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Profiles of Epitope-Defined Markers in Sera from Patients with Testicular Germ Cell Tumors

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Summary. Epitope-defined tumor markers of AFP (FA), HCG (PM), PLAP (H7) and CEA (D/AH) were determined by monoclonal antibodies in sera of patients with germ cell tumors of the testis. Characteristic profiles of PLAP (H7) were seen in localized and metastatic seminoma and in sera of patients with mixed tumors with seminoma components. PLAP (H7) levels started to rise 10 months before clinical detection of recurrence in one case. Persisting elevated levels of PLAP (H7) in several cases were indicative of metastatic seminoma. PLAP (H7) occurred rarely in sera of patients with metastasing non-seminomatous tumors. AFP (FA) detected in seminoma sera led to identification of non-seminomatous disease in one case. High AFP (FA) alone occurred in yolk sac tumors, HCG (PM) with AFP (FA) or PLAP (H7) in patients where the tumors had components of teratoma and/or embryonal carcinoma, moderately elevated levels of AFP (FA) and sometimes also HCG (PM) occurred.

Key words: Seminoma – Tumor markers – Antigenic epitropes

Introduction

The availability of markers for testicular germ cell tumors has improved the clinical management concerning diagnosis, prediction and monitoring [8, 12, 21, 30]. Alphafetoprotein (AFP) with molecular weight of 70 kD, is normally synthesized by the fetal liver and the yolk sac [1]. In adult life hepatomas, yolk sac tumors and non-seminomatous germ cell tumors produce AFP [24]. Circulating AFP from tumors has a half-life of 4–5 days, and a deflected or rising serum concentration indicates residual AFP-secreting tumor cells. Human chorionic gonadotrophin (HCG) consists of subunit α and β chains [28], forming a molecule of 40 kD. Only the complete molecule exhibits biological activity. HCG is synthesized by syncytiotrophoblasts of the human placenta and serves to activate corpus luteum and to stimulate placenta to secrete steroids.

Human placental alkaline phosphatase (PLAP; E.C. 3.1.3.1) is a membrane-bound enzyme of 120 kD, normally synthesized by syncytiotrophoblasts from the 12th week of pregnancy [3]. The placental enzyme is heat-stable and catalyzes phosphate transfer. Inhibition by several tripeptides is characteristic of the placental enzyme, while the PLAP-like enzyme in normal testis or in seminomas is instead inhibited by L-leucine [11, 18]. Very high amounts of PLAP-like enzyme have been found in seminoma and certain other testicular tumors [11, 30], and in serum of patients with seminoma [9, 10, 14, 21, 29]. The fraction of elevated PLAP-like serum levels in patients with seminoma is about 50–60%. In contrast, small amounts of PLAP have been described in normal human cervix [4] and the normal testes [2, 5, 17, 18].

Carcinoembryonic antigen (CEA) is a glycoprotein of 180 kD, sharing epitopes with several normally occurring glycoproteins. CEA is mainly found in the fetal gut and occurs in large amounts in localized and metastatic colorectal tumors and in serum from these patients [26].

AFP and HCG have been helpful in early diagnosis, in staging, and in the detection of recurrence [8, 13, 16, 21, 22]. Seminomas and about 10–20% of non-seminomas do not produce AFP or HCG [8, 16]. The 10% of patients with seminoma who show a fatal course may benefit from better methods of monitoring the disease. Monoclonal antibodies to the above-mentioned proteins were used to define serum profiles of marker epitopes in patients with localized, metastatic and recurrent germ cell tumors.

Materials and Methods

Sera were derived from patients aged 15–59 years with testicular tumors treated 1979–1984 at the Departments of Urology and Radiumhemmet, Karolinska Hospital. Serum samples were collected

Table 1. PLAP (H7), AFP (FA), HCG (PM) and CEA (D/AH) contents in sera of patients before treatment

Serum from patients with	No. of patients	Mean values (range) and frequency of levels above cut-off			
		PLAP (H7) μg/l	AFP (FA) kIU/l	HCG (PM) IU/l	CEA (D/AH) μg/l
Seminoma (localized or metastases)	21	27.0 (8.3–48) 18/21	41.0 (11–100) 3/21	76.0 (65–87) 2/21	<5 0/21
Seminoma component	6	27.0 (9.6–47) 3/6	1015 (145–2875) 6/6	4848 (140–9500) 4/6	<5 0/6
Non-seminoma (localized or metastases)	13	15.7 (10.8–43) 2/13	2728 (52–20000) 11/13	4840 (115–9500) 5/13	<5 0/13
Choriocarcinoma component	3	43 1/3	1422 (120–2400) 3/3	1120 (85–2050) 3/3	<5 0/3
Yolk sac tumor	4	<5 0/4	3781 (175–1950) 4/4	6650 1/4	<5 0/4
No tumor, healthy males	10	<5	<5	<5	<5

within one week before primary treatment, within a week after orchiectomy, within a week before prophylatic radiation or chemotherapy and 1 month to several years after treatment. Fortyseven germ cell tumors were classified according to AFIP (Table 1; 20): The patients had typical seminoma without metastases (11 patients), typical seminoma with metastases (10 patients), combined tumors consisting of seminoma with components of embryonal carcinoma, teratocarcinoma or both (6 patients), non-seminoma without metastasis (4 patients), non-seminoma with metastasis (9 patients), choriocarcinoma with or without other components (3 patients), and yolk sac tumors with or without other components (4 patients). 10 healthy non-smoking males aged 15–57 years and 7 women pregnant in weeks 28–40 provided normal sera.

Catalytic Assay for PLAP (CAT Assay)

The catalytic assay for PLAP was performed as described [25]. The enzyme activity was calculated in μmoles x min^{-1} x l^{-1} (= IU/l). The cut-off value was 0.2 IU/l.

PLAP Immunoassay

Coating for enzyme linked immunosorbent assay (ELISA) was performed with protein A purified rabbit anti-PLAP IgG [19] and a horse-radish peroxidase conjugated mouse monoclonal antibody (Mab) produced against the purified 2-1 phenotype of PLAP [17]. Our detection level was 1 μg/l, the cut-off level of PLAP (H7) in healthy males 5 μg/l.

AFP Immunoassay

Immunoradiometric assay (IRMA) for AFP (FA) with tubes coated with Mab anti-AFP (code F) and another ^{125}I-labelled Mab (code name A) was used (Behringwerke AG, Marburg, FRG). The detection limit was 0,1 kIU AFP/l, our cut-off value for healthy males 5 kIU/l.

HCG Immunoassay

IRMA for HCG (PM) was performed in tubes coated with Mab anti-HCG β-chain (code P) and the ^{125}I-labelled tracer Mab directed to HCG α-chain (clone M; Behringwerke). The detection limit was 0.4 IU/l, our cut-off value for healthy males 5 IU/l.

CEA Immunoassay

The CEA Maria radioimmunoassay of Pharmacia Diagnostics (Uppsala, Sweden) was used. It is composed of one Mab I-38S1 specific for CEA epitope D for coating and ^{125}I-labelled Mab II-17 reactive with epitope A and II-10 reactive with epitope H of CEA [7]. The assay thus detects CEA (D/AH). An assay with increased specificity for CEA in tumors is thus obtained [6, 7]. Our detection level was 0.1 μg/l and the cut-off value 5 μg/l.

Results

A comparison of preoperative PLAP (H7), AFP (FA), HCG (PM), and CEA (D/AH) serum levels of patients with different testicular tumors is shown (Table 1) together with cut-off values of ten healthy males calculated by $\bar{x}$ + 3 SD. Of 21 patients with seminoma, 18 (86%) had elevated serum levels of PLAP (H7). PLAP elevation occurred alone in 14 patients or was accompanied by small elevations of AFP (FA) in three or HCG (PM) in two patients. Elevated serum levels of PLAP (H7) were infrequent in patients with non-seminomatous disease, occurring in three out of altogether 20 patients. Instead elevations of AFP (FA), HCG (PM) or both were seen. With choriocarcinoma one out of three patients had PLAP (H7) but all had high HCG (PM) values. Four patients with yolk sac tumors had elevated (AFP) (FA), and one an elevated HCG (PM). CEA

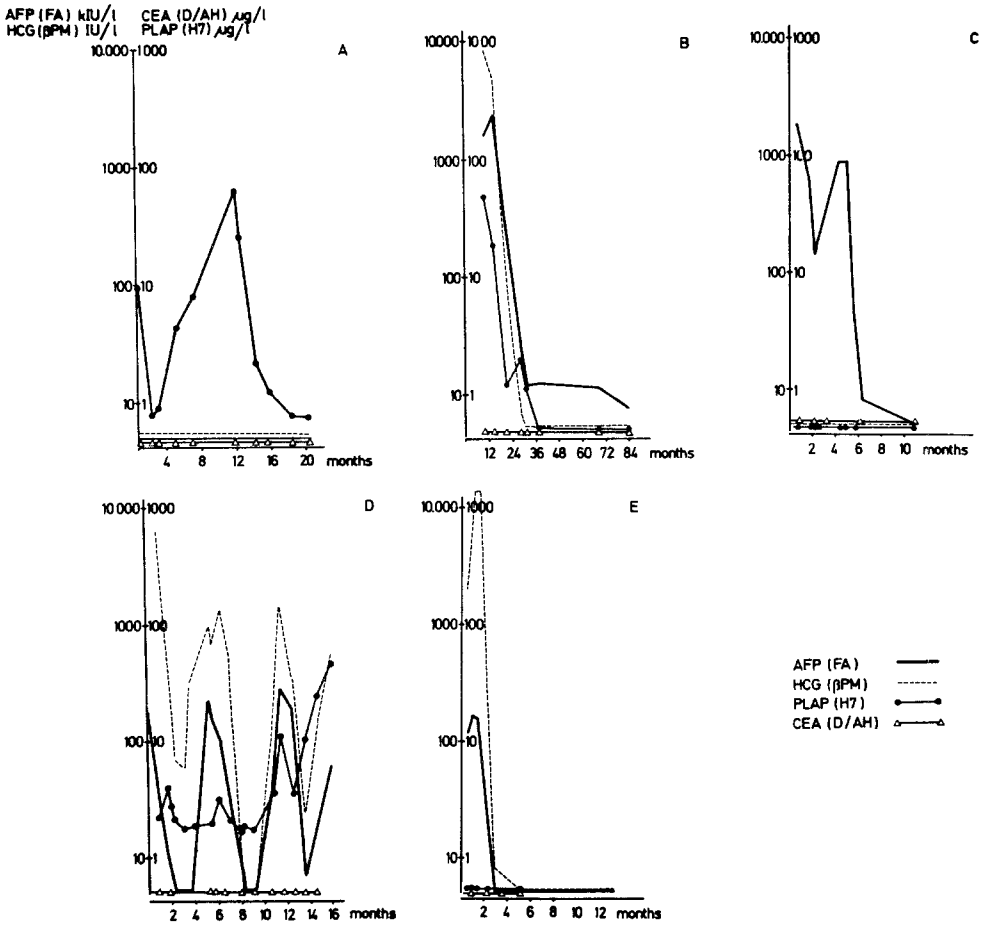

Fig. 1. A A 40-year-old male had a radical orchiectomy showing pure seminoma. He had elevated serum PLAP (H7) with the primary tumor and at mediastinal recurrence but no AFP (FA), HCG (PM) or CEA (D/AH). At a routine check-up this patient was found to have metastatic growth. After radiation for mediastinal metastasis, PLAP (H7) levels were again below detection limit. B A 16-year-old male had a radical orchiectomy, histopathology showing seminoma and teratocarcinoma. Initially PLAP (H7), AFP (FA) and HCG (PM) were elevated. After three courses of combination chemotherapy with Cisplatinum, Vinblastine and Bleomycin (CVB) he was clinically tumor free and all tumor markers had normalized. Six years later he remained free of tumor. C A 24-year old male had a radical orchiectomy showing yolk sac tumor and embryonal carcinoma. Only AFP (FA) was elevated initially, and started to decline with 3 courses of CVB. Three months after primary operation pulmonary metastases were detected and the patient was given 4 further courses of CVB. He remained tumor-free for a follow-up of 2 years. D This 21-year-old man had a radical orchiectomy. The tumor was composed of embryonal carcinoma and yolk sac tumor. The preoperative PLAP (H7), AFP (FA), and HCG (PM) levels were elevated but not CEA (D/AH). After CVB, tumor markers started to decrease but 4 months later were again elevated. After 6 courses of CVB and 2 courses of CB and VP-16 chemotherapy, AFP (FA) and HCG (PM) levels were normal but later AFP (FA), HCG (PM) and PLAP (H7) levels were elevated again. At that time he was found to have metastatic growth in the abdomen, bilateral inguinal lymph node and liver metastases. With addition of two CB and VP-16 courses, AFP (FA) and HCG (PM) levels decreased temporarily. 18 months later AFP (FA), HCG (PM) and PLAP (H7) were elevated and the patients died from massive metastases. E A 21-year-old male had a radical orchiectomy. The tumor was composed of choriocarcinoma and teratocarcinoma. HCG (PM) and AFP (FA) levels were elevated but not PLAP (H7) or CEA (D/AH). After 4 courses of CVB chemotherapy, HCG (PM) and AFP (FA) levels became normal. No recurrences were subsequently found

(D/AH) levels were low in all patient sera. Figure 1 shows serum profiles characteristic of the tumor types.

The relation of PLAP (H7) to various stages of seminomas was analysed (Table 2). Ten patients out of 11 with localized seminomas (91%) had elevated serum levels of PLAP (H7) before orchiectomy. With metastatic seminoma, eight out of 10 (80%) had elevated values. Elevated preoperative PLAP (H7) levels in patients with localized seminoma decreased after orchiectomy (Table 2). In patients who later would have a recurrence (localized seminoma 3/11, metastatic seminoma 9/10) the post-treatment PLAP (H7) levels were all above the cut-off level (means 13.8 and 14.3 µg/l respectively, not shown in tables). When the recurrence was clinically diagnosed it had risen further (means 37.5 and 63.1 µg/l respectively; Table 2). Measurement of serum PLAP (H7) after primary therapy thus may help anticipating recurrent seminoma. In recurrent metastatic non-seminoma, three out of 13 patients had elevated PLAP (H7) in addition to AFP (FA) and HCG (PM) which did not change after orchiectomy. They decreased following chemotherapy.

Table 2. Distribution of PLAP (H7) serum values in various phases of disease

	Frequency of patients with elevated PLAP levels	Mean PLAP (H7), μ/l				Frequency of recurrence	PLAP (H7), μg/l	
		Orchiectomy		Radiation and/or chemotherapy			Treatment for recurrence	
		before	after	before[a]	after		before	after
Localized seminoma	10/11	26.3	17.7	20.9	13.8	3/11	37.5	13.5
Metastatic seminoma	8/10	28.1	25.0	25.0	14.3	9/10	63.1	27.5
Seminoma[b] component	3/6	27.0	29.6	29.3	7.5	0/6	–	–
Localized[c] non-seminoma	0/7	<5	<5	<5	<5	1/7	<5	<5
Metastatic non-seminoma	3/13	24.8	25.1	25.1	12.3	5/13	11.0	<5

[a] Directly following orchiectomy
[b] Including teratoma, embryonal carcinoma, teratocarcinoma and mixtures thereof
[c] Including tumors with yolk sac tumor and choriocarcinoma components

Table 3. Enzymatic activity in PLAP-positive sera

Sera from persons with	No. of samples with elevated levels	Mean levels ± SD		
		CAT IU/l	PLAP (H7) μg/l	CAT/H7 IU/mg
Seminoma	18	1.6 ± 0.7	27.0 ± 12.3	58.1 ± 20.6
Seminoma component	3	1.4 ± 1.0	27.0 ± 18.8	50.5 ± 1.3
Non-seminomatous germ cell tumor	3	1.2 ± 0.7	24.8 ± 16.5	52.1 ± 10.5
Pregnancy	7	18.6 ± 9.7	180 ± 125	112 ± 22

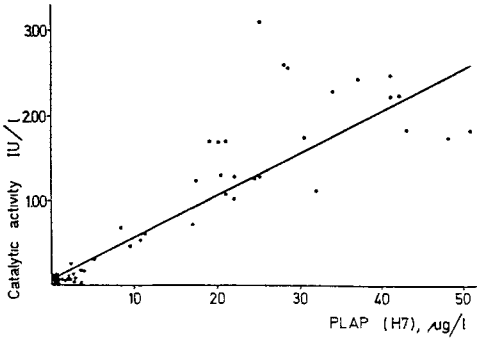

Fig. 2. Comparison of catalytic activity and ELISA of PLAP (H7) in sera before orchiectomy from 47 subjects with testicular tumors and 3 non-smoking healthy men. A correlation coefficient of 0.92 was found

The relation between catalytic activity and protein concentration as measured by ELISA of male sera showed a high correlation of r = 0.92 (Fig. 2). The same 10 out of 11 patients who had elevated PLAP (H7) values (Table 2) also had CAT assays above 0.2 IU/l, the mean value being 1.43. The mean value for ten non-smoking healthy men was 0.03 ± 0.01 IU/l. The relation of enzyme activity to PLAP-like (H7) protein (CAT/H7) was compared between seminoma sera and sera from pregnant women. The specific activity of the seminoma enzyme appeared to be only half of that in pregnant women (Table 3).

Elevated serum levels of PLAP (H7) and in a few cases of AFP (FA) were seen in patients with seminoma at the time of a recurrence. Elevated PLAP (H7) levels were shown in 11 (92%) patients before treatment for recurrence, with a mean of 58.5 μg/l. Increased AFP (FA) were shown in 2 patients with a diagnosis of seminoma before and after treatment for recurrence. It is likely that these two patients also had non-seminomatous disease, and in fact one of them was later operated for a mature abdominal teratoma. It is of interest to note that in patients who were not going to show a clinical recurrence, elevated PLAP (H7) levels were seen after orchiectomy and before the ensuing radio- or chemotherapy (Table 4). This may indicate remaining tumor, not detected by other means. After therapy these levels decreased to normal.

Table 4. Serum PLAP (H7) levels in various phases of patients with a treated testicular tumor and no recurrence (NED)

	No. of patients with		PLAP (H7) of all non recurrent patients, μg/l ± SD (no. of patients in the NED group who had elevated PLAP (H7)			
	recurrence	no recurrence	Orchiectomy		Radiation and/or chemotherapy	
			before	after	before	after
Localized seminoma $n = 11$	3	8	26.6 ± 13.9 (8)	11.1 ± 12.3 (4)	9.7 ± 13.7 (2)	2.8 ± 2.4 (0)
Metastatic seminoma $n = 10$	9	1	37 (1)	12 (1)	12 (1)	2.4 (0)
Seminoma component $n = 6$	0	6	14.7 ± 18.1 (2)	11.7 ± 17.8 (2)	15.6 ± 21.4 (2)	2.8 ± 3.1 (1)
Localized non-seminoma $n = 7$	1	6	1.3 ± 0.8 (0)	2.4 ± 2.8 (0)	1.9 ± 1.9 (0)	2.6 ± 2.9 (0)
Metastatic non-seminoma $n = 13$	5	8	3.5 ± 6.9 (1)	3.3 ± 7.0 (1)	3.3 ± 7.0 (1)	2.3 ± 4.1 (1)

Discussion

In testicular tumors, effective therapy has become available, which enhances the utility of specific and sensitive markers for residual disease. The availability of specific epitope determinations, has an advantage at the analytical level: due to selection for high affinity monoclonal antibodies AFP, HCG and PLAP can be rapidly determined. We found that two patients with a diagnosis of seminoma from the orchiectomy specimen also had non-seminomatous disease [23]. Three patients with an initial diagnosis of non-seminomatous disease had elevated PLAP. One of them had a choriocarcinoma, one embryonal carcinoma and the third teratocarcinoma. In our mind the elevated PLAP levels are indicative of an occult seminoma component of the localized tumor or residual seminomatous disease. CEA was not raised in any of the present cases. A two-monoclonal assay and the three-monoclonal assay for CEA-unique epitopes used here have however shown selective advantages with gastrointestinal tumors [7].

PLAP is a membrane-bound enzyme, which may be the reason that rather small amounts of PLAP-like enzymes are released into the serum from tumor cells in contrast to e.g. HCG which is secreted in large amounts. Other factors that have been reported to give raised levels of PLAP are smoking [15, 27] and Buergers disease [29]. None-the-less, release of PLAP-like enzymes seems to be indicative of tumor disease in males. It is of interest to note that the testicular enzyme appears to have a lower specific enzymatic activity than the enzyme found in sera of pregnant women.

For PLAP determinations H7 Mab was used, since most of the PLAP-like substances in seminoma sera [29] and tissues [11] could be typed to belong to PLAP types 1–4 (H7 elevated types). In ten patients with localized seminoma and elevated PLAP (H7) before orchiectomy, four (40%) still had a positive PLAP (H7) level after orchiectomy but before radiation therapy. This indicates that the operation had not been radical and that there was a clinical staging error. After radiation therapy, two out of four patients had continuously positive PLAP (H7) levels. These two patients also had a recurrence. The half-life of PLAP is 2–7 days and beyond that time elevation of PLAP-like enzyme is a strong indication of residual disease or recurrence. In eight patients with metastatic seminoma and elevated PLAP (H7) before orchiectomy, six had positive PLAP (H7) levels after orchiectomy but before radiation and or chemotherapy. Four out of the six patients were treated only by radiation therapy and the others had a combination of radiation and chemotherapy. One out of four in the former group and all in the latter group had normalized PLAP (H7) levels after therapy.

In patients with testicular tumors the addition of PLAP measurement to AFP, HCG and clinical examination gives an improved selective screening and accuracy of staging. It also appears useful prognostically and for follow up.

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