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TUMOUR MARKERS IN TESTICULAR GERM CELL TUMOURS

Five-year experience from the DATECA Study 1976–1980

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Abstract

Since Jan. 1, 1976, nearly all new cases of testicular germ cell tumours have been included in the Danish Testicular Carcinoma Study (DATECA), and have been monitored by the tumour markers alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG). During the first five years, 1 058 patients participated in the investigation, but only 603 of these patients were followed by preoperative as well as postoperative marker determinations in serum. The overall prevalence of marker positivity, i.e. elevated preoperative values for AFP and/or HCG, was 8 per cent for seminoma patients and 60 per cent for non-seminoma patients. Elevated levels of serum AFP and HCG were correlated to the presence of endodermal sinus tumour and choriocarcinoma elements, respectively, in the primary tumour. The presence of increased marker concentration in serum was correlated to stage (higher percentage in higher stages) and to prognosis (marker negative patients had a better prognosis than marker positive patients). Marker production by seminoma patients seems to indicate a poor prognosis, especially for HCG producing seminomas.

The clinical use of tumour markers in the diagnosis and management of germ cell tumours is now well established (11) and an extensive literature has developed in the past few years. International recommendations and collaborative studies on the clinical use of alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) have also appeared in recent years (12).

From the inception of the Danish Testicular Carcinoma Study (DATECA) on Jan. 1, 1976, nearly all of the patients have been monitored by AFP and HCG determinations in blood. The DATECA tumour marker material includes all patients with testicular tumours irrespective of histology and stage, and the size of this unselected material is larger than in any other investigation on tumour markers, and testicular malignancy. As described elsewhere (17), a uniform protocol has been used for pathology, staging and clinical trials. The purpose of the present report is to describe five years' experience on the clinical relevance of AFP and HCG in the diagnosis, prognosis and treatment of 1 058 consecutive patients with testicular tumours.

Material and Methods

The staging procedure and histologic classification have been described by SCHULTZ et coll. (18). In nearly all of the 1 058 patients, postoperative determinations of AFP and HCG were carried out on admission to one of the five oncologic centres in Denmark. Only 603 of these patients, however, had also had preorchiectomy blood samples taken at local hospitals (Table 1).

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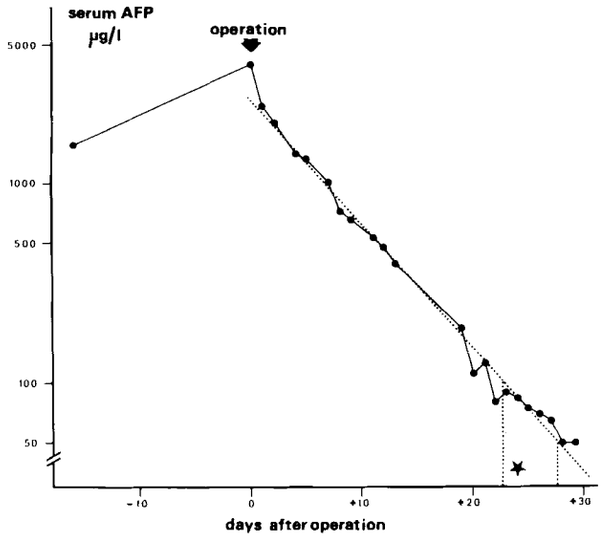

Fig. 1. Observed half-life of AFP in a patient after radical orchiectomy calculated by regression analysis. Observed half-life: 4.90 days (★).

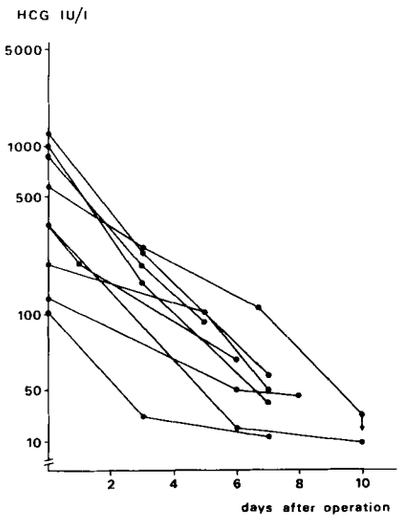

Fig. 2. Observed half-lives of serum HCG in 8 patients (non-seminoma stage I) after radical orchiectomy calculated by regression analysis. Mean value 2.1 days (range 1.4–3.2 days).

The AFP assay was carried out as a polyethylene glycol radioimmunoassay with a sensitivity of 5 µg/l (3 KIU/l). The analytic coefficient of variation calculated from 80 determinations on serum pools with AFP concentrations of 25 and 150 µg/l was 6.1 and 8.4 per cent, respectively. The upper limit of AFP in normal adults is about 20 µg/l.

The HCG assay was carried out as a double antibody radioimmunoassay with a sensitivity of 30 IU/l. The analytic coefficient of variation calculated from 116 determinations on serum pools with HCG concentrations of 45 and 155 IU/l was 10 and 12 per cent, respectively. The levels in normal adults are less than 30 IU/l. The WHO international standard, and later international reference preparation, was used as standard for HCG. The assay was HCG-specific, since an antiserum against the β-subunit was used in the assay.

Results

Pre- as well as postoperative marker determinations were carried out in 603 of the total number of the five-year material (1058 patients). As seen in Table 1, 307 of these patients had pure seminoma (S), and AFP and/or HCG were elevated in only 8 per cent. In 296 non-seminoma (NS) patients the corresponding percentage was 60. Preoperative marker values in serum for these 603 patients in relation to stage and histology are shown in Tables 2 and 3. The group of non-seminoma patients with disseminated disease (stages II and III) showed a statistically significant ($p<0.01$) higher percentage of marker elevation than in the patients with localized disease (stage I; Table 3). A similar stage dependency was not found in the patients with pure seminoma (Table 2).

Table 1

Preoperative HCG and AFP levels and histology in 603 DATECA patients

Histology	Preoperative serum HCG levels		Preoperative serum AFP levels	
	Normal	Increased	Normal	Increased
Seminoma (n=307)	286	21	304	3
Non-seminoma (n=296)*	204	92	141	155**

* 179 non-seminoma patients were marker positive, i.e. 60 per cent.

** 68 had increased levels of both HCG and AFP.

Table 4 shows the correlation between the percentage of elevated preoperative marker values and histologic type for both pure and mixed tumours.

Elevated AFP values were found in all NS tumour types except pure choriocarcinoma (CC). The frequency of elevated values was especially high in tumours with endodermal sinus tumour (EST) com-

Table 2

Preoperative HCG and AFP levels and stage in 307 seminoma patients

Stage	Preoperative serum HCG levels		Preoperative serum AFP levels		Increased levels of HCG or AFP or both
	Normal	Increased	Normal	Increased	
I (n=245)	232	13	242	3	16
II (n=56)	49	7	56	0	7
III (n=6)	5	1	6	0	1

Table 3

Preoperative HCG and AFP levels and stage in 296 non-seminoma patients

Stage	Preoperative serum HCG levels		Peroperative serum AFP levels		Increased levels of HCG or AFP or both	Increased levels of both HCG and AFP
	Normal	Increased	Normal	Increased		
I (n=170)	128	42	89	81	91 (54%)	32 (19%)
II (n=77)	53	24	33	44	50 (65%)	18 (23%)
III (n=49)	23	26	19	30	38 (78%)	18 (37%)

Table 4

Preoperative serum values of HCG and AFP related to histology

	Pure types				Mixed types					
	EST	EC	T	CC	EC+S	EC+T	EC+CC	EC+EST	EC+T+CC	EC+T+EST
HCG, percentage elevated	0	25.9	17.6	100	12.5	27.0	55.5	25.0	52.9	42.3
AFP, percentage elevated	100	42.9	37.5	0	24.3	62.9	50.0	90.0	50.0	80.8

EC: Embryonal carcinoma, T: Teratoma, CC: Choriocarcinoma, S: Seminoma and EST: Endodermal sinus tumour.

ponents. The HCG values were most markedly correlated to the presence of CC tumour components.

Marker half-life for AFP and HCG. The half-life for AFP was estimated in one patient, in whom an AFP producing tumour was removed radically by orchiectomy (Fig. 1). Fig. 1 also shows a marked increase in AFP concentration during a 14-day preoperative period. After orchiectomy the postoperative fall in AFP concentration was almost linear, and by regression analysis the half-life was calculated to be 4.9 days. In 10 randomly selected patients with non-seminoma stage I, and with at least three samples taken during the first postoperative week, the mean half-life was calculated to be 4.8 days (range 2.6–5.9 days). The mean half-life for HCG, calculated by regression analysis for 8 patients with non-seminoma stage I, was 2.1 days (range 1.4–3.2 days; Fig. 2).

Pre- and postoperative marker levels. Marker levels pre- and postoperatively in relation to the course of the disease are shown in Table 5 for 141 NS patients with residual disease (stage II or III) or with tumour recurrence. The 3-year survival was significantly ($p<0.01$) higher among marker negative patients than among marker positive patients. Among the 141 patients the following tumour marker profiles were found:

(1) Twenty-five patients (18%) were never marker positive in serum.

(2) Thirteen patients (9%) were all marker positive, but they all died within about one month and therefore the marker profiles were difficult to evaluate.

Table 5

Marker production (AFP and/or HCG) and outcome in 141 non-seminoma patients with recurrent or residual disease. Numbers in parentheses indicate patients with residual disease

	Marker positive	Marker negative	Total
Dead	84	8	92
Alive	32 (7)	17 (0)	49 (7)
Total	116	25	141

(3) The remaining 103 patients (73%) all had regular marker determinations during the course of the disease. In 33 of these patients the marker levels closely reflected the clinical disease, whereas the levels in 63 patients showed patterns which deviated from the clinical and radiologic findings on some occasion. Among these patients the following 71 asynchronic episodes were observed: (a) 21 cases with marker normalization, but residual diseases, (b) 15 cases with persistently elevated marker levels but apparently no clinical disease. However, within an interval of one to four months, clinical and radiologic examination nevertheless indicated disease in all these 15 patients, (c) 35 episodes of tumour progression, but with declining or negative marker levels.

Finally, in 8 of the 103 patients, the clinical information was too scarce for detailed evaluation with regard to marker levels.

In 23 (22%) of the 103 NS patients a rise in marker levels was the first sign of tumour recurrence and usually occurred several months before clinical signs of disease.

Among the 80 remaining patients 18 never showed regression in clinical disease and markers. In 57 patients increased marker levels were not the first evidence of disease, and finally, in 8 patients the clinical information was insufficient.

Among the same 103 NS patients 14 (14%) showed dissociation in marker levels of AFP and HCG during the course of the disease, whereas 74 patients did not show any marker dissociation. In 15 patients the details in clinical information were insufficient for evaluation of marker dissociation.

Postoperative marker levels 2 to 3 weeks after orchiectomy in relation to survival are shown in Fig. 3 for 146 NS patients in stages II and III, i.e. patients who all had residual disease after orchiectomy. A statistically significant ($p<0.01$) higher mortality was found in patients with postoperative marker levels, when compared with the patients without increased postoperative marker levels.

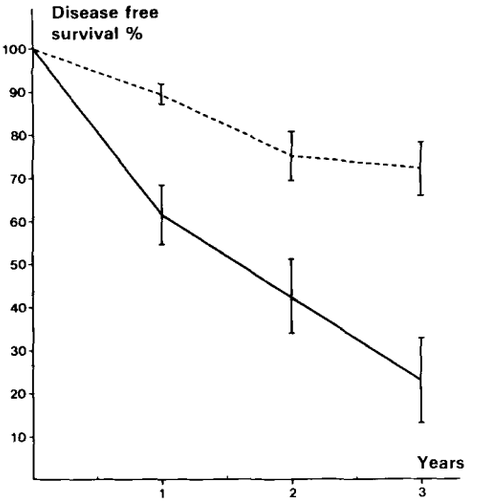

Fig. 3. Advanced non-siminomatous germ cell tumours of the testis (stages II and III). Disease-free survival and postoperative serum marker (AFP and/or HCG) status. Marker negative (n=92 ---), marker positive (n=54 ——).

Special marker findings. Twenty-one patients with clinicoradiologic stage I showed increasing or stationary postoperative marker values or prolonged marker half-life, indicating metastatic disease. In 14 patients with normal preoperative marker values, marker production was found later on during progression of the disease or recurrence. Another group of 14 patients had slightly elevated preoperative AFP values (range 9–20 μg/l), but postoperative values of 5 μg/l or less.

Discussion

The clinical value of AFP and HCG determination in germ cell tumours of the testis is well documented. Several recent investigations have confirmed the diagnostic and clinical significance of these markers (1, 2, 3, 4, 6, 7, 8, 10, 14, 15, 16). However, as shown in Table 6, most of these investigations were based on small and selected groups of patients usually with more advanced disease. In many parts of the world, only patients with stages II and III disease are referred to an oncologic centre, whereas patients with stage I disease are treated at local surgical departments. This was not the case with the DATECA Study, where all patients with testicular tumours were referred after orchiectomy to one of the five oncologic centres in Denmark for further

Table 6

Elevated tumour marker levels in patients with testicular tumours

Reference	AFP		HCG		AFP or HCG	
	Non-seminoma	Seminoma	Non-seminoma	Seminoma	Non-seminoma	Seminoma
LANGE et coll. (8)	46/68	0/21	36/68	5/21	57/68	5/21
SCARDINO et coll. (15)	21/36	0/9	28/36	2/9	33/36	2/9
BOSL et coll. (2)					53/67	
KOHN et coll. (7)	6/27					
SCARDINO et coll. (14)	50/139		34/110		60/136	
HENRY (4)	28/49	0/20	23/49	0/20		
JAVADPOUR et coll. (6)		1/60		4/60		
GRIGOR et coll. (3)	56/86	0/16				
SCHULTZ et coll. (16)	12/34	1/33	13/34	0/33	20/34	1/33
MANN et coll. (10)	17/64		9/64		56/64	
ANDERSON et coll. (1)	16/100		14/100		89/100	
Total	252/603 (42%)	2/159 (1%)	157/461 (34%)	11/143 (8%)	368/505 (73%)	8/63 (13%)
DATECA						
Stage I	81/170 (48%)	3/245 (1%)	42/170 (25%)	13/245 (5%)	91/170 (54%)	16/245 (7%)
Stage II+III	74/126 (59%)	0/62 (0%)	50/126 (40%)	8/62 (13%)	88/126 (70%)	8/62 (13%)
All stages	155/296 (52%)	3/307 (1%)	92/296 (31%)	21/307 (7%)	179/296 (60%)	24/307 (8%)

examination and treatment. Most other investigations can also be criticized for not giving precise information about the time for blood sampling. In most instances blood samples were not taken preoperatively, but only postoperatively. Therefore, tumour marker data from most investigations (Table 6) correspond to the tumour marker data from stages II and III in the DATECA Study. Of non-seminoma patients pooled from the literature 73 per cent had elevated markers (AFP and/or HCG), which corresponds to 70 per cent found in non-seminoma patients stages II and III in the DATECA Study.

For seminoma the corresponding percentages were 13 from the pooled literature, and 13 in the DATECA Study. The percentages of elevated markers for stage I in the DATECA Study were significantly lower than reported in the literature, namely 54 for non-seminoma patients, and 7 for seminoma patients.

As shown in Table 5, elevated preoperative values for AFP and HCG were correlated to the presence of EST and CC elements, respectively, in the primary tumour. The correlation was not perfect, and there may be several explanations for this. It is a well-known fact that pure yolk sac tumour of the infantile testis always produces AFP, and that there is a close relationship between tumour mass, and serum AFP in these patients (11). In adults, pure EST is very rare. Only 3 patients out of 1058 DATECA patients had pure EST, and they all produced AFP. As described by TEILUM (21), EST in adult testis tumours is present as a specific neoplastic entity but in varying amounts and patterns. Even examination of many tissue sections from the same tumour will not always reveal a small EST component. Furthermore, AFP seems to be synthesized by other elements than EST, such as some groups of cells in EC, and different epithelial cells in T (5). On the other hand, some tumours with a histologically recognizable EST pattern may not always synthesize or release AFP to such an extent that elevated levels can be demonstrated in blood, i.e. levels above 20 μg/l. By analysing pre- and postoperative samples from such patients in the same analytic batch it was possible to demonstrate higher values preoperatively than postoperatively in 14 patients with preoperative AFP values below 20 μg/l.

Elevated HCG levels are always found in pure CC tumours, and in about half of the patients with CC elements in the tumour. Also for HCG the correlation between morphology and function is therefore not perfect. Increased HCG levels in blood are found in many patients without typical trophoblast elements in the tumour. However, it has been demonstrated by immunohistology that some giant cells

also stain for HCG and other placental proteins, and therefore may be of trophoblast origin (11).

Immunohistologic examinations are not yet a routine in the DATECA Study, but such analysis should be able to give further information about the correlation between morphology and function.

AFP and HCG producing pure seminomas represent a special group of testicular tumours (Tables 1, 3). Significantly elevated serum AFP levels were found in about one per cent, and very careful histologic examination could not demonstrate any NS elements in the primary tumour. However, some of the patients later developed NS metastases, and AFP producing seminomas should therefore be classified and treated as NS patients. It should be added that we have seen three pure seminoma patients with a slightly, but persistently elevated AFP level of about 30 μg/l, but without apparent clinical disease. These patients are still under close observation, but without any treatment. Elevated HCG levels were found in about 10 per cent of all patients with pure seminoma. In most of these patients it was possible to demonstrate giant cells, but not typical CC elements. Elevated HCG levels may furthermore be seen in several other tumour forms without any trophoblastic elements. Slightly elevated levels of HCG in seminoma patients should therefore be interpreted with caution even after the use of the HCG specific assay. However, marker elevation per se, in seminoma patients, seems to indicate a poor prognosis (Fig. 4) in these patients, although the different patient materials have until now been too small for a definite conclusion (22).

The prognostic value of AFP and HCG determination in testicular tumours, with regard to survival, is somewhat difficult to evaluate, since about 90 per cent of the patients can be radically cured with the present highly active chemotherapy regimens. However, as shown in Table 5, marker profiles appear to be correlated to prognosis. In 141 NS patients with recurrence or residual disease 92 died from active disease, and 91 per cent of these patients were marker positive in pre- and postoperative samples, whereas in 49 patients who were alive, only 65 per cent were marker positive and of these patients with positive markers 7 still had residual disease. The prognostic significance of AFP and HCG is even more evident from Fig. 3, where marker status two or three weeks postoperatively for 146 NS patients stages II and III is correlated to three-year survival. Marker negative patients had a significantly better

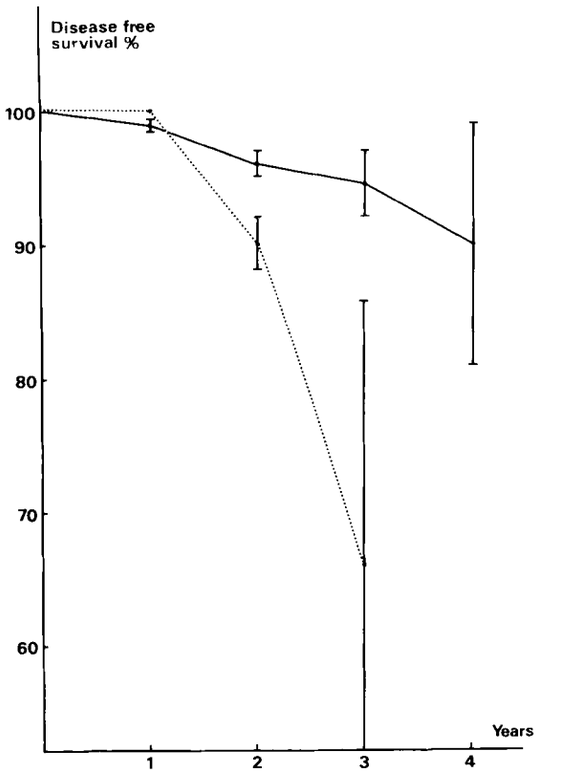

Fig. 4. Survival of seminoma, all stages, and preorchiectomy. HCG values. Marker negative (n=293 ---), marker positive (n=23 —).

prognosis than marker positive patients. Testicular tumours, like other germ cell tumours, imitate embryonal and fetal as well as extra-embryonal tissues, and may thus contain various cell populations with different functions. Some tumours may therefore produce AFP or HCG or both, and some may produce neither AFP nor HCG. Because of the cellular heterogeneity of these teratomas no complete assessment of tumour can be made from marker data alone. The markers are most often produced only by some of the tumour cells. This is the explanation for the following different marker patterns in germ cell tumours:

(1) A large group of patients are marker negative throughout the course of the disease.

(2) Another large group is marker positive either preoperatively and shortly after operation, or remains positive throughout the course of their disease.

(3) A small heterogeneous group of patients in whom considerable variation in marker pattern takes place with or without any relation to therapy.

(4) A few patients in whom marker estimations

are initially negative, but who subsequently develop elevated levels.

(5) A small group of patients showing a terminal rise in marker levels. These patients are otherwise marker negative throughout the course of the disease.

In order to evaluate and identify the different marker patterns a certain minimum number of blood samples needs to be taken from the individual patient. In the DATECA Study we have recommended that samples be taken preoperatively, and 3 and 7 days postoperatively. Furthermore, new samples are always taken on admission to the oncologic department 2 to 3 weeks after orchiectomy. This represents fewer samples than was recommended by an international panel in 1979 (12), but is enough for routine initial marker status. During the course of the disease, marker positive patients should be followed at weekly intervals until the marker levels are normal, whereas marker negative patients should be followed at least every month during the first year after orchiectomy.

In conclusion, the 5-year experience regarding AFP and HCG in the DATECA Study has shown that these markers are a valuable aid in the management of testicular malignancy, and contribute significantly to the improved treatment results. It should be especially underlined that some patients in clinical stage I have elevated marker levels, that increase in marker levels may be the first sign of recurrence, and that there is good accordance between marker pattern and clinical course in many patients.

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