Method Validation of Human Chorionic Gonadotropin and α-Fetoprotein in Cerebrospinal Fluid: Aiding the Diagnosis of Intracranial Germ Cell Tumors

Zahra Shajani-Yi, Isabella W. Martin, Abigail A. Brunelle, and Mark A. Cervinski

Background: Our study objective was to validate 2 individual methods to measure α-fetoprotein (AFP) and human chorionic gonadotropin (hCG) in cerebrospinal fluid (CSF) on the Roche cobas® 6000 analyzer. A 3-year retrospective chart review of CSF samples analyzed for AFP and hCG was also conducted.

Methods: Serum samples with high concentrations of AFP or hCG were added to aliquots of pooled CSF. Precision, linearity, detection limit, recovery, carryover, stability, and interference studies of the AFP and hCG+β assays were performed.

Results: Within-day and day-to-day assay imprecision for AFP and hCG assays were <5% at all concentrations tested. The linear range of the AFP assay was established as 1.0–1100 μg/L, and limit of quantification (LOQ) was <1.0 μg/L. The linear range of the hCG assay was established as 1.0–9500 IU/L and LOQ 0.7 IU/L. There was no demonstrable matrix effect, and neither assay was affected by the presence of hemolysis or xanthochromia. AFP in CSF was stable at room and refrigerated temperatures for up to 48 h at concentrations of 19 and 306 μg/L but increased by 24 h at a concentration of 908 μg/L. AFP in CSF was stable frozen (−20 °C) for up to 7 days. hCG in CSF at all concentrations tested was stable at room, refrigerated, and frozen temperatures for up to 7 days.

Conclusions: The Roche cobas 6000 AFP and hCG+β assays accurately quantify AFP and hCG in CSF, facilitating rapid and accurate diagnosis as well as monitoring of intracranial germ cell tumors.

IMPACT STATEMENT

These validation studies will increase awareness of the utility of the measurement of AFP and hCG in CSF for the diagnosis and management of intracranial germ cell tumors. These studies were undertaken to improve the turnaround time associated with this testing as it is typically performed at reference laboratories. This report will provide other clinical laboratories a guide of the studies required to validate these assays in CSF for use in their own laboratories.
Intracranial germ cell tumors (GCTs)\(^3\) are a rare, heterogeneous, and diagnostically challenging group of complex neoplasms that comprise approximately 2%–3% of pediatric brain tumors and are most prevalent in young adult males between the ages of 10 and 12 (1). WHO classifies these tumors into 2 subgroups: germinomas (secreting and nonsecreting) and nongerminomatous germ-cell tumors (NGGCTs) with germinomas accounting for approximately 65% of GCTs (1). NGGCTs include embryonal carcinomas, yolk sac tumors, choriocarcinomas, and mixed tumors that contain more than one type of histology. Teratomas are sometimes classified as NGGCTs; however, they have also been considered as a separate entity and categorized as either mature teratomas, immature teratomas, or teratomas with malignant transformation.

Neuroimaging often cannot distinguish GCTs from other tumors such as pineoblastomas, pineocytomas, and gliomas, and verification via histology is usually required. Increased concentrations of the tumor markers α-fetoprotein (AFP) and/or human chorionic gonadotropin (hCG) in cerebrospinal fluid (CSF) combined with imaging can bypass the need for additional tissue confirmation in certain GCTs and aid in the discrimination between germinomas and NGGCTs. This result subsequently helps direct appropriate treatment (2–4) and reduces the risk of surgery-related complications in this subset of patients (5). Increased concentrations of AFP in CSF support the diagnosis of yolk sac tumors/yolk sac components of mixed GCTs, whereas high concentrations of hCG in CSF support the diagnosis of choriocarcinomas/choriocarcinomatous components of mixed GCTs (6, 7).

Detection of an increased concentration of AFP or hCG in CSF is diagnostically more sensitive and reliable than serum measurement and can be used to monitor response to treatment (7–9). After successful treatment, new-onset elevations of AFP and hCG in CSF are sensitive markers of tumor recurrence and may precede radiologic or symptomatic detection, allowing for better management. Recurrence has been observed years after treatment (10), and patients require long-term monitoring. For these reasons, it has been recommended that all patients with possible intracranial GCTs have AFP and hCG measured in CSF unless medically contraindicated (2). An accurate and reliable assay to determine AFP and hCG in CSF is therefore vital for managing and monitoring these patients.

Measurement of AFP and hCG in alternate sample matrices such as CSF need to be validated for the alternative matrix before clinical laboratory use to determine how the matrix will affect accuracy. A previous study detailed the validation of AFP and hCG in CSF on the Siemens Centaur platform (11). Here we report the validation of AFP and hCG+β assays in CSF on the Roche cobas® 6000 (Roche Diagnostics).

**MATERIALS AND METHODS**

**CSF samples**

Institutional Review Board approval was obtained before beginning the study. Validation was performed on pooled residual CSF samples from Dartmouth-Hitchcock Medical Center. Samples were included if they were clear, colorless, and contained at least 1 mL CSF. Medical records were reviewed to ensure that patients with abnormal CSF values, known CSF infections, or blood-CSF barrier breakdown were excluded. To generate CSF samples with known concentrations of AFP and hCG, serum samples containing either high concentrations of AFP or hCG were added to the

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\(^3\) Nonstandard abbreviations: GCT, germ cell tumor; NGGCT, nongerminomatous germ-cell tumor; AFP, α-fetoprotein; hCG, human chorionic gonadotropin; CSF, cerebrospinal fluid; LOB, limit of blank; LOD, limit of detection; LOQ, limit of quantitation.
pooled CSF, and baseline AFP and hCG concentrations were determined before use. The ratio of serum to CSF did not exceed a 1:10 dilution except where specified. Precision studies, limit of blank (LOB), limit of detection (LOD), limit of quantification (LOQ), linear range, recovery, and carryover studies were performed, and data were analyzed using available templates in an EP evaluator (Data Innovations).

**AFP and hCG assays**

AFP concentrations were measured using the Roche cobas AFP assay, and hCG concentrations were measured using the Roche hCG+β assay on the Roche cobas 6000. Both assays are sandwich-electrochemiluminescence assays that use biotinylated monoclonal mouse capture antibodies and monoclonal mouse detection antibodies that recognize 2 separate epitopes on either AFP or hCG. The AFP assay has a serum measuring range of 0.605–1210 μg/L with a LOD of 0.605 μg/L. The hCG+β assay has a measuring range of 0.100–10000 IU/L with a LOD of <0.100 IU/L in serum.

**Precision studies and linearity**

Serum containing high concentrations of AFP or hCG was added to residual CSF to generate pools of CSF containing 2 concentrations for each analyte. To assess within-day assay imprecision, the 2 pools were assayed 20 times in 1 day with mean target concentrations of 12 and 600 μg/L of AFP and 10 and 120 IU/L of hCG. To determine day-to-day imprecision, individual aliquots were assayed once a day for 20 nonconsecutive days at mean target concentrations of 12 and 375 μg/L of AFP and 12 and 175 IU/L of hCG. Linearity was surveyed by preparing 5 CSF samples with varying concentrations of AFP and hCG spanning the linear range for each assay as described above. Each sample was evaluated in duplicate. The final concentrations ranged from 11 to 1130 μg/L for AFP and from 11 to 9500 IU/L for hCG.

Precision targets were selected from the publicly available Data Innovations allowable total error table (12). Precision goals were set to be equal to one-half of the total allowable error. Total allowable errors for AFP and hCG were 20% and 15%, making the acceptable precision goals 10% and 7.5%.

**LOB, LOD, and LOQ**

The LOB was calculated using the following formula: \( \text{LOB} = \text{mean}_{\text{blank}} + 1.65 \times (\text{SD}_{\text{blank}}) \), where the blank sample was pooled residual CSF as described above. The LOD was calculated as: \( \text{LOD} = \text{LOB} + 1.65 \times (\text{SD}_{\text{low concentration sample}}) \), where the low concentration samples were 12 μg/L and 10 IU/L for AFP and hCG, respectively. Samples were assayed once a day for 12 nonconsecutive days. The LOQ was defined as the lowest concentration measured with a CV <10% and was determined by assaying 5 CSF specimens with AFP and hCG concentrations ranging from 0 to 30 μg/L and 0 to 11 IU/L, respectively, once a day for 14 days. The LOQ was calculated by plotting the CV against the mean and fitting a curve to the data using an EP Evaluator.

**Stability studies and interference studies**

To perform the stability studies, serum containing high concentrations of AFP or hCG was added to residual CSF to generate CSF pools containing 3 concentrations for each analyte (low, intermediate, high) to be held at 3 sample storage conditions: room temperature (18 °C–25 °C), refrigerated (4 °C–8 °C), and frozen (−20 °C). The target concentrations were 20 μg/L for AFP and 20 IU/L for hCG (low), 300 μg/L for AFP and 300 IU/L for hCG (intermediate), and 900 μg/L for AFP and 3000 IU/L for hCG (high). Baseline measurements of AFP and hCG were conducted immediately. Room temperature and refrigerated samples were then measured in triplicate at the following time intervals: 2 h, 4 h, 6 h, 24 h, 48 h, 72 h, and 7 days. To test the stability of frozen samples, aliquots were distributed into separate tubes and frozen. A new aliquot
was thawed and measured in triplicate at the following times: 24 h, 48 h, 72 h, and 7 days. The stability limits were determined to be equivalent to 3-fold the CV of the individual marker as determined at a mean AFP concentration of 371.6 μg/L and a mean hCG concentration 174.8 IU/L from our day-to-day precision experiment (Table 1). The same limits were used for the interference studies below. Samples were deemed stable if the percent recovery was within 9.9% for AFP and 8.7% for hCG. Percent recovery was determined for both analytes at each storage temperature and time interval using the following formula: percent recovery = (mean measured value/mean baseline value) × 100, where the baseline value is the analyte concentration at time = 0.

To perform the interference studies, 2 CSF pools containing AFP and hCG were prepared as described for the stability studies: the first pool to investigate the effect of xanthochromia and the second to investigate the effect of hemolysis. To investigate the effect of xanthochromia, 5 mg bilirubin (Sigma Aldrich) was reconstituted in 5 mL of 0.1 mol/L NaOH for a final concentration of 1000 mg/dL. The reconstituted bilirubin was added to CSF and independently evaluated by 2 experienced technologists to determine which concentrations corresponded to the visual assessment of mild, moderate, and marked xanthochromia. CSF pools containing AFP and hCG at 3 concentrations (low, intermediate, high) were mixed with the bilirubin to target concentrations that corresponded visually to mild (0.033 mg/dL), moderate (0.067 mg/dL), and marked (0.1 mg/dL) xanthochromia. To investigate the effects of hemolysis, a hemolysate was prepared from EDTA anticoagulated blood. The hemolysate was prepared by pelleting the cellular components via centrifugation at 2100g and discarding the plasma supernatant. The pelleted cells were then resuspended in isotonic saline and inverted gently 10 times. The washed red blood cells were again pelleted by centrifugation and the saline was discarded. These washing and pelleting steps were repeated 3 times. The final pellet was again resuspended in type 1 water and frozen overnight to lyse the cellular components. The hemolysate was added to CSF and independently evaluated by 2 experienced technologists to determine which concentrations corresponded to the visual assessment of mild and gross hemolysis as assessed in CSF. CSF pools containing AFP and hCG at 3 concentrations (low, intermediate, high) were mixed with the hemolysate to target concentrations that corresponded visually to mild (20 mg/dL) and gross hemolysis (134 mg/dL). Each sample pool was analyzed in triplicate. Percent interference was calculated as: percent interference = [(mean measured value-mean baseline value)/mean baseline value] × 100, where baseline is the analyte concentration before the addition of bilirubin or hemolysate.

### Recovery and carryover studies

To perform the matrix recovery study, serum samples with a high concentration of AFP or hCG were diluted into pooled CSF at 3 different concentrations. The expected concentrations for AFP

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**Table 1. Imprecision of AFP and hCG in CSF.**

<table>
<thead>
<tr>
<th></th>
<th>Mean, μg/L</th>
<th>CV, %</th>
<th>Mean, IU/L</th>
<th>CV, %</th>
</tr>
</thead>
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<tr>
<td>AFP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within-day imprecision, level 1</td>
<td>12.0</td>
<td>2.4</td>
<td>10.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Within-day imprecision, level 1</td>
<td>602.8</td>
<td>4.7</td>
<td>118.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Day-to-day imprecision, level 1</td>
<td>12.5</td>
<td>3.6</td>
<td>11.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Day-to-day imprecision, level 1</td>
<td>371.6</td>
<td>3.3</td>
<td>174.8</td>
<td>2.9</td>
</tr>
</tbody>
</table>

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were 8, 462, and 924 μg/L. The expected concentrations for hCG were 7, 93, and 5786 IU/L. Each concentration was analyzed in triplicate. To assess recovery of AFP and hCG above the stated linear range of the assay, we prepared a CSF pool as above with target concentrations of AFP or hCG of 2220 μg/L and 12900 IU/L, respectively. The ratio of serum added to the pooled CSF samples was 1:8 (12.5%) for this study. The pooled CSF sample was analyzed and automatic instrument dilutions using the Roche Diluent Universal were performed at 1:50 for AFP and 1:100 for hCG. Additional manual dilutions were performed using the same diluent at 1:50 and 1:100 for both analytes and measured. Carryover was assessed by preparing and measuring CSF samples with a low concentration (1 μg/L for AFP; 4 IU/L for hCG) and a high concentration (1040 μg/L for AFP; 9000 IU/L for hCG) using the template in EP Evaluator in the following order: 3 low, 2 high, 1 low, 2 high, 4 low, 2 high, 1 low, 2 high, 1 low, 2 high, and 1 low.

**Retrospective analysis**

We retrospectively reviewed the medical charts of patients who had AFP and hCG measurements in CSF between January 1, 2014, and February 20, 2017.

**RESULTS**

Within-day repeatability studies demonstrated imprecision of 2.4% and 4.7% for AFP mean concentrations of 12 and 603 μg/L and imprecision of 1.8% and 3.6% for hCG mean concentrations of 11 and 119 IU/L. Day-to-day reproducibility studies demonstrated imprecision of 3.6% and 3.3% for AFP mean concentrations of 13 and 372 μg/L and imprecision of 2.5% and 2.9% for hCG mean concentrations of 12 and 175 IU/L (Table 1).

The LOB, LOD, and LOQ (10% CV) for AFP were 0.8, 0.9, and <1.0 μg/L (Fig. 1). The LOQ of AFP exceeded the precision target of 10%, and at a mean concentration of 0.9 μg/L, the CV was 9%. The linear range for the AFP assay was established as 1.0–1100 μg/L (Fig. 2). The LOB, LOD, and LOQ (10% CV) for hCG were 0.4, 0.5, and 0.7 IU/L (Fig. 1). The linear range for the hCG assay was established as 1.0–9500 IU/L (Fig. 2). The recovery experiment demonstrated that AFP and hCG concentrations were within 10% of the target concentration, with the exception of the level 1 AFP sample (118%) (Table 2). A dilution study of a pooled CSF sample with AFP and hCG concentrations above the stated...
assay linear range (target concentrations, AFP = 2220 μg/L, hCG = 12 900 IU/L) using the Roche Di-
luent Universal was performed. The AFP concen-
tration determined by automated dilution was
2341 μg/L (117% of target) and the AFP concentra-
tion of the 1:50 and 1:100 manual dilutions were
2517 μg/L (113%) and 2470 μg/L (111%), respec-
tively. The hCG concentration determined by auto-
mated dilution was 12 342 IU/L (96% of target), and
the hCG concentration of the 1:50 and 1:100 man-
ual dilutions were 12 485 IU/L (97%) and 12 780
IU/L (99%), respectively. Carryover experiments
demonstrated no appreciable specimen carryover
for either assay (data not shown).

The stability of AFP and hCG differed at room
and refrigerated temperatures (Table 3). AFP at a
mean concentration of 19 and 306 μg/L was stable
at both room and refrigerated temperature for up
to 48 h. The AFP in the high pool (AFP = 908 μg/L)
increased in concentration by 24 h of incubation.
The cause of this increase in the high AFP concen-
tration pool could not be elucidated and was not
explored further. Conversely, hCG at all tested
concentrations remained stable at room temper-
ate and at 4 °C–8 °C for up to 7 days. Both AFP
and hCG were stable when frozen at −20 °C for up
to 7 days. Both assays were unaffected by marked
xanthochromia (bilirubin = 0.1 mg/dL) and gross
hemolysis (hemoglobin = 134 mg/dL) (Table 4).

Our retrospective chart review identified 6 pa-
tients who had CSF collected for measurement of
AFP and hCG analyzed at a reference laboratory
Testing was requested because of an elevation in
serum tumor markers or the presence of an intra-
cranial mass. Patient 1 was a 6-month-old female
with a 9-mm enhancing pineal tumor that could
not be biopsied because of its location. Serum AFP

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**Table 2. Recovery of AFP and hCG in CSF.**

<table>
<thead>
<tr>
<th></th>
<th>Expected (n = 3)</th>
<th>Observed (n = 3)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP, μg/L</td>
<td>8.4</td>
<td>9.9</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>461.5</td>
<td>469.9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>923.0</td>
<td>981.2</td>
<td>106</td>
</tr>
<tr>
<td>hCG, IU/L</td>
<td>6.7</td>
<td>6.4</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>92.9</td>
<td>90.9</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>5785.9</td>
<td>5375.3</td>
<td>93</td>
</tr>
</tbody>
</table>
measurements were increased, but hCG and AFP concentrations in CSF were undetectable. The tumor was diagnosed as a complex pineal cyst and is currently stable. Patient 2 was a 36-year-old female who presented with sudden-onset diabetes insipidus. MRI revealed a thickened pituitary stalk. hCG in CSF and AFP concentrations were below the reference range. Patient 3 was a 19-year-old male with a 15-mm lesion in the pineal region. Serum and CSF hCG and AFP measurements were undetectable. Patient 4 was a 19-year-old male with a 12-mm pineal tumor. AFP and hCG measurements in CSF were undetectable. Pathological examination after total resection confirmed the mass as a pineal tumor. Patient 5 was a 26-year-old male who presented with central diabetes insipidus and near-panhypopituitarism. MRI revealed a 13-mm pituitary adenoma that was pathologically confirmed, after total resection, as a germinoma. AFP and hCG measurements in CSF were sent out 8 weeks after surgery as part of his monitoring program and were undetectable. Patient 6 was a 15-year-old male who presented with 2 weeks of diplopia, intermittent headaches, nausea, and vomiting. MRI revealed a 32 × 27 × 22-mm pineal complex cystic mass with moderate hydrocephalus. Serum and CSF AFP measurements were 47 and 0.7 μg/L, respectively, and serum and CSF hCG measurements were 181 and 272 IU/L, respectively. Biopsy results indicated only a germinoma; however, the increased concentrations of hCG in both serum and CSF suggested a diagnosis of NGGCT. MRI imaging coupled with the serum and CSF led to a working diagnosis of a mixed teratoma with both benign and malignant elements.

Of these patients, CSF samples from patients 5 and 6 were analyzed both in our laboratory and a reference laboratory. The CSF sample for patient 5 had no detectable AFP or hCG at both laboratories, while the AFP and hCG concentrations for patient 6 were 1.1 μg/L and 297 IU/L at our institution and 0.7 μg/L and 272 IU/L at the reference laboratory.

**DISCUSSION**

In this report, we detail the laboratory validation of the Roche cobas AFP and hCG+β assays for use in CSF. Our validation studies included precision, accuracy, linearity, LOB, LOD, and LOQ as well as assessment of sample stability and interferences due to xanthochromia and hemolysis. Our studies demonstrate that mild, moderate, and marked xanthochromia and mild and gross hemolysis did not demonstrate interference with either assay. Our studies also demonstrated no appreciable matrix effect for AFP and hCG with recovery differing <10% of the target concentration, with the exception of the level 1 AFP sample, which had an over-recovery of 18%. This over-recovery was likely

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**Table 3. Stability studies of AFP and hCG.**

<table>
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<tr>
<th></th>
<th>Percent recovery</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>168 h</th>
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<tr>
<td><strong>AFP, μg/L</strong></td>
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<tr>
<td>19 RT</td>
<td>103 106 109 108</td>
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<td></td>
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<td><strong>hCG, IU/L</strong></td>
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* All measurements were performed in duplicate and mean percent recovery compared to time 0 was recorded.
* RT, room temperature (18 °C–25 °C); 4C, refrigerated temperature (4 °C–8 °C).
* The time point at which the stability limit listed in Materials and Methods was exceeded.
Table 4. Interference effects on AFP and hCG in CSF.\textsuperscript{a}

|                  | Baseline AFP, \(\mu g/L\) | Mild xanthochromia, \(\mu g/L\ | Moderate xanthochromia, \(\mu g/L\ | Marked xanthochromia, \(\mu g/L\ | Baseline hCG, IU/L | Mild hemolysis, IU/L | Gross hemolysis, IU/L |
|------------------|---------------------------|---------------------------------|---------------------------------|---------------------|---------------------|----------------------|
|                  | Baseline AFP, \(\mu g/L\) | Mild xanthochromia, \(\mu g/L\ | Moderate xanthochromia, \(\mu g/L\ | Marked xanthochromia, \(\mu g/L\ | Baseline hCG, IU/L | Mild hemolysis, IU/L | Gross hemolysis, IU/L |
| Xanthochromia interference | 21\textsuperscript{b} | 21 (0%) | 21 (0%) | 21 (0%) | 21 (0%) | 298 (0%) | 314 (0%) |
|                  | 315\textsuperscript{b} | 308 (−2%) | 311 (−1%) | 311 (−1%) | 300 (1%) | 298 (0%) | 314 (0%) |
|                  | 802\textsuperscript{b} | 808 (1%) | 795 (−1%) | 821 (2%) | 821 (2%) | 298 (0%) | 314 (0%) |
| Hemolysis interference | 24 | 24 (0%) | 24 (0%) | 24 (0%) | 24 (0%) | 24 (0%) | 24 (0%) |
|                  | 289 | 287 (−1%) | 287 (−1%) | 287 (−1%) | 287 (−1%) | 287 (−1%) | 287 (−1%) |
|                  | 846 | 846 (0%) | 846 (0%) | 846 (0%) | 846 (0%) | 846 (0%) | 846 (0%) |

\textsuperscript{a} All measurements were performed in triplicate, and mean value was reported as whole numbers. Values in parentheses represent the percent interference as described in Materials and Methods. Mild, moderate, and marked xanthochromia were assessed by visual inspection and correspond to target bilirubin concentrations of 0.03, 0.07, and 0.1 mg/dL, respectively. Mild and gross hemolysis were assessed visually and corresponded to target hemoglobin concentrations of 20 and 134 mg/dL, respectively.

\textsuperscript{b} The baseline samples contained a volume of dilute NaOH equivalent to the marked xanthochromia pool to account for any possible effects of the NaOH on assay performance.
due to a combination of alternate CSF matrix and assay imprecision. To our knowledge, only one other publication reported the measurement of hCG in CSF using the Roche assay (13). In that study, only a functional sensitivity assessment of the Roche hCG assay in CSF was performed. Our study details many additional analytical aspects of the performance of both hCG and AFP assays in CSF.

The management, treatment, and prognosis of GCTs vary by subtype. Correct identification is therefore extremely important. Prognosis of pure germinomas and mature teratomas treated with radiation is favorable, with 5-year survival rates of >90% (14–16). Mature teratomas are usually benign and total resection is often curative (2). Germinomas with an increased β-hCG concentration, immature teratoma, and mixed tumors that are composed of mainly germinoma or teratoma tissues have an intermediate prognosis with reported 5-year survival rates of 70% (14–16). The remaining NGGCTs are associated with poorer prognosis, and treatment consists of chemotherapy, radiation, and surgery depending on the location and tumor stage. Patients with yolk sac, embryonal carcinomas, and choriocarcinomas or mixed tumors composed of these malignant histologies have a 5-year survival rate of 30%–40% (14–16). Mild elevations of hCG in CSF can also be detected in a subpopulation of germinomas, limiting the specificity of hCG as a tumor marker for NGGCTs. Regardless, quantification is still important, since increased concentrations of AFP and hCG have been associated with overall poorer prognosis (17).

In a study of more than 300 patients, Tian et al. (18) established a reference interval (97.5th percentile) for hCG in CSF of 0.77 and 1.42 IU/L in male and female patients, respectively. An additional study of 351 patients established the reference interval for AFP in CSF as 1.042 μg/L (97.5th percentile) (19). The reported decision limits for diagnosis and discrimination between secreting and nonsecreting intracranial GCTs is varied and protocols vary. Three different criteria to differentiate secreting from nonsecreting tumors are prevalent in the literature with values of (a) hCG ≥50 IU/L or AFP ≥10 μg/L (3), (b) hCG ≥50 IU/L or AFP ≥25 μg/L (20), and (c) hCG ≥100 IU/L or AFP ≥50 μg/L (5), indicating active secretion. These criteria do not distinguish whether the sample tested is CSF or serum. The decision limits were recently reassessed in a study comparing hCG and AFP values in 58 GCTs confirmed by pathology. This study concluded that serum and CSF should have different decision limits, and lower decision limits of ≥8.2 IU/L for hCG and ≥3.8 μg/L AFP in CSF were proposed (7).

A potential limitation of our study is that the serum samples used for the hCG studies were obtained from pregnant patients. We attempted to obtain samples from patients with known GCTs but were unsuccessful. GCTs may produce different hCG variants from those produced by the placenta during pregnancy. Free β-subunit was reported to be the dominant variant in some GCTs (21). The Roche cobas hCG+β assay recognizes these alternate isoforms and has equimolar detection of the free β-subunit, the holo-hormone, and nicked forms of hCG (22, 23). The Roche assay also detects the β-core fragment, albeit at a lower recovery (22, 23). Because the Roche cobas hCG+β assay detects these variants, it is an excellent choice for measuring hCG in the setting of GCTs. One additional limitation to our study is that we assessed xanthochromia via visual inspection. Assessment of xanthochromia is subjective and suffers from poor interobserver variability.

Our retrospective study is limited by (a) the number of samples analyzed during our study period and (b) the fact that only 1 of these samples had detectable concentrations of hCG and AFP in CSF. The results of the 2 patient samples tested at both our institution and the reference laboratory were concordant.
The analytical performance of hCG and AFP assays on the Roche cobas platform was similar to the reported performance on the Siemens Centaur platform (11). The Roche cobas assays did demonstrate lower LOD as well as larger analytic measuring ranges for both hCG and AFP than the Siemens Centaur assays. The clinical significance of these differences is negligible and demonstrates that laboratories with either of these platforms could validate these tests for clinical use.

Our validation demonstrates that the Roche cobas 6000 AFP and hCG+β assays can accurately quantify AFP and hCG in CSF, facilitating rapid and accurate diagnosis and monitoring of intracranial GCTs.

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