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Urological Research

Simultaneous Determination of Six Tumor Markers in Patients With Prostatic Carcinoma and Bladder Tumors

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Summary. Serum levels of fucosyltransferase (FT), phosphohexoseisomerase (PHI), tissue polypeptide antigen (TPA), Tennessee antigen (TAG), carcinoembryonic antigen (CEA) and prostatic acid phosphate (PAP) were determined in 75 healthy individuals and in 86 patients with prostatic carcinoma and 38 patients with bladder tumors. The discrimination capacities of the different markers were compared by using inverse distribution plots. At a rate of 5% false positive values the sensitivities for bladder tumors were: FT 30%, TPA 24%, CEA 16%, TAG 15%. The sensitivities for prostatic carcinoma were: PAP 63%, PHI 36%, TPA 18%, CEA 14%, TAG 14%.

Key words: Tumor marker, Prostatic carcinoma, Bladder carcinoma.

Introduction

Tumor markers are potentially useful as screening tests for cancer, to monitor the course of disease and to detect relapse. Future aspects include targeting isotopes to localize tumors and directing therapeutic agents. The term tumor marker refers to normal body constituents which undergo quantitative or qualitative alterations during tumor growth. Another group includes substances that appear de novo during cancer growth, such as oncofetal antigens or tumor associated antigens. Despite the wealth of reports on tumor markers, there are only few standarized comparisons of markers in different types of cancer and of different markers in a single tumor type. Usually, the marker has a particular serum level compared with controls in patients with a particular type of tumor. The sensitivity (% test positive among true positives) of markers should only be compared by considering identical false positive rates. Since authors use different serum levels for their normal values, it is impossible to compare their results. This study was undertaken to give a standardized comparison of six tumor markers in patients with prostatic carcinoma and with bladder tumors.

Material and Methods

Assays. At least two fucosyltransferases (FT) have been identified in plasma. FT_1 catalyzes transfer of fucose onto n-acetylglycosamine residues. FT_2 transfers fucose onto terminal galactose residues of desialated fetuin or analogous acceptors. In our study we measured FT_2 according to the method of Chou [1] with minor modifications. A 210 µl volume contained 50 µl plasma, 10 mM $MgCl_2$, 10 mM EGTA, 10 µl GDP-(^{14}C) fucose (0.5 nmol), 50 mM cacodylate buffer, ph 7.0 and 0.5 mg of a fetuin-derived fucose acceptor. N-ethylmaleimide, a specific inhibitor of FT_2 was added to duplicate samples. The level of this transferase could be calculated by the difference, thereby correcting for the action of other fucosyltransferases on endogenous acceptors. Incubation was at 37° for 60 min. The product was separated from the radioactive precursor on columns of Dowex 1 (OH). Results were calculated in terms of activity units = cpm of radioactivity incorporated into product per 50 µl of plasma.

Phosphohexoseisomerase (PHI) was assayed using a kit supplied by Behringwerke, Marburg, Germany (Testomar^R-PHI). Prostatic acid phosphatase (PAP) was measured by using a radioimmunoassay kit supplied by NEN Chemicals, Frankfurt, Germany. Carcinoembryonic antigen (CEA) was determined by an Enzyme-Immuno-Assay (EIA), Abbot. Tissue polypeptide antigen (TPA) was measured by a radioimmuno assay obtained from Sangtec Medical, Sweden. For Tennessee antigen (TAG) determination a haemaglutination-inhibition method was employed. The reagents were supplied by Oxford Lancer.

Subjects. The control group consisted of 75 men, aged 24 to 89 years, without evidence of malignant disease. 86 patients with prostatic carcinoma, aged 48 to 86 years, were included in the study. 28 patients were previously untreated, and 58 patients had undergone high voltage radiation therapy or were receiving conventional hormone therapy. Patients were grouped as follows: stage B (n = 23), stage C (n = 17), stage D (n = 46). 38 patients with bladder tumors were also studied. The different tumor stages were grouped according to the UICC as follows: T_A (n = 8), T_1 (n = 13), $T_{2,3,4}$ (n = 17). 4 patients had G_1-, 17 G_2- and 17 G_3-tumors.

Treatment of Data. As recommended by Oehr [2] we used inverse distribution plots to compare data of controls and patients with prostatic carcinoma or bladder tumors. From these plots information on the specifity and sensitivity of the tests can easily be obtained.

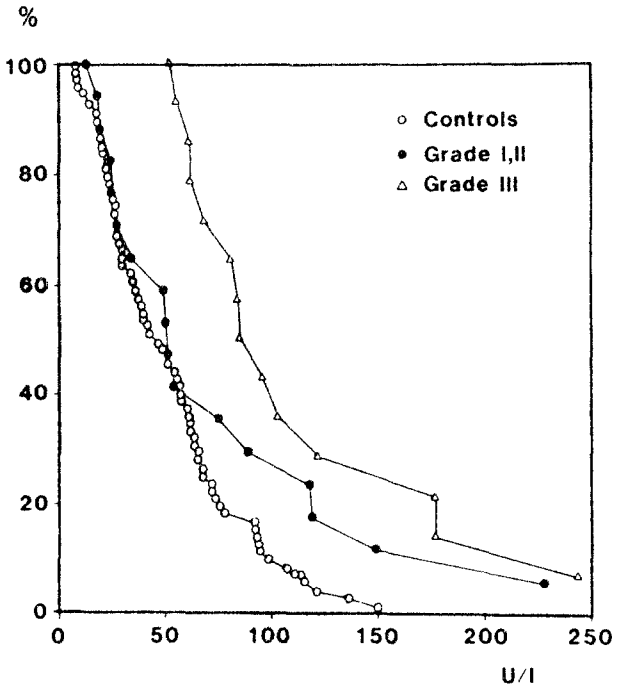

Fig. 1. Inverse distribution function of FT_2 concentrations in sera of patients with bladder carcinoma (Grade I, II: n = 17; Grade III: n = 14) and controls (n = 67)

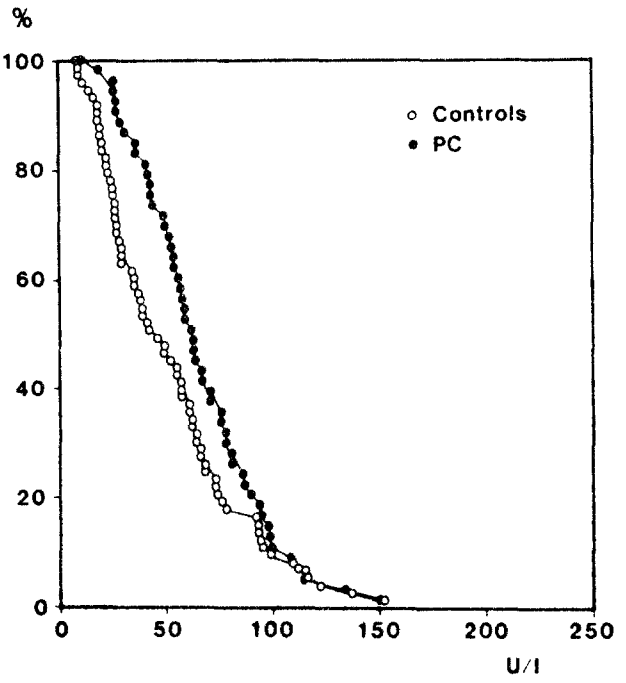

Fig. 2. Inverse distribution function of FT_2 concentrations in sera of patients with prostatic carcinoma, stage B, C, D (n = 52) and controls (n = 75)

Results

Figure 1 shows the distribution of the results on FT_2 in an inverse distribution plot. FT_2 concentrations in patients with bladder tumors were elevated as compared to controls. Moreover, patients, with poorly differentiated tumors had considerably higher FT_2 levels than patients with well differentiated tumors. The sensitivity of FT_2 for bladder carcinoma was 30% at a 5% false positive rate. Figure 2 represents the data on FT_2 for patients with prostatic carcinoma. The distribution curve of patients with prostatic carcinoma is similar to that of controls. Thus, in contrast to the high discriminatory power of FT_2 in bladder tumors FT_2 determination seems to offer no additional information in patients with prostatic carcinoma.

The sensitivity of PHI for prostatic carcinoma was 36% (Fig. 3). The graph for treated patients lies well below the curve which represents untreated patients. In Fig. 4 the inverse distribution of PHI for patients with carcinoma of the urinary bladder and controls is depicted. The graphs for both groups were almost identical. This indicated a very low discriminatory efficiency of PHI for bladder tumors.

Figure 5 compares the values of TPA obtained from patients with bladder tumors and prostatic carcinoma. The graph for patients with bladder tumors lies above the graph for patients with prostatic carcinoma. The sensitivity at 5% false positive rate was 24% for bladder tumors and 18% for prostatic carcinoma.

Table 1 summarizes the results of carcinoembryonic antigen (CEA) and Tennessee antigen (TAG) determinations.

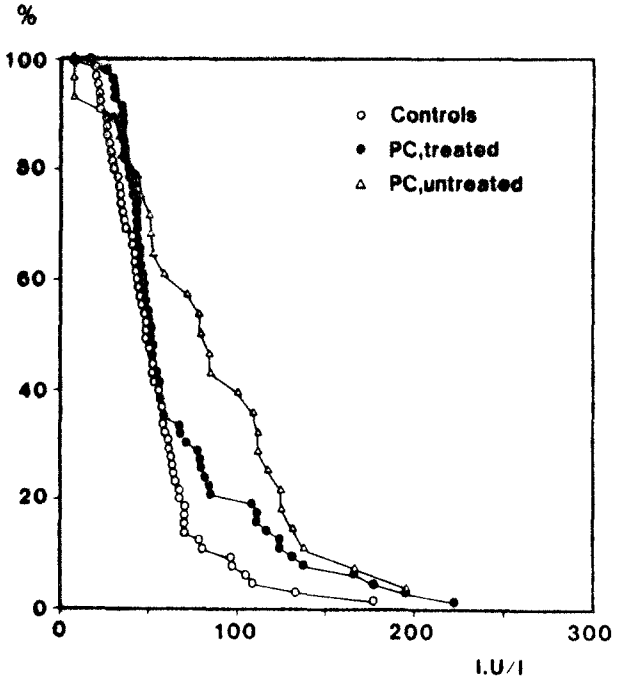

Fig. 3. Inverse distribution function of PHI concentrations in sera of patients with untreated (n = 28) and treated (n = 58) prostatic carcinoma and controls (n = 62)

Both, TAG and CEA were less sensitive for both cancer sites than TPA.

As expected, prostatic acid phosphatase (PAP) discriminated between controls and patients with prostatic carcinoma. The sensitivity was 63%.

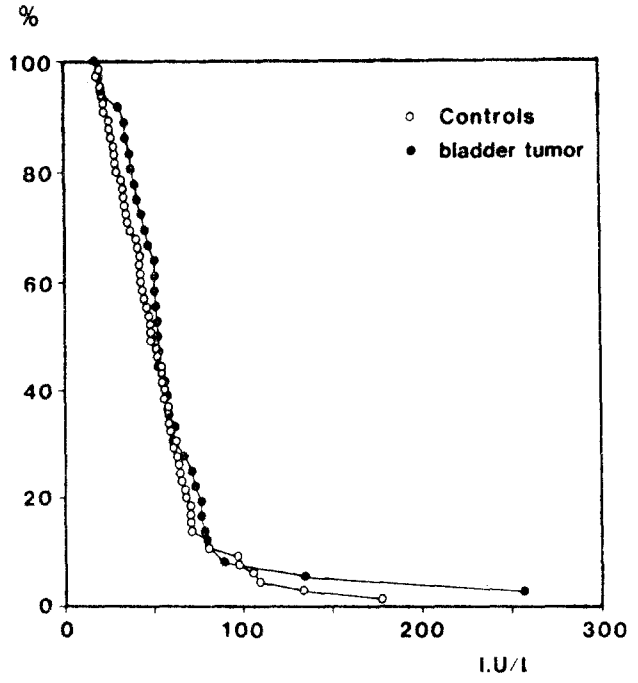

Fig. 4. Inverse distribution function of PHI concentrations in sera of patients with bladder carcinoma (n = 38) and controls (n = 63)

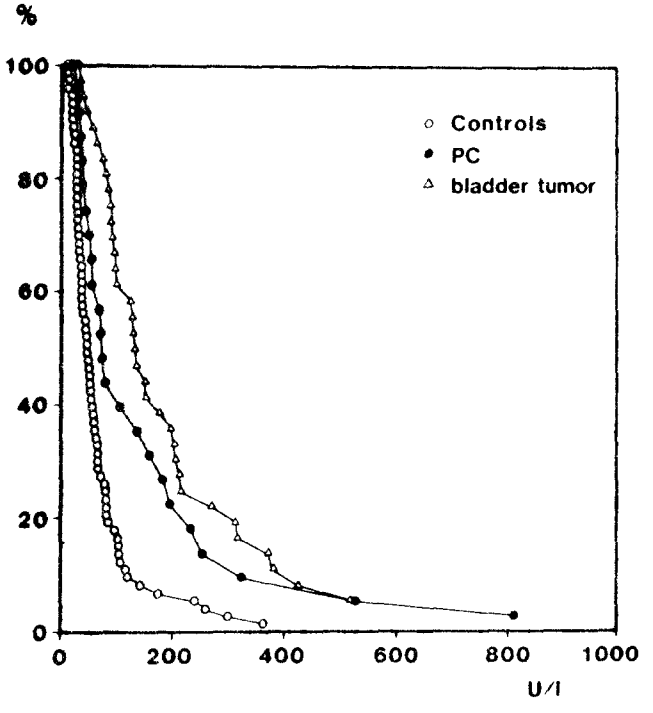

Fig. 5. Inverse distribution function of TPA concentrations in sera of patients with prostatic carcinoma (n = 23) and patients with bladder carcinoma (n = 38) and controls (n = 73)

The most sensitive markers for bladder tumors were FT_2 and TPA. Thus, it seems reasonable to combine these two markers to increase diagnostic accuracy. In Fig. 6 the inverse distribution for the product values of FT_2 and TPA is plotted. The sensitivity of the combined markers was 40% at 5% false positive rate. Thus, the simultaneous determination of FT_2 and TPA is superior to single determinations of either FT_2 or TPA. The highest capacity to discriminate between patients with prostatic carcinoma and controls was by PAP and PHI. The marker product values (PAP x PHI) were calculated, but the sensitivity of the combined markers was not superior to the sensitivity of single PAP determination.

Table 1. Sensitivity of CEA and TAG for prostatic carcinoma and carcinoma of the urinary bladder at 5% false positive rate

	prostatic carcinoma n = 28	bladder carcinoma n = 38
CEA	14%	16%
TAG	14%	15%

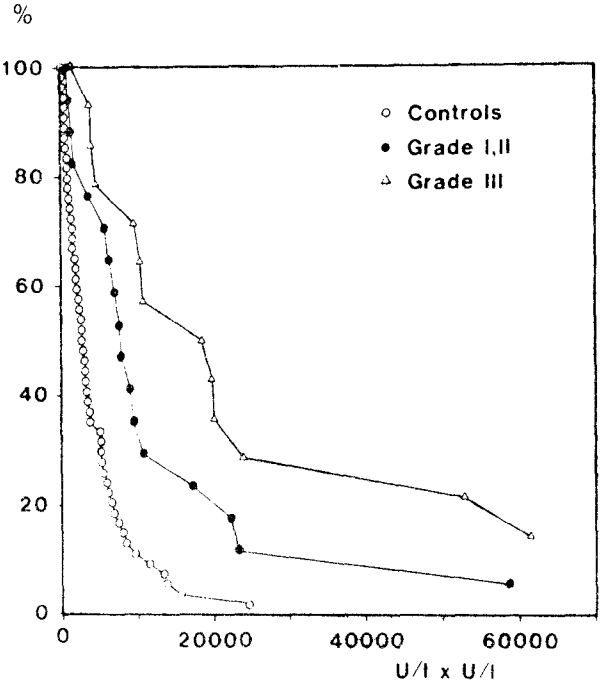

Fig. 6. Inverse distribution for the product values of TPA x FT_2 from serum determinations of patients with bladder carcinoma (Grade I, II: n = 17; Grade III: n = 13) and controls (n = 54)

Discussion

The fucosyltransferases are enzymes which catalyze the transfer of fucose from guanosine diphosphate (GDP)-fucose onto appropriate glycoprotein receptors. Elevated levels of plasma fucosyltransferases have been found in tumor bearing animals [3] and patients with myelogenous leukemias [4]. The observed elevation may be related to shedding of membrane components into plasma since many glycosyltransferases are associated with cell-surface components. Among the markers studied FT_2 exhibited the

highest sensitivity for bladder tumors. Moreover, the levels of FT_2 in patients with poorly differentiated tumors were higher than in patients with well differentiated carcinomas. Interestingly, experimental work has directly related the shedding of glycocalyx constituents to the growth properties and metastatic behaviour of tumors [5]. Therefore, the further study of FT_2 as a marker of carcinoma of the urinary bladder seems to be warranted.

PHI is a glycolytic enzyme which catalyzes the conversion of glucose-6-phosphate into fructose-6-phosphate. Bodansky [6] found PHI useful in monitoring the evolution of malignant breast disease. From Bodansky's studies it would seem that serum levels of PHI reflect a combination of tumor mass and tumor metabolic activity. This corresponds to our observation of reduced PHI-levels in treated patients with prostatic carcinoma as compared to PHI-levels in previously untreated patients. Besides, Schwartz [7] reported on rises in serum PHI-levels during episodes of clinical worsening in patients with advanced prostatic carcinoma. We therefore believe that PHI determination can provide additional information besides standard PAP determiniation in monitoring prostatic cancer. It may produce supportive evidence as to the effectiveness of palliative treatment in advanced prostatic cancer and may provide a biochemical estimate of the extent of tumor mass.

TPA is a membrane-bound polypeptide which is shed into the circulation by cancerous tissue of different origins. In agreement with the study of Huber and co-workers [8], the sensitivity of TPA for prostatic carcinoma was low. TPA is inferior to PHI for monitoring prostatic cancer. In patients with bladder tumors TPA levels were more often elevated than in patients with prostatic carcinoma. The sensitivity of TPA was lower than that reported by Oehr [9, 10]. Our finding of lower TPA levels might be explained in part by the lower rate of advanced tumors in our group.

TAG was first described by Potter and Jordan [11]. It is mainly associated with carcinoma of the colorectal and gastrointestinal tract [12]. In our study the percentage of raised TAG values in bladder carcinoma is considerably less than published values [9]. The sensitivity for prostatic carcinoma was even smaller. Thus, in our hands TAG estimation in patients with prostatic or bladder cancer is not clinically useful.

AT present, CEA is the most widely studied tumor associated antigen. Sequential assay of CEA in plasma has been proved to be a valuable method of detecting patients with recurrent or metastatic cancer of the colorectal tract and carcinoma of the breast [13]. As far as serum CEA measurement in patients with bladder tumors is concerned, some investigators do not consider this test to be of any value [14]. Other authors conclude that elevated serum levels occur only in advanced disease [15]. In our study the sensitivity of CEA for bladder tumors was 16% at 5% false positive rate and therefore we consider serum CEA determinations to be of limited value in monitoring patients with bladder cancer.

In conclusion, the most promising markers for bladder carcinoma seem to be FT_2 and TPA. For monitoring prostatic carcinoma PHI and standard PAP determinations should be further evaluated. Additional studies are needed to determine the prognostic relevance of the different markers.

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