First Experiences with Low-dose Anti-angiogenic Treatment in Gliomatosis Cerebri with Signs of Angiogenic Activity

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Abstract. *Background: Gliomatosis cerebri is a rare primary cerebral tumour entity characterized by diffuse infiltrative growth patterns representing a WHO grade III malignancy. The prognosis is dismal and therapeutical options are still controversial. In contrast to other high-grade gliomas, angiogenesis is thought to be absent in gliomatosis cerebri. Patients and Methods: Despite this assumption, histopathological analyses of samples of six patients with gliomatosis cerebri were performed and surprisingly there was angiogenic activity with expression of vascular endothelial growth factor and cyclooxygenase 2. It was therefore decided to administer continuous low-dose chemotherapy with temozolomide and celecoxib for antiangiogenic treatment in the four patients that were in good clinical condition following external radiotherapy. Results: In all patients, treatment was well tolerated and MRI follow-up showed no tumour progression for at least six months. One patient died due to pulmonary embolism 9 months after diagnosis; another patient survived 15 months after diagnosis with progressive disease in the last follow-up MRI before death. Two other patients at the present time are still in a stable clinical condition without signs of tumour progression in MRI (12 and 18 months). Conclusion: From our initial experience in a small number of patients with gliomatosis cerebri with signs of angiogenic activity, we conclude that low-dose chemotherapy might provide a promising approach for treatment of these patients and that overexpression of angiogenic factors such as VEGF or COX-2 seems to be more frequent than hitherto reported.*

Gliomas constitute the most common primary brain tumour. Infiltrative growth is a hallmark of these neoplasms, especially in astrocytic and oligodendroglial tumours. According to criteria of the WHO, gliomatosis cerebri (GC) is a rare glioma entity defined as a lesion with an extraordinary diffuse infiltrative growth affecting at least three cerebral lobes, often bilateral or with extension into the infratentorial space (1, 2) representing a grade III malignancy. The term gliomatosis cerebri was first introduced by Nevin in 1938 describing a diffuse cellular overgrowth of neuroglial cells throughout wide areas of the cerebral hemispheres (3), but involvement of the spinal axis has also been reported (4, 5). The prognosis of GC remains dismal. According to a review by Sanson *et al*., the median survival is 12 months with supportive care alone (6). Due to the extent of the infiltrative growth, resection of the tumour frequently is considered impossible. The diagnostic approach and frequently used treatment modalities include stereotactic biopsy or partial tumour resection followed by radiotherapy and chemotherapy or initial chemotherapy with procarbazine, lomustine and vincristine (PCV) or temozolomide (6-9). With regard to treatment options in GC, it is of importance whether neo-angiogenesis is as relevant for its growth as it is for the growth of other high-grade gliomas. A recently published study described angiogenesis to be completely absent from GC (10). This theory is supported by the fact that during magnetic resonance imaging (MRI) or computed tomography (CT) contrast enhancement as a surrogate parameter for angiogenic activity in these tumours can rarely be detected (11).

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It is known that increased expression of vascular endothelial growth factor (VEGF) or cyclooxygenase 2 (COX2) is associated with increased angiogenic activity in high-grade gliomas (12, 13). As recently demonstrated, an antiangiogenic regimen featuring a combination of continuous low-dose chemotherapy with temozolomide and COX-2 inhibitors showed good response in patients with glioblastoma multiforme (14). Furthermore, functional and clinical improvement after chemotherapy with temozolomide have been reported for patients with GC (9). Based on these reports and our own new findings that suggest angiogenic activity in GC, we describe our first promising experiences of an antiangiogenic approach for the treatment of GC in four patients.

Patients and Methods

Clinical data. A series of six patients (median age 59,5 years with a range from 40 to 72 years) presented in our department between May 2005 and August 2007 with seizures or psychomotoric deterioration and in one case with a progressive left-sided hemiparesis (Table I). All patients underwent MRI leading to the suspected diagnosis of GC. Subsequent histopathological analyses were performed after obtaining histological specimens by stereotactic biopsy in 5 out of the 6 patients. In one case, histological specimens were obtained by partial tumour resection. In all cases, neuropathological examination of specimens from different localizations revealed nearly homogenous astrocytic tumour tissue with focally increased mitotic activity, pleomorphism and cell density. The morphological criteria in two cases corresponded with a diffuse astrocytoma WHO grade II; the other four cases showed criteria corresponding to WHO grade III. In most specimens, there were regions with parallel rows of elongated tumour cells. In consideration of the radiological findings suggestive of GC and meeting the requirement of extensive infiltration of the central nervous system and localization in at least three lobes, all lesions were classified as GC.

Neuropathological examination. Formalin-fixed tissue was embedded in paraffin, deparaffinized and 4 μm sections were HE stained according to standard laboratory protocols. Immunohistochemistry was performed using the Ventana BenchMark XT™ system and ultraView™ alkaline phosphatase red detection kit (Ventana Medical Systems, USA), followed by counterstaining with haematoxylin. Pretreatment with buffer CC1 (Ventana) was followed by incubation with a 1:200 dilution of mouse antibody against human VEGF (sc 7269; Santa Cruz, CA, USA), a 1:20 dilution of COX-2 antibody (Novocastra, Leica Microsystems, Germany), or with a 1:25 dilution of an antibody against CD 31 (DAKO, Germany) at 37˚C. Slides treated with antibodies against VEGF or COX-2 were scored according to the method described by Reiner *et al*. for both intensity (negative, weak, moderately, strongly positive) and proportion (0, 1-9% , 10-50% , 51-80% , >81%) of tumour cells stained (15). Integer values were assigned to the scores of intensity (0-3) and percentage of positive cells (0-4). These values were totalled to provide a single immunoreactive score (IRS).

Treatment and follow-up examinations. After histopathological confirmation of the diagnosis of GC, all patients except for one received external radiation therapy. Radiation therapy was performed in 5 patients and varied initially (different combinations of whole brain radiotherapy, WBRT, and a focal boost were used), including one patient in whom therapy was stopped after application of 45 Gy because of clinical deterioration. A protocol was used consisting of 45 Gy WBRT in single doses of 1.8 Gy and continuing on the predominant lesion focally up to a resulting dose of 59.4 Gy/1.8 Gy, under which the last two patients of this series were treated. After radiation therapy the four remaining patients in good clinical condition (KPS >70%) received continuous low dose chemotherapy with temozolomide (20 mg/m^2 daily) and celecoxib (200 mg daily). Blood count was monitored weekly and patients were followed by clinical examination and MRI every three months after initiation of chemotherapy.

Results

Expression of VEGF and COX-2 in gliomatosis cerebri. Intracellular COX-2 expression was found in samples from four out of the six patients (Figure 1).

Cellular VEGF expression was divided into reaction in the cytosol and nuclei due to variable reaction patterns (Table II). Intracellular VEGF was detected by immunohistochemistry in GC samples from five out of six patients. Interestingly, we observed weak (in one patient) and moderate (in three patients) intranuclear VEGF expression (*i.e*. in samples from four out of our six patients, Figure 1), which has been found to be associated with tumour hypoxia (16).

Vascular proliferation. Immunohistochemical staining with a CD31 antibody showed a dense network of branching capillaries in parts of all analyzed tumors. In three patients, conglomerated vessels with prominent endothelia were observed focally (Figure 2).

Survival time and clinical progression. As described above, one patient deteriorated rapidly and, therefore, radiation therapy was not performed. Another patient deteriorated during radiotherapy which was discontinued after 45 Gy had been applied. The four remaining patients received full course radiotherapy, followed by continuous low-dose chemotherapy with temozolomide and celecoxib. MRI follow-up scans showed tumour control for at least six months, with a range from six months to 18 months (Figures 3 and 4) and stable clinical conditions. Of treated patients, one patient died due to pulmonary embolism 9 months after diagnosis, with the last MRI being performed 6 months after diagnosis. At that time point, the clinical course was stable and the tumour was locally controlled based on follow-up MRI. Time to radiological progression with simultaneous clinical deterioration and death was 15 months after diagnosis in another patient. The other two treated patients show tumour control on follow-up MRI and

Table I. *Characteristics of patients included in this study.*

Patient no.	Gender	Age (years)	KPS (%)	Symptoms	Enhancement in MRI	Histology*	Surgical approach	Treatment
1	Male	56	80	Seizure/Aphasia	Absent	A II	STX	RT + LDC
2	Male	63	90	Seizure	Absent	AA III	Partial resection	RT + LDC
3	Female	72	40	Hemiparesis	Absent	A II	STX	RT
4	Female	71	30	PM deficits	Absent	AA III	STX	None**
5	Female	40	90	Seizure	Absent	AA III	STX	RT + LDC
6	Female	51	70	PM deficits	Absent	AA III	STX	RT + LDC

A II: Diffuse astrocytoma WHO II; AA III: anaplastic astrocytoma WHO III; STX: stereotactic biopsy; PM deficits: psychomotoric deterioration; RT: radiation therapy; LDC: low-dose chemotherapy. *Histology based on tissue specimens without regard to the radiological diagnosis of gliomatosis cerebri. **Patient died soon after biopsy after progressive clinical deterioration without further neuroimaging.

Table II. *Neuropathological examination scores. Stained slides were scored for both intensity (negative, weak, moderately, strongly positive) and proportion (0, 1-9% , 10-50% , 51-80% , >81%) of positively stained tumour cells. Integer values were assigned to the scores of intensity (0-3) and percentage of positive cells (0-4). These values were totalled to provide a single immunoreactive score (IRS).*

Patient no.	COX-2 intensity	IHC-positive	Score	VEGF intensity		IHC-positive		Score	
				Cytosol	Nucleus	Cytosol	Nucleus	Cytosol	Nucleus
1	2	2	4	1	2	3	2	5.5	4
2	2	4	6	2	0	1	0	3	0
3	2	3	5	2	2	2	1	4	3
4	1	2	3	1	1	2	1	3	2
5	2.5	3	5.5	0	0	0	0	0	0
6	3	2	5	3	2	2	1	5	3

stable clinical conditions for 12 and 18 months at the time of writing. Chemotherapy was well tolerated; no relevant side-effects were observed.

Discussion

The best treatment option for patients suffering from GC is still a matter of debate. Although no firm conclusions can be drawn from the literature consisting of case reports and series with rather low patient numbers, a pattern does seem to emerge: For patients undergoing no treatment (clearly being a cohort with a bad prognosis to begin with) survival is <5 months (17); for patients treated with RT alone, survival of 10 to 30 months has been reported, although the median seems to be closer to 10 months rather than 30 months (17-19). On a case by case basis, adding chemotherapy seems to improve survival (9, 19, 20). External radiotherapy has shown good results in patients with GC just like in other malignant glioma. Since the prognosis remains poor with a median survival of 12 months according to a literature review of Sanson *et al*. (67 published cases receiving supportive therapy alone) additional therapeutical attempts are therefore justified to possibly increase survival times of these patients (6). Recent studies have shown promising results of chemotherapy either with temozolomide or with PCV (procarbazine, lomustine and vincristine) regime in patients with GC. Temozolomide as first-line treatment in two larger series with 46 and 11 patients showed good response rates, with a low toxicity profile and good quality of life (6, 9). Especially because of this low toxicity profile, but also because of the possibility of long-term administration and comparable results, temozolomide is favourable compared to a scheme with PVC alone (6). Metronomic chemotherapy with temozolomide and COX-2 inhibition provided good effects on progression-free survival and even lower side-effects in patients with highly vascularized glioblastoma multiforme (14).

In five out of our six patients, a strong expression of VEGF was detected, and four out of six patients presented with increased expression of COX-2. VEGF was not only detected in the cytosol, but in nuclei as well, in the samples of four out of our six patients. The appearance of VEGF in the nucleus is in accordance with the literature (16) and, as suggested here, might be regarded as a sign of a role of VEGF in hypoxia. Moreover, conventional histology and immunohistochemistry with a CD 31 antibody substantiated vascular proliferation in gliomatosis. This, together with evidence of VEGF and COX-2 expression, demonstrates a possible target for antiangiogenic therapy of GC.

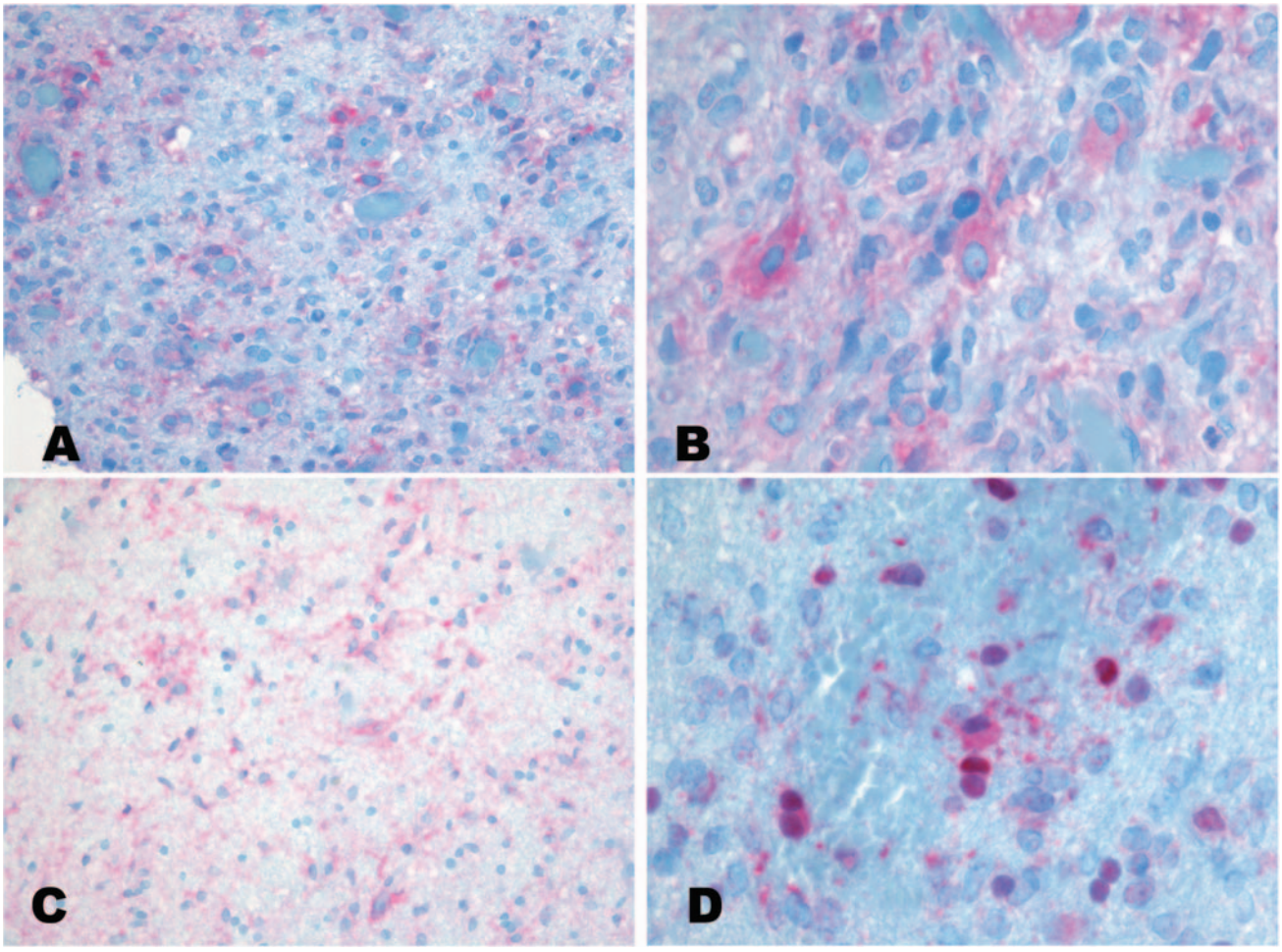

Figure 1. *Immunohistochemical findings in biopsy specimens (incubation as indicated in the text). Cytosolic COX-2 detection in fibrillary astrocytic cells, as exemplary shown here, was detected at varying degrees in specimens of four patients (panel A 20-fold, panel B 40-fold magnification). Panel C shows an example of cytosolic VEGF reaction, again highlighting the astrocytic nature of positive cells, as found in five patients (40-fold magnification). In four of these patients, additional intranuclear staining was observed as shown in panel D (40-fold magnification).*

To the best of our knowledge, this is for the first time that VEGF and COX-2 expression was found in GC. A recent publication denied any angiogenic activity in GC (10). Their finding in only one patient led the authors to the assumption that neo-angiogenesis is absent in all cases of GC. They stated that vessels were recruited from the pre-existing vascular bed. In tumour cells of the patient in this article, no evidence of VEGF expression was shown (10).

Although contrast enhancement is commonly absent in GC (11), this does not exclude the presence of tumour angiogenesis and VEGF expression since Ginsberg and colleagues reported that up to 40% of high-grade gliomas do not enhance on gadolinium MRI (21). According to our findings, VEGF expression in GC is not as strong as in other high-grade gliomas. Hence, due to sampling error, it is possible to miss areas with VEGF expression in stereotactic procedures. There might also be a threshold that is actually not defined for VEGF-mediated vascular changes leading to contrast enhancement in neuroimaging. In a recent study, White *et al*. reported that the presence of contrast enhancement in oligodendrogliomas is not a statistically significant finding for discriminating between high-grade and low-grade tumours and that in both enhancing and non-enhancing tumours, angiogenic activity is demonstrable (22). Abdulrauf *et al*. showed angiogenic patterns in fibrillary diffuse low-grade astrocytomas with increased microvessel count and VEGF expression defining these parameters as independent markers for an unfavourable outcome in these patients. These findings show that these tumours can be divided into 'angiogenic' and 'non-angiogenic' forms with regard to their microvascular density and/or VEGF staining (23). Maia *et al*. showed three supratentorial low-grade astrocytomas out of 20 patients without contrast enhancement in MRI to have VEGF expression and higher rCBF values compared to the contralateral white matter (24).

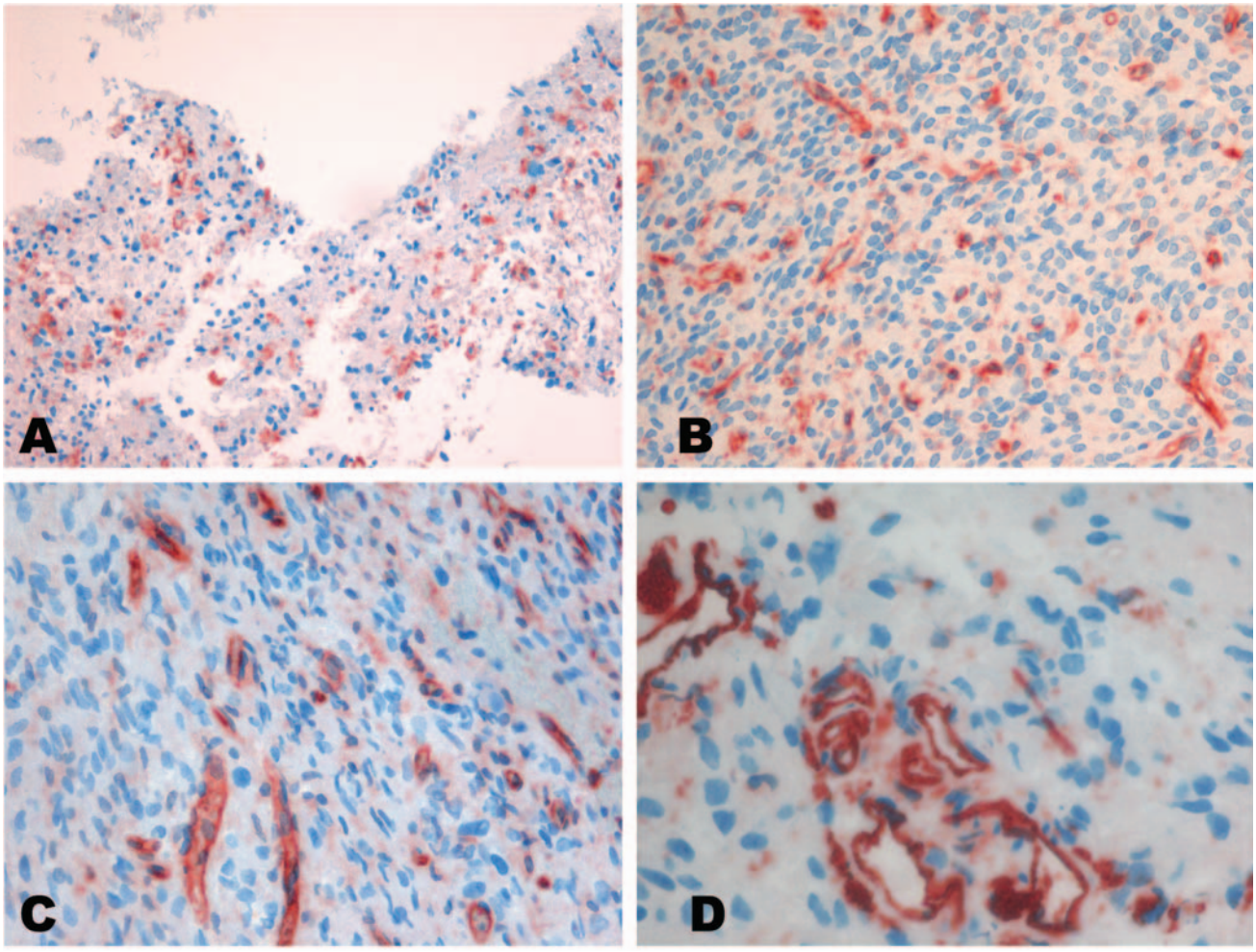

Figure 2. *Immunohistochemical staining with a CD31 antibody highlights a dense network of branching capillaries (A-C). D, Conglomerated vessels with prominent endothelia. (A-C, 200-fold magnification; D, 400-fold magnification).*

COX-2 expression is also frequently found in low-grade gliomas. Even though expression is less pronounced than in high-grade gliomas, it is still a negative prognostic factor regarding survival (25, 26). Additionally, COX-2 expression is strongly correlated with angiogenic activity, not only in astrocytic tumours (25), but also in other tumour entities (27-29). Therefore, angiogenic activity and malignancy of tumours can only be evaluated in immunohistopathological workup. For diagnostic purposes, in a diffuse infiltrative lesion such as GC, an additional pre-surgical work-up such as MR spectroscopy, perfusion-weighted MRI or PET should be implemented to better detect relevant targets and reduce the possibility of sampling errors.This leads us to the conclusion that angiogenesis very likely plays a role in the pathogenesis of GC in our patients, even in those without contrast enhancement in preoperative galodinum-enhanced MRI scans. In this series of six patients suffering from GC, we demonstrated angiogenic patterns by immunohistochemical detection of COX-2 and VEGF expression combined with a dense microvascular intratumoural bed. Furthermore, the response to an antiangiogenic therapy in four patients with stable tumour control after at least 6 months to 18 months with metronomic chemotherapy following external radiotherapy might also underline the importance of angiogenic activity in the progression of GC. Especially in patients with an already impaired clinical condition at the time of diagnosis, metronomic antiangiogenic chemotherapy might be an appropriate alternative treatment given the very low toxicity profile.

Conclusion

The expression of VEGF and COX-2 in our patients with GC leads to the assumption that angiogenesis plays a role in the progression of the disease and therefore

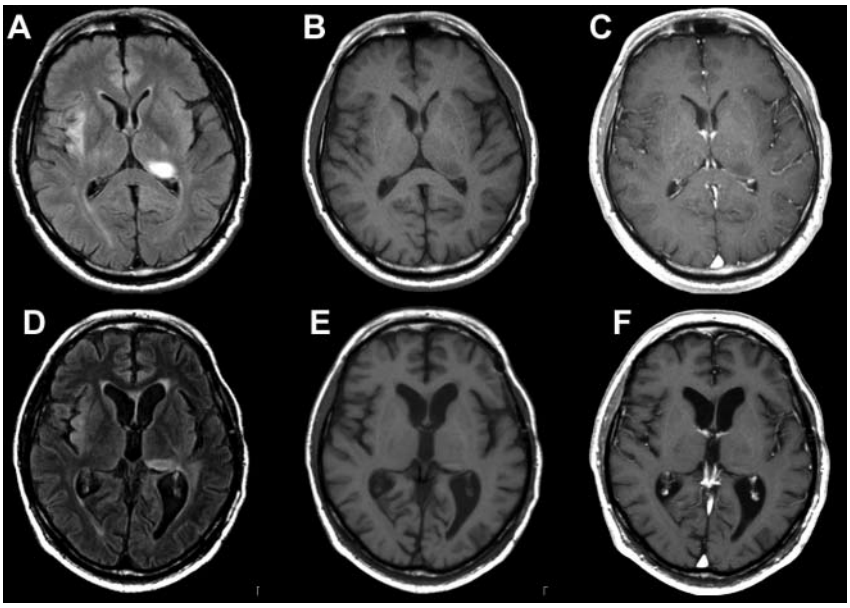

Figure 3. *Time course of gliomatosis-associated cerebral lesions in T2-weighted imaging of patient 2. No progression of gliomatosis cerebri was noted over a period of one year: A-C: February 2006; D-F: February 2007. All images were acquired on a 1.5 Tesla MRI-unit. A, D: FLAIR sequence; B, E: non-enhanced T1-weighted imaging; C, F: contrast-enhanced T1-weighted imaging.*

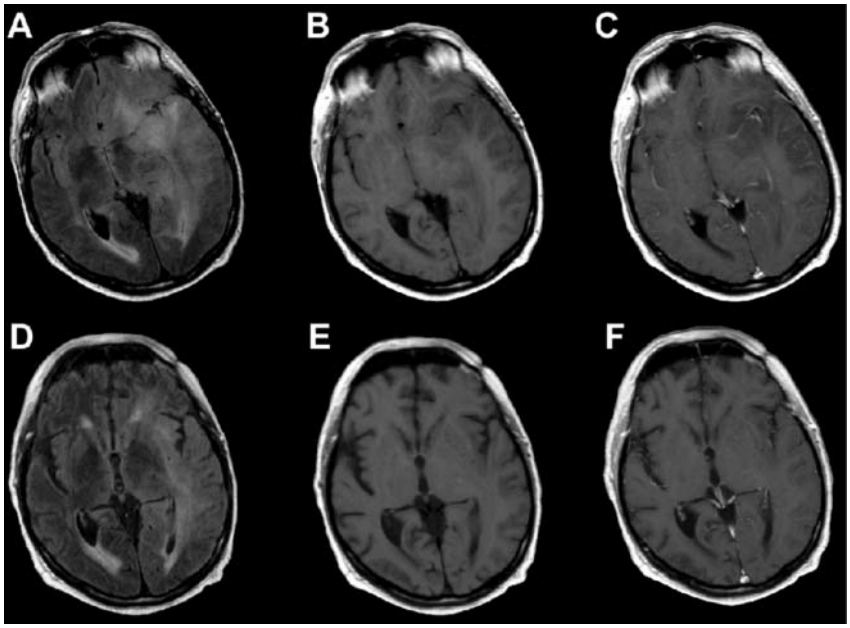

Figure 4. *Time course of gliomatosis-associated cerebral lesions in T2-weighted imaging of patient 1. Gliomatosis cerebri-induced space-occupying effects such as compression of the left lateral ventricle and midline shift are clearly regressive between June 2006 (A-C) and February 2007 (D-F). All images acquired on a 1.5 Tesla MRI-unit: A, D: FLAIR sequence; B, E: non-enhanced T1-weighted imaging; C, F: contrast-enhanced T1-weighted imaging.*

antiangiogenic chemotherapy might be a valuable treatment option in selected patients. The real benefit of this treatment has of course to be confirmed in a larger series of patients with longer follow-up. In order to provide a more targeted therapy, more information about the pathology of this rare entity with regard to the receptor status and the differentiation between the various cell types has to be gathered.

References

1 Kleihues P and Cavanee WK: Pathology and Genetics of Tumours of the Nervous System. Lyon: IARC Press, 2000.
2 Louis DN, Ohgaki H, Wiestler OD and Cavanee WK (eds.): WHO Classification of Tumours of the Central Nervous System. IARC: Lyon, 2007.
3 Nevin S: Gliomatosis cerebri. Brain *61*: 170-191, 1938.
4 Cummings TJ, Hulette CM, Longee DC, Bottom KS, McLendon RE and Chu CT: Gliomatosis cerebri: cytologic and autopsy findings in a case involving the entire neuraxis. Clin Neuropathol *18*: 190-197, 1999.
5 Yaguchi M, Nakasone A, Sohmiya M, Saitoh F, Ohya N, Yoshida T, Nakazato Y and Okamoto K: Gliomatosis cerebri involving the lumbosacral spinal cord. internal Med *42*: 615-618, 2003.
6 Sanson M, Cartalat Carel S, Taillibert S, Napoletano L, Diafani J, Cougnard J, Gervais H, Laigle F, Carpentier A, Mokhtari K, Taillandier L, Chinot O, Duffau H, Honnorat J, Hoang Xuan K and Delatore JY: Initial chemotherapy in gliomatosis cerebri. Neurology *63*: 270-275, 2004.
7 Herrlinger U, Felsberg J, Kuker W, Bornemann A, Plasswilm L, Knobbe CB, Strik H, Wick W, Meyermann R, Dichgans J, Bamberg M, Reifenberger G and Weller M: Gliomatosis cerebri: molecular pathology and clinical course. Ann Neurol *52*: 390-399, 2002.
8 Kannuki S, Hondo H, Ii K, Hirose T and Matsumoto K: Gliomatosis cerebri with good prognosis. Brain Tumor Pathol *14*: 53-57, 1997.
9 Levin N, Gomori JM and Siegal T: Chemotherapy as an initial treatment in gliomatosis cerebri: Results with temozolomide Neurology *63*: 354-356, 2004.
10 Bernsen H, Van der Laak J, Küsters B and Van der Ven P: Gliomatosis cerebri: quantitative proof of vessel recuitment by cooptation instead of angiogenesis. J Neurosurg *103*: 702-706, 2005.
11 Freund M, Hähnel S and Sommer C: CT and MRI findings in gliomatosis cerebri: a neuroradiologic and neuropahtologic review of diffuse infiltrating brain neoplasms. Eur Radiol *11*: 309-316, 2001.
12 Ezhilarasan R, Mohanam I, Govindarajan K and Mohanam S: Glioma cells suppress hypoxia-induced endothelial cell apoptosis and promote the angiogenic process. Int J Oncol *30*: 701-707, 2007.
13 Onguru O, Gansizkan M, Ulutin C and Gunhan O: Cyclooxygenase-2 (Cox-2) expression and angiogenesis in glioblastoma. Neuropathology *28*: 29-34, 2008.
14 Tuettenberg J, Grobholz R, Korn T, Wenz F, Erber R and Vajkoczy P: Continuous low-dose chemotherapy plus inhibition of cyclooxygenase-2 as an antiangiogenic therapy in glioblastoma multiforme. J Cancer Res Clin Oncol *131(1)*: 31-40, 2005.
15 Reiner A, Spona J, Reiner G, Schemper M, Kolb R, Kwasny W, Függer R, Jakesz R and Holzner JH: Estrogen receptor analysis on biopsies and fine-needle aspirates from human breast carcinoma. Correlation of biochemical and immunohisto-chemical methods using monoclonal antireceptor antibodies. Am J Pathol *125*: 443-449, 1986.
16 Lejbkowicz F, Goldberg-Cohen I and Levy AP: New horizons for VEGF. Is there a role for nuclear localization? Acta Histochem *106*: 405-411, 2005.
17 Elshaikh MA, Stevens GH, Peereboom DM, Cohen BH, Prayson RA, Lee SY, Barnett GH and Suh JH: Gliomatosis cerebri: treatment results with radiotherapy alone. Cancer *95*: 2027-2031, 2002.
18 Perkins GH, Schomer DF, Fuller GN, Allen PK and Maor MH: Gliomatosis cerebri: improved outcome with radiotherapy. Int J Radiat Oncol Biol Phys *56*: 1137-1146, 2003.
19 Vates GE, Chang S, Lamborn KR, Prados M and Berger MS: Gliomatosis cerebri: a review of 22 cases. Neurosurgery *53*: 261-271; 2003.
20 Glas M, Rasch K, Wiewrodt D, Weller M and Herrlinger U: Procarbazine and CCNU as initial treatment in gliomatosis cerebri. Oncology *75*: 182-185, 2008.
21 Ginsberg LE, Fuller GN, Hashmi M, Leeds NE and Schoemer DF: The significance of lack of MR contrast enhancement of supratentorial brain tumors in adults: Histopathological evaluation of a series. Surg Neurol *49*: 436-440; 1998.
22 White ML, Zhang Y, Kirby P and Ryken TC: Can tumor contrast be used as a criterion for differentiating tumor grades of oligodendrogliomas? AJNR *26*: 784-790, 2005.
23 Abdulrauf SI, Edvardsen K, Ho KL, Yang XY, Rock JP and Rosenblum ML: Vascular endothelial growth factor expression and vascular density as prognostic markers of survival in patients with low-grade astrocytoma. J Neurosurg *88*: 513-520, 1998.
24 Maia AC Jr, Malheiros SM, da Rocha AJ, da Silva CJ, Gabbai AA, Ferraz FA and Stávale JN: MR cerebral blood volume maps correlated with vascular endothelial growth factor expression and tumor grade in nonenhancing gliomas. AJNR *26*: 777-783, 2005.
25 Hara A and Okayasu I: Cyclooxygenase-2 and inducible nitric oxide synthase expression in human astrocytic gliomas: correlation with angiogenesis and prognostic significance. Acta Neuropathol *108*: 43-48, 2004.
26 Shono T, Tofilon PJ, Bruner JM, Owolabi O and Lang FF. Cyclooxygenase-2 expression in human gliomas: prognostic significance and molecular correlations. Cancer Res *61*: 4375-4381, 2001.
27 Cianchi F, Cortesini C, Fantappiè O, Messerini L, Sardi I, Lasagna N, Perna F, Fabbroni V, Di Felice A, Perigli G, Mazzanti R and Masini E: Cyclooxygenase-2 activation mediates the proangiogenic effect of nitric oxide in colorectal cancer. Clin Cancer Res *10*: 2694-2704, 2004.
28 Kim HS, Youm HR, Lee JS, Min KW, Chung JH and Park CS: Correlation between cyclooxygenase-2 and tumor angiogenesis in non-small cell lung cancer Lung Cancer *42*: 163-170, 2003.
29 Li N, Sood S, Wang S, Fang M, Wang P, Sun Z, Yang CS and Chen X: Overexpression of 5-lipoxygenase and cyclooxygenase 2 in hamster and human oral cancer and chemopreventive effects of zileuton and celecoxib. Clin Cancer Res *11*: 2089-2096, 2005.

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