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The Influence of Volumetric Tumor Doubling Time, DNA Ploidy, and Histologic Grade on the Survival of Patients with Intracranial Astrocytomas

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PURPOSE: To improve the prediction of individual survival in patients with intracranial astrocytomas through the analysis of volumetric tumor doubling time (VD_t) and DNA ploidy. **METHODS:** A pilot study was retrospectively conducted on a group of 25 patients with intracranial astrocytomas in whom recurrent and/or progressive disease was observed on serial contrast-enhanced CT or MR examinations. VD_t was computed using two or more data points from a semilogarithmic plot of tumor volume versus time. Size-adjusted survival was calculated using a method based on VD_t and initial tumor volume to decrease the lead time bias attributable to differing tumor sizes at presentation. **RESULTS:** Slower VD_t was associated with significantly longer survival and size-adjusted survival as determined by a univariate Cox proportional hazard analysis. Aneuploidy was a significant indicator of poor survival. Aneuploid and multiclonal astrocytomas had poor size-adjusted survivals compared with diploid astrocytomas. Grade IV astrocytomas had significantly poorer survival and size-adjusted survival compared with lower grades (I to III), which individually were not significantly correlated. However, grade IV histology was not a significant independent predictor of size-adjusted survival in a multivariate Cox model, whereas VD_t and DNA ploidy remained significant. VD_t also had a significant direct linear correlation to survival and size-adjusted survival. **CONCLUSIONS:** VD_t and DNA ploidy were more sensitive than histologic grading as indicators of individual survival. Initial tumor size needs to be considered when staging and assessing survival in patients with intracranial astrocytomas.

Index terms: Astrocytoma; Brain, neoplasms

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The survival of patients with intracranial astrocytomas has been studied extensively with respect to histologic grade, DNA ploidy, proliferative index (as measured by bromodeoxyuridine and thymidine labeling indices and flow cytometry), and more recently by oncogene analysis (1–21) in an attempt to improve prognostic accuracy. Despite improved methods for determining prognosis from histologic grading of astrocytomas in groups of patients (1, 2), the outlook for an individual patient has remained uncertain. The use of DNA ploidy as measured by flow cytometry and image cytometry in conjunction with histologic grade to predict individual survival is a subject of controversy in the literature (3–11), and other studies have shown an inconsistent relationship between labeling indices of cellular proliferation and survival (12–17).

Two studies have attempted to correlate volumetric tumor doubling time (VD_t) with histologic grade, with limited success (22, 23). Our study focused on a group of 25 patients with intracranial astrocytomas that radiographically demonstrated growth of the primary and/or recurrent tumor after initial resection

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and adjuvant therapy. Our purpose was to correlate survival, DNA ploidy, and histologic grade with VD_t in an effort to improve prognostication and understanding of the cellular dynamics in patients with intracranial astrocytomas.

Materials and Methods

Patient and Specimen Selection

Twenty-five patients with intracranial astrocytomas who had serial computed tomography (CT) or magnetic resonance (MR) examinations demonstrating residual and/or recurrent tumor were retrospectively studied in cooperation with the Division of Neuroradiology at Stanford University Hospital and the Department of Pathology (Neuropathology Section) of the University of California at Davis. Twenty-four patients underwent primary resection and/or diagnostic biopsy of their brain tumors from July 1979 to April 1991. One additional patient (patient 4) did not undergo biopsy or therapy but was included, because the clinical presentation, course, and radiographic appearance were consistent with a high-grade astrocytoma (glioblastoma multiforme). The average age was 43.5 years with a range of 5 months to 76 years; there were a total of 15 female and 10 male patients; 23 patients had supratentorial astrocytomas, one a cerebellar astrocytoma, and one a pontine astrocytoma.

Histology and DNA Image Cytometry

Eighteen patients had their primary histologic material available for review by W.E. and S.S., who were blinded to the results of previous pathologic interpretation, DNA ploidy, VD_t, and survival. Six patients were initially evaluated by the Division of Neuropathology at Stanford University Hospital; however, no paraffin-embedded tissue from their primary biopsy or resection was available for review. One patient (patient 4) did not undergo biopsy but was included in the study as mentioned above. Histologic grading followed the criteria of Dumas-Duport et al (1), which emphasize the presence of mitoses, necrosis, and endothelial proliferation. In this study, pilocytic astrocytomas were classified as grade I along with fibrillary astrocytomas that lacked all criteria for anaplasia. The designation *gemistocytic astrocytoma* indicated that the majority of the neoplastic astrocytes had a gemistocytic form. The reference histologic diagnosis used for each case was based on the primary surgical biopsy and/or resection. Seventeen patients had sufficient paraffin-embedded material from their primary tumors for determination of DNA ploidy. DNA image cytometry was used for the estimation of DNA ploidy in a technique described by Teplitz et al (24). The DNA histograms were interpreted in a blinded fashion by R.L.T. Studies were performed on 7-μm sections of suitable paraffin blocks, stained specifically for DNA by the Cell Analysis (CAS, Elmhurst, Ill) procedure and examined with the CAS model 200 image cytometer. A 280-nm filter was used with a 20-nm band pass and the CAS thickness correction program. To avoid the possibility of detecting only clonal expansion within these tumors, a minimum of 25 nuclei were analyzed on four separate quadrants of each tumor. No cases were included when the minimum number of determinations (100 determinations) could not be obtained. In most cases several hundred nuclei were quantitated. DNA ploidy results were classified in the following manner: D, diploid, that is, normal somatic cell DNA content (7.18 pg/cell); E, euploid, that is, diploid or an even multiple of diploid; A, aneuploid, that is, abnormal somatic cell DNA content; and MC, multiclonal, that is, two or more major clones.

Radiographic Tumor Volume Estimation and Calculation of VD_t

Eleven patients had contrast-enhanced serial CT examinations to determine residual and/or recurrent tumor growth. Nine patients had serial MR examinations, five with serial T1-weighted images (600–800/20/2 [repetition time/echo time/excitations]) with or without gadolinium enhancement and four with serial T2-weighted images, (2000–2500/30, 80/2), and five patients had a combination of contrast enhanced CT and/or MR T1-weighted images with or without gadopentetate dimeglumine. The margins of only enhancing solid tumor tissue as seen on CT or MR imaging were used for determination of tumor volume. When only T2-weighted images were available, vasogenic edema (ie, white matter edema) was not included in the volume determinations. Although the actual margins were more difficult to determine on nonenhanced T2-weighted MR images, we were able to define the bulk of a tumor based on its abnormal signal and mass effect. A similar set of methods for determination of tumor margins for radiation therapy planning has been described by Galloway et al (25) and others (26, 27).

The calculation of the volume of gross tumor was done retrospectively from the images of different scanners using the method described by Breiman et al (28). Briefly, the cross-sectional area of a lesion was calculated by planimetry. The absolute cross-sectional area of each square (in centimeters) was determined from the centimeter scale on the filmed image. Tumor volumes were calculated by multiplying the absolute cross-sectional area on each MR or CT section by section thickness and summing the resultant products. To adjust for intersection gaps on MR scans, the average cross-sectional area of two contiguous sections was multiplied by the section gap thickness; the product was then added to the total volume. Tumor volumes were performed by F.B., who was blinded to the histologic grade, DNA ploidy, and survival data.

Volumetric tumor doubling time of the residual primary or recurrent disease was calculated using two or more data points separated by at least 1 month from a semilogarithmic plot of tumor volume versus time as described by Collins (29) and others (30–32). Only periods in which there was clear tumor progression demonstrated on serial imaging studies before or between tumor resections were used for the calculation of VD_t. Patients with three or more data points had VD_t calculated from the slope of the best-fit line generated by a simple linear regression. Ten patients had two serial volume determinations, eight had three ($r \geq .789$), and two had six ($r \geq .972$). Five patients had two or more periods of clinical progression or disease recurrence separated by surgical resection and/or radiation therapy in which the resultant VD_t of the recurrent and/or residual disease of each time period was averaged. The intervals in which it was possible to measure VD_t were as follows: 11 patients after primary resection and radiation therapy, three after a second resection, one after the third resection, three after primary radiation therapy without resection, two before and after radiation therapy without resection, two after the primary and third resections, one after the primary and second resections, and two patients after radiation therapy only.

Size-adjusted survival was computed using a method previously described by Collins et al (29) and others (30–32) to eliminate the lead-time bias for primary tumors of differing sizes at diagnosis. The volume of each tumor at diagnosis was converted into the approximate number of tumor doublings from its theoretical origin as a single neoplastic cell (assuming an average cell diameter of 10 μm). An arbitrary number of 30 tumor doublings (a 1-cm-diameter tumor) was subtracted from the observed volume of each tumor at diagnosis (also expressed as the number of tumor doublings). A volume of 30 doublings was chosen, because this usually represents the smallest size at which a central nervous system tumor can be detected clinically (33). This was then multiplied by VD_t, and the resultant product (ie, the extrapolated number of months that were needed for a tumor to reach the observed size at diagnosis from an initial size of 1 cm) was then added to the observed survival to obtain size-adjusted survival. The volume of tumor at death was extrapolated in a similar fashion from the last radiographically determined tumor volume after completion of all therapy to the time of death. The formulas for the calculation of the number of tumor doublings, size-adjusted survival, and estimated tumor volume at death are given in the Appendix.

Statistical Analysis

Cox proportional hazard models were fitted for survival and size-adjusted survival using various subsets of the possible predictor variables as covariates. Likelihood ratio tests were performed for assessing the significance of models, whereas Wald statistics and coefficient/SE were used to evaluate the significance of particular coefficients in models involving more than one covariate by comparison with standard normal quantities. Survival analyses were performed using S-PLUS software (Statistical Sciences). All *P* values are based on asymptotic approximations. Survival, size-adjusted survival, VD_t, and tumor volume were all converted to natural logarithms before computation of their respective averages mentioned in the text assuming log normal distributions for each of the above quantities, according to Spratt et al (34, 35). The average values mentioned in the text represent the geometric means of survival, size-adjusted survival, and VD_t. Comparisons of averages (ie, geometric means) were performed using a two-tailed Student's *t* test. Linear regressions of survival and size-adjusted survival on VD_t were calculated by the method of Buckley and James for regression with censored data, that is, patients still alive at the completion of the study (36), using a function written in S-PLUS by one of the authors (D.B.B.).

TABLE 1: Analysis of survival and size-adjusted survival with respect to VD_t, histologic grade, and DNA ploidy

Univariate Models	Survival, *P*	Size-adjusted Survival, *P*
Tumor growth rate		
VD_t	.02	<.001
Histology		
Grades I–IV	.007	.03
Grades I–III (excluding IV)	.77, NS	.47, NS
Grade IV (vs non–Grade IV)	.007	.006
DNA ploidy		
Diploid, euploid, aneuploid, and multiclonal	.14, NS	.007
Aneuploid (coeff)*	.05	.003
Multiclonal (coeff)	NS	.02
Best-fit multivariant models		
VD_t and Grade IV	.001	···
VD_t (coeff)	.04	···
Grade IV (coeff)	.01	···
VD_t and diploid	···	<.001
VD_t (coeff)	···	<.001
Diploid (coeff)	···	.03

* Coefficient of significance of a single variable in models involving more than one covariate. NS indicates not significant.

Results

Survival

Overall, VD_t and the presence or absence of grade IV histology were the most important predictors of (unadjusted) survival in the univariate and as multivariate Cox models (Table 1). Longer survival was seen in patients with longer VD_t (ie, slowly growing tumors), and shorter survival was seen in patients with grade IV tumors. A univariate Cox model including only grade I to III tumors, excluding grade IV astrocytomas, did not show any significant differences between the survival of each grade (nb, patients evaluated as grades I–II were considered grade II in our analysis). The presence of aneuploidy was of borderline significance with respect to survival in a univariate model, but this did not persist after the adjustment of the other covariates in a multivariate model.

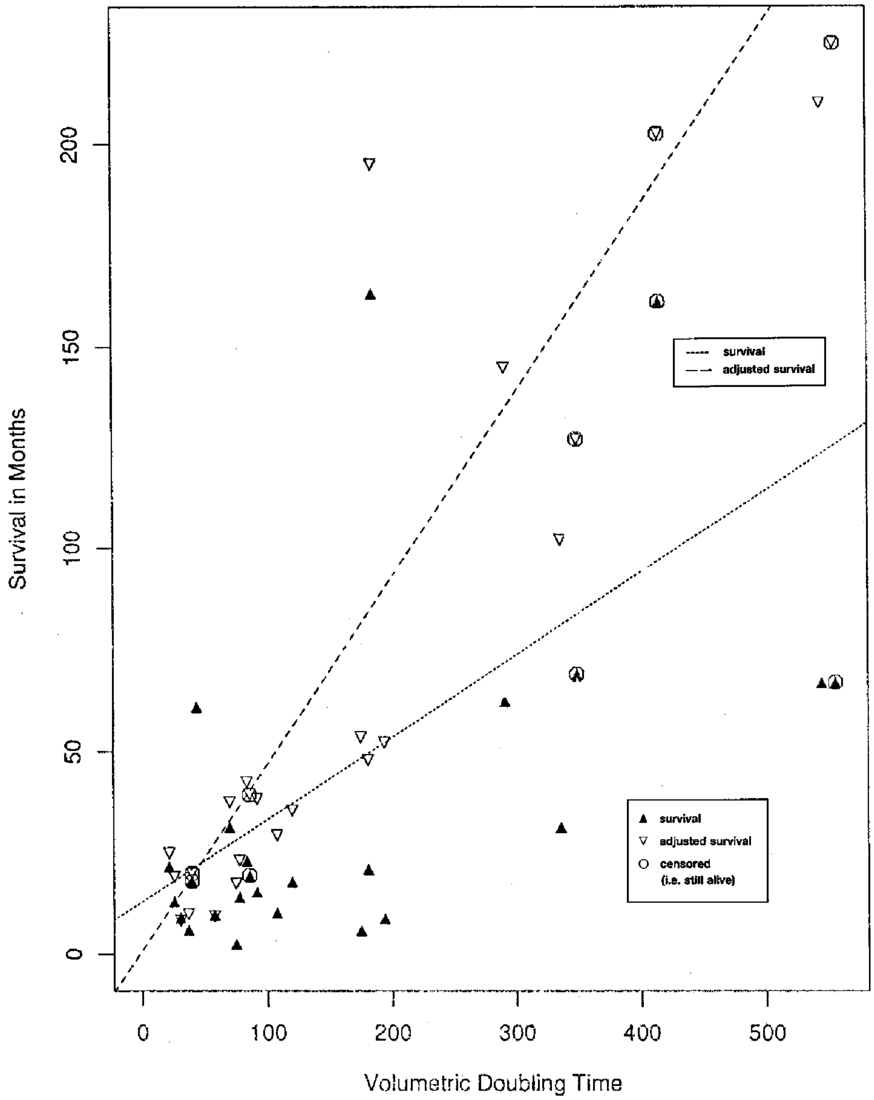

Fig 1. Relationship of survival time (*solid triangle*) and adjusted survival (*inverted triangle*) in months, to VD_t in days for each patient. Also shown are fitted (Buckley-James) regression lines for both survival responses. Censored patients (ie, patients who were still alive at the end of the study) are *circled*.

Size-Adjusted Survival

The analysis of size-adjusted survival was performed on 24 of 25 patients, because one patient (patient 6) did not have sufficient documentation of the size of the primary tumor available.

Overall, VD_t and DNA ploidy were the most important predictors of size-adjusted survival both in the univariate and multivariate Cox models; long VD_t and tumor diploidy were correlated with a reduced hazard (see Table 1). The presence or absence of grade IV histology was of significance in a univariate model, but this did not persist after adjusting for the other covariates in a multivariate model. In addition, a univariate model including only grade I to III astrocytomas did not reveal any significant differences among the size-adjusted survivals of each grade.

VD_t and Tumor Volume

There was a significant linear relationship between VD_t and survival ($P = .0011$) and also with size-adjusted survival ($P < .001$) as shown in Figure 1. The five patients who were alive at the end of the study (censored data) were included in the analysis. The slopes of survival and size-adjusted survival lines obtained using Buckley and James' method were 6.09 ± 1.98 and 13.8 ± 1.89 tumor doublings, respectively (ie, number of tumor doublings = survival [or size-adjusted survival]/VD_t). More than one VD_t was able to be calculated in five patients at

TABLE 2: Measurement of VD_t during different clinical periods

Patient	Average VD_t, d	VD_t	Clinical Period
8	70	75 d (2 points)	pre–radiation therapy (5/84–12/84)
		79 d (3 points, $r = .878$)	7 mo after 1st radiation therapy (8/85–9/85)
		56 d (5 points, $r = .789$)	2 mo after 2nd radiation therapy (12/85–5/86)
13	92	102 d (3 points, $r = .971$)	6 mo after 1st resection/radiation therapy (12/88–4/89)
		84 d (4 points, $r = .936$)	1 mo after 2nd resection/radiation therapy (4/89–8/89)
17	181	182 d (2 points)	during and immediately after radiation therapy (7/86–10/86)*
		181 d (2 points)	4 months after radiation therapy (1/87–8/87)
22	349	327 d (2 points)	4 mo after 1st resection/radiation therapy (7/88–7/89)
		370 d (4 points, $r = .977$)	5 mo after 3rd resection (7/90–12/91)
25	556	530 d (4 points, $r = .88$)	2 mo after 1st resection/radiation therapy (8/87–6/88)
		582 d (3 points, $r = .863$)	1 mo after 3rd resection (7/89–4/91)

Note.—Patient 17 did not undergo a primary resection of tumor.

different periods of tumor progression (primary or secondary tumor) as listed in Table 2. In these patients, VD_t of the primary or recurrent disease changed little despite intervening resection and/or radiation therapy over a time interval ranging from 8 to 44 months as shown in Figure 2.

Diploid and euploid tumors had a significantly longer average VD_t ($P < .001$) compared with aneuploid and multiclonal astrocytomas (Table 3). No tumor in the diploid group had a VD_t of less than 84 days. Conversely, no tumor of the aneuploid and multiclonal groups had a VD_t greater than 92 days (Table 4). Grade IV

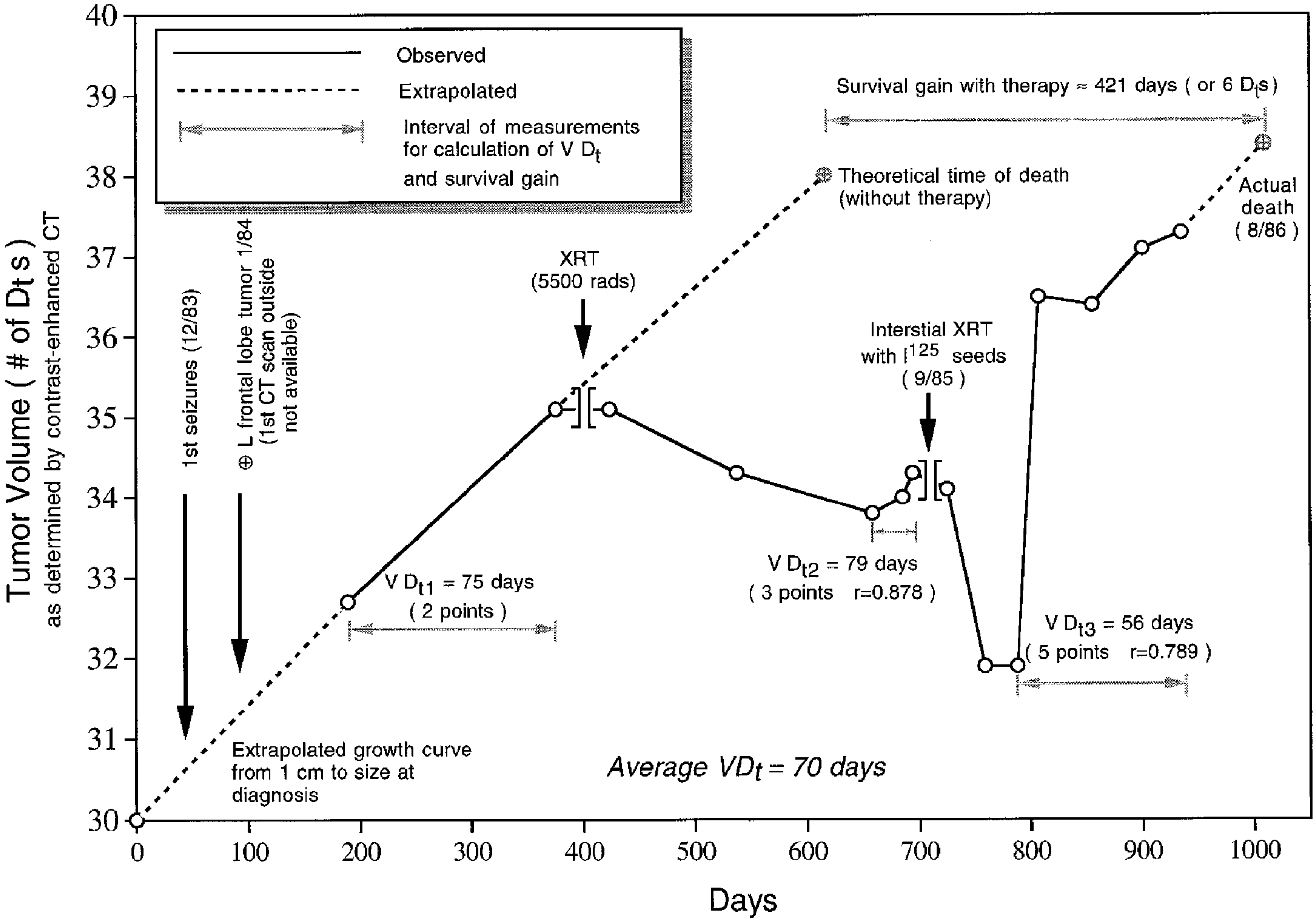

Fig 2. Tumor volume measurements of patient 8 from diagnosis to death. *XRT* indicates radiation therapy.

TABLE 3: Histologic grade and DNA ploidy correlated to VD_t and tumor volume

Group	Average VD_t, d	Average, in VD_t
All patients	110.3 (105)*	4.7 ± 0.94, n = 25 (4.65 ± 0.82, n = 16)*
Grades I–II	145 (108.3)	4.98 ± 0.93, n = 9 (4.69 ± 0.45, n = 4)
Grade III	140 (173.7)	4.94 ± 1.25, n = 7 (5.16 ± 1.23, n = 4)
Grade IV	69.7 (80.4)†	4.24 ± 0.77, n = 9 (4.38 ± 0.68, n = 8)
Diploid and euploid	179 (156)‡	5.19 ± 0.59, n = 11 (5.05 ± 0.39, n = 8)
Aneuploid and multiclonal	44.3 (48.9)	3.79 ± 0.60, n = 6 (3.89 ± 0.63, n = 4)
Group	Average Tumor Volume, cm^3	Average No. of Tumor Doublings
At diagnosis		
Supratentorial astrocytomas	20.5 (22.1)*	35.3 ± 2.4, n = 22 (35.4 ± 2.47, n = 16)*
Grades I–II	19.2 (15.6)	35.2 ± 2.29, n = 8 (34.9 ± 2.26, n = 4)
Grade III	19.2 (19.2)	35.2 ± 3.48, n = 6 (35.2 ± 4.2, n = 4)
Grade IV	26.6 (29.1)	35.7 ± 1.66, n = 8 (35.8 ± 1.71, n = 8)
At death		
Supratentorial astrocytomas	181 (165)*	38.4 ± 0.87, n = 17 (38.3 ± 1.08, n = 16)*
Grades I–II	233 (165)	38.8 ± 1.31, n = 5 (38.3 ± 0.37, n = 4)
Grade III	203 (203)	38.6 ± 1.34, n = 4 (38.6 ± 1.34, n = 4)
Grade IV	143.5 (143)	38.1 ± 1.26, n = 8 (38.1 ± 1.26, n = 8)

Note.—Numbers in parentheses are averages of the 16 patients with supratentorial gliomas with both preoperative and antemortem scans.

* Only 16 patients with supratentorial astrocytomas had scans available after last therapy before death in addition to scans before resection and/or radiation therapy of the primary tumor.

† Not significantly different, $P = .08$ ($P < .10$) from grades I and II.

‡ Significantly different, $P < .001$ ($P < .005$) from the aneuploid and multiclonal group of astrocytomas.

tumors tended to have a shorter average VD_t than grade I and II astrocytomas, but this difference was not significant. In addition, there were no significant differences between the average VD_t of grade IV tumors compared with grade III tumors or non–grade IV tumors. The two infratentorial tumors in our study demonstrated similar sizes at diagnosis compared with cerebral astrocytomas of approximately 35 tumor doublings. However, the two infratentorial astrocytomas demonstrated a markedly smaller final tumor size at death of 35.3 tumor doublings compared with the final volume of 38.4 tumor doublings for the cerebral tumor group (see Table 3).

Histologic Grade and DNA Ploidy

Of the 17 patients with DNA ploidy data, 6 patients (35.3%) had aneuploid or multiclonal tumors, 2 (11.8%) had euploid tumors, and 9 (52.9%) had diploid tumors (see Table 4). Of the seven patients with grade IV tumors, four (57%) had aneuploid or multiclonal tumors with an average VD_t of 45.1 days, and three (43%) were diploid with an average VD_t of 151 days. Of the four grade III patients, one (25%) had an aneuploid tumor with a rapid VD_t of 31 days, one (25%) had a euploid tumor with a relatively slow VD_t of 291 days, and two (50%) were diploid with an average VD_t of 126.5 days. Of the six grade I and II patients, one (17%) had a multiclonal tumor with a relatively rapid VD_t of 58 days, one (17%) had an euploid tumor with an extremely slow VD_t of 556 days, and four (66%) were diploid with an average VD_t of 161.6 days.

In the 12 patients in whom one or more biopsies were performed after initial resection, the relationship of DNA ploidy and histology with respect to time was unclear (Table 5). Generally when tumors recurred and were subsequently rebiopsied, they had progressed in histologic grade and in the degree of aneuploidy and multiclonality. One patient (patient 2), who initially had a grade IV tumor with an initially aneuploid DNA histogram, converted to a grade III diploid tumor after resection, radiation therapy, and systemic chemotherapy.

Discussion

To gauge therapy and advise patients with intracranial astrocytomas, an accurate measure of prognosis is needed. Histologic grading has not been adequate to determine individual outcome. Our study demonstrates that VD_t and DNA ploidy may be better prognosticators than histologic grade. The importance of the growth rate of residual or recurrent tumors in patients

TABLE 4: Patients with intracranial astrocytomas

Patient (Age at Diagnosis)	Initial Histology before Therapy	Primary Tumor Location	Survival, mo	Size-adjusted Survival, mo	VD_t, d	DNA Ploidy
1(58 y)	Grade IV	P	21.6	24.9	22	MC D/T_3T_4
2(28 y)	Grade IV (gemistocytic)	P	13.0	19.1	26	A Hypodiploid
3(77 y)	Grade III	T	9	8.3	31	A SubD/necrosis
4(72 y)	Grade IV*	T	6.0	9.9	37	···
5(40 y)	Grade III	P	>18	>20	40	···
6(36 y)	Grade II	F/T/P	61	61+	44	···
7(7 y)	Grade I–II	Pontine	9.7	9.4	58	MC D/T_3Sub T_3
8(52 y)	Grade I, oligodendro/astrocytoma	F	31.3	37.6	70	···
9(76 y)	Grade IV	T	2.5	17.5	75	···
10(43 y)	Grade IV	P	14.1	23.2	78	MC D/A/H
11(30 y)	Grade II	F	23	42.6	84	D D/5.72% T_4
12(8 mo)	Grade III	T/SS	>19.4	>39.5	86	D D/9/10% supra D
13(50 y)	Grade IV	F	15.4	38.4	92	MC D/A/H
14(51 y)	Grade IV	T/P	10.3	29.4	108	D D/11% - A
15(67 y)	Grade I	P/O	17.9	35.5	120	D D/6% supra T_4
16(61 y)	Grade IV	T	5.8	53.6	175	D D/a few >4N clones
17(71 y)	Grade IV	F/P	21.0	48.0	181	D D/sub T_3
18(52 y)	Grade III (gemistocytic)	P	163	195	185	D D/10% A
19(62 y)	Grade I	T	8.9	52.3	194	D D
20(32 y)	Grade III	F	62.4	144.9	291	E D/T_4/6.79% hyper T_4
21(35 y)	Grade III	Cerebellar	31.3	102	336	···
22(5 mo)	Grade I, pilocytic	SS	>69	>127	349	D D
23(45 y)	Grade I–II (fibrillary)	F	>161	>202.5	415	···
24(23 y)	Grade III	F(Bifrontal)	67	210	545	···
25(11 y)	Grade II	F	>67	>225	556	E E/<3% H

Note.—> indicates patient still alive; +, unknown size of primary tumor; %, percentage of a particular subclone in the total population of nuclei studied; MC, multiclonal tumor; A, aneuploid; D, diploid; T_3, triploid; T_4, tetraploid; E, euploid; H, hyperploid; F, frontal; P, parietal; O, occipital; T, temporal; and SS, suprasellar.

* Probable histology based on radiographic appearance.

with intracranial astrocytomas is underscored by the significance of VD_t with respect to survival and size-adjusted survival. Furthermore, there seems to be a significant direct relationship between VD_t and survival as well as size-adjusted survival based on our study. It is important to note, however, that our study dealt with patients who were not cured by primary therapy and who subsequently progressed or developed recurrent disease, the imaging of which allowed for the computation of VD_t. Therefore, our results cannot necessarily be generalized to all patients with intracranial astrocytomas, particularly the lower-grade astrocytomas, which may be cured by simple excision and postoperative radiation therapy.

Hoshino et al (15) and others (16), although concluding that both the size and the growth rate of astrocytomas were important determinants of survival, thought that radiographically determined VD_t did not truly represent tumor proliferative potential and, therefore, was not prognostic. Several reasons were provided, one of which was that astrocytomas tend to be poorly marginated and that the entire extent of a tumor cannot be reliably measured from CT and/or MR. However, others suggest that the determination of the location and volume of gross tumor, however, can be reliably defined by CT and/or MR (25–27). Furthermore, Yamashita and Kuwabara (22) found that the ratio of tumor volumes of gross tumor estimated from CT scanning was consistently similar to the ratio of tumor volumes determined at surgery. Estimation of microscopic infiltration and extension, manifested usually as nonspecific peritumoral white matter edema, however, can be challenging and, therefore, was not included in our volume determinations.

A number of prior studies of radiographically determined VD_t (of primary and metastatic breast and lung carcinoma) have demonstrated the following: (*a*) that VD_t is directly related to survival, time to recurrence, and the percentage of patients with recurrent disease at clinical follow-up; and (*b*) that VD_t and survival are distributed in an exponential fashion in a patient population (ie, both VD_t and survival have log-

TABLE 5: Histology and DNA ploidy in patients with one or more biopsies after initial resection

Patient	Pathologic Specimen	Date	Histology	DNA Ploidy
2	1st resection	12/87	Grade IV	A hypodiploid
	2nd resection	4/88	Grade III	D D
6	1st resection	1981	Grade II	· · ·
	2nd resection	9/85	Grade IV	MC D/T4/A
8	1st resection	1/84	Grade I, oligodendro/astrocytoma	· · ·
	2nd resection	8/85	Grade III	· · ·
10	1st resection	6/86	Grade IV (gemistocytic)	MC D/A/H
	2nd resection	2/87	Grade IV (gemistocytic)	· · ·
11	1st resection	2/89	Grade II	D D/5.72% T_4
	2nd resection	11/90	Grade IV	MC D/4% A and H
13	1st resection	6/88	Grade IV	MC D/A/H
	2nd resection	4/89	Grade IV	· · ·
16	1st resection	12/86	Grade IV	D D/A few > 4N clones
	2nd resection	3/87	Grade IV	MC D/T_3/T_4 ? octaploids
20	1st resection	4/85	Grade III	E D/T4/6.79% Hyper T_4
	2nd resection	7/89	Grade IV	MC D/T_3/T_4
21	1st resection	5/87	Grade III	· · ·
	2nd resection	3/89	Grade III	· · ·
22	1st resection	3/87	Grade I, pilocytic	D D
	2nd resection	8/89	Grade I, pilocytic	D D
	3rd resection	2/90	Grade I, pilocytic	· · ·
23	1st resection	9/79	Grades I–II (fibrillary)	· · ·
	2nd resection	8/84	Grade III (fibrillary)	D D/0.75% A
	3rd resection	7/87	Grade III	MC D/4.8% A and H
	4th resection	11/88	Grade III	MC D/T_4/A
25	1st resection	6/87	Grade II	E E (<3% H)
	2nd resection	6/88	Grade III	MC T_3/T_4/small supra T_4 population

Note.—Abbreviations are as in Table 4.

normal frequency distributions) (37–53). Because tumor growth rate is also an exponential function with respect to time, several authors have concluded that survival is primarily dictated by VD_t in patients in whom therapy is not curative (50, 51). This hypothesis is bolstered by the observation that VD_t is directly proportional to survival and the time to recurrence as noted in a recent critical review (54). Our current study establishes a link between radiographically determined VD_t and survival in patients with intracranial astrocytomas.

A point worth emphasizing is that our data both directly and indirectly demonstrate that VD_t must be relatively constant during the periods of clinical observation. When VD_t was able to be measured directly, over two or more periods of disease progression, it changed little despite intervals between estimates of VD_t of 8 or more months during which there was surgical resection and/or radiation therapy. The slope analysis of survival versus VD_t as depicted in Figure 1 provides an indirect confirmation of the relative constancy of VD_t over the clinical period of observation. If survival were linearly independent of VD_t, then the slope of the best-fit line generated by a linear regression analysis would be zero. However, the slope of survival versus VD_t is 6.09 ± 1.98 tumor doublings. Stated slightly differently, the slope is greater than 3 SD ($P = .0011$) above zero (ie, no linear association between VD_t and survival). The slope of adjusted survival, 13.8 ± 1.89 tumor doublings (ie, greater than 6 SD from zero), further underscores the fact that the linear relationship between VD_t and survival in our study is not coincidental.

The exceptions to the above were patients 6 and 18, who had unusually long survivals. Patient 6 initially had a grade II tumor, which recurred 5 years later as a rapidly enlarging mass (VD_t of 44 days), of which the patient quickly died. Presumably the recurrent tumor was a distinctly different (ie, dedifferentiated) clone with a rapid VD_t compared with the original tumor. Patient 18 had a relatively slowly growing grade III astrocytoma (VD_t of 185 days) who survived for an exceptionally long period. Presumably the initial therapy for this slowly growing tumor was markedly more cytoreductive compared with other cases in this series. A marked degree of cytoreduction coupled with a relatively slow VD_t could have accounted for a long survival.

At first glance our analysis seems to ignore the effect of therapy (surgery, radiation therapy, or chemotherapy); however, this is not the case. In fact, the cumulative amount of cytoreduction can be directly inferred when the VD_t, tumor size at diagnosis and at death, and survival are known. For example, survival, as expressed as the number of tumor doublings, should be equal to the difference between the volume of tumor at death and diagnosis, also expressed as the number of tumor doublings, if no therapy was undertaken. If these quantities are different (ie, the patient survived more tumor doublings than expected), then there must have been a reduction of tumor volume due to therapy in which the patient gained x number of tumor doubling times (x = survival [number of doublings] − tumor volume at death [number of doublings] − tumor volume at diagnosis [number of doublings]). Therefore, the average gain in number of tumor doubling times in our group was three tumor doublings (3.0 doublings = 6.09 doublings [slope] − 38.4 doublings [volume at death] − 35.3 doublings [volume at diagnosis]). This gain is equivalent to a 90% reduction in tumor volume. Because the average VD_t in our study was 110 days, the therapeutic gain of three doubling times is equal to 1 year of extended survival. The gain, in terms of time, however, would be greater for patients with longer VD_ts and far less for the rapidly growing (shorter-VD_t) tumors.

These arguments apply only to cerebral astrocytomas in which mass effect is usually the cause of death. The two infratentorial tumors in our group were similar to each other in size at the time of diagnosis and at the time of death, which may indicate that the invasion of vital brain stem structures by recurrent disease, not gross mass effect, was the cause of death.

In an individual patient, the benefit of a single therapeutic intervention can be estimated from the following example regardless of its anatomic location. For example, if a patient has an intracranial astrocytoma with an initial tumor volume of 30 VD_ts (0.523 cm^3) growing at a VD_t of 1 month, which recurs with a volume of 30 VD_ts 12 months after resection, then the reduction of tumor burden must have been equivalent to 12 VD_ts. A 12-VD_t decrease in tumor burden is equivalent to an approximately 0.999% reduction in tumor volume (ie, less than 0.0005 cm^3 of residual tumor after resection), which would be undetectable when the patient is scanned in the immediate postoperative period.

A surprising result of our study was the relative insensitivity of histologic grade with respect to survival. In a multivariate analysis, histology only became a significant independent predictor of survival if grade IV was compared with non–grade IV tumors. In contrast, VD_t (a continuous variable) based on a multivariant analysis was a highly significant independent predictor of survival. In addition, histology ceased to be a significant independent predictor as shown by the multivariate analysis of histology, DNA ploidy, and VD_t with respect to size-adjusted survival. Histologic grade is clearly significant in large registry-based studies (1, 9, 10). Based on our study, however, histology is a relatively insensitive predictor of survival with respect to VD_t and requires relatively large patient populations to see significant differences between individual grades.

One possible bias not explored in our study was the use of the histologic grade of the primary tumor as the reference diagnosis for each case. VD_t, on the other hand, was determined from a period(s) of progression of the primary tumor or from the growth of recurrent tumor sometime after initial diagnosis. As stated previously, VD_t in our patient population was relatively constant from diagnosis to death. In contrast, in the 12 patients with two or more follow-up biopsies, 6 increased in histologic grade, and 1 decreased. Furthermore, in two of the five patients with two or more measurements of VD_t at different clinical periods (patients 8 and 25), histologic grade increased, whereas VD_t remained constant. Because of the changes of histologic grade over time and with therapy, it is unlikely that the grade of recurrent disease would be as helpful as VD_t in the prediction of survival, although this issue was not directly addressed in our study.

The relationship between DNA ploidy and survival has been a subject of controversy in the literature. One large study (10, 11) claims a significantly longer survival in patients with hypertriploid DNA histograms, whereas another large series (9) claims a significantly poorer survival in patients with aneuploid and multiclonal tumors. Our study showed that aneuploid and multiclonal tumors had a significantly shorter survival than the remainder of the group only in the univariate analysis (see Table 1). An aneuploid and multiclonal DNA histogram was not a significant independent predictor of survival as shown in a multivariate analysis. The differences in the various studies of DNA ploidy may in part be attributable to the lack of correction for the lead time bias because of differing tumor sizes at diagnosis. We, therefore, attempted to decrease lead time bias by the calculation of size-adjusted survival, thereby establishing a baseline tumor volume (a 1-cm-diameter tumor) from which the survival of patients could be more reasonably compared. When size-adjusted survival was used in place of survival, DNA ploidy (diploid and euploid versus aneuploid and multiclonal) became a highly significant independent variable as shown by a multivariate analysis. The identification of patients destined to have poor survival within each histologic grade was also possible through the analysis of DNA ploidy (as well as VD_t). Furthermore, we found that aneuploid and multiclonal tumors in our study grew significantly faster than the diploid and euploid tumors. This conceivably could be another confounding factor in correlating DNA ploidy with survival if the issue of lead time bias because of differing tumor sizes at diagnosis is not addressed. The lead time bias in our study ranged from 0 to 12.7 years with an average lead time of 2.3 years.

DNA ploidy and histologic grade, however, progressed with time as seen with multiple sequential biopsies with one exception. The exception may have been attributable to sampling or to radiation therapy and chemotherapy, which can be expected to select against the aneuploid clones of the tumor, leaving the diploid clones as in other malignancies (55). Timing, therefore, seems to be a critical factor in obtaining the most predictive value from estimates of DNA ploidy. Analysis of the primary tumor tissue before therapy correlates best with survival. The frequency of aneuploid tumors also increased with higher histologic grades as noted by Zaprianov and Christov (6), Ahayi (3), and others (7–11). In addition, these authors also noted the same relationships of DNA ploidy, histologic grade, timing of biopsied material, and survival as were found in our study.

With the exception of the study by Zaprianov and Christov (6), another problem with previous studies is that flow cytometry or image cytometry (after tumor disaggregation and preparation of nuclei) were used to estimate DNA ploidy. When performing flow cytometry, tumor cells are not separated from normal cells, and the estimates of DNA ploidy may, therefore, be unreliable, as noted in two critical reviews (56, 57). A similar problem may also occur when nuclei analyzed by image cytometry are prepared from suspensions (11). In this type of preparation, distinction between normal and pathologic isolated nuclei may be uncertain.

Conclusions

VD_t and tumor size are important prognostic variables in patients with intracranial astrocytomas who have recurrent or progressive disease. VD_t demonstrated a significant direct linear relationship to survival as well as size-adjusted survival and, therefore, is generally predictive of an individual astrocytoma patient's prognosis. DNA ploidy also becomes significant when survival is adjusted for tumor size at presentation. Histologic grade is a relatively insensitive predictor of individual survival compared with in vivo tumor growth rate (VD_t). Based on our results, we recommend that VD_t and DNA ploidy as well as primary tumor size be further studied to refine current staging as well as the assessment of therapy in patients with intracranial astrocytomas.

Appendix

The method of Collins et al (29) was used to calculate tumor volume in terms of the number of tumor doublings from a single neoplastic cell origin (with an average diameter of 10 μm) assuming exponential tumor growth. Briefly, a 1-cm-diameter tumor has a volume of 0.523 cm^3, which corresponds to 2^{30} cells or 30 tumor doublings. Therefore:

$$\frac{2^{30}\ \text{cells}}{0.523\ \text{cm}^3} = \frac{2^x\ \text{cells}}{\text{observed tumor volume (cm}^3\text{)}},$$

where x represents the number of tumor doublings. Solving for x:

$$1)\quad x = 30 + \frac{\ln\ \text{(observed tumor volume (cm}^3\text{)}}{\ln 2},$$

where ln is a natural logarithm, and $ln\ 2 \approx 0.693$.

Adjusted survival was calculated using the formula:

$$2)\quad \text{adjusted survival} = (x - 30) \times (VD_t) + \text{observed survival},$$

where x represents the observed tumor volume at diagnosis expressed as the number of tumor doublings as calculated from equation 1.

Tumor volume at death was determined by first calculating the number of months from the last imaging study until death and dividing by VD_t (in months). This was then added to the last observed tumor volume (also as expressed as the number of tumor doublings) to obtain the estimated tumor volume at death. The estimated tumor volume at death was then converted into units of cubic centimeters by the following relationship:

$$3)\quad \text{tumor volume}(\text{cm}^3) = \frac{2^z \text{ cells}}{2^{30} \text{ cells}} \times (0.523 \text{ cm}^3),$$

where z is the estimated tumor volume at death in terms of the number of tumor doublings.

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