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Dynamic-susceptibility contrast agent MRI measures of relative cerebral blood volume predict response to bevacizumab in recurrent high-grade glioma

Kathleen M. Schmainda, Melissa Prah, Jennifer Connelly, Scott D. Rand, Raymond G. Hoffman, Wade Mueller, and Mark G. Malkin

Department of Radiology, Medical College of Wisconsin, Milwaukee Wisconsin (K.M.S., M.P., S.D.R.); Department of Biophysics, Medical College of Wisconsin, Milwaukee Wisconsin (K.M.S.); Translational Brain Tumor Research Program, Medical College of Wisconsin, Milwaukee Wisconsin (K.M.S., J.C., S.D.R., W.M., M.G.M.); Department of Neurology, Medical College of Wisconsin, Milwaukee, Wisconsin (J.C., M.G.M.); Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin (R.G.H); Department of Neurosurgery, Medical College of Wisconsin, Milwaukee, Wisconsin (J.C., W.M., M.G.M.)

Corresponding author: Kathleen M. Schmainda, PhD, Department of Biophysics, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226 (kathleen@mcw.edu).

Background. The anti-VEGF antibody, bevacizumab, is standard treatment for patients with recurrent glioblastoma. In this setting, traditional anatomic MRI methods such as post-contrast T1-weighted and T2-weighted imaging are proving unreliable for monitoring response. Here we evaluate the prognostic significance of pre- and posttreatment relative cerebral blood volume (rCBV) derived from dynamic susceptibility contrast MRI to predict response to bevacizumab.

Methods. Thirty-six participants with recurrent high-grade gliomas who underwent rCBV imaging 60 days before and 20–60 days after starting bevacizumab treatment were enrolled. Tumor regions of interest (ROIs) were determined from deltaT1 maps computed from the difference between standardized post and precontrast T1-weighted images. Both pre- and posttreatment rCBV maps were corrected for leakage and standardized (stdRCBV) to a consistent intensity scale. The Kaplan–Meier method was used to determine if either the pre- or post-bevacizumab stdRCBV within the tumor ROI was predictive of overall survival (OS) or progression free survival (PFS).

Results. The OS was significantly longer if either the pre- (380d vs 175d; P=.0024) or posttreatment stdRCBV (340d vs 186d; $P = .0065$) was <4400. The posttreatment stdRCBV was also predictive of PFS (167d vs 78d; $P = .0006$). When the stdRCBV values were both above versus both below threshold, the OS was significantly worse (100.5d vs 395d; $P < .0001$). With a 32.5% decrease in stdRCBV, the risk of death was reduced by about 68% but increased by 140% with a 29% increase in stdRCBV.

Conclusions. Standardized rCBV is predictive of OS and PFS in patients with recurrent high-grade brain tumor treated with bevacizumab.

Keywords: bevacizumab, brain tumor, DSC, MRI, rCBV, perfusion.

High-grade gliomas remain one of the most fatal tumors with median survivals ranging from 3–5 years for WHO (World Health Organizaiton) grade III anaplastic astrocytoma and 12–14 months for WHO grade IV glioblastoma.[1] Until recently, few treatment options were available for patients once standard chemoradiation therapy with temozolomide had failed. With the identification of multiple signaling pathways and growth factors essential for tumor angiogenesis, several new anti-angiogenic drugs have been developed. One of these, bevacizumab, is a recombinant humanized monoclonal IgG_1 antibody that binds to human vascular endothelial growth factor (VEGF) and inhibits angiogenesis.[2] Bevacizumab received accelerated FDA approval for the treatment of recurrent glioblastoma on May 5, 2009, and has since become the standard of care for treatment of high-grade glioma.

Patients with recurrent high-grade glioma treated with bevacizumab have demonstrated an excellent radiographic response rate and improved clinical outcome when compared with historical data.[3] In 2 of the early prospective phase II clinical trials,[4,5] the rate of progression-free survival (PFS) at 6 months was 29%–42.6% compared with the 15% historical control rates. However, a statistically significant increase in overall survival (OS) was not

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demonstrated. In these trials, the traditional approach for assessing response, the MacDonald Criteria,[6] were used. The MacDonald criteria are based on the 2-dimensional measurement of enhancing tumor on MRI or CT. In later studies using a modified MacDonald criteria that included fluid-attenuated inversion recovery (FLAIR) imaging, increases in both PFS and OS were reported.[3] Subsequently, the RANO (Response Assessment in Neuro-Oncology) criteria were published in 2010 as an update to the MacDonald criteria.[7] The RANO criteria newly incorporates FLAIR imaging to assess the degree of peritumoral edema and is therefore the standard approach used by many institutions for assessing disease progression and treatment response in glioblastoma.

Though these assessment criteria are considered standard, it is becoming increasingly clear that these anatomic measures of tumor response to bevacizumab are often unreliable. Contrast-agent enhancement and FLAIR hyperintensities on imaging primarily reflect the breakdown of the blood-brain-barrier. Bevacizumab acts as a powerful corticosteroid decreasing the permeability of the blood-brain-barrier.[8] Thus, rapid decreases in the degree of contrast enhancement and FLAIR hyperintensity may not necessarily reflect true changes in tumor biology or cellular burden. As such, measures derived from standard imaging may neither be able to predict OS reliably nor be an accurate representation of PFS. The antivascular permeability effect of VEGF inhibition likely accounts for the high initial radiographic response rate, with profound reductions in enhancement on MRI being observed as soon as 24 hours after the first dose of bevacizumab.[5,9] Yet, as demonstrated in a recent study,[5] this is clearly too early for an equally profound cellular antitumor effect since rapid subsequent progression developed in nearly half of the initial responders. Still, patients do derive clinical benefit from treatment as manifested by decreased cerebral edema, improved neurological symptoms, and decreased requirement for corticosteroids. In addition, some bevacizumab-mediated antumor effect likely occurs in at least a subpopulation of patients because nearly half of the initial radiographic responders were progression free for more than 6 months. Consequently, biomarkers that more directly measure the biologic effects of these agents are vital for better stratification into various treatment arms, as well as the improvement of research pursuing new therapies and strategies for high-grade tumors.

Relative cerebral blood volume (rCBV) imaging, which is derived from dynamic susceptibility contrast (DSC) MRI, has the potential to serve as a predictive biomarker of response. With DSC-MRI, T2 or T2*-weighted images are acquired with high temporal resolution during the bolus adminstration of a gadolinium (Gd) contrast agent.[10] The resulting rCBV image maps have demonstrated the ability to predict tumor grade[11–16] and survival[17] and to distinguish posttreatment radiation effects from recurrent tumor.[18–20] In preliminary studies the, potential of rCBV to predict response to antiangiogenic therapy more reliably than standard MRI has been demonstrated.[21]

Though DSC-MRI has shown great promise for the evaluation of brain tumors, its role as a prognostic measure for response to bevacizumab has not been fully evaluated. Consequently, the goal of this study is to investigate the utility of rCBV, or more specifically standardized rCBV (stdRCBV), to predict the overall survival (OS) and progression free survival (PFS) in response to bevacizumab in comparison with FLAIR-hyperintense and contrast-agent enhancing tumor volumes.

Materials and Methods

Patients

All patients with grade III or IV gliomas who underwent rCBV imaging were retrospectively considered for this study and were included if they underwent rCBV imaging within 60 days before and 20–60 days after starting treatment with bevacizumab (Avastin, Genentech), and had a confirmed diagnosis of high-grade glioma at the time of treatment initiation. Patients were excluded from this study if the rCBV data were not collected within the time frame described above, if they did not have the full cohort of imaging data necessary for analysis, if a repeat surgery was performed between the pre- and post-bevacizumab scans, or if the data were of insufficient quality resulting from such things as motion or poor contrast injection. A total of 36 participants satisfied these criteria and were enrolled in this study. Bevacizumab was typically given along with another chemotherapeutic agent, the choice of which was based on the prior chemotherapy received by the participant. All patients gave informed written consent under HIPAA-approved guidelines approved by our Institutional Review Board.

The participant characteristics are shown in Table 1. Glioblastoma accounted for 64% (23/36) of the cases at the time of original diagnosis, with secondary glioblastoma comprising 11% (4/36). Anaplastic astrocytoma and anaplastic oligodendroglioma (pure or mixed) constituted 17% (6/36) and 8% (3/36), respectively, of the remaining cases. The median KPS score of all participants was 70, with a range of 40–100. All participants had undergone previous resection(s) and/or biopsies and adjuvant therapy including radiation and/or chemotherapy.

Imaging

Studies were performed on either 1.5T or 3T MRI systems. Standard pre- and postcontrast MRIs were acquired including FLAIR (TE/TR = 151 ms/10 s) and T1-weighted spin-echo imaging (TE/TR = 20 ms/450 ms). To reduce T1 leakage effects, a loading dose of contrast agent (0.05 mmole/kg when using Multihance [Bracco Diagnostics Inc.] and 0.1 mmole/kg when using Omniscan [GE Healthcare]) was administered prior to the DSC study. Since Multihance has twice the T1 relaxivity as Omniscan, only half of the standard dose was given so that the T1 effects would be equivalent. Next, GRE-EPI images (TE/TR = 30 ms/1100 ms, matrix = 96 × 96, FOV = 24 cm) were acquired for 1 minute before and 2 minutes after a 0.1 mmol/kg bolus injection of contrast agent.

Image and Data Analysis

The rCBV maps were corrected for leakage effects and standardized (stdRCBV) using OsiriX open-source software with the IB Neuro (Imaging Biometrics, LLC) plug-in. Standardization was a process by which rCBV values were mathematically transformed to a consistent intensity scale regardless of MR scanner vendor, model, or field strength (1.5T or 3 T).[22–24] All images within a study were registered to the study's FLAIR images. Registration was performed using FSL (FMRIB FLIRT [FMRIB's Linear Image Registration Tool]),[25,26] with 6 degrees of freedom and normalized mutual information cost function. The regions of interest (ROIs) and 3D volumes of contrast-enhancing (T1 + C) tumor and FLAIR hyperintensities were determined using a supervised, automatic, threshold-based algorithm.[27,28] Specifically, files were converted to AFNI[29] (http://afni.nimh.nih.gov/afni) format and standardized using a user-created AFNI script. Delta T1 maps, including thresholded enhancing ROIs and thresholded FLAIR ROIs were all created using AFNI software. The delta T1 maps were determined from the difference between standardized post- and standardized precontrast T1-weighted images,[27] from which thresholded enhancing ROIs and thresholded FLAIR ROIs were determined. The resulting deltaT1 image map, an example of which is shown in Fig. 1, shows only areas with true contrast enhancement and precludes bright T1 signal that might result, for example, from blood products. From deltaT1 images, the enhancing tumor ROIs

Table 1. List of participants included in this study

Participant No.	Gender	Age (years)	Surgical Diagnosis	Previous Treatment
1	Male	60	Anaplastic oligoastrocytoma	Resection, RT with TMZ, Adj TMZ
2	Male	48	Anaplastic astrocytoma	Resection, RT with TMZ, Adj TMZ
3	Male	63	Anaplastic astrocytoma	Biopsy, RT with TMZ, Adj TMZ
4	Male	44	Mixed astrocytoma/oligodendroglioma	Resection, RT, TMZ, re-resection, TMZ
5	Female	36	Secondary anaplastic astrocytoma	Resection, redo surgery, RT, TMZ, Tamoxifen
6	Female	39	Anaplastic mixed glioma	Biopsy, RT with TMZ, Adj TMZ, beta-interferon
7	Female	61	Anaplastic astrocytoma	Biopsy, RT with TMZ, low dose Adj TMZ
8	Male	40	Anaplastic astrocytoma	Resection, RT with TMZ, Adj TMZ
9	Female	34	Anaplastic astrocytoma	Biopsy (x2), RT, redo surgery, TMZ
10	Female	45	Recurrent glioblastoma	Resection, RT with TMZ, Adj TMZ
11	Male	34	Recurrent glioblastoma	Resection, Gliadel wafers, RT with TMZ, Adj TMZ
12	Male	30	Secondary glioblastoma	Resection (grade II), RT, Adj TMZ, redo resection (grade IV)
13	Female	67	Secondary glioblastoma	Resection (gr III), RT with TMZ, Adj TMZ, redo resection
14	Male	52	Glioblastoma	Resection, RT with TMZ, Adj TMZ
15	Male	58	Glioblastoma	Resection, RT with TMZ, Duke ERADICATE (CMV-dcs vaccine trial), with TMZ, redo resection
16	Male	44	Recurrent glioblastoma	Resection, RT with TMZ
17	Male	39	Secondary glioblastoma	Resection (grade II), RT with TMZ, Adj TMZ, redo resection (grade IV), Adj TMZ
18	Male	59	Glioblastoma	Resection, RT with TMZ, Adj TMZ
19	Female	60	Glioblastoma	Resection, RT with TMZ, Adj TMZ
20	Female	64	Glioblastoma	Resection, RT with TMZ
21	Female	40	Recurrent glioblastoma	Resection, RT, redo resection, RT, interferon, Adj TMZ, redo resection
22	Female	63	Glioblastoma	Resection, RT with TMZ, Adj TMZ
23	Female	68	Glioblastoma	Resection, RT only (myelodysplastic syndrome)
24	Female	50	Glioblastoma	Resection, RT with TMZ, Adj TMZ
25	Male	32	Secondary glioblastoma	Resection (mixed grade II), RT, Adj TMZ, redo resection (grade IV)
26	Male	61	Glioblastoma	Resection, RT with TMZ, Adj TMZ
27	Male	47	Glioblastoma	Resection, RT with TMZ, Adj TMZ
28	Male	62	Glioblastoma	Biopsy, RT with TMZ & Motexafin Gadolinium, Adj TMZ
29	Female	49	Glioblastoma	Biopsy, resection, RT with TMZ, Adj TMZ, Accutane
30	Male	60	Glioblastoma	Biopsy, RT with TMZ, Adj TMZ
31	Male	48	Recurrent glioblastoma	Resection, RT with TMZ, redo resection
32	Male	52	Recurrent glioblastoma	Resection, RT with TMZ, Adj TMZ, redo resection,
33	Female	62	Glioblastoma	Resection, RT with TMZ, Adj TMZ
34	Male	35	Glioblastoma	Resection, RT with TMZ, Adj TMZ
35	Female	64	Glioblastoma	Resection, RT with TMZ, Adj TMZ
36	Male	41	Glioblastoma	Resection, RT with TMZ, Adj TMZ

Abbreviations: Adj TMZ, adjuvant temozolomide; RT, radiation therapy; TMZ, temozolomide.

were determined separately for each pre and post-treatment scan. From these the enhancing volumes were computed and median stdRCBV values extracted. For one participant where there was no contrast agent enhancement on the post-bevacizumab scan, the pre-bevacizumab ROI was used. Similarly, hyperintense FLAIR volumes were computed from standardized FLAIR images. The ROIs were determined separately for each pre- and post-bevacizumab scan. For the patient who did not have post-treatment enhancement the pre-treatment ROI was used.

To determine the FLAIR ROIs, the FLAIR images were first standardized using the standardization files created for either 3T or 1.5T field strengths, respective to the scan. From these standardized FLAIR images, the hyperintense regions were chosen using a visual, semiautomated approach that included all abnormal regions including cystic or radiation-induced changes. The approach was the same for both pre- and post-bevacizumab scans for all participants.

Receiver operator curve (ROC) analysis was performed using GraphPad Prism v4.03 (GraphPad Software Inc.) to determine the sensitivity and specificity of stdRCBV in order to distinguish an overall survival of greater than and less than a certain number of days. The empirically chosen stdRCBV value was evaluated for its prognostic power to predict response to bevacizumab. The OS was measured from the date of bevacizumab therapy to the study end date or the time of death. At the end of the study, 7 participants were still alive. The same empirical approach was used in an attempt to choose values for enhancing tumor and FLAIR volumes that might distinguish survival. Finally, the Kaplan–Meier method was used to determine if stdRCBV, enhancing tumor volumes, or hyperintense FLAIR volumes (obtained before or after treatment), or percent changes in these values were predictive of survival.

The ability of stdRCBV to predict PFS was also determined. In order to determine the time to progression, the longitudinal MRI exams acquired for all

participants in this study were assessed. Specifically, for each participant, all available MRI exams that included FLAIR and T1-weighted pre- and post-contrast images from pre-bevacizumab treatment to time of death or study end date were coregistered across time and scored by a board-certified radiologist with more than 20 years of experience (SDR) and blinded to all participant information including exam dates, time between exams, and pathology. FLAIR and T1-weighted images were scored using a Likert scale from +2 to −3, where representative scores are stable (0), moderate improvement or decline (+/−1), significant improvement or decline (+/−2), and treatment failure (−3). PFS was then calculated using the Kaplan–Meier method (GraphPad Prism). All participants were considered to have had progression at the first exam date that showed either a moderate or significant decline or treatment failure when compared with the previous exam date. Participants who did not have a progressive event at the last available MRI exam were censored if the time between the last exam and date of death was >60 days or if the participant was still alive. Six participants were censored for the following reasons: 3 participants were alive at the study end date (censor date is study end date), 2 participants died more than 60 days after their last MRI exam and were lost to follow-up imaging (censor date is last MRI exam), and 1 participant was still alive but lost to follow-up imaging (censor date is last MRI exam). Participants were censored at 60 days to prevent the results from being skewed

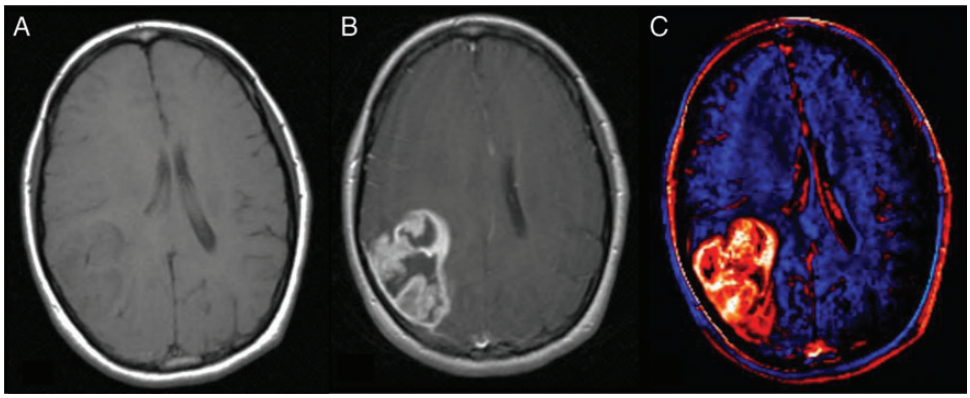

Fig. 1. Creation of deltaT1 map (C) requires subtraction of a standardized pre-contrast T1-weighted image (A) from a standardized post-contrast T1-weighted image (B).

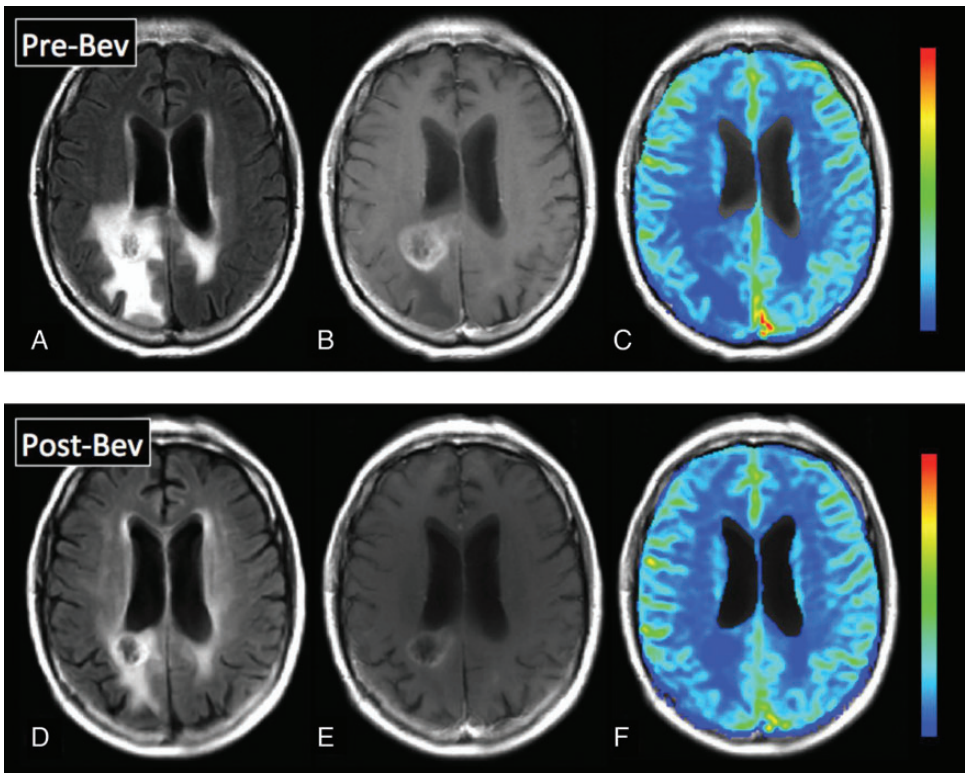

Fig. 2. A male participant aged 62 years with glioblastoma 2 days before and 32 days after treatment with bevacizumab. Included are the pre- and post-bevacizumab FLAIR (A and D), T1 + C (B and E) and standardized rCBV maps (C and F).

towards date of death as an event in cases where participants were lost to follow-up or transferred patient care. Of the participants who were not censored, 10 died within 60 days of their last MRI, having never had a progressive event on imaging; their date of death was used as their progressive event. The remaining 20 uncensored participants had a progressive event on imaging.

Results

Shown in Fig. 2 are examples of T1 + C and FLAIR images with corresponding stdRCBV maps obtained 2 days before and 32 days after beginning treatment with bevacizumab. This participant showed a 46% decrease in standardized rCBV with a survival of 175 days after initiation of bevacizumab treatment. Using ROC analysis, a stdRCBV threshhold of 4400 was determined to distinguish an OS of $<$ or $>$300 days with a sensitivity and specificity of 81% and 60%, respectively, and an AUC of 0.78. While other values for number of days for survival were tested, it was a survival of 300 days that gave the best sensitivity and specificity. Using this threshold, both the pre- and post-bevacizumab stdRCBV predicted a significant difference in overall survival ($P = .0024$ and $P = .0065$, respectively) as shown in Fig. 3a and b. Specifically, if the pre-bevacizumab stdRCBV was $>$4400, the mean OS was 175 days compared with 380 days for stdRCBV $<$4400 (Fig. 3a). Likewise, for a post-bevacizumab stdRCBV of $>$4400, the OS was 186 days compared with 340 days if the stdRCBV was $<$4400 (Fig. 3b). Using the same threshold, the pretreatment stdRCBV was not predictive of PFS ($P = .48$), but the posttreatment stdRCBV was ($P = .0006$; 167d vs 78d). Alternatively, if we divide the data into 4 classes (Fig. 4) where both the pre- and post-bevacizumab stdRCV $<$4400, only pre-rCBV $>$4400, only post-rCBV $>$4400, or both pre- and post-rCBV $>$ 4400, we find that having both the pre- and post-bevacizumab rCBV $>$ 4400 are significantly worse than either pre- rCBV only or post-rCBV alone being $<$ 4400 (HR = 20.2, $P < .0005$ for both greater and HR = 2.8, $P < .016$ for either greater. The reference for both is $<$4400.) The number of participants in each group was $n = 15$, 8, 7, and 6, respectively.

Shown in Fig. 5a are the stdRCBV before (time-point 1 [TP1]) and after (time-point 2 [TP2]) the initiation of bevacizumab treatment. The groups are distinguished by whether the pre-bevacizumab stdRCBV (TP1) was less than (open circles) or greater than (open squares) 4400. A clear pattern of change based on the pretreatment values could not be distinguished. Furthermore, no significant difference in OS could be predicted based on whether there

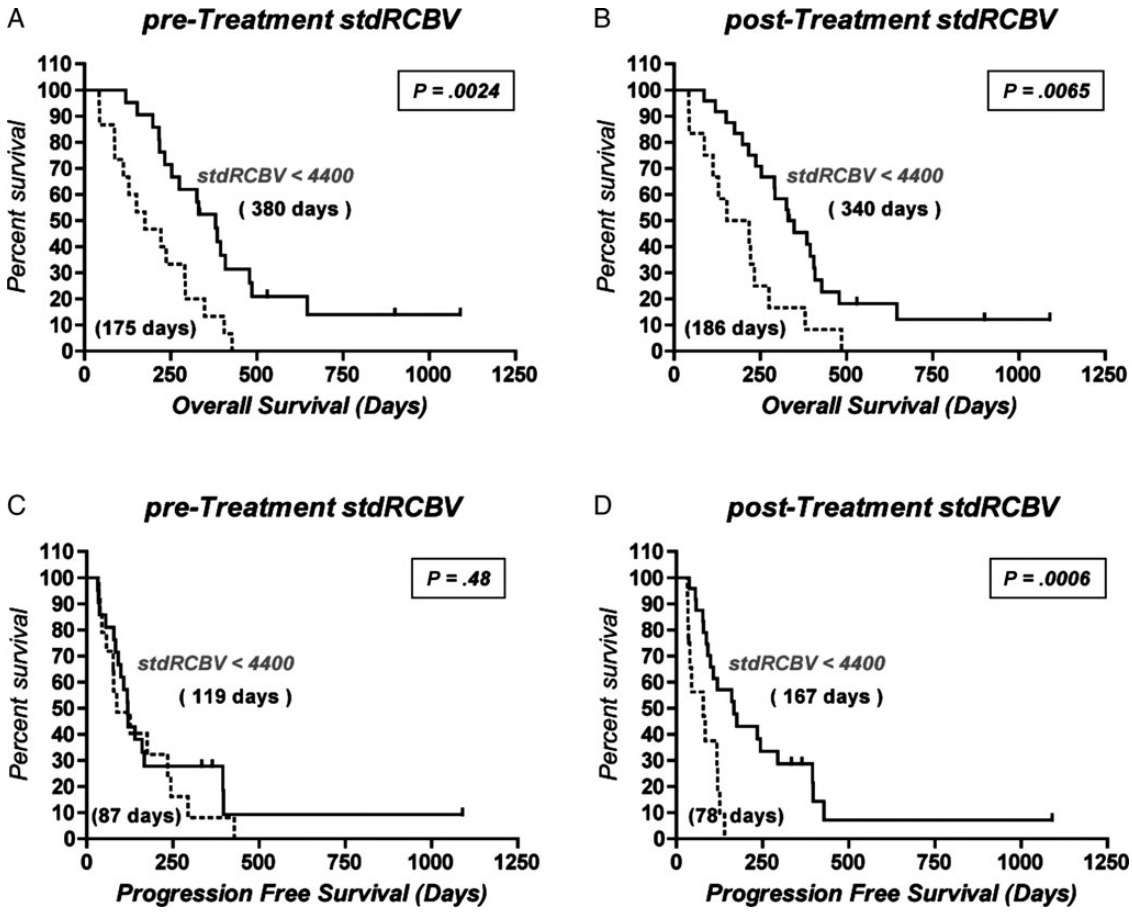

Fig. 3. Overall survival (OS) (A and B) and progression free survival (PFS) (C and D) for participants with high-grade tumors using a stdRCBV threshold of 4400 for the pre-bevacizumab and post-bevacizumab stdRCBV values. The percent survival is designated by a solid black line for stdRCBV $<$4400 and a dashed line for stdRCBV $>$ 4400. Using this threshold results in significant differences in OS based on both pretreatment (175d vs 380d; $P = .0024$) and posttreatment (186d vs 340d; $P = .0065$) stdRCBV values. For PFS, only the posttreatment stdRCBV was predictive of outcome with the stdRCBV $>$4400 having a significantly shorter PFS (78d compared with 167d; $P = .0006$).

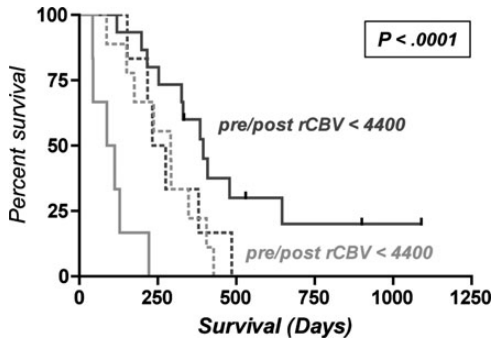

Fig. 4. If both the pre-rCBV and post-rCBV are <4400, the median survival is 395 days. This is significantly longer than the median survival of 100.5 days when both pre-rCBV and post-rCBV >4400. Consistent with this trend, if either pre-rCBV or post-rCBV is >4400, the survival is likewise diminished with median survivals of 291 and 254 days (dashed lines).

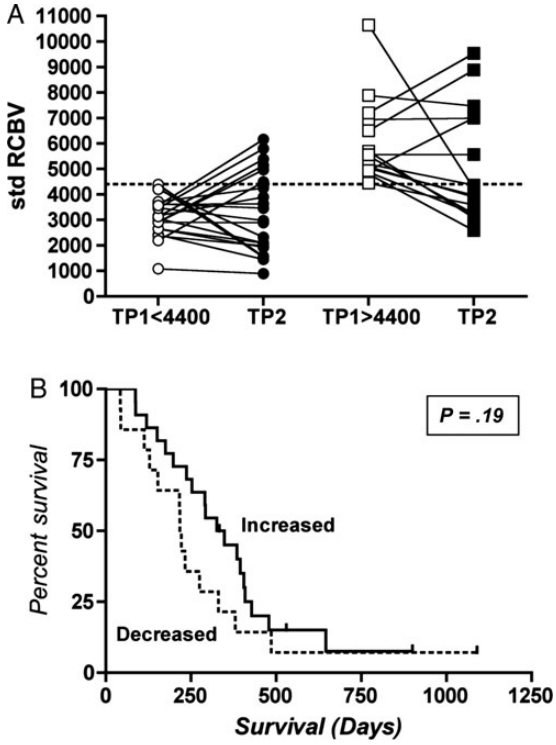

Fig. 5. Shown are (A) the stdRCBV at time point 1 (TP1), before treatment initiation, and at (time-point 2(TP2)) following bevacizumab treatment. The groups are distinguished by whether the pre-bevacizumab stdRCBV (TP1) was less than (open circles) or greater than (open squares) 4400. (B) Whether the stdRCBV decreases (dashed line) or increases (solid line) from TP1 to TP2 is not predictive of overall survival.

was a positive or negative change in stdRCBV values ($P = .19$) (Fig. 5b). However, if either the pretreatment or posttreatment stdRCBV 4400, both the percent change and absolute change corresponded to risk of death. Table 2 shows the reduction in risk of death for the lower quartile, median, and upper quartile of change, assuming a pretreatment stdRCBV < 4400. Being at the lower quartile, for which there is a bigger difference in percent change, results in a reduction of the risk of death by 0.678 times (about 68%); numerically this is a 32.5% decrease from pre-bevacizumab stdRCBV. On the other hand, there is a 1.4 times increased risk of death with a 29% increase in rCBV, which corresponds to the upper quartile. The absolute change works the same way, other than the change being in the absolute value of the stdRCBV and not the percent change. The same prediction of risk holds true if the posttreatment stdRCBV is <4400. Essentially, treatment efficacy depends on both the stdRCBV value and the degree of change induced.

The predictive values of deltaT1 and FLAIR hyperintense volumes were likewise evaluated. The use of either median values or a threshold to distinguish various lengths of survival did not result in any clear separation of groups. For example, when using a survival of < or >300 days, which proved to be useful for the stdRCBV analysis, the ROC analysis gave an AUC of 0.56 with a sensitivity of 81% and specificity of 25% for deltaT1 volumes. Likewise, for FLAIR volumes, the AUC was 0.50 with a sensitivity of 81% and specificity of 20% to distinguish participants with shorter versus longer median survival. Furthermore, comparison of the deltaT1 and FLAIR volumes between the low and high stdRCBV groups did not reveal any significant differences (Fig. 6).

Discussion

This study demonstrates that the stdRCBV, and not contrast-agent enhancing or FLAIR-enhancing tumor volumes, is predictive of response to bevacizumab. The OS was significantly longer if either the pre- or posttreatment stdRCBV was <4400. When the stdRCBV values were both above threshold, the OS was significantly worse compared with the case where either or both values were less than threshhold. While, at the time-point measured, a positive versus negative change in stdRCBV was not predictive of OS, the risk of death could be predicted by the degree of change with treatment when the stdRCBV level was less than a threshold of 4400. Finally, the posttreatment stdRCBV was also shown to be predictive of PFS, while the pretreatment stdRCBV was not.

The findings in this study showing an improvement in OS of 11–16 months seem to contradict the earlier studies showing improvement in PFS but not OS.[4,5] However, subsequent studies indeed showed that the median OS for high-grade tumors treated with bevacizumab was 9–11 months from treatment initiation,[3,30] which is superior to the historically documented survival of 3–6 months for recurrent malignant glioma.[31] The even longer median survival shown in this study is likely the result of further subdividing the participants into categories based on rCBV levels.

Despite the promising result of this study, there are some limitations. First, when trying to summarize imaging data for analysis purposes, the proper choice of a tumor ROI always poses a challenge. For this study, we chose an ROI based primarily on area of enhancing tumor. With this approach, however, it is possible that some tumor existing outside the enhancing regions is missed. Alternatively, using areas of hyperintensity on FLAIR images for the tumor ROIs may include much more non-tumor tissue, and thereby dilute the results, leading to inaccurate conclusions regarding the utility of rCBV. Consequently, as a first order assessment of the role of rCBV to predict outcomes, we chose to

Table 2. Reduction in risk of death as a function of change in rCBV

Quartile of change	Effect of % Change in rCBV on Risk	Corresponding % Change in rCBV	Effect of Absolute Change in stdRCBV on Risk	Corresponding Absolute Change in stdRCBV
Lower	0.678 X	−32.5%	0.735 X	−740.5
Median	0.834 X	−15.2%	0.896 X	−264
Upper	1.397 X	+29.0%	1.464 X	+919.7

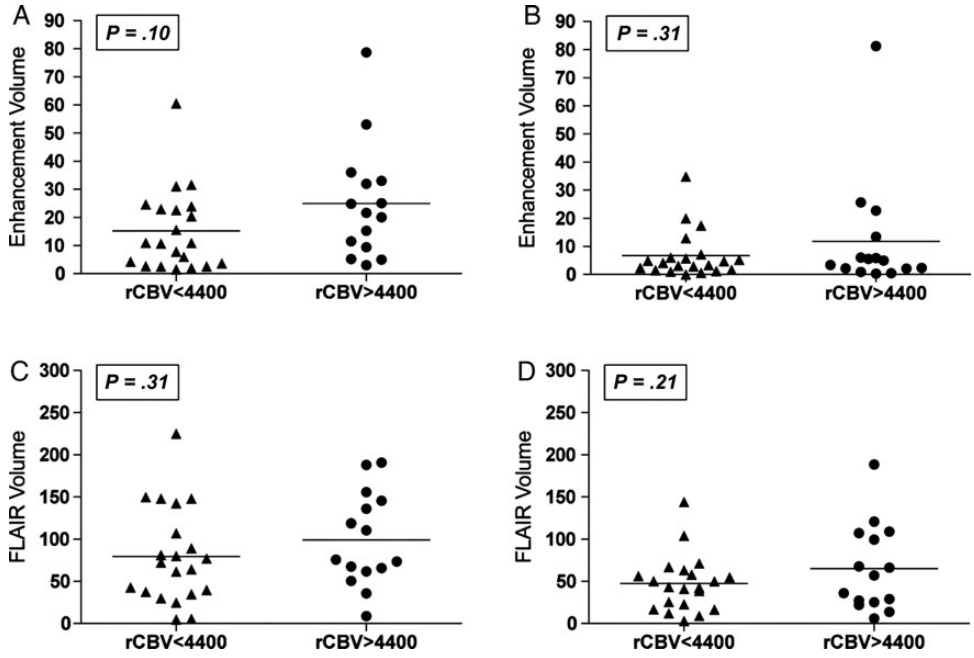

Fig. 6. Contrast-agent enhancing (deltaT1) tumor and FLAIR volumes measured before (A and C) and after (B and D) the initiation of bevacizumab treatment. The groups are distinguished by a median stdRCBV value > or <4400. No significant difference, as defined by $P < .05$ in deltaT1 or FLAIR volumes, exists between these groups.

include only enhancing areas that clearly contained tumor. Additional analyses using other types of "tumor" ROIs are warranted.

A second potential limitation to this study is that the population size was small and comprised of both grade III and grade IV gliomas. Additional questions could have been addressed if there had been more participants. Specifically, there is some evidence to suggest that bevacizumab may be more effective in the early stages of cancer when VEGF may be the predominant pro-angiogenic factor secreted.[32] Alternatively, large tumors may produce very large amounts of angiogenic factors, which theoretically may overwhelm anti-angiogenic agents.[33] It is therefore necessary to perform larger studies with correlative tissue analysis to address these questions. Each of these factors, considered separately, may help to further refine our assessment of bevacizumab response and perhaps determine when and for which patients it would be most beneficial.

The use of stdRCBV is based on previous reports demonstrating that standardization of rCBV improves the consistency of rCBV values over time without altering any of the underlying information.[24] This approach also eliminates the need to draw reference ROIs and thereby eliminates errors resulting from inter- and intraobserver differences. While use of standardization requires an initial training step using 10–15 imaging datasets, it is only necessary to perform this step once. For all subsequent rCBV datasets, only the transformation step is required. The transformation step takes only seconds, therefore saving significant processing time on a routine basis. As such, it is ideal for routine use or for clinical trials. Further study is needed, however, to determine the stdRCBV threshold that most accurately identifies the patients who will receive a survival benefit from bevacizumab versus those who will not. The threshold of 4400 determined in this study is based on a small patient population. Consequently, studies are planned that include a larger cohort of patients to either confirm or further refine the choice of this threshhold.

While this study shows that stdRCBV is more predictive of response than standard anatomical measures of contrast-agent enhancing and FLAIR hyperintense volumes, measures of tumor angiogenesis alone may not be sufficient for full assessment of response to bevacizumab. One unanswered question is whether VEGF-targeted therapy can be harmful in the long run by evoking evasive resistance.[34] For this reason, we and others are also

working to develop diffusion-based methods, which have shown promise in the detection of non-angiogenic invading tumor cells.[35–37]

Several other possible biomarkers are being evaluated to predict and detect response to targeted therapies such as bevacizumab. For example, measurements of circulating levels of VEGF and basic fibroblast growth factor have proven helpful in some instances. However, the multitude of angiogenic factors involved in tumor-associated angiogenesis implies that relying on any single angiogenic factor may be impractical and misleading.[33] For this reason, looking at tumor biology more directly, as we are doing with images of rCBV, may continue to prove to be the best way to predict and monitor response even when using targeted therapies.

In summary, when bevacizumab treatment maintains or decreases the stdRCBV to a value <4400, it will positively impact OS and PFS. Thus, an early measure of stdRCBV should translate into a more rational approach to bevacizumab therapy and lead to enhanced efficacy, a reduction in the number of participants required for statistically informative clinical trials, and the potential for more rapid integration of promising novel agents into clinical practice.[33] Furthermore, this information should aid in the optimization of the clinical response and survival of patients on an individualized basis and decrease the frequency of unnecessary treatment, associated morbidities, and expense if a given patient is shown to be an unlikely responder.

Conclusion

The findings of this study suggest that rCBV in general and stdRCBV specifically may help to improve the prediction of treatment outcome and improve the outcome itself by identifying patients at high risk of treatment failure for whom alternative treatment may be instituted earlier.

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