On-line Appendix A: Semiautomatic Segmentation Algorithm

The threshold analysis is a semiautomatic one, in which a rough ROI is manually defined in the region of tumor occurrence (excluding areas of obvious blood products) and then segmentation is applied, as explained below:

T1GdVs. First, image histograms (representing the normalized number of pixels in the sections on T1WI at each range of signal-intensity values) were plotted and smoothed, and a basic peak-detection algorithm was applied. This histogram typically consisted of 2 distinct peaks: surrounding noise at the lower intensities and the brain tissue at higher intensities. Then, the brain peak was fitted to a Gaussian function, and the threshold for enhancing regions was set as the signal intensity at which the number of pixels reached half of their value at the smoothed-peak height after removal of the background level. Pixels above this threshold were considered enhancing, and pixels below the threshold were considered nonenhancing. The enhancing lesion volume was calculated as the number of enhancing pixels in the rough ROI multiplied by the volume of a single pixel.

In this analysis, a rough ROI is delineated manually prior to the automatic segmentation; this step prevents the need for user-dependent manual corrections.

The advantage of the adaptive thresholding method compared with a fixed empiric threshold is demonstrated in the On-line Figure: A responding patient was scanned before initiation of bevacizumab treatment on an Optima MR450w 1.5T open MR imaging system (GE Healthcare), and the first follow-up after treatment was acquired on a Signa HD 3T system (GE Healthcare). The empiric threshold used in the baseline scan was not suited to the dynamic range of images acquired in the second scan; therefore, the calculated enhancing volume at the first follow-up was not accurate. Manual corrections are often applied, but the result in such cases is user-dependent and not necessarily reproducible.

FLAIRVs were calculated by applying the same threshold analysis to precontrast T2-FLAIR MRIs.

BlueVs and red volumes were calculated from the TRAMs with the following threshold analysis: A mask consisting of all the pixels within the signal intensities within the full width at half maximum of the brain Gaussian from the T1 MRIs was copied to the TRAMs, and a histogram of the TRAM intensities in these pixels was plotted. This histogram typically consisted of a Gaussian distribution centered at zero, where the center of the distribution represents normal brain and the negative and positive tails represent blue and red abnormal tissues. Again, the threshold was set to 50% of the Gaussian peak height so that values within ±50% were considered normal brain. The pixels within the ROI below the low threshold (50% of peak height on the left side of the peak) were considered blue, and the pixels within the ROI above the high threshold (50% of peak height on the right side of the peak) were considered red.

HPVs were calculated from relative CBV (rCBV) maps that were calculated from the DSC MRIs with a leakage correction using commercially available software (FuncTool 5x2.1.08; GE Healthcare). rCBV maps were normalized to the average rCBV value of an ROI chosen in the contralateral normal-appearing white matter.[1] The average value of rCBV in the enhancing-lesion ROI was then calculated by registering the T1 MRIs to the rCBV maps and copying the enhancing ROIs from the T1 MRIs to the rCBV maps. HPV was defined as rCBV > 1.8 based on previously published thresholds.[1,2]

Mean ADCs were calculated from ADC maps that were calculated from the DWI array spatial sensitivity encoding technique sequence using commercially available software (FuncTool 5x2.1.08; GE Healthcare). The mean ADC value in the enhancing-lesion ROI was then calculated by registering the T1WI to the ADC maps and copying the enhancing ROIs from the T1WIs to the ADC maps.

REFERENCES

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On-line Appendix B: Comparison of the TRAMs with Conventional MRI

To further study the added value of the TRAMS over conventional MRI, the correlation between BlueV and the parameters calculated from conventional MRI, T1GdV, FLAIRV, HPV, and mean ADC were calculated for all the follow-up MRI examinations of all patients ($n = 94$ imaging sessions). The results are summarized in the On-line Table.

BlueVs were found to be strongly correlated with T1GdVs and moderately correlated with HPVs. There was no correlation with FLAIRVs or with mean ADC values. The slope of the correlation function implied that on average, ~50% of the T1GdVs were blue in the TRAMs and, on average, ~90% of the HPVs were blue in the TRAMs.

The slope of 0.52 for the correlation between BlueVs and T1GdVs is consistent with previous data from patients with brain tumors undergoing standard treatments, suggesting that ~50% of the enhancing lesions on contrast-enhanced T1WI is due to morphologically active tumor, while the rest consists of nontumoral tissues.[1]

To further study the different contributions of the TRAMs and DSC MRI and DWI, we registered the TRAMs to the DSC MRIs and DWIs to compare HPV and ADC values in red volumes with those in BlueVs with a Wilcoxon matched-pairs signed rank test.

HPVs were calculated separately within BlueVs and red volumes for each MRI session. Average HPV in BlueVs was found to be significantly higher (4.8 mL; 95% CI, 3.6–6.0 mL) than the average HPV in red volumes (2.8 mL; 95% CI, 2.0–3.6 mL) ($P < .0001$). This result is expected under the assumption that active tumor is depicted in blue in the TRAMs and shows hyperperfusion in rCBV maps. Still, the information in the TRAMs is not redundant to rCBV because red regions in the TRAMs, assumed to represent nontumor tissues, still showed hyperperfused regions.

Similar analysis comparing ADC maps with the TRAMs

showed no significant difference between mean ADC values in BlueVs and mean ADC values in red volumes ($P = .38$).

REFERENCE

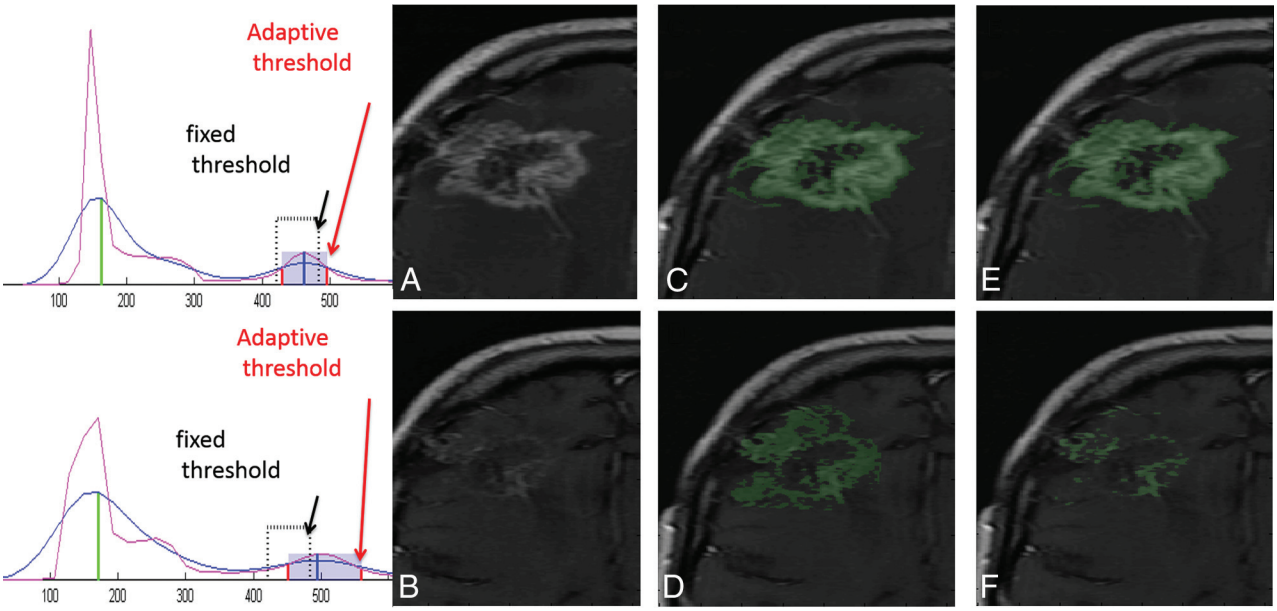
1. Zach L, Guez D, Last D, et al. **Delayed contrast extravasation MRI for depicting tumor and non-tumoral tissues in primary and metastatic brain tumors.** *PLoS One* 2012;7:e52008 CrossRef Medline

On-line Appendix C: Effects of Re-Irradiation during Bevacizumab Treatment

As mentioned above, the role of bevacizumab on OS is controversial; therefore, there is an ongoing search for treatments to combine with bevacizumab to maximize the effect on OS. A phase II study, aimed at establishing an improvement in OS in patients with recurrent GBM undergoing bevacizumab and re-irradiation compared with patients undergoing bevacizumab alone is currently ongoing (Bevacizumab With or Without Radiation Therapy in Treating Patients With Recurrent Glioblastoma; ClinicalTrials.gov, RTOG1205). Here, we present preliminary data collected from a subgroup of 7 patients who underwent re-irradiation and bevacizumab treatment, following progression under bevacizumab treatment alone.

The change (ratio) in T1GdVs, BlueVs, red volumes, and FLAIRVs 48 days on average after initiation of re-irradiation was the following: 1.0 ± 0.2, $P = .64$; 0.7 ± 0.1, $P = .05$; 1.9 ± 0.4, $P = .06$; and 2.0 ± 0.7, $P = .16$, respectively. Five of the 7 patients showed significant reduction in BlueVs (down to $56\% \pm 5\%$ of the preirradiation volumes, $P = .03$), suggesting a response to re-irradiation under bevacizumab. An example is shown in Fig 4.

The observed increase in average FLAIRVs is consistent with the effects of postradiation changes on FLAIR images, preventing early detection of response using this sequence. T1GdVs did not change significantly after re-irradiation, which may be explained either by no response to radiation or by insensitivity to changes from tumor-to-treatment effects (both depicted as enhanced on T1WI). The significant decrease in BlueVs and increase in red volumes in the TRAMs are consisted with response to treatment. These marked changes suggest that the TRAMs may provide sensitive radiologic markers for the response to re-irradiation under bevacizumab unattainable by conventional MRI. The significant reduction of BlueVs to 56% of the initial volume in 70% of the patients suggests that re-irradiation under bevacizumab may be an effective treatment and should be further studied. Unfortunately, we were not able to assess the efficacy due to the limited number of patients.



ON-LINE FIGURE. Contrast-enhanced T1-weighted MR images (CE-T1) of a responding patient before and after treatment. The upper row shows images before initiation of bevacizumab treatment, acquired using an Optima MR450w 1.5T open MR imaging system (GE Healthcare): CE-T1 image without thresholding (*A*), after applying a fixed empiric threshold (*C*), and after applying our adaptive threshold (*E*). The corresponding histogram of signal intensities (upper left) shows that the fixed (*gray dotted line*) and adaptive (*red line*) thresholds are close in this case and result in similar segmentation. The lower row shows images acquired after initiation of bevacizumab treatment using a Signa HD 3T MR imaging system (GE Healthcare): CE-T1 image without thresholding (*B*), after applying the fixed empiric threshold (*D*), and after applying our adaptive threshold (*F*). The corresponding histogram of signal intensities (lower left) shows that this time the fixed and adaptive thresholds differ significantly. The result is a clearly inaccurate volume measurement when using the fixed-threshold method. The OS of this patient is >2 years (the patient is still alive), consistent with the significant decrease in lesion volume calculated using the adaptive threshold.

On-line Table: The correlation between blue volumes in the TRAMs and MRI parameters calculated from conventional MRI[a]

MRI-Based Parameter	Slope	95% CI	R^2	*P* Value
T1GdV	0.52	0.48–0.55	0.9	<.0001
FLAIRV	0.06	0.02–0.09	0.1	.003
Mean ADC	0.0008	−0.0001–0.0017	0.03	.08
HPV	0.92	0.76–1.01	0.64	<.0001

[a] The correlation between the blue volume calculated from the TRAMs and T1GdV, FLAIRV, ADC calculated from diffusion-weighted MRI, and HPV was studied with linear regression analysis for all patients at all time points ($n = 94$).