Morphology of Tumor Cell Nuclei Is Significantly Related with Survival Time of Patients with Glioblastomas

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ABSTRACT

Purpose: To investigate whether histomorphology of tumor cell nuclei has a significant and independent relation to survival time of patients with glioblastomas.

Experimental Design: Seventy-two tumors from 72 patients were investigated by means of digital image analysis. Proliferating and nonproliferating nuclei were separately measured and parameters of nuclear size, shape, texture, and spatial relationships (topometric parameters) were detected. Survival analysis was done regarding morphometric data together with the patients’ age, the amount of resection (total or subtotal), and the classification of the tumor as a “primary” (de novo) or “secondary” glioblastoma.

Results: The overall relation of all morphometric data to the time of survival was highly significant (Cox analysis, P < 0.0001). Apart from the extent of surgical resection, parameters of nuclear shape and topometric variables, such as the distance between two nuclei lying nearest to each other, showed an independent and significant relation to survival time. The patients’ age had also a significant but comparably slight relation to survival time.

Conclusions: The morphology of tumor cell nuclei, as represented by morphometric data, shows a significant relation to survival time of patients with glioblastomas. This relation is statistically independent from the amount of surgical resection, from the patients’ age and from the classification of the glioblastoma as being primary or secondary. The results support the view that histomorphometry of tumor cell nuclei is a valuable prognostic marker for patients with glioblastomas. We believe that such a marker ought to be incorporated into the formation of individual therapeutic decisions.

INTRODUCTION

Together with meningiomas, glioblastomas belong to the most frequent types of primary tumors of the central nervous system. In contrast to the frequency and, thus, the clinical importance of this tumor type, only little is known about its significant prognostic factors. Glioblastomas have a generally worse prognosis, but survival time can show a considerable variation with unexpectedly long survival in individual cases (1). Therefore, a detailed investigation of potential prognostic factors is worthwhile even for glioblastomas. It is generally accepted that gross surgical resection and young age of the patients are predictors of a more favorable clinical outcome (2). There are only few reports about additional factors showing an independent and significant influence on survival time. Recently, some reports addressed a significant association of patient survival with specific genomic aberrations and gene expression profiles (3). Up to now, there are only few reports about the association of histomorphologic aspects with patient survival (1, 3, 4).

Tumor cell nuclei belong to the most important histologic structures for the assessment of tumor biology including typing and grading of gliomas (4, 5), but it is unknown up to now whether their morphology provides independent information on biological behavior of glioblastomas. Determination of the proliferation index using proliferation markers, such as Ki67, has been done in many studies, but a significant association with prognosis of glioblastoma patients remains controversial (4). However, it seems worthwhile to test whether cytomorphology of proliferating and nonproliferating tumor cell nuclei could yield prognostic and thus biologically relevant information when considering both groups of nuclei separately. In previous studies, we have shown that quantitative morphologic data describing nuclear morphology correlated with tumor grade, molecular biological data, and even prognosis of different types of human gliomas (5–8). From the clinical point of view, application of histomorphometric methods could provide important prognostic information, also with relevance for subsequent therapeutic decisions. Recently, we have developed a new computer program for a detailed morphometric analysis of the four main histomorphologic characteristics of tumor cell nuclei: nuclear size, nuclear shape, nuclear texture (gray value distribution), and spatial relationships between the nuclei (topometric analysis). In the present study, we used this program for the morphometric investigation of proliferating and nonproliferating tumor cell nuclei in surgical specimen from human glioblastomas to contribute to the discussion of the following four questions:

1. Does a detailed morphometric analysis of tumor cell nuclei in glioblastomas provide independent information concerning survival of the patients besides the extent of surgical resection, the patient’s age, and the classification of the tumor as “primary” or “secondary” glioblastoma?

2. If so, has the separate investigation of the morphology of proliferating and nonproliferating tumor cell nuclei any advantage regarding the prognostic significance of nuclear morphology?

3. Which of the morphometric aspects of tumor cell nuclei show the most prominent association with clinical outcome (survival time)?
4. Do the results provide evidence that histomorphometry is a valuable diagnostic approach for the assessment of the prognosis of patients with glioblastomas?

**MATERIALS AND METHODS**

Seventy-two primary surgical specimens from 72 patients (mean age: 58 years, range 27-79) were investigated. All patients underwent open surgery in the Department of Neurosurgery, Johann Wolfgang Goethe University in Frankfurt/Main, Germany. The standard postsurgical treatment was local radiation with 60 Gy. Fifty patients had received total surgical resection of the tumors as confirmed by postsurgical magnetic resonance imaging and/or computed tomography. The remaining 22 patients had received subtotal resection. Inclusion criteria for the study were the following: (a) only tumor specimen from first surgical resection after initial diagnosis were investigated (no recurrent tumors), (b) sufficient histologic quality was required, (c) the diagnosis of a glioblastoma must have been confirmed by two histopathologists, (d) only patients dying from the tumor disease were included in the study and no patients dying for other reasons. Every tumor specimen was embedded in paraffin and was typed and graded according to the WHO classification of tumors of the central nervous system as a glioblastoma grade 4 (4). It was also investigated whether the tumor was likely to be a primary (de novo) glioblastoma or if there was evidence for a secondary genesis of the glioblastoma out of a glioma with lower tumor grade. The latter was found to be likely in nine cases because there were large tumor areas of less malignancy resembling gliomas of WHO tumor grades 2 or 3. For morphometric investigations, paraffin sections of 4 μm thickness stained with a monoclonal antibody against the Ki67 antigen (DAKO, Hamburg, Germany) and hematoxylin counterstaining were used. As in our previous morphometric studies (5, 9, 10), the tumor region being studied in each case was the centrally located tumor region with the highest proliferative activity (highest percentage of Ki67-positive tumor cell nuclei in relation to the total number of tumor cell nuclei in that area). This region must have been clearly from the tumor center and not from the tumor border showing areas of less malignancy.

A minimum of 300 tumor cell nuclei per tumor case were measured by means of a digital image analysis system (KS400, Zeiss, Oberkochen, Germany). This system consisted of a personal computer with a black/white video camera mounted on a microscope. Measurements were done using a ×40 objective with numerical aperture 0.75 and a tube factor ×1.25. For calibration of the densitometric measurements, microscopic light was adjusted to a constant gray value of 245 in an empty slide region for every case. All nuclei were measured as described in previous reports (8–10). Briefly, the microscopic image was projected on the computer screen and all nuclei were manually traced using the computer mouse. Every nucleus was given the category “Ki67-positive” or “Ki67-negative” by the observer. Afterwards, mean value and the SD of each morphometric parameter were calculated for the entire set of tumor cell nuclei, as well as for all Ki67-positive and all Ki67-negative tumor cell nuclei separately. Even the proliferation index was calculated automatically as the ratio between the number of Ki67-positive nuclei and all nuclei measured in each case. Variables of nuclear size were nuclear area, maximum nuclear diameter, and largest and smallest elliptic axe. Variables of nuclear shape were the roundness factor and the ellipse shape factor, which have a range of values between 0 and 1 with the value 1 for a regular circle and smaller values for irregular nuclei. As further shape variables, Fourier amplitudes 1 to 15 were determined invariate to nuclear size. Fourier analysis is a mathematical method for decomposition of a closed two-dimensional shape into several harmonics, each being represented by a “Fourier amplitude.” Each amplitude can be considered as an independent morphometric parameter. Therefore, this method provides a more detailed analysis of nuclear shape when compared with simple shape factors like the roundness factor. In general, the amplitudes have as higher values as the shape deviates more from a regular circle. As described in more detail elsewhere, the first 15 amplitudes were shown to be sufficient for the morphometric description of a nuclear shape in light microscopy (6, 7). Figure 1 gives an example for Fourier analysis of three tumor cell nuclei (Fig. 1). As densitometric parameters, mean, SD, skewness, and kurtosis of the gray value histogram, as well as several textural parameters were calculated by means of the morphometric analysis system (5, 9, 10), the tumor region being studied in each case was the centrally located tumor region with the highest proliferative activity (highest percentage of Ki67-positive tumor cell nuclei in relation to the total number of tumor cell nuclei in that area). This region must have been clearly from the tumor center and not from the tumor border showing areas of less malignancy.

**Fig 1** Three examples for the Fourier analysis of tumor cell nuclei. Values of Fourier amplitudes 1 to 15 (dimensionless, invariant to rotation and size of nuclei). Amplitude 1 is a good measure for axial symmetry: High value for nucleus 1 with distinct symmetry, lowest value for nucleus 3 that shows a distinct asymmetry. Amplitudes 2 to 15 are good measures for irregularities of nuclear outlines: Nucleus 1 has a nearly circular shape and thus rather low values for these amplitudes; higher values of most amplitudes for nucleus 2. Due to distinct irregularities of nucleus 3, most amplitudes have considerably high values. Each Fourier amplitude is an independent parameter of nuclear shape. Therefore, Fourier analysis provides a more detailed quantitative analysis of nuclear shape than simple shape factors.
features according to Haralick, were determined as previously described (9, 11). As topometric variables, several distance measures, such as the distance from a nucleus to the nucleus lying nearest to it or second nearest to it, were determined. Determination of the number of neighbors per nucleus and the percentage of nuclei with 2, 3, ..., 8 neighbors is based on distances and angles and is described in detail in a previous pilot study (10). For each morphometric parameter, mean and SD were calculated from the 300 tumor cell nuclei in each case. These values (means and SD) then served as single values in statistical analysis describing one single tumor case. As described in a review of statistical methods, this is an appropriate method for data analysis in histomorphometry (12). Reproducibility of this morphometric procedure has been tested in a previous study by measuring each of two test cases five times. In this study, examination of at least 300 tumor cell nuclei per measurement provided a mean coefficient of variation of 2.16% and 2.68% in the two test cases for all morphometric parameters and thus constituted optimal reproducibility (13).

Statistical analysis was done using the SPSS software (SPSS, Chicago, IL). Morphologic differences between Ki67-positive and Ki67-negative nuclei were tested using the t test for paired samples. To test for a significant and independent influence of morphometric parameters on survival time and on the time until recurrence, Cox analysis was done for all cases (n = 72) regarding all morphometric data, age of the patients, as well as two categorical variables for each case: The amount of surgical resection (total/subtotal) and the histopathologic classification of the tumor as a primary (de novo) or a secondary glioblastoma. This analysis was done considering the three sets of morphometric data separately: data from the entire set of nuclei, data from Ki67-positive nuclei, and data from Ki67-negative nuclei. As a further test for the influence of morphometric data on survival time, linear discriminant analysis was done in original form and cross-validated for cases with total surgical resection of the tumor but considerably short survival (<8 months) and for cases with long survival (>30 months). This analysis provided a test on the number and percentage of cases classified into the correct groups (short or long survival) by means of the morphometric data. In contrast to original discriminant analysis, cross-validated analysis provided a “leave-one-out classification,” which was a good estimate for the prognostic significance of the morphometric data (12). Principal component analysis was done to provide a descriptive data analysis of the histomorphology for all cases. This procedure transformed a set of highly correlated morphometric parameters into a set of uncorrelated factors (“factorial analysis”). Morphometric parameters belonging to the same factor were highly correlated with each other, whereas parameters belonging to different factors were weakly correlated or even not correlated. The whole statistical procedure was done as described in a review on statistical methods in quantitative histopathology (12).

RESULTS

Compared with Ki67-negative nuclei, Ki67-positive nuclei were significantly larger and more regular, as confirmed by significantly higher values for area, maximum diameter, roundness factor, and ellipse shape factor (Table 1). This showed that both types of nuclei represented two morphologically distinct populations of tumor cell nuclei. Therefore, it was justified to perform survival analysis based on the three sets of morphometric data independently: morphometric data for Ki67-positive nuclei, data for Ki67-negative nuclei, and data for the entire set of tumor cell nuclei. Cox analysis showed a significant influence of the morphometric data on survival time (Table 2). The strongest influence was observed for the morphometric data from the entire set of tumor cell nuclei (independently of the Ki67 staining reaction) because this data showed larger \( \chi^2 \) values. Cox analysis for the time until recurrence was done for cases with total surgical resection (n = 50). Only the morphometric data for the entire set of nuclei and for Ki67-negative nuclei showed a significant influence on this time span, whereas data for Ki67-positive nuclei did not (Table 2). The low \( \chi^2 \) values indicated that the influence of histomorphometric data on the time until recurrence was only slight when compared with the strong influence of morphometric data on survival time (Table 2).

Because morphometric data from the entire set of tumor cell nuclei showed the strongest influence on survival time, we used this data set to test which parameters were independent prognostic factors according to Cox analysis. Cox analysis automatically calculated a so-called “B coefficient” for each

Table 1 Morphometric data for all cases (n = 72) regarding all tumor cell nuclei (irrespective of Ki67 status), as well as Ki67-positive and Ki67-negative tumor cell nuclei

<table>
<thead>
<tr>
<th>Variable</th>
<th>All tumor cell nuclei</th>
<th>Ki67-positive tumor cell nuclei</th>
<th>Ki67-negative tumor cell nuclei</th>
<th>Significant difference, Ki67-positive/negative tumor cell nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear area, mean (( \mu m^2 ))</td>
<td>45.71 ± 10.68</td>
<td>53.27 ± 15.86</td>
<td>44.05 ± 10.11</td>
<td>**</td>
</tr>
<tr>
<td>Maximum nuclear diameter, mean (( \mu m ))</td>
<td>10.12 ± 1.08</td>
<td>10.61 ± 1.55</td>
<td>10.01 ± 1.04</td>
<td>**</td>
</tr>
<tr>
<td>Nuclear roundness factor, mean</td>
<td>0.815 ± 0.032</td>
<td>0.833 ± 0.037</td>
<td>0.812 ± 0.032</td>
<td>**</td>
</tr>
<tr>
<td>Nuclear ellipse shape factor, mean</td>
<td>0.619 ± 0.041</td>
<td>0.642 ± 0.046</td>
<td>0.615 ± 0.042</td>
<td>**</td>
</tr>
<tr>
<td>Number of neighbors per nucleus, mean</td>
<td>4.26 ± 0.15</td>
<td>4.27 ± 0.20</td>
<td>4.26 ± 0.15</td>
<td>–</td>
</tr>
<tr>
<td>Percentage of nuclei with six neighbors (%)</td>
<td>5.96 ± 2.71</td>
<td>6.51 ± 4.36</td>
<td>5.78 ± 2.71</td>
<td>–</td>
</tr>
<tr>
<td>Distance between two nuclei lying nearest to each other, mean (( \mu m ))</td>
<td>9.44 ± 1.52</td>
<td>9.50 ± 1.52</td>
<td>9.44 ± 1.54</td>
<td>–</td>
</tr>
<tr>
<td>Distance between two nuclei lying nearest to each other, SD (( \mu m ))</td>
<td>2.86 ± 0.89</td>
<td>2.86 ± 0.83</td>
<td>2.84 ± 0.91</td>
<td>–</td>
</tr>
</tbody>
</table>

Note. Data are means ± SD of important morphometric variables. **. Significant difference between Ki67-positive and Ki67-negative nuclei (P < 0.01); –, no significant difference (P > 0.05); t test for paired samples.
morphic parameter. This coefficient had the value of 0 if the corresponding parameter showed no statistical relationship to survival time. In contrast, the $B$ coefficient would have a high absolute value if the corresponding parameter had a strong statistical relation to survival time. A positive $B$ coefficient meant that the parameter had a negative influence on survival time (shorter survival time with larger value for that parameter).

A negative $B$ coefficient meant that the corresponding parameter had a positive influence on survival time (longer survival with larger value for that parameter). The first independent factor was the categorical variable “total surgical resection” (Table 3). This was supported by Kaplan-Meier analysis, which confirmed a significantly longer survival for patients having had total surgical resection (Fig. 2). Nuclear shape analysis by means of Fourier analysis revealed several parameters with an independent influence on survival time, such as the SDs of Fourier amplitudes 1, 5, 7, 9, and 13. These parameters had a positive $B$ coefficient and thus a negative influence on survival time (Table 3). A strong influence on survival time was confirmed for the mean value of Fourier amplitude 7, which has a negative $B$ coefficient and thus a positive influence on survival time (significantly longer survival for cases with a larger value for this parameter).

Even topometric parameters were independent predictors of survival time: the percentage of nuclei with six neighbors, as well as SD and mean of the distance between two nuclei lying nearest to each other. The first two parameters had a negative $B$ coefficient; the latter one had a positive $B$ coefficient. Finally, the patients’ age had also an independent influence on survival time. Due to the positive $B$ coefficient, this influence was also negative (shorter survival with increasing age). The low value of the $B$ coefficient showed that the influence of the patient’s age on survival time was comparably small when compared with the strong influence of the morphometric parameters (Table 3). Principal component analysis extracted 11 factors from the morphometric data. Each factor provided independent morphologic information (Table 4). From all morphometric parameters showing an independent influence on survival time (Table 3), the SD of Fourier amplitude 13 belongs to the first factor. This variable was negatively correlated with parameters of nuclear size. Because the first factor showed cumulative morphologic information of 20.1% only (Table 4), the conclusion could be drawn that the other morphometric parameters provided additional prognostic information on the tumor cases to a considerable degree. This was further emphasized by the fact that the other parameters showing an independent influence on survival time (Table 3) belonged to different factors and were thus not correlated with each other (Table 4).

In descriptive terms, the strong influence of Fourier amplitude 7 on survival time and its correlation with mean values of many other Fourier amplitudes (Table 4, factor 4)
confirmed that a tendency toward more irregularly shaped tumor cell nuclei (larger mean values for these amplitudes) had a positive influence on survival time (i.e., there was a tendency toward longer survival for cases with more irregularly shaped nuclei). In contrast, a more pronounced variation of nuclear shape, which was represented by larger values for the SDs of the Fourier amplitudes, had a negative influence on survival time (i.e., there was a tendency toward shorter survival for cases with a pronounced intratumoral variation of nuclear shapes; Tables 3 and 4). According to Cox analysis (Table 3), a larger distance between two tumor cell nuclei lying nearest to each other was negatively correlated with survival time. However, a more pronounced variation of that distance, as represented by the SD of the distance between two tumor cell nuclei, showed a positive influence on survival, as indicated by a negative value for the B coefficient (Table 3). The influence of our set of morphometric parameters on survival time was also confirmed by discriminant analysis comparing the following two groups of patients: cases with considerably long survival (>30 months, n = 5) and cases with considerably short survival despite total surgical resection of the tumor (<8 months, n = 6). Based on the morphometric data, original discriminant analysis provided a 100% correct reclassification of all patients, whereas cross-validated analysis led to a correct reclassification of 90.9% of all cases (Table 5). Microscopic reevaluation of the cases after performance of statistical analysis made it possible to understand the significant influence of many morphometric aspects on survival time. For example, in some cases with considerably short survival, a distinct intratumoral variation of nuclear shape was confirmed, which was morphometrically represented by large SDs of the Fourier amplitudes (Fig. 3A). In a previous pilot study, we have shown a significant and negative influence of nuclear size (mean nuclear area) on survival time for patients with total surgical resection of a primary glioblastoma (8). Therefore, we repeated this analysis for those patients from our present set of cases with total surgical resection of primary glioblastomas (n = 44). As a result, the significant and negative influence of mean nuclear area on survival time could be confirmed (Cox analysis, B coefficient = 0.0381, P = 0.0484). Regarding all cases (n = 72)...

Table 4  Principal component analysis for morphometric data of all cases (n = 72)

<table>
<thead>
<tr>
<th>No. factor</th>
<th>Variables correlated with this factor</th>
<th>Cumulative morphologic information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(−) Means of all variables of nuclear size (area, maximum diameter, elliptic axe, SD of area and the smallest elliptic axes)</td>
<td>20.1%</td>
</tr>
<tr>
<td>2</td>
<td>(+) Variables of nuclear shape (means of FA 9-15; SD of FA 10-15)</td>
<td>36.4%</td>
</tr>
<tr>
<td>3</td>
<td>(+) All variables describing distances between nuclei, percentage of nuclei with two neighbors</td>
<td>59.5%</td>
</tr>
<tr>
<td>4</td>
<td>(+) Further variables of nuclear shape (means of FA 2 and 4-8; SD of FA 2-9)</td>
<td>63.0%</td>
</tr>
<tr>
<td>5</td>
<td>(+) Further variables of nuclear size (SD of maximum diameter and largest elliptic axe)</td>
<td>73.8%</td>
</tr>
<tr>
<td>6</td>
<td>(+) Further variables of nuclear shape (mean and SD of FA 1, mean of FA 3, mean of roundness factor and ellipse shape factor; SD of roundness factor and ellipse shape factor)</td>
<td>77.8%</td>
</tr>
<tr>
<td>7</td>
<td>(+) Mean number of neighbors per tumor cell nucleus, percentage of nuclei with 3, 4, 5, 6, and 7 neighbors</td>
<td>80.9%</td>
</tr>
<tr>
<td>8</td>
<td>(+) One variable of gray value distribution (SD of skewness of the gray value distribution)</td>
<td>83.0%</td>
</tr>
<tr>
<td>9</td>
<td>(+) SD of the number of neighbors per tumor cell nucleus</td>
<td>84.9%</td>
</tr>
<tr>
<td>10</td>
<td>This factor is not characterized by a single variable</td>
<td>86.8%</td>
</tr>
<tr>
<td>11</td>
<td>(+) One variable of gray value distribution (Haralick textural feature 3)</td>
<td>88.3%</td>
</tr>
</tbody>
</table>

NOTE. Data are from all tumor cell nuclei irrespective of the Ki67 staining reaction. List of variables correlated with the corresponding factor and cumulative morphologic information provided by these factors [sum of the squared factor loadings (%)]; (−), variables negatively correlated with the corresponding factor; (+), variables positively correlated with the corresponding factor. Abbreviation: FA, Fourier amplitude.
and the complete set of morphometric parameters, however, nuclear area, as well as the other parameters of nuclear size (maximum nuclear diameter, largest and smallest elliptical axe), did not reveal an independent significant influence on survival time as described above (Tables 2 and 3).

DISCUSSION

In contrast to major advances concerning the understanding of molecular biological pathways or new therapeutic strategies (14–16), comparably scarce attention has been paid to the histopathology of glioblastomas during the last 10 years. Up to now, there are only few reports about prognostic significance of histomorphologic aspects (Table 6). Previous studies did not show an independent correlation of histomorphometric data with survival of glioblastoma patients (5, 17–19), which can be explained in part by the inclusion of heterogeneous sets of gliomas and a limited number of morphometric parameters. In our study, we have shown that histomorphometric procedures have significant clinical relevance for patients with glioblastomas for several reasons. First, the morphometric results showed that nuclear morphology correlated significantly with survival time of patients with glioblastomas. This correlation was independent of other prognostic factors: total surgical resection, patients’ age, and classification of the tumor as a primary or secondary glioblastoma. Of note, because this correlation cannot be shown by conventional (nonquantitative) histomorphologic examination alone, a detailed morphometric examination is indispensable. Second, the morphometric procedure showed good reproducibility (13), which is an important prerequisite for its use as a diagnostic tool for the clinical evaluation of glioblastoma patients.
investigation of individual cases. Third, the results showed a more pronounced influence of quantitative nuclear morphology on survival compared with two frequently used criteria: the patient’s age and the Ki67 proliferation index. The influence of the patient’s age was comparably slight and the proliferation index showed no independent correlation with survival.

The major clinical indication of the present histomorphometric procedure is the prognostic assessment of individual patients and it is likely to contribute to the planning of individual therapeutic strategies. Postsurgical differential therapy of glioblastomas is a topic in progress due to fast-changing standards of knowledge concerning molecular biology and new strategies. There are differing opinions concerning the treatment of individual patients depending on the patient’s age, clinical course, localization, and molecular biological characteristics of the tumor. Therefore, it is too early to claim that an individual histomorphometric profile can provide definitive information concerning the patient’s suitability for a specific treatment (e.g., with gene therapy or slow-release drugs). Nevertheless, the prognostic information provided by histomorphometry could support therapeutic decision making, which encompasses well-established differential therapeutic strategies in individual clinics. For further improvement of the clinical relevance of histomorphometry, we recommend that it should be integrated in prospective studies and clinical trials to study the correlation between histologic characteristics of tumor cell nuclei and the efficacy of specific treatments. The goal is to define the role of histomorphometry in clinical neuropathologic diagnostics with respect to prognostic assessment and therapeutic consequences. Furthermore, it seems worthwhile to integrate histomorphometry of tumor cell nuclei into a spectrum of additional diagnostic procedures with prognostic significance. Within the field of molecular biology, recent reports showed a significant association of specific genetic alterations in glioblastomas with survival time (20, 21). From the viewpoint of histopathology, the expression of tumor-suppressor genes, as well as the presence of necroses, vascular pattern, and apoptotic index were shown to be associated with survival time (Table 6). Due to these results, it seems likely that a combined study of genetics, expression profile, and histomorphologic aspects of glioblastomas could provide more useful prognostic information even with respect to therapeutic strategies. The intraoperative recognition of tumor borders in cryostat sections could be another clinical application in the near future. The crucial point is the combined examination and the subsequent comparison of intraoperative cryostat histology and paraffin histology because the latter is still the gold standard for the histopathologic evaluation of tumors.

An important point is that the selection of a specific part of the tumor, the region with the highest proliferative activity in each tumor case, is sufficient to determine prognosis. The prognostic and diagnostic significance of morphometric data determined in that region was confirmed in previous studies of different brain tumors (5, 6, 8, 10). Glioblastomas may show a pronounced regional variation of histomorphology, which has been investigated morphometrically (13). In that study, a significant association was found between histomorphology and molecular biological alterations in different tumor regions but the clinical course of the patients was not emphasized (13). Because regional heterogeneity is an important aspect in tumor biology, it must be investigated in additional studies, whether morphometric analysis of more than one tumor region could further improve the statistical relationship between histomorphology and the clinical course of patients with glioblastomas.

It is even worthwhile to test if such an analysis could provide more biological information: the individual age and growth potential of the tumor. Up to now, however, the present results already confirm that selection of the region with the highest proliferative activity for the examination of tumor cell nuclei can be regarded as highly representative for the assessment of the biological behavior of the individual tumor. Due to the standardized postsurgical treatment with local radiation (60 Gy), we can exclude that different outcome of the patients had been influenced by different therapeutic strategies. To establish a statistically relevant relationship between histomorphology and survival, we believe that multivariate analysis of all morphometric parameters (by means of Cox analysis) is mandatory. We have found that the limited inclusion of morphometric parameters is far from sufficient. An instructive example is the significant and negative influence of nuclear area on survival for cases with total surgical resection, as previously reported (8). In that study, only a limited number of morphometric parameters were investigated and the question has not yet been answered if these parameters provided prognostic information independent of other prognostic factors.

In the present study, multivariate analysis showed no independent prognostic value for nuclear size in contrast to a distinct prognostic significance of parameters describing nuclear shape and spatial relationship. Up to now, our histomorphometric procedure in combination with multivariate data analysis confirmed a significant influence of nuclear morphology on survival time, which is statistically independent from total tumor resection, from the patients’ age and from the classification of the tumor as a primary or secondary glioblastoma. Therefore, nuclear morphology must be considered a strong indicator of tumor biology even for this type of brain tumor.

### Table 6

Reports about histopathologic criteria with significant influence on survival time of patients with glioblastomas

<table>
<thead>
<tr>
<th>Association with better clinical outcome:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Immunohistologic expression of tumor suppressor genes</td>
</tr>
<tr>
<td>Deleted in colorectal cancer gene (DDC gene; ref. 22)</td>
</tr>
<tr>
<td>Annexin VII gene (23)</td>
</tr>
<tr>
<td>p27 gene (24)</td>
</tr>
<tr>
<td>• Presence of multinucleated giant cells (25)</td>
</tr>
<tr>
<td>• High apoptotic index (26)</td>
</tr>
<tr>
<td>• Low content of bizarre vascular formations in combination with a prominent classic (nonbasil) vascular pattern (27)</td>
</tr>
<tr>
<td>• Especially for a group of long-term survivors (&gt;36 mo), fewer mitoses, a lower Ki67 proliferation index, as well as a higher frequency of p53 and mdm2 overexpression was shown (1, 3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Association with worse clinical outcome:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Large extent of necroses (28)</td>
</tr>
<tr>
<td>Criteria with contradictory reports concerning their prognostic significance:</td>
</tr>
<tr>
<td>• Epidermal growth factor receptor expression, p53 expression, Ki67 proliferation index (4, 29–32)</td>
</tr>
</tbody>
</table>

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REFERENCES


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