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The Pattern of Gamma-glutamyl Transpeptidase, Alkaline Phosphatase, Serum Glutamyl Oxalate Transaminase and Serum Glutamyl Pyruvate Transaminase in Patients with Disseminated Non-seminomatous Testicular Tumors

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Abstract—*The serum enzyme activities of gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (AP), serum glutamyl oxalate transaminase (sGOT) and serum glutamyl pyruvate transaminase (sGPT) were determined longitudinally in 51 patients with a disseminated non-seminomatous testicular tumor. Elevated levels of one or more enzymes before chemotherapy were observed in 13 patients, all with stage III disease. If, after two cycles of chemotherapy, the established tumor markers alpha-fetoprotein (AFP), human chorionic-gonadotropin (HCG) and/or lactate dehydrogenase (LDH) were normalized, the initially increased enzyme activities were declined to normal values as well. Peaking concentrations of one or more of the tumor markers during induction chemotherapy, probably due to tumor cell lysis, were found in 34 of 45 marker-positive patients (76%). In addition, increases of one or more of the investigated enzyme activities were also noticed in 20 patients. In 76% of these patients the highest point of the tumor marker concentration coincided well with that of the enzyme activities. Indications are given that the peak activities were probably not caused by liver damage. Enzyme elevations were also found in 3 out of 7 patients with progressive disease. The behaviour of the enzyme activities of GGT, AP, sGOT and sGPT in patients with a disseminated non-seminomatous testicular tumor coincided with the known tumor markers. It favors the hypothesis that these enzymes are synthesized in the tumor. The mortality amongst stage III patients with or without initially raised GGT levels differed significantly ($P < 0.02$). Finally, it is concluded that in patients with a non-seminomatous testicular tumor, sGOT, sGPT, GGT and AP cannot be used to diagnose liver function.*

INTRODUCTION

DURING the last decade the prognostic value of biochemical data in cancer therapy has been discerned more and more. For non-seminomatous testicular tumors alpha-fetoprotein (AFP), human chorionic-gonadotropin (HCG) and serum lactate dehydrogenase (LDH) have been approved as markers of importance [1–9]. These markers are used in early detection of tumor growth, staging and in following the effect of chemotherapy. In 70–90% of the patients with a non-seminomatous testicular tumor one or more of these markers are elevated. A decrease of these tumor markers during chemotherapy usually indicates complete remission. The marker levels, however, also declined in about 50% of the patients with residual tumor [9]. Moreover, 10–30% of the patients have normal initial levels of AFP, HCG

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or LDH [1, 9, 10]. Therefore investigations for more markers are requested. We recently demonstrated that pregnancy-specific β_1-glycoprotein (SP_1), suggested as a potential marker [11, 12], is not useful as far as detection of tumor relapse after chemotherapy is concerned [13]. SP_1 may be a valuable marker, in addition to HCG, to detect choriocarcinomatous elements in non-seminomatous testicular cancer [14]. Carcino-embryonic antigen (CEA), alpha$_1$-antitrypsin (α_1AT) and alpha$_2$-macroglobulin (α_2M) have been explored, however, without success [15].

Previously, indications were given that alkaline phosphatase (AP) might be a marker for seminomatous testicular cancer [16]. Yokosama *et al.* reported the synthesis of gamma-glutamyl transpeptidase (GGT) by a yolk sac tumor [17]. Furthermore, GGT and/or AP are known as markers for several other human tumors, e.g. ovarian carcinoma [18], renal carcinoma [19] and malignant melanoma [20]. We therefore wondered whether AP and GGT might be useful as tumor markers in patients with a disseminated non-seminomatous testicular tumor. In addition, we investigated two other enzymes usually synthesized in the liver, *viz.* serum glutamyl oxalate transaminase (sGOT) and serum glutamyl pyruvate transaminase (sGPT). In this study we compared the course of these four enzymes and the known tumor markers before, during and after chemotherapy.

MATERIALS AND METHODS

Fifty-one patients with a non-seminomatous testicular tumor were included in this study. They were treated in the University Hospital of Groningen between January 1979 and June 1982. Sixteen patients had stage II and 35 stage III disease. The classification and determination of the stages have been published previously [13]. Liver metastases were excluded by means of CT scanning, isotope liver scanning and echography. Twenty-five stage III patients had lung metastases. Thirty patients (59%) were serum positive for AFP, 31 patients (61%) for HCG and 30 patients (59%) for LDH. Six patients (12%) had none of these tumor markers.

All patients were treated with four cycles of combination chemotherapy consisting of *cis*-platinum, vinblastine and bleomycin according to Einhorn and Donohue [21]. Complete remission was defined as complete disappearance of all evidence of tumor, including serum tumor markers, for a period of at least 3 months. Forty-four patients met these requirements. Seven had a partial remission, including 6 patients who died as a result of tumor progression. Blood samples were collected before and during (1–3 times a week) chemotherapy. AFP (normal levels <20 μg/l) and HCG (normal levels <4 μg/l) have been determined as published before [9]. LDH (normal levels <235 U/l), GGT (normal levels <65 U/l), AP (normal levels <120 U/l), sGOT (normal levels <40 U/l) and sGPT (normal levels <30 U/l) were determined with the SMAC (Sequential Multiple Analyser plus Computer) (Technicon, Perritown, NY, U.S.A.).

The statistical significances of different distributions of discrete variables were determined by the χ^2 test.

RESULTS

Enzyme levels before and after the first two cycles of chemotherapy

First we examined the activities of the enzymes GGT, AP, sGOT and sGPT before chemotherapy. One or more of the enzyme activities were elevated in 13 men with stage III disease (37%) and in none with stage II disease. This difference between the stages of disease is significant ($P < 0.02$). For the single enzyme activities only the number of patients with an elevated GGT was high enough for a significant difference ($P < 0.05$). Table 1 shows which of the investigated enzyme activities and which tumor markers were increased. In 9 patients the tumor markers as well as the elevated enzyme activities normalized within two cycles of chemotherapy, despite the presence of vital tumor in one of them (case 7). The other 4 patients had persistent tumor after two cycles of chemotherapy, as was concluded from the elevated marker levels. In 2 of the latter patients (cases 6 and 11) the initially elevated enzyme activities also remained high. Taking these data together, the initially elevated enzymes behave in a similar way as the known tumor markers in these patients.

Peaking levels of tumor markers and enzymes during chemotherapy

Considering the tumor markers in the whole group of 51 patients, a temporary elevation was found within the first 2 weeks of therapy in 34 of the 45 marker-positive patients (76%), 20 of the 30 patients with AFP (67%), 16 of the 31 patients with HCG (52%) and 21 of the 30 patients with LDH (70%) as marker. This sudden increase in marker level during the initial phase of therapy, followed by a rapid decline of concentration, should probably be attributed to tumor lysis [22, 23].

Surprisingly, in 20 of the 51 patients (39%) a temporary increase of one or more of the enzyme activities in the first 2 weeks of therapy was found as well (Table 2). Two patients had stage II disease

Table 1. The patients with increased activities of GGT, AP, sGOT and/or sGPT before chemotherapy (cases 1–13)

	Case	Established tumor markers AFP	HCG	LDH	Enzyme activities GGT	AP	sGOT	sGPT	Deceased
	1	+	+/+	+	+		+	+	+
	2	+	+	+				+	
	3	+	+	+	+	+			
	4	+	+	+	+	+			
	5	+	+	+	+	+			
	6	+/+		+/+	+/+				+
	7			+	+	+		+	+
	8				+				
	9		+	+		+			
	10	+	+	+	+	+	+	+	
	11	+/+		+/+	+/+	+/+			+
	12		+/+	+	+	+		+	+
	13	+	+	+		+			
n_{tot}	13	9	9	12	10	9	2	5	5

Elevated tumor markers and elevated enzyme activities are indicated with +. In those cases where the activities or marker concentrations remained high after two cycles of chemotherapy, a second + is given. All 13 patients had stage III disease.

Table 2. The patients with elevated peak activities of GGT, AP, sGOT and/or sGPT in the first 2 weeks *of therapy (cases 2–21)*

	Case	Established tumor markers AFP	HCG	LDH	Enzyme activities GGT	AP	sGOT	sGPT	Tumor stage II	III	Deceased
	2	P	P	+				P		+	
	3	+	+	P	P	P	P	P		+	
	4	P	P	P	P	P	P	P		+	
	5	P	P	P	P	P				+	
	6	P		+	+	P				+	+
	7			P	P	P		P		+	+
	8				P	P		P		+	
	9		P	P		P	P	P		+	
	10	P	P	P	P	P	+	P		+	
	11	P		P	P	P		P		+	+
	12		+	P	P	P		P		+	+
	13	P	+	P		P				+	
	14							P		+	
	15							P	+		
	16	P	+					P		+	
	17	P		P		P				+	
	18		P	P	P	P		P		+	
	19	P		P				P	+		
	20		P				P	P		+	
	21			P			P	P		+	
n_{tot}	20								2	18	4
n_P		10	7	13	9	13	5	16			

Peaks in tumor markers and/or enzyme activities are indicated with P. The tumor markers or enzyme activities which are elevated without any peak during therapy are indicated with +. The line n_P gives the total number of peaks in each column. Cases 2–13 are the same as in Table 1.

(2/16 = 13%) and 18 stage III disease (18/35 = 51%). This difference between both stages of disease is significant ($P < 0.02$). For the peaks of the marker concentrations no significant differences between the two stages of disease were found. Twelve of the 13 patients with increased enzyme activities before chemotherapy (cases 2–13) are also in this group of 20. It should be noticed that the enzyme activities do not fluctuate together in every case.

Seventeen of these 20 patients have tumor marker producing cells. Table 2 shows that these tumor markers have peaks in the first 2 weeks of chemotherapy as well. In 13 of these 17 patients (76%) the day of the highest concentration of the markers coincided well with the day of the highest enzyme activities. Figures 1 and 2 are illustrations of this. Figure 3 gives a divergent course of the markers and AP. If the rise in enzyme activities is

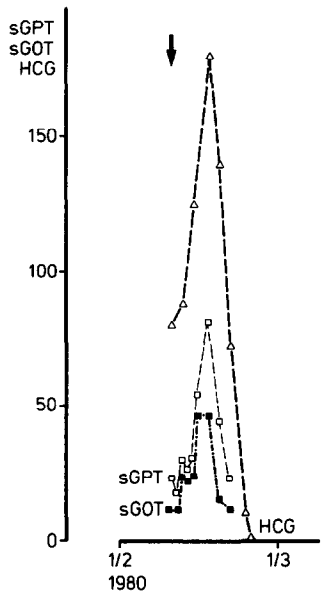

Fig. 1. Profiles of elevated enzyme activities (U/l) and tumor marker (μg/l) in one patient (case 20). The arrow indicates the start of chemotherapy. No elevated values were found during further therapy.

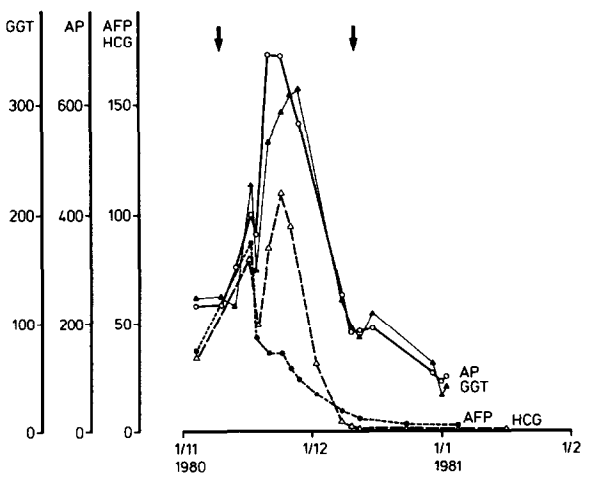

Fig. 2. Profiles of elevated enzyme activities and tumor markers in one patient (case 5) (explanation as in Fig. 1). The LDH profile has been omitted to keep a surveyable figure.

caused by liver damage as a consequence of therapy, one might expect a repeat at every cycle of drug administration. However, if the peaks are due to tumor cell death, a repeat has to be expected only occasionally, namely in cases of rest tumor. It was found that in 2 patients (cases 5 and 18) the activities of GGT and AP increased temporarily above normal values in the second cycle only. sGPT peaks were found in 3 patients (cases 14–16) in the second and third cycles but not in the fourth. An example is given in Fig. 4. No further peaks in enzyme activities were found. Also, peaks of AFP were found in the further treatments with chemotherapy in 6 patients (20%). For LDH no

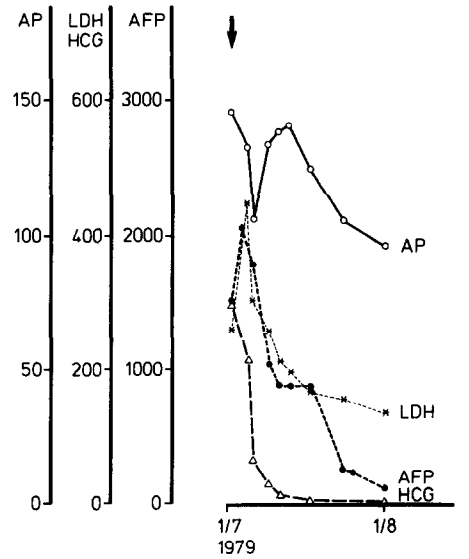

Fig. 3. Profiles of elevated enzyme activities and tumor markers in one patient (case 13) (explanation as in Fig. 1).

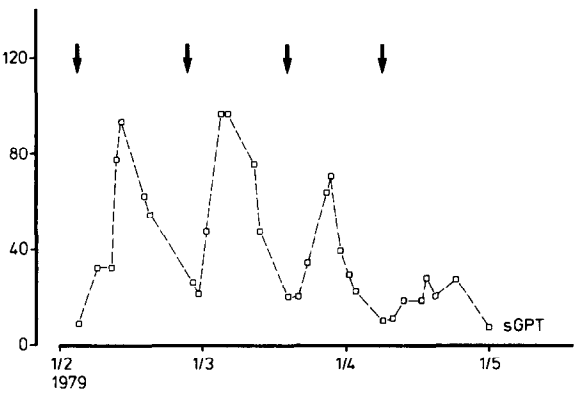

Fig. 4. Profile of sGPT in one patient (case 15) (explanation as in Fig. 1).

clear elevations were found in the last 3 cycles, probably since the activity had reached the normal range in most cases after one course. Only once was a clear reaction of the HCG concentration seen during further treatment. Hence there is a striking similarity between the course of the enzyme activities and the course of the tumor marker concentrations during chemotherapy.

Marker and enzyme levels in case of tumor progression

We next examined the level of enzyme activities during progressive tumor growth. As indicated in Table 1, 5 patients with initially elevated enzyme activities died. In 3 of the patients (cases 6, 7 and 11) GGT and AP activities and also the tumor markers increased later on and remained high (Fig. 5). In cases 1 and 12 only the HCG level increased.

In the group of 30 patients in whom increased enzyme activities were never found, 2 cases of tumor progression occurred. One patient had

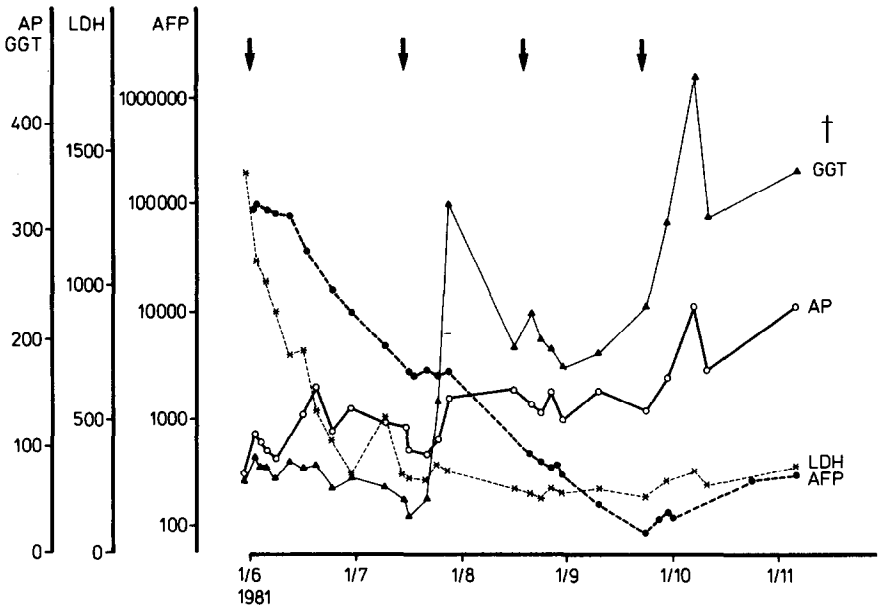

Fig. 5. Profiles of elevated enzyme activities and tumor markers in one patient (case 6) (explanation as in Fig. 1).

only an elevation of HCG at the end. In the other patient no increases of enzyme activities or tumor markers have been found.

Further aspects of the elevated enzyme activities

It is known that lung cancer cells are able to synthesize GGT and AP [24]. We wondered whether the observed GGT and AP elevations could be attributed to lung metastases. Nine of 11 patients with GGT elevations and 9 of 13 patients with AP elevations had lung metastases. These distributions are not significantly different from the group of stage III patients without lung metastases. Hence a direct relationship between the increased activities of the enzymes and lung metastases cannot be proven.

A striking phenomenon is that in 9 of 11 cases (82%) of GGT elevation the course of the enzyme activity is very similar to that of AP (Fig. 2). LDH is also elevated in these 9 patients; however, the fluctuations are less similar.

Eight of 9 patients with peaks in GGT activity during chemotherapy had an initially elevated GGT, whereas it was observed for sGPT that only 4 of 16 patients and for sGOT that none of 5 patients with rises during therapy showed initial elevations. This latter enzyme is always increased together with sGPT.

It should be noticed that the mortality of patients with an initially increased GGT activity was significantly higher than the other stage III patients ($P < 0.02$). Five of 10 patients died compared to 1 patient with progressive tumor growth and 1 deceased patient amongst the other 25 patients. For the other enzyme activities a similar phenomenon was not found.

Finally, no relation between elevated enzyme activities and histology was observed.

DISCUSSION

The results presented in this paper are suggestive for the hypothesis that non-seminomatous testicular tumors are able to produce in addition to LDH several enzymes which usually are predominantly synthesized in the liver. These enzymes are GGT, AP, sGOT and sGPT. The hypothesis is based on our observations that the serum levels of these enzymes behave similarly to the known tumor markers. Firstly, 37% of the patients with stage III disease have an elevated activity of one or more of the enzymes. After two cycles of chemotherapy, when tumor markers were normalized, these enzyme activities were decreased to normal values as well. Secondly, temporary increases in the enzyme activities after the first administration of chemotherapeutics were found in 51% of the patients with stage III disease. A similar phenomenon was found for the known tumor markers. Thirdly, in some cases of tumor progression after chemotherapy, elevations were found for enzyme activities as well as for tumor markers.

In our opinion the elevated serum enzyme activities cannot be due to synthesis in the liver. In our group of patients no liver metastases were found. Also, liver damage, for instance by chemotherapy, is not a plausible explanation since: (1) in 13 patients increased enzyme activities were found before chemotherapy; (2) the sudden rise in enzyme activity within the 2 weeks after the start of chemotherapy is not equally divided over both stages of disease ($P < 0.02$); (3) these rises in

activity were not often found in the second to fourth cycles of chemotherapy; (4) in some cases tumor progression coincided with elevated enzyme activities; (5) in the literature no studies concerning high incidence of liver toxicity have been reported for the drugs used in our study; (6) to our knowledge none of the patients with increased enzyme activities is or was an alcoholic; (7) during the period of this study none of the patients received drugs that might induce the GGT activity.

It should be emphasized that based on our data, GGT, AP, sGOT and sGPT cannot be used to diagnose liver function in patients with a non-seminomatous testicular tumor.

As mentioned above, the temporary rise in tumor marker concentration during therapy is supposed to be due to tumor cell lysis [22, 23]. We hypothesize that the same holds true for the enzyme activities. In this respect some phenomena are striking. The fact that the course of the enzyme GGT activity is very similar to that of AP suggests that these enzymes are synthesized in the same cells. The same can be hypothesized for sGOT and sGPT.

Since most of the elevations of these latter enzymes were found during chemotherapy, we suggest that sGOT and sGPT are not usually secreted from the tumor cells, in contrast to GGT, AP and the tumor markers. During chemotherapy the cancer cells will disintegrate, resulting in a release of cell content.

Yokosama and co-workers [17] gave indications for the production of GGT in a yolk sac tumor. Moreover, Benham *et al.* [25] found that 7 of their 8 cell lines of testicular teratocarcinoma synthesize high amounts of AP. It has been reported that even the normal testicle produces AP, although the amounts are very low [26]. The synthesis by testicular germ cell tumors of proteins predominantly produced by the liver is not unexpected. The production of AFP and LDH by these tumors are examples of the same phenomenon. Probably the ability for synthesis is due to the common embryonic origin. Germ cells as well as liver cells are derived from the yolk sac.

Finally, one may wonder whether the enzymes may be useful as tumor markers for non-seminomatous testicular tumors. For this purpose those cases in which the increased enzyme activities give more information than the markers AFP, HCG and LDH are important. This was found in only 1 of 51 patients. Here the absence of the common tumor markers was accompanied by the presence of an initially elevated GGT activity (Table 1, case 8). In this patient the decrease of GGT activity to a normal value indeed seemed to be accompanied by the disappearance of tumor. However, as shown above, the normalization of increased enzyme activities does not imply a warranty for tumor regression (cases 7, 10 and 12). Putting these data together, we conclude that the enzyme activities of GGT, AP, sGOT or sGPT may have only a limited value in diagnosis of patients with non-seminomatous testicular tumors. Maybe isoenzyme patterns of GGT and AP will improve the usefulness of these enzymes since the determinations are probably more sensitive. Extra enzyme bands might also be found in cases in which no elevations of enzyme activity have been noticed (e.g. high normal levels). Furthermore, isoenzyme profiles could further prove that part of the (increased) enzyme activities are due to synthesis in the tumor. To this end the isoenzyme patterns of GGT and AP are currently under investigation in our laboratory.

REFERENCES

1. SCARDINO PT, COX HD, WALDMANN TA, MCINTIRE KR, MITTEMEIJER B, JAVADPOUR N. The value of serum tumor markers in the staging and prognosis of germ cell tumors of the testis. *J Urol* 1977, **118**, 994–999.
2. SCHULTZ H, SELL A, NØRGAARD-PEDERSON B, ARENDS J. Serum alphafetoprotein and human chorionic gonadotropin as markers for the effect of postoperative radiation and/or chemotherapy. *Cancer* 1978, **42**, 2182–2186.
3. EDLER VON EYBEN F. Biochemical markers in advanced testicular tumors. *Cancer* 1978, **41**, 648–652.
4. THOMPSON DK, HADDOW JE. Serial monitoring of serum alphafetoprotein and chorionic gonadotropin in males with germ cell tumors. *Cancer* 1979, **43**, 1820–1825.
5. JAVADPOUR N. The value of biological markers in diagnosis and treatment of testicular cancer. *Semin Oncol* 1979, **6**, 37–47.
6. LIPPERT MC, JAVADPOUR N. Lactate dehydrogenase in the monitoring and prognosis of testicular cancer. *Cancer* 1981, **48**, 2274–2278.
7. NARAYANA AS, LOENONG S, WEIMAR G, CULP DO. Serum markers in testicular tumors. *J Urol* 1979, **121**, 51–53.
8. BOSL GJ, LANGE PH, NOCHOMOVITS LE *et al.* Tumor markers in advanced non-seminomatous testicular cancer. *Cancer* 1981, **47**, 572–576.

9. WILLEMSE PHB, SLEIJFER DTH, SCHRAFFORDT KOOPS H *et al.* Tumor markers in patients with non-seminomatous germ cell tumors of the testis. *Oncodev Biol Med* 1981, **2**, 117–128.
10. FRALEY EE, LANGE PH, KENNEDY BJ. Germ cell testicular cancer in adults. *N Engl J Med* 1979, **301**, 1370–1377.
11. LANGE PH, BREMMER RD, HORNE CHW, VESSELLA RL, FRALEY EE. Is SP-1 a marker for testicular cancer? *Urology* 1980, **15**, 251–255.
12. SZYMENDERA JJ, ZBORZIL J, SIKOROWA L, KAMINSKA JA. Value of five tumor markers (AFP, CEA, HCG, HPL and SP-1) in diagnosis and staging of testicular germ cell tumors. *Oncology* 1981, **38**, 222–229.
13. DE BRUIJN HWA, SUURMEIJER AJH, SLEIJFER DTH *et al.* Evaluation of pregnancy specific β_1-glycoprotein in patients with nonseminomatous testicular germ cell tumors. *Eur J Cancer Clin Oncol* 1982, **18**, 911–916.
14. SUURMEIJER AJH, DE BRUIJN HWA, OOSTERHUIS JW, SLEIJFER DTH, SCHRAFFORDT KOOPS H, FLEUREN GJ. Non-seminomatous germ cell tumors of the testis. Immunohistochemical localization and serum levels of human chorionic gonadotropin (HCG) and pregnancy-specific beta-1 glycoprotein (SP-1): value of SP-1 as a tumor marker. *Oncodev Biol Med* 1982, **3**, 409–422.
15. MARRINK J, WILLEMSE PHB, SLEIJFER DTH *et al.* AFP, HCG, α_1AT, α_2M, CEA and SP-1 profiles in patients with nonseminomatous testicular tumors. *Oncodev Biol Med* 1981, **2**, 200 (Abstr.).
16. LANGE PH, MILLAN JL, STIGBRAND T, VESSELLA RL, ROUSLAHTI E, FISHMAN WH. Placental alkaline phosphatase as a tumor marker for seminoma. *Cancer* 1982, **42**, 3244–3247.
17. YOKOSAMA N, TANIGUSHI N, TSUKADA Y, MAKATI A. Physicochemical and immunochemical characterisation of gamma-glutamyl transpeptidase from yolk sac tumor and ascites hepatoma (AH-66) cells. *Oncodev Biol Med* 1981, **2**, 165–177.
18. SKILLEN AW, HARRISON J, GUTHRIE D, TURNER GA. Serum enzyme changes during chemotherapy for ovarian cancer. *Clin Biochem* 1982, **15**, 41–45.
19. HADA T, HIGASHINO K, YAMAMOTO H, OKOCHI T, YAMAMURA Y. Phenotypic changes of alkaline phosphatase in association with the novel gamma-glutamyl transpeptidase in renal cell carcinomas. In: LEHMANN F-G, ed. *Carcino-embryonic Proteins.* Amsterdam, Elsevier, 1979, Vol. 2, 689–692.
20. MURRAY JL, LERNER MP, NORDQUIST RE. Elevated gamma-glutamyl transpeptidase levels in malignant melanoma. *Cancer* 1982, **49**, 1439–1443.
21. EINHORN LH, DONOHUE J. *Cis*-diammine-dichloroplatinum, vinblastine and bleomycin combination chemotherapy in disseminated testicular cancer. *Ann Intern Med* 1977, **87**, 293–298.
22. VOGELZANG NJ, LANGE PH, BOSL GJ, FRALEY EE, JOHNSEN K, KENNEDY BJ. Paradoxal tumor-marker elevations during induction chemotherapy for testicular tumor. *Proc Am Assoc Cancer Res* 1980, **21**, 431.
23. VOGELZANG NJ, LANGE PH, GOLDMAN A, VESSELLA RH, FRALEY EE, KENNEDY BJ. Acute changes of α-fetoprotein and human chorionic gonadotropin during induction chemotherapy of germ cell tumors. *Cancer Res* 1982, **42**, 4855–4861.
24. DEMPO K, ELLIOTT KAC, DESMOND W, FISHMAN WH. Demonstration of gamma-glutamyl transpeptidase, alkaline phosphatase, CEA and HCG in human lung cancer. *Oncodev Biol Med* 1981, **2**, 21–38.
25. BENHAM FJ, ANDREWS PW, KNOWLES BB, BRONSON DL, HARRIS H. Alkaline phosphatase isoenzymes as possible markers of differentiation in human testicular teratocarcinoma cell lines. *Dev Biol* 1981, **88**, 279–287.
26. GOLDSTEIN DJ, ROGERS C, HARRIS H. A search for trace expression of placental-like alkaline phosphatase in non-malignant human tissues: demonstration of its occurrence in lung, cervix, testis and thymus. *Clin Chim Acta* 1982, **125**, 63–75.