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CYTOKINE AND GROWTH FACTOR RESPONSES AFTER RADIOTHERAPY FOR LOCALIZED EPENDYMOMA

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Abstract

Purpose—To determine the time course and clinical significance of cytokines and peptide growth factors in pediatric patients with ependymoma treated with postoperative radiotherapy (RT).

Methods and Materials—We measured 15 cytokines and growth factors (fibroblast growth factor, epidermal growth factor, vascular endothelial growth factor [VEGF], interleukin [IL]-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, interferon- γ , tumor necrosis factor- α , granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1, and macrophage inflammatory protein- α) from 30 patients before RT and 2 and 24 h, weekly for 6 weeks, and at 3, 6, 9, and 12 months after the initiation of RT. Two longitudinal models for the trend of log-transformed measurements were fitted, one during treatment and one through 12 months.

Results—During RT, log IL-8 declined at a rate of $-0.10389/\text{wk}$ ($p = 0.0068$). The rate of decline was greater ($p = 0.028$) for patients with an infratentorial tumor location. The decline in IL-8 after RT was significant when stratified by infratentorial tumor location ($p = 0.0345$) and more than one surgical procedure ($p = 0.0272$). During RT, the decline in log VEGF was significant when stratified by the presence of a ventriculoperitoneal shunt. After RT, the log VEGF declined significantly at a rate of $-0.06207/\text{mo}$. The decline was significant for males ($p = 0.0222$), supratentorial tumors ($p = 0.0158$), one surgical procedure ($p = 0.0222$), no ventriculoperitoneal shunt ($p = 0.0005$), and the absence of treatment failure ($p = 0.0028$).

Conclusion—The pro-inflammatory cytokine IL-8 declined significantly during RT and the decline differed according to tumor location. The angiogenesis factor VEGF declined significantly during the 12 months after RT. The decline was greater in males, those without a ventriculoperitoneal shunt, and in those with favorable disease factors, including one surgical procedure, supratentorial tumor location, and tumor control.

Keywords

Radiotherapy; Pediatrics; Ependymoma; Cytokines; Central nervous system tumors

INTRODUCTION

Ependymoma describes a diverse group of central nervous system tumors that can develop at any age (1). Successful therapy requires aggressive surgical intervention, accurate evaluation to determine the neuraxis extent of the tumor, and radiotherapy (RT) administered using methods that minimize the risk of side effects (2). The clinical and laboratory research performed during the past decade has resulted in important discoveries related to the biology of this tumor and the clinical and treatment factors that are prognostic for disease control (3). Future advances in the treatment of ependymoma, similar to other brain tumors, will require objective clinical and molecular information to identify patients at greatest risk of failure after optimal treatment, patients with a good prognosis who would be eligible for a reduction in adjuvant therapy, and markers for side effects so that early intervention could be provided.

Ependymoma most often affects the very young, and because the requirement for RT has been viewed with trepidation because of the risk of side effects, we focused on studying the side effects of RT in the context of modern treatment that includes aggressive surgery, with the goal of gross total resection, and postoperative RT. Cytokines and growth factors have been identified as predictors or correlates of radiation effects in a number of systems; however, limited data are available to characterize the induction of these markers in the central nervous system (4, 5). We have shown a time-dependent change in the central nervous system tissue levels of tumor necrosis factor- α in mice. A significant elevation was observed for this cytokine within 2 h of a single dose of RT (6). This elevation preceded an inflammatory response, determined by a leukocyte–endothelial cell interaction and an increase in intercellular adhesion molecule-1 mRNA and protein levels, which we have shown to peak in the cerebral vessels of the rat during the first 24–48 h after RT (6, 7). Because vascular permeability is also increased in a similar fashion after RT, we postulated that the cytokines induced by the act of RT attract inflammatory cells to the extravascular compartment where tissue destruction is initiated that could eventually be clinically significant.

The time course and clinical significance of cytokines, peptide growth factors, and other biologic markers of central nervous system injury after RT is unknown; we chose to study these response markers in children with ependymoma. In searching for clinical biomarkers that would monitor the normal tissue and tumor response to postoperative RT, we prospectively measured the serum levels of specific growth factors and cytokines during and after RT in children with localized ependymoma.

METHODS AND MATERIALS

Patients

The study included 30 patients with intracranial ependymoma prospectively treated with focal RT between June 7, 2001 and February 5, 2003. All patients underwent conformal or intensity-modulated RT using a 1-cm clinical target volume margin. Of the 30 patients, 59.4 Gy was prescribed in 24 and 54 Gy in 6. The latter prescription was determined from the protocol-specified dose for patients with the characteristics of gross total resection and age <18 months at RT. The main results of the analysis detailed in this report concern interleukin (IL)-8 and vascular endothelial growth factor (VEGF). Because the 27 patients contributing to the IL-8 and the 27 patients contributing to the VEGF measurements were not identical, we have provided the patient characteristics for the combined and separate groups in Table 1.

Cytokine evaluation

We measured 15 cytokines from 30 ependymoma patients before RT and at 2 and 24 h, Weeks 1–6, and 3, 6, 9, and 12 months after the initiation of RT. The 15 cytokines included fibroblast growth factor, epidermal growth factor, VEGF, IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, interferon- γ , tumor necrosis factor- α , granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1, and macrophage inflammatory protein- α . Serum was drawn for cytokine evaluation 2 h before RT, 2 and 24 h after RT, weekly during RT, and 3, 6, 9, and 12 months after the start of RT. Each patient had the potential to contribute 13 samples between the start of RT and their 12-month follow-up visit. The median number of samples was 12 (range, 6–13). Only 2 patients had <10 samples. The missed samples generally occurred during RT.

Multiplexed cytokine assay

The serum samples were transported at room temperature, allowed to coagulate, and then centrifuged at 1,000g, at –4°C, for 20 min within 30 min of collection. The serum was then removed and stored in microtubes at temperatures of –80°C. The samples were analyzed using the Beadlyte Human Multi-Cytokine Flex Kit (15-Plex; Upstate, Waltham, MA) using the company's protocol and the FlowMetrix system with multiplexing capabilities (Luminex, Austin, TX). The principal advantage of the assay is its multiplexing capability, which allows for accurate measurement of 15 cytokines simultaneously in a 100- μ L sample volume (8, 9). The units of measurement were pg/ml

Statistical analysis

The objective of this study was to explore the longitudinal trend of the cytokines in ependymoma patients and to investigate whether the longitudinal trend of cytokines was associated with the clinical variables. A linear mixed effects model with random intercept and slope were used to explore the longitudinal trends of the 15 cytokines. In the longitudinal model, the response variable cytokine values were transformed by taking the natural log of the original values. For cytokines that showed statistically significant changes either during the period that included RT or the interval that included treatment and follow-up for 1 year, we also estimated the statistical associations between the longitudinal trend of cytokines and the specific clinical variable, including the primary tumor location (infratentorial vs. supratentorial), number of procedures (one vs. more than one), permanent ventriculoperitoneal (VP) shunt (yes vs. no), patient gender (male vs. female), and treatment failure (yes vs. no). Multivariate linear mixed effects models were also performed by applying the Purposeful Selection of Covariates (10) to the linear mixed effect model as follows. For this analysis, we included the variables whose *p* value of the slope term was < 0.25 on univariate analysis, by including them in both the intercept and the slope terms. We then removed the terms that had a *p* value > 0.05 by the likelihood ratio test of the reduced model vs. the full model; however, if deleting a term would change the value of any other model estimates by 50%, the term was not removed. Using this method, a temporary model was created. The other covariates that were not significant on the univariate models were added to the temporary model, one at a time, and the significant terms were kept in the model. We added all clinically plausible interactions to the temporary model and selected them using the aforementioned process. For each cytokine in the analysis, we investigated two situations according to the follow-up time: the interval that included RT only and the interval that included RT and follow-up for 1 year. In each situation, we report both the univariate and the multivariate regression results. The models of some cytokine levels during RT showed a curve linear relationship when the quadratic term of the time was included in the model.

RESULTS

For each cytokine, two longitudinal models for the trend of log-transformed measurements were fitted. The first model included measurements during treatment including before RT and at 2 and 24 h, and Weeks 1–6 of RT. The second model included measurements during and after treatment including before RT, the end of treatment (Week 6), and at 3, 6, 9, and 12 months after the initiation of RT. The parameters of the longitudinal model estimates are presented in Tables 2 and 3.

As shown in Table 2, during treatment, log IL-8 declined significantly at a rate of $-0.10389/\text{wk}$ ($p = 0.0068$). The log macrophage inflammatory protein- α and log VEGF both declined at a marginally significant rate of $-0.07460/\text{wk}$ ($p = 0.087$) and $-0.06611/\text{wk}$ ($p = 0.068$), respectively. The log tumor necrosis factor- α showed an increasing trend of $0.05378/\text{wk}$ ($p = 0.13$), and the 11 remaining cytokines did not show any significant longitudinal change.

As shown in Table 3, during the interval that included RT and follow-up for 12 months, log VEGF declined significantly at a rate of -0.06207 units/mo ($p = 0.0057$). The remaining 14 cytokines did not show significant longitudinal change during the same period.

Because of the significance of their respective changes during and after RT, the trends of IL-8 and VEGF were further modeled to determine any statistically significant associations with the clinical variables other than time. The following clinical variables were chosen: primary site location (infratentorial vs. supratentorial), number of surgeries (one vs. more than one), permanent VP shunt (presence vs. absence), gender (male vs. female), and treatment failure (failure vs. no failure). The actual measurements of IL-8 and VEGF are presented in Fig. 1.

IL-8 during conformal RT (before RT to week 6)

The longitudinal trend of log IL-8 during RT fit a quadratic curve (Fig. 2A) with the following expression:

$$\text{Log IL-8} = 0.912 - 0.274 \times \text{week} + 0.0315 \times \text{week}^2$$

Time was measured in weeks from the initiation of RT (Weeks 0–6), and the coefficients were significantly different than 0 (intercept, 0.912 ± 0.2352 , $p = 0.0006$; first order coefficient, -0.2743 ± 0.08095 , $p = 0.0024$; second order coefficient, 0.0315 ± 0.01352 , $p = 0.0286$).

Univariate analysis was performed using clinical variables. Clinical variables found to have some level of statistical significance in estimating the linear term are presented in Table 4. None had a significant effect on the quadratic term. Example models using the covariate of gender are presented in which time is measured in weeks (Week 0–6) from the initiation of RT.

$$\text{Female: Log IL-8} = 0.6207 - 0.2169 \times \text{weeks} + 0.03042 \times \text{weeks}^2$$

$$\text{Male: Log IL-8} = 1.0929 - 0.3034 \times \text{weeks} + 0.3042 \times \text{weeks}^2$$

The two intercepts, 0.6207 and 1.0929, were not significantly different statistically ($p = 0.33$). The two first order time terms, -0.2169 and -0.3034 , were not different statistically significant ($p = 0.23$). Both first order terms decreased with statistical significance ($p = 0.024$ and $p = 0.0005$). The quadratic term was significant ($p = 0.034$). All clinical variables were considered in building a multiple regression model, as well as confounding and interactions between variables. The fitted multiple regression model could not be generated.

IL-8 after conformal RT (before RT to 12 months)

For the long-term analysis, six points were also used to estimate the trend in log IL-8. With the long-term analysis, the estimated trend of log IL-8 was linear, because the quadratic term was not significant. The linear model is given by the equation:

$$\text{Log IL-8} = 0.8483 - 0.02448 \times \text{month}$$

The intercept 0.8483 ± 0.2226 ($p < 0.0009$) was significantly different than 0 but the slope -0.02448 ± 0.02720 ($p = 0.38$) was not. A statistically significant decline in log IL-8 was observed among patients with the following characteristics: infratentorial tumor location and more than one surgical procedure (Table 5). A statistically significant multivariate model could not be created from the covariates included in the present study.

VEGF during conformal RT (before RT to 6 weeks)

We found that the longitudinal trend of log VEGF during RT fit a quadratic curve (Fig. 2B) with the following expression:

$$\text{Log VEGF} = 3.442 - 0.337 \times \text{week} + 0.0493 \times \text{week}^2$$

The time was measured in the weeks from the start of RT (Weeks 0–6), and the coefficients were significantly different than 0 (intercept 3.4424 ± 0.2593 , $p < 0.0001$; first order coefficient -0.3368 ± 0.1065 , $p = 0.004$; second order coefficient 0.04929 ± 0.01838 , $p = 0.0126$).

Univariate analysis was performed using clinical variables. The clinical variables found to have some level of statistical significance in estimating the linear term are presented in Table 6. None had a significant effect on the quadratic term. The example models using the covariate of VP shunt are presented in which the time is measured in weeks (Weeks 0–6) from the initiation of RT.

$$\begin{aligned} \text{Noshunt} &= \log \text{VEGF} \\ &= 3.354 - 0.2872 \times \text{weeks} + 0.04992 \times \text{weeks}^2 \end{aligned}$$

$$\begin{aligned} \text{Shunt present} &= \log \text{VEGF} \\ &= 3.686 - 0.4744 \times \text{week} + 0.04992 \times \text{weeks}^2 \end{aligned}$$

The two intercepts, 3.354 vs. 3.686, were not significantly different ($p = 0.55$); the first order time terms, -0.2872 vs. -0.4744 , were significantly different statistically ($p = 0.0081$). Both first order time terms were significantly different statistically than 0 ($p = 0.0079$ and $p < 0.0001$). The quadratic term was significant ($p = 0.0115$). All clinical variables were

considered in building a multiple regression model, as well as confounding and interactions between variables. The fitted multiple regression model included the variables of the presence of a VP shunt and the number of surgical procedures.

VEGF after conformal RT (before RT to 12 months)

For the long-term analysis, six points were used to estimate the trend in log VEGF. The chosen points were before RT, Week 6, and Months 3, 6, 9, and 12. With the long-term analysis, the estimated trend of log VEGF was linear, because the quadratic term was not significant. The linear model is given by the equation

$$\text{Log VEGF} = 3.3157 - 0.06207 \times \text{month}$$

The intercept 3.3157 ± 0.2183 ($p < 0.0001$) and slope -0.06207 ± 0.02063 ($p = 0.0058$) were significantly different than 0 (Fig. 2C). A statistically significant decline in log VEGF was observed among the patients with the following characteristics: male gender, supratentorial tumor location, one surgery, the absence of a permanent VP shunt, and no treatment failure (Table 7). A statistically significant multivariate model could not be created from the covariates included in this study.

Effect of mean dose and planning target volume

The mean dose $\pm$ standard deviation to the entire brain was determined for the patients contributing to the IL-8 (2142.6 ± 331.22 cGy) and VEGF (2125.2 ± 331.25 cGy) analyses. The mean dose did not have any effect on baseline values or change over time. The planning target volume did not have an effect on baseline values but did have a significant and positive effect on change over time for IL-8 that was limited to the interval during RT. The following model included the planning target volume measured in milliliters with the time measured in weeks. The coefficient 0.001246 was statistically significant ($p = 0.0404$).

$$\begin{aligned} \text{Log IL-8} = & 0.84.72 + \text{planning target volume} \\ & \times 0.00046 + (\text{planning target volume} \\ & \times 0.001246 - 0.4465) \times \text{weeks} \\ & + 0.03406 \times \text{weeks}^2 \end{aligned}$$

Tumor control

In light of the association between the lack of treatment failure and the declining serum VEGF concentration (Fig. 2D), the follow-up for the study cohort was updated: 10 patients had treatment failure, 6 with neuraxis dissemination and local tumor control and 4 with local failure and no evidence of metastases. The median time to failure was 21 months (range, 6–32). The median follow-up was 67 months (range, 56–75) for patients who had achieved disease control. One patient died 3 months after RT following a seizure. None of the patients were lost to follow-up.

DISCUSSION

Cytokines have been investigated as factors that might individualize the risk profiles for injury in patients treated with RT (11). The earliest investigations associating cytokine expression with radiation-induced cerebral injury were immunohistochemical and demonstrated moderate levels in peritumoral tissues of patients with malignant glioma (12). Cytokines were then shown to be elevated in the serum of brain tumor patients and most

declined after treatment (13). Considering their pro-inflammatory and profibrotic nature, determining their contribution to treatment-related morbidity has been a primary objective in a number of investigations, including ours, and resulted in the design of the present study.

We examined 15 cytokines and growth factors, focusing on two different periods, to evaluate the early and late responses to treatment. The change during treatment was only significant for IL-8, which declined during the course of 6 weeks. Although declines in macrophage inflammatory protein- α and VEGF were observed, in a linear model, they were not significant. Considering the period from before RT to 12 months after RT, only the change in VEGF was statistically significant. We also investigated the association of the clinical factors with changes in IL-8 and VEGF during and after treatment, considering the primary tumor location, number of surgical procedures, permanent VP shunt placement, patient gender, and treatment failure.

Interleukin-8

All the clinical factors, analyzed as categorical variables and requiring a quadratic term in time, contributed to the significant decline in IL-8 during RT. IL-8 has a number of known functions, including the regulation of angiogenesis, enhancement of endothelial cell survival, proliferation, migration, and matrix metalloproteinases production (14–16). In response to RT, IL-8 is considered to be involved in pro-inflammatory and thrombotic activities, because suppression of these activities has been observed after downregulation of IL-8 expression (17). The change in serum cytokine concentrations after neurosurgical procedures was investigated by Osuka *et al.* (18). IL-8 was among the cytokines that did not change comparing the pre- and postoperative values. These same investigators found that IL-6 concentrations were greater for patients with a supratentorial tumor location after brain tumor surgery, suggesting IL-6 as an early marker of brain injury and that levels of IL-6 after surgery exceeded those observed after shunt procedures.

Although all the categorical variables included in the analysis were modeled to show a statistically significant decline, only the difference between the infratentorial and supratentorial patients was significant. The infratentorial patients had greater baseline values, perhaps owing to a greater frequency of multiple surgical procedures and a greater frequency of VP shunt requirement. In returning to lower levels of IL-8, the in-fratentorial patients had a greater range of decline. When viewed for a longer period, only the infratentorial patients and those treated with multiple surgical procedures had a decline that was significantly different than 0. The angiogenic properties of IL-8 notwithstanding, this cytokine has the potential as a marker of inflammation in this tumor system and will require additional evaluation and correlation with objective treatment effects.

Vascular endothelial growth factor

When all the patients were included together in the model, the VEGF level did not change significantly during RT. When the change in VEGF was modeled according to the clinical factors, the fitted change was highly significant, provided that a quadratic term in time was included. All clinical factors contributed to a statistically significant decline during RT. When considering the period 12 months after RT, the significance was limited to male gender, supratentorial tumor location, a single surgical procedure, no requirement for a VP shunt, and achieving tumor control. The decrease in VEGF was significant for patients with a supratentorial tumor location or male gender ($p = 0.02$). In contrast, the decrease was highly significant ($p < 0.003$) for patients who had undergone a single surgical procedure, the absence of a VP shunt, or the lack of treatment failure.

Vascular endothelial growth factor has been investigated for its ability to monitor the tumor response to treatment in a number of settings, and a number of factors might enhance or suppress the expression of this important cytokine. VEGF was shown, nearly two decades ago, to be secreted by malignant brain tumors (19) and to be a measurable element of brain tumor angiogenesis (20). Even though the local release of VEGF was not reflected in the serum of brain tumor patients according to some groups (21) and not detectable in patients with low-grade glioma (22), others have shown that VEGF is often elevated in cancer in the presence of distant metastases (23) from epithelial tumors.

It is not surprising that the decline in VEGF was greater in patients with supratentorial tumors because these patients are known to have a larger area of vasogenic edema compared with those with infratentorial tumors. The slightly larger intercept for the patients with supratentorial tumors served as additional evidence. None of these brain tumor patients required corticosteroids because both VEGF expression and vasogenic expression can be suppressed by corticosteroids (24).

Vascular endothelial growth factor expression has been investigated in ependymoma as a marker of tumor grade and a predictor of outcome. Early studies showed no correlation among VEGF expression, vascular proliferation, and tumor grade in ependymoma (25). More recently, VEGF has been shown to be distinctly expressed in ependymoma and associated with angiogenesis (26), vascular proliferation, and necrosis (27). The results from studies designed to correlate the immunohistochemical expression pattern of VEGF an outcome have been mixed. Preusser *et al.* (26) demonstrated the association of VEGF and other hypoxia-related factors with angiogenesis in ependymoma; however, the association was not predictive of the outcome compared with proliferation and the extent of resection. Korshunov *et al.* (28) considered the expression of VEGF and other factors on progression-free survival stratified by tumor grade. Progression-free survival for low-grade ependymoma was predicted by a variety of proliferation indexes and growth factors, including VEGF; however, VEGF was not among the factors predictive of outcome for patients with high-grade tumors (28).

Our results suggest that VEGF is an important marker of tumor response in ependymoma because a single surgical procedure, lack of VP shunt requirement, and the lack of treatment failure might not be independent. The requirement of the model fitted during the treatment period required a quadratic term in time, indicating the strong nature of VEGF expression from residual disease to maintain the high levels of this marker. In our larger series of patients, considering that our rate of gross total resection before RT exceeded 85%, those who underwent resection of their tumor in one surgical procedure tended not to have gross residual tumor. These patients fared better in the long term. Those who did not require a VP shunt tended to be patients who had required an uncomplicated single surgery procedure. We have demonstrated a statistically significant association between a VP shunt requirement and a second surgical procedure (29).

CONCLUSION

Balancing the effects of RT to achieve tumor control and the goal of limiting side effects requires objective measures. Tumor- and normal tissue-derived growth factors and cytokines might serve that role if controlled for important clinical factors, as we have demonstrated in the present study. The number of patients included in this study was small, an important limitation that precluded meaningful multivariate analysis and an assessment of prominent prognostic factors in ependymoma such as tumor grade and resection extent. The strength of this study was in the relatively homogenous patient population in the treatment and environment of care, which helped to strengthen our conclusion that serial assessment of

cytokines during and after RT should be considered for additional investigation. Targeting inflammation is considered to be an important approach to further minimizing the side effects of RT. Targeting angiogenesis is considered to be a promising therapeutic approach, and the combination of RT and anti-angiogenesis agents is being investigated for a variety of pediatric and adult solid tumors. We hoped that demonstrating the importance of VEGF might lead to new treatment approaches in ependymoma. RT produces a serum VEGF decline in patients with head-and-neck cancer (30), and VEGF receptor inhibition is considered a potential approach to improve the effect of RT (31).

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Fig. 1.

Interleukin (IL)-8 and vascular endothelial growth factor (VEGF) during and after conformal radiotherapy (RT). Each connected line represents the same patient.

Fig. 2.

(A) Model and actual data (local sample mean and standard error) for log interleukin (IL)-8 trend during radiotherapy (RT). (B) Model and data (local sample mean and standard error) of log vascular endothelial growth factor (VEGF) during radiotherapy. (C) Model estimate and data (local sample mean and standard error) of log vascular endothelial growth factor from beginning of radiotherapy to 12 months of follow-up. (D) Log vascular endothelial growth factor stratified by treatment failure. Observations plotted as local sample mean and standard error.

Table 1

Patient characteristics

Characteristic	IL-8 (n = 27)	VEGF (n = 27)	All patients (n = 30)
Age at RT start (y)			
Mean	4.70	4.88	4.88
SD	4.38	4.55	4.35
Median	2.91	2.91	2.92
Minimum	1.10	1.10	1.10
Maximum	18.87	18.87	18.87
Gender (n)			
Female	10 (37.0)	10 (37.0)	12 (40)
Male	17 (63.0)	17 (63.0)	18 (60)
Tumor grade (n)			
AEP	19 (70.4)	19 (70.4)	21 (70)
ED	8 (29.6)	8 (29.6)	9 (30)
Tumor location (n)			
Infratentorial	17 (63.0)	16 (59.3)	18 (60)
Supratentorial	10 (37.0)	11 (40.7)	12 (40)
Surgical procedures (n)			
1	15 (55.6)	15 (55.6)	17 (56.7)
2-4	12 (44.4)	12 (44.4)	13 (43.3)
Extent of resection before RT (n)			
GTR	24 (88.9)	23 (85.2)	26 (86.7)
NTR	2 (7.4)	2 (7.4)	2 (6.7)
STR	1 (3.7)	2 (7.4)	2 (6.7)
Shunt placement (n)			
No	17 (63.0)	18 (66.7)	19 (63.3)
Yes	10 (37.0)	9 (33.3)	11 (36.7)
Treatment failure (n)			
No	20 (74.1)	17 (63.0)	20 (66.7)
Yes	7 (25.9)	10 (37.0)	10 (33.3)

Abbreviations: IL-8 = interleukin-8; VEGF = vascular endothelial growth factor; RT = radiotherapy; SD = standard deviation; AEP = anaplastic ependymoma; ED = differentiated ependymoma; GTR = gross tumor resection; NTR = near total resection; STR = subtotal resection.

Data in parentheses are percentages.

Table 2

Longitudinal trend (log values) of cytokines during conformal RT

Cytokine	<i>n</i>	Baseline estimate (intercept) $\pm$ SE	Change in estimate each week (slope) $\pm$ SE	<i>p</i> [*]
EGF	29	3.26 $\pm$ 0.14	0.00060 $\pm$ 0.02992	0.98
FGF	27	3.44 $\pm$ 0.25	0.02848 $\pm$ 0.03201	0.38
GM-CSF	25	2.77 $\pm$ 0.32	0.01463 $\pm$ 0.05157	0.77
IL-10	28	1.28 $\pm$ 0.25	0.00693 $\pm$ 0.03541	0.84
IL-1b	24	1.17 $\pm$ 0.33	-0.04060 $\pm$ 0.04536	0.38
IL-2	22	0.92 $\pm$ 0.40	-0.00035 $\pm$ 0.05895	0.99
IL-4	25	1.10 $\pm$ 0.32	-0.02678 $\pm$ 0.04381	0.54
IL-5	24	0.33 $\pm$ 0.26	-0.01310 $\pm$ 0.02290	0.57
IL-6	21	1.35 $\pm$ 0.32	-0.04013 $\pm$ 0.06747	0.56
IL-8	27	0.85 $\pm$ 0.23	-0.10389 $\pm$ 0.03514	0.0068 [†]
INF- γ	19	2.19 $\pm$ 0.38	-0.00281 $\pm$ 0.04857	0.95
MCP-1	30	4.04 $\pm$ 0.22	-0.00173 $\pm$ 0.02255	0.93
MIP- α	15	4.72 $\pm$ 0.20	-0.07460 $\pm$ 0.04013	0.087 [‡]
TNF- α	30	0.84 $\pm$ 0.30	0.05378 $\pm$ 0.03513	0.13
VEGF	27	3.32 $\pm$ 0.26	-0.06611 $\pm$ 0.03473	0.068 [‡]

Abbreviations: RT = radiotherapy; SE = standard error; EGF = epidermal growth factor; FGF = fibroblast growth factor; GM-CSF = granulocyte-macrophage colony-stimulating growth factor; IL = interleukin; INF = interferon; MCP-1 = monocyte chemoattractant protein-1; MIP- α = macrophage inflammatory protein- α ; TNF- α = tumor necrosis factor- α ; VEGF = vascular endothelial growth factor.

* Significant *p* value indicates slope is not 0.

[†] *p* < 0.05.

[‡] *p* < 0.1 but > 0.05.

Table 3

Longitudinal trend (log values) of cytokines during and after conformal RT

Cytokine	<i>n</i>	Baseline estimate (intercept) $\pm$ SE	Change in estimate each month (slope) $\pm$ SE	<i>p</i> [*]
EGF	29	3.48 $\pm$ 0.10	-0.01501 $\pm$ 0.01638	0.36
FGF	29	3.80 $\pm$ 0.17	0.02781 $\pm$ 0.02225	0.22
GM-CSF	25	3.03 $\pm$ 0.25	0.00110 $\pm$ 0.03351	0.97
IL-10	28	1.26 $\pm$ 0.24	-0.01383 $\pm$ 0.02445	0.57
IL-1b	26	1.56 $\pm$ 0.23	0.02276 $\pm$ 0.03089	0.46
IL-2	28	1.31 $\pm$ 0.36	-0.00305 $\pm$ 0.04045	0.94
IL-4	26	1.12 $\pm$ 0.31	0.01189 $\pm$ 0.03032	0.69
IL-5	21	0.14 $\pm$ 0.23	-0.03072 $\pm$ 0.02437	0.22
IL-6	22	1.24 $\pm$ 0.47	0.00959 $\pm$ 0.04744	0.84
IL-8	27	0.85 $\pm$ 0.23	-0.02448 $\pm$ 0.02720	0.37
INF- γ	18	2.59 $\pm$ 0.46	-0.06518 $\pm$ 0.08560	0.45
MCP-1	30	4.09 $\pm$ 0.21	-0.02137 $\pm$ 0.01756	0.23
MIP- α	13	4.69 $\pm$ 0.27	-0.02111 $\pm$ 0.01760	0.26
TNF- α	30	1.13 $\pm$ 0.31	0.01842 $\pm$ 0.02616	0.48
VEGF	27	3.32 $\pm$ 0.22	-0.06207 $\pm$ 0.02063	0.0057 [†]

Abbreviations as in Table 2.

^{*} Significant *p* value indicates slope is not 0.[†] *p* < 0.05.

Table 4

Trend of log IL-8 during RT

Variable	n	Baseline score		Week from RT			Week ² from RT	
		Estimate ± SE	P1	Estimate ± SE	P2	P3	Estimate ± SE	P4
Gender								
Female	10	0.6207 ± 0.3852	0.33	-0.2169 ± 0.0946	0.024	0.23	0.03042 ± 0.01353	0.034
Male	17	1.0929 ± 0.2986		-0.3034 ± 0.0837	0.0005			
Tumor location								
Infratentorial	17	1.2312 ± 0.2882	0.073	-0.3329 ± 0.08394	0.0001	0.028*	0.03357 ± 0.01356	0.021
Supratentorial	10	0.3768 ± 0.3756		-0.1868 ± 0.08872	0.0375			
Surgical procedures								
1	15	0.7147 ± 0.313	0.34	-0.2731 ± 0.08733	0.0022	0.95	0.03146 ± 0.01353	0.0292
2-4	12	1.1684 ± 0.3573		-0.2774 ± 0.09048	0.0027			
VP shunt								
No	17	1.0101 ± 0.2997	0.58	-0.2452 ± 0.0853	0.0048	0.28	0.0311 ± 0.01352	0.0308
Yes	10	0.7401 ± 0.3877		-0.3238 ± 0.0931	0.0007			

Abbreviations: P1 = level of significance in mean of baseline scores comparing subgroups; P2 = level of significance comparing value for subgroup over time; P3 = level of significance comparing change in values for subgroups over time; P4 = quadratic term is significant; VP = ventriculoperitoneal; other abbreviations as in Table 2.

* $p < 0.05$.

Table 5

Trend of log IL-8: before RT to 12 months after RT

Variable	<i>n</i>	Baseline score (intercept)		Value change per month (slope)		
		Estimate ± SE	P1	Estimate ± SE	P2	P3
Tumor location						
Infratentorial	17	0.97 ± 0.29	0.50	-0.0724 ± 0.0335	0.0345 *	0.0310 *
Supratentorial	10	0.65 ± 0.38		0.0411 ± 0.0390	0.29	
Surgical procedures						
1	15	0.68 ± 0.30	0.34	0.0183 ± 0.0323	0.57	0.0365 *
2-4	12	1.11 ± 0.35		-0.0945 ± 0.0418	0.0272	

Abbreviations as in Tables 2 and 4.

* Statistically significant.

Table 6

Trend of log VEGF during RT

Variable	N	Baseline score		Week from RT		Week ² from RT	
		Estimate ± SE	P1	Estimate ± SE	P2	Estimate ± SE	P4
Gender							
Female	10	3.873 ± 0.419	0.19	-0.3327 ± 0.1181	0.0057*	0.04890 ± 0.01843	0.0136*
Male	17	3.189 ± 0.320		-0.3383 ± 0.1090	0.0027*		
Tumor location							
Infratentorial	16	3.363 ± 0.341	0.71	-0.3245 ± 0.1114	0.0042*	0.04921 ± 0.0184	0.0130*
Supratentorial	11	3.559 ± 0.409		-0.3544 ± 0.1142	0.0024*		
Surgical procedures							
1	15	3.729 ± 0.344	0.20	-0.2916 ± 0.1101	0.0092*	0.04894 ± 0.0182	0.0127*
2-4	12	3.078 ± 0.382		-0.3911 ± 0.1121	0.0007*		
VP shunt							
No	18	3.354 ± 0.318	0.55	-0.2872 ± 0.1064	0.0079*	0.04992 ± 0.0183	0.0115*
Yes	9	3.686 ± 0.458		-0.4744 ± 0.1174	<0.0001*		

Abbreviations as in Tables 2 and 4.

* Statistically significant.

Table 7

Trend of log VEGF before RT to 12 months after RT

Variable	n	Baseline value (intercept)		Value change per month (slope)		
		Estimate ± SE	P1	Estimate ± SE	P2	P3
Gender						
Female	10	3.58 ± 0.35	0.35	-0.0597 ± 0.0314	0.061 *	0.91
Male	17	3.16 ± 0.28		-0.0644 ± 0.0275	0.0222 *	
Tumor location						
Infratentorial	16	3.15 ± 0.29	0.37	-0.0504 ± 0.0275	0.070	0.51
Supratentorial	11	3.55 ± 0.34		-0.0776 ± 0.0314	0.0158 *	
Surgical procedures						
1	15	3.56 ± 0.28	0.19	-0.0843 ± 0.0265	0.0021 *	0.20
2-4	12	3.00 ± 0.32		-0.0298 ± 0.0330	0.36	
VP shunt						
No	18	3.43 ± 0.27	0.34	-0.0854 ± 0.0235	0.0005 *	0.050 *
Yes	9	2.97 ± 0.40		0.0077 ± 0.0405	0.84	
Treatment failure						
No	17	3.37 ± 0.28	0.73	-0.0809 ± 0.0261	0.0028 *	0.24
Yes	10	3.22 ± 0.37		-0.0313 ± 0.0335	0.35	

Abbreviations as in Tables 2 and 4.

* Statistically significant.