ABSTRACT

A sensitive and specific enzyme-linked immunoabsorbent assay was used in a retrospective study of serum levels of placental alkaline phosphatase (PLAP) in testicular cancer. Sixteen of 28 men with active seminoma had elevated PLAP levels, and 71% had elevated levels of either PLAP, human chorionic gonadotropin, or both. Only four of 22 men with active nonseminomatous cancer had elevated PLAP levels, and the levels were normal in all control patients, including 33 men apparently cured of testicular cancer. In six of ten serial studies, PLAP levels provided information not otherwise available that would have been useful clinically, and the levels never were elevated inappropriately. Our data suggest that PLAP is a clinically useful serum tumor marker for seminoma.

INTRODUCTION

In patients with NSGCT, the serum markers AFP and HCG often improve staging accuracy, signal recurrences weeks before they are detectable by anatomical methods, and may show whether a chemotherapy regimen will be effective (11, 14, 18). This success contrasts with the relative lack of markers for seminoma, which accounts for 40% of testicular cancers. Between 10 and 30% of men with seminoma have elevated HCG levels (12, 15), but the AFP levels are always normal, and no other marker has been proven reliable.

PLAP (phosphomonoesterase, alkaline optimum, EC 3.1.3.1) is an oncodevelopmental protein which is expressed by placental syncytiotrophoblastic cells by the 12th week of pregnancy but is also produced ectopically by a variety of malignant tumors including seminoma (5, 6, 9, 17, 20, 21, 23).

We found that, when serum PLAP levels were measured with a new sensitive ELISA (4, 16), PLAP appears to be a clinically useful marker for seminoma.

MATERIALS AND METHODS

Serum. From among the serum specimens preserved in our liquid nitrogen tumor resources bank at the University of Minnesota, we selected 209 specimens representing various stages and clinical circumstances of testicular cancer, particularly seminoma. These sera came from 88 patients, and in all, the AFP and HCG levels had been determined with reliable RIA. Sera were obtained from 28 patients with pathologically confirmed, untreated (active) seminoma and from 22 with active nonseminomatous cancer. When a specimen was found to have an elevated PLAP level, we retrieved other samples obtained from the same patient to study changes during the clinical course. Appropriate samples were available in 14 cases.) In 27 patients, seminoma was thought to be cured (inactive) when the serum was obtained, and in 6, inactive nonseminomatous cancer was present. Six further control specimens came from patients with benign intrascrotal masses. All sera were tested for PLAP as coded specimens in La Jolla and later decoded in Minneapolis for evaluation.

Staging. In this article, the stages of testicular cancer are defined as follows: Stage I, tumor limited to the testis, epididymis, and spermatic cord; Stage II, small retroperitoneal metastases (by bipedal lymphangiography or lymphadenectomy); Stage IIC, bulky retroperitoneal metastases; Stage III, metastases outside the retroperitoneum.

ELISA. PLAP serum levels were determined by a sandwich ELISA (4, 16). The lower limit of detection of this assay is 0.4 ng/ml. The upper limit of normal, defined in 128 men and nonpregnant women, is 1.85 ng/ml.

RESULTS

Chart 1 illustrates the PLAP levels in our patients with active testicular tumor. Serum PLAP levels were elevated in 16 of 28 men with active seminoma (average, 10.6 ng/ml; range, 1.9 to 33.2 ng/ml). Of these 16, 10 also had elevated HCG levels. An additional 4 had only HCG elevated. Thus, in 20, the level of at least one of the proteins was elevated. There were no serum PLAP elevations among patients with an inactive testicular tumor or among any patients who had benign scrotal masses at the time serum was obtained.

Serial studies were performed in 10 seminoma cases in which appropriate specimens were available. In 4 cases (one Stage I, 3 Stage III), the HCG levels were elevated also; and the PLAP levels, although reflecting the clinical course, were either not as good as or no better than HCG levels in this regard. In 3 cases (Stages I, II, and IIC), the HCG levels were elevated, but PLAP reflected the clinical course more accurately at some point. In 3 cases (Stages I, II, and III), HCG levels were normal throughout, and the changing PLAP levels would have provided clinically useful information. In no instance in our serial studies was the PLAP level elevated inappropriately. Four cases are illustrative.

Case 1. This 40-year-old man had a radical orchiectomy which on pathological analysis was pure seminoma. Postoperatively, he was classified as clinical Stage I disease on the basis of bipedal lymphangiography, full lung tomography, and abdominal computerized axial tomography. Accordingly, 3 weeks after surgery, the patient began a course of 2500 R of radiation therapy to the abdomen and remains free of disease 3 years later.

Analysis of sera obtained before surgery and subsequently revealed normal AFP and HCG levels as determined by RIA throughout his clinical course. However, PLAP determinations

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3 The abbreviations used are: NSGCT, nonseminomatous germ cell tumor; AFP, a-fetoprotein; HCG, human chorionic gonadotropin; PLAP, placental alkaline phosphatase; ELISA, enzyme-linked immunoabsorbent assay; RIA, radioimmunoassay.

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of stored samples revealed a significantly elevated level of 33.2
ng/ml preoperatively, which returned to normal levels 8 days
after surgery and remained normal as revealed in 4 serum
samples obtained over the next 3 years.

**Case 2.** This 50-year-old man underwent right and left
orchiectomies 35 and 25 years previously and had received
extensive abdominal and chest irradiation for seminoma. He
came to us with a large retroperitoneal mass which was found
at exploratory laparotomy to be unresectable seminoma. There
was no evidence of other metastases. He received 3000 R to
the upper abdomen, and the mass shrank dramatically. How-
ever, 4 months postoperatively, he returned with lung, mediastinal,
and left-neck masses found to be pure seminoma. He
was given 3000 R to the chest, and the masses disappeared.
He has had no evidence of tumor in the ensuing 4 years.

Except for a borderline abnormal level immediately after
laparotomy, this man's HCG level was normal throughout (Chart
2). However, the PLAP level clearly reflected the disease
process, beginning to rise 3 months before the chest and neck
metastases were discovered and declining to normal during irradiation.

**Case 3.** Four years earlier, this 55-year-old man had had a
left radical orchiectomy for teratocarcinoma and seminoma and
refused further treatment. He then later presented with a ret-
roperitoneal mass and elevated HCG levels. At lymphadenectomy,
the mass, a pure seminoma, was excised, apparently
completely, and the HCG level fell to normal values. The patient
declined further treatment but agreed to have serial determi-
inations of his serum AFP and HCG levels. Seven months later,
the HCG level became abnormal, but when the tumor could not be
located, the patient again refused treatment. The HCG level
continued to rise, and when the patient was finally persuaded
to return for reevaluation 8 months later, he had retroperitoneal
and pulmonary metastases. He received vinblastine, bleomy-
cin, and cis-platinum (5), and the masses shrank. He discontin-
ued chemotherapy in midcourse and was lost to follow-up with
a rising HCG level.

Our retrospective analysis showed that the PLAP levels
closely paralleled the HCG levels throughout much of this
patient's course (Chart 3). However, after lymphadenectomy,
5 months before the HCG levels became abnormal, the PLAP
levels were elevated, thereby indicating residual tumor.

**Case 4.** This 36-year-old man had a right radical orchiectomy
at another hospital. Because the tumor was an anaplastic
seminoma and the HCG level had been elevated preoperatively,
we performed a retroperitoneal lymphadenectomy. No tumor
was found in the resected lymph nodes. The HCG level was
normal for more than 5 months before a rapid rise was noted
(Chart 4). No tumor was evident clinically, but 1 month later,
there was a mass in the abdominal wall above the orchiectomy
scar. It was excised and found to be anaplastic seminoma.
Intraoperative palpation of the retroperitoneum revealed no tumor, but the HCG level did not return to normal until the abdomen was irradiated (3000 R). Forty-five months after orchietomy, the HCG level began rising, although whole-lung tomography, computerized tomography, and abdominal echography revealed no tumor. Our experience has shown that, provided other causes of elevated marker levels are eliminated, rising marker levels are always a sign of recurrent tumor (14); and therefore, we gave the patient vinblastine, bleomycin, and cis-diaminedichloroplatinum. After 3 courses, the HCG level was normal, but 2 months later, it was again elevated, although no tumor was detectable. He is now receiving cyclophosphamide. Retrospective study of the sera showed that the PLAP levels were normal early in the course but warned of the second recurrence 6 months before the HCG level did (Chart 4).

PLAP appeared less useful in patients with active NSGCT, being elevated in only 4 of 22 (Chart 1). One of these men had an elevated HCG level, one had an elevated AFP level, and the other 2 had elevated AFP and HCG levels. Serial PLAP data in these 4 patients did not provide new information. Four of the 16 men had seminomatous elements in their tumors, and 3 had falsely negative AFP and HCG levels. PLAP levels were normal in all these patients.

DISCUSSION

The survival rates of men with NSGCT improved dramatically in the last decade because of improved chemotherapy and the better monitoring made possible by the RIA for the markers AFP and HCG. At several medical centers, including ours, long-term survival rates are now > 90% in Stages I and II and > 60% in Stage III (2). The rates often are lower in seminoma, which was once considered the most curable type of testicular cancer. In Stages IIIC and III, the rate is < 40% (15, 18). Sensitive tumor markers might help improve the prognosis of these patients.

The value of AFP and HCG as markers of pure seminoma has been much debated. Now, it is believed that elevated AFP levels always indicate nonseminomatous cancer, whereas elevated HCG levels, although they also may indicate nonseminomatous cancer, can accompany pure seminoma (12, 15). The frequency with which this occurs is being explored; the estimates are 15 to 20% of cases in all stages combined. In these few patients, HCG is a clinically useful marker for seminoma.

PLAP has been distinguished from the common tissue alkaline phosphatases by its heat resistance, its inhibition by L-phenylalanine, and its immunological properties (17). It resembles in many properties the alkaline phosphatase called Regan isoenzyme found by Fishman et al. (5) in nonthromboplastic tumors. PLAP has been reported in the sera of about 20% of patients with various cancers, although some have reported a prevalence as high as 95% (1, 5, 8-10, 17, 19, 21, 22). Substantial evidence of clinical utility has been lacking.

One problem in developing PLAP as a tumor marker has been variations in the sensitivity and specificity among the RIA and catalytic assays used in these studies (9, 21, 22). Also, several PLAP variants have been recognized recently. For example, a heat-sensitive PLAP is present in patients with Hodgkin's disease (3). The extent to which certain assays detect these variants is unknown.

The importance of PLAP in testicular tumor has therefore been unclear. Early studies of testicular tumor sera revealed detectable PLAP levels infrequently (5, 9, 17, 21). However, Fishman et al. (7), using a sensitive catalytic assay, studied the sera of 27 treated and untreated seminoma patients and found elevated PLAP levels in 4, all of whom also had elevated HCG levels. Subsequently, Wahren et al. (23) studied PLAP levels in the tumor and sera of testicular tumor patients using an RIA with a minimum detectable level of 12 ng/ml. They found that seminoma tumors contain more PLAP than do nonseminomatous tumors and that serum values were more frequently elevated in seminoma patients than in nonseminoma patients unless the latter disease was far advanced. They reported elevated PLAP levels in 10 of 19 seminoma patients, but horizontal studies were not presented. Finally, Uchida et al. (20) analyzed recently a variety of testicular tumor specimens using indirect immunoperoxidase techniques and reported the oncodevelopmental enzyme to be present in 8 of 9 seminomas but in none of the 9 NSGCTs examined.

In the present clinical study, we measured serum PLAP levels with a sensitive sandwich ELISA which has a minimal detectable level of 0.4 ng/ml. We found PLAP elevations in 16 of 28 active seminoma patients (57%) but in only 4 of 22 patients with nonseminomatous cancer. Moreover, PLAP was the only marker elevated in 6 of 16 seminoma patients, and in all 4 of these cases in which serial studies were possible, PLAP levels provided clinical information of importance. In these serial studies, it is noteworthy that, although PLAP levels were high in some instances, other clinically relevant abnormal levels were found in the range of 2 to 10 ng/ml, stressing the need for a very sensitive assay if PLAP determinations are to be of clinical value.

In the 14 seminoma patients with elevated HCG levels, serum PLAP was also elevated in 10. While PLAP often paralleled the HCG level, in 3 of the 7 cases where both markers were elevated and serial studies were possible, PLAP levels were at certain times better than HCG at either reflecting or predicting tumor behavior, while at other times in these same patients, HCG levels were more valuable. Thus, the simultaneous measurement of both HCG and PLAP would have been necessary for accurate serum monitoring. Such a situation is reminiscent of nonseminomatous cancer where discordance between HCG and AFP is a common occurrence, and both of these markers must be measured together for optimal accuracy (14). Discordance between HCG and PLAP has been encountered in ovarian cancer also (5). Finally, among our seminoma patients, either HCG and/or PLAP was elevated in 68%. Again, this is similar to the prevalence of AFP and/or HCG elevations in nonseminomatous cancer (14). Hence, the impression that seminoma is usually a marker-negative tumor may need to be revised.

Obviously, further study is needed before PLAP can be accepted as a clinical marker for seminoma with the same faith one has in AFP and HCG as markers for nonseminomatous testicular cancer. It is perhaps reassuring that, in previous studies using the sandwich ELISA, PLAP was never greater than 1.8 ng/ml among over 100 men and nonpregnant women. Furthermore, in this study, there were no PLAP elevations among 39 men (including 27 with seminoma), who were considered cured of their testicular tumor or had benign scrotal masses. Moreover, among our seminoma patients who had elevated PLAP levels and serial studies, PLAP was never
elevated inappropriately.

Our data provide evidence that PLAP levels can give useful clinical information in patients with seminoma and suggest that both PLAP and HCG should be monitored in these patients. This may be particularly true for men with bulky or widespread metastases, as PLAP and HCG levels may provide a much-needed guide to appropriate use of the various treatment options (15). Our results also are relevant to an understanding of the pathogenesis of seminoma, in that PLAP has been added to the list of trophoblastic products known to be secreted by some seminomas, a list which already includes HCG and the pregnancy protein SP-1 (13).

REFERENCES

Placental Alkaline Phosphatase as a Tumor Marker for Seminoma

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