Acta Radiologica Oncology 22 (1983) Fasc. 3

FROM THE DEPARTMENT OF ONCOLOGY, THE ONCOLOGIC CENTER, AND THE DEPARTMENT OF CLINICAL CHEMISTRY, UNIVERSITY HOSPITAL, S-90185 UMEÅ, AND THE DEPARTMENTS OF MICROBIOLOGY, BACTERIOLOGY AND PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF UMEÅ, S-90187 UMEÅ, SWEDEN.

TUMOR MARKERS IN BRONCHOGENIC CARCINOMA

An evaluation of carcinoembryonic antigen, tissue polypeptide antigen, placental alkaline phosphatase and pseudouridine

T. RASMUSON, G. R. BJÖRK, L. DAMBER, S. E. HOLM, L. JACOBSSON, A. JEPPSSON, T. STIGBRAND and G. WESTMAN

For some malignant diseases biologic markers can serve as tools in diagnosis and also to monitor the effect of treatment. Biologic markers can also be useful as prognostic parameters (SUGARBAKER et coll. 1982). In order to demonstrate the possible diagnostic and prognostic value 4 different biologic markers were analysed in patients with bronchogenic carcinoma. Carcinoembryonic antigen (CEA) has been thoroughly investigated as a marker for malignant diseases including bronchogenic carcinoma. However, its prognostic value for this disease has been questioned (CONCANNON et coll. 1974, VINCENT et coll. 1975). For tissue polypeptide antigen (TPA), elevated levels were found in patients with bronchogenic carcinoma (MENENDEZ-BOTET et coll. 1978). Placental alkaline phosphatase (PLAP, Regan isoenzyme) was first demonstrated in a patient with bronchogenic carcinoma (FISHMAN et coll. 1968). An increased excretion of pseudouridine, which is a degradation product of transfer-ribonucleic acid (t-RNA), has also been demonstrated in urine from patients with bronchogenic carcinoma (WAALKES et coll. 1975). In small cell carcinoma of the lung elevated levels of modified nucleosides have been correlated to poor prognosis (WAALKES et coll. 1982).

The aim of this investigation was to evaluate the 4 mentioned biologic markers with regard to their diagnostic value and their usefulness in control and treatment of patients in different clinical stages.

Material and Methods

Reference material. A reference group of 80 healthy adults was chosen with even distribution according to sex in 4 different age groups, 15–30, 31–45, 45–60 and above 60 years.

Clinical material. Sixty-two patients (57 men, 5 women) with primary bronchogenic carcinoma were chosen among those admitted to the Oncologic department at the University hospital. The patients were primarily examined at the departments for pulmonary diseases in the region, and operable patients were excluded. The diagnoses were based upon histologic examination in 43 cases, and in 19 upon cytologic examination. The median age at admission was 67 years, varying from 42 to 83 years. Nine patients had previous surgical intervention with exploration, lobectomy or pulmectomy. Subdivided in histologic types, 36 patients had squamous cell carcinoma, 13 patients had undifferentiated carcinoma of small cell type (oat cell), 8 had adenocarcinoma, 4 large cell anaplastic carcinoma and one had a pulmonary blastoma.

Samples of serum and urine were collected with an interval of 1 to 3 months and sampling within 2 weeks after cytostatic therapy was avoided. From 55 of the patients specimens could be collected before treatment was initiated. A total of 170 samples

Accepted for publication 3 March 1983.

Table 1

Biologic markers in normal adults

Marker	n	Mean	Median	Frequency of elevated levels	Per cent	Reference level, literature data	Units	Body fluid
CEA	80	–	–	3/80	4	2.5	µg/l	Plasma
TPA	70	86	62	5/70	7	180*	U/l	Serum
PLAP	79	0.05	0.03	2/79	3	0.2*	µmol/min × l	Serum
Pseudo-uridine	80	23.5	21	3/80	4	35*	nmol/µmol of creatinine	Urine

* Own results.

Table 2

Frequency of samples with elevated levels of biologic markers before treatment

Clinical stage	CEA		TPA		PLAP		Pseudouridine	
	n	Per cent	n	Per cent	n	Per cent	n	Per cent
1	0/1	–	0/1	–	0/1	–	0/1	–
2	14/39	36	9/32	28	4/36	11	5/37	14
3	8/13	62	3/10	30	1/14	7	4/13	31
Total	22/53	42	12/43	28	5/51	10	9/51	18
Reference group		4		7		3		4

were collected, giving an average of 2.7 samples per patient (range 1–10). At each observation the patients were examined and allocated into 3 different clinical stages, depending upon the extension of the disease: (1) no evidence of disease, (2) loco-regional disease and (3) generalized disease with infiltration of the pleura, the contralateral lung or with distant metastases.

From the stored samples the analyses of CEA were performed with commercial kits from Hoffmann-La Roche (HANSEN et coll. 1974, HAAGENSEN et coll. 1978). Determination of TPA was performed with a radioimmunoassay with reagents purchased from Sangtec Medical Co. (Bromma, Sweden). The method is described by WIKLUND et coll. (1979). The level of PLAP was determined with an enzymatic reaction and quantified by spectrophotometry at 505 nm at pH 9.8 as described by STIGBRAND et coll. (1983).

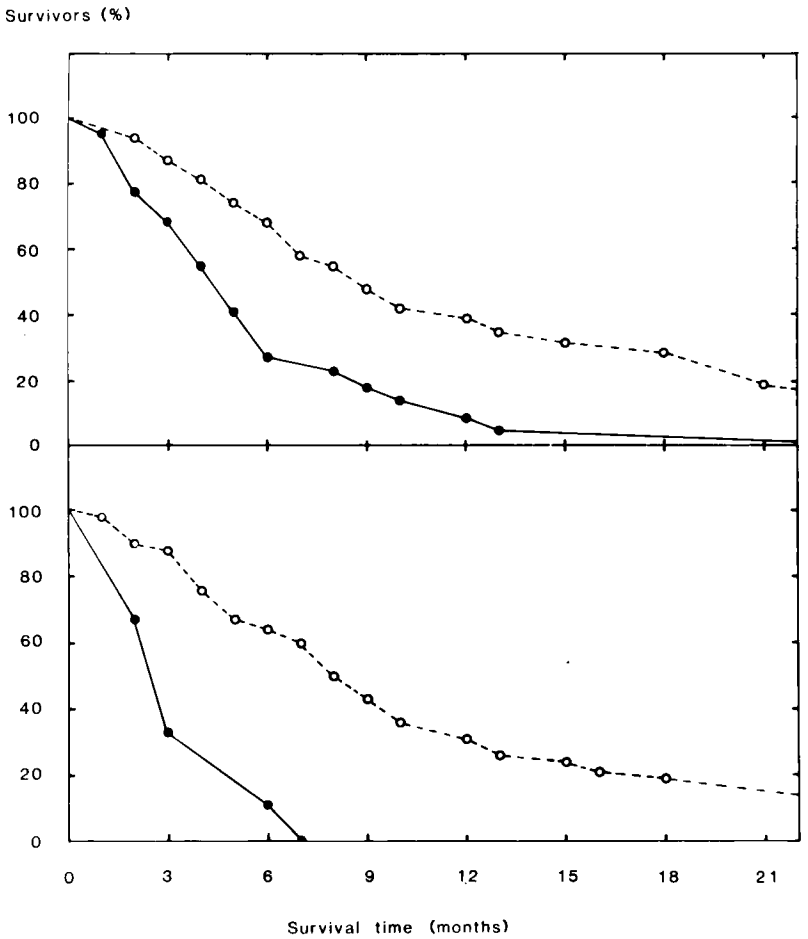
In order to determine the concentration of pseudouridine, the urine samples were divided into 2 aliquots and the amount of creatinine was analysed immediately according to the Technicon procedure. The rest of the urine was stored at −80°C until processed. The analysis of pseudouridine was performed according to GEHRKE et coll. (1978) and KUO et coll. (1978). After initial purification on a boronate column the eluate was separated using high performance liquid chromatography (HPLC). The quantification was based upon UV-absorbance at 254 and 280 nm.

The level of pseudouridine was then correlated to the level of creatinine in the sample.

The statistical analysis of differences in survival was performed with the Mann-Whitney test (DANIEL 1978).

Results

Reference material. Table 1 shows the levels of CEA, TPA, PLAP and pseudouridine from 80 healthy adults. The frequency of samples with elevated values for CEA was 3/80 which is in accordance with literature data, and these 3 individuals were all above 60 years of age (HANSEN et coll. 1974). For TPA 21 per cent of the healthy individuals had levels above 90 U/l, which is the reference



Actuarial survival for 22 patients with elevated (●), and 31 with ordinary levels (○) of CEA before treatment (upper graph) and for 9 patients with elevated (●), and 42 with ordinary levels (○) of pseudouridine before treatment (lower graph).

level according to literature data (BJÖRKLUND 1980). For this reason a higher limit of 180 U/l was chosen as reference in this investigation. With this elevated limit still 7 per cent of the individuals had elevated values. The reference levels of PLAP in serum and pseudouridine in urine were based upon the present material and set to 0.2 μmol/min × liter, and 35 nmol/μmol of creatinine, respectively. For PLAP and pseudouridine no variation with age group could be found.

Clinical material. Table 2 demonstrates data obtained from patients before radiation therapy or cytostatic treatment was initiated. There was no increased frequency of elevated values observed for TPA and PLAP with more advanced disease. For CEA the frequency of elevated values increased parallel to the clinical stage and the same tendency was demonstrated for pseudouridine. Taking all clinical stages together 42 per cent of the CEA samples and 18 per cent of the pseudouridine samples had elevated levels.

Table 3 demonstrates the total amount of samples analysed, i.e. also samples taken after treatment. The same qualitative and quantitative results were obtained as with samples taken only before treatment (cf. Table 2). Monitoring the levels of the different biologic markers for individual patients did not demonstrate any significant decrease of the level. Thus it can be concluded that the treatment does not have any greater influence on the frequency of elevated levels of these markers.

The results are based upon samples from patients with different histologic types of bronchogenic carcinoma. When the samples are subdivided into different histologic diagnoses, the results for TPA and PLAP were the same independent of the histologic type of tumor. For CEA, on the other hand, there is a higher proportion of elevated values seen in pa-

Table 3

Frequency of samples with elevated levels of biologic markers before and after treatment

Clinical stage	CEA		TPA		PLAP		Pseudouridine	
	n	Per cent	n	Per cent	n	Per cent	n	Per cent
1	3/22	14	6/17	35	3/20	15	1/21	5
2	28/102	27	26/81	32	14/96	14	14/93	15
3	22/44	50	11/35	31	1/41	2	9/40	22
Total	53/168	32	43/133	32	18/157	11	24/154	16

Table 4

Survival data (months) for patients with bronchogenic carcinoma and their levels of biologic markers before treatment

Marker	Number of patients	Survival			Patients still alive
		Mean	Median	Min–max	
CEA					
Ordinary level	31	13.7	9	2–41	4
Elevated level	22	6.2	5	1–25	1
TPA					
Ordinary level	31	10.7	7	2–41	4
Elevated level	12	5.7	5	1–12	–
PLAP					
Ordinary level	46	10.5	7.5	1–42	5
Elevated level	5	12.6	7	3–25	–
Pseudouridine					
Ordinary level	42	11.6	8.5	1–42	4
Elevated level	9	3.8	3	2–7	–

Differences in median survival for CEA ($p=0.002$) and for pseudouridine ($p=0.001$).

tients with adenocarcinoma (57%, 13/23) than with other types (28%, 40/145). Pseudouridine is more often elevated in patients with small cell carcinoma (24%, 10/41) compared with the other diagnoses (12%, 14/113).

During the observation time 56 of the patients died due to bronchogenic carcinoma. The surviving 6 patients were observed for 17 to 41 months after admission with a mean observation time of 25 months. With this outcome it was possible to determine the prognostic value of the marker. As seen from the Figure, patients with elevated levels of CEA and pseudouridine before treatment had a shorter life expectancy than patients with normal levels of these markers. The observed survival was less than half if the level of CEA or pseudouridine was elevated (Table 4). For TPA and PLAP no such correlation could be found (Table 4). No difference existed in age or type of tumors in the compared groups.

Discussion

Four tumor markers—CEA, TPA, PLAP and pseudouridine—were evaluated as diagnostic as well as prognostic tools for patients with bronchogenic carcinoma. Only patients not suitable for operation were included in this investigation. About 60 per cent of the patients had squamous cell carcinoma, and 20 per cent had small cell carcinoma. The frequency of elevated values for CEA was 42 per cent and showed a positive correlation to clinical stage (Tables 2, 3). Also pseudouridine had a posi-

tive correlation with clinical stage, but only 18 per cent of the patients had elevated levels. The other 2 markers, TPA and PLAP did not increase with clinical stage, indicating their limited value as biologic marker for this disease (Tables 2, 3). All 4 tumor markers had limited diagnostic value, since either the frequency of elevated values was low (TPA, PLAP, pseudouridine), or the marker is also elevated in patients with other malignancies (CEA; HANSEN et coll.). However, CEA and pseudouridine had a significant prognostic value (Table 4, Figure).

Only 32 per cent of the patients had elevated values of TPA using 180 U/l as reference. This is much lower than the reported 80 to 90 per cent (MENENDEZ-BOTET et coll., BJÖRKLUND 1980). However, these authors used 90 U/l as reference based on their reference material. Using the lower reference of 90 U/l in the present material, 73 per cent of the patients would get elevated levels of TPA before treatment. Such a low reference value would lead to an unreasonable frequency of 21 per cent elevated values of TPA among healthy adults, and consequently the higher reference value was chosen. Except for the reference value, the present results are in agreement with data published from patients with bronchogenic carcinoma (MENENDEZ-BOTET et coll., BJÖRKLUND). Irrespective of which of the 2 reference values that is used the results show that TPA has limited value as tumor marker for this disease, since it does not reflect the tumor burden (Tables 2, 3).

PLAP was first recognized in a patient with bronchogenic carcinoma of squamous cell type (FISHMAN et coll. 1968). More extended investigations showed that PLAP could be demonstrated only in 14 per cent of the patients with bronchogenic carcinoma (NATHANSON & FISHMAN 1971). The results of the present investigation are in agreement with that report, and confirm its limited value as marker for bronchogenic carcinoma.

The frequency of elevated values of both CEA and pseudouridine increased with clinical stage. However, for pseudouridine only 31 per cent of the patients had elevated levels in stage 3. This is in agreement with results from WAALKES et coll. (1975) and the frequency is too low to make it useful in clinical routine. For CEA the frequency of elevated values was as high as 62 per cent in clinical stage 3. Previous investigations concerning the prognostic value of CEA in bronchogenic carcinoma were contradictory. CONCANNON et coll. (1974) could not demonstrate any prognostic value, although VINCENT et coll. later were able to do so. The latter is in agreement with the present results (Figure, Table 4) which also strengthens the NIH consensus statement that the level of CEA is of prognostic value (NIH 1981).

For malignant lymphoma, RASMUSON & BJÖRK (1983) were unable to demonstrate any prognostic value for pseudouridine, but that investigation was based on a limited material. No previous evaluation of pseudouridine as prognostic factor for bronchogenic carcinoma has to our knowledge been presented. However, for small cell carcinoma of the lung, WAALKES et coll. (1982) found that the urinary excretion of several modified nucleosides was increased. A composite score for 5 modified nucleosides, including pseudouridine was of prognostic value. Although the present clinical material is different from theirs, the results clearly indicate that an elevated level of pseudouridine is correlated to shorter survival. Since the analyses of pseudouridine and CEA are fast, relatively simple and not costly, they could be included in the clinical practice, i.e. as a tool to evaluate the prognosis for patients with bronchogenic carcinoma. This was also suggested by WAALKES et coll. (1982).

SUMMARY

From 62 patients with bronchogenic carcinoma, carcinoembryonic antigen (CEA), tissue polypeptide antigen (TPA), placental alkaline phosphatase (PLAP) in serum and pseudouridine, a modified nucleoside, were analysed in urine. About 60 per cent of the patients had squamous cell carcinoma, and 20 per cent had small cell carcinoma. The patients were allocated into 3 different clinical stages based upon tumor burden, and the markers were analysed before treatment and thereafter. TPA and PLAP had limited value as biologic markers. For both CEA and pseudouridine the frequency of elevated values increased parallel to clinical stage. Elevated levels of these 2 markers were also correlated to shorter survival.

ACKNOWLEDGEMENTS

The investigation was supported by grants from the Swedish Cancer Society (Project No. 1280) and Lion's Research Foundation, Department of Oncology, University Hospital, Umeå (Project No. 233/82).

Request for reprints: Dr T. Rasmuson, Department of Oncology, University Hospital, S-901 85 Umeå, Sweden.

REFERENCES

BJÖRKLUND B.: On the nature and clinical use of tissue polypeptide antigen (TPA). Tumor Diagnostik 1 (1980), 9.

CONCANNON J. P., DALBOW M. H., LIEBLER G. A., BLAKE K. E., WEIL C. S. and COOPER J. W.: The carcinoembryonic antigen assay in bronchogenic carcinoma. Cancer 34 (1974), 184.

DANIEL W. W.: Applied nonparametric statistics. Houghton & Mifflin, Boston 1978.

FISHMAN W. H., INGLIS N. I., STOLBACH L. L. and KRANT M. J.: A serum alkaline phosphatase isoenzyme of human neoplastic cell origin. Cancer Res. 28 (1968), 150.

GEHRKE C. W., KUO K. C., DAVIS G. E., SUITS R. D., WAALKES T. P. and BOREK E.: Quantitative high-performance liquid chromatography of nucleosides in biological materials. J. Chromatogr. 150 (1978), 455.

HAAGENSEN D. E., EASTERDAY R. L., STOLLE C. A. and WELLS S. A.: Buffer-exchange column for rapid separation of carcinoembryonic antigen from perchloric acid. Clin. Chem. 24 (1978), 135.

HANSEN H. J., SNYDER J. J., MILLER E., VANDEVOORDE J. P., MILLER O. N., HINES L. R. and BURNS J. J.: Carcinoembryonic antigen (CEA) assay, a laboratory adjunct in the diagnosis and management of cancer. Human Pathol. 5 (1974), 139.

KUO K. C., GEHRKE C. W., MCCUNE R. A., WAALKES T. P. and BOREK E.: Rapid, quantitative high-performance liquid column chromatography of pseudouridine. J. Chromatogr. 145 (1978), 383.

MENENDEZ-BOTET C. J., OETTGEN H. F., PINSKY C. M. and SCHWARTZ M. K.: A preliminary evaluation of tissue polypeptide antigen in serum or urine (or both) of patients with cancer or benign neoplasms. Clin. Chem. 24 (1978), 868.

NATHANSON L. and FISHMAN W. H.: New observations on the Regan isoenzyme of alkaline phosphatase in cancer patients. Cancer 27 (1971), 1388.

NIH: Carcinoembryonic antigen. Its role as a marker in the management of cancer. Brit. Med. J. 282 (1981), 373.

RASMUSON T. and BJÖRK G. R.: Pseudouridine, a modified nucleoside as biological marker in malignant lymphomas. Cancer Detect. Prevent. (1983, in press.)

STIGBRAND T., HOLMGREN P. Å., JEPPSSON A., DAMBER M.-G. and VON SCHOULTZ B.: On the value of placental alkaline phosphatase as a marker for gynecological malignancy. Acta Obstet. Gynecol. Scand. (1983, in press.)

SUGARBAKER P. H., DUNNICK N. R. and SUGARBAKER E. V.: Cancer, principles and practice of oncology, p. 248. Edited by V. T. DeVita, S. Hellman and S. A. Rosenberg. Lippincott, Philadelphia 1982.

WIKLUND B., KALLIN E., ANDERSSON K. and BJÖRKLUND B.: Radioimmunoassay of tissue polypeptide antigen versus hemagglutination technique. *In:* Protides of the biological fluids, p. 243. Edited by H. Peeters. Pergamon Press, Oxford 1979.

VINCENT R. G., CHU T. M., FERGEN T. B. and OSTRANDER M.: Carcinoembryonic antigen in 228 patients with carcinoma of the lung. Cancer 36 (1975), 2069.

WAALKES T. P., ABELOFF M. D., ETTINGER D. S., WOO K. B., GEHRKE C. W., KUO K. C. and BOREK E.: Modified ribonucleosides as biological markers for patients with small cell carcinoma of the lung. Eur. J. Cancer Clin. Oncol. 18 (1982), 1267.

— GEHRKE C. W., ZUMWALT R. W., CHANG S. Y., LAKINGS D. B., TORMEY D. C., AHMANN D. L. and MOERTEL C. G.: The urinary excretion of nucleosides of ribonucleic acid by patients with advanced cancer. Cancer 36 (1975), 390.