Changes in Serum α-Fetoprotein and Chorionic Gonadotropin in Response to Cancer Therapy

JEROME S. NISSELBAUM, P.H.D.,*
GEORGE J. BOSL, M.D.†
ROBERT B. GOLBEY, M.D.†
DELIA C. SCHWARTZ, R.N.*
and MORTON K. SCHWARTZ, P.H.D.*

*Department of Clinical Chemistry and the †Department of Medicine
Memorial Sloan-Kettering Cancer Center,
New York, NY 10021

ABSTRACT

The usefulness of measuring both α-fetoprotein and hCG in following the progress of patients with testicular cancers and germ cell tumors is demonstrated. In almost half the cases of testicular cancers studied, only one tumor marker was elevated; in at least one instance, the source of α-fetoprotein was eliminated while the source of hCG persisted. A comparison of the hCG method in use at Memorial Sloan-Kettering Cancer Center (MSKCC) with a commercial kit (Corning) showed that the methods were equivalent for monitoring both testicular cancers and gestational trophoblastic disease. All 17 patients with hepatocellular carcinoma had elevated levels of α-fetoprotein.

Introduction

Over the past 20 years, there has been intense interest in studies of a special group of proteins that may be associated with cancers. Some are oncofetal proteins such as carcinoembryonic antigen (CEA) and α-fetoprotein (αFP). Others may be normal proteins observed at abnormal levels or under abnormal circumstances such as prolactin, ACTH, or chorionic gonadotropin (hCG). These proteins may present abnormally in serum or other body fluids of patients with certain cancers. Kurman et al and Morinaga et al have demonstrated by immunoperoxidase staining of cancer tissues and radioimmunoassay of serum that the cancer tissues are the source of elevated serum αFP and/or hCG. Romero and Schwartz have shown the importance of measuring serum αFP in the management of ovarian germ cell tumors, while Talerman et al have shown the correlation between increased serum αFP and endodermal sinus tumors. Thompson and Haddow have emphasized the strong association between increased serum levels of αFP and hCG with nonsemenomatous testicular cancers and have suggested that cases of apparent seminoma with elevations of these
markers are probably cases of mixed tumor and should be treated as nonseminoma patients.

The demonstration or failure to demonstrate these proteins in the absence of other criteria, i.e., histology, cannot be taken to be diagnostic. However, once it has been established that a patient has an elevated level of one of these so-called tumor markers in a body fluid, usually serum or plasma, the changes in levels are of great utility in assessing progress of disease and effectiveness of therapy. In addition, as has been pointed out by Thompson and Haddow in the case of serum αFP, if after surgical removal of the tumor the patients serum markers do not decline with the expected half life, it is a strong indication that there is residual tumor. The half life of α-fetoprotein is four to six days and that for hCG is about 36 hours.

The present study was designed to show the various patterns of serum αFP and hCG that may occur in patients with germ cell tumors, gestational trophoblastic disease, and hepatocellular carcinoma. In addition, the present authors compared a commercial hCG kit† that utilized an immobilized polyclonal antibody with the polyclonal, double antibody method.

Materials and Methods

Blood serum was obtained by salvage of samples that were submitted to the Department of Clinical Chemistry for either αFP or hCG measurements after all clinical testing has been completed. Patients selected for the study included 68 males with testicular germ cell tumors, four males with nontesticular germ cell tumors, six females with germ cell tumor, and 12 with gestational trophoblastic disease. Patients with cancers other than germ cell tumors included 17 with hepatocellular carcinoma, and 12 males and eight females with a variety of cancers including bladder cancer, pancreatic cancer, meningo, epidermoid cancer, pulmonary blastoma and colon cancer. Many patients had multiple analyses for αFP and hCG during the course of therapy. Some typical responses are shown in the figures.

Beta-chorionic gonadotropin and whole molecule hCG for use as immunogens and standards, respectively, were obtained from National Institute of Arthritis, Metabolic, and Digestive Diseases (NIAMDD). In addition, ¹²⁵I-hCG was obtained commercially, as was normal rabbit serum, and goat-antirabbit gamma globulin, (1 ml of antisem is capable of precipitating 3 mg of rabbit gamma globulin). Polyethylene glycol 6000 (PEG) was also obtained commercially.

Human Chorionic Gonadotropin

Human chorionic gonadotropin was measured by two methods—our own MSKCC method and with kits obtained from Corning. The Corning kit was used exactly as described in the manufacturer’s directions. However, it was noticed by the present authors that the method is linear up to 200 IU per L and routinely included a 200 IU per L point on the standard curve.

The MSKCC method utilized rabbit antiserum to be β-subunit of hCG. Beta-human chorionic gonadotropin (250 μg) was mixed with Freund adjuvant and injected subcutaneously into male New Zealand Red rabbits in four doses; three doses were given one week apart and the
fourth dose two weeks later. The animals were exsanguinated 20 days after the last injection, the serum collected, titrated, and frozen in five mL aliquots at —20°C.

A stock 1000 fold dilution of antiserum in water was made, aliquoted in 1.0 mL portions, and kept at —20°C. A further 10 fold dilution of PBS-NRS (0.01 M phosphate, pH 7.8, 0.15% NaCl, 0.25% percent normal rabbit serum) was made at the time of assay. Standard hCG, obtained as a lyophilized powder of known weight from NIAMDD, was diluted to approximately 25 μg per L in one percent bovine serum albumin and stored at —80°C. Five serial dilutions were made in PBS-NRS. Each tube contained 0.2 mL of sample: standard, control or patient sample. Tubes containing undiluted patient samples or controls also received 0.4 mL of a cocktail of three parts PBS-NRS and one part of 0.1 M ethylenediamine tetraacetic acid (EDTA), pH 7.8. Tubes containing standards, cerebrospinal fluid, or diluted patient samples received 0.4 mL of a cocktail of one part PBS-NRS, one part 0.1 M EDTA, and two parts normal male plasma. Exactly 0.2 mL of the 10,000 fold dilution of rabbit anti-αhCG was added to all tubes except total count tubes and nonspecific binding tubes, and they were incubated at 37°C for one hour (volume = 0.8 mL). Precisely 0.2 mL of 125I-hCG containing 20,000 CPM diluted in PBS-NRS was added (volume now = 1.0 mL) and incubation at 37°C was continued for 30 minutes. Exactly 0.2 mL of goat anti-rabbit gamma globulin diluted 1:15 in PBS (no rabbit serum) was added to all tubes except the total count tubes. They were incubated at room temperature for 10 minutes, and 1.0 mL of 12.5 percent polyethylene glycol (PEG) (in PBS) was added, and incubation at room temperature was continued for 10 minutes. All tubes except the totals were centrifuged at 2500 g for 30 minutes. The supernatants were decanted and the pellets were counted for three minutes in a Packard 5266 Autogamma scintillation spectrometer. When this method was compared with the original method of Vaitukaitis et al13 using antibody SB6 and standard hCG, CR115 from NIAMDD, the result were identical.

α-FETOPROTEIN

Alpha-fetoprotein was measured with kits obtained commercially.* The upper range of the standard curve was extended to 300 μg per L. Any samples that assayed higher than 300 μg per L were diluted in the borate buffer supplied with the kits.

Results

The ratio of IU measured with the Corning kit to μg measured with MSKCC method was determined by assaying MSKCC standards in the Corning kit and Corning standards in the MSKCC method. The average value from 23 measurements at several levels was 15.1 ± 2.9 IU per μg. This agreed well with the value of 15.7 ± 4.4 IU per μg that was obtained with 177 patient samples. It should be noted that this ratio may vary with different sources of tracer, standard, and antibody. The correlation between the two methods was r = 0.918.

The records of each patients were reviewed in order to determine diagnosis, pathology, and dates of therapy. In table I are shown the pattern of elevations of serum markers in the 127 patients who were entered into this study between November 1, 1982 and March 11, 1983. Values that were slightly elevated on first analysis and were normal on repeat are listed as not elevated.

Of the 12 women with gestational trophoblastic disease, all had elevated

---

* Wampole Laboratories, Division of Carter-Wallace, Cranbury, NJ.
Elevations of α-Fetoprotein and Human Chorionic Gonadotropin in the Serum of Patients with Gender Related Cancers and Other Cancers

<table>
<thead>
<tr>
<th>Type of Cancer</th>
<th>Only αFP</th>
<th>Marker Elevated*</th>
<th>Only hCG</th>
<th>Both</th>
<th>Neither</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germ cell tumors in males [72]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testicular</td>
<td>18 (25)†</td>
<td>14 (19)</td>
<td>27 (37.5)</td>
<td>9 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Non-testicular</td>
<td>1 (1.4)</td>
<td>2 (2.8)</td>
<td>0</td>
<td>1 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Germ cell tumors in females [6]</td>
<td>2 (33)</td>
<td>2 (33)</td>
<td>0</td>
<td>2 (33)</td>
<td></td>
</tr>
<tr>
<td>Gestational trophoblastic disease [12]</td>
<td>0</td>
<td>12 (100)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Non-germ cell cancers males [12]</td>
<td>3 (25)</td>
<td>5 (42)</td>
<td>2 (17)</td>
<td>2 (17)</td>
<td></td>
</tr>
<tr>
<td>Non-germ cell cancers female [6]</td>
<td>1 (12.5)</td>
<td>3 (37.5)</td>
<td>0</td>
<td>4 (50)</td>
<td></td>
</tr>
<tr>
<td>Hepatomas male &amp; female [17]</td>
<td>15 (88)</td>
<td>0</td>
<td>2 (12)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Elevated αFP is any value above 10 pg/L.
Elevated hCG is any value above 1.0 μg/L when measured in our MSKCC method or above 3.12 IU/L when measured with the Corning Kit. There were six borderline positive hCG by Corning that were negative by our MSKCC method and five that were borderline positive by our MSKCC method and negative with the Corning Kit.
†Values in parentheses are percentages of total number of patients (in brackets) represented by the figures.

serum hCG levels. Four of six women with germ cell tumors had either elevated serum hCG or elevated αFP.

Ten of 12 males and four of eight females with cancers other than those of germ cell had either elevated serum hCG or αFP.

Alpha-fetoprotein is a product of the fetal liver and has been known for many years to be a marker for hepatocellular carcinoma. This was born out in the current study where all of the 17 patients with diagnosed hepatocellular carcinoma had elevated serum αFP levels. One patient with a diagnosis of chronic active hepatitis had an initial serum level of αFP of 1590 μg per L. This fell to within normal limits in about six months after treatment with prednisone.

**Longitudinal Studies**

Human chorionic gonadotropin and αFP are most useful for following the course of disease during therapy. The rises and falls in the serum levels of these markers indicate the success or lack of success of therapy and provide indications of when therapy should be changed or repeated. These changes in marker levels frequently occur weeks or even months in advance of overt clinical symptoms.

In figures 1 through 6 are illustrated the types of changes in markers that have been observed in several patients with testicular cancers.

Patient # 5 in figure 1 had a germ cell tumor of the right testis with retroperitoneal node involvement. It contained seminoma, choriocarcinoma, teratoma, and embryonal elements. Blood obtained shortly after orchietomy contained elevated hCG and αFP. Both decreased rapidly following surgery and the last traces of αFP were eliminated following chemotherapy.

In contrast, patient # 20 in figure 2, who had embryonal carcinoma with teratoma elements and elevated serum marker levels, showed only a moderate fall in serum markers following chemotherapy. The subsequent rise in serum hCG and αFP reflected progress of disease despite several changes in therapeutic strategies.

Patient # 98 in figure 3 also had a mixed type of testicular cancer that contained elements of choriocarcinoma, embryonal carcinoma and teratoma. Disease
was judged to be stage III with massive involvement of retroperitoneal nodes. The markers were elevated following orchiectomy and fell, but not to normal levels, after retroperitoneal node resection. The subsequent rises and falls of serum marker levels reflected the efficacy of chemotherapy.

Patient #6 in figure 4 had embryonal carcinoma and choriocarcinoma. His serum markers fell initially in parallel in response to chemotherapy. However, only his αFP fell to the normal range. Human chorionic gonadotropin never reached normal levels and, indeed, rose despite additional chemotherapy. The rise in serum hCG reflected a worsening of his disease.

Not all patients with testicular tumors have elevations in both serum hCG and αFP. Patient #3 in figure 5 had a stage III non-seminomatous germ cell tumor metastatic to lung. At no time during the course of the disease did his αFP rise above normal. However, his hCG was elevated and reflected progression of disease despite vigorous chemotherapy.

Patient #14 in figure 6 had stage II testicular teratoma and never had an elevated hCG by either of the methods used in this study. Serum αFP, however, was elevated and kept rising throughout the course of disease despite several courses of chemotherapy.

In figure 7 are shown the patterns of serum hCG in two patients with trophoblastic disease. In one case, patient #1, treatment with actinomycin D, metho-
trexate, and chlorambucil was successful in eliminating the disease and this was reflected in the fall in serum hCG to normal with no rebound for the duration of this study.

Despite several changes in chemotherapy to patient # 19 (figure 7), the disease persisted. It is noteworthy that the liver and spleen scans were normal at a time when serum hCG levels were still elevated and indicated that there was persistent disease that was resistant to several drugs and other agents.

In figure 8 the course of disease in a typical patient with hepatocellular carcinoma is illustrated. Values of serum αFP are greatly elevated, up to $2.8 \times 10^6 \mu g$ per L in one patient. Serum αFP levels did not return to normal levels in any of the 17 cases of hepatocellular carcinomas entered into this study despite surgical and/or chemotherapeutic interventions. The case illustrated in figure 8 is interesting because it is the only one that had an increase in serum hCG. This increase was borderline and detectable only with the Corning reagents. However, it did persist for two months of the study and appeared to reflect the detection of a cross reacting antigen that did not react with MSKCC reagents.

In figure 9 is shown the response to chemotherapy of an ovarian endodermal sinus tumor in a one year old child. A T-6 protocol (Actinomycin D, Mtx, Cyclophosphamide, Vincristine, Adriamycin) was administered. The serum αFP level reflected the positive effect of therapy, and the child was sent home. There has been no recurrence six months later after initiation of therapy. Maintenance dosage is being continued.
Discussion

There is no one substance that when found in blood or other body fluid indicates the presence of cancer and that when absent indicates the absence of cancer. There are, however, several substances which are grouped under the title of "tumor markers." These may be present or absent in the blood of known cancer patients and are, therefore, not diagnostic. When they are present, however, they are of great usefulness in charting the course of the disease and in judging the effectiveness of different modes of therapy. Furthermore, different substances may be of great utility in following some types of cancer and of no use in others. Thus, carcinoembryonic antigen (CEA) is of great value in following patients with colorectal, breast, and lung cancers but of little use in following patients with melanoma, leukemias, or germ cell tumors.

The results reported in this paper demonstrate the effectiveness of measuring both αFP and hCG in following the progress of patients with cancers of the testes, germ cell tumors in general, in trophoblastic disease (choriocarcinoma), and αFP in hepatocellular carcinoma where elevations were observed in all of the 17 patients.

Examination of the pathology reports on the patients with testicular tumors revealed that most patients with this disease had mixed tumors that contain elements of embryonal carcinoma, endodermal sinus tumors (yolk sac tumors), seminoma, choriocarcinoma, and tera-
toma in varying combinations. According to Kurman et al., hCG is a product of syncytiotrophoblastic giant cells, embryonal carcinoma, and choriocarcinoma while aFP is produced by embryonal carcinoma and endodermal sinus tumors. Thus, it is not surprising that 59 of the 68 patients with testicular cancers unselected for stage, and including some patients with recurrence or resistant disease, had at least one elevated marker. Frequently both aFP and hCG are elevated. However, it is not sufficient to measure only one marker since in almost half of the cases only one tumor marker was elevated. Similar results were obtained in patients with germ cell tumors that were not of testicular origin. Seven of the 10 serums from patients in this category contained only one elevated tumor marker. In the case of women with gestational trophoblastic disease (choriocarcinoma), only hCG was elevated.

In the case of non-germ cell tumors, both male and female, 14 specimens were found that had either one or both tumor markers elevated. In most instances, these elevations were of only borderline significance; however, substantial elevations were observed in serum hCG of four patients with epidermoid carcinoma of the bladder, one with metastatic adenocarcinoma of unknown origin, and one with pulmonary blastoma. Elevations in aFP were observed in serum from a patient with epidermoid carcinoma of the neck and alcoholic hepatopathy (aFP is probably from the hepatopathy), a pulmonary blastoma with liver and bone metastases, a colon cancer...
with liver metastases, and a patient with no diagnosis.

The longitudinal studies of patients in all categories shown in figures 1 through 9 demonstrate the reflection of the patients' disease state by the degree of elevation of serum tumor markers. Changes accurately conform to the patients' clinical status and may indicate a change in tumor type, i.e., patient # 6, figure 4. The tumor produced both αFP and hCG at the onset of disease but produced only hCG after two months of treatment. Such changes may indicate the need for changes in therapy.

The current practice at MSKCC is to monitor tumor markers, including lactic dehydrogenase in the case of testicular cancers,² monthly for the first year after diagnosis and treatment, at bimonthly intervals during the second year, and every four to six months thereafter. In addition, it is highly desirable to obtain values before surgery or other treatment is initiated to serve as a point of reference and to calculate half life of the marker if frequent samples are available.

During the course of this study the Corning hCG kit was compared with the MSKCC method. Our investigations found the two to be in excellent agreement as is shown in several of the figures where longitudinal data are plotted. The mean relationship between the Corning values and the MSKCC values was 15.1 IU per μg when the two sets of standards were compared. Patient values were in fairly good agreement with this relationship. The average of 177 patient samples was 15.7 ± 4.4 IU per μg. Since each manufacturer of hCG kits has a different source of hCG and since the biological...
potency of the various preparations may differ according to the method of purification used, it is strongly recommended by the present authors that all kits be calibrated by weight, i.e., ng per ml. Adoption of this recommendation will facilitate comparison between results obtained by different laboratories.

Acknowledgments

Thanks are extended to Corning Medical and Scientific Division of Corning Glass Works for their generous gift of βhCG Immunophase RIA kits.

References