Defining pseudoprogression in glioblastoma multiforme


Keywords: chemotherapy, glioblastoma, pseudoprogression, radiotherapy, temozolomide

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Background and purpose: Pseudoprogression is a frequent phenomenon observed since the introduction of postoperative therapy with radiotherapy and temozolomide (RT/TMZ) in glioblastoma multiforme (GBM) patients. However, the criteria defining pseudoprogression, its incidence, the time of occurrence and its impact on therapy and outcome remain poorly defined.

Methods: The objective of this study is to compare two sets of criteria (liberal and stringent), defining pseudoprogression, in a cohort of patients treated before and after the introduction of RT/TMZ in the standard postoperative treatment. This retrospective review includes 136 unselected and consecutively treated patients with pathologically diagnosed GBM.

Results: Pseudoprogression was observed in 10 (12%) cases applying the stringent criteria, and in 18 (23%) patients when using the liberal criteria, in the cohort treated with RT/TMZ. Pseudoprogression was observed in only one patient treated with RT alone. The median time to pseudoprogression was 4 weeks after the end of RT. Patients with pseudoprogression had a median survival time of 28 months, compared with 12 months for patients without pseudoprogression.

Conclusions: The incidence of pseudoprogression after RT/TMZ strongly depends on the applied criteria. However, regardless of the stringency of the criteria, the impact on survival remains the same.

Introduction

Glioblastoma multiforme (GBM) is the most frequent and devastating primary malignant brain tumor in adults. Despite all efforts made in recent years, the prognosis remains very poor, with a median overall survival (OS) of 14 months [1]. The current standard of care was set with a randomized phase III trial conducted by the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC). Progression-free survival and OS of patients treated with adjuvant temozolomide (TMZ) and radiotherapy (RT) were longer than for patients treated with RT alone [1]. Pseudoprogression is a phenomenon that is recognized with increasing frequency since the introduction of concomitant RT/TMZ [2–5]. It is detected during post-irradiation imaging, suggesting either a relapsing disease or progression of remaining tumor foci, often accompanied by clinical symptoms. In contrast to a true progression, these lesions resolve spontaneously without further therapy or might evolve into radiation necrosis [6]. The use of non-invasive diagnostic criteria, the actual incidence, the time of occurrence of pseudoprogression and its impact on the clinical outcome remain poorly defined. In a seminal report by Chamberlain et al., tumor specimens of re-operated patients with progressive disease within the first 6 months were analyzed according to Macdonald criteria [7,8], thereby
providing an insight into the pathology underlying pseudoprogression. Based on these data, at least 14% of patients treated with the RT/TMZ regimen are expected to develop pseudoprogression [8]. Although pathological confirmation of pseudoprogression is the gold standard, such an approach is not useful in clinical practice, as it requires second surgery. The Rotterdam group defined pseudoprogression as a 25% increase in tumor size according to the Macdonald criteria, followed by a partial response or stable disease for at least 6 months after completing the concomitant phase of the RT/TMZ regimen [9]. Using this definition, the pseudoprogression occurs in 25% of patients with either glioblastoma or anaplastic glioma. Other definitions of pseudoprogression are the absence of further progression within a period of 2 months after an initial growth of the lesion has occurred within 4 weeks after the end of RT/TMZ [10]. Applying these criteria, the phenomenon is observed in 31% of the patients. Criteria accepting the absence of further progression within a short period of time inevitably overestimate the incidence of pseudoprogression.

Applying the aforementioned definitions of pseudoprogression requires several months of follow-up in order to increase diagnostic accuracy. This can certainly impact the clinical management of these patients. Perfusion magnetic resonance imaging (MRI) and positron emission tomography (PET) are often used to predict pseudoprogression [11,12]. Despite the technical advances, no standard imaging technique is currently validated for this purpose. The use of these techniques has therefore not been incorporated into the latest response assessment criteria [13,14], which means the diagnosis of pseudoprogression remains a challenging issue, even for the experienced clinician. The variability of the criteria defining pseudoprogression adds additional complexity to the matter.

Glioblastoma multiforme usually spreads by local growth and infiltration. Only 5–10% of recurrent or progressive GBMs are observed outside the primary tumor field [15,16]. Exocranial and contralateral lesions are very uncommon and extracranial metastases are extremely rare. Since the introduction of the concurrent regimen, however, such ‘unusual’ relapses have been reported to occur more often [17], although this finding is not confirmed by observations of other groups [18,19].

In order to explore whether the use of different (liberal or more stringent) criteria for pseudoprogression has an impact on survival in patients with GBM, a retrospective study was conducted. Criteria for pseudoprogression were correlated with OS, the incidence of pseudoprogression, and the pattern of recurrence in patients treated with or without concomitant RT/TMZ.

Methods
A retrospective review was conducted of all pathologically proven GBM cases treated in our center between 2004 and 2008. Concurrent RT with TMZ became available for patients in Belgium in May 2006. OS and time to progression (TTP) were calculated in months from the time of diagnosis. Early progression was defined as increase in tumor or appearance of new lesions occurring within 3 months after completing RT. Estimation of tumor size was done by measuring the largest diameter of the contrast-enhancing lesion multiplied by its largest perpendicular diameter on T1-weighted MRI images. In case of multiple lesions, the sum of these diameters was calculated. Treatment decisions were always made during a multidisciplinary consultation involving a neurosurgeon, a radiation oncologist and a medical oncologist. Multimodality treatment consisted of surgical resection of the tumor to a feasible extent, concomitant RT/TMZ followed by adjuvant TMZ. RT consisted of fractionated focal irradiation at a total dose of 60 Gy, given as 2 Gy per fraction/day, 5 days/week for a period of 6 weeks. Concomitant chemotherapy consisted of TMZ 75 mg/m² once daily, given 7 days/week from the first day of RT. After a break of 4 weeks, patients started the maintenance treatment phase, consisting of six cycles of TMZ (150–200 mg/m² body surface area) for 5 days of every 28-day cycle. Tumor staging was performed by MRI and, where there was a contraindication for MRI, by a computed tomography (CT). Tumor evaluations were routinely done before and after surgery, 1 month after completion of RT, and every 3 months thereafter.

Pseudoprogression was assessed by applying two different criteria. The stringent criteria defined pseudoprogression as a ≥ 25% increase in tumor size or the occurrence of a new contrast-enhancing lesion that spontaneously regressed to baseline. The liberal criteria were the criteria described by Taal et al. [9], including patients showing a stable disease for at least 6 months after the initial occurrence of ≥ 25% increase in tumor volume. Therefore the major difference between the stringent and liberal criteria is the fate of the lesion after the initial increase which, in one case, should resolve, but in the other may still persist unchanged for a certain period of time.

Recurrence patterns were described as ‘usual’ (on the initially affected side) or ‘unusual’ for cases were it occurred on the contralateral side of the brain or outside the cerebrum.

This retrospective study was approved by the Ethical Committee of University Hospitals Leuven, Belgium.
**O6-methyl guanine methyl transferase (MGMT) promoter methylation status**

Material for molecular analysis was available from 112 tumors. The assessment of MGMT promoter methylation was done using the methyl-specific PCR (MSP) approach on DNA after bisulfate treatment as previously described [20]. DNA was isolated from 51 fresh frozen and 61 formalin-fixed, paraffin-embedded (FFPE) samples using the QiAmp DNA Mini Kit and EpiTect Plus FFPE Bisulfate Kit (both Qiagen, Venlo, the Netherlands). Bisulfate treatment was conducted with EpiTect Plus Bisulfate Kit (Qiagen) according to the manufacturer’s procedure, followed by PCR amplification, using two pairs of primers specific for either methylated or unmethylated promoter fragment. Amplicons were separated on 3% agarose gels and visualized by ethidium bromide staining (Bio-Rad, Nazareth Eke, Belgium).

**Statistical analysis**

Continuous data were reported with median and ranges or means and SDs when appropriate. Comparison of continuous data were performed with Student’s *t*-test. Chi-squared and Fisher’s exact tests were used for comparison of categorical data as appropriate. OS and TTP were analyzed by Kaplan–Meier method, with use of the log-rank test for comparison of two groups. Cox proportional hazards model was used to identify independent variables for OS and TTP (not reported here). The statistical software used were SAS version 9.2 (Cary, NC, USA) and Statistica version 9 (Tulsa, OK, USA).

**Results**

Over a period of 4 years, 136 consecutive patients (81 males and 55 females) with a histopathologically proven GBM were treated in our center. The median (range) age at diagnosis was 62 (17–81) years and the median (range) Karnofsky performance status at diagnosis was 70 (20–90). There were 123 primary and 13 secondary GBMs. Thirteen patients presented with multifocal disease at diagnosis. In total, 109 patients underwent surgery. A macroscopically complete resection was achieved in 69 patients. Twenty-four (27%) patients underwent biopsy alone and three patients had no primary surgery, but were diagnosed by biopsy later. The median (range) tumor size at diagnosis was 15.7 (1.1–56.1) cm².

Seventy-three patients were intended to be treated with concurrent chemoradiation followed by six cycles of TMZ, of whom 37 (51%) completed the full treatment as planned. The median TTP for the group that received the combination therapy was 8.5 months, compared with 4.4 months for the RT group (*P* = 0.0004). The median OS for the group that received concurrent RT/TMZ was 16.5 months, compared with 8.0 months for those receiving RT without concomitant TMZ (*P* < 0.0001) (Fig. 1a). Because more patients underwent surgery in the combination therapy group, a separate analysis was carried out in relation to those patients who underwent a resection (*n* = 109), comprising 67 patients in the combined therapy group and 42 patients in the RT group. The characteristics of this population are described in Table 1. In this population, RT/TMZ was still associated with a significantly better OS compared with the RT patients (17.0 vs. 11.6 months, respectively) (Fig. 1b). Among variables showing significant prognostic values in univariate analysis, only the concur-
rent RT/TMZ therapy \((P = 0.041)\), unifocal disease \((P = 0.048)\), the occurrence of pseudoprogression assessed using liberal criteria \((P = 0.001)\) and good Karnofsky performance status at the time of diagnosis \((P = 0.004)\) were revealed as independent parameters for good prognosis by Cox multivariate regression analysis (Table 2).

Early progression was observed in 81 patients (60%) in the total population, with an equal distribution between both treatment groups (59% in the RT/TMZ group and 60% in the RT group). Pseudoprogression occurred in 10 cases (14%) in the combined therapy group compared with no cases in the RT group, using the stringent criteria \((P = 0.0018)\). According to the liberal criteria, pseudoprogression was found in 17 cases (23%) in the RT/TMZ group compared with one in the RT group \((P = 0.0002)\). The median time to occurrence of pseudoprogression was 4 weeks after completion of RT. In the group of patients who had pseudoprogression defined by the stringent criteria, the median OS was 27.7 months, compared with 12.0 months for patients in whom this phenomenon was not observed \((P = 0.0798)\) (Fig. 2a). OS in the group with pseudoprogression according to the liberal criteria was 32.4 months, compared with 11.5 months for the group without pseudoprogression \((P < 0.001; \text{Fig. 2b})\). Those patients who were treated with the TMZ/RT combination and having pseudoprogression also had a better prognosis than those without pseudoprogression. OS was 28.1 months compared with 15.6 months \((P = 0.1365)\) when stringent criteria were applied, and 28 months compared with 13.9 months \((P < 0.001)\) when pseudoprogression was assessed according to the liberal criteria. Twelve out of the 18 pseudoprogression cases occurred without clinical deterioration or neurological symptoms. However, six patients required high-dose corticosteroids (>5 mg dexamethasone/day) for several months because of clinical deterioration. Patients developed symptoms ranging from headache to dysphasia, hemianopsia and worsening paresis. The symptomatology was completely reversible with corticosteroids in four of these six patients. The other two only partially recovered. None of the patients underwent second surgery for pseudoprogression in our series.

### Table 2 Clinical and molecular variables as risk factors for death in the analyzed cohort of glioblastoma patients. The analysis used the Cox regression model; results indicated in bold are significant

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
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<tr>
<td>Age (&gt;) vs. (\leq) median (62 years)</td>
<td>1.5</td>
<td>1.02–2.14</td>
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<tr>
<td>Gender M vs. F</td>
<td>1.4</td>
<td>0.96–2.04</td>
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<tr>
<td>RT/TMZ vs. no</td>
<td>0.4</td>
<td>0.30–0.64</td>
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<tr>
<td>Multifocal disease yes vs. no</td>
<td>2.5</td>
<td>1.37–4.43</td>
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<tr>
<td>Pseudoprogression – stringent criteria yes vs. no</td>
<td>0.6</td>
<td>0.31–1.22</td>
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<tr>
<td>Pseudoprogression – liberal criteria yes vs. no</td>
<td>0.3</td>
<td>0.16–0.57</td>
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<td>MGMT promoter methylation yes vs. no</td>
<td>0.7</td>
<td>0.46</td>
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<tr>
<td>Karnofsky performance status (WHO) 0–1 vs. (\geq 2)</td>
<td>0.5</td>
<td>0.30–0.69</td>
</tr>
</tbody>
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CI, confidence interval; HR, hazard ratio; M, male; F, female; n/a, not analyzed; RT/TMZ, concomitant radiotherapy and temozolomide.
MGMT promoter methylation was found in 49/112 (44%) of the samples analyzed. The presence of the promoter methylation correlated with a better OS (median, 14.6 vs. 11.1 months), although this difference did not reach statistical significance ($P = 0.07$).

Pseudoprogression tended to occur more frequently in patients with MGMT promoter methylation than in those without methylation, regardless of the use of stringent (12.2% vs. 3.2%) or liberal criteria (18.4% vs. 6.3%; Fig. 3).

Relapse outside of the radiation field was found in 37 patients (32%). An unusual pattern of relapse was observed in 22 patients, with two cases of dural metastasis, two subcutaneous scalp lesions, one malignant meningitis, three cases of distant metastasis, one pial metastasis and 13 contralateral lesions. Such an unusual pattern of relapse was seen in 16 (22%) patients who were treated with RT/TMZ compared with six (10%) in the control group ($P = 0.099$).

**Discussion**

Pseudoprogression, a phenomenon that is caused by a transient blood–brain barrier disruption, is not distinguishable from tumor progression by conventional imaging techniques, and is recognized with increasing frequency since the establishment of concomitant RT/TMZ as a standard of care in GBM patients. Our data unequivocally support this notion, with a statistically significant difference between the incidence of pseudoprogression in patients treated with RT/TMZ and patients treated with RT alone. Whether the criteria used to define pseudoprogression were stringent or more liberal did not seem to matter, irrespective of the observation that the incidence of pseudoprogression between these two groups differed 1.5-fold. Despite these differences, the presence of pseudoprogression remains associated with an improved survival. Median OS time was more than two-fold longer in both criteria groups. Nevertheless our data strongly suggest that whether or not an enlarged contrast-enhancing lesion decreases again does not affect the patient’s prognosis.

The criteria defined by Taal et al. [9] were preferred by the authors of this study, requiring a 6-month progression-free period following documented progressive disease on MRI, rather than a 2- or 3-month interval, because the latter definition would obviously identify a considerable number of true progressions as pseudoprogression. Regardless of the interval, the interpretation of survival data in a cohort of patients with pseudoprogression inevitably implies a lead-time bias. In order to meet the criteria for pseudoprogression, a patient has to survive the defined interval after the first observation of an increased contrast-enhancing lesion. Another complicating fact is that some authors have included cases who received active treatment upon observed progression, in series describing pseudoprogression [21]. The lack of further progression could, in these cases, be explained by a treatment effect. Here, it was preferred not to label such cases as pseudoprogression. This way, the real incidence of pseudoprogression may have been underestimated. However, the low response rates in second-line treatment do not suggest that this occurred frequently based on the stringent criteria.

It has been suggested that pseudoprogression is a sign of treatment response, and more specifically it is a response to TMZ treatment. In this study, as well as in the work published by Brandes et al. [10], pseudoprogression occurred predominantly in patients with MGMT promoter methylation, a subgroup thought to
benefit more from TMZ treatment as part of the combined modality treatment [20]. Disrupted DNA repair machinery is likely to render cells more susceptible to genotoxic stress caused by chemotherapy and irradiation. Secondary immunological reactions striving to contain and eliminate tissue necrosis would cause a disruption of the blood–brain barrier, hence leading to the radiographic signs of pseudoprogression. This is certainly of clinical importance, as all commonly used response criteria are image-based and could therefore lead to treatment cessation upon suspected progression. It should be noted that our analysis is not affected by a pseudo-response phenomenon, because none of the patients in this analysis were treated with bevacizumab or agents targeting vascular endothelial growth factor receptor-mediated signal transduction.

In two-thirds of the patients where pseudoprogression was observed, this phenomenon was not associated with clinical deterioration. However, in the remaining third, pseudoprogression was associated with a worsened clinical condition. These patients required treatment with high-dose corticosteroids for several months, and the symptomatology was not always completely reversible.

Our data suggest that the improved survival associated with pseudoprogression is independent of continuation of TMZ, because the vast majority of patients stopped treatment after six cycles of TMZ. The unusual pattern of progression seen in the concurrent group may be related to improved local control of the disease due to increased efficacy of radiation with TMZ, which probably acts as a radiation sensitizer. This supports the hypothesis that the bulk of the benefit of adding TMZ to standard therapy may come from its 6-week use together with RT rather than its use as adjuvant therapy. This hypothesis is endorsed by the observation that concomitant and adjuvant treatment improves survival of GBM patients, whereas the use of adjuvant alkylating treatment alone fails to show any benefit [1,22].

Although more unusual recurrences were observed in the combined treatment group, the difference in incidence was not statistically significant. Our results are partially in line with those of Wick et al. [23], who used a slightly different definition of unusual relapses (e.g. >50% of tumor volume at recurrence outside the radiation field). They observed distant relapses in approximately 20% of cases, a number similar to what was observed in the combined treatment group of the present study, but twice as high as what was expected [16] and observed in the control group in this study.

In conclusion, our data confirm that the addition of TMZ to RT early in the treatment of GBM provides a clinically meaningful OS benefit that is more pronounced in cases of pseudoprogression. In addition, our study shows that the predictive effect of pseudoprogression on survival is not affected by the stringency of the applied defining criteria; an unusual
pattern of relapse was seen in more than twice as many patients who received the combined therapy. The introduction of TMZ in the postoperative treatment of GBM clearly improves survival, but also impacts treatment decisions when progression is observed on MRI. Future studies should include advanced imaging techniques in order to facilitate the discrimination between true progression and pseudoprogression. Neuro-oncologists should be aware of the possibility of unusual sites of relapse, and the concept of pseudoprogression.

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Disclosure of Conflict of Interest

The authors declare no financial or other conflict of interests.

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