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Evaluation of Pregnancy-specific β_1-Glycoprotein in Patients with Non-seminomatous Testicular Germ Cell Tumors*†

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Abstract—*Serum SP-1 levels were measured serially in 94 patients with non-seminomatous germ cell tumors to evaluate its clinical significance as a tumor marker. In 12 out of 80 patients (15%) with active tumors serum SP-1 was found to be elevated, whereas serum HCG and AFP in the same sample were raised in 53 and 45% respectively. Elevation of serum SP-1 levels was always associated with raised HCG levels, and with AFP in 7 patients. During chemotherapy, serum SP-1 and HCG disappeared when a complete remission was obtained. In contrast to HCG, serum SP-1 failed to detect tumor progression in two patients. Serum HCG and AFP are superior as tumor markers to serum SP-1.*

INTRODUCTION

THE TUMOR-associated markers alpha-foetoprotein (AFP), human chorionic gonadotropin (HCG) and lactate dehydrogenase (LDH), which are produced by non-seminomatous germ cell tumors of the testis, have now gained an important role in following the course of the disease [1–10]. Their application involves staging, monitoring chemotherapy and early detection of tumor progression. The sera of 10–30% of the patients with this tumor, however, show normal levels of AFP and HCG [1, 9–11]. In search for other parameters, a variety of potential tumor-associated markers are under investigation, in particular, carcino-embryonic antigen (CEA) [9, 12, 13] and the pregnancy-specific β_1-glycoprotein (SP-1) [14–17].

The few reports on serum SP-1 as a tumor marker come to different conclusions with respect to the incidence of SP-1 elevations and its inter-relationship with other tumor markers in patients with non-seminomatous germ cell tumors.

The incidence of serum SP-1 elevation varies from 18 [16] to 52% [15]. Concordant elevation of serum SP-1 and other tumor-associated markers was found [17], in contrast to cases in which SP-1 was the only marker elevated [15]. Only scarce information on the value of serum SP-1 for the clinical management of the patients can be derived from these studies.

This article describes our experience with serial evaluation of serum SP-1 in patients with advanced non-seminomatous testicular cancer as compared with HCG and AFP.

PATIENTS AND METHODS

Between August 1977 and December 1980, 94 patients with a non-seminomatous testicular cancer were seen at the University Hospital of Groningen. The majority of the patients had their primary tumor removed elsewhere. The

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clinical stage of the disease was determined by physical examination, chest X-rays, lung tomography, bipedal lymphangiography, intravenous urography, isotope scans and computed tomography of the retroperitoneal lymph nodes. Based on these data, patients were classified as stage I—tumor confined to the testis; stage II—metastatic disease in retroperitoneal lymph nodes; or stage III—visceral or distance metastases.

In patients with stage I and II, a laparotomy and bilateral retroperitoneal lymph node dissection was performed. Patients with bulky, non-resectable stage II tumors and patients with stage III were treated with combination chemotherapy, including *cis*-platinum, vinblastine and bleomycin [18].

Blood samples were collected two or three times a week during hospitalisation and thereafter at every outpatient visit. During remission–induction chemotherapy the actual half-life of HCG and SP-1 were calculated as described previously [19]. High serum AFP levels were subjected to an identity reaction by counter-immunoelectrophoresis following the method of Kohn [20]. All samples were subsequently assayed by means of a radioimmunoassay (Behring Werke A. G., Marburg, Germany) with a sensitivity of 0.5 μg/l. As an upper limit of normal, 20 μg/l of AFP was determined. Serum HCG was measured with a radioimmunoassay (Institute National des Radioéléments, Fleurus, Belgium) as described by Franchimont *et al.* [21]. Cross-reactivity with the β-HCG subunit was 5% compared to native HCG. LH showed a cross-reactivity of 2%. In normal, non-pregnant subjects serum HCG levels were found to be lower than 2 μg/l; in 1.8% levels up to 4 μg/l were measured. The upper level of normal was established at 4 μg/l.

Serum levels of SP-1 were measured in a double antibody radioimmunoassay. Reagents were supplied by Behring Werke A. G., Marburg, Germany. The [^{125}I]-labeled SP-1 (sp. act. 40 mCi/mg) was used without further purification. The mean binding capacity B_0 was $35 \pm 9\%$ (S.D., $n = 16$). The mean SP-1 levels at B/B_0 values of 90 and 50% were respectively 4.7 ± 1.1 and 39 ± 6 μg/l, and an interassay variation of 10% was obtained. In 42 healthy male blood donors the mean B/B_0 value was $100.3 \pm 3.4\%$, indicating the absence of SP-1, and all values were below 5 μg/l.

RESULTS

The serum SP-1 level was found elevated in 12 out of 80 patients with active tumors prior to treatment, and varied from 20 to 525 μg/l. The incidence of SP-1 elevations (15%) is lower than for the other tumor markers. Serum HCG was elevated in 42 (53%) and AFP in 36 (45%) patients and 15 (19%) did not produce any of these markers.

Tumor markers and stage

The relation between tumor stage and tumor markers is given in Table 1. Most patients underwent orchiectomy before referral. In 14 patients with stage I disease, SP-1 levels were below 5 μg/l during the follow-up period after orchiectomy. In the 3 patients with a stage I tumor who were examined before orchiectomy, SP-1 was not detectable in serum.

In stage II disease, serum levels of AFP and HCG were increased in 11 and 9 patients respectively. One of these patients showed an elevated serum SP-1 level of 105 μg/l. Most of the patients in stage II with positive tumor markers had bulky non-resectable retroperitoneal lymph nodes.

In stage III disease, 11 patients (24%) showed increased SP-1 levels, 24 patients (52%) had elevated serum AFP levels and serum HCG was detectable in 32 of them (70%).

Table 2 shows the pre-treatment serum levels of tumor markers in the 12 patients with increased serum SP-1 levels. We saw that all these men also had elevated serum HCG levels. However, no correlation between the concentrations of HCG and SP-1 was found ($r = 0.21$, $P > 0.1$).

Table 1. *Tumor markers in relation to the stage of tumor growth*

Stage	No.	No. of patients with increased levels of: SP-1	HCG	AFP
I	3 (before orchiectomy)	0	1	1
	14 (after orchiectomy)	0	0	0
II	31	1	9	11
III	46	11	32	24
Total	94	12	42	36

Table 2. Serum levels of tumor markers in patients with SP-1 producing non-seminomatous testicular tumors before treatment

Patient No.	SP-1 (μg/l)	HCG (μg/l)	AFP (μg/l)
1	525	300	<20
2	450	38,000	2000
3	320	35,000	<20
4	230	1200	800
5	200	90,000	40
6	140	4200	110
7	105	9500	<20
8	38	700,000	<20
9	29	2700	1600
10	25	1700	<20
11	25	28	2000
12	20	1450	160

A relation between raised serum SP-1 and AFP in stage II and III disease could not be demonstrated: in 12 SP-1-positive patients serum AFP was raised in 7 of them, and in 65 SP-1-negative patients 28 showed raised AFP levels ($\chi^2 = 0.092$, $P > 0.1$).

Tumor marker levels during chemotherapy and follow-up

All 12 patients with elevated serum SP-1 levels had advanced tumor; 11 of them were treated with combination chemotherapy and the other patient died before therapy was instituted. During treatment serum levels of SP-1 normalized simultaneously with HCG and, when present, with AFP. In all cases SP-1 levels were below 5 μg/l before serum HCG reached normal levels.

Two patients (Nos. 5 and 10) showed tumor progression after initial remission which first became evident by rising serum HCG levels. In contrast to the high initial SP-1 level of 200 μg/l, patient No. 5 showed no evidence of SP-1 production during the period of tumor progression (Fig. 1), and his serum AFP level, 40 μg/l before chemotherapy, remained normal, too.

In patient No. 10 a rise in serum SP-1 to 10 μg/l first became detectable 7 months after tumor relapse. In both patients serum HCG values were rising above 100 μg/l and gave definite evidence for tumor progression.

As can be derived from Table 1, in 65 patients with stage II and III disease no SP-1 was detectable before therapy was instituted. Their sera were screened for the presence of SP-1 during treatment. In three of these men a relatively small elevation of their serum SP-1 level (up to 32 μg/l) could be measured during a period of tumor relapse, but not in their pre-treatment samples (one example is given in Fig. 2). In all three cases, however, raised levels of serum HCG (varying from 14 to 100 μg/l), and in one case serum AFP (over 1000 μg/l),

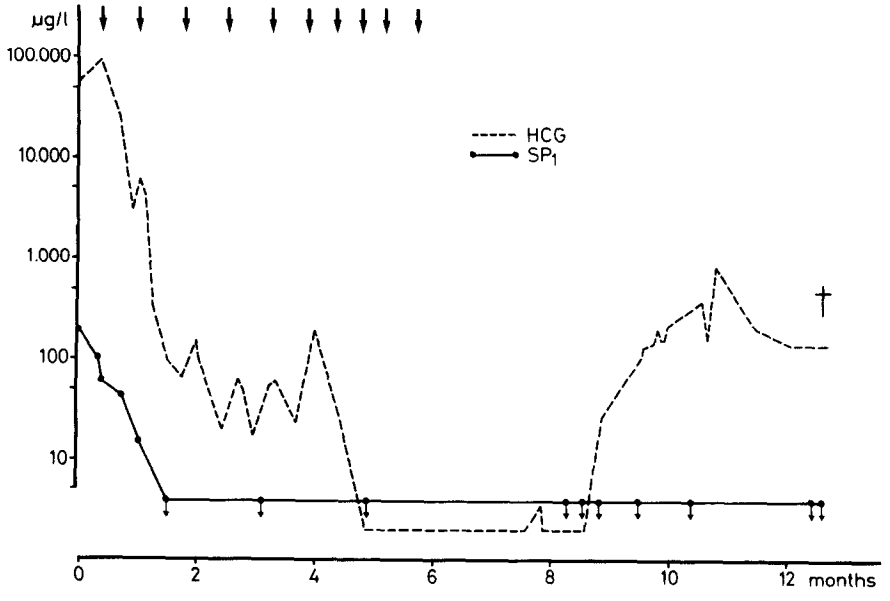

Fig. 1. Course serum SP-1 and HCG levels in patient No. 5. He had advanced retroperitoneal and pulmonary disease. During chemotherapy (indicated by arrows on top of the figure) the SP-1 level becomes lower than 5 μg/l (arrows at bottom line). At the time of tumor progression and development of cerebral metastases only serum HCG increased. Serum AFP (40 μg/l in the initial serum sample) remained normal.

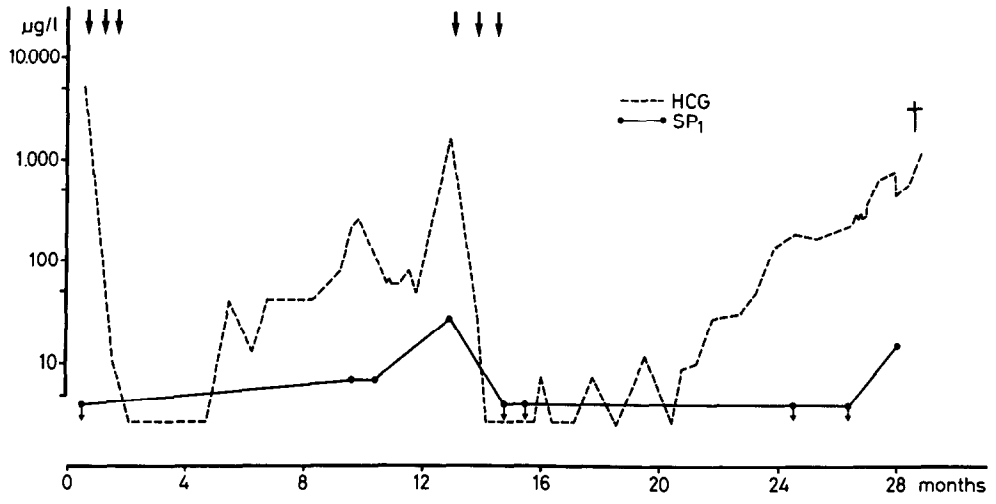

Fig. 2. Follow-up serum SP-1 and HCG levels in a patient with a non-detectable SP-1 level in the initial serum sample (explanation as in Fig. 1). During two episodes of tumor progression (both marked by a rise in serum HCG) the presence of SP-1 could be demonstrated. Serum AFP was normal.

indicated that their tumor had already relapsed.

Serum SP-1 half-life during chemotherapy

The decline of serum SP-1 was analysed in more detail in search for a parameter indicating incomplete remission. The apparent half-life of serum SP-1 during chemotherapy varied from 4 to 13 days, a mean value of 8.0 ± 2.5 days being calculated from the data from 10 patients. The corresponding value for serum HCG was 2.0–4.2 days, with a mean value of 3.0 ± 0.8 days. In two patients (Nos. 5 and 10) with tumor relapse after initial remission the apparent half-life of serum SP-1 was within the range mentioned: 8 and 8.5 days respectively. Apparent half-life for HCG was delayed in patient No. 5, after a normal initial half-life time of 4.0 days (Fig. 1).

DISCUSSION

Germ cell tumors of the testis synthesize proteins which can be assayed in the serum of the patients and serve as markers for the presence of active tumor or residual tumor mass. AFP, HCG and LDH have proven to be valuable parameters in the management of patients with non-seminomatous testicular tumors [1–10]. CEA appears to have no value as a marker in these patients because elevated levels are seldom found and changes in CEA levels do not correlate with tumor activity [9, 13, 22]. Nevertheless, it has been suggested that this marker may be of value for the diagnosis of differentiated teratoma in metastatic tumor [17, 23].

Improvement of treatment requires an early detection of new metastases or tumor progression. Some patients (19%) escape surveillance because no tumor markers could be recognised—the reason why SP-1 was evaluated. SP-1 is a glycoprotein produced by the syncytiotrophoblast of the placenta. It contains about 28% carbohydrate and has a molecular weight of 90,000 daltons [24–26].

The incidence of 15% found for serum SP-1-positive patients is comparable to the figures reported by Rosen *et al.* [16] and Szymendera *et al.* [17], 18 and 21% respectively, but is lower than the 52% described Lange *et al.* [15]. This discrepancy can be accounted for by differences in the sensitivity of radioimmunoassay systems and in the upper limit of normal established systems by each laboratory (Lange *et al.* [15], 1 µg/l, as compared to 3–5 µg/l by the others).

Elevated levels of serum SP-1 have always been associated with increased levels of HCG, but have never been found in combination with AFP alone. Using an indirect immunoperoxidase technique, SP-1 has been detected in the syncytiotrophoblastic component of choriocarcinoma and syncytial (syncytiotrophoblastic-like) giant cells in association with embryonal carcinoma and teratocarcinoma [5, 27–29].

The same localization has been reported for HCG [27, 29], and this gives an explanation for concordance of these tumor markers in serum.

However, in an additional study of 41 non-seminomatous germ cell tumors of the testis with bulky stage II and stage III disease we

found a striking quantitative difference in tissue localization and serum concentrations between HCG and SP-1. SP-1 revealed to be mainly produced by the syncytiotrophoblastic component of choriocarcinoma since both high serum concentrations and considerable tissue positivity of this marker were mainly found in primary tumors with admixture of choriocarcinoma or pure choriocarcinoma. HCG, on the other hand, showed a broader spectrum of tissue localization than SP-1, namely in the syncytiotrophoblastic cells of choriocarcinoma as well as in syncytial giant cells associated with embryonal carcinoma or teratoma and mononuclear embryonal carcinoma cells [29]. This sheds further light on some clinical observations: the broader spectrum of tissue localization of HCG is reflected in the higher percentage of serum positivity of HCG in different patient groups as compared to SP-1 [17, 27–29]. The higher serum specificity of SP-1 for choriocarcinoma is reflected in the predominance of elevated SP-1 serum levels in stage III disease since choriocarcinoma disseminates hematogenously and has a worse prognosis as compared to other histological components of non-seminomatous germ cell tumors of the testis. Also, the discordance between HCG and SP-1 in patient No. 5 during the period of tumor progression can be explained in terms of a quantitative difference in tissue localization or production of these markers.

We cannot confirm the observation of Lange *et al.* [15, 30], who found elevated SP-1 levels in association with AFP alone, and SP-1 as a single elevated tumor marker.

In conclusion, our study shows that serum SP-1 partially fulfils criteria for a tumor marker for non-seminomatous germ cell tumors. It failed to indicate tumor progression. Serum SP-1 levels gave no additional information to serum HCG values, which were superior in detecting a tumor relapse.

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