Multicentric Tumor Manifestations of High Grade Gliomas: Independent Proliferation or Hallmark of Extensive Disease?

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Abstract
Objective: Improvements in microneurosurgical techniques, radiotherapy and chemotherapy for the treatment of high grade gliomas resulted in better local tumor control and longer progression-free survival. Multicentric (MC) lesions located distant from the initial resection area contribute to treatment failure in a growing number of patients. These MC lesions may develop within the course of the disease (meta-chronous) or may already be present at the time of first tumor manifestation (synchronous). To look for mechanisms and regular patterns behind MC glioma manifestations and to investigate whether they are “a second primary tumor” or the result of continuous diffuse glioblastoma cell invasion, we retrospectively analyzed the initial and all follow-up MR studies of our high grade glioma (HGG) patients.

Patients and Methods: MR studies of 247 consecutive patients treated for HGG at a single institution were analyzed. MC tumor manifestation was defined as more than one gadolinium enhancing lesion within the brain on MRI without a connecting signal alteration in T2 sequences and/or without a connecting hypointense mass in T1 sequences. The minimal distance to define two solitary lesions was set at >10 mm. According to these specifications 40 patients showed MC tumor manifestations in their MR studies on admission or during treatment of their disease. The MR studies of these cases were retrospectively analyzed for patterns in MC tumor manifestation and progression. Topographical specifications and delay in manifestation were used to explain possible pathways of development. Kaplan Meyer survival graphs for metachronous and synchronous MC disease were calculated.

Results: 24 patients showed MC tumor manifestation at the time of admission. 16 cases developed MC manifestation within a follow-up period of 3–57 months. The location of all lesions could be categorized into one of three distinct patterns (white matter, subependymal, intraventricular). The patterns showed individual and location-specific time gaps to metachronous manifestation. Calculated from the time of first tumor diagnosis, the median survival was longer for patients with metachronous MC lesions (353 days, p<0.05) compared to patients with synchronous MC lesions (110 days) or patients without multicentricity (234 days). Patients with metachronous lesions showed a similar survival (72 days) as patients with synchronous MC lesions (110 days) once they developed MC disease.

Conclusion: The topographical patterns and temporal characteristics of MC disease suggest that all manifestations share common mechanisms such as an active migratory process. Our data therefore do not support the concept of an independent MC development of multiple gliomas.

Introduction
Despite progress in local tumor control the prognosis for high grade glioma patients continues to be dismal. Resection of the gadolinium enhancing tumor often results in local recurrence within a margin of approximately 2–3 cm [12]. In follow-up brain images, almost all glioma patients show continuous tumor growth and infiltration into the surrounding brain parenchyma along axonal fibers, basal membrane-like structures (perivascular or subpial) or on a subependymal route along the cerebral ventricles of the brain [29]. In rare cases, however, tumor spread appears to be non-continuous and the apparent gap between isolated tumor lesions shows no glioma infiltr-
tion in the histopathological examination. Multicentric tumor manifestations in gliomas have been recognized as early as 1880 [7, 17], and numerous individual cases and series of multicentric gliomas have been reported [5]. With increasing local tumor control, satellite manifestations, often far away from the initial resection area, define treatment failure in a growing number of patients. According to previous reports, multicentricity occurs in up to 20% of malignant gliomas [4, 9, 5]. Willis introduced the hypothesis of “initiation” and “promotion” that allows for unlinked proliferation of neoplastic cells at different topographical locations [31]. Fitting this hypothesis, Batzdorf and Malamud defined criteria for multicentricity in glioma lesions in 1963 [4]. According to them, multifocal tumors are the result of dissemination or growth via an established route, e.g. spread via commissural axon fibers or other pathways, spread via CSF. Multicentric tumors on the other hand were defined as widely separated glioma centers (in different lobes or hemispheres) that find no ready explanation for their dissemination through known pathways of distribution. This definition of multicentricity also includes tumors not only separated by location but also by time. These metachronous lesions are opposed to synchronous lesions, which already present as multicentric at the time of the initial diagnosis [21]. We retrospectively analyzed our own high grade glioma patients for multicentric disease. We compared life expectancy, patterns of topographical tumor distribution, and time-to-manifestation of multicentric lesions to monocentric tumor behavior, to find evidence for independent glioma proliferation at two centers within the brain.

Materials and Methods

Between January 2000 and December 2005, 256 patients were treated at a single institution for malignant glioma. Treatment was tailored to each individual case and included biopsy, partial resection or gross total resection, radiotherapy and chemotherapy. Patients were followed up clinically, and MR studies were obtained at least every three months or at the time of clinical deterioration. Multicentric tumor manifestation was defined as more than one gadolinium enhancing lesion within the brain, without connecting signal alteration in T2 sequences and/or connecting hypointense lesion in T1 sequences. The minimal distance chosen to define two solitary lesions was at least 10 mm to correct for different angles and varying slice thicknesses between scans. All other tumors which did not meet these criteria were termed ‘monocentric lesions’. MR studies determined the time elapsed prior to the development of metachronous tumor foci. Medical records were reviewed for age at diagnosis, histology, treatment and time of death. Survival for synchronous and metachronous lesions was calculated using the Kaplan Meyer method.

Results

In 9 cases either the initial or the follow-up scans were not available for analysis, which left us with 247 patients with malignant glioma. Out of a total of 7835 films, MR studies of 40 patients demonstrated multicentric malignant glioma disease. The clinical data of these patients are presented in Table 1.

### Table 1: Clinical data.

<table>
<thead>
<tr>
<th>Gender/Age</th>
<th>Multicentric</th>
<th>Surgical intervention at first admission</th>
<th>chemotherapy</th>
<th>Survival in days from diagnosis</th>
<th>Survival in days from diagnosis</th>
<th>Histology</th>
<th>Radiotherapy</th>
<th>Treatment Failed</th>
<th>Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synchronous</td>
<td>11 female/13 male</td>
<td>5 GTR</td>
<td>23 GBM</td>
<td>100 (median) (range: 8-1703)</td>
<td>110 (median) (range: 8-1703)</td>
<td>23 GBM</td>
<td>4 completed</td>
<td>8 aborted</td>
<td>17 none</td>
</tr>
<tr>
<td>Metachronous</td>
<td>9 female/7 male</td>
<td>14 BIO</td>
<td>3 GTR</td>
<td>72 (median) (range: 10-146)</td>
<td>72 (median) (range: 10-146)</td>
<td>3 GTR</td>
<td>2 aborted</td>
<td>2 none</td>
<td>2 none</td>
</tr>
<tr>
<td>Monocentric</td>
<td>79 female/128 male</td>
<td>52 BIO</td>
<td>198 GBM</td>
<td>234 (median) (range: 0-2111)</td>
<td>234 (median) (range: 0-2111)</td>
<td>198 GBM</td>
<td>91 completed</td>
<td>131 aborted</td>
<td>35 other drug</td>
</tr>
</tbody>
</table>

Histological diagnosis was anaplastic oligoastrocytoma in 3 patients (mean age 27 years), anaplastic astrocytoma in one, glioblastoma multiforme (GBM) in 35 patients (mean age 58.9 years), and gliosarcoma in one patient.

Mean time to metachronous tumor manifestation was calculated to be 8 months (range 2–27) for GBM and 21 months (range 5–57) for anaplastic oligoastrocytoma. The mean follow-up time was 6.5 months (range 1–27) for GBM and 26.8 months (range 13–44) for anaplastic oligoastrocytoma patients. We found 67 synchronous lesions in 24 patients and 58 metachronous lesions in 16 patients. The lesions showed three distribution patterns: manifestation within the white matter tracts (ipsilateral, contralateral), subependymal location in one or more ventricles, solid manifestation within the optic and pituitary recess of the third ventricle and/or lateral recess of the fourth ventricle.

12 patients had lesions fitting more than one pattern; one patient had lesions corresponding to all patterns. One patient showed spinal seeding 3 months after the first sign of intraventricular multicentricity, 4 patients had infratentorial tumor manifestations, one of which was synchronous.

Ipsilateral multicentricity was observed in 53 lesions of which 33 were synchronous and 20 were metachronous. Contralateral multicentricity was observed in 33 lesions of which 18 were synchronous and 20 metachronous. 17 lesions were subependymal or ventricular, 9 synchronous and 8 metachronous. 8 lesions were found within the third ventricle, 3 synchronous and 5 metachronous. 6 lesions could be detected that were congruent at identical locations in both hemispheres; this pattern was found in 3 out of 247 patients reviewed.

There was a trend for ipsilateral metachronous GBM lesions to manifest early during the follow-up period (mean 6.8 months, range 1–22) compared to contralateral metachronous lesions (mean 11.25 months, range 6–17). Subependymal metachronous lesions developed within an average of 12 months (range 7–22). Intraventricular metachronous lesions developed after 8.4 months (range 2–12).

Median survival of patients with synchronous lesions was 110 days. Patients who developed metachronous lesions showed a longer median survival (median 353 days) (Fig. 1a). The survival time for metachronous lesions calculated from the time of detection of multicentricity was similar to that of patients with synchronous MC disease (Fig. 1b).

17% of patients with synchronous high grade glioma manifestation completed radiotherapy. In 33% of synchronous patients, radiotherapy had to be terminated early due to rapid clinical deterioration. In view of their clinical state, half of the patients were not offered radiotherapy at all. Only about one third of the patients in the synchronous group received chemotherapy (mean: 3.9 cycles TMZ).

12 out of 16 metachronous patients received radiotherapy, 11 patients with metachronous lesions had TMZ as adjuvant chemotherapy. However, once they developed multicentricity their median survival proved to be identical to their synchronous counterparts (median 72 days, p < 0.05) (Fig. 1b). Additional therapy for their second distant glioma manifestation was seldom given, as clinical performance scores usually prohibited further treatment.

Discussion

We found multicentric glioma lesions to be located along the well known preferential pathways of tumor cell migration within the brain. In patients with metachronous lesions the time to manifestation of MC was related to these regions (e.g. white matter tracts, ventricular lining). Patients who developed metachronous lesions had a longer median survival than patients with monocentric lesions, but MC disease meant a significantly shorter life expectancy for all patients. These data suggest that MC manifestations share common mechanisms such as an active migratory process for their development. Supporting these findings, similarities in the genetic profiles of primary and secondary tumors in a total of 3 patients with MC disease have been previously described by other authors [20, 30]. As recurrences of high grade glioma can share genetic alterations with the primary tumor, these chromosomal data of corresponding genetic alterations present very strong arguments for the concept of invasion in these multicentric tumors.
Recently, however, completely dissimilar genetic profiles were described in the multicentric GBM lesions of two patients. The authors concluded that the development of MC disease in their patients were independent processes [26,32]. The patients they described showed satellite lesions in topographically corresponding “mirrored” areas within the brain, a pattern we registered in 1.2% of our own GBM patients. We hypothesize that these “mirrored” lesions could account for “true” MC disease, as proliferative zones within the germinal region define the topographical destination of precursors [24], and cancer stem cells from the same proliferative zone could end up in corresponding “mirrored” destinations within the brain. Various autopsy studies report an overall incidence of between 4.9 and 10% for multicentric gliomas [3]. Higher incidences were observed with MR studies [22]. Invading glioma cells change the extracellular matrix (ECM) through enzymatic modification, receptor-mediated adhesion of tumor cells to matrix proteins, and metalloproteinase degradation of ECM, thereby increasing water content in the ECM [6,15]. These ECM degrading activities not only create space into which invading cells can move, but are also likely to change the signal on MR sequences. Accordingly, growth patterns in glioblastoma patients correlate positively with the configuration of T2-weighted MR images in up to 75% of all patients [18]. We therefore excluded all lesions linked in T2 as well as bridges with gadolinium enhancing structures to define multicentric gliomas [11]. Nevertheless we found multicentric disease in 16.2% of our high grade glioma patients. The infiltrative behavior of gliomas was recognized very early by neurosurgeons [10]. Satellite lesions at a distance from the initial presentation were observed even in the contralateral hemisphere and a significantly higher incidence in later stages of the disease has been postulated [15,29]. It has been assumed that glioma dissemination in the brain could be the result of growth pressure in the perivascular space or a passive cell distribution via CSF [24]. Yet glioma cells inherently are capable of rapid and distant migration. When plated on ECM proteins, the speed of locomotion of individual cells increases [13]. Migrated distances were consistently higher along white matter tracts than along blood vessels or other basal membrane-like structure [16]. As glioblastoma cells have been observed to move mostly in isolation along these fiber tracts [8], the changes they create within their adjacent environment while migrating in this fashion might be very subtle, all the more so, as axonal fiber tracts offer a plane where even CSF bulk flow transport of microspheres and nanoparticles is feasible [16]. Here glioma cells move along the path of least resistance and supposedly no extensive ECM degradation is needed for this type of migration. CT and MRI imaging therefore cannot reliably identify isolated tumor cells outside of contrast-enhanced areas of the brain, so changes in T2 images most likely depict ECM degradation due to large cell clusters following the isolated migrating cell. Thus, seemingly topographically independent glioma centers on MR could still be the result of isolated migrating tumor cells that proliferate at a later date. If so, there ought to be a time-related dependency for multicentricity accounting for the speed of migration of individual cells and the time needed to develop into a detectable tumor size. Thus, in this setting the development of ipsilateral lesions in the white matter (Fig. 2) should hypothetically take less time than their contralateral counterparts (Fig. 3) and therefore ipsilateral lesions should be seen more often in disease where a very short life expectancy is the norm. In comparison to contralateral multicentricity, ipsilateral lesions should not only appear more frequently but should also be encountered earlier. Indeed we found the highest number of multicentric lesions within the white matter corresponding to the ipsilateral hemisphere. Most of these lesions were already multicentric at the time of diagnosis. Ipsilateral metachronous white matter lesions developed within approximately 7 months; about 4.25 months earlier than the contralateral metachronous lesions.
We found a total of 17 multicentric lesions located in close vicinity to the ependyma (Fig. 4). Contralateral, ipsilateral and midline locations could be observed. Approximately half of these lesions were synchronous.

Subependymal spread of glioma cells was first described by Scherer in 1940 [29]. The strong support of the subependymal layer for the spread of glial tumors has been extensively studied and the adhesion behavior and kinetics are thought to be linked to ECM and basal membrane-like structures. Adhesion to these structures is rapid and in experimental settings precedes attachment to white matter [14]. We expected metachronous subependymal lesions to manifest early, given the rapid adhesion of glial cells to the structures of the ependyma. Nevertheless it took these lesions a mean of 12 months to develop, which was significantly longer than white matter-type metachronous lesions. A large number of non multicentric glioma patients, however, showed early continuous spread along the subependymal layer. So we conclude that the avid adhesion of glial cells to ependymal structures and the migration speed in this area generates continuous growth patterns rather than skip lesions, and multicentric disease is thus observed less frequently.

The long interval until the development of subependymal lesions suggests that adhesion, locomotion, and growth of secondary lesions are individual biological processes with specific time scales. Factors immanent to the location of the different glioma centers might play a major part in their development.

The flow of brain interstitial fluid (bulk flow, 0.1–0.3 μl/min) [1] via preferential pathways such as perivascular spaces and axonal tracts as well as subependymal layers has long been regarded as the main cause of tumor dissemination in gliomas. Evans blue dye has demonstrated flow through the interstitium [13]. By injecting cell sized latex beads along with cell suspensions in experimental animal models, a passive component for tumor cell spread could be demonstrated. Dissemination of malignant glioma cells through CSF pathways often is associated with leptomeningeal spread and continuous growth along intracranial cisterns [2].

Immunohistochemically proven poor astrocytic differentiation is attributed to earlier and more vigorous shedding of tumor cells into the CSF [25]. These findings suggested that CSF distribution phenomena might reflect biological mechanisms (loss of intercellular adhesions and cell shedding) which are more closely related to classical metastatic mechanisms than to mechanisms of active tumor locomotion [15].

We found non continuous intraventricular lesions (Fig. 5) in 8 patients. All patients showed tumors within the third ventricle, filling the recess of the optic chiasm or pituitary stalk. Two patients showed tumor in the fourth ventricle about two months later, and one of these developed spinal seeding. Assuming CSF distribution to be a non migratory, passive metastatic process, we expected bulk flow to transport and deposit glioma cells mainly into regions of slow flow and low pressure within the ventricular system. There gravity effects are maximal at creep flow velocities (where the diameter of the CSF system widens significantly, or alteration of flow direction creates backwater pools) and cells above a certain size and density will precipitate and accumulate. Sedimentation, nidation and later in loco proliferation are the steps involved, if CSF dissemination is considered a passive metastatic process.

Studies with polyamide seeding particles in a model of the human ventricular system showed the highest velocity for CSF in the aqueduct and near the inlets to the third ventricle. A dead velocity area within the third ventricle, where the flow tends to take a 90° turn to the rear, could be demonstrated (Ammourah S, Aroussi A, Vloeberghs M: A PIV study of the cerebrospinal fluid dynamics inside a model of the human ventricular system. Unpublished data, 2004). Anatomically this area lies exactly in the region of the optical recess and infundibulum of the pituitary stalk. The fourth ventricle was shown to have the lowest CSF pressure and a very slow CSF velocity with two distinctive “dead velocity” areas within the lateral parts (Ammourah S, Aroussi A, Vloeberghs M: A PIV study of the cerebrospinal fluid dynamics inside a model of the human ventricular system. Unpublished data, 2004; Ammourah S, Aroussi A, Vloeberghs M: Cerebrospinal fluid dynamics in a simplified model of the human ventricular system. Unpublished data, 2003). The bottom of the third ventricle and the fourth ventricle (fastigium and lateral recess) therefore appear to be the most likely spots for cell sedimentation and intraventricular tumor formation in CSF-attributed multicentricity. All our 8 patients showed sedimentation at the base of the third ventricle. Two of these patients developed non continuous tumor manifestation within the fourth ventricle at a later date with additional spinal seeding in one case.

Germinal regions such as the subventricular zone have long been proposed as sources for glioma cells [16]. Multiple regions of the adult brain contain both neural stem cells and glial progenitor cells [28]. These are regulated by cellular pathways that are also active in gliomas [27]. Subpopulations with various cancer stem cell characteristics have been identified for malignant gliomas; their distinct migratory and metabolic behavior is often very close, if not identical to, embryologic neuronal stem cell locomotion [19, 23, 28]. Different proliferative zones exist for the
distinct generation of specific classes of cortical neurons, their
topographical destination being already defined within the ger-
minal region [24].

To assume that the malignant transformation of progenitors and
stem cells within specific proliferative germinal zones generates tumors in topographically corresponding areas within the brain would hence not be far fetched. Glioma lesions in mirrored topographical location of the left and right hemisphere (Fig. 6) could thus originate from a single specific proliferative zone within the germinal region. If so, only these tumors would account for true multicentric disease. We found 3 cases of mirror-like multicentricity among our 247 high grade glioma patients (1.2%).

Conclusions

Most multicentric lesions correspond to known and long estab-

lished migrational pathways of glioma cells. The topography of
multicentric lesions and the time needed to develop meta-
chronous multicentricity indicate an active migratory process for
the development of seemingly isolated glioma centers.

Metachronous manifestation is more common among patients
with a long progression-free survival. With growing evidence in
favor of radical tumor resection and better local control through
individualized and specific adjuvant therapies in glioma disease,
increased life expectancy will see more multicentric glioma
manifestations. Once multicentricity is established, patients have a short median life expectancy of 72 (metachronous) to
110 (synchronous) days.

Distant satellite lesions thus are the hallmark of extensive gli-
omat disease, their topographical and chronological patterns of
manifestation closely linked to the biology of invasion.

Conflict of interest: None

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