Serum acid phosphatase activities in patients with lung cancer: a biochemical and immunohistochemical analysis of 25 cases

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SUMMARY A series of 25 cases of lung cancer are presented in which total (TAcP) and nonprostatic serum acid phosphatase (NPAcP) activities were measured. Of these cases, 36% had raised TAcP and NPAcP activities in their serum. However, the serum activities of TAcP and NPAcP did not correlate with either the presence of lung cancer nor with the morphological tumour type. This fact indicates that, despite isolated reports of raised serum acid phosphatase activities in cases of lung cancer, acid phosphatase is of no value as a marker for lung cancer. We sought alternative explanations for the raised TAcP and NPAcP activities observed in our series in the hope that this enzyme might prove useful as a marker for early metastatic disease in lung cancer patients. This possibility is not substantiated, and the findings are analysed and discussed. It is tentatively suggested that raised NPAcP activities in patients with lung cancer may relate to haemostasis.

Acid phosphatase is a lysozomal enzyme¹ of molecular weight 13 400 to greater than 200 000.² Acid phosphatase activity is demonstrable in various tissues, especially prostate where this knowledge is applied in the diagnosis of metastatic carcinoma of prostatic origin. Other tissues rich in acid phosphatase include liver,² platelets,³ bone and red blood cells,⁴ and mammary epithelium.¹ Apart from prostatic malignancy, other conditions may have associated increased activities of serum acid phosphatase including thrombocythaemia,³⁻⁵ multiple myeloma and metastatic pancreatic carcinoma,⁵ Gaucher's disease,⁵⁻⁶ leukaemia, Paget's disease, renal disease and liver disease,⁶ and carcinoid tumour of rectum.⁷ Raised serum acid phosphatase has also been reported in bronchogenic carcinoma.^{6,8} Our index case, which was erroneously diagnosed as metastatic carcinoma of prostate mainly on the basis of raised TAcP activities, proved at necropsy to be a case of widely disseminated bronchogenic oat cell carcinoma with only benign hyperplastic changes in the prostate gland. This finding prompted us to explore the significance of serum acid phosphatase activities in patients with lung cancer. Twenty-five cases are presented and analysed biochemically and immuno-

histochemically. These include our index case (No 11 in this series) and all were studied with emphasis on tumour morphology, liver function, bone metabolism, platelet count, and haemolysis.

Material and methods

Twenty-five consecutive cases of lung cancer were studied. They were all newly diagnosed cases and all had full routine investigations including full blood count, urea and electrolytes, urinalysis, chest x-ray examination, and sputum cytology.

Diagnosis of lung cancer was obtained by various means including sputum cytology, bronchial biopsy, open lung biopsy, lymph node biopsy, x-ray, necropsy and pneumonectomy. One patient had lung cancer but precise diagnosis was not obtained (case 15).

SERUM ACID PHOSPHATASE

As soon as a diagnosis was established, blood was obtained by venepuncture into a dry 10 ml tube, was immediately centrifuged, and the serum then removed and buffered with 5 M acetate buffer at pH 5.3 (20 microlitres per ml of serum) and frozen at -20°C for later analysis for acid phosphatase. Acid phosphatase was assayed in serum samples in our biochemistry department with the method of Andersch and Szczypinski,⁹ and employing tartrate-stability test

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TISSUES

Histological diagnosis was made by routine haematoxylin and eosin staining of formalin-fixed, paraffin-embedded tissue sections.

Immunohistochemistry of tumours was performed on sections from these same paraffin-embedded blocks in 15 cases. This was carried out by means of the indirect immunoperoxidase technique with acid phosphatase antiserum donated by Dr A Leatham of Ludwig Cancer Institute, Royal Marsden Hospital, London. This antiserum was raised in rabbits by injection of acid phosphatase purified from pooled human semen. Normal postmortem prostate was used as a control in this series. The conjugates were obtained commercially, and two runs were carried out. The first run used swine antirabbit IgG conjugated with horseradish peroxidase (Dako) and the second run employed alkaline phosphatase-conjugated goat antirabbit IgG obtained from Miles-yeda. Tissue was not available for study of 10 of our cases.

LIVER

Liver function tests included serum bilirubin, transaminases (SGOT and SGPT), alkaline phosphatase (including electrophoresis on cellulose acetate for identification of isoenzymes in patients with raised enzyme activities in their serum), and serum protein (total, albumin, and globulin).

RED CELLS

Haemolysis was excluded clinically and by measurement of serum bilirubin and potassium.

BONE

Bone metabolism was assessed biochemically by measurement of serum calcium and phosphate.

PLATELETS

Platelet counts were performed in the routine way in our haematology department with a standard counting chamber.

Results

The results of this study are shown in Tables 1 & 2.

The series includes 25 patients, 18 men and seven women. Their ages ranged from 33 to 86 yr, mean 62.7 yr. The diagnosis in the series included oat-cell carcinoma, 12 cases (48%); epidermoid (squamous) carcinoma, 6 cases (24%); fibrosarcoma and undesignated sarcoma, 2 cases (8%); mesothelioma, 1 case (4%); metastatic tumour, 1 case (4%); and indeterminate morphological type, 3 cases (12%).

SERUM ACID PHOSPHATASE

Serum acid phosphatase (AcP) activity was measured

in all 25 cases. The total serum acid phosphatase activity (TAcP) was the only estimation in five cases, otherwise both TAcP and the NPAcP fraction (tartrate-stable) were measured. The five cases in which TAcP alone was measured each gave results within the normal reference range. Nine of the remaining 16 cases (36% of total number studied) had raised TAcP values. Of these nine cases, all AcP activities were mostly tartrate-stable (NPAcP). Five of the cases were oat-cell carcinomas (56%), two were epidermoid carcinomas (22%), one was a primary lung sarcoma (11%), and one was a metastatic tumour of undetermined origin (11%). Because of the small numbers involved, statistical analysis of these figures was not carried out. It is apparent, however, that raised serum NPAcP activity does not correlate with tumour morphology. It is also noteworthy that three of the nine patients with raised serum NPAcP were women (see Table 1).

TISSUE SECTIONS

Tissue sections from 15 cases were stained for AcP by the indirect immunoperoxidase technique. All 15 available tumours were negative in the first run and this finding was confirmed in the second run. The control tissues stained positively in each run, thereby confirming activity of the antiserum. Suitability of the antiserum was confirmed by positive staining of red blood cells and macrophages in the tumour sections. These findings were interpreted as indicating that the tumour cells themselves were not the source of the raised serum AcP observed. Seven further cases studied by immunohistochemistry did not contain any tumour tissue when the blocks were cut. There was no tissue available for study from three cases (Nos 15, 16, and 17 in the Tables). Of the other cases from which tumour tissue was not available in the blocks for immunohistochemistry, cases 4 and 5 had raised serum activities of NPAcP. This reduced the number of cases with raised serum NPAcP studied histochemically to seven. The remainder of this study was devoted to correlation of raised serum NPAcP in lung cancer patients with tests of function of other tissues (see Tables 1 & 2).

LIVER

Liver function tests were carried out in all 25 cases in this study. Of the nine cases with raised serum NPAcP activities, complete liver function tests were available in five, but abnormalities were apparent in all nine cases (100%). These ranged from minor disturbances of individual parameters to severe upset in the full range of tests. However, similar degrees of abnormality were noted in 14 of the 16 cases with normal serum NPAcP or TAcP activities (87%) or both. The single case with grossly abnormal liver

function tests is the index case (No 11 in Table 2) in which metastases were demonstrated in liver, bones, brain, marrow, spleen, and kidneys, and in which abnormalities of bone metabolism were also demonstrated (vide infra). Of particular note is the fact that the level of serum NPACP activity does not correlate with any single parameter or group of parameters of liver function in the entire series.

Isoenzymes of alkaline phosphatase were studied in seven of eight cases in which raised serum alkaline phosphatase was noted during assessment of liver function. (Index case serum was not available for this examination.) All seven cases revealed liver isoenzyme with a trace of bone isoenzyme in one case (this case had normal indices of bone metabolism). These findings do not parallel the serum NPACP activity in the respective patients and thus fail to support the concept of liver as the source of the raised serum NPACP. Neither do these tests correlate with tests of bone metabolism.

The possibility that liver dysfunction may precede a rise in serum NPACP in cases of hepatic metastases was considered but rejected, as the activity of serum alkaline phosphatase fails to correlate with or parallel its AcP counterpart. (See Table 2.)

BONE

Serum calcium and phosphate concentrations were

used as indices of bone metabolism and were measured in 24 of the 25 cases in this study. Abnormalities were observed in four cases (17%). These were associated with raised serum activities of NPACP in two cases, but did not correlate with the AcP activities in the other six cases in which calcium and phosphate concentrations were also measured, and further, abnormal calcium and phosphate concentrations were noted in two cases with normal serum activities of AcP. Neither did the serum concentrations of calcium and phosphate correlate with alkaline phosphatase activities (see Tables 1 & 2).

HAEMOLYSIS

None of the cases in this study showed any clinical evidence of haemolysis. Serum potassium was marginally raised in two cases but each of these had normal serum TACP activities. The serum bilirubin concentration was raised in one of these two cases but this was accompanied by raised serum transaminases and was attributed to liver dysfunction rather than haemolysis (see Tables).

PLATELETS

Platelet counts were available in nine cases. Four of these showed raised platelet counts and two of these (50%) were associated with raised serum activities of TACP and NPACP, one in a female patient and one in

Table 1 (Reference values given in parentheses)

Case No and type	Age	Sex	Acid phosphatase (serum)		*Immunohistochemistry		Haemolysis		Bone metabolism	
			Total (0-9 IU/l)	Non- prostatic	Alkaline phosphatase	Horseradish peroxidase	Serum potassium (3.6-5.0 mmol(Eq)/l)		Serum calcium (2.20-2.63 mmol/l)	Serum phosphate (0.65-1.62 mmol/l)
1 Oat cell	73	M	8.4	—	—	—	Nil	4.4	2.65	1.32
2 Oat cell	52	M	6.3	—	—	—	Nil	3.5	2.60	1.10
3 Oat cell	37	M	5.2	3.8	No tissue	—	Nil	4.7	2.50	1.07
4 Oat cell	57	F	13.8	10.7	No tissue	—	Nil	4.8	3.00	1.03
5 Oat cell	63	F	17.5	16.9	No tissue	—	Nil	3.9	2.35	1.39
6 Oat cell	52	M	32.1	30.3	—	—	Nil	4.0	2.40	0.94
7 Oat cell	86	F	8.9	8.3	No tissue	—	Nil	3.3	2.35	1.07
8 Oat cell	61	M	9.8	9.7	—	—	—	—	—	—
9 Oat cell	64	M	7.6	7.5	—	—	Nil	4.2	2.25	1.29
10 Oat cell	71	F	6.0	3.8	—	—	Nil	4.1	2.57	0.93
11 Oat cell	73	M	11.7	9.6	—	—	Nil	4.5	3.00	1.36
12 Oat cell	70	M	7.4	5.9	No tissue	—	Nil	4.4	2.18	1.03
13 Metastasis	73	M	18.9	14.7	—	—	Nil	4.4	2.33	1.20
14 Sarcoma	69	M	11.0	8.3	—	—	Nil	4.8	2.15	0.87
15 Not defined	67	M	2.8	0	No tissue	—	Nil	3.9	2.35	1.20
16 Not defined	73	M	8.5	6.2	No tissue	—	Nil	4.1	2.30	1.00
17 Not defined	73	F	4.9	2.6	No tissue	—	Nil	3.9	2.20	1.07
18 Fibrosarcoma	62	M	5.8	—	—	—	Nil	4.6	2.30	0.90
19 Mesothelioma	33	M	4.2	—	No tissue	—	Nil	4.6	2.40	1.03
20 Epidermoid	63	M	7.7	6.7	—	—	Nil	4.0	3.13	1.03
21 Epidermoid	66	M	7.8	6.9	—	—	Nil	3.9	2.35	1.03
22 Epidermoid	53	F	10.2	8.3	—	—	Nil	3.9	2.23	1.29
23 Epidermoid	74	F	8.3	7.5	No tissue	—	?	5.7	2.40	1.23
24 Epidermoid	44	M	14.0	11.6	—	—	Nil	4.0	2.38	0.84
25 Epidermoid	58	M	3.5	—	—	—	?	5.2	2.50	0.74

*Immunohistochemistry: — = negative. Conversion: SI to traditional units Calcium 1 mmol/l $\approx$ 4.0 mg/100 ml. Phosphate 1 mmol/l $\approx$ 3.1 mg/100 ml.

Table 2

Case No and type	Platelets (150-400 × 10 ⁹ /l)	Bilirubin (0-14 μmol/l)	SGOT (8-40 IU/l)	SGPT (5-35 IU/l)	Alkaline phosphatase isoenzyme level (35-150 IU/l)	Proteins		
						Total (58-80 g/l)	Albumin (35-53 g/l)	Globulin (18-36 g/l)
1 Oat cell	—	10	—	—	60	81	41	40
2 Oat cell	253	5	22	14	94	78	39	39
3 Oat cell	570	5	25	36	60	76	36	40
4 Oat cell	—	9	—	—	179 Liver	71	39	32
5 Oat cell	424	10	41	26	77	70	40	32
6 Oat cell	—	9	21	17	81	83	41	42
7 Oat cell	—	9	17	18	112	63	38	25
8 Oat cell	—	—	—	—	112	—	—	—
9 Oat cell	—	15	28	18	69	72	35	37
10 Oat cell	282	8	—	—	67	72	36	36
11 Oat cell	—	46	200	200	> 1000	65	29	36
12 Oat cell	393	5	—	—	105	65	31	34
13 Metastasis	—	5	27	26	297 Liver + bone	71	32	39
14 Sarcoma	—	10	—	—	261 Liver	65	23	42
15 Not defined	—	7	—	—	130	72	35	37
16 Not defined	—	12	—	—	157 Liver	76	37	39
17 Not defined	545	10	15	22	91	69	31	38
18 Fibrosarcoma	260	24	40	27	103	64	34	30
19 Mesothelioma	—	7	23	34	100	75	41	34
20 Epidermoid	—	10	—	—	102	75	34	41
21 Epidermoid	—	10	20	19	159 Liver	75	36	39
22 Epidermoid	—	5	—	—	153 Liver	68	31	37
23 Epidermoid	—	9	—	—	153 Liver	68	25	43
24 Epidermoid	470	5	23	18	52	76	39	37
25 Epidermoid	181	65	45	36	62	69	35	34

Conversion: SI to traditional units Bilirubin 1 μmol/l ≈ 0.0585 mg/100 ml.

a male. All of these four cases were shown to have normal indices of bone metabolism, normal serum potassium, normal serum bilirubin in three, and virtually normal liver function tests. As the serum alkaline phosphatase was also normal in the case with slightly raised serum bilirubin, it is unlikely that the raised serum NPACP activity here is emanating from the liver. Therefore, some correlation seems to exist between the blood platelet count and serum NPACP activity (see Tables 1 & 2).

Discussion

Serum AcP activities in patients with lung cancer became of great importance to us because of an erroneous diagnosis of metastatic cancer of prostate in a man with raised serum AcP who was shown at necropsy to have disseminated bronchial oat-cell carcinoma.

Raised activities in serum AcP have been recorded previously in isolated cases of lung cancer, as well as in other malignancies especially prostatic cancer, as mentioned earlier (vide supra). We decided to test the possible association between lung cancer and serum AcP activity.

This series of 25 cases of lung cancer revealed nine cases with raised serum NPACP activity (36% incidence). Of these, 56% were associated with oat-cell carcinoma, but raised activities were also noted in

cases of epidermoid carcinoma, metastatic carcinoma, sarcomatous and mesotheliomatous neoplasms. This raised NPACP activity in serum was observed in three female patients (33%). Immunohistochemical study failed to demonstrate AcP in any of the tumours available for study. Consequently, it is clear that, despite the high incidence of raised serum NPACP in lung cancer patients, this NPACP does not originate in the tumours themselves, nor does the raised serum activity of NPACP correlate with tumour morphology but may be observed in any type of lung tumour, even metastatic tumour. In addition seven of the nine lung cancer cases have biochemical evidence of dysfunction of liver or bone metabolism or both. Case No. 8 has incomplete tests of liver function, and case No. 24 is one with a raised platelet count (vide infra), so that eight of the nine cases with raised serum AcP offer immediate alternative explanations other than tumour product.

Other tissues known to contain AcP (apart from prostate) include liver, bone, red blood cells, and platelets. It was attempted, on the basis of this knowledge, to correlate the observed raised serum activities of NPACP in our nine patients with biochemical evidence of dysfunction of these tissues. However, no clear association could be found between liver dysfunction, haemolysis or bone metabolism and raised serum NPACP activity. Therefore, serum NPACP activity cannot be held to reflect the possible pre-

sence of tumour metastases in these organs (liver or bone). It was considered that raised serum alkaline phosphatase activity might indicate hepatic metastasis of lung cancer, but even if this assumption were correct, then from our study it would clearly have to precede any rise in serum NPAcP and so invalidate the latter as a screening test for early hepatic metastasis.

One aside noted was the association of raised serum NPAcP activities in two of four patients with raised platelet counts. The numbers involved here are too small to allow conclusions to be drawn, but if this association were confirmed in a larger series, it could add to our knowledge of thrombosis and embolism in patients with malignant disease.

The main points to emerge from this series, however, are

(1) despite individual case reports, raised serum acid phosphatase activities are of no value as a marker of lung cancer and the state of biochemical markers of lung cancer remains as recently reviewed;¹⁰

(2) Serum acid phosphatase activities do not reflect the presence or absence of distant metastases of lung cancer, and are of no value as a screening device for early detection of metastases.

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