Evaluation of Beta-Subunit Chorionic Gonadotropin as an Aid in Diagnosis of Trophoblastic Disease

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ABSTRACT

The advent of the radioimmunoassay of Beta subunit chorionic gonadotropin (BhCG) has eliminated a major problem in following the patient with gestational trophoblastic neoplasia (GTN). Whole hCG cross reacts with luteinizing hormone (hLH) causing difficulties when GTN patient titers fall to levels coincidental with pituitary levels of hLH. The use of the Beta subunit of hCG in radioimmunoassay has eliminated this problem because there is essentially no cross reaction between the B-subunit of the hCG and hLH. A review of the use of this sensitive assay in the management of patients with GTN is presented.

Introduction and Historical Perspective

Thirty-one years ago, Hertig and Sheldon classified gestational trophoblastic neoplasms (GTN) in terms of morphologic characteristics which could be used to predict the potential for aggressive disease. At that time, approximately 40 to 50 percent remission could be expected in patients with choriocarcinoma, only if the disease were confined to the uterus, and then only with hysterectomy. The mortality in patients with metastatic disease was 95 percent.

Chemotherapy induced remissions in metastatic GTN were first reported by Li and associates in 1956. In 1961, Hertz and associates reported complete and sustained remissions in 47 percent of patients with metastatic GTN, again with chemotherapy. Ross and co-workers in 1967 achieved 95 percent complete remission in non-metastatic GTN with preservation of reproductive function.

Monitoring levels of human chorionic gonadotropin (hCG) became important in following GTN patients, but the available methods in the 1950's were the bioassays which were cumbersome and insensitive. In the 1960's when further purification of the hCG molecule made it possible to raise antibodies in rabbits, a proliferation of immunologic methods using red cell or latex particle agglutination soon became readily available. The relative insensitivity of these methods (750 to 3500 IU per L) would allow a 20 to 25 percent false negative rate if used to follow hCG titers in patients with GTN.

The next generation of hCG determinations combined the technique of radioimmunoassay and the availability of a
TABLE I

Sensitivities of HCG* Determinations

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivities IU/L</th>
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<tbody>
<tr>
<td>Yes-No pregnancy tests</td>
<td>1500 - 3500</td>
</tr>
<tr>
<td>Slide tests</td>
<td>750 - 1250</td>
</tr>
<tr>
<td>Tube tests</td>
<td>200* (6)</td>
</tr>
<tr>
<td>Radioreceptor assay, HCG</td>
<td>6</td>
</tr>
<tr>
<td>Radioimmunoassay, HCG</td>
<td>5-6</td>
</tr>
<tr>
<td>Radioimmunoassay, β-HCG</td>
<td>5-6</td>
</tr>
</tbody>
</table>

*Human chorionic gonadotropin
†Commercial kit

highly purified hCG molecule, making possible a radioimmunoassay to whole hCG with sensitivity to 6 IU per L. The practical sensitivity of this assay is, however, limited by the cross reaction between the intact hCG molecule and luteinizing hormone (hLH). Unless specifically depressed, menstrual midcycle hLH can range from an average 5 to 50 IU per L to 200 IU per L. At this level, if all reactivity were due to hCG from GTN and not due to endogenous hLH, approximately one million viable tumor cells could be present.† The newer radio-receptor assay for hCG is also very sensitive, but cross reacts with hLH.23 It is for this reason that the commercial "kit" has a sensitivity adjusted to 200 IU per L.

In 1972, Vaitukaitus and co-authors29 introduced the radioimmunoassay for the Beta subunit of hCG (β-HCG). β-HCG assays allows measurement of the chorionic gonadotropin titers in the presence of hLH because there is not a significant cross reaction between these two substances. The use of β-HCG as a marker permits patient follow-up until a true negative value has been achieved. Further, this assay has obviated the practice of giving a single course of "prophylactic" chemotherapy at the time of molar evacuation. The method used in our laboratory is a modification of a double antibody of Vaitukaitus and co-workers2,29 for serum determinations with a similar technique used for urine assays. The results of the assay are reported at 5 IU per L although the true sensitivity of the assay is 2 IU per L.

**BhCG is Uncomplicated Disease**

The general protocol used is modeled after that of Pastorfide, Goldstein and Kosasa20 and is briefly outlined in table II. BhCG determinations are done at the time of mole evacuation and then at weekly intervals until the level falls to non-pregnant range for three consecutive weeks (less than 5 IU per L). Monthly determinations are done for the next six months. If the BhCG levels remain below 5 IU per L for six months, the patient is considered disease-free and allowed to become pregnant.

The regression curve of the serum BhCG in uncomplicated molar pregnancies, post evacuation, is shown in figure 1. The curve based on multiple patients closely parallels that previously published by Morrow and co-workers.19 Eighty percent of patients will have this course and will require no chemotherapy.16

**BhCG and Decisions Regarding Chemotherapy**

The 20 percent of patients requiring chemotherapy (including the 3 to 5 percent who have or will progress to choriocarcinoma) will in 16 percent have persistent disease locally, and in 4 percent will develop metastases.7,9,11 Certain findings on physical examination and characteristics of the BhCG titers are important in
predicting which patient will need chemotherapy after molar evacuation (table III). Approximately 60 percent of patients with the combined finding of large for date uterus and bilateral ovarian enlargement (over 5 to 8 cm by ultrasound), presumably owing to theca lutein cysts, will require chemotherapy. The occurrence of bilateral ovarian enlargement alone is seen in 14 percent of patients and approximately one-half of these will require chemotherapy.\(^7\)\(^\text{19}\) The occurrence of large for date uterus alone is associated with a slightly increased incidence of aggressive GTN, but to a much lesser extent.

Patients with a flattened BhCG regression curve during the initial few weeks (i.e., a BhCG level greater than 500 IU per L in four weeks) or those in which there is failure to reach non-pregnant levels in 10 to 12 weeks will, in 50 percent of the cases, develop an invasive mole in choriocarcinoma.\(^16\) Any patient with a residual titer at six months, regardless of prior regression, should be considered for chemotherapy. The use of prophylactic chemotherapy must also be considered in the patient who cannot be followed either because of unreliability or lack of adequate facilities.

**BhCG During Chemotherapy**

Chemotherapy (table IV) is instituted in all patients with histologic choriocarcinoma and in all patients with metastatic disease. Those patients in whom the titer to BhCG plateaus for two weeks or in whom an increase in titer is demonstrated are also started on chemotherapy. Since the one-half life of hCG is 24 to 36 hours, such a change in the pattern of titer regression is interpreted as indicative of a parallel failure of trophoblastic regression or an increase in the numbers of trophoblastic cells.

If titers are measured during the initial 12 to 24 hours of chemotherapy, there may be a transient "surge" in BhCG titers,\(^18\) believed to be due to tumor necrosis. The significance of this transient increase in predicting tumor responsiveness to a given agent is at present not resolved. The BhCG titer is measured weekly during chemotherapy, and additional therapy can be withheld as long as the titer continues to decrease. Therapy is usually continued until non-pregnant levels are achieved for three consecutive weeks. BhCG is then measured at monthly intervals for six consecutive months, and then at three to six month intervals for five years.\(^11\) BhCG is detectable in the circulation for a longer period than is whole hCG, possibly contributing to fewer cases in which chemotherapy is discontinued prematurely.\(^25\)

![Figure 1. Regression curve of the serum BhCG in uncomplicated molar pregnancies, post evacuation.](image)

**TABLE III**

<table>
<thead>
<tr>
<th>Patients in Whom Prophylactic Chemotherapy Should Be Considered</th>
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<tbody>
<tr>
<td>1. In an unreliable patient.</td>
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<tr>
<td>2. If facilities for monitoring g-HCG are not available.</td>
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<tr>
<td>3. If uterine size is larger than expected for date.</td>
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<tr>
<td>4. If there is bilateral ovarian enlargement (physical and ultrasound).</td>
</tr>
<tr>
<td>5. If there is flattened regression curve.</td>
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<tr>
<td>6. If g-HCG fails to reach non-pregnant levels by 10 to 12 weeks.</td>
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<tr>
<td>7. If g-HCG is demonstrable at six (6) months.</td>
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</table>
Chemotherapy is instituted:
1. If \( \beta \)-HCG level plateaus for more than two (2) consecutive weeks.
2. If \( \beta \)-HCG level rises.
3. If metastases are clinically demonstrable.
4. If there is morphologic choriocarcinoma.

It must be emphasized that prophylactic chemotherapy and even hysterectomy at the time of molar evacuation do not absolutely prevent persistent or metastatic disease. All patients must be monitored by the best method available, preferably with sequential assays for \( \beta \)-HCG.

**Problems with \( \beta \)-HCG Assay**

There are cross reacting substances, including an up to five percent cross reaction with hLH (which is equivalent to 3 IU per L) in most assay systems. Human chorionic gonadotropin—like substances which cross react *in vitro* are present in normal colon and liver and have been isolated in pituitaries, plasma and urine in post menopausal women; obviously, this does not cause a problem in GTN. A long list of gynecologic conditions is associated with a demonstrable \( \beta \)-HCG titer, including such problems as adenomyosis, myomas and single cysts of the ovary (but not endometriosis). These patients, however, together with the group having nontrophoblastic gynecologic tumors, including cervical, endometrial and ovarian carcinomas, are older with mean ages of 57 and 62 years. A long and well known list of extragenital nontrophoblastic tumors can also produce hCG-like material but again do not pose a clinical problem.

Conversely, the rare patient can have absent titers to \( \beta \)-HCG after initially elevated levels, with continuing disease, perhaps in part accounting for the 5 to 7 percent relapse rate described by Hertz. At this point, it is presumed that this is due to gestational trophoblastic neoplasia.

*Choriocarcinoma treated at time of histologic diagnosis.*

**FIGURE 2.** Scheme of method provided by \( \beta \)-HCG radioimmunoassay for following patients with gestational trophoblastic neoplasia.
to a focus of tumor producing free alpha subunits and fragments of the hCG molecule which are not antigenic in the BhCG assay system. Recently, a method using urine concentrates and a modified antigen-antibody system has been described which detected urinary BhCG when serum levels were negative.24

Summary

The reasons for the greatly improved mortality statistics in GTN are multiple and include such modalities as ultrasound in early diagnosis, chemotherapy and the accurate method provided by BhCG radioimmunoassay for following these patients (figure 2). Work continues in the problems of GTN. Karyotyping of molar tissue allows separation of the 46XX complete mole (with no fetal parts or membranes) in which one can anticipate a propensity for problems, from the several types of partial moles and as of yet unclassified conceptuses26,30 in which the tissue allows separation of the 46XX complete mole (with no fetal parts or membranes) in which one can anticipate a propensity for problems, from the several types of partial moles and as of yet unclassified conceptuses26,30 in which the propensity toward proliferative trophoblastic disease is not known. Morphologic differences in the types of GTN are again being studied, not only in terms of predicting aggressive disease but also in an attempt to define those tumors which will be resistant to chemotherapy.16,27 Perhaps with these additional modalities, those patients with potentially aggressive disease can be detected before the development of brain, liver and bowel metastases which at present are still associated with a 60 to 70 percent mortality.11

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References

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