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CLINICAL STUDY

Prolonged treatment with bevacizumab is associated with brain atrophy: a pilot study in patients with high-grade gliomas

Asim K. Bag · Hyunki Kim · Yi Gao · Mark Bolding · Paula P. Warren · Hassan M. Fathallah-Shaykh · Demet Gurler · James M. Markert · John Fiveash · Timothy M. Beasley · Ayaz Khawaja · Gregory K. Friedman · Philip R. Chapman · Louis B. Nabors · Xiaosi Han

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Abstract Bevacizumab is widely used for treatment of high-grade gliomas and other malignancies. Because bevacizumab has been shown to be associated with neurocognitive decline, this study is designed to investigate whether prolonged treatment with bevacizumab is also associated with brain atrophy. We identified 12 high-grade glioma patients who received bevacizumab for 12 months at the first recurrence and 13 matched controls and blindly compared the volumes of the contralateral hemispheres and contralateral ventricle in these two groups at baseline and after 12 ± 2 months of the baseline scan by two independent analyses. The volumes of the contralateral hemispheres and ventricles did not differ significantly between the two groups at baseline. Whereas, in the control group the volumes of the contralateral hemisphere changed subtly from baseline to follow-up ($p = 0.23$), in the bevacizumab-treated group the volumes significantly decreased from baseline to follow-up ($p = 0.03$). There was significant increase in the contralateral ventricle volume from base line to follow-up scans in both the control group ($p = 0.01$) and in the bevacizumab group ($p = 0.005$). Both the absolute and the percentage changes of contralateral hemisphere volumes and contralateral ventricular volumes between the two patient groups were statistically significant ($p < 0.05$). Results of this study demonstrate prolonged treatment with bevacizumab is associated with atrophy of the contralateral brain hemisphere.

Keywords Bevacizumab · Brain atrophy · MRI

Introduction

Vascular endothelial growth factor (VEGF) is a subfamily of growth factors that are involved in both vasculogenesis

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A. K. Bag · H. Kim · M. Bolding · D. Gurler · P. R. Chapman
Department of Radiology, The University of Alabama at Birmingham, Birmingham, USA

Y. Gao
Department of Biomedical Informatics, Stony Brook University, New York, USA

P. P. Warren · H. M. Fathallah-Shaykh · A. Khawaja · L. B. Nabors · X. Han (✉)
Department of Neurology, The University of Alabama at Birmingham, FOT 1020; 510 20th Street South, Birmingham, AL 35294, USA
e-mail: xhan@uab.edu

J. M. Markert
Department of Neurosurgery, The University of Alabama at Birmingham, Birmingham, USA

J. Fiveash
Department of Radiation Oncology, The University of Alabama at Birmingham, Birmingham, USA

T. M. Beasley
Department of Biostatistics, The University of Alabama at Birmingham, Birmingham, USA

G. K. Friedman
Department of Hematology Oncology, The University of Alabama at Birmingham, Birmingham, USA

and angiogenesis. VEGFA, which is often referred to as VEGF, is the most studied factor regulating angiogenesis. It activates VEGF receptors (VEGFR1 and VEGFR2) and plays a critical role during embryonic development, as well as in homeostatic functions in fully developed brain.

In the adult brain, VEGF plays an essential role in homeostasis of normal neuronal and astrocyte functions. The most prominent site of VEGF expression is in the choroid plexus, and VEGF plays an important role in choroid plexus endothelial cell survival and fenestrae formation [1]. In brain parenchyma, astrocytes are the primary source of VEGF [2–6], where VEGF plays a major role in upregulation of different genes in response to altered cerebral perfusion and metabolism [7]. Recent evidence shows that VEGF is also expressed in the adult brain in a region specific manner and executes nontraditional homeostatic functions that are not angiogenesis-dependent or even perfusion dependent. [8] Of the many known functions, VEGF has an important role as a neurogenic factor that is essential for neuronal stem cell production and neuronal differentiation in the hippocampus [3, 9–11], and in the subventricular zone [10–14], and it also functions in neuroblast migration and neuronal maturation [8]. Additionally, it has been shown that upregulation of VEGF in the hippocampus improves memory as a result of increasing plasticity of mature hippocampal neurons [3], and hippocampal neurogenesis [9]. In addition VEGF has been shown to enhance hippocampal memory by stabilization of hypoxia inducible factor (HIF) in the hippocampus [15].

VEGF plays a crucial role in the physiology of high-grade gliomas. There is upregulation of VEGF production in high-grade gliomas mediated by autocrine and paracrine production in the hypoxic microenvironment of the tumor tissue [16–19]. Excessive production of VEGF leads to new blood vessel formation. However, VEGF-induced tumor vessels in glioblastoma (GBM) are disorganized and highly permeable, resulting in loss of blood brain barrier (BBB) integrity. The impaired BBB results in brain edema, which often causes serious clinical symptoms in GBM patients. Furthermore, higher VEGF expression in gliomas correlates with increased tumor invasiveness and higher histological grade [20]. These findings provide a rationale for the development of anti-angiogenesis therapy. Of the many developed antiangiogenic drugs, bevacizumab, a humanized monoclonal antibody against VEGF, is most commonly used for the treatment of recurrent GBM [21, 22].

With the wide spread use of bevacizumab in GBM and in other cancers, new adverse biological effects are gradually being recognized. Bevacizumab treatment can significantly change the glioma physiology by promoting tumor cell invasiveness through activation of MET signaling that is induced by inhibition of VEGF [23, 24]. Also, a recently completed phase III clinical trial comparing the efficacy of bevacizumab with the standard of care treatment in newly diagnosed GBM patients showed a significant neurocognitive decline in patients treated with bevacizumab [25]. In clinical practice, we have noticed that more prominent enlargement of brain ventricles in patients treated with bevacizumab, as compared to the patients treated with the standard of care. These observations led to our hypothesis that treatment with bevacizumab is associated with brain atrophy.

In this retrospective study, we compared the volume of the contralateral (non-tumor side) brain hemisphere and contralateral lateral ventricular volume in patients treated with bevacizumab plus standard of care treatment (bevacizumab group) with patients treated only with standard of care treatment regimen including radiation and temozolomide (control group) in a blinded fashion.

Methodology

Study set up and patient selection

The University of Alabama at Birmingham Institutional Review Board approved this Health Insurance Portability and Accountability Act (HIPAA) compliant study and waived the requirement of informed consent. Patients with histopathologically confirmed high-grade gliomas were included in the study. Higher-grade gliomas developing from previously known WHO grade II tumors were excluded from the study because these tumors have a different time course of disease and heterogeneous treatment experience. In addition, we excluded all patients who were treated with more than one course of fractionated radiation therapy or who received a second dose of radiation therapy at tumor recurrence (Table 1). The inclusion criteria were: (1) overall survival longer than 1 year from the initiation of bevacizumab therapy, (2) availability of a baseline MRI that included a 3D post contrast T1 weighted sequence within prior 1 month of initiation of bevacizumab therapy, (3) availability of a follow-up MRI that included a 3D post contrast T1 weighted sequence after 12 ± 2 months initiation of the bevacizumab treatment, (4) treated with standard Stupp protocol [26] at the diagnosis and (5) bevacizumab treatment was started at first recurrence. All patients with bi-hemispheric involvement, tumor extension to the ventricles, midline tumor (corpus callosum involvement), midline deviation and significant mass effect to the ventricles were excluded. All the volumetric analysis were performed on the contralateral hemisphere (non tumor bearing), not in the ipsilateral (tumor containing) brain hemisphere in order to avoid treatment-related changes of brain and or ventricular volumes such as anti-edema effect of bevacizumab, steroids and variable size of the resection.

Table 1 Details of the patients: demographics and treatment history

Bevacizumab group (n = 12)	Control group (n = 13)
Sex of the patient	
F = 6	M = 6
M = 6	F = 7
Age of the patient at diagnosis	
Average = 47.5	Average = 50.8
Range = 30–61	Range = 29–67.5
Hemispheric distribution of the tumors	
Right = 9	Right = 6
Left = 3	Left = 7
Treatment	
Initial treatment	
Resection (n = 11)	Resection (n = 12)
Biopsy (n = 1)	Biopsy (n = 1)
Treatment before Initiation of bevacizumab therapy	Treatment before baseline scan
Concurrent tem + rad followed by tem (n = 9)	Radiation followed by tem (n = 2)
	Concurrent tem + rad followed by tem (n = 10)
Concurrent tem + rad followed by tem + CCNU (n = 3)	Concurrent tem + rad followed by tem + CCNU (n = 1)
Radiation dose	
60 Gy (n = 5)	60 Gy (n = 8)
50 Gy (n = 1)	Not documented (n = 5)
Not documented (n = 6)	

* *Rad* fractionated radiation, *tem* temozolomide, *CCNU* lomustine

Bevacizumab group

We retrospectively evaluated all the patients with high grade gliomas (n = 154) who received bevacizumab therapy in the Division of Neuro-oncology in the Neurology Department at the University of Alabama at Birmingham (UAB) between December 2007 and January 2013. Only 62/154 patients lived longer than 1 year. Details of the bevacizumab therapy were not known in 6 patients because they were treated at different hospitals after initiation of their treatment. Of the remaining 56 patients, only 12 patients (Table 1) met the inclusion and exclusion criteria: 7 patients had primary GBM, 3 patients had anaplastic astrocytoma (AA) and 2 patients had anaplastic oligoastrocytoma (AO). Most of the patients were excluded because the 3D post contrast T1 weighted sequence was not available for analysis either at the baseline or at the second follow-up scan (n = 28). Furthermore, 7 patients had bihemispheric tumors, 4 patients had tumor extension to the ventricles, and 5 patients had midline involvement (Fig. 1).

Of the 12 patients in the bevacizumab group, 11 patients were initially treated with tumor resection and 1 patient had a biopsy only (thalamic tumor). After initial resection or biopsy, all patients were treated with concurrent radiation and temozolomide therapy (75 mg/m^2) followed by maintenance temozolomide therapy (150–200 mg/m^2) for 5 consecutive days every month). Of the included 12 patients, 8 patients received ≥12 cycles of temozolomide therapy at the time of follow-up MRI and the remaining 4 patients received ≤12 cycles of temozolomide therapy at

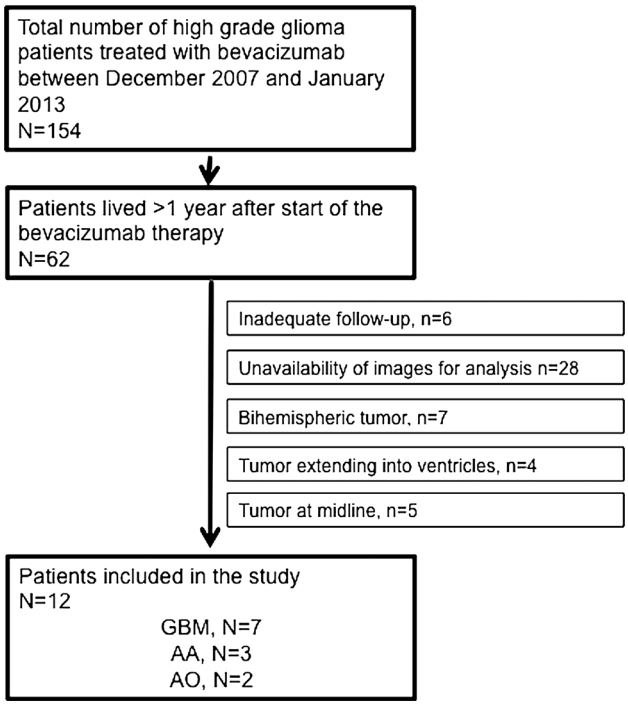

Fig. 1 Flow chart of patients who met inclusion and exclusion criteria for the bevacizumab group

the time of follow-up MRI. Fractionated radiation therapy was delivered at UAB in 6 patients (60 Gy = 5, 50 Gy = 1). The remaining 6 patients were treated at different hospitals and the dose of radiation was not documented. Only 4 patients were treated with oral dexamethasone at a daily dose varying between 0.5 and 4 mg at the time of baseline scan and 5 patients were treated with oral dexamethasone at a daily dose varying between 0.5 and 8 mg at the time of follow-up scan.

Control group

In the control group, we included primary GBM patients who were treated at UAB between January 2006 and December 2007. Diagnosis of primary GBM was based on clinico-radiological findings that met the following three criteria: (1) neurological symptoms for less than 3 months with no clinical or histopathological evidence of a preexisting less malignant precursor lesions, (2) brain imaging findings at presentation that are suggestive of high-grade infiltrative tumor, and (3) histopathologically proven GBM [27, 28]. As the median time difference between the diagnosis and initiation of bevacizumab treatment in the bevacizumab group was 9 months, the baseline scan for the control group was obtained 9 ± 2 months after the initial diagnosis and the follow up scan was obtained 12 ± 2 months after the base line scan. One patient had tumor recurrence before the baseline scan and additional 2 patients had recurrence between the baseline and follow-up scans. All the recurrent tumors were treated with resection and chemotherapy. Radiation therapy was not used to treat any of the recurrent tumors.

All 13 patients (Table 1) in the control group were treated with standard treatment protocol) at diagnosis: resection followed by concurrent radiation and temozolomide therapy (75 mg/m^2) followed by maintenance temozolomide therapy (200 mg/m^2) for 5 consecutive days every month. Radiation therapy was delivered at UAB in 8 patients. The remaining 5 patients were treated at different hospitals and the dose of radiation was not documented. Of the 13 patients, 8 patients received ≥12 cycles of temozolomide therapy at the time of follow-up MRI and the remaining 5 patients received ≤12 cycles of temozolomide therapy at the time of follow-up MRI. The steroid dose at the time of baseline scans and at the time of follow-up scans was not documented in the medical record in 12 of 13 patients. One patient was treated with 2 mg of dexamethasone at the baseline and 3 mg at follow-up.

Image acquisition

Post contrast gradient echo 3D T1 weighted MRI was used for brain segmentation with administration of 0.1 mmol/kg of gadoteridol (ProHance, Bracco Diagnostic Inc, Princeton, NJ). MRI was performed either on 1.5 T magnets (Echospeed, GE Medical Systems, Milwaukee, Wisconsin and Achieva, Phillips Medical System, Netherlands) or 3 T magnet (Achieva, Phillips Medical System, Netherlands). 3D fast spoiled gradient echo sequence (fSPGR) in the axial plane (TR = 16.528 ms, TE = 7.472 ms, FOV = 25 cm, slice thickness = 1.8 mm) was used in the GE magnets, high resolution sensitivity encoded T1 weighted sequence (sT1 W 3D HR SENSE) in the axial plane (TR = 25 ms, TE = 4.339 ms, FOV = 25 cm, slice thickness = 1.8 mm) was used in the 1.5 T Phillips magnet and 3D T1 turbo field echo (TFE) (TR = 15.0888 ms, TE = 4.60005 ms, FOV = 25 cm, slice thickness = 1.8 mm) was used in the 3 T Philips magnet. The image matrix size of both the control group and the bevacizumab varied from 0.79–1.86 mm^3.

Image analysis

All images were processed and analyzed by an imaging scientist blinded to patient treatment history and then validated by another imaging scientist who was also blinded to the initial results as well as the treatment history. The entire brain was segmented using an automated brain segmentation tool, Object Extractor, which is supported by a commercial image processing software package, Analyze (version 11.0; Biomedical Imaging Resource, Mayo Clinic, Rochester, MN). The ipsilateral hemisphere region (tumor containing) was removed first, and then the brainstem and cerebellum remaining in the contralateral hemisphere region were excluded manually using ImageJ (version 1.48o; National Institutes of Health, Bethesda, MD). A global threshold technique was applied to deselect regions that appeared darker than grey matter, such as ventricles and fissures. 3-dimensional median filtering (7 × 7 × 7) was applied to suppress noise and to smooth out the object boundary. The brain volume was calculated as the sum of all voxels within the boundary multiplied by the unit voxel size. The lateral ventricle in the contralateral hemisphere region was segmented using a variational region-growing method [12]. The ventricular volume was segmented excluding the volume of the choroid plexus. The quality of segmentation was checked for all the images. During the validation analysis, all segmented images were compared with the original brain images, and any segmentation errors were fixed. Finally, a neuroradiologist manually reassured the quality of the segmentation.

Statistical methods

Differences in the change in absolute brain and ventricle volume and percent change in these values were analyzed

with repeated measures ANOVA. Data are presented as mean ± standard deviation, and p values less than 0.05 were considered significant. To assess inter-rater agreement, we calculated intra-class correlations initial analysis and the validation analysis for each time point both for the control group and for the bevacizumab group. SAS, version 9.4 (SAS Institute Inc., Cary, NC) were used to analyze all data.

Results

Supplementary Fig. 2 demonstrates 3D volume rendered images of contralateral hemispheres and ventricles in the baseline and follow-up scans of one representative patient from the bevacizumab treated group (Fig. 2a) and one representative patient from the control group (Fig. 2b). Contralateral hemisphere volumes in the control group at the baseline and follow-up scans were 536.1 ± 50 cm^3 (range 568.3–623.3 cm^3) and 541 ± 46 cm^3 (range 462.0–601.0 cm^3) respectively. This change of volume from baseline to follow-up imaging was not statistically significant ($p = 0.23$). Contralateral ventricular volumes at baseline and at follow-up scans of the same patient group were 13 ± 8 cm^3 (range 5.5–29.1 cm^3) and 16 ± 9 cm^3 (range 6.7–33.6 cm^3) respectively (Supplementary Table 1). This change of volume was statistically significant ($p < 0.01$). In the bevacizumab treated group, the mean hemisphere volumes at the baseline and follow-up scans were 579 ± 59 cm^3 (range 491.4–672.8 cm^3) and 559.7 cm^3 (range 409.6–668.4 cm^3) respectively. The ventricular volumes at the baseline and at the follow-up scan of the same patient group were 11 ± 7 cm^3 (range 3.0–26.5 cm^3) and 15 ± 10 cm^3 (range 4.6–34.7 cm^3). There was no statistical difference in the base line contralateral hemisphere ($p = 0.0678$) and contralateral ventricular volume ($p = 0.3878$) between the patients in the control group and the bevacizumab-treated group. Supplementary Fig. 1 shows box plots of the contralateral brain hemisphere and contralateral ventricle volumes.

Mean absolute contralateral hemisphere volume change in the bevacizumab group was −18.3 ± 25.8 cm^3. Mean absolute contralateral hemisphere volume change in the control group was 4.4 ± 13.2 cm^3 (Table 2). The difference in change of absolute contralateral hemisphere volume between the two treatment groups was statistically significant ($p = 0.0093$). Mean absolute contralateral ventricle volume change in the bevacizumab group was (4.1 ± 4.1 cm^3) compared to the control group (1.4 ± 1.7 cm^3). This difference in change of ventricle volume was also statistically significant ($p = 0.0388$).

The percentage change of volume of the contralateral hemisphere and contralateral lateral ventricle in each patient from both the control group and the bevacizumab treated group is shown in Fig. 2 and Table 2. Mean of the percentage change of brain volume in the control group was 1 ± 0.6 % compared to −3.2 ± 1.5 % in the bevacizumab group. Mean of the percentage change of ventricle volume in the control group was 11.0 ± 2.6 % versus 33.2 ± 9 % in the bevacizumab group. The percentage changes in brain volume ($p = 0.0114$) and ventricle volume ($p = 0.0144$) between the base line scan and the

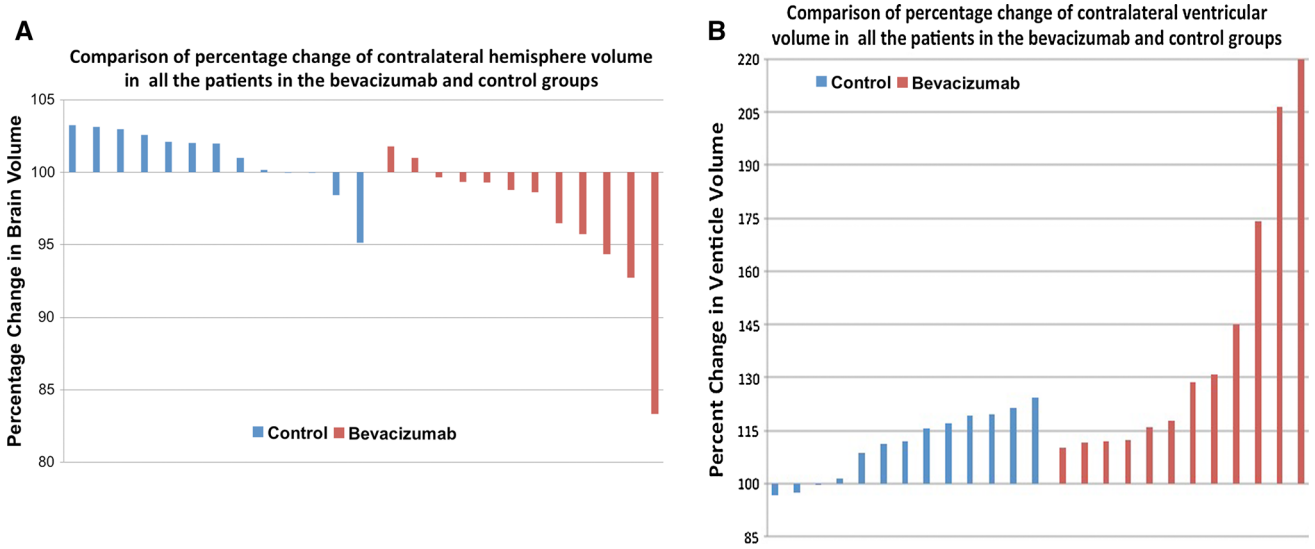

Fig. 2 *Bar* diagram showing percentage change of volumes of contralateral hemisphere and contralateral ventricle from baseline scan to follow-up scan from the initial analysis. **a** Percentage change of contralateral hemisphere volume in each patient in the control group (*blue bars*) and in the bevacizumab group (*red bars*). Contralateral hemisphere volume is considered 100 % for each patient at the baseline. **b** Percentage change of contralateral ventricle volume in each patient in the control group (*blue bars*) and in the bevacizumab group (*red bars*). Contralateral ventricle volume is considered 100 % for each patient at the baseline

Table 2 Results. Absolute and percentage change of brain volumes between the bevacizumab group and control group

	Control group [mean (in cm^3) ± SD]	Bevacizumab group [mean (in cm^3) ± SD]	p value
Absolute contralateral hemisphere volume change	4.4 ± 13.2	−18.3 ± 25.8	0.0093
Absolute contralateral ventricular volume change	1.4 ± 1.7	4.1 ± 4.1	0.0388
Percentage contralateral hemisphere volume change	1.0 ± 2.2	−3.2 ± 4.9	0.0114
Percentage contralateral ventricular volume change	11.1 ± 9.5	40.4 ± 38.7	0.0144

Table 3 Results of the validation analysis

	Control group (mean ± SD)	Bevacizumab group (mean ± SD)	p value
Contralateral hemisphere volume (cm^3) at baseline	536.1 ± 50.1	578.1 ± 59.3	0.0646
Contralateral ventricular volume ($cm^{3)}$ at baseline	14.1 ± 8.1	11.4 ± 8.1	0.3876
Percentage change of contralateral hemisphere volume from baseline to follow-up	1.0 ± 2.2 %	−3.4 ± 4.9 %	0.0106
Percentage change of contralateral ventricle volume from baseline to follow-up	10.0 ± 11.5 %	40.3 ± 38.6 %	0.0126

follow-up scan are significantly different between bevacizumab and the control group.

The results of the validation analysis (Table 3) by the second imaging scientist were similar to the original analysis (Supplementary Table 2). At baseline contralateral hemisphere volume of the control group was 535 ± 14 cm^3 and of the bevacizumab group was 578 ± 16 cm^3 ($p = 0.0646$). At baseline contralateral ventricle volume of the control group was 14 ± 2 cm^3 and of the bevacizumab group was 11 ± 2 cm^3 ($p = 0.3876$). Percentage change of the contralateral hemisphere from baseline to follow-up MRI in the control group was 0.79 ± 0.61 % ($p = 0.2061$) and in the bevacizumab group was −3.4 ± 1.42 % ($p = 0.0106$). Percentage change of the contralateral ventricle from baseline to follow-up MRI in the control group was 10.01 ± 3.19 % and in the bevacizumab group was 40.28 ± 11.13 % ($p = 0.0126$). There was almost perfect agreement between the initial analysis and the validation analysis with intra-class correlation coefficient of >0.99 in all the measurements except for the measurement of the contralateral hemisphere volume measurement at follow-up that demonstrates intra-class correlation coefficient of 0.85 (Supplementary Table 3).

Discussion

VEGF is one of the key proangioangemic molecules expressed in high-grade gliomas [29, 30]. In addition to angiogenesis, recent studies have discovered that autocrine and paracrine VEGF signaling contribute to key aspects of tumorigenesis, independently of angiogenesis in high grade glioma [31, 32]. This led to the development of anti-angiogenic therapy, either VEGF- or VEGFR-targeted agents, for the treatment of high grade gliomas [33] In 2009, the US Food and Drug Administration (FDA) granted accelerated approval of bevacizumab for treatment of recurrent GBM based on two phase II trials showing improved response rates and 6-month progression-free survival compared to historical controls [21, 22]. Bevacizumab-mediated inhibition of VEGF is currently the predominant mode of anti-angiogenic therapy in high-grade gliomas and in cancers of many other body parts [33, 34]. We believe that the results of our study identifies a potential adverse event of long term bevacizumab therapy and should be validated with a large number of patients. The results of our study are particularly important to patients who have longer overall survival.

Two recently published large phase III trials aimed at determining the efficacy of first-line bevacizumab in newly diagnosed GBM show somewhat different outcomes. Radiation Therapy Oncology Group Study 0825 (RTOG8025) found no improvement in overall survival nor a significant prolongation of PFS, while the industry-sponsored AVAglio trial found improved PFS and patient-reported outcomes, but no improvement in overall survival [25, 35].

Interestingly, RTOG8025 study also incorporated assessment of neurocognitive function, and confirmed a significantly decline in neurocognitive function in the bevacizumab treated group in a large cohort of patients with appropriate control group. The authors did not propose any biologic explanation of neurocognitive decline. Neurocognitive decline in patients with

GBM can be related to treatment such as radiation therapy, chemotherapy or even surgery (if medial temporal lobe or frontal lobe have been removed) or can be due to tumor infiltration of the key areas brain responsible for memory and executive functions. It is difficult to precisely identify the cause of neurocognitive decline. In the RTOG 0825 clinical trial, patients in both the control and the bevacizumab groups were treated with radiation, chemotherapy and surgery. The only difference between the two groups was use of bevacizumab versus placebo. This raises a concern if neurocognitive decline is related to bevacizumab treatment particularly with the known fact that VEGF has many critical memory functions. Our study did not include any neuropsychiatric evaluation and the relationship of neurocognitive decline and brain atrophy cannot be established from our study. As atrophy of different brain regions is strongly associated with neurocognitive decline in many neurodegenerative diseases such as Alzheimer's dementia, frontotemporal dementia, it may be possible that bevacizumab induced brain atrophy is associated with bevacizumab induced neurocognitive decline. This hypothesis needs to be validated in a properly designed clinical trial, preferably in patients with cancers of other body parts without brain metastasis undergoing treatment with bevacizumab.

Our study demonstrates that patients who received bevacizumab had a significant decrease in brain volume and a significant increase in ventricular volume from baseline to follow-up imaging as compared to a control group patients who did not receive bevacizumab. We analyzed the volume of the contralateral hemisphere to avoid the anti-edema effect of bevacizumab and steroids that are frequently co-administered. Although there was an increase in the brain volume in the control group from baseline to follow-up imaging, the difference was not statistically significant ($p = 0.23$). The underlying cause of this apparent increase of the brain volume over time is not clear. This could be related to different hydration status [36], different dose of steroids [37], different image acquisition techniques or different magnets, between the baseline and follow-up scan or due to limitation in the segmentation technique.

Astrocytes are the major producers of VEGF in adult brain parenchyma [5], but VEGF is also expressed in other cells including CA1 pyramidal neurons in the hippocampus [38, 39], pyramidal neurons in the cortex [38], and Purkinje cells in the cerebellum [1, 40]. While the physiological function of VEGF in the adult brain is not well understood, recent evidence showed that VEGF is functionally important in maintaining neural stem cells, in neuroblast production and in neuronal differentiation in the hippocampus [3, 9–11] and in the subventricular zone [10–14]. It is noteworthy that all of these sites of constitutive VEGF expression are angiogenically quiescent, thus suggesting non-angiogenic roles of VEGF. Perturbation of VEGF by bevacizumab could have a direct effect on these neurogenic processes. The atrophy of the brain could be related to blockade of homeostatic functions of VEGF by bevacizumab.

The current study is limited by its retrospective nature and small sample size. The small sample size can be explained by very short overall survival of patients with recurrent high-grade gliomas and strict inclusion criteria. Only 40.25 % of the patients in the bevacizumab arm lived >1 year. Neurocognitive data and exact steroid dose in the control group were not available. Another limitation is radiation dose spills to the contralateral hemisphere could not be assessed in all the patients. In addition, we are unable to segment grey matter, white matter, or hippocampus due to poor contrast to define the region boundary. Different image acquisition protocols and different magnetic field strength might be other concerns, although the slice thickness and image resolution were comparable throughout all images. Future analysis of a larger data set with concurrent image acquisition and neurocognitive assessment will help to overcome the limitations.

In conclusion, the results of this pilot study suggest that prolonged administration of bevacizumab is associated with brain atrophy. This study sets the stage for prospective clinical trials that will include a large number of patients at many different time points, simultaneous neurocognitive assessment and regional brain volume assessment to evaluate temporal as well as regional variation of brain volume loss associated with bevacizumab therapy.

Conflict of interests AKB: Consultant to Dotarem Advisory board. Guerbet LLc. HK: No disclosure. YG: No disclosure. MB: No disclosure. PPW: No disclosure. HMF: No disclosure. DG: No disclosure. JMM: Leadership role, Stocks, Consultancy, Intellectual property and Research Funding: Catherex Inc. & Aettis Inc.; Research funding: ICT. JF: Research funding: Varian Oncology. TMB: No disclosure. AK: No disclosure. GKF: No disclosure. PRC: No disclosure. LBN: Consultant, Merck Inc.; Travel allowance: BMS. XH: No disclosure.

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