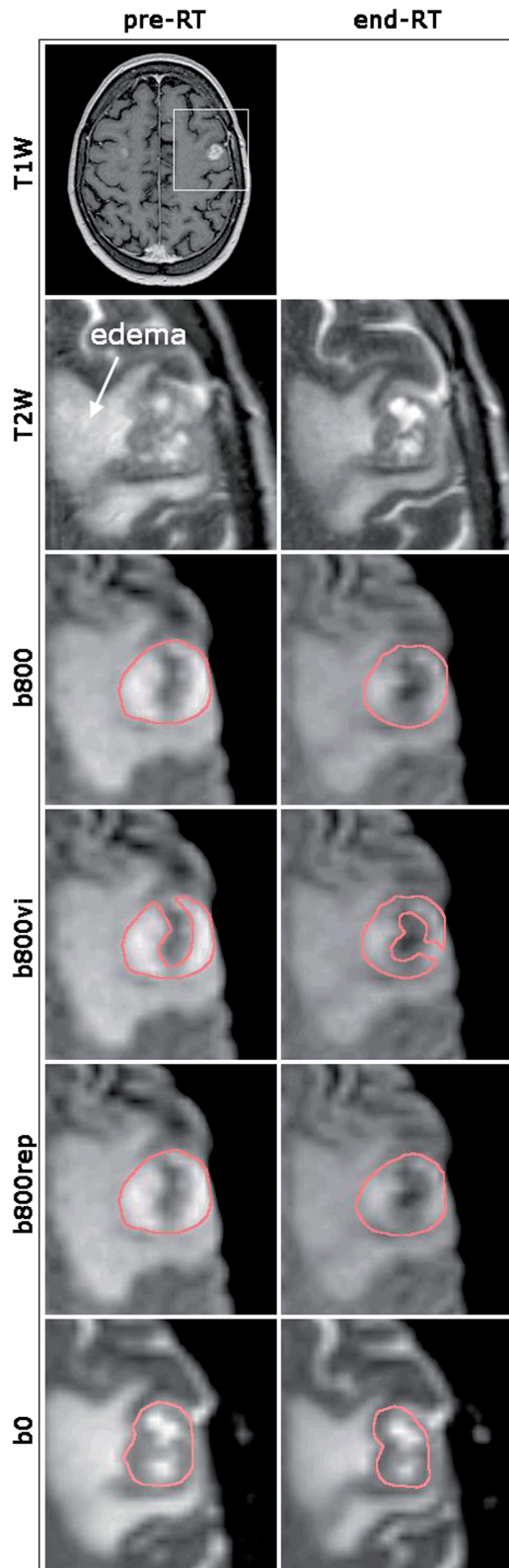


Figure 1. Axial MRI slices for Patient 7. The white box on T1W image shows the location of the brain metastasis and the images below show the corresponding magnification. The pink contours are different ROI strategies for the same metastasis.

Mono exponential fit – high b-values: Data from the high b-values (400–800 s/mm²), avoiding potential signal contribution from tissue perfusion, were fitted as above.

Bi-exponential fit: Data was fitted to a bi-exponential function $S(b) = S(0) \cdot (f \cdot \exp(-b \cdot D_p) + (1-f) \cdot \exp(-b \cdot \text{ADC}))$. In this model part of the irreversible signal loss is assumed to originate from the capillary perfusion giving rise to the so-called pseudo-diffusion D_p . Another compartment is assumed to be the extracellular, extracapillary water volume of the tissue, where something approaching *true diffusion* is taking place. The factor f is the perfusion fraction, and hence $1-f$, the *true diffusion* fraction. Only ADC values were compared in this study. The ADC estimate was the median of solutions based on different initial guesses of f , D_p and ADC. The initial guess of the parameters was qualified by the method described in [8].

Statistics

Two-way repeated measures ANOVA was used to compare relative ADC changes between pre-RT and end-RT scans of pooled data from different ROI strategies and different fitting methods, and possible interaction between the factors. The non-parametric Friedman test was used to test for the difference in absolute ADC estimates between ROI strategies and fitting methods individually. Multiple comparisons using the Wilcoxon signed-rank test with Holm's Bonferroni correction were used to identify pairs of significantly different groups. Significance level was chosen as 0.01.

Results

The ADC estimates of the pooled data from all 22 metastases (Figure 2) showed no clear difference in ADC across ROI strategies, neither between the pre-RT and end-RT scans. End-RT ADC values were indeed increased compared to pre-RT ADC values (except for ROI_{b800rep}, pre-RT) but not significantly (Supplementary Table II, available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2015.1061211>).

The mono exponential fit with all b-values yielded the highest ADC (Figure 2). The mono exponential fit with high b-values and the bi-exponential fit estimated significantly lower ADC values ($p < 10^{-7}$) compared to the mono exponential fit with all b-values, for all ROI strategies. The differences between the mono exponential fit with high b-values and bi-exponential fit were all non-significant; although the latter generally showed lower ADC values (Supplementary Table II available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2015.1061211>).

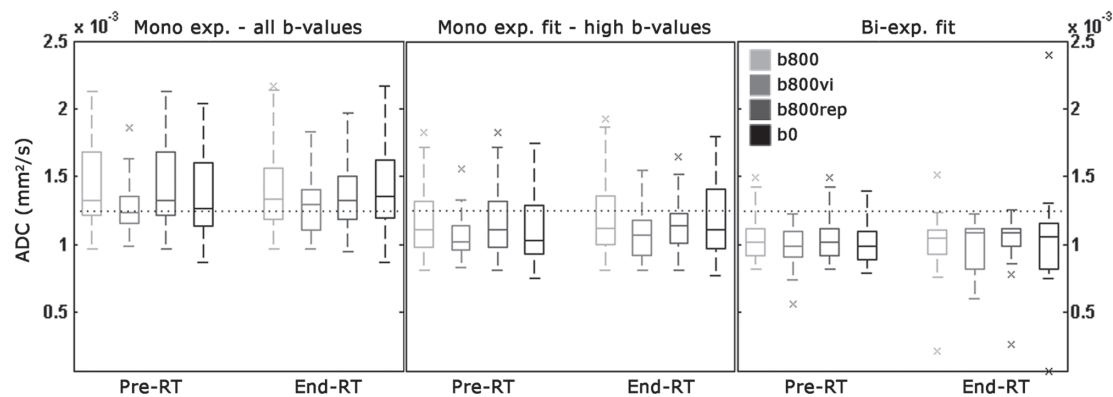


Figure 2. Boxplots of pooled data showing ADC for different ROI strategies for pre-RT and end-RT scans. The horizontal line indicates the median and the edges of the box are the 25th and 75th percentiles. Data points outside $\pm 2.7\sigma$ are defined as outliers and shown individually.

Data also showed that the variation is slightly lower with the mono exponential fit when only the high b-values are used as compared to all b-values being used. The variation decreases further when the full range of data is modeled with the bi-exponential fit. However, the bi-exponential fit introduces more outliers (Figure 2).

Individual data points allowed assessment of the agreement in the relative change in ADC from pre-RT to end-RT scans, between the different ROI strategies (Figure 3). Generally, about two thirds of the data points were below $y=1$, indicating an increased ADC, which corresponds to a positive treatment effect, and in agreement with absolute ADC estimates (Supplementary Table II available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2015.1061211>). Disagreement in the relative ADC change across ROIs was seen in six metastases for the mono exponential models (metastases no. 1, 14, 18, 20, 21, 22 and 1, 3, 14, 20, 21, 22, respectively) and nine metastases for the bi-exponential model (metastases no. 1, 2, 4, 7, 14, 17, 19, 20, 21). Ignoring ROI_{b800rep}, these numbers dropped to three to four and eight, respectively. The largest variation in relative ADC change was seen in the bi-exponential data, with no specific relation to ROI strategy. The two-way ANOVA showed no difference in the relative ADC between ROI strategies ($p < 0.51$) or between fitting methods ($p < 0.40$).

Discussion

We have analyzed how the selected ROI strategy affects the ADC assessment in brain metastases of patients treated with whole brain RT. It was not possible to demonstrate any significant effect of ROI strategy in a population based (pooled data) analysis (Figure 2), regardless of ADC fitting method (no interaction). Interestingly, the individual data points revealed that in 13 of the 22 metastases, the relative ADC change was

indeed affected by the choice of ROI strategy when all fitting methods are added up (Figure 3). As the change in ADC from pre-treatment scan to end-treatment scan is assumed to be correlated to treatment response, this level of disagreement indicates that the prognostic value of DW-MRI is critically dependent on the choice of ROI and fitting method for individual patients (metastases). Although our results cannot be generalized, studies of the ROI strategy's impact on ADC in other body parts report similar findings [9,10].

An increase in ADC is expected in patients responding well to the treatment [11,12] although evidence is still lacking [13,14]. Increase in ADC, especially with ROI_{b800}, is a reflection of a larger portion of the tumor becoming necrotic and due to cell lysis during the RT course [4,5]. Our data did not show a clear increase in ADC at the end-RT scan. One possible explanation is that data were not stratified into response groups (based on volumetric change in post-RT follow-up scan), as the objective in this study was simply to relate ADC to ROI strategy regardless of treatment response. Studies show that ADC is not increased in non-responders [15]. Another explanation may relate to the concomitant treatment with corticosteroid (Prednisolone) which reduces vasogenic edema, i.e. the water content in the extracellular and extracapillary space. In a study of low dose corticosteroid (10 mg/day) no effect was found on the ADC [16] with a DW-MRI sequence similar to ours. No report on high dose corticosteroid, as in our study, was found and we therefore cannot discard potential effects of this on both the absolute ADC estimates and the relative ADC change.

The mono exponential fit with all b-values yielded the highest ADC values compared to the other fitting methods. This is probably due to the signal at low b-values, where the pseudo-diffusion contributes considerably to the total signal loss. As pseudo-diffusion is almost eliminated at b-values above 200 s/mm² [3], the result is a steeper overall falloff (hence higher ADC

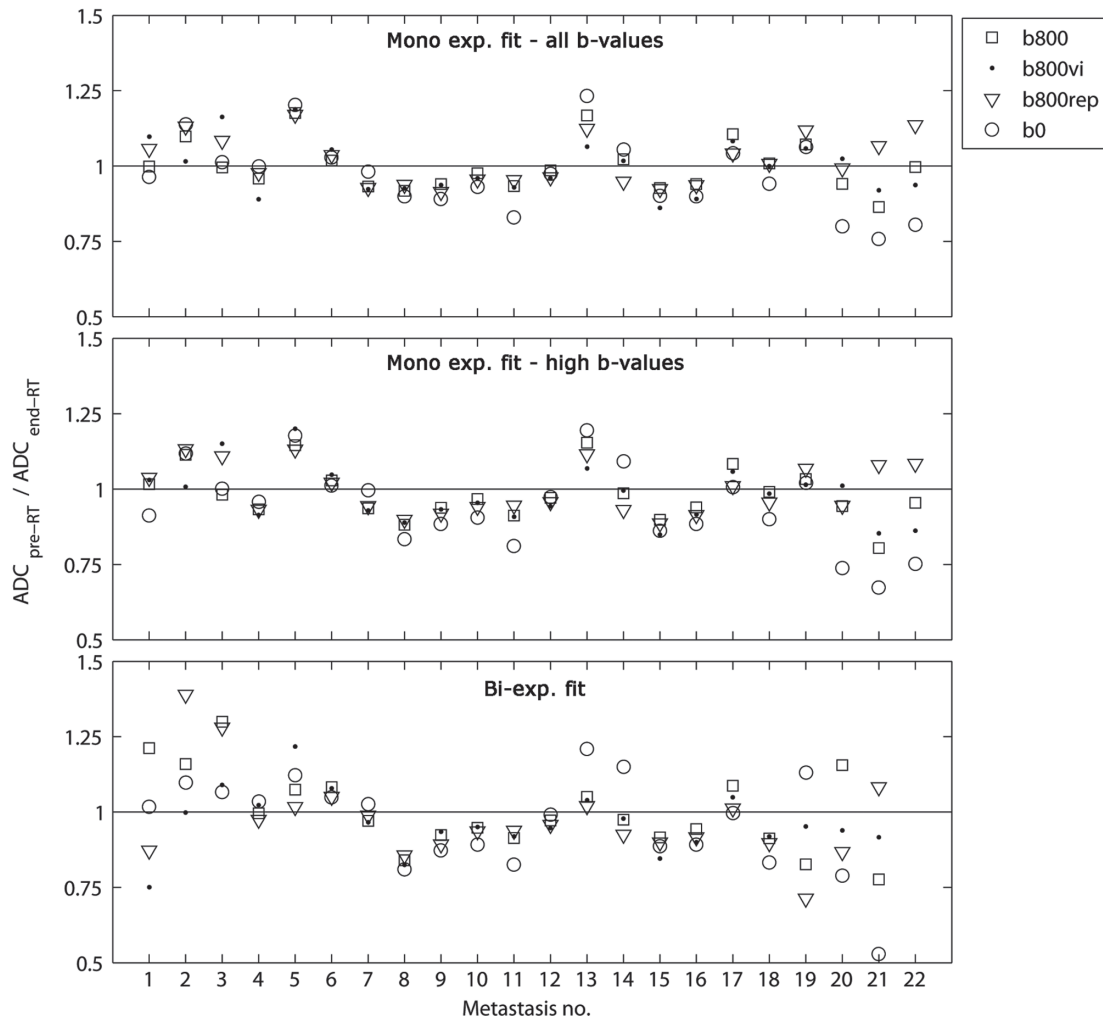


Figure 3. The relative ADC change for individual metastases when different ROI strategies are applied. Metastasis 22 is outside of scale for the bi-exponential fit (all values were above 1).

estimate). An overestimation of ADC of up to 40% has been reported when low b -values are included in a mono exponential fit [17]. Further, data from the mono exponential fit with all b -values has the highest degree of variation (Figure 2), indicating that the underlying biology is not correctly modeled by a single exponential function, i.e. a single compartment. Accordingly, the lower level of variation with the bi-exponential fit may indicate that this model represents more accurately diffusion in the tissue (metastasis). In contrast, the larger variation in the relative ADC change seen with the bi-exponential fit may be explained by the higher number of outliers, probably caused by the ill-conditioned underlying mathematical model. It could also imply that the bi-exponential model, assuming it explains data better, is more sensitive to changes in the tissue, i.e. to the changes in ROI.

The signal to noise ratio (SNR) in DW-MRI is not simply quantifiable in parallel-imaging sequences as used in the present study [18] and may, if too low, lead to an underestimation of the ADC [19]. The

magnitude of this effect may be both dependent on the ROI strategy, since SNR is dependent on tissue type, and on the selected fitting method, as SNR is also dependent on the b -value. (SNR is also dependent on the distance of the metastasis to the receiver coil, but this is presumed negligible since a dedicated head coil was used). We found SNR to be around 10 using a simplistic approach, comparing the signal in the skull with the average signal of sample metastases at $b = 800 \text{ mm}^2/\text{s}$. The DW-MRI sequence DWIBS in this study is combined with a short inversion time inversion recovery (STIR) fat suppression pre-pulse, which is known to reduce SNR. A better alternative could be a chemical shift selective (CHESS) pre-pulse. Also, with DWIBS the ADC estimate is known to be affected in moving ROIs [20]. In this study although patients were not scanned with fixation mask motion was not considered an issue because patient alignment and comfort was secured through the use other RT fixation accessories, such as head support and cushions.

The purpose of this study was not to advise one particular ROI strategy, rather to investigate how the choice affected the ADC. We conclude that median ADC is unaffected by the choice of ROI in a heterogeneous population. However, we did observe large differences in individual metastases across ROI strategies, and also in ADC estimates across fitting methods. This suggests that research DW-MRI data should be pre-analyzed prior to selecting a method for final calculations, with different fitting methods in conjunction with the ROI strategy selected for the specific response study. Our focus was the ROI strategies and analysis methods commonly used in clinical studies, however, other ROI strategies and fitting methods are possible, and also different analysis methods are in use, e.g. histogram analysis, voxel-based analyses etc. [21]. For further investigation we would suggest subgrouping data into responders/non-responders, primary disease etc., which also requires larger sample sizes.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Supplementary material available online

Supplementary Appendix and Table I-II available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2015.1061211>