J Neurooncol (2010) 96:277–285
DOI 10.1007/s11060-009-9961-x

CLINICAL STUDY - PATIENT STUDY

Assessment of tumor angiogenesis as a prognostic factor of survival in patients with oligodendroglioma

H. Quon · A. Hasbini · J. Cougnard · L. Djafari · C. Lacroix · B. Abdulkarim

Received: 5 December 2008 / Accepted: 6 July 2009 / Published online: 19 July 2009

Abstract According to World Health Organization (WHO) and Daumas-Duport grading systems, progression of oligodendrogliomas (ODGs) to a higher grade (WHO grade III, grade B) is associated with increased angiogenesis. Based on multivariate assessment of molecular, pathological, and radiological parameters, we further assessed the influence of tumor angiogenesis on tumor progression and patient survival. Patients with a diagnosis of ODG, consecutively treated in a single institution, were reviewed and reclassified according to WHO and Daumas-Duport grading systems. MRI scans were reviewed to assess contrast enhancement and necrosis. Tissue sections were used for pathology review and to evaluate immunostaining of vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor (VEGF-R), Ki-67, and CD34. Multivariate analysis was performed to assess the impact of tumor angiogenesis-related pathological and radiological factors on patient survival. One hundred thirty-four patients with pure ODG were included in this study. Multivariate analysis identified four independent poor prognostic factors: necrosis, absence of seizure, increased vascularization, and age >55 years. A subgroup of patients with tumor necrosis, increased vascularization, and absence of seizures had a significantly worse outcome than predicted, with a median overall survival of 14.2 months. VEGF expression was significantly higher in this subgroup and correlated with disease progression regardless of histologic grade. Based on the presence of radiological or pathological necrosis, contrast enhancement or endothelial hyperplasia, and absence of seizures, a high risk group of ODG can be identified with significantly worse overall survival. Also, VEGF overexpression in ODG constitutes an early marker for predicting tumor progression.

Keywords Oligodendroglioma · Angiogenesis · Survival

Harvey Quon and Ali Hasbini contributed equally as first authors. Study was accepted for "poster viewing" at the 50th ASTRO annual meeting, September 21–25, 2008, Boston, Massachusetts.

H. Quon · B. Abdulkarim (✉)
Department of Oncology, Cross Cancer Institute & University of Alberta, 11560 University Avenue, Edmonton, AB T6G 1Z2, Canada
e-mail: bassamab@cancerboard.ab.ca

A. Hasbini · B. Abdulkarim
Department of Oncology, Institut Gustave Roussy, 39 Rue Camille Desmoulins, 94805 Villejuif, France

J. Cougnard · L. Djafari
Department of Statistic and Clinical Research, Schering-Plough, 92 rue Baudin, 92 307 Levallois-Perret Cedex, France

C. Lacroix
Department of Pathology, Hôpital Kremlin Bicetre and Universite Paris XI, 78, rue du General-Leclerc, 94275 Le Kremlin-Bicetre, France

Introduction

Oligodendrogliomas (ODGs) represent the third most common type of glioma [1, 2]. Refinements of pathological criteria, distinguishing diffuse astrocytomas from ODGs, oligoastrocytomas, and pilocytic astrocytomas, have indicated that ODGs comprise up to 5% of brain tumors [3]. Tumor grade remains the most important prognostic survival parameter for ODGs. However, for anaplastic (grade

III) ODG, two recent randomized studies have shown that combined loss of chromosomes 1p and 19q have been associated with a significantly improved prognosis [4–6]. In addition to such molecular analysis, other factors including tumor angiogenesis may provide additional prognostic information. However, the significance of other prognostic factors has been intensively examined in numerous studies but have yielded conflicting results [7–13].

Tumor angiogenesis is considered to be a prerequisite for tumor growth to a size greater than a few millimeters [14]. Interestingly, ODGs appear to be an exception [1]. Low-grade ODGs may grow slowly for several years by spreading isolated tumor cells over a large volume of the brain, with the blood supply being provided only by the microvasculature of the host brain parenchyma. Tumor angiogenesis eventually occurs at later stages of tumor progression within areas of parenchyma showing high tumor cell density and hypoxia [15], and is often accompanied by malignant degeneration to a higher-grade tumor. The important role of tumor angiogenesis in high-grade gliomas led to the hypothesis that the intensity of angiogenesis in ODGs may correlate with tumor grade and aggressiveness [16]. Daumas-Duport et al. [1] found that pathologic endothelial hyperplasia, especially when associated with contrast enhancement on CT or MRI, was significantly associated with a poor prognosis. They subsequently developed a grading system for ODG [15] based on pathologic and radiologic criteria which distinguished between two grades, A and B, depending on the absence or presence of endothelial hyperplasia and/or contrast enhancement, respectively. In another grading system, the revised World Health Organization (WHO) criteria include five parameters (high cellularity, nuclear atypia, mitosis, necrosis, and endothelial proliferation) to identify malignant or grade III ODGs [17]. The WHO has guidelines for grading "pure" ODGs when tumors do not contain malignant astrocytes [1, 15] and are considered to be low-grade (WHO grade II), while anaplastic ODGs (WHO grade III) are associated with frequent mitosis, endothelial proliferation, and/or conspicuous necrosis [18].

The criteria used in both the Daumas-Duport and WHO grading [15, 17] systems suggest that vascular parameters play a crucial role in the outcome of ODGs. According to both systems, progression of ODGs to a higher grade (grade B, WHO grade III) is associated with increased tumor angiogenesis. We hypothesize that radiological with pathological analysis of tumor angiogenesis may add important prognostic information. We report on a multivariate assessment of tumor angiogenesis using molecular, pathological, and radiological parameters in a cohort of 134 ODG patients.

Patients and methods

Patient population

Consecutive patients diagnosed with ODG between January 1995 and December 2001 were identified from a single institutional database. Charts were reviewed and information regarding patient and tumor characteristics were recorded. All tumor specimens were reviewed by a neuropathologist and only patients diagnosed with pure ODGs [17] were enrolled. This study took advantage of a standardized imaging protocol using prospective brain MRI at the time of diagnosis and for subsequent follow up. The preoperative and postoperative MRI images were reviewed prospectively in all patients and several tumor characteristics such as contrast enhancement and necrosis were recorded. The degree of contrast enhancement was measured as follows: absent or present (low and high-signal).

Treatment modalities

All patients were reviewed by a multidisciplinary group and offered guideline-based surgery and adjuvant radiation therapy (RT). The extent of surgical resection was determined post-operatively by T1-weighted contrast enhancement on MRI for WHO grade III and T2-weighted images for WHO grade II ODG. In the event of non-contrast enhancing WHO grade III tumors, these were identified by the T2-weighted images. Tumor resection was considered to be subtotal if less than 70% was removed. Patients over 40 years old, with tumor size $\geq$5 cm and midline shift were classified as high-risk grade II and offered adjuvant 3D-conformal RT (3D-CRT) to a dose of 54 Gy in 30 fractions. According to these criteria, 32 high-risk WHO grade II patients were offered RT. All WHO grade III ODG patients were offered adjuvant RT using conventional fractionation (2 Gy/fraction daily, 5 days/week) to a 2 cm margin surrounding the tumor volume, including peritumoral edema to 45 Gy (T2-weighted MRI) with a subsequent boost to a total dose of 60 Gy to gross tumor (contrast-enhanced T1-weighted MRI). High energy photons and a rigid immobilization system were used. Chemotherapy was given at the time of tumor progression, which was defined as a $\geq$ 25% increase in the cross-sectional area of tumor on consecutive MRI scans.

Immunohistochemical analysis for tumor proliferation and angiogenic factors

From paraffin-embedded specimens, 5-μm slides were prepared with H&E for diagnostic confirmation and tumor classification according to WHO criteria. After confirmation, slides with 3-μm sections of paraffin-embedded

material were mounted for immunohistochemical (IHC) evaluation. The primary antibodies used included anti-vascular endothelial growth factor (VEGF) (clone C-1, Santa Cruz), anti-vascular endothelial growth factor receptor 1 (VEGF-R1) (MF-1, ImClone Systems), anti-VEGF-R2 (DC101, PharMingen), anti-CD34 (clone ICO115, Santa Cruz), and anti-Ki-67 (MIB-1, Dako) and staining was performed by the streptavidin–biotin method. The sections on slides were deparaffinized, rehydrated, and antigenic recovery was performed by heating the slides in microwaves. Endogenous peroxidase activity was blocked by immersing the slides in H_2O_2 5% (2 × 10 min). Subsequently, the slides were rinsed twice with PBS and then incubated in solution to block nonspecific binding (Protein Block Serum, DakoCytomation). The sections were then covered with the specific antibodies and kept overnight at 4°C. After an additional PBS rinse, the histological sections were then incubated with secondary antibody for 40 min, in biotin-peroxidase complex (Novocastra Laboratories) for another 40 min, and then rinsed in PBS again. The antigen–antibody was visualized with diaminobenzidine tetrachloride (DAB). Placental tissue was used as a positive control for the anti-VEGF antibodies and tonsil tissues for the anti-CD34 antibodies. Negative controls were established by eliminating the primary antibody, which was replaced by bovine albumin.

The expression of VEGF and VEGF-R1/R2 were assessed according to the percentage of immunoreactive cells. Stained cells were counted in four randomly selected sites on a 200× field and averaged. The degree of immunostaining was considered low (1+) when the proportion of immunostained cells was less than 20%, intermediate (2+) if between 20 and 50%, and high (3+) if greater than 50%. The vessels labeled with anti-CD34 were counted under light microscopy with 20-fold magnification. The average counts were recorded as the CD34-microvessel density for each case.

Statistical methods

The primary endpoint of this study was overall survival, as measured from the date of surgery to last follow-up or death. Univariate analysis was performed using the log rank test. All factors reaching statistical significance with $P < 0.05$ were included in a multivariate analysis (Cox model). Overall survival was constructed according to the method of Kaplan–Meier. In addition, multivariate classification and regression tree (CART) models for censored data, as modified by Leblanc and Crowley, were performed to identify interactions between independent baseline variables and to classify patients into clinically relevant categories (low vs. high-risk) [19]. Chi-square tests and hazard-ratios along with corresponding 95% confidence intervals were calculated. All reported P values are two-sided and differences were considered statistically significant when $P < 0.05$. The SAS program (Version 9.1, SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Results

Patients, tumor grade and survival

The median follow-up was 60 months (48–80 months). Forty-six percent of patients were diagnosed with partial or generalized seizures and 90% of the population had Karnofsky performance status (KPS) ≥90%. Eighty-four patients (62%) had undergone subtotal resection (debulking) and 50 patients (38%) had stereotactic biopsies. Patient characteristics are listed in Table 1. At the time of analysis, 101 patients (75%) had tumor progression, 82 patients (61%) died of disease progression, and 19 patients (14%) were alive with disease progression. The median overall survival for the whole population was 25 months. Subtotal resection was associated with statistically longer survival than biopsy in patients with WHO grade III ($P = 0.03$) but not grade II ODG ($P = 0.88$).

According to the Daumas-Duport grading system, patients with grade A (25 patients) and B (109 patients) ODGs had median survivals of 62 and 20 months, respectively. Based on the WHO system, grade II ODGs had a median survival of 48 months while grade III had a median survival of 18 months. As shown in Table 1, there is excellent concordance between the WHO and Daumas-Duport grading systems for higher grade tumors with 95% (83/87) of WHO grade III ODGs being diagnosed as grade B according to the Daumas-Duport system. However, discrepancies were found between the WHO grade II and Daumas-Duport grading systems. Only 44% (21/47) of WHO grade II patients were classified as Daumas-Duport grade A while 56% (26/47) were classified as Daumas-Duport grade B. These discrepancies between grading systems have been previously described by others investigators [20].

Univariate analysis of prognostic factors

Consistent with previous studies [20], older age was associated with significantly shorter survival. Patients younger than 55 years had a median survival of 32.6 months compared to 14.2 months in older patients ($P = 0.002$, Table 2). Occurrence of seizure was associated with significantly longer median survival as compared to absence of seizure (70 vs. 19 months, respectively, $P = 0.0001$). Median survival of patients with WHO grade

Table 1 Population characteristics of 134 ODG patients

Variables	WHO grade II $N = 47$	WHO grade III $N = 87$	Whole population $N = 134$
Age			
≤55	40	62	102
>55	7	25	32
Seizure			
No	25	62	87
Yes	22	25	47
Radiological criteria			
Tumor location			
Superficial	33	72	105
Deep	14	15	29
Contrast enhancement			
No	26	21	47
Yes	21	66	87
Radiological necrosis			
No	38	67	105
Yes	9	20	29
Extent of surgery			
Biopsy	24	26	50
Subtotal resection	23	61	84
Pathological criteria			
Endothelial hyperplasia			
No	24	2	26
Yes	23	85	108
Pathological necrosis			
No	47	45	92
Yes	0	42	42
Nuclear atypia			
No	30	22	52
Yes	17	65	82
Mitosis			
No	28	20	48
Yes	19	67	86
Daumas-Duport grading			
A	21	4	25
B	26	83	109

II and III ODGs with seizures were 58 and 40 months, respectively ($P = 0.002$).

As shown in Table 2, evidence of contrast enhancement correlated with poorer median survival. Patients with and without contrast enhancement had an overall survival of 19 and 46 months, respectively ($P = 0.021$, Fig. 1a). Also, the presence of contrast enhancement correlated with poorer outcome in patients with WHO grade III ODG ($P = 0.04$) but not WHO grade II ($P = 0.10$). As shown in Fig. 1b, the presence of endothelial hyperplasia was strongly correlated with shorter patient survival ($P = 0.001$). The influence of endothelial hyperplasia on survival was statistically significant in WHO grade III patients ($P = 0.0049$) but not in WHO grade II ($P = 0.128$).

The presence of necrosis predicted for poor survival in the overall population. Patients without radiological or pathological (Fig. 1c, d) necrosis have a significantly better overall survival (48 months) compared to patients with either radiological or pathological necrosis (15 months, $P = 0.0001$). This effect was restricted to patients with WHO grade III ODG, where patients with either radiological or pathological necrosis had a worse overall survival than patients without necrosis ($P = 0.0032$). It was not statistically significant for patients with WHO grade II ODG ($P = 0.39$).

Multivariate analysis of prognostic factors

In multivariate analysis, age, seizure, contrast enhancement, endothelial hyperplasia, and necrosis were found to be independent prognostic factors for survival (Table 2). Multivariate classification and regression tree (CART) analysis was performed to identify subsets of patients with distinctly different survival distribution. CART analysis (Fig. 2a) generates a tree that consists of nodes (subsets of patients) by successively splitting each node into two nodes. This technique can identify interactions between independent baseline variables. Using the CART model, three distinct groups of patients were identified with different patient characteristics (Table 3) and outcomes (Fig. 2b). A high risk subgroup of patients was identified based on evidence of necrosis (radiological or pathological), angiogenesis (contrast enhancement or endothelial hyperplasia), and no past history of seizure (Fig. 2b, c). These patients had a median survival of 14.2 months. Similarly, a low risk subgroup was identified based on the absence of necrosis and age <55 years. Median survival in this favorable subgroup was not reached after 5 years of follow-up.

Angiogenesis-related factors and prognostic significance

We analyzed the expression of VEGF in tumors and endothelial cells. Tumor cells exhibited a strong membranous/intracytoplasmic VEGF expression, which tended to be concentrated around the vessels with endothelial hyperplasia in anaplastic ODG patients (Fig. 3a, b). In this analysis, the cut-off of VEGF expression was 20% of positive cells and we found no statistical difference in overall survival between patients with moderate (2+) or strong (3+) VEGF intensity. VEGF intensity (2+ and 3+) in tumor cells (VEGFt) was significantly higher among patients with grade III as compared to grade II ODGs

Table 2 univariate and multivariate analysis of whole population

Variables	Univariate analysis			Median survival (Months)	Multivariate analysis		
	HR	95% CI	*P*		HR	95% CI	*P*
Age							
≤55	2.3	[1.2–3.0]	0.002	32.6	1.6	[1.2–2.6]	0.002
>55				14.2			
Seizure							
Absent	2.3	[1.6–3.2]	0.0001	19.0	2.1	[1.7–3.1]	<0.001
Present				69.6			
Contrast enhancement							
None	1.8	[1.3–2.6]	0.021	46.5	1.6	[1.1–2.5]	0.021
Present				19.0			
Endothelial hyperplasia							
None	2.1	[1.5–2.8]	0.001	41.0	1.9	[1.4–2.4]	0.001
Present				17.0			
Radiological necrosis							
None	2.7	[2.2–3.4]	0.0001	32.0	2.4	[1.9–3.2]	<0.001
Present				14.0			
Pathological necrosis							
None	2.4	[1.9–3.1]	0.0001	39.8	2.1	[1.9–3.1]	0.001
Present				14.5			
Nuclear atypia							
None	1.9	[1.3–2.6]	0.02	60.0	3.9	[1.2–2.3]	NS
Present				17.7			
Mitosis							
None	2.5	[1.3–2.6]	0.007	62.7	2.3	[1.0–2.1]	NS
Present				19.4			
Daumas-Duport grading							
A	2.5	[1.5–4.3]	0.007	62.7	1.9	[1.1–3.9]	0.005
B				19.4			
Extent of surgery							
Biopsy	1.3	[1.2–1.3]	0.66	19.0	1.1	[0.9–1.3]	NS
Subtotal resection				21.0			

($P = 0.017$). Similarly, VEGF expression (2+ and 3+) in vascular cells (VEGFv) was more prevalent among patients with WHO grade III than those with grade II ($P = 0.01$). A similar pattern of increased CD34 expression was observed in WHO grade III as compared to grade II ODGs ($P = 0.0001$, Fig. 3c). In contrast, VEGF-R1 and VEGF-R2 staining were low (1+) and no statistically significant difference was observed between WHO grade II and III (data not shown). Median survivals were 17 and 43 months for patients with and without VEGFt over-expression (2+ and 3+) and 18 and 48 months with and without VEGFv over-expression. The intensity of VEGF staining in tumor and endothelial cells was found to be strongly associated with WHO grade III (67 and 68%, respectively) or Daumas-Duport grade B (69 and 67%, respectively, Fig. 3c) ODG.

The VEGF intensity in tumor and vessel (2+ and 3+) correlated with contrast enhancement and was significantly increased in the high-risk subgroup of patients identified by CART analysis compared to other subgroups (Fig. 3c; $P = 0.0023$). On multivariate analysis, none of the biological markers (VEGF, VEGF-R, CD34 and Ki-67) emerged as an independent prognostic factor for survival. However, VEGF was found to be an independent prognostic factor for tumor progression regardless of tumor grade ($P = 0.02$).

Discussion

Determining prognosis accurately is an important step in the treatment decision making process. To date, most

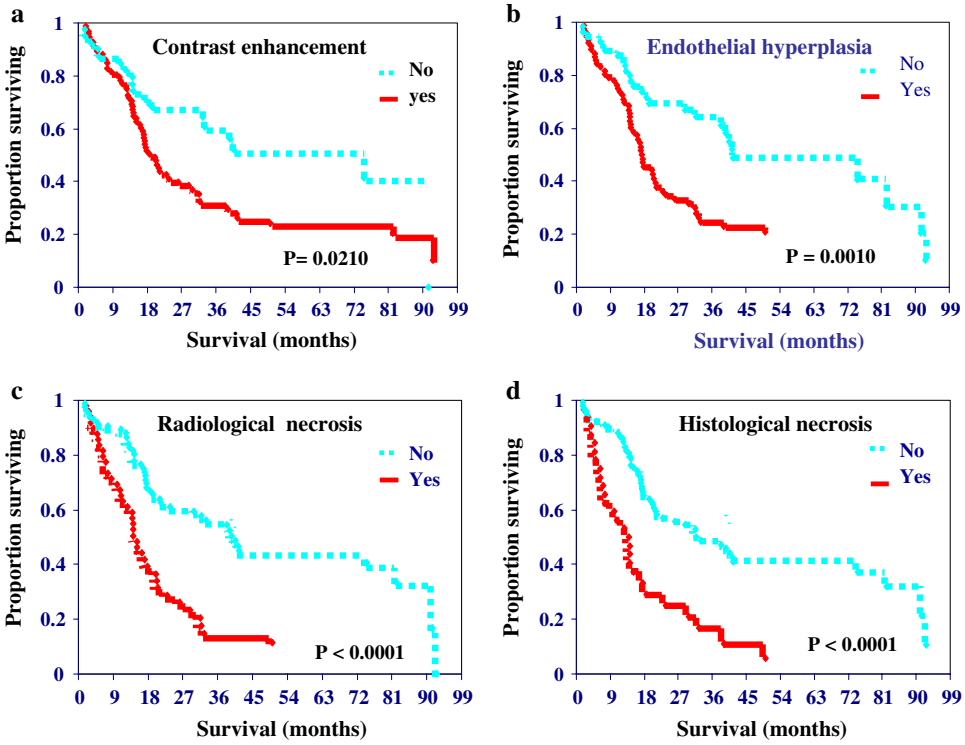
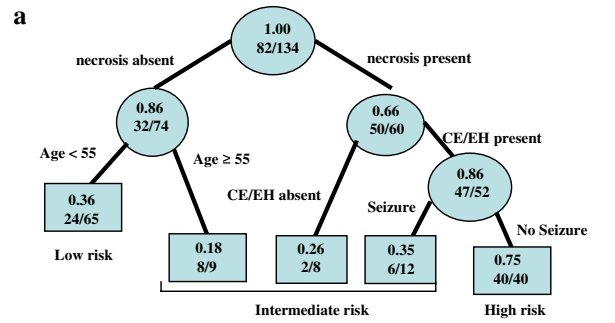


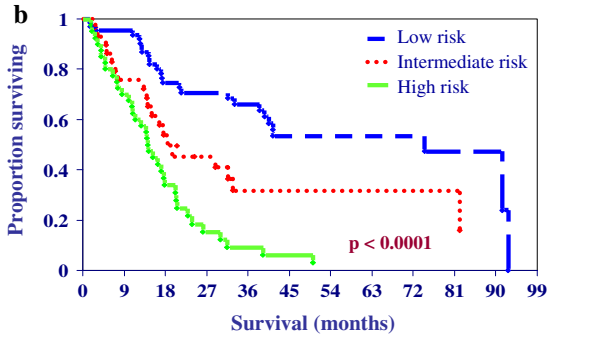
Fig. 1 Overall survival based on the presence or absence of contrast enhancement (**a**), endothelial hyperplasia (**b**), radiological necrosis (**c**) or pathological necrosis (**d**)

prognostic factors for ODG have been based on pathologic criteria, which have their limitations [7, 9, 10]. Nevertheless, recent studies suggest that among many pathological features, endothelial hyperplasia should be considered one of the most reliable prognostic factors in predicting survival in patients with ODGs [11, 12, 15]. Angiogenesis (i.e., endothelial hyperplasia) is a hallmark of high-grade gliomas [21]. However, the precise role of angiogenesis and its predictive value in ODG progression and patient survival remain to be demonstrated. In our study, we evaluated the prognostic significance of tumor angiogenesis at the microscopic level using pathological examination, and at the macroscopic level using imaging techniques. We showed significant correlation between endothelial hyperplasia and/or contrast enhancement and the clinical outcome of ODGs. Moreover, the presence of necrosis was also shown to be an independent prognostic factor associated with poorer overall survival.

In our study, clinical, pathological, and radiological data subjected to multivariate CART-survival modeling showed that the most significant poor prognostic factor was the presence of either pathologic and/or radiologic tumor necrosis. This is consistent with the results of a phase III trial of adjuvant procarbazine, lomustine, and vincristine in anaplastic ODG or anaplastic mixed oligoastrocytoma which found that necrosis was an independent prognostic factor for survival [5]. In contrast, Miller et al. [22] reported in a series of 1093 patients that necrosis was not an independent prognostic factor for survival in patients with grade III pure ODG, but it was prognostic for patients with anaplastic mixed oligoastrocytoma. This difference may have been due to differences in treatment, as patients in our study were managed in a single institution according to a standardized protocol, while 85% of anaplastic pure ODG reported by Miller et al. were treated at different institutions and could have had different standards of care for treatment.

We identified a high-risk subgroup of patients who have particularly poor outcomes, even worse than for anaplastic oligodendrogliomas. Patients with necrosis (radiological or pathological), angiogenic markers (contrast enhancement or endothelial hyperplasia), and no past history of seizure had a poor median overall survival of 14.2 months. Interestingly, this high-risk subgroup of ODG had pathological and radiological characteristics similar to anaplastic astrocytomas but their survival was much worse than expected. The overall survival of this high-risk group is more similar to the emerging subset of "glioblastoma multiforme with oligodendroglial features" [22] than for traditional anaplastic ODG. In their analysis, Miller et al. [22] found that necrosis was an independent prognostic factor, even after correcting for age and 1p/19q status. In addition, a recent report from EORTC 26951 confirmed our finding that histopathological necrosis and 1p and 19q loss are independent prognostic factors in anaplastic oligoastrocytomas. *Kouwenhoven* et al. [23] suggested that anaplastic oligoastrocytomas with necrosis should be considered as grade IV tumors. The prognostic significance





c

Risk groups	1 year	3 years	5 years
Low (n=65)			
No. at Risk	61 (94%)	46 (70%)	41 (63%)
Intermediate (n=29)			
No. at Risk	22 (76%)	11 (38%)	9 (31%)
High (n=40)			
No. at Risk	24 (60%)	6 (15%)	4 (10%)

Fig. 2 **a** Classification and regression tree (CART) survival analysis to identify independent prognostic factors most strongly associated with survival. The upper number is the hazard ratio. The numerator represents the number of deceased patients in each node and the denominator represents the total number of patients at risk. **b** Overall survival for risk groups identified by CART analysis. **c** Overall survival of risk groups at 1, 3, and 5 years

of necrosis in identifying high-risk ODG patients is confirmed by our study. Thus, both necrosis and tumor angiogenesis, as evaluated by pathological and radiological criteria, may be used in combination with other clinical characteristics and genetic analysis of 1p and 19q deletions to determine prognosis of ODG patients [24, 25]. Furthermore, as the favorable prognostic significance of 1p and 19q co-deletion appears restricted to ODG with a "classic" appearance [26], the identification of high-risk ODG patients by our criteria may have increased prognostic importance in patients with non-classical ODG histology.

Table 3 Summary of risk group characteristics according to CART analysis

Variables	Low $N = 65$	Int $N = 29$	High $N = 40$	P
Age				
<55	65	12	25	0.0001
>55	0	17	15	
Seizure				
No	35	19	33	0.014
Yes	30	10	7	
Contrast enhancement				
No	36	11	0	0.0001
Yes	29	18	40	
Endothelial hyperplasia				
No	22	4	0	0.02
Yes	43	25	40	
Radiological necrosis				
No	65	20	20	0.001
Yes	0	9	20	
Pathological necrosis				
No	65	19	8	0.0001
Yes	0	10	32	
Pathological grade				
WHO II	32	8	7	0.002
WHO III	33	21	33	

The discrepancies found between the WHO and Daumas-Duport grading systems illustrate the difficult and subjective nature in classifying ODG. As the grading of these tumors directly impacts on treatment, the importance of a more objective scheme is needed. Molecular testing is one way in which this could be achieved, especially with recent trials confirming the favorable prognostic value of 1p and 19q co-deletions [4–6]. Also, WHO grade III ODGs with microvascular proliferation and/or necrosis have recently been linked to 9p deletion, a region which contains tumor suppressor genes such as p16 (CDKN2A) involved in the regulation of glioma angiogenesis and frequently implicated in tumor progression [27]. Tumor angiogenesis is known to be modulated by various growth factors, including VEGF. VEGF is upregulated in most cases of glioblastoma multiforme and was found as an independent prognostic marker for survival in low-grade astrocytoma patients [29, 30]. However, the significance of VEGF expression in ODGs is controversial. Pietsch et al. [31] did not show VEGF expression in ODGs and Christov et al. [32] found that VEGF expression could be located in tumor vessels rather than in tumor cells. We and others [33–36] have shown that VEGF expression was associated with microvascular proliferation in anaplastic ODGs. In our study, VEGF expression in anaplastic ODG patients was

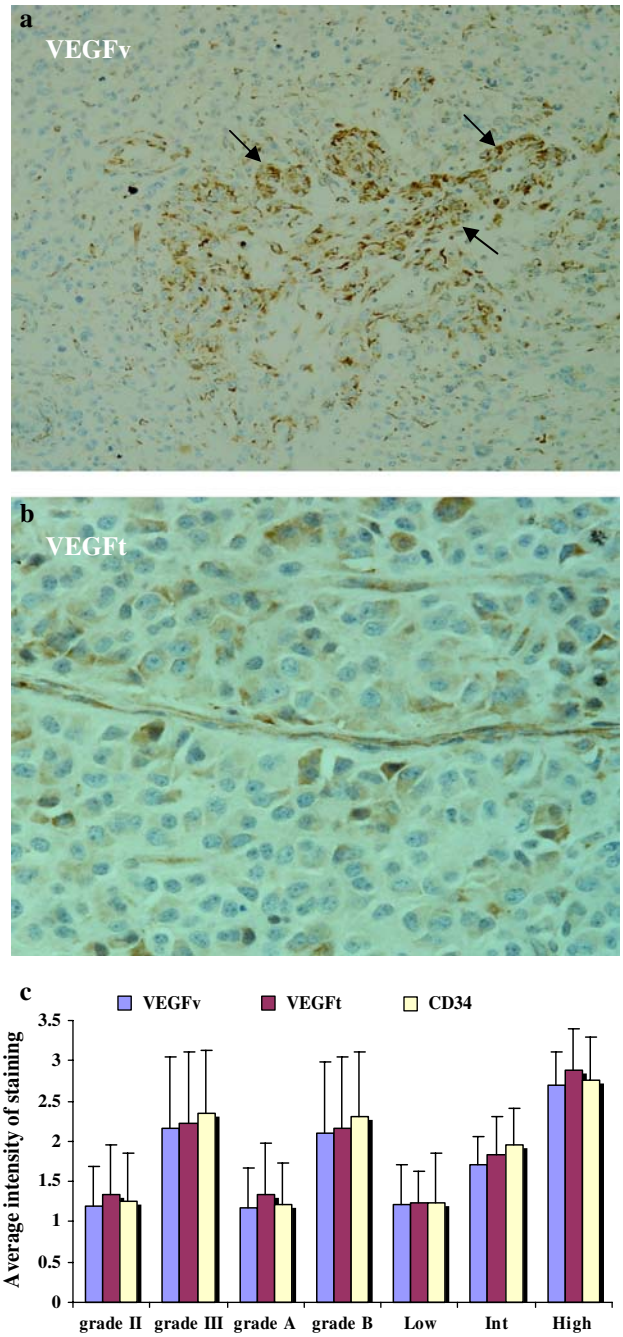


Fig. 3 Immunohistochemical detection of VEGF in endothelial cells (VEGFv) (**a**) and tumor cells (VEGFt) (**b**). Average intensity of staining in patients separated by WHO grade, Daumas-Duport grade, and CART analysis risk groups (**c**)

concentrated around the vessels in large areas of tumor necrosis and high mitotic index (Ki-67). We also found a high correlation between VEGF and endothelial hyperplasia. Interestingly, we identified VEGF over-expression as an independent predictive factor for tumor progression regardless of tumor grade. Taken together, these findings suggest that VEGF expression constitutes an early marker for identifying a group of WHO grade II ODG at increased risk for tumor progression. This also suggests that VEGF expression in WHO grade II ODGs mandates a careful review of the pathological diagnosis as well as close radiologic monitoring in order to detect the earliest events of angiogenesis and tumor progression.

Attempts to evaluate prognostic factors that predict for survival in ODGs have typically been based on the study of genetic markers [24, 37]. Combined loss of 1p/19q is present in 25–60% of ODGs [4, 5, 38, 39] and has been associated with improved survival as well as response to cytotoxic therapy [4, 5, 40–42]. However, relying only on the 1p/19q status to determine outcomes may not encompass all available prognostic data. Although our study is potentially limited by the lack of assessment of 1p/19q deletions, the multivariate assessment of histological and radiological markers of tumor angiogenesis may complement 1p/19q co-deletion analysis in determining prognosis. Indeed, some reports have already emerged suggesting that the prognostic significance of certain pathologic factors, such as necrosis, as independent factor of the 1p/19q status in anaplastic oligoastrocytomas [22]. Additional research in a separate population is needed to validate these findings and is currently underway in a population-based study in our institution. In addition, the authors are examining the prognostic significance of dynamic contrast-enhanced (DCE)-MRI in identifying angiogenesis in ODG patients.

In summary, we have identified a new high-risk group of ODGs based on the presence of radiological or pathological necrosis, angiogenesis (contrast enhancement or endothelial hyperplasia), and absence of seizures. These patients have a shorter overall survival compared to WHO grade III ODG. Additional studies are required to better identify this high-risk subgroup and to define the optimal treatment, as they may be candidates for more aggressive therapy used for high-grade gliomas such as concurrent temozolomide and radiotherapy.

References

1. Daumas-Duport C, Varlet P, Tucker ML et al (1997) Oligodendrogliomas. Part I: patterns of growth, histological diagnosis, clinical and imaging correlations: a study of 153 cases. J Neurooncol 34:37–59
2. Shaw EG, Scheithauer BW, O'Fallon JR et al (1992) Oligodendrogliomas: the Mayo Clinic experience. J Neurosurg 76:428–434
3. Ries LAG, Melbert D, Krapcho M, Mariotto A, Miller BA, Feuer EJ, Clegg L, Horner MJ, Howlader N, Eisner MP, Reichman M, Edwards BK (eds). SEER Cancer Statistics Review, 1975–2004, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2004/, based on November 2006 SEER data submission, posted to the SEER web site, 2007
4. Cairncross G, Berkey B, Shaw E et al (2006) Phase III trial of chemotherapy plus radiotherapy compared with radiotherapy

alone for pure and mixed anaplastic oligodendroglioma: Intergroup Radiation Therapy Oncology Group Trial 9402. J Clin Oncol 24:2707–2714
5. van den Bent MJ, Carpentier AF, Brandes AA et al (2006) Adjuvant procarbazine, lomustine, and vincristine improves progression-free survival but not overall survival in newly diagnosed anaplastic oligodendrogliomas and oligoastrocytomas: A Randomized European Organisation for Research and Treatment of Cancer Phase III Trial. J Clin Oncol 24:2715–2722
6. Quon H, Abdulkarim B (2008) Adjuvant treatment of anaplastic oligodendrogliomas and oligoastrocytomas. Cochrane Database Syst Rev, Issue 2. Art. No.: CD007104. DOI: 10.1002/14651858.CD007104
7. Schiffer D, Dutto A, Cavalla P et al (1999) Prognostic factors in oligodendroglioma. Can J Neurol Sci 24:313–319
8. Saito A, Nakazato Y (1999) Evaluation of malignant features in oligodendroglial tumors. Clin Neuropathol 18:61–73
9. Burger PC, Rawlings CE, Cox EB, McLendon RE, Schold SC Jr, Bullard DE (1987) Clinicopathologic correlations in the oligodendroglioma. Cancer 59:1345–1352
10. Celli P, Nofrone I, Palma L et al (1994) Cerebral oligodendroglioma: prognostic factors and life history. Neurosurgery 35:1018–1034
11. Fortin D, Cairncross GJ, Hammond RR (1999) Oligodendroglioma: an appraisal of recent data pertaining to diagnosis and treatment. Neurosurgery 45:1279–1291
12. Giannini C, Scheithauer BW, Weaver AL et al (2001) Oligodendrogliomas: reproducibility and prognostic value of histologic diagnosis and grading. J Neuropathol Exp Neurol 60:248–262
13. Vaquero J, Zurita M, Morales C et al (2001) Prognostic significance of the endothelial surface in low-grade resected oligodendrogliomas. Br J Neurosurg 15:247–260
14. Folkman J (1990) What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 82:4–6
15. Daumas-Duport C, Tucker ML, Kolles H et al (1997) Oligodendrogliomas. Part II: a new grading system based on morphological and imaging criteria. J Neurooncol 34:61–78
16. Brem S, Cotran R, Folkman J (1972) Tumor angiogenesis: a quantitative method for histologic grading. J Natl Cancer Inst 48:347–356
17. Kleihues P, Cavenee WK, eds (2000). Pathology and genetics: tumors of the nervous system. In: WHO classification of tumors. Lyon (France): IARC press, 56–61
18. Engelhard HH, Stelea A, Cochran EJ (2002) Oligodendroglioma: pathology and molecular biology. Surg Neurol 58:111–117
19. LeBlanc M, Crowley J (1992) Relative risk trees for censored survival data. Biometrics 48:411–425
20. Leighton C, Fisher B, Bauman G et al (1997) Supratentorial low-grade glioma in adults: an analysis of prognostic factors and timing of radiation. J Clin Oncol 15:1294–1301
21. Plate KH, Breier G, Weich HA, Risau W (1992) Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 359:845–848
22. Miller CR, Dunham CP, Scheithauer BW, Perry A (2006) Significance of necrosis in grading of oligodendroglial neoplasms: a clinicopathologic and genetic study of newly diagnosed high-grade gliomas. J Clin Oncol 24(34):5419–5426
23. Kouwenhoven MC, Gorlia T, Kros J, et al. (2009). Molecular analysis of anaplastic oligodendroglial tumors in a prospective randomized study: a report from EORTC study 26951. Neuro-oncology. 2009 February 17
24. Bigner SH, Matthews MR, Rasheed BK et al (1999) Molecular genetic aspects of oligodendrogliomas including analysis by comparative genomic hybridization. Am J Pathol 155:375–386
25. Smith JS, Perry A, Borell TJ et al (2000) Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. J Clin Oncol 18:636–645
26. McDonald JM, See SJ, Tremont IW et al (2005) The prognostic impact of histology and 1p/19q status in anaplastic oligodendroglial tumors. Cancer 104:1468–1477
27. Bortolotto S, Chiado-Piat L, Cavalla P et al (2000) CDKN2A/p16 inactivation in the prognosis of oligodendrogliomas. Int J Cancer 88:554–567
28. Perry A, Aldape KD, George DH, Burger PC (2004) Small cell astrocytoma: an aggressive variant that is clinicopathologically and genetically distinct from anaplastic oligodendrogliomas. Cancer 101:2318–2326
29. Chan AS, Leung SY, Wong MP et al (1998) Expression of vascular endothelial growth factor and its receptors in the anaplastic progression of astrocytoma, oligodendroglioma, and ependymoma. Am J Surg Pathol 22:816–826
30. Abdulrauf SI, Edvardsen K, Ho KL et al (1998) Vascular endothelial growth factor expression and vascular density as prognostic markers of survival in patients with low-grade astrocytoma. J Neurosurg 88:513–520
31. Pietsch T, Valter MM, Wolf HK et al (1997) Expression and distribution of vascular endothelial growth factor protein in human brain tumors. Acta Neuropathol 93:109–117
32. Christov C, Adle-Biassette H, Le Guerinel C et al (1998) Immunohistochemical detection of vascular endothelial growth factor (VEGF) in the vasculature of oligodendrogliomas. Neuropathol Appl Neurobiol 24:29–35
33. Godfraind C, Rousseau E, Ruchoux MM et al (2003) Tumour necrosis and microvascular proliferation are associated with 9p deletion and CDKN2A alterations in 1p/19q-deleted oligodendrogliomas. Neuropathol Appl Neurobiol 29:462–471
34. Harada H, Nakagawa K, Iwata S et al (1999) Restoration of wild-type p16 down-regulates vascular endothelial growth factor expression and inhibits angiogenesis in human gliomas. Cancer Res 59:3783–3789
35. Jen J, Harper JW, Bigner SH et al (1994) Deletion of p16 and p15 genes in brain tumors. Cancer Res 54:6353–6358
36. Varlet P, Guillamo JS, Nataf F et al (2000) Vascular endothelial growth factor expression in oligodendrogliomas: a correlative study with Sainte-Anne malignancy grade, growth fraction and patient survival. Neuropathol Appl Neurobiol 26:379–389
37. Louis DN, Holland EC, Cairncross JG (2001) Glioma classification: a molecular reappraisal. Am J Pathol 159:779–786
38. Reifenberger J, Reifenberger G, Liu L et al (1994) Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. Am J Pathol 145:1175–1190
39. Burger PC, Minn AY, Smith JS et al (2001) Losses of chromosomal arms 1p and 19q in the diagnosis of oligodendroglioma. A study of paraffin-embedded sections. Mod Pathol 14:842–853
40. Cairncross JG, Ueki K, Zlatescu MC et al (1999) Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst 90:1473–1479
41. Thiessen B, Maguire JA, McNeil K et al (2003) Loss of heterozygosity for loci on chromosome arms 1p and 10q in oligodendroglial tumors: relationship to outcome and chemosensitivity. J Neurooncol 64:271–278
42. Hashimoto N, Murakami M, Takahashi Y et al (2003) Correlation between genetic alteration and long-term clinical outcome of patients with oligodendroglial tumors, with identification of a consistent region of deletion on chromosome arm 1p. Cancer 97:2254–2261