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The Role of Serum Tumour Markers to Aid the Selection of Lung Cancer Patients for Surgery and the Assessment of Prognosis

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Abstract—*We have measured the following ten serum proteins in a sample of 290 patients presenting with possible lung cancer: carcinoembryonic antigen (CEA), α_1-acid glycoprotein (AGP), C-reactive protein (CRP), ferritin (FER), prealbumin (PAB), third component of complement (C3), immunoglobin E (IgE), α_2-pregnancy-associated glycoprotein (PAG), β_2-microglobulin (β_2-m) and retinol binding protein (RBP). It is found that, with the exception of PAG, C3 and IgE, there are significant differences between protein concentrations in the subsequently diagnosed cancer and non-cancer patients. However, protein concentrations in the cancer patients who were suitable for surgery do not differ significantly from the concentrations in inoperable patients. The prognostic significance of the proteins in the inoperable and operable cancer patients is also envisaged. In the operable group C3 appears to be useful, whilst AGP and RBP are prognostic indicators in the inoperable group.*

INTRODUCTION

THE RECOGNITION that malignancies are often associated with non-specific changes in the levels of various plasma proteins has prompted many studies to define the role of such measurements in the diagnosis, prognosis and treatment of malignant disease [1, 2]. There is no agreement, however, as to which proteins, individually or in combination, are useful in the overall management of lung cancer. The following ten serum proteins have been implicated as potentially useful: carcinoembryonic antigen (CEA), α_1-acid glycoprotein (AGP), C-reactive protein (CRP), ferritin (FER), prealbumin (PAB), third component of complement (C3), immunoglobin E (IgE), α_2-pregnancy-associated glycoprotein (PAG), β_2-microglobulin (β_2-m) and retinol-binding protein (RBP). A raised CEA has been found to be associated with a shortened survival in lung cancer [3–7], and so too have the acute-phase reactant proteins AGP and CRP [8, 9]. An elevated FER is associated with an impaired prognosis in various tumours, notably in germinal cell malignancies [10, 11]. IgE levels have been of considerable interest in cancer [12, 13]. Some patients seem to have an increased IgE concentration in response to the tumour whereas others have depressed levels, presumably as a sign of immune disturbance which is considered to be associated with a poor prognosis [14]. The protein C3 has properties of an acute phase reactant, and raised levels may be indicative of distant metastases [15]. The role of PAG as a tumour marker is not entirely clear but it has immunosuppressive effects on T lymphocytes and may therefore have an impact on the prognosis of cancer patients [16]. The speed of progression of lung cancer has been linked to serum β_2-m levels [17]. The levels of PAB and RBP are indicators of the nutritional state of the patient [18]. A low RBP reflects a low serum vitamin A, which recently has attracted much attention as a factor in the development of lung cancer [19, 20]. We have measured these proteins at presentation in a study of a sample of patients referred for surgery with possible lung cancer. The patients in the study have been followed for up to 4 yr and the sample affords the opportunity to assess the usefulness of these proteins in the diagnosis, treatment and prognosis of lung cancer.

MATERIALS AND METHODS

The study involved 290 patients, of whom 230 were male and 60 were female, with possible lung cancer presenting during the period September 1980 to July 1981. Each patient had been referred for surgical opinion to the thoracic surgery services of Leeds General Infirmary, Killingbeck Hospital

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Leeds, Bradford Royal Infirmary or Castle Hill Hospital Cottingham. In 215 patients the diagnosis of a primary lung cancer was confirmed. Among these patients, 113 were found to have non-resectable tumours owing to extensive local growth in 72 patients, as indicated by bronchoscopic and/or mediastinoscopic findings; previously undetected metastatic disease in 19 patients; and the discovery of non-resectable tumours following exploratory thoracotomy in 22 patients. Surgically complete resections were carried out on 102 patients, who are henceforth referred to as the 'operable' group; the remaining 113 cancer patients are the 'inoperable' group. In the operable group 45 patients had a pneumonectomy and 57 had a lobectomy, and their post-operative TNM staging revealed 44 patients with stage 1 disease, 31 patients at stage 2 and 27 patients at stage 3. For the inoperable group of patients definitive staging was not possible. Table 1 gives the histological classification of the cancer patients.

A blood sample was taken from each patient at presentation, clotted at room temperature, the serum separated and stored at −20°C until tested. Serum CRP, PAB, AGP, C3 and RBP were measured by single radial immunodiffusion [21] using antisera and standards supplied by Behringwerke AG, Marburg/Lahn, F.R.G. for CRP, PAB and RBP and by using antisera and standards obtained from Seward, London for AGP and C3. CEA was measured by using an enzyme immunoassay obtained from Abbott (CEA–EIA). PAG was determined by an enzyme immunoassay developed by Behringwerke AG. The FER, IgE and β_2-m radioimmunoassay kits were supplied by Pharmacia Diagnostics, Uppsala, Sweden.

Statistical methods

Statistical procedures were considered to assess the use of biochemical responses to indicate malignancy suitability for resective surgery and survival. A statistical test for differences in the biochemical levels in each of the 'non-cancer', 'operable cancer' and 'inoperable cancer' patient groups was done using the Kruskal–Wallis non-parametric one-way analysis of variance [22]. An alternative multivariate analysis was also used by fitting a trichotomous logistic regression model to discriminate between the three groups and statistical tests concerning the significance of the biochemical variables in the model were made [23, 24].

Table 1. *Histological classification of lung cancer patients*

Histology	Operable	Inoperable
Squamous	68	67
Adeno	24	11
Small cell	8	19
Undifferentiated large cell	0	3
Unconfirmed	2	13

The second objective, concerning the survival of patients, was investigated in three ways: (a) by comparing survival rates to 1 or 2 yr; (b) by the 'log-rank' test statistic [25] for differences in survival; and (c), by using Cox's proportional hazards survival model [26]. The patients have been followed for up to 4 yr, at the time of writing. Patients whose deaths were due to a cause not directly related to the cancer or whose deaths were immediately post-operative have been excluded from the survival analysis. This amounted to 20 patients, 12 in the operable group and eight in the inoperable group, so that for the survival analyses the sample sizes were reduced to 90 and 105 respectively.

All analyses were done using either the Statistical Analysis System (SAS) or the BMDP statistical packages, with the exception of the logistic regression, which was carried out using a computer program developed by one of us [23]. Unless stated otherwise, the level for 'statistical significance' in the tests to follow is 1%.

RESULTS

Malignancy and suitability for surgery

Table 2 lists the median values of the ten biochemical variables in each of the three patient groups, non-cancer, operable and inoperable, together with the 'normal' limits for each variable. With the exception of PAG, IgE and C3, there are significant differences in the distributions in the three groups by the Kruskal–Wallis test. Similar tests for differences between the operable and inoperable groups show only a significant difference for AGP, but several borderline significant cases. A tentative conclusion is that, while there is some evidence that some biochemical variables might be useful to distinguish between cancer and non-cancer, there is little evidence to show that differences in the biochemical levels in the operable and inoperable cancer patients are sufficiently large to be clinically useful. A trichotomous logistic regression analysis seems to confirm this: an overall test that the three groups are distinguishable with respect to biochemical variables is highly significant, having a P value <0.0001, but a test to distinguish between only the inoperable and operable groups has a P value of 0.02, and the test is not significant at the 1% level. Thus the evidence for the ability for the biochemical variables to discriminate between the operable and inoperable groups is tenuous; their value lies overwhelmingly

Table 2. Median protein values in each group and generally accepted normal limits

Protein	Cancer			Normal limits	*P* values†	
	Non-cancer	Operable	Inoperable		Overall	Operable vs inoperable
PAG (mg/l)	17 (3–29)*	13 (4–27)	16 (6–35)	<30	0.234	0.085
CEA (μg/l)	1.6 (0.8–2.7)	2.7 (1.8–4.7)	3.9 (2.1–8.1)	<5	0.0001	0.025
CRP (mg/l)	2 (0–8)	16 (3–42)	25 (9–53)	<10	0.0001	0.032
AGP (g/l)	1.0 (0.8–1.4)	1.4 (1.1–2.1)	1.8 (1.3–2.2)	0.4–1.3	0.0001	0.006
FER (g/l)	110 (56–259)	167 (77–291)	222 (104–367)	130–400	0.0015	0.032
IgE (IU/ml)	48 (13–128)	40 (12–130)	51 (17–129)	<100	0.640	0.370
PAB (mg/l)	280 (230–360)	230 (160–280)	200 (150–260)	100–400	0.0001	0.021
RBP (mg/l)	53 (40–65)	42 (30–54)	38 (27–51)	30–60	0.0001	0.281
C3 (g/l)	1.05 (0.9–1.3)	1.14 (1.0–1.3)	1.21 (1.0–1.4)	0.5–1.2	0.026	0.187
β_2-m (mg/l)	not recorded	2.6 (1.2–3.1)	2.5 (2.0–3.2)	0.8–2.4	—	0.969

*Figures in parentheses give the inter-quartile range.
†Using the Kruskal–Wallis test for significant differences between groups.

in discriminating between cancer and non-cancer. In constructing these tests we have omitted β_2-m, PAG and PAB since a high proportion of these values were missing in the non-cancer group. This difficulty can be avoided by doing an alternative analysis in which a binary logistic model is fitted to discriminate between only the operable and inoperable groups. The test statistic to distinguish the groups is not significant ($P = 0.045$), which again confirms these findings.

It is instructive to note that some biochemical variables are associated. The correlation matrix (Table 3) shows a strong correlation between the acute phase reactant proteins CRP and AGP ($r = 0.73$). They are also weakly correlated with FER (AGP–FER: $r = 0.41$) and C3 (AGP–C3: $r = 0.35$), both of which are known to have acute-phase reactant properties. PAB and RBP are markers of nutritional status and are well correlated ($r = 0.73$). They correlate inversely with the acute-phase proteins, indicating that these two groups of variables relate to the activity of the disease process and the present state of health of the patient.

Survival

There is no doubt that surgically treated patients have a better prognosis; the 2-yr survival rate and median survival in the operable group are 38/90 (43%) and 20.5 months compared to 9/105 (9%) and 5.7 months in the inoperable group.

In the operable group the factors that individually are statistically related to survival are given in Table 4. Of the ten biochemical indices only C3 is significant. For histology we have combined the adenocarcinoma and the squamous cell types since the 2-yr survival rates, 7/20 and 31/61 respectively, do not differ significantly. Surgical procedure was not a prognostic factor: the 2-yr survival rates in the lobectomy and pneumonectomy groups, 16/43 and 24/47 respectively, are not statistically different. In fitting Cox's survival model only the factors stage and histology had any influence on survival. There was no improvement to the model ($P = 0.30$) by adding in the third significant factor C3. This is because C3 and stage appear to be closely associated ($P = 0.02$ by a chi-square test); for example, at stage I 28%

Table 3. Correlation coefficients between the ten biochemical variables for the combined inoperable and operable patient groups

	CEA	CRP	AGP	FER	IgE	PAB	RBP	C3	β_2-m
PAG	−0.03	0.04	0.13	−0.08	−0.08	−0.12	−0.09	0.16	0.05
CEA		0.08	−0.01	0.02	0.01	−0.09	−0.01	−0.04	0.05
CRP			0.76*	0.38*	0.06	−0.57*	−0.44*	0.29*	0.18
AGP				0.38*	0.04	−0.55*	−0.36*	0.35*	0.22
FER					0.01	−0.18*	−0.16	0.21*	0.07
IgE						−0.08	−0.11	−0.04	0.01
PAB							0.75*	−0.13	−0.12
RBP								−0.04	0.12
C3									0.02

*Correlation coefficient significantly different from zero.

Table 4. Significant prognostic factors in the operable group of patients

Factor and level	2-yr survival rate	Median survival (months)	P
Stage			
I	22/36	39.15	0.01,* 0.0002†
II	11/28	20.07	
III	6/26	7.90	
Histology			
Non-small cell	38/81	21.67	0.02, 0.0002
Small cell	0/7	6.43	
C3(g/l)			
≤1.2	29/55	26.43	0.024, 0.0028
>1.2	10/35	11.34	

*Associated with a chi-square test for differences in 2-yr survival.
†Associated with the 'log-rank' test statistic for overall differences in survival.

(10/36) have raised C3 >1.2 g/l whilst at stage III the rate is 62% (16/26).

In the inoperable group the factors that are associated with survival are listed in Table 5. The survival rate for males is significantly worse than for females, and an elevated AGP and a depressed RBP also carry an adverse prognosis. Further, if neither AGP is elevated nor RBP depressed, prognosis is relatively favourable (Table 5). In fitting Cox's model to the inoperable group only sex and AGP are significant factors.

DISCUSSION

As expected, various biochemical changes are observed in lung cancer patients at the time of presentation but differences may not be sufficiently specific for diagnostic purposes. They include raised CEA, raised acute-phase reactant proteins CRP, AGP and FER and raised β_2-m. Both RBP and PAB are depressed in cancer patients, which is probably an indication of a poor nutritional state. With the exception of some marginal differences for CRP, AGP and CEA, there are no differences between the operable and inoperable cancer groups with regard to the concentration of biochemical variables. If one assumes that the right decision to operate had been made in these patients, then this analysis suggests that the prospects for using biochemical factors as a guide to suitability is poor. However, a more fruitful way to view the significance of the biochemical variables is by a survival analysis.

With the exception of C3, none of the preoperative levels of the biochemical variables appears to have any bearing on survival in the operable group of patients and stage is the strongest determinant of survival. The complement C3 is itself associated with stage and a raised level may be indicative of distant metastases [15], which might explain its bearing on prognosis since the

Table 5. Significant prognostic factors in the inoperable group of patients

Factor and level	1-yr survival rate	Median survival (months)	P
Sex			
F	10/23	9.48	0.008,*0.02†
M	14/82	4.36	
AGP (g/l)			
<1.4	11/27	9.80	0.01, 0.01
>1.4	13/78	3.97	
RBP (mg/l)			
<42	7/56	3.77	0.007, 0.002
>42	17/49	9.80	
AGP–RBP			
AGP↑ or RBP↓	14/87	3.84	0.0003, 0.001
AGP↓ and RBP↑	10/18	13.41	

*†See Table 4.

patients who have not responded to surgery may be those with undetected metastases. None of the other pre-operative levels of acute-phase reactant proteins are associated with survival. In contrast, in the inoperable group AGP and RBP are significant prognostic indicators whilst C3 is not. In particular, a normal AGP in conjunction with a normal RBP is the most favourable combination with regard to survival. AGP and RBP respectively reflect tumour size, or aggressiveness, and nutritional status so that prognosis is as good as can be reasonably expected in a patient with both of these favourable signs without surgery. Since neither pre-operative AGP nor RBP are prognostic factors in the operable group, their association with subsequent survival is removed once the tumour is removed. In view of the many clinical factors which determine the suitability for surgery, it is in 'doubtful' cases that the prognostic significance of C3 in the operable group and AGP and RBP in the inoperable group may be considered to be most useful. For example, the pre-operative concentration of these proteins could be used to predict survival both with and without surgery and the clinician could choose the course that appears to be most favourable.

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