Serum and Cellular Biologic Tumor Markers in Testicular Cancer

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During the past two decades, a dramatic improvement has been made in the treatment of testicular germ cell tumor. This progress has been due to finding more efficacious systemic chemotherapeutic agents and the availability of specific and sensitive biologic tumor markers to detect early recurrence and monitor the therapy. In this review, I will update my 15 years of experience in establishing and utilization of these serum and cell markers in testicular cancer.

Historical Landmarks

My interest in these markers initiated in 1967 when I was serving at the American Urological Association in the Armed Forces Institute of Pathology in Washington, D.C. The development and utilization of testicular tumor models in syngeneic strain 129J mouse helped to understand the original and biological characteristics of this tumor[1]. In 1963, Abeleve and associates demonstrated an alphafetoprotein (AFP) in serum of the mouse embryo[2]. In 1972, a radioimmunoassay (RIA) was developed that measured serum hCG in the presence of physiological amounts of luteinizing hormone with minimal cross reactivity[3]. Also, the development of a RIA to measure AFP in minute amounts prompted us to study the frequency and usefulness of these markers in testicular cancer. Although we found a number of specific and nonspecific serum and cell markers in patients with testicular cancer (Table 1). However, none were more accurate than serum AFP and/or hCG when measured by sensitive and specific RIAs.

Table 1 — Testicular tumor markers.

Specific Markers
1. Alpha-fetoprotein (AFP)
2. Human chorionic gonadotropin (hCG)
3. Placental alkaline phosphatase (PLAP)
4. Gamma-glutamine transpeptidase (GGT)
5. Placental proteins number 5, 10, 15
6. Placental lactogen
Nonspecific markers
1. Lactic dehydrogenase(LDH)
2. Polyamines (putrescine, spermine, spermicline)
3. Carcinoembryonic antigen

Serum AFP

Human AFP is a glycoprotein having a molecular weight of 70,000 and containing about 41% carbohydrate. It is produced in the liver, yolk sac, and gastrointestinal tract of the fetus. AFP is present in human fetal serum at a concentration of 3ng/ml by the 12th week of gestation. At birth, the concentration is approximately 30ng/ml and drops to much lower levels by 1 year of age; in normal adults it is found in concentrations of approximately 1-16ng/ml. AFP has been clinically useful primarily as a diagnostic tool for hepatoma and certain other malignant diseases.

In a prospective study, data from our clinical program of testicular cancer the following distributions for AFP and hCG were obtained.

One hundred and two of 145 (70%) patients with embryonal carcinoma, 36 or 56 patients (69%) with embryonal carcinoma with or without teratoma (64%), 3

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of 4 patients with yolk sac tumors (75%) and none of 5 patients with choriocarcinomas had an elevated level of serum AFP. Eighty-seven of 145 patients (60%) with embryonal carcinoma, 32 of 56 patients (57%) with embryonal carcinomas with/without teratoma, one of 4 patients (25%) with yolk sac tumors. None of the patients with seminoma and/or choriocarcinoma had elevated serum AFP.

Serum hCG

hCG is a glycoprotein secreted by the normal placenta. It is normally found in the serum only during pregnancy, hCG has a molecular weight of 38,000 and is composed of two dissimilar subunits. Alpha and beta subunit is the basic subunit of the pituitary glycoprotein hormones; luteinizing follicle-stimulating, and thyrotropin. The beta subunit comprising two thirds of the molecular weight, is unique to the hCG and is distinct from the subunits of luteinizing, follicle-stimulating and thyrotropic hormones particularly in the terminal 29 amino acids. The subunit was isolated, purified and used to immunize rabbits to produce an antibody specific for hCG which does not cross-react with physiologic concentrations of the other glycoprotein hormones[3].

Data from our laboratory showed that 14 out of 140 (9%) of patients with seminoma, 4 out of 16 (25%), 87 of 145 (60%) of embryonal carcinoma and 1 out of 4 yolk sac tumor and all with choriocarcinoma had elevated serum hCG.

Combined Serum AFP and hCG

When both markers were considered, 9.0% of seminoma, 44% of teratoma, 88% of embryonal carcinoma, 86% of embryonal carcinoma with teratoma, 75% of yolk sac tumors had elevated levels of serum hCG and/or AFP[6] (Table 2).

The frequency of markers in testicular seminoma was unsatisfactory. Therefore, we embarked in studying multiple markers in seminoma.

Multiple Serum Markers in Seminoma

We studied the role of placental alkaline phosphatase (PLAP), gammaglutamyl transpeptidase (GGT), and hCG in testicular seminoma. In 89 seminoma patients with negative beta-glycoprotein, total serum GGT was measured and values about 30 IU per liter were considered abnormal[7]. Serum PLAP was measured by enzyme-linked immunoabsorbent assay and values greater than 1.85mg per ml were considered abnormal. Serum hCG and AFP were measured by double antibody radioimmunoassays normal less than 1ng/per ml and greater than 20ng per ml, respectively. At the time of this study, 30 patients had detectable seminoma, 10 were histologically unconfirmed and the remaining 49 had no evidence of tumor. Only six of 30 patients (20%) with active tumor had elevated levels of serum hCG. Twelve of 30 patients with active tumor (40%) had elevated serum PLAP, and 10 of 30 (33%) of these patients had elevated serum levels of GGT. When these three serum markers were considered together, over 80% of the patients with clinically active tumours had detectable serum levels of one or more of these biochemical serum markers. It should be emphasized that the false positive, false negative rates of these markers, especially false positive rates for GGT, due to occasional concomitant liver disease and the biologic half-lives of these markers should be taken in consideration.

A nonspecific marker that may be useful in the management of seminoma is lactic dehydrogenase (LDH) (Figures 1 and 2). Serum lactic dehydrogenase is a nonspecific enzyme made up of five heterogenous isoenzymes in man that can be measured electrophoretically. Cancer cells have increased glycolysis leading to an increased synthesis of lactate, and it may be utilized as a nonspecific tumor markers in several cancers[7].

Role of AFP and hCG in Staging

Clinically, when these markers were considered in staging testicular tumor the staging errors decreased

Table 2 — Frequency of Elevated hCG and AFP in Patients with Testicular Cancer.

	AFP		hCG		AFP and/or hCG	
	No. of patients	Percent of patients	No. of patients	Percent of patients	No. of patients	Percent of patients
Seminoma	0/160	0	14/160	9.0	14/160	9.0
Teratoma	6/16	37.5	4/16	25,0	7/16	43.7
Embryonal carcinoma	102/145	70.3	87/145	60.0	127/145	87.5
Embryonal carcinoma with teratoma	36/56	64.2	32/56	57.0	48/56	85.7
Choriocarcinoma	0/5	0	5/5	100.0	5/5	100.0
Yolk sac tumor	3/4	75.0	1/4	25.0	3/4	75.0

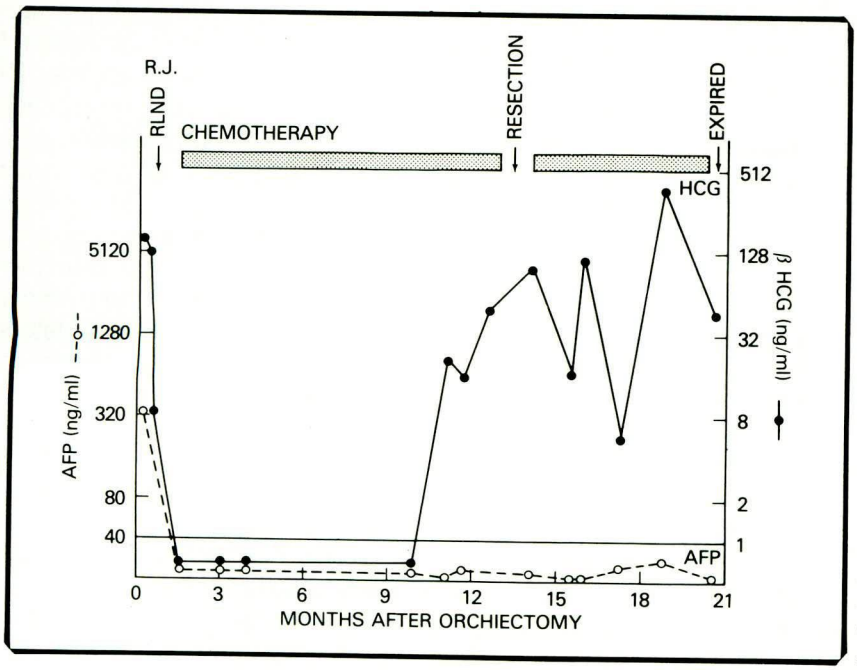

Figure 1 — hCG and AFP in monitoring a patient with embryonal carcinoma and an element of choriocarcinoma. Note the discordance between the serum hCG and AFP.

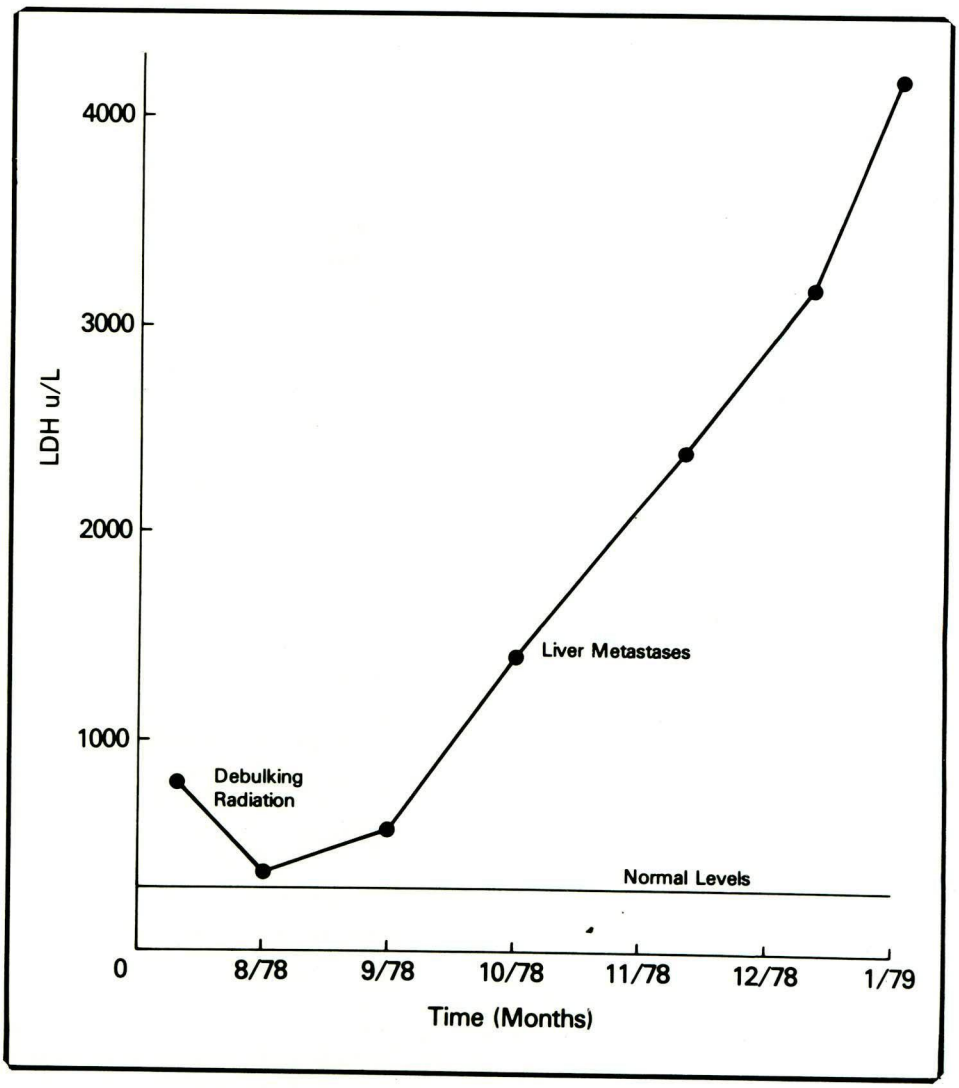

Figure 2 — LDH in monitoring of a patient with bulky testicular seminoma.

to 5-14%, an improvement when compared to a high clinical staging error of 15-20%.

The important feature which tumor markers add to the understaging of testicular cancer are as follows:

(1) Improved staging based on clinical investigation markers, therapy and pathological findings.
(2) Persistently elevated serum markers after orchiectomy for testicular cancer invariably indicate stage II or III disease.
(3) Persistently elevated serum markers after lymphadenectomy indicate stage III disease or an inadequate lymphadenectomy.
(4) When lymphadenectomy is negative for tumor but postlymphadenectomy serum markers are persistently elevated, patients invariably have stage III disease. However, surgery still remains the most accurate means of assessing retroperitoneal metastases.
(5) Perhaps, the most important application of these markers are in monitoring of testicular tumor when serially measured.

Monitoring the Response to Therapy

Serial measurements of serum hCG and AFP by RIA reflect the efficacy of surgical, radiation, and/or chemotherapeutic regimens in patients with testicular tumor. When these therapies are effective, they produce an immediate decrease in serum levels of hCG and AFP that reflects the decrease in tumor size and could be as rapid as the catabolic rate for these markers. In our series, elevated markers were found, often months before the patients were symptomatic or recurrence was detectable by any other clinical tests. Consequently, the markers proved to be sensitive indicators of the presence of otherwise undetectable metastases.

It is important to consider the biologic half-lives of the markers (AFP 5 days and hCG 18 to 24 hours) to avoid any confusion from the progressively decaying markers of the already excised tumor. Preorchiectomy serum markers are not always available but this should not disturb the proposed system since the original orchiectomy specimen is usually available and immunohistologic techniques, such as immunoperoxidase, can determine the presence of cellular markers when serum is not available.

Discordance, Limitations and Clinical Guidelines

The discordance between various testicular tumor markers is well known and may be explained on the basis of the findings that different cells produce these various markers. Also, during chemotherapy of a patient with elevated levels of serum hCG and AFP, one may return to normal whilst the other remains elevated (Figure 1). This may occur if some of the cells producing a given marker are resistant to the therapy. To avoid errors, currently we submit the following guidelines. (1) The physician should discuss the sensitivity and specificity of a given commercial assay with the laboratory, and perhaps, occasional inclusions of normal serum or serum with known levels of AFP and/or hCG may serve as negative and positive controls when blindly coded.

(2) These markers should not replace scrotal exploration for histopathologic diagnosis of the primary tumor and retroperitoneal lymphadenectomy to detect or exclude the presence of retroperitoneal metastases. However, the elevated levels of tumor markers are indicative of the presence of tumor and the necessity for further treatment. They are also helpful in monitoring the efficacy of and the need for changes in therapy.

(3) The problem of impurity of certain antisera against the subunit of the hCG of the possibility of high levels of luteinizing hormones (LH) in patient undergoing orchiectomy and/or chemotherapy causing a false positive result should also be kept in mind. The false positive results may be clarified by the testosterone suppression test, determination of serum LH, and measurement of hCG on urinary concentrate utilizing a carboxy-terminal RIA that is currently available to all urologists through the NCI laboratories as a courtesy.

(4) In monitoring the therapy or following the patients with testicular tumor, one should utilize frequent clinical examination, chest x-rays, and other tests as physicians find them necessary, along with determination of serum AFP and hCG. In patients on chemotherapy the normalization of these serum markers does not mean tumor-free status; as a matter of fact, on exploration of the retroperitoneum and chest, it is not unusual to find cystic fibrotic markers should not deter the surgeon from looking for tumor. Appropriate utilization of chemotherapy, surgery, radiotherapy, and tumor markers can make a dramatic improvement in the prognosis and survival of these patients.

Other Placental Proteins

Over the past several years, we have studied a number of placental proteins including pregnancy specific beta glycoprotein and placental proteins number 5, 10 and 15 utilizing immunoperoxidase. We have localized these markers in syncytiotrophoblastic components of the human placenta, choriocarcinoma and syncytiotrophoblastic giant cells associated with testicular cancer.

Cellular Localization of AFP and hCG

In 1972, as we embarked on serum measurements of AFP and hCG we also concentrated on detecting cellular localization of these markers[5,10,11]. First, utilizing

immunofluorescence technique, we were able to localize AFP and hCG to their cellular origin. However, this technique is mainly a research tool requiring fresh tissue and needs storing in dark areas. Later, we utilized immunoperoxidase.

Immunoperoxidase methods have much in common with established immunofluorescence procedures. Both have the potential for demonstration of specific cell and tissue antigens with similar limitations demanding rigorous control of specificity. In any study, the choice of an immunofluorescence method of an immunoperoxidase method can be and on rational grounds, according to the desired objective, the degree of morphologic detail required, the materials available for study, and the ease of access to specialized ultraviolet microscopy. The major advantage of immunoperoxidase is that it can be utilized in either a prospective or a retrospective study, since the tissue to be stained can be fixed in formaldehyde and this is usually available as opposed to immunofluorescence requiring fresh or frozen tumor specimens. Therefore, these properties of immunoperoxidase have the necessary features that make immunocytochemistry more convenient and practical.

This technique utilizes a 4 to 6 micron thick section of formal formaldehyde fixed tumor that is deparaffinized in xylene and cleared in the usual fashion. The section is incubated in a humid chamber for 30 to 60 minutes with appropriate antisera to a given marker. The second antibody is a gamma-globulin that is conjugated with horse-radish peroxidase. The section is washed again and exposed to Diaminobenzidine. The slides are counter-stained with hematoxyline. Utilizing this technique, we reported for the first time that the yolk sac tumor is mainly responsible for AFP and syncytiotrophoblastic giant cells (STGC) are producers of hCG (Table 3). Later utilizing antisera to hCG and AFP tagged to $I^{(3)}$ we were able to detect and localize tumors producing these markers by gamma scintillation cameras as a specific radioimmunodetection technique[12].

Table 3 — Immunohistologic Classification of Germ Cell Tumors.

Tumor	AFP	hCG	SP_1
Placenta	—	+	+
Yolk Sac Tumor	+	—	—
Seminoma	—	—	—
Seminoma with STGC	—	+	+
Embryonal carcinoma	+	+	—
Choriocarcinoma	—	+	+
Teratoma	—	—	—

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