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Original Article

Prognostic value of histological features in diffuse astrocytomas WHO grade II

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Abstract: The histopathological diagnosis of diffuse astrocytoma is challenging. As the WHO classification system is based on subjective assessments, the prognosis for the individual patient is somewhat uncertain. The aim of this study was therefore to investigate the prognostic value of various histological features, Ki-67/MIB-1 labeling index (LI), and clinical factors. The study was designed as a retrospective study of 109 patients consecutively operated for their primary diffuse astrocytoma WHO grade II. Clinical data was collected from patient files. All routine stained sections were revised, and 20 different histological features were recorded, including cell density, atypia, mitoses, apoptoses, secondary structures (of Scherer), microcysts, and lymphocytic infiltration. Ki-67/MIB-1 LI was determined by conventional immunohistochemistry. Using uni- and multivariate analyses, the prognostic value of these factors was assessed as well as clinical parameters. Median age at primary surgery was 40 years (range 18-75). The median overall survival was 70 months with a minimum follow-up of 3 months. Neither histopathological features nor Ki-67/MIB-1 LI (median value of 4.5% (range 0.1-16%) indicated unfavorable prognosis. However, age > 40 years, gender (male), poor preoperative performance score, and biopsy rather than resection were significant negative prognostic factors in both uni- and multivariate analyses. Among diffuse grade II astrocytomas neither any histopathological trait nor Ki-67/MIB-1 LI achieved prognostic significance, whereas clinical parameters were shown to serve as the major prognostic factors for these patients.

Keywords: Brain neoplasms, brain tumours, gliomas, histopathology, proliferation, survival

Introduction

Astrocytomas are the most common primary tumour in the CNS. The diagnosis is based primarily on histopathological criteria defined by the World Health Organisation (WHO) that grades astrocytomas as pilocytic astrocytoma (grade I), diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III), and glioblastoma (grade IV) [1]. Although grade II astrocytoma is a relatively slow growing tumour with a median survival time of 5-8 years, they have a high recurrence rate due to diffuse infiltration of brain tissue and an inherent malignant potential to transform into high-grade astrocytoma, such as anaplastic astrocytoma and secondary glioblastoma [2, 3]. As long as the WHO classification system is based on subjective assessment of histopathological features, there will be a problem of interobserver variability, in such a way that the prognosis for the individual patient will remain somewhat uncertain.

The histopathological diagnosis of diffuse grade II astrocytoma can be challenging due to their pronounced heterogeneity. Typically, they are characterized by moderately increased cellularity, little nuclear atypia, low mitotic activity, absence of necrosis, and microvascular proliferation [4]. They also forms so-called "secondary structures of Scherer", which includes perineuronal satellitosis, subpial growth, and perivascular spread [5]. The literature is sparse and ambiguous regarding histopathological features and prognosis in well-differentiated astrocytomas, however, cellularity, mitoses, microcysts, lymphocytic infiltration, and vascular density have been shown to have impact on survival [6-8].

To supplement the histopathological diagnosis new techniques have been introduced, for in-

stance methods designed to detect proliferating cells, and immunohistochemical determination of Ki-67/MIB-1 labeling index (LI) has become widely used. Ki-67 is an antigen expressed during all the active phases of the cell cycle [9]. In human astrocytoma a positive association between survival and Ki-67/MIB-1 LI has been established. The clinical role, however, is not straightforward due to great spread of indices between astrocytomas of different malignancy grades, so distinct threshold values have not been established [10, 11].

The aim of this study was to investigate the prognostic value of various histopathological features in a series of 109 consecutively operated patients with diffuse astrocytoma grade WHO II diagnosed according to the latest WHO classification (2007). In addition we extended our previous study to investigate the prognostic value of Ki-67/MIB-1 LI [12] as well as for clinical factors.

Material and methods

Histological and clinical data

Surgical removed specimens obtained from 109 patients with diffuse astrocytoma WHO grade II treated between 1987 and 2007 at St. Olavs Hospital of Trondheim, Norway, were investigated. Of these, 22 cases have been included in a previous study [12]. The material was collected from the electronic database at the pathology department. The search profile encompassed gliomas and astrocytomas, and the inclusion criteria were grade II astrocytomas, intracranial localization, and age > 16 years old. Clinical data regarding gender, performance score, main presenting symptoms, age at the time of surgery, tumour location (supra- or infratentorial), treatment (surgery (gross total resection, partial resection, biopsy), radiotherapy, chemotherapy), and follow-up/survival, were obtained either from medical records at St. Olavs Hospital of Trondheim or at the local hospitals. The date and cause of death were obtained from the Norwegian Population Registry.

Histological sections were collected and revised by one senior pathologist (SHT), and the diagnoses were made according to the latest WHO classification [1]. Subtypes (fibrillary, gemistocytic (> 20% gemistocytes), and protoplasmic) and twelve histological features were recorded and graded in each sample. Regarding cellularity, calculation was performed using the mean cell count from three high power fields (HPF), each of 0.0576 mm^2, using an eye grid. The cell density was judged subjectively as low, moderate, or high. Cellular/nuclear atypia was assessed as mild, moderate, or severe. Further, different degenerative features (Rosenthal fibres, eosinophilic granular bodies, microcysts, myxoid matrix, and microcalcification), perivascular lymphocytic infiltrate, and secondary structures of Scherer (perineural satellitosis, angiocentric and subpial growth) were registered as present or not.

Proliferation measurements

Proliferative activity was determined on 4 µm thick paraffin sections from representative tumour tissue. They underwent antigen retrieval by means of pressure-cooking and were thereafter incubated with the monoclonal Ki-67-equivalent antibody MIB-1 (DakoCytomation, Glostrup, Denmark), dilution 1:100, for 40 min at room temperature using an automatized immunohistostainer (Dako Techmate 500). A standard avidin-biotin-peroxidase technique was used. The sections were developed with diaminobenzidine and counterstained with haematoxylin. Human tonsils served as positive controls, and in the negative controls MIB-1 was omitted. Microscopic areas with highest labelling intensity were chosen for calculation; in each case either at least 1000 tumour cell nuclei were counted or three HPFs were examined using an eye-grid. The Ki-67/MIB-1 LI was defined as the percentage of immunoreactive tumour cell nuclei. Due to sparse material only 104 out of 109 cases underwent immunohistochemical analyses.

Statistical analysis

Survival was calculated from date of diagnosis to either date of death or last follow-up. Survival was assessed and compared using Kaplan-Meier curves and log-rank test. In the multivariate analyses Cox regression was applied. Kappa statistic was used to determine interobserver agreement in tumors with Ki-67/MIB-1 LI higher than 5%. P-values < 0.05 were considered being significant.

The study was approved by the Regional Committee for Medical Research Ethics, and the study protocol adhered to guidelines by Helsinki Convention.

Results

Clinical data

The clinical data are summarized in **Table 1**. The male/female ratio was 1.22, and median age was 40 years with range 18-75 years. The majority of the tumours were located supratentorially (102/109, 94%). Seizure was the most frequent first clinical symptom (70/109, 64%). Tumour resection (radical and partly) was performed in 85 cases (85/109, 78%), while biopsy was performed in 24 patients (24/109, 22%). The treatment modalities used were radiotherapy and chemotherapy given to 65 and 21 patients, respectively. Minimum follow-up was 3 months. The median survival was 70 months (range 3-277 months), and the overall actuarial survival at 2, 5, and 10 years was 82%, 56%, and 22%, respectively. Seventy patients (70/109, 64%) had tumour recurrence verified by either MRI, CT, or in need for treatment with corticosteroids. The relapse treatment was re-surgery (41/109, 38%), palliative chemotherapy (16/109, 15%), radiotherapy (3/109, 3%), or no treatment (9/109, 8%) (information about one patient was not accessible).

Histopathological data

Mitoses were found in 29 samples (29/109, 27%), mean and median number of 0.39 (SD 0.758) and 0 (range 0-3), respectively. Nineteen cases had one mitosis, 6 had two, and 4 had three mitoses per 10 HPFs. Apoptoses were detected in 49 samples (49/109, 45%). Most cases had moderate cell density. In about half of the tumours atypia was assessed as moderate. Fibrillary astrocytoma was the most common subtype (95/109, 87%), followed by gemistocytic (13/109, 12%) and protoplasmic (1/109, 1%) variants. Both secondary structures and microcysts were commonly observed, 61% (60/109) and 41% (38/109), respectively. Degenerative features such as Rosenthal fibres and eosinophilic granula were found in less than 10% of the cases. The mean and median values of Ki-67/MIB-1 LI were 5.2% and 4.5% (range 0.1-16%), respectively (**Table 1**).

Survival analysis

Kaplan-Meier plots of survival were examined with log-rank tests of significance for patient age, gender, surgical removal, performance score, Ki-67/MIB-1 LI, and various histopathological features (**Figure 1**). In univariate analyses no significant association between survival and histopathological features (mitoses, apoptoses, cellularity, atypia, secondary structures, lymphocyte infiltration, and microcysts) was achieved. On the other hand, significance was obtained between age ($p = 0.016$), gender ($p = 0.050$), tumour resection ($p = 0.005$), and pre-surgical performance score ($p = 0.001$) (**Table 2** and **3**). The cut-off value for the different parameters was under vs over 40 years, total resection vs partly/biopsy, and zero and one vs patients with performance score

Table 1. Clinical data

Clinical variables	n (%)
Age yrs	
18-40	53 (48.6)
40-60	40 (36.7)
>60	16 (14.7)
Gender	
Male	60 (55%)
Female	49 (45%)
Tumor location	
Supratentorial	102 (93.6)
Infratentorial	5 (4.6)
Combination	2 (1.8)
Performance score	
0	86 (78.9)
1	19 (17.4)
2	2 (1.8)
3	2 (1.8)
4	0 (0)
Main symptoms	
Epilepsy	70 (64.2)
Headache	16 (14.7)
Mental changes	3 (2.8)
Neurological outcome	13 (1.9)
Pressure symptoms	6 (5.5)
None	1 (0.9)
Surgery	
Radical resection	27 (24.8)
Partly resection	58 (53.2)
Biopsy	24 (22.0)
Treatment modalities	
Radiotherapy	64 (58.7)
Chemotherapy	21 (19.3)
Gamma knife radiosurgery	1 (0.9)
Ki-67/MIB-1 (n, median (range))	104/109, 4.45% (0.1-16%)
<2% (n, %)	15/104 (14.4%)
2-5% (n, %)	41/104 (39.4%)
5-10% (n, %)	34/104 (32.7%)
10-15% (n, %)	10/104 (9.6%)
>15% (n, %)	2/104 (1.9%)

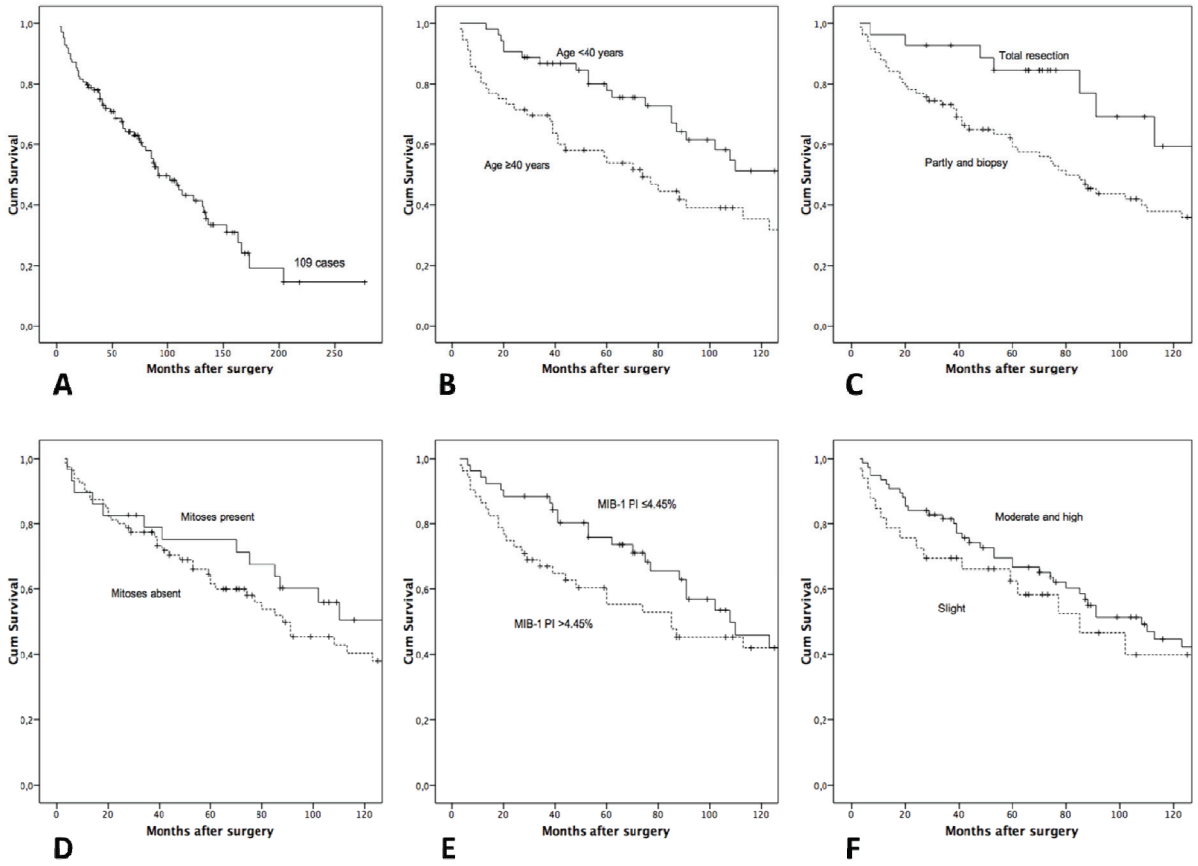

Figure 1. Selected Kaplan-Meier-plots showing overall survival (A), relation to age (log-rank test, p=0.016) (B), grade of resection (log-rank test, p=0.005) (C), mitoses (log-rank test, p=0.09) (D), Ki-67/MIB-1 LI (log-rank test, p=0.255) (E), and cell density (log-rank test, p= 0.386) (F).

Table 2. Prognostic significant factors in univariate analyses

Age at surgery	<40 years vs >40 years	p=0.016
Gender	Female vs male	p=0.050
Preoperative Performance score	0 and 1 vs >1	p=0.001
Extension of surgical removal	total vs partial and biopsy	p=0.005
Ki-67/MIB-1 LI	<median vs >median	p=0.255

beyond one. Interobserver reproducibility of Ki-67/MIB-1 immunostaining gave a Kappa score of 0.413 indicating moderate agreement. Cox regression analyses showed that age (p = 0.007), gender (p = 0.050), performance score (p = 0.010), and tumour resection (p = 0.037) all were significant factors related to survival (**Table 4**).

Discussion

In the present study the prognostic value of clinical data, relevant histopathological features, and Ki-67/MIB-1 LI was investigated in diffuse astrocytomas WHO grade II. We found that only clinical factors such as age at surgery, gender, performance score, and grade of resection had prognostic significance.

Only clinical factors emerged as robust prognostic factors both in univariate and multivariate analyses and are in agreement with the literature [13, 14]. Thus, early diagnosis and radical surgery are important in the management of

Table 3. Univariate analyses of the association of factors with survival

Factor	Group	Number of patients	Deaths	Mean survival months	Five year survival	p- value
Ki-67/MIB-1 LI	<4.45	52	28	86	65%	0.255
	>4.45	52	31	70	46%	
Age	<40	53	25	88	64%	0.016
	>40	56	37	66	48%	
Gender	Male	60	23	69	48%	0.050
	Female	49	39	85	65%	
Tumor location	Supratentorial	102	59	77	58%	0.032
	Infratentorial	5	3	27	0%	
	Combination	2	0	164	100%	
Resection	Total	27	7	86	74%	<0.001
	Partly	58	36	82	55%	
	Biopy	24	19	53	38%	

Table 4. Cox model results of the factors that contributed toward predicting survival.

Age at surgery	p=0.007
Gender	p=0.050
Performance score	p=0.010
Tumour resection	p=0.037

patients with low grade astrocytomas. Interestingly, female patients had a longer survival compared with males, a fact other authors has not been able to confirm [15].

The investigated histopathological features known for their involvement in tumour progression and importance in the grading process (mitoses, apoptoses, high cell density, atypia, and secondary structures of Scherer), did, however, not reach statistically prognostic significance in our series of tumours. This observation may be interpreted as clinical parameters have a more profound impact on prognosis than histopathology.

Regarding mitotic activity, our findings demonstrated that up to three mitoses in cases with adequate tumour samples, did not have any influence on survival. This may correspond to cases as Schiffer et al [6] designed without malignancy with number of mitoses less than 5/10 HPFs. Further, it supports the hypothesis that few mitoses, when adequate biopsy material is present, does not affect the survival of patients with grade II astrocytoma [16, 17]. On the other hand, one should suspect a more aggressive astrocytoma when a solitary mitosis is present when sparse material is available [16, 17].

According to WHO, cell density in grade II astrocytoma is described to be moderately increased compared with normal brain tissue [1]. We estimated cell density both subjectively and by the number of cells per mm^2, and neither ways showed any impact on survival. This is in contrast to others that have shown that a high cell density is the only histological feature with influence on survival besides the numbers of mitoses [6]. However, the fact that cell density is not included in the St Anne Mayo system of classification, is in agreement with our finding that cell density and cell count may not serve as major prognostic factors for diffuse astrocytomas grade II [8].

Secondary structures of Scherer are regarded as malignant properties of neoplastic astrocytes representing infiltrative and migratory characteristics of the tumour cells. They were observed in as much as 60% of our cases, and could thus well substantiate their influence on the high recurrence rate of diffuse astrocytomas [18]. We could, however, not establish any correlation to prognosis, probably due to the strong influence of clinical factors. As microcysts are not found in neither normal brain tissue nor in gliosis, supports the hypothesis that this process is linked to the gliomagenesis [19]. Some has also found that microcysts serve as a positive prognostic factor in diffuse astrocytomas [6]. Neither this histological feature reached statistical significance in our study. Another feature suggested to be associated with better outcome, is lymphocytic infiltration [6], which we neither could confirm. As the lymphocyte infiltration was in general sparse, we are in doubt of the significance of this phenomenon. It can, however, be linked with any immune response

against the tumour cells [20].

Several studies have shown that higher Ki-67/MIB-1 LIs in human astrocytomas correlate with tumour grade and prognosis [10, 11]. There are, however, some disadvantages linked to this immunohistochemical procedure, such as overlap of values between the various malignancy groups, considerable interlaboratory variations, and lack of standardization. In one of our previous study we showed that Ki-67/MIB-1 LI had prognostic significance with a proposed cut-off value of 2.7% [12]. The present study could, however, not verify these findings, in accordance with other studies as well [21]. This discrepancy can be explained by the larger number of patients in the present study, underlining the importance of larger tumour series. The considerable spread of indices makes it hard to suggest a cut-off value for this astrocytoma group. Accordingly, in the individual case a Ki-67/MIB-1 LI alone cannot be regarded as prognostic. For these reasons Ki-67/MIB-1 LI has not been included in the latest WHO classification system [22]. Still, in the daily routine, however, Ki-67/MIB-1 immunostaining is useful, both to distinguish between gliosis and astrocytoma, to display areas with high proliferative activity to ease the search of mitoses, and to identify astrocytomas with an increased proliferative potential with an otherwise low-grade histological appearance. Such cases is recommended to be signed out as astrocytomas with elevated proliferative index, and these patients might be in the need of a more closely follow-up [22].

In conclusion, the histopathological examination still serves as the gold standard for making the diagnosis of astrocytic tumours, and is thus fundamental for prognostication and therapeutic managements of these patients. However, the current classification scheme is not optimal because of several parameters including tumour heterogeneity, sampling error, poor reproducibility, and that tumours with common histology can be genetically different. For these reasons, also because of small tumour specimens, additional markers are needed to improve the diagnostic and prognostic accuracy. In this connection, Ki-67/MIB-1 is a useful tool. Furthermore, molecular genetic analyses will gradually be included in the diagnostics as well. Nevertheless, since clinical factors stand out as fundamental prognosticators, it just illustrates the multidisciplinary in the diagnosis of astrocytomas based on clinical, radiological, and pathological data.

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