Prognostic scoring in adult astrocytic tumors using patient age, histopathological grade, and DNA histogram type

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High-grade astrocytic tumors constitute the most serious as well as the most common group of primary brain tumors. Although several prognostic factors have been proposed, little is known about the prognostic value of deoxyribonucleic acid (DNA) ploidy in adult astrocytic tumors. In a series of 146 adult patients, aged 16 to 82 years, the individual prognostic values of six variables were studied, namely: tumor histopathological grade, treatment, patient age, extent of tumor, ploidy level, and DNA histogram type. Cox's proportional hazard model was then applied to the data to ascertain which factors might independently determine patient survival. Univariate analyses revealed that histopathological grade, age, and DNA histogram type were very powerful prognostic factors. The statistical significance of the influence of adjuvant radiotherapy and chemotherapy was at a borderline level, and the two remaining variables (tumor extent and ploidy level) had no prognostic relevance. Multivariate analyses showed that age, histopathological grade, and DNA histogram type were independent, statistically significant prognostic factors.

A prognostic score was calculated from Cox's polynomial function in which those factors were introduced. The best score corresponded to a patient aged 16 years with a hypertriploid low-grade astrocytoma, while the worst score corresponded to a patient aged 82 years with a diploid high-grade astrocytoma. The worst score:best score ratio revealed a risk 71 times higher for a bad prognosis. It is concluded that patient age, histopathological grade, and DNA histogram type are very powerful prognostic factors for adult astrocytic tumors. A prognostic score including those factors could be used to characterize astrocytic tumor aggressiveness presurgically on fine-needle aspirates, and to monitor the patient's postsurgical evolution to define the appropriate therapy.

Key Words: ploidy · prognostic score · astrocytoma · age · grading system · DNA histogram

Wilson reported that primary brain tumors account for slightly less than 10% of all nontraumatic neurological diseases and for the same percentage of deaths from all neoplastic diseases. He and others estimated that high-grade astrocytic tumors constitute the most serious as well as the most common group of primary brain tumors. The histopathological classification of astrocytic tumors is still unclear and, as a consequence, their clinical management remains complex. The search for prognostic factors that might help predict the outcome of these brain neoplasms is therefore of primary interest. VandenBerge reported that, during the past decade, the neuropathological analysis of astrocytic tumors has been markedly affected by the application of special techniques to study tumor histogenesis and growth, particularly cytoskeletal and membrane proteins, growth factors, oncogene expression, cytogenetics, and cell-cycle kinetics. Several prognostic factors have been proposed: patient age, histopathological grade, sex, tumor extent, and preoperative mental status. In the case of neuroblastomas, the most common primary brain tumor in children, tumor ploidy level also appears to have great prognostic significance. We recently found such a relationship between survival of adult patients and their astrocytic tumor ploidy level.

The aim of this study was to identify a new prognostic scoring system that could predict the outcome in a patient with an astrocytic tumor. We studied the importance of six clinicohistopathological variables: histopathological grade; type of treatment; patient age; the number of intracranial lobes affected by the tumor; ploidy level; and the deoxyribonucleic acid (DNA) histogram type.
Clinical Material and Methods

Prognostic Variables

The study population for this series consisted of 146 adult patients. A complete clinical follow-up evaluation was obtained in 142 patients and an incomplete follow-up review, with one of the six variables missing, in four.

Extent of Tumor. One hundred eight patients had only one intracranial lobe affected by an astrocytic tumor; these were given a code of 1. The remaining 34 had two lobes affected and received a code of 2.

Age of Patient. The patients ranged in age from 16 to 82 years and were placed in one of three groups. Eighteen were aged less than 40 years and received a code of 1; 48 were aged between 40 and 60 years and received a code of 2; and 80 were more than 60 years of age and were coded as 3.

Type of Treatment. All 146 patients underwent surgery at the Erasmus Hospital between March, 1982, and December, 1989. The patients treated with radiotherapy received conventional postoperative treatment consisting of 6000 cGy delivered in 33 daily fractions. Radiotherapy was started within 4 weeks after surgery. All patients were treated with photons in the megavoltage range (10 to 23 MV x-rays). Only the tumor was irradiated; however, the volume targeted corresponded to the tumor volume as revealed by computerized tomography or magnetic resonance imaging, with an additional 2 to 3 cm for security. Corticosteroids were administered postsurgically in decreasing doses during radiotherapy, and were stopped in the middle of the radiation therapy. The patients treated with chemotherapy received intravenous carmustine (BCNU, 150 mg/sq m) 3 weeks after the end of radiotherapy. Chemotherapy was administered every 6 weeks for 1 year according to a previous protocol with lomustine (CCNU). The treatment code was assigned as follows: 1, surgery alone (40 cases); 2, surgery and radiotherapy (81 cases); and 3, surgery, radiotherapy, and chemotherapy (22 cases).

Histopathological Grading System. All 146 astrocytic tumors were classified as normal infiltrating astrocytomas. The histopathological diagnosis was performed as described previously, according to the classifications proposed by Burger, et al. Two histopathological groupings of the 146 tumors under study were made (see Discussion). Group I (20 cases) included the low-grade astrocytic tumors (astrocytomas) and Group II (126 cases) included the high-grade astrocytic tumors (anaplastic astrocytomas and glioblastomas multiforme).

Ploidy Determination. An archival collection of 146 formalin-fixed paraffin-embedded gliomas was used for ploidy determination. Deoxyribonucleic acid index and histogram type were assessed on Feulgen-stained nuclei from cell suspensions. The cell suspensions were obtained as described elsewhere. Depending on how much material was available, between one and three paraffin blocks were studied for each case. Five sections were cut from each block; the first, third, and fifth (5 µm thick) were subjected to histological verification after routine hematoxylin and eosin staining. The second and fourth sections (80 µm thick) were enzymatically digested to obtain single-cell nucleus suspensions that were centrifuged onto glass histological slides. The slides were then stained by the Feulgen reaction according to the procedure described previously.

Ploidy determination was performed by means of a microscope image processor with a × 100 magnification lens (numerical aperture 1.30). In total, 400 to 1200 nuclei were analyzed in a high-resolution mode for each astrocytic tumor under study. The nuclear integrated optical density was computed on 256 densitometric levels; integrated optical density measures the amount of absorbant material (nuclear DNA content).

We determined the DNA index from the integrated optical density, the value of which is 1.00 in the case of a normal G0 to G1 diploid population and corresponds to 2100 arbitrary units of integrated optical density. Normal human brain cell nuclei were used as an external standard. Group I (Group 1, 80 cases) was defined as those astrocytic tumors with a DNA index of less than 1.30, and aneuploid (Group 2, 66 cases) was defined as those with a DNA index higher than 1.30.

DNA Histogram Type. We recognized six types of DNA histograms, as defined in Table 1. In our analysis, we took into account that a tumor sample may be "contaminated" by normal cells. Regarding the selection of Feulgen-stained nuclei for DNA ploidy assessment, some errors may appear in the recognition of normal as compared with low-grade cell nuclei. Therefore, we considered that a true diploid astrocytic tumor must show a DNA histogram type with a G0 to G1 DNA peak containing at least 70% of its cell nuclei population. In contrast, we considered that a proportion of 15% of cell nuclei in the G0 to G1 peak was enough to define a triploid, a tetraploid, a hypertriploid, and a hyper-

<table>
<thead>
<tr>
<th>Type</th>
<th>Definition</th>
<th>DNA Index</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>diploid</td>
<td>0.90–1.15†</td>
</tr>
<tr>
<td>II</td>
<td>triploid</td>
<td>1.40–1.60</td>
</tr>
<tr>
<td>III</td>
<td>tetraploid</td>
<td>1.90–2.20</td>
</tr>
<tr>
<td>IV</td>
<td>hyperdiploid</td>
<td>1.16–1.39</td>
</tr>
<tr>
<td>V</td>
<td>hypertriploid</td>
<td>1.61–1.89</td>
</tr>
<tr>
<td>VI</td>
<td>polymorphic</td>
<td>‡</td>
</tr>
</tbody>
</table>

* Characterization is defined as the value of the deoxyribonucleic acid (DNA) index from the major G0-G1 DNA peak.
† A polymorphic DNA histogram type must contain at least two of the nondiploid DNA histogram types described above.

I. Salmon, et al.
Prognostic scoring in adult astrocytic tumors

![Graphs illustrating assessment by means of univariate analyses of survival by tumor extent (A) and by histopathological grade (B).](image)

**Fig. 1.**

Graphs illustrating assessment by means of univariate analyses of survival by tumor extent (A) and by histopathological grade (B). We coded patients whose tumor was located in one lobe as 1 and those whose tumor extended into more than one lobe as 2. Patients less than 40 years of age were coded as 1, those whose age ranged between 40 and 60 years as 2, and those more than 60 years of age as 3. Asterisks = two codes with same value.

triploid tumor. This estimation is based on the fact that $G_2 + M$ fractions changed when the $G_2$ to $G_1$ fractions had attained 15% (data not shown). Briefly, Type I histograms correspond to diploid tumors, that is, astrocytic tumors with $G_0$ to $G_1$ DNA peaks possessing a DNA index from 0.90 to 1.15. Type II histograms correspond to triploid tumors with $G_0$ to $G_1$ DNA peaks possessing a DNA index from 1.40 to 1.60. In the same way, Type III histograms are tetraploid astrocytic tumors (DNA index 1.90 to 2.20), Type IV histograms are hyperdiploid tumors (DNA index 1.16 to 1.39), and Type V histograms are hypertriploid tumors (DNA index 1.61 to 1.89). Type VI histograms correspond to polymorphic tumors, astrocytic tumors containing at least two of the four non-diploid DNA histogram types described in this section. We coded the hypertriploid astrocytic tumors as 1 and all of the astrocytic tumors corresponding to the five other DNA histogram types as 2 (see Discussion).

**Statistical Analyses**

Survival curves corresponding to the various levels of the prognostic variables were analyzed using the actuarial method of Mantel, and differences between those curves were assessed using the Lee-Desu statistic. Variables that achieved statistical significance ($p < 0.05$) in those univariate analyses were subsequently included in Cox's proportional hazard model. Variables were entered stepwise or removed from the model. Preassigned $p$ values of 0.10 and 0.05 controlled the stepping of entry and removal, respectively. Regression coefficients and their standard error were computer-generated for those variables that entered and remained in the regression polynomial. From this polynomial, a prognostic score was then computed for each patient in the study.

**Results**

**Univariate Analyses**

No statistical significance was found for the prognostic value of the tumor extent variable ($p = 0.9345$, Fig. 1A). Patient age had a highly significant prognostic value ($p = 0.0001$, Fig. 1B). Because the patient's survival appears to be universally correlated with age, this variable should be included with its primary continuous form and not with its categorically derived form in further multivariate analyses. The statistical significance of the treatment variable was at borderline level ($p = 0.04$, Fig. 2A). We obtained a very high level
I. Salmon, et al.

Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Decreasing Rank</th>
<th>Regression Coefficient</th>
<th>Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>patient age (yrs)</td>
<td>2</td>
<td>0.026 ± 0.0074</td>
<td>3.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>histological grade</td>
<td>3</td>
<td>1.1618 ± 0.3485</td>
<td>3.33</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DNA histogram type</td>
<td>1</td>
<td>1.3418 ± 0.3204</td>
<td>4.19</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*In this example, the prognostic score from the polynomial function of Cox's model may be calculated as 0.026 (patient age) + 1.1618 (histological grade) + 1.3418 (DNA histogram type), where 0.026, 1.1618, and 1.3418 are the regression coefficients corresponding to patient age, histological grade, and DNA histogram type, respectively. DNA = deoxyribonucleic acid.

† Regression coefficients are expressed as means ± standard error of the means.

The first variable that we studied was the histopathological grade, the definition of which is not well established in the literature. Several authors agree that there are two groups of astrocytic tumors: benign astrocytoma and malignant astrocytic tumors. North and coworkers, using Kernohan's classification, have reported that tumor grade did not influence survival in their series of 77 patients with Grade I and
Prognostic scoring in adult astrocytic tumors

II astrocytic tumors. In contrast, Burger, et al., proposed a three-grade classification for high-grade astrocytic tumors and showed in a series of 1440 malignant astrocytic tumors that patients with glioblastoma multiforme exhibited worse clinical behavior than those with anaplastic astrocytoma. Larson, et al., also stated that the pathological distinction between anaplastic astrocytoma and glioblastoma multiforme is of prognostic significance. Indeed, they found that external beam radiation therapy produced significant remission in 20% to 25% of patients with anaplastic astrocytoma but was not curative in those with glioblastoma, although it did prolong median survival time. In contrast, Black reported that increasing the fractionation of the dose or adding the sensitizing agent misonidazole did not increase survival, a result also shown by the EORTC Brain Tumor Group. The survival rate is still 8% at the end of 2 years for anaplastic astrocytoma as well as for glioblastoma multiforme.

In a previous study, we noted a significant decrease in survival time, ranging from the group of patients with astrocytomas, through those with anaplastic astrocytomas, to those with glioblastomas multiforme. There was only a slight statistically significant difference between the mean survival period of patients with anaplastic astrocytomas and that of patients with glioblastomas multiforme. These results together with the data reported above led us to place astrocytomas into Group I and anaplastic astrocytomas along with glioblastomas multiforme into Group II. Our current results demonstrate that a very high level of statistical significance (p = 0.0001) is obtained with respect to the prognostic value of histopathological grading into two groups.

Treatment is the second variable that should be important for patient survival. Black noted that the treatment of astrocytic tumor usually begins with surgery performed for diagnosis or excision. In his opinion, at the time of initial diagnosis, radiation directed first to the area surrounding the tumor and then to the tumor itself prolongs survival. In contrast, Shenouda, et al., reported that, although postoperative irradiation of high-grade gliomas has been shown to prolong median survival time from 14 weeks (surgery alone) to 37 weeks, the long-term survival rates nevertheless remain unaffected by such treatment, a finding that we also observed in this study. With respect to low-grade astrocytomas, Leibel, et al., and Fazeek have shown that radiation therapy improves survival time, while Laws, et al., and Uhllein, et al., failed to demonstrate any such benefit.

In addition to radiotherapy, many oncologists have also used chemotherapy for astrocytic tumors. Today, no drug evaluated by clinical trial has exceeded the established effectiveness of carmustine. Black claimed that a meta-analysis conducted over the past 10 years on 884 patients treated with radiation therapy alone, as opposed to 1538 patients who received radiation therapy and chemotherapy, shows that chemotherapy has a significant benefit (p < 0.0005). He also suggested that clinical trials with smaller numbers of patients have a high likelihood of a statistical Type II (beta) error, which may mask any beneficial effect. Black emphasized that individual studies therefore only show a limited effect of chemotherapy. Our present investigation is in fact such an individual study. It is not surprising, therefore, that we obtained only a slight (but nevertheless significant) benefit from treatment adjuvant to surgery.

Some authors have reported that the sex of the patient is an important prognostic indicator, but others disagree. We obtained no statistically significant values related to sex in terms of patient survival (data not shown).

The same feature was observed with the variable describing the number of intracranial lobes affected by a tumor. Indeed, we obtained no statistical significance for the prognostic value of the extent of tumor. In contrast, North, et al., did find such a significant prognostic value; however, their study included only low-grade astrocytic tumors, and the level of statistical significance that they obtained was relatively weak (p = 0.016).

In sharp contrast to the variables of sex and tumor extent, patient age was a strong prognostic factor (p < 0.0001) for survival. This finding is in accordance with reports by other groups.

To our knowledge, the prognostic value of the ploidy level in adult astrocytic tumors is not yet well established, unlike its value in children's brain tumors and, more precisely, in neuroblastoma. Bourhis, et al., reported that, of the clinical factors found to be of prognostic value in neuroblastomas, patient age at diagnosis and tumor stage have been the most reliable. Bourhis, et al., and many others stated that the DNA ploidy index predicts clinical outcome because aneuploid neuroblastomas have generally been associated with a favorable evolution of the disease. With adult astrocytic tumors, the situation remains confused. Aply, et al., hypothesized that the aggressive clinical course of malignant glial neoplasms may be related to an abnormal DNA stemline and/or an alteration in cell proliferation activity. This opinion is not supported by Giangaspero, et al., who showed that 12 of 16 cases of glioblastoma multiforme have a main population that is diploid or near diploid. They claimed that in contrast to observations made on other solid malignant tumors such as bladder cancers, in which aneuploidy may be associated with a poor prognosis, no relationship has been established in malignant astrocytic tumors. In contrast, in a recent study of 206 adults with astrocytic tumors, six types of DNA histograms were recognizable. In this study of the use of Feulgen-stained nucleus cytophotometry from archival materials, that is, formalin-fixed paraffin-embedded tumors, we studied both the DNA ploidy level and the DNA histogram type of each tumor. In our opinion, these two indices provide complementary information. In the literature, the ploidy level of a tumor is generally assessed through the DNA index. However, the DNA index only measures the mean nuclear DNA content of the tumor and gives no information about the nuclear heteroge-
neity of the tumor analyzed. As a consequence, two tumors that possess two completely distinct nuclear DNA distributions or, in our opinion, two completely distinct biological and/or clinical patterns of potential evolution may lead to identical DNA index values. We think that the DNA index measurement is valuable only when the tumor being analyzed is composed exclusively of a monostemline cell population. If this is not the case, the DNA index measurements must be completed by the DNA histogram type characterization. In our previous study, we observed that, in the group of patients with astrocytomas, 75% of the patients with hypertriploid tumors survived longer than the mean survival period associated with this histopathological group. In the group of patients with anaplastic astrocytomas, there were also patients with hypertriploid tumors who survived longer. The most striking result appeared in the group of patients with glioblastomas multiforme in which all patients with hypertriploid tumors survived longer than the mean survival time associated with this group. Accordingly, the present study reveals that the DNA histogram type represents a very strong prognostic factor; therefore, these six DNA histogram types (Table I) were divided into two groups: Group I corresponds to the hypertriploid Type V DNA histogram and Group II includes the five other types.

We think that the absence of prognostic value associated with ploidy level, as assessed by the DNA index, relates to the fact that aneuploid tumors contain not only the Type V DNA histogram, but also Type III (tetraploid), Type IV (hyperdiploid), and Type VI (polymorphic) DNA histograms. A Type V DNA histogram might indicate a marked improvement in survival compared to the other five DNA histogram types because it is associated with a significantly lower proliferation than the other five types.

In conclusion, when the Cox multivariate analysis was used, the three variables that appeared to be the most significant of the six analyzed here, histopathological grade, patient age, and DNA histogram type, retained their statistical significance. They were introduced into the polynomial function of Cox’s model, and this process enabled a prognostic score to be obtained. In our series of 142 patients, the worst score: best score ratio reveals that the patient with the worst score has a 71 times higher risk of a poor prognosis than the patient with the best score. Such a risk calculation can be applied at two levels. The first level might correspond to the characterization of astrocytic tumor aggressiveness. This characterization should be based on cytological material obtained prior to surgery. Thus, to obtain the prognostic score, three variables are required: 1) patient age (known at the time of surgery); 2) histopathological grade (from cytological specimens); and 3) DNA histogram type. The DNA histogram type can be assessed from individual cell nuclei, which may be taken from archival material. The fact that we reduce our “histopathological grading variable” to two values markedly decreases the risk of pathologists or cytologists making diagnostic errors. In a given patient, we can now compare our score obtained from presurgical cytological material (fine-needle aspirates) with postsurgical specimens, such as imprint smears, that reveal the histology.

The second level of application of the present score should correspond to the clinical monitoring of the patient. Indeed, we hope that this score will make it possible to distinguish those patients who will benefit from intensive care (patients who should receive surgery, radiotherapy, and chemotherapy) from those who should undergo surgery alone. We have begun a large-scale study to test this scoring system from the clinical point of view.

References

Prognostic scoring in adult astrocytic tumors


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