The Importance of Flow Cytometric DNA Analysis in Glial Tumors

Glial Tümörlerde Flow Sitometrik DNA Analizinin Önemi

CELAL BAĞDATOĞLU, GÖKALP SİLAV, ARZU KANIK,
HABİBULLAH DOLGUN, GÜLŞAH BADEMCİ, HALUK DEDA

Mersin University, Faculty of Medicine, Department of Neurosurgery (CB), Ankara University Faculty of Medicine Department of Neurosurgery (GS, HD, GB, HD), University of Mersin Faculty of Medicine, Department of Biostatistics (AK)

Received : 13.7.2000 ⇔ Accepted : 22.10.2000

Abstract: Neoplasms of glial origin constitute 40-50% of all primary tumors of the central nervous system. Although a number of clinical features are related to prognosis, tumor grade is currently used to predict outcome. However, at present, grading is a subjective process based on tumor morphology and quantitation of microscopic features such as cellularity, cytologic atypia, mitotic figures, and endothelial proliferation. Distinguishing between low- and high-grade lesions is sometimes subjective, and results may vary depending on the observer. We studied fresh tissue samples that were surgically resected from 30 patients (16 males, 14 females) between April 1996 and February 1998. Patient age ranged from 6 to 73 years, and the mean age was 43.7 years. Pathologic examination revealed 3 pilocytic astrocytomas, 3 grade I astrocytomas, 10 grade II astrocytomas, 9 grade IV astrocytomas, 2 malignant ependymomas, and 3 oligodendrogliomas. We studied relationships between patient survival time and tumor ploidy, patient age, S-phase, and histological subtype. Follow-up ranged from 2 to 31 months, with an average of 13.1 months. Sixteen of the 30 tumors were aneuploid (53.3%) and 14 were diploid (46.7%). The mean coefficient of variation value was 4.36%. Patients with diploid cell populations had a longer period of disease-free survival compared to those with aneuploid cell populations. Also, individuals with low S-phase fractions survived longer than individuals with higher S-phase fractions. The study showed that patient survival time was correlated with DNA ploidy, histological subtype (except the grade II astrocytomas), and S-phase fraction,

Özet: Glial tümörler tüm santral sinir sistemi tümörlerinin % 40-50'sini oluşturur. Hernekadar birçok klinik yöntem prognoz ile ilişkili olsa bile, tumor gradelemesi tumor geleceğine ilişkin halen kullanılmaktadır. Bununla beraraber, günümüzde gradeleme endotel proliferasyonu, mitozun varlığı, sitolojik atipi, selülarite gibi nicelliğe bağlı mikroskopik özellikler ve tumor morfolojisi temelinde subjektif olaydır. Bazı zamanlar yüksek ve düşük grade'li tümörlerde ayırım subjektiftir, ve sonuçlar değerlendiren kişiye bağlı olabilir. Nisan 1996- Şubat 1998 tarihleri arasında açık cerrahi olarak opere edilen 30 hastadan (16 erkek, 14 bayan) taze doku örneklerinde çalışma yapıldı. Hasta yaşı 6-73 arasında ve ortalama 43.7'dir. Patolojik inceleme 3 pilositik astrositoma, 3 grade 1 astrositoma, 10 grade 2 astrositoma, 9 grade IV astrositoma, 2 malignnt ependimoma, ve 3 oligodendroglioma olarak tespit edildi. Çalışmamızda hasta survive'I ile tumor ploidi, hasta yaşı, S-phase ve histolojik subtip arasındaki ilişkiyi araştırdık. Hasta takip süresi 2-31 ay ve ortalama olarak 13.1 ay olarak tespit edildi. 30 tümör örneğinin 16'sı (% 53.3) aneuploid ve 14'ü (%46.7) diploid olarak tespit edildi. Ortalama varyasyon katsayısı % 4.36 olarak bulundu. Diploid hücre popülasyonu tespit edilen hasta grubunda hastalıksızlık dönemi aneuploid gruba gore uzun bulunmuştur. Ayrıca, düşük S-fazlı hastalarda survive yüksek olanlara gore uzun bulunmuştur. Çalışma sonucunda S-faz fraksiyonu, DNA ploidi ve Histolojik subtip (Grade 2 astrositoma hariç) ile survive arasında korelasyon var iken, hasta yaşı ile survive

but there was no correlation between survival time and patient age. In our study, besides histologic subtype, flow cytometric DNA analysis is found to be necessary for determining prognosis in glial tumors.

Key Words: Flow cytometry, glial tumors, prognosis

arasında korelasyon olmadığı gözlenmiştir. Çalışmamızda, histolojik subtip'in yanısıra, flow sitometrik DNA analizinin glial tümörlerin prognozunun belirlenmesinde gerekli olduğu gösterilmiştir.

Anahtar Kelimeler: Flow sitometri, cytometry, glial üumörler, prognoz

INTRODUCTION

Tumors of glial origin represent 40-50% of all primary tumors of the central nervous system. Although a number of clinical features are related to prognosis, the trait that is currently used to predict outcome is tumor grade. At present, grading is a subjective process based on tumor morphology and quantitation of microscopic features, including cellularity, cytologic atypia, mitotic figures, and endothelial proliferation. Distinguishing between low- and high-grade lesions is sometimes subjective, and findings may vary with the observer. In contrast, flow cytometric studies of tumor DNA content is a more objective method of characterizing tumors, and may be a valuable means of establishing prognoses for glial neoplasms.

Flow cytometry has been used to study the DNA content of solid tumors for 20 years. In the past, DNA content has also been measured using a variety of other techniques. Until fairly recently, the most popular method was static cytophotometry (9). This is a microscopy-based technique in which approximately 100-200 cells are examined after staining for DNA. In contrast to this, more than 100,000 tumor cells can be analyzed in just a few minutes using flow cytometry. This and other advantages make this method a more objective, more efficient, and less labor intensive means of measuring cellular DNA content (9). Hedley and co-workers developed a method for flow cytometric DNA analysis of paraffin-embedded tissue in 1983 (14,15,16). The results using flow cytometry on fresh and paraffin-embedded tissues are comparable in terms of accuracy (6).

DNA measurements can be divided into the three basic cell cycle compartments: G1, S, and G2+M (21). DNA duplication occurs during the S-phase of the cell cycle. In the G2 and M phase, the cellular DNA content is double that measured in the G0/G1 phase. Comparison of the DNA content of tumor cells in the G0/G1 phase to the DNA content of non-malignant cells in G0/G1 identifies tumor ploidy (14,21,28). Tumors in which the stem line DNA contents are not measurably different from the content of non-malignant reference cells are referred to as diploid, and those in which the stem line DNA content is higher than that of reference cells are labeled aneuploid (2,8,9,10,11). The numerical ratio of the mean DNA content of phase G0/G1 tumor cells compared to that of normal cells is termed the "DNA index" (DI) (2). The DI of diploid cells is 1.00 (9,10). It follows that hyperdiploid tumors have a DI > 1.00, hypodiploid types have a DI < 1.00, and that the DI for tetraploid tumors is 2.00 (9,10).

The DNA histogram derived from flow cytometry is essentially a map of the changes in cellular DNA content throughout the cell cycle. On the histogram, the first peak represents the G0/G1 population, the second peak represents the G2+M population (6), and the values for the S-phase cells are located between these two peaks (7,21,35). Some investigators have chosen to report tumor proliferative activity as the percentage of cells in the S and G2+M phases combined (10,25). Thus, the "proliferation index" (PI) represents data from S phase and G2+M phase. The validity of the DNA histogram can be assessed using the coefficient of variation (CV) of the G0/G1 peak (10). If the G0/G1 CV on a histogram is > 5, the results are considered uninterpretable (10).

Tumor ploidy, which is a close relative of proliferative activity, is one of the most interesting and potentially important observations in flow cytometric study of tumor cell DNA content. Early investigations compared these findings with established prognostic factors, such as clinical stage, histologic grade, or age, but more recent studies have tested correlations between tumor ploidy, or proliferative activity, and the individual patient's clinical course (27,28,29).

Our aim in this study was to determine whether histological subtype alone is an accurate predictor of disease-free and overall survival in glial tumor patients, or whether flow cytometric DNA analysis is also necessary.

PATIENTS AND METHOD

Surgical tumor samples from 30 patients (16 males, 14 females) with intracranial neoplasms who underwent surgery between April 1996 and February 1998 were studied with flow cytometry (Table 1). Patient age ranged from 6 to 73 years, and the mean was 43.7 years. Gross total excision was achieved in 22 patients, and subtotal excision was performed in 8 cases. The tumors were classified and graded according to the World Health Organization grading system for tumors of the central nervous system (16), and the same pathologist examined all surgical specimens. The pathological findings for the group were 3 pilocytic astrocytomas, 3 grade I astrocytomas, 10 grade II astrocytomas, 9 grade IV astrocytomas, 2 ependymomas, and 3 oligodendrogliomas.

All patients were consulted regarding radiation oncology and medical oncology. Fifteen underwent both radiation and chemotherapy, and 12 received radiation therapy only. Neither treatment was administered in three cases (patients no. 19, 20, and 21) because these individuals had already undergone radiation and chemotherapy. For radiotherapy, a

Table 1. Summary of diagnosis and study findings. (Oligo; oligodendroglioma, Astro; astrocytoma, Pil Astro; pilocytic astrocytoma, Mal Epend; malignant ependymoma, DI; DNA index, PI; proliferation index, Recur; recurrence, MF; months of follow-up)

Patient	Pathology	Recur	age (yrs)	S-phase %	DI	PI	MF
1-HY	Oligo I	+	43	3.48	1	4.11	31
2-CA	Oligo II	+	47	2.81	1.48	3.89	21
3-HG	Oligo II	+	55	0.96	1.31	19.35	12
4-HÖ	Astro I	-	67	2.08	1	3.85	20
5-TA	Astro I	-	47	4.33	1	4.33	26
6-NA	Astro I	-	9	2.52	1	2.89	9
7-FE	Astro II	-	47	1.19	1	2.07	19
8-SP	Astro II	-	33	1.31	1	1.71	18
9-HE	Astro II	-	51	4.61	1	7.02	17
10-AE	Astro II	-	21	4.00	1	16.14	17
11-SA	Astro II	-	32	1.66	1	4.37	11
12-HÇ	Astro II	+	37	3.25	1.82	4.62	19
13-ÇF	Astro II	+	60	7.02	1.73	7.02	15
14-NG	Astro II	+	52	4.62	1.69	6.25	12
15-OB	Astro II	+	35	11.74	1.18	14.03	9
16-İC	Astro II	+	48	1.53	1.93	1.53	9
17-DK	Astro IV	+	72	17.39	1	18.32	9
18-SN	Astro IV	+	49	2.38	1	2.71	5
19-SSA	Astro IV	+	49	11.14	1.55	11.14	2
20-NU	Astro IV	+	43	2.75	1.85	5.13	5
21-HK	Astro IV	+	57	25.91	1.40	59.89	9
22-TÖ	Astro IV	+	56	35.47	1.65	35.47	14
23-NG	Astro IV	+	54	18.49	1.30	18.49	10
24-MB	Astro IV	+	58	4.34	1.74	6.45	9
25-AG	Astro IV	+	51	6.78	1.78	1.12	9
27-CY	Pil. Astro	-	13	0.64	1	0.64	16
26-BT	Pil. Astro	-	13	2.20	1	3.45	24
28-SA	Pil. Astro	-	6	0	1	1.67	9
29-ZG	Mal.Epend	+	17	6.60	1.37	6.60	9
30-HS	Mal.Epend	+	26	22.93	1.61	22.93	9

dose of 45 Gy was applied to the whole brain for 6-7 weeks. The chemotherapeutic agent CCNU (Lomustine) was used for at least two 8-week cycles.

Flow Cytometry Technique:

A tissue sample of approximately 500 mg was placed in 0.9% NaCl immediately after each patient's surgery. In preparation for flow cytometry, each specimen was removed from the saline solution, placed in a special medium (RPMI 1640, Sigma), and mechanically fragmented to suspend the cells. Next, 2 ml of 0.5% pepsin-HCl was added, and the mixture was continuously agitated for 5-10 min at room temperature to produce a single-cell suspension. The full volume of the single-cell suspension was transferred to another tube and allowed to rest for 5 min at room temperature. It was then centrifuged twice (250 g for 10 min each time), the supernatant was removed, and the resultant solution was washed with citrate buffer (BDIS, California, USA). The cells in this solution were initially counted in a cell counter (STKS, Coulter), after which the concentration was adjusted to approximately 2×10^6 tumor cells/ml.

A 250-µl aliquot of the tumor cell solution was transferred to a new tube. In a second, tube, another 150-µl aliquot of the cell solution was combined with 100 µl of a separate solution that contained an equal concentration of normal cells taken from healthy individuals as a control. Both of the tubes were centrifuged (250 g for 5 min), the supernatant was drawn off, and 250 µl of L Trypsin solution (Cycle test plus DNA reagent kit, BDIS, USA) was added to each pellet. The reaction was allowed to progress undisturbed for approximately 10 min at room temperature. Next, 200 µl of Trypsin inhibitor containing RNAase (Cycle test plus DNA reagent kit, BDIS, USA) was added to each tube, followed by another wait of roughly 10 min at room temperature. Finally, 200 µl of cold propidium iodide (Cycle test plus reagent kit, BDIS, USA) was added to each tube, and the tubes were placed in the refrigerator for approximately10 min. Once cooled, the tubes were inserted in the flow cytometry apparatus and a DNA histogram was derived.

Statistical Analysis

Multivariate survival Kaplan-Meier and Cox regression analysis, the Student's t-test, ANOVA and Chi square testing were used to compare differences in survival among the various tumor types, and when tumors were grouped as aneuploid/diploid or low-grade/high-grade (SPSS for Windows, Version 10.0).

RESULTS

The follow-up period ranged from 5 to 31 months (mean, 16.4 months) for the diploid cases, and 2 to 21 months (mean, 10.2 months) for the aneuploid cases. Overall follow-up for all patients was 2 to 31 months, with a mean of 13.1 months. Fourteen of the 30 tumors were diploid (46.7%) (Figure 1) and 16 were aneuploid (53.3%) (Figure 2). The mean CV was 4.36%. None of the tumors was hypodiploid or tetraploid. The mean DI value for the aneuploid tumors was 1.56, and the individual index values in this group ranged from 1.18-1.93.

Of the grade IV astrocytomas, seven were aneuploid and two were diploid. The seven patients with aneuploid cell populations had a mean disease-free survival of 8 months (range, 2-14 months), and the two with diploid cell populations had the same mean disease-free survival time (range, 5-9 months).

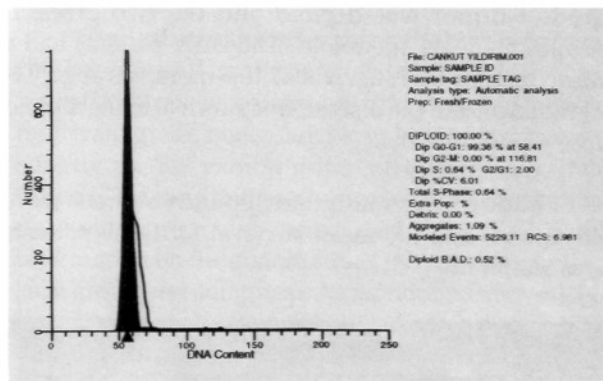

Figure 1: A DNA histogram showing a diploid cell population.

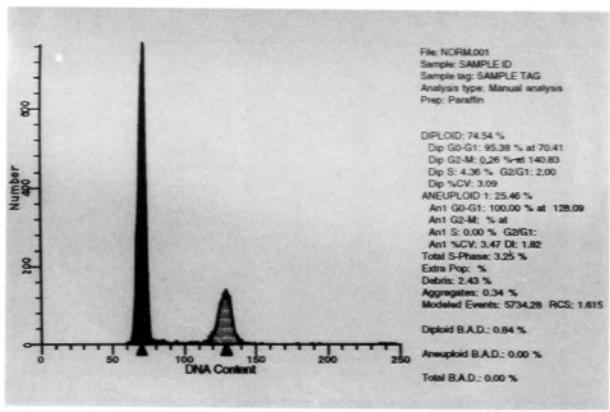

Figure 2: A DNA histogram showing an aneuploid cell population.

All nine of these patients died within 14 months of their surgery.

Of the 10 grade II astrocytomas, 5 were aneuploid and 5 were diploid. The patients with aneuploid tumors had a mean disease-free survival of 12.8 months (range, 9-19 months), and those with diploid tumor cells had a mean disease-free survival of 16.4 months (range, 11-19 months).

All three grade I astrocytomas were diploid, and the mean disease-free survival in this group was 18.3 months (range, 9-26 months). Figure 3 shows a preoperative computed tomography (CT) scan of one of the grade I astrocytoma patients, and Figure 4 is a scan of the same patient at 26 months postoperatively.

The pilocytic astrocytomas were also diploid in all three cases, and the mean disease-free survival in this category was 16.3 months (range, 9-24 months).

In the oligodendroglioma patients, the single grade I tumor was diploid and the two grade II neoplasms were aneuploid. The latter patients had a mean disease-free survival of 16.5 months (range, 11-21 months), and the disease-free survival in the diploid case was 31 months.

Both of the malignant ependymomas were aneuploid, and the mean survival for these patients was 9 months.

Our results showed no significant correlation between age and survival ($p = 0.803$, $r = -0.048$). The mean patient age in our study population was 47 years, and we observed a trend towards longer disease-free survival in individuals below this age. We have established the survival times of groups 1 (grade II oligodendroglioma), 2 (grade I astrocytoma), 3 (grade II astrocytoma), 4 (grade IV astrocytoma), 5 (pilosytic astrocytoma), 6 (malignant ependymoma), as 16.50 ± 4.50, 18.33 ± 4.97, 14.60 ± 1.26, 8.00 ± 1.16, 16.33 ± 4.33, 9.00 ± 0.00 respectively. The survival times were found to be significantly different among the groups. According to statistical analysis there were two different subset [4,6] and [1,2,3,5] ($p=0.015$).

When cases were grouped according to tumor ploidy, we found that recurrence occurred earlier and in more patients in the aneuploid group, and that there were only three cases of recurrence in the diploid group. The mean survival time in the diploid group was 28 months (95% confidence interval [CI] of 23 to 32 months), whereas mean survival in the aneuploid group was 11 months (95% CI of 9 to14 months). The diploid group survived significantly longer than the patients with aneuploid tumors ($p=0.0003$).

A significant relationship between S-phase fraction and patient survival time was found ($p=0.0192$). When cases were divided according to presence of low- and high-grade tumors, the mean S-phase fraction values were 3.15 ± 0.62 and 14.01 ± 3.28,

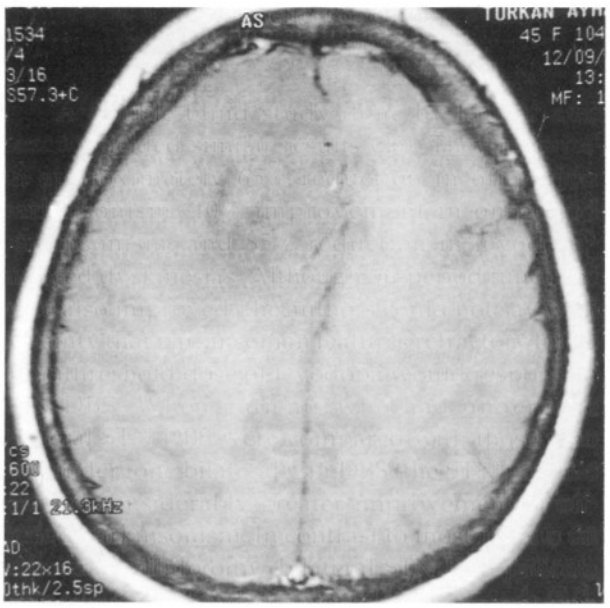

Figure 3: A preoperative CT scan of a patient with a grade 1 astrocytoma.

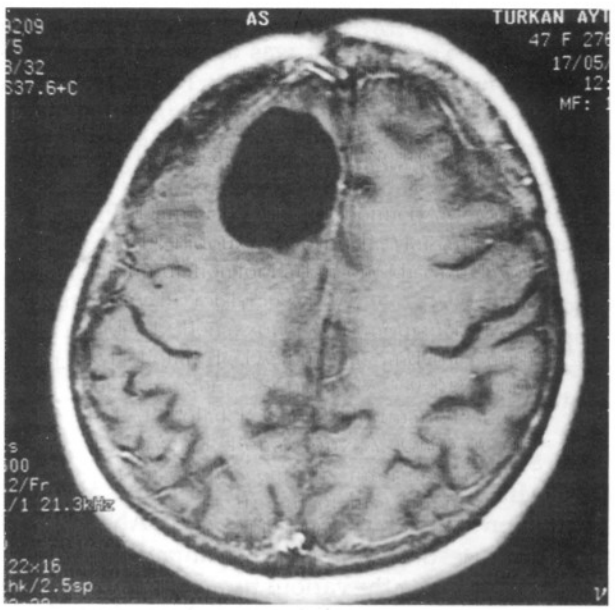

Figure 4: A postoperative CT scan of the same patient at 26th month of surgery.

respectively, it was found statistically significant (P=0.008). There was also a significant difference in the survival times for the low- and high-grade tumor groups (p=0.000).

When only the low- and high-grade tumors with DI =1 were compared, again there was a significant difference in S-phase fraction (p=0.023). Further, the mean survival time for patients with low-grade tumors of DI =1 was 25 months (95% CI of 21-29 months), whereas survival for those with high-grade tumors of DI=1 was significantly lower, at 8 months (95% CI of 6 to 10 months) (p=0.000). when the patients were grouped as low and high grade for pathology , DI=1 and DI>1 for DI. After cross tabulation, We also found a **statistical relation** between pathology groups and DI (p=0.017).

DISCUSSION

Currently, the prognosis for patients with neuroepithelial tumors is mainly determined on the basis of histological characteristics. However, none of the classification systems that are in use are very accurate for predicting clinical outcome, and more precise indices are needed (29). It is well known that patients with highly differentiated astrocytomas (particularly grade I tumors) have a more favorable prognosis than those who have poorly differentiated astrocytomas or glioblastoma multiforme. The latter patients survive for a significantly shorter time.

Zaprianov and Christov analyzed 62 astrocytoma cases using flow cytometry (38). They found diploid cell populations in the grade I astrocytomas, and noted aneuploid cell populations in grade III astrocytomas and glioblastoma multiforme tumors. The authors found that abnormal DI and high S-phase fractionation were strongly correlated with prognosis, in addition to glial tumor differentiation. Our findings also indicate that abnormal DI and S-phase fraction are important for prognosis in glial tumor cases.

In contrast, Struikmans and colleagues' multivariate analysis indicated that neither bromodeoxyuridine labeling index, S-phase fraction, nor age were significant prognostic factors in patients with these tumors (33). Another study reported that, in patients with grade II or III astrocytoma, age, grade of malignancy, DNA ploidy, and S-phase fraction were all independent prognostic factors (36). Other investigations have also found patient age to be prognostic (29,31); however, our results did not support the above mentioned data regarding age and disease-free survival.

Danova and coauthors investigated correlations between survival and parameters such as flow cytometric DNA analysis, age, gender, histologic type, and anatomic region affected in 153 neuroepithelial tumor cases (7). They noted the importance of histologic subtype as a prognostic factor, in addition to DNA ploidy. Our results indicated that histologic subtype is an accurate predictor for patient survival in association with flow cytometric DNA analysis.

Taylor et al. did a retrospective study of 89 cases of ganglioneuroma and neuroblastoma (34), and identified aneuploidy in 60% of the tumors. The authors established that the DNA analysis results were good predictors of tumor progression and maturation, and good bases for planning therapeutic strategy in their aneuploid cases. They also found a correlation between survival time and flow cytometric DNA analysis in aneuploid tumor cases. Our results concur with these findings.

Grade II astrocytomas are on the border between the benign and malignant states (40). Histologic investigations have shown that astrocytomas of moderate-grade malignancy can be divided into two subgroups that exhibit different degrees of cellular atypia (32). Using this grading system for astrocytomas, flow cytometry was introduced to give objective information on the quantitative DNA abnormalities in each group and subgroup. As mentioned above, DNA distribution analysis of solid tumors has been studied for 20 years. Recently, numerous clinical research studies have suggested that nuclear DNA content, as quantified by DNA flow cytometry, has important prognostic significance (15,16,17,19,39). Our results indicate that the DNA abnormalities in all astrocytomas except for the grade II type are strongly correlated with morphological grade of malignancy.

Some reports have indicated that grade I astrocytomas are composed of strictly diploid cell populations (12,13,18,20,23,24,26,39), whereas the ploidy abnormalities in grade II and III astrocytomas can only be established with certainty in 30-60% of cases (12,22). Our study group did not include any patients with grade III astrocytoma. Two patients with glioblastoma multiforme had diploid tumor cell populations, which is in line with the findings in Zaprianov and Christov's series (39). However, our two glioblastoma multiforme patients who had diploid tumor cell populations did not survive as long as the individuals in that series. In their group of 24 patients

with grade II astrocytomas, Zaprianov and Christov found diploid tumor cells in 12 of the patients, and aneuploid cell populations in the other 12 and noted statistically longer survival in their diploid group with grade II astrocytomas (39). The authors explained this flow cytometric difference in two ways: first, they remarked that the histological peculiarities of some astrocytomas (5,40) make it difficult to accurately determine the degree of differentiation; and second, they noted the differences in tumor ploidy. In our study, five of the grade II astrocytomas were diploid and five were aneuploid. There was no tumor recurrence in the diploid group until 16.4 months of follow-up, whereas the mean recurrence time in the aneuploid group was 12.8 months. There was no statistical difference in the survival times for these two grade II astrocytoma groups, but the patients with diploid tumor cell populations survived longer than the aneuploid group. This difference was not statistically significant, however, and we attribute this to the small number of patients that were studied.

Our results indicate that patients with aneuploid cell clones in the neoplasms we studied survive for a shorter time than patients with diploid cell clone populations (p=0.0158). These results support recent data that has been reported for glial tumors (4,30,39) and other human tumors, such as mammary adenocarcinoma and non-small cell lung carcinoma (1,7,37). The percentage of S-phase fraction is an important indicator of the proliferative activity of meningioma and glial tumor cell populations (3,12,39). Many authors have suggested that the percentage of S-phase fraction in human tumors is an important prognostic indicator (13,20,37). Our flow cytometry results support this claim.

In conclusion, histological subtype alone is not an accurate predictor of disease-free and overall survival in patients with glial tumors. The added information from flow cytometric DNA analysis is necessary for determining prognosis in this group.

This study was presented as an oral presentation at the National Neurosurgery Congress in May 2000.

Correspondence: Yrd. Doç. Dr. Celal Bağdatoğlu
Mersin Üniversitesi Tıp Fakültesi
Nöroşirurji ABD
Mersin / Türkiye
Tel: + 90 324 336 77 32
Fax: + 90 324 337 36 87

REFERENCES

1. Auer G, Eriksson E, Azavedo E, et al: Prognosticsignificance of nuclearDNA content in mammary adenocarcinomas in humans.Cancer Res 44: 394–396,1984
2. Barlogie B, Gohde W, Johnson DA, et al.:Determination of ploidy andproliferative characteristics of human solid tumors bypulsecytophotometry. Cancer Res 8: 3333–3339,1978
3. Barlogie B, Raber MN, Schumann J, et al: Flow cytometry in clinicalcancer research. Cancer Res 43: 3982–3997,1983
4. Ben Arush MW, Linn S, Ben-Izhak O, et al:Prognostic significance ofDNAploidy in childhood astrocytomas. Pediatr HematolOncol 16(5): 387–96,1999
5. Daneshbod K, Yusefi M: Rapid frozen sectiondiagnosis of centralnervoussystem tumors : Study of 230 cases. Pathol Res Pract180, 3,262-266,1985(Abstract)
6. Danova M, Ricardi A, Mazzini G, et al: Ploidy andproliferativeactivityof human brain tumors. A flow cytometric study.Oncology 44:102–107,1987
7. Danova M, Giaretti W, Merlo F, et al: Prognosticsignificance ofnuclearDNA content in human neuroepithelial tumors. Int JCancer 48,663–667,1991
8. Dean PN: A simplified method of DNA distribution analysis. CellTissueKinet 13: 299–308,1980
9. Douglas EM, Dressler LG, McGuine WL: Flowcytometry, cellular DNAcontentand prognosis in human malignancy. J of Clin Onc5(10),1690–1703,1990.
10. Douglas EM, McGuire WL: Ploidy, proliferative activity andprognosis.DNA flow cytometry of solid tumors. Cancer 65: 1194–1205,1990
11. Dressler LG, Seawer L, Owers MA, et al: Evaluationof a modellingsystemfor S-phase estimation in breast cancer by flowcytometry. Cancer Res47: 5294–5302,1987
12. Feichter G, Schwechheimer K, Mylz H, et al: Assistierendeimpulszytometrische beurteilung bei derroutine-diagnostik von meningiomenund von gliomen. Pathologie 4, 294–297,1983
13. Frederiksen P, Reske-Nielsen E, Bichel P: Flowcytometry in tumorsofthe brain. Acta Neuropath. (Berlin) 41, 65–69,1979
14. Friedlander ML, Hedley D, Taylor IW: Clinical and biologicalsignificance of aneuploidy in human tumors. J. ClinPathol. 37; 961–974,1984
15. Hedley DW, Friedlander ML, Taylor IW: Preparationof tissues for DNAflow cytometric analysis. Cytometry 1: 229–232, 1981
16. Hedley DW, Friedlander ML, Taylor JW: Method foranalysis ofcellularDNA content of paraffin-embedded pathological materialusing flowcytometry.J Histochem Cytochem 31: 1333–1338, 1983
17. Gaetani P, Danova M, Butti G, et al: Cell kineticaspect of humanneuroepithelial tumors: a follow-up study. Tumori 74,145–150, 1988

18. Hoshino T, Nomura K, Wilson CB: The distributionof nuclear DNAfromhuman brain tumor cells. J Neurosurgery 49, 179–186,1978
19. Kasai H, Kawamoto K: Cytogenical analysis of braintumors by FISH(fluorescence in situ hybridisation) and FCM (flowcytometry). NoshuyoByori12(1): 75–82, 1995 (Abstract)
20. Kawamoto K, Herz F, Wolley RC: Flow cytometricanalysis of the DNAdistribution in human brain tumors. Acta Neuropath(Berlin) 46:39–43, 1979
21. Koss LG, Czernyak B, Herz F, et al: Flowcytometric measurement ofDNAand other cell components in human tumors: A criticalappraisal. HumanPathology 20(6):528–548, 1989
22. Laerum OD, Forsund T: Clinical application of flowcytometry: Areview.Cytometry 2(1): 1-13, 1981
23. Lehmann J, Krug H: Flow-throughfluorocytophotometry of differentbraintumors. Acta Neuropath (Berlin) 49: 123–132, 1980
24. Mathev P, Look T, Luo X, et al: DNA index of glialtumors inchildren.Correlation with tumor grade and prognosis. Cancer 15;78(4):881–6, 1996
25. Mcdivitt RW, Stone KR, Craig RB, et al. A comparison of humanbreastcancer cell kinetics measured by flow cytometry and thymidinelabelling. LabInvests 52:287–291, 1985
26. Mörk SJ, Laerum OD: Modal DNA content of humanintracranialneoplasmsstudied by flow cytometry. J.Neurosurgery53(2):198-204, 1980
27. Nishizaki T, Orita T, Furutani Y, et al: Flowcytometric DNAanalysisand immunohistochemical measurement of Ki-67 and BudR labelling indicesinhuman brain tumors. J Neurosurgery 70:379–384, 1989
28. Nishizaki T, Ohshita N, Nagatsugu Y, et al:Clinical evaluation ofDNAindex in human brain tumors. J of Neuro-oncology 17:9–13, 1993
29. Perry A, Jenkins RB, O'Fallon JR, et al: Clinicopathologic study of 85 similarly treated patients with anaplastic astrocytictumors. An ananalysisof DNA content (ploidy), cellular proliferation, andp53 expression.Cancer15; 86(4): 672–83, 1999
30. Salmon I, Kiss R, Dewitte O, et al:Histopathologic grading and DNAploidy in relation to survival among 206 adultastrocytic tumorpatients.Cancer 15; 70(2): 538–46, 1992.
31. Salmon I, Dewitte O, Pasteels JL, et al:Prognostic scoring inadult astrocytic tumors using patient age, histopathologicalgrade, and DNAhistogram type. J Neurosurgery 80(5): 877–883, 1994
32. Singer H, Zang KD: Cytologische und cytogenetischeuntersuchugen ahirntumoren. Humangenetik 9(2): 172-184, 1970
33. Struikmans H,Rutgers DH, Jansen GH, et al.Prognostic relevance ofcell proliferation markers and DNA-ploidy in gliomas. ActaNeurochir (Wien)140(2): 140–7, 1998
34. Taylor A, Leviton A, Fulchiero A: Flow cytometricanalysis of DNA content in neuroblastoma tumors. ASCP Weekend ofPathology July;p 64, 1989
35. Vavruch L, Enestrom S, Carstenser J, et al: DNAindex and S-phaseinprimary brain tumors. A comparison between fresh anddeparaffinizedspecimens studied by flow cytometry. J of Neurosurgery80: 85–89, 1994
36. Vavruch L, Nordenskjold B, Carstensen J, et al:Prognostic value offlowcytometry and correlation to some conventionalprognostic factors: aretrospective study of archival specimens of 134astrocytomas. JNeurosurg85(1): 146–51, 1996 Jul
37. Volm M, Hattern J, Vogt-Schaden M, Wayss K: DNAdistribution innon-small-cell lung carcinomas and its relationship toclinicalbehaviour.Cytometry 6: 348–356, 1985
38. Zaprianov Z, Christov K: Flow cytometry in braintumors. 1. Ploidyabnormalities. Neoplasma 33(1): 49–55, 1986
39. Zaprianov Z, Christov K: Histological grading, DNAcontent, cellproliferation and survival of patients with astroglialtumors.Cytometry9: 380–386, 1988
40. Zülch KJ: Histological typing of tumors of thecentral nervous system. Geneva: World Health Organization 1979.

COMMENT

In this report, authors have used flow cytometriy to predict the prognotic value in evaluating glial tumors not only in low grade astrocytomas but also in high grade gliomas. That is why it would be better if the paper's title is "The prognotic value of flow cytometric DNA analysis in glial tumors". Clinical correlations of aneuploidy in astrocytomas are still contrversial. Several authors agree on the prognestic value of flow sytometry parameters in evaluating astrocytomas, namely S-phase fraction and DNA ploidy. However, some others didn't find any correlation between flow cytometry parameters and clinical outcome in patients with malignant neuroepithelial tumors. In this study, unfortunately because of the small number patient, it is diffucult to come to decision on this subject.

Prof. Dr. İzzet ÖVÜL