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Potential Role of Ventricular Tumor Markers in CNS Germ Cell Tumors

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Background. There is increasing reliance on oncoprotein assays such as the β-subunit of human chorionic gonadotropin (β-hCG) and alpha-fetoprotein (AFP) for diagnosis or confirmation of histology of central nervous system (CNS) germ cell tumors (GCT), but the relative diagnostic sensitivity and reliability of assays from serum (S), lumbar (L), and ventricular (V) cerebrospinal fluid (CSF) are uncertain. ***Procedure.*** A total of 86 patients with CNS GCT were identified from our database. Fourteen patients had contemporaneous β-hCG and/or AFP measurements from serum, ventricular, and lumbar CSF at diagnosis (n = 13) or relapse (n = 1), constituting the subjects for this report. Their primary tumor sites were: pineal (n = 8), suprasellar (n = 1), or both (n = 5). Their mean age at diagnosis was 16.0 years (range 9.1–25.9). The male:female sex ratio was 13:1. ***Results.*** For the germinoma-treated patients (n = 8), the median (range) β-hCG values (S, V, L) were 0 (0–6.9), 7.0 (0–57.4), 8.3 (0–34.0) mIU/ml. For patients managed as mixed malignant GCT (MMGCT) (n = 6), the median (range) β-hCG values (S, V, L) were 3.9 (0–58.0), 3.6 (0–147.0), 61.8 (0–358.0) mIU/ml. The median (range) AFP values were 7.5 (0–27,400.0), 2.0 (0–2,981.0), 3.0 (0–14,015.0) ng/ml. Lumbar CSF β-hCG values were equal or greater than those in ventricular CSF or serum in 12 of 13 cases (92.3%). All patients with MMGCT had lumbar AFP equal or greater than the ventricular CSF values, while serum AFP values remained highest. ***Conclusions.*** Ventricular CSF values cannot be considered a replacement for lumbar CSF. Lumbar CSF is the most reliable source of tumor markers to establish baseline and follow-up diagnostic endpoints. Pediatr Blood Cancer 2013;60:1647–1650. © 2013 Wiley Periodicals, Inc.

Key words: alpha-fetoprotein; cerebrospinal fluid; germ cell tumor; germinoma; human chorionic gonadotropin; tumor marker

INTRODUCTION

Current potentially curative therapy for patients with malignant central nervous system (CNS) germ cell tumors (GCT) combines selected aspects of surgery, chemotherapy, and radiation therapy. The specific components of these therapies vary according to clinical diagnostic category, that is, germinoma or mixed malignant GCT (MMGCT). Germinoma is the most common tumor type, comprising 65% of all CNS GCT [1], while the latter group may consist of single but usually a combination of different histologies such as germinoma, embryonal carcinoma, choriocarcinoma, endodermal sinus tumor, and occasionally mature or immature teratoma [2]. The intensity of treatment required for cure is much greater in the MMGCT category.

The methods of distinguishing between these two clinical categories have evolved. Initially when surgery and radiation therapy were the only therapies employed, the distinction was based on either histology or a radiographic response to 2 weeks of radiation therapy. If the tumor persisted, an attempt at radical resection of these midline tumors was usually employed. As medical therapies including platinum-based chemotherapy combined with radiation therapy evolved, and progression-free survival rates increased, the indication for radical surgical resection became reserved for medically refractory tumors such as immature or growing teratomas. The preferred diagnostic procedures were biopsies, either open or endoscopic, replacing open craniotomy and resection, thereby reducing the peri-operative morbidity. The added benefits included control of hydrocephalus with an endoscopic third ventriculostomy for pineal region tumors and access to ventricular cerebrospinal fluid (CSF) analysis. The limitations of this approach included an increased potential for inaccurate histologic diagnosis related to small amounts of biopsy material of a heterogeneous tumor [3].

Concurrent with this trend towards lesser morbid neurosurgery procedures was the identification of GCT markers in serum and lumbar CSF and the correlation of their profile with histology. It became recognized that elevations of alpha-fetoprotein (AFP) in either serum or lumbar CSF correlated closely with the diagnosis of a MMGCT and germinomas were usually associated with levels of β-subunit of human chorionic gonadotropin (β-hCG) <200 mIU/ml in either serum or lumbar CSF. Current cooperative group protocols accept serum and lumbar tumor markers as surrogate diagnostic tools in lieu of histology for MMGCT (elevated AFP, very high β-hCG) and in classic germinoma presentations such as bifocal or suprasellar/pineal region disease (normal AFP and low levels of β-hCG) for protocol enrollment. In a recent Canadian national review, a presumptive diagnosis of an MMGCT was made in 66% based on markers alone without histologic confirmation [4].

An emerging challenge is the determination of the diagnostic reliability and sensitivity of ventricular CSF assays for tumor markers obtained during an initial endoscopic procedure. To our knowledge, only two reports totaling three patients exist in the literature with regard to specifically comparing ventricular and lumbar CSF tumor marker assays [5,6]. The objective of this retrospective study was to ascertain the relationship between the values of oncoproteins (β-hCG and AFP) assays obtained prior to initial treatment or at recurrence from contemporaneously derived ventricular CSF, lumbar CSF, and serum in a cohort of patients with intracranial CNS GCT from our clinical database.

METHODS

We queried our institutional database of patients followed at our pediatric neuro-oncology clinic at NYU Langone Medical Center (NYULMC) between 2000 and 2013 to identify all patients with CNS GCT. All patients who had been evaluated and/or treated by physicians at our institution for a confirmed intracranial CNS GCT with available results for AFP and/or β-hCG tumor markers in the

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serum, as well as lumbar and ventricular CSF were eligible for participation, with no additional exclusion criteria. Normal ranges for AFP were: serum <7.5 ng/ml and lumbar CSF <1.0 ng/ml; and for β-hCG: serum <5.0 mIU/ml, lumbar CSF <3.0 mIU/ml. There are no published normative values for ventricular CSF oncoproteins.

The study was conducted under a protocol approved by the Institutional Review Board of NYULMC and was performed in accordance with institutional privacy and confidentiality policies. Data were systematically abstracted by a single investigator (G.L.) by reviewing medical charts and electronic health records, as available.

Patients were grouped according to their treatment assignment by their original treating team, based on the combined information from the histopathological evaluation and tumor marker assessments. By definition, none of the germinoma patients were to have elevated AFP.

RESULTS

Patient Demographics

From the 86 reviewed charts, our cohort of 14 patients fulfilled our inclusion criteria outlined above (Table I). The data were collected in 13 patients at diagnosis and 1 at relapse. Their mean age was 16.0 years at diagnosis. Affected sites included the pineal region only (n = 8), including one with subependymal and leptomeningeal extension (Case 3), the sellar/supra-sellar location (n = 1), or both regions from a bifocal GCT (n = 5).

Tumor Histological Diagnosis

Diagnostic pathology was available for 11 patients. Germinoma alone was found in eight patients, although elevations of lumbar β-hCG raised concerns about MMGCT components in two (Cases 3 and 4). Two patients had inconclusive pathology: Case 6 had normal AFP levels and minimally elevated β-hCG levels in both the ventricular and lumbar CSF, compatible with a pure germinoma, and was treated as a pure germinoma; Case 14 had normal AFP and β-hCG levels, and was treated on the most current Children's Oncology Group protocol as pure germinoma given his bifocal disease. One subject (Case 12) forewent tumor biopsy prior to treatment as tumor marker elevation alone confirmed a diagnosis of MMGCT. Overall, this cohort consisted of eight patients (57.1%) treated as pure germinoma and six (42.3%) as MMGCT.

Tumor Marker Analysis

As per our study inclusion criteria, results for AFP and/or β-hCG tumor markers in the serum, as well as lumbar and ventricular CSF were required. Initial ventricular CSF values for one subject (Case 5) performed at an outside institution were unavailable. However, he underwent an Ommaya reservoir puncture prior to treatment, rendering readily evaluable ventricular CSF. Both the ventricular and the lumbar CSF values were obtained on the same day for that patient. For all other patients, ventricular CSF was obtained intraoperatively at the time of surgery for endoscopic third ventriculostomy and/or tissue biopsy. One patient (Case 13) had his lumbar CSF sampled 7 days prior to ventricular CSF. The exact dates of CSF tumor markers measurements were not available for one patient (Case 6). For all other patients (n = 11), ventricular CSF was measured at a median of 9 days (range 3–50) prior to the lumbar CSF. Tumor marker assays from serum (S), lumbar (L), and ventricular (V) CSF for each subject are indicated in Table I. For the germinoma-treated patients (n = 8), the median β-hCG values (S, V, L) were 0, 7.0, and 8.3 mIU/ml. For patients managed as mixed malignant GCT (MMGCT) (n = 6), the median β-hCG values (S, V, L) were 3.9, 3.6, and 61.8 mIU/ml and the median AFP values were 7.5, 2.0, and 3.0 ng/ml.

DISCUSSION

Therapy assignments for CNS GCT are based on histology and/or tumor markers. Serial ventricular CSF assays are not practical and not available at diagnosis in all patients so most of the published guidelines on the use of these markers in CSF relate to lumbar CSF values. A marked elevation of AFP level alone in either serum or lumbar CSF is usually the signature of yolk sac or endodermal tumor component. Elevations of AFP and β-hCG are usually

TABLE I. Patient Characteristics and Tumor Marker Values From Serum (S), Lumbar (L), and Ventricular (V) Cerebrospinal Fluid

Patient	Age at diagnosis (years)	Sex	Tumor location	Histology	AFP values (S, V, L)	β-hCG values (S, V, L)
1	15.0	M	Pineal	G	0, 0, 0	6.9, 57.4, 8.4
2	17.6	M	Pineal + S/SS	G + E + MT	12.4, 2.0, 9.0	5.0, 6.0, 116.0
3	14.5	M	Pineal	G	2.3, 1.0, 1.0	6.0, 147.0, 244.0
4	10.0	M	Pineal + S/SS	G	0, NA, 0	58.0, 81.0, 358.0
5	17.3	M	Pineal + S/SS	G	3.2, 0, 0	0, 7.0, 16.0
6	18.7	M	Pineal + S/SS	I	1.6, 0, 0	0, 10.0, 15.0
7	9.1	F	S/SS	G	0, 0, 0	0, NA, 6
8	17.8	M	Pineal	MMGCT including Y	36.5, 2.0, 5.0	4.7, 1.1, 7.6
9	17.0	M	Pineal	G	3.5, 0, 0	0, 8.1, 8.1
10	21.6	M	Pineal	G	1.9, 0, 0.1	0, 0, 0
11	11.1	M	Pineal	G	1.0, 0, 0	1.4, 3.0, 34.0
12	25.0	M	Pineal	NA	27,400.0, 2,981.0, 14,015.0	0, 0, 0
13	10.8	M	Pineal	G + IT	2.1, 0, 0	0, 0, 0
14	18.7	M	Pineal + S/SS	I	7.8, 0, 0	0, 1.0, 5.0

F, female; M, male; S/SS, sellar/supra-sellar; G, germinoma; E, endodermal sinus tumor; MT, mature teratoma; IT, immature teratoma; I, inconclusive; Y, yolk sac tumor; AFP, alpha-fetoprotein; β-hCG, the β subunit of human chorionic gonadotropin; NA, not available (for histology, NA indicates no biopsy was obtained). Values of AFP are ng/ml, while values of β-hCG are mIU/ml.

associated with embryonal carcinoma components and high values of β-hCG alone occur in choriocarcinoma. The diagnosis of a pure germinoma can only be entertained with a normal AFP value in serum and CSF and relatively low levels of β-hCG. Most contemporary European and North American clinical trials have arbitrarily set an upper limit of CSF or serum β-hCG level of 50–100 mIU/ml above which any tumor, even in the setting of a biopsy-proven pure germinoma, has been regarded as containing MMGCT elements and treated as such. The upper limit of serum or CSF β-hCG still compatible with germinoma is, however, arbitrary and has become even more controversial with recent Japanese studies. Indeed, the Japanese Pediatric Brain Tumor Study Group found no statistically significant difference in 5-year survival and recurrence rates between low-secreting pure germinoma and high secreting β-hCG either in the serum or CSF with levels $\leq$200 mIU/ml in children with pathologically confirmed pure germinoma treated with combinations of chemotherapy and radiation therapy [7,8]. A debate therefore remains about the upper limit of β-hCG level in serum or lumbar CSF which can be safely attributed to a pure germinoma. Moreover, since treatment recommendations have used serum and lumbar CSF values, it is unclear if using ventricular CSF measurements to make a clinical decision about diagnosis and management is appropriate.

Individual protein levels in the CSF are known to vary along the neuraxis. Albumin, a serum-derived protein, has been previously shown to exhibit a rostrocaudal ratio of 2.2- to 2.8-fold increase in concentration [9–11]. In addition, the ventricular to lumbar CSF concentration gradients of brain-derived proteins such as tau protein, cystatin C, β-trace protein, and S-100, show source-dependent differences, with some proteins being higher in ventricular than lumbar CSF (tau protein, S-100) while the reverse can be observed with other proteins (cystatin C and β-trace protein) [10].

The purpose of this retrospective descriptive study was to compare the levels of tumor marker assays (β-hCG and AFP) in serum and CSF, sampled in two distinct sources (lumbar and ventricular CSF), when establishing a diagnosis of CNS GCT and assigning treatment regimen to patients with CNS GCT. This constitutes to date the largest series reporting contemporaneous measurements of intracranial CNS GCT tumor markers in both ventricular and lumbar CSF, as well as serum.

Averting sampling the lumbar CSF for tumor marker assays when such values are readily available from ventricular CSF obtained at surgery is based on the assumption that values are equivalent or equally informative. However, our data suggest that lumbar CSF is more informative.

Lumbar CSF sampling was performed at least 3 days following the neurosurgical procedure and ventricular CSF collection. Given the half-life of β-hCG of less than 24 hours, this allowed for sufficient time to avoid erroneous interpretations of lumbar CSF β-hCG related to a surgical procedure.

When detectable, β-hCG values in our cohort of patients treated for germinoma were higher in the lumbar than in the ventricular CSF, with the exception of one germinoma subject whose β-hCG value was superior in the ventricular than in the lumbar CSF. Although our numbers are small, the trend suggests that as a consequence, evaluation at diagnosis might be underestimating the tumor marker values for a given patient when measuring tumor marker values in the serum and ventricular CSF only and by foregoing a lumbar CSF assessment, evidence of a MMGCT may be missed. One of our subjects (Case 4) exemplified this, as the sampling of lumbar CSF tumor markers (β-hCG value of 358.0) modified our initial treatment recommendation based on a histologically confirmed germinoma with serum and ventricular CSF β-hCG $<$100 and normal AFP values in serum and ventricular CSF. This patient presented with a 3-month history of symptoms related to progressive diabetes insipidus followed by acute hydrocephalus, and was diagnosed with a bifocal midline tumor with accompanying non-communicating hydrocephalus. He underwent placement of a ventriculoperitoneal (VP) shunt and an endoscopic biopsy of the pineal region tumor was performed, consistent with a pure germinoma. Tumor markers from the ventricular CSF were examined at that time and a cytology examination from ventricular CSF was positive for malignant cells. To complete staging, a lumbar puncture was performed 16 days following his neurosurgical procedure. Cytology evaluation of the lumbar CSF was negative for the presence of malignant cells, and a spine magnetic resonance imaging (MRI) did not disclose any evidence of spinal metastases. Given his increased β-hCG tumor marker value in the lumbar CSF, he was treated as an MMGCT.

For MMGCT, AFP values were higher in the serum than the CSF (serum $\geq$ lumbar CSF $\geq$ ventricular CSF) in all of our subjects. Since any elevation of AFP will assign a patient to an MMGCT protocol, the source of fluid is not as critical in the staging. However, the number of patients with elevated AFP in our study group was very small, and it is unknown at this time if normal lumbar CSF AFP level can be assumed with a normal ventricular CSF AFP level. A major limitation of ventricular CSF analysis is that it cannot be repeatedly sampled unless a VP shunt is placed with an accessible reservoir. Thus, serum and lumbar CSF assays are the primary source of oncoprotein determinations in clinical trials and the only sources available to confirm the completeness of response or likelihood of recurrence.

Multiple studies comparing serum and lumbar CSF β-hCG values have shown that values are higher in the CSF than in the serum [6,12–15], mandating CSF sampling prior to treatment initiation. In contrast, there are only three case descriptions documenting concomitant levels of tumor markers in the ventricular and lumbar CSF [5,6]. One study reported on two patients [5]. The first patient with histological evidence of endodermal sinus and embryonal carcinoma compatible with MMGCT had decreasing but persistently elevated AFP after radiation therapy, with S, V, and L levels of 211, 139, and 608 ng/ml, respectively [5]. These results were similar to our finding of AFP values being higher in the lumbar than the ventricular CSF, although this patient had lower serum than lumbar CSF values, in contrast to all patients from our series. The second patient had β-hCG S, V, and L levels of 4.4, 2.1, and 26.0 ng/ml, respectively [5]. In agreement with our findings, ventricular was lower than lumbar CSF β-hCG, although in this particular instance, the serum β-hCG value was higher than ventricular CSF, and lower than the lumbar CSF value. Another published patient by Rogers et al. [6] consist of a patient with a pineal region MMGCT with β-hCG S, V, L values of 19, 0, and 350 IU/L, respectively. Again, lumbar CSF tumor markers analysis yielded the highest value [6], although similarly to Allen et al. [5], the serum value was higher than the ventricular CSF and lower than the lumbar CSF value.

In conclusion, the role of ventricular CSF oncoprotein assays is evolving but lumbar CSF remains the most sensitive and reliable source of fluid for clinical guidance in the management of CNS

GCT. We suggest that lumbar CSF continue to be obtained on all patients suspected of a CNS GCT within the limitations of safety. However, more prospective data should be obtained on the relative diagnostic sensitivities of ventricular and lumbar CSF, not only for existing oncoproteins but also new surrogate markers such as various miRNA species [16,17].

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