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Circulating immune complexes in patients with lung cancer

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ABSTRACT Sera from 41 patients with lung cancer and 41 age and sex-matched hospital in-patients with non-malignant disease were tested for the presence of immune complexes using the ^{125}Iodine Clq binding test. All patients were untreated or had recurrences after surgery. An increased Clq binding activity was found in 34% of patients with lung cancer and 24% of patients with non-malignant disease. There was no significant association between increased serum Clq binding activity and histological tumour type or survival but there was an association with the extent of malignant disease. No patient with limited (stage 1) disease had raised Clq binding activity but in 42% of patients with extensive disease it was increased. Increased Clq binding activity did not correlate with either an increased total white cell count or ESR. Measurement of Clq binding activity may be of value in serial monitoring of disease progress and response to treatment.

Considerable interest has been shown in the measurement of circulating immune complexes in patients with malignant disease as a possible approach to immunodiagnosis or prognosis.[1-4] This was originally stimulated by the description of factors in the serum of tumour-bearing patients and animals which interfere with cell-mediated immunity.[5] It has also been suggested that circulating or cell-bound immune complexes may be responsible for the relatively high frequency of serum antiglobulin responses in cancer patients,[6 7] and signs and symptoms of so-called "immune complex disease" may be seen in patients with cancer.[9] More recently it has been suggested that immune complexes cause synthesis and release of prostaglandins and release of lysosomal acid hydrolysases from macrophages.[10]

Various methods have been used for the detection and quantitation of immune complex (CIC) levels in serum including the Raji cell binding assay and tests measuring binding of the Clq component of complement.[4 11] Using these tests raised CIC levels have been reported in patients with lymphomas as well as many types of solid tumour.[3 4] In many studies, however, patients with well established disease were studied and reports from different groups have been contradictory. We describe here the measurement of serum CIC by radioprecipitation of ^{125}I-Clq in patients with lung cancer compared with a group of hospital in-patients with non-malignant disease and normal young healthy control subjects.

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Methods

Blood samples were taken from 41 patients with histologically confirmed lung cancer and 41 age and sex-matched hospital inpatients with benign conditions (excluding patients with diseases known to be associated with high levels of immune complexes), and allowed to clot at room temperature for one hour. Serum was separated, the lipid layer removed after centrifugation at 1500 g for 15 min at 4°C and then 30 000 g for 30 min at 4°C and the samples stored at −70°C. Immune complexes were assayed in duplicate by a modified ^{125}I-Clq binding test in which ^{125}I-Clq is added to heparinised serum and complexes precipitated with polyethylene glycol.[4 12] Histology was provided by the routine hospital pathology service and patients classified retrospectively as limited disease (stage 1) or extensive disease (stages 2 and 3 or deemed inoperable because of tumour spread). Survival at one year after diagnosis and sampling was deter-

mined from the case notes and by contacting the patient's general practitioner where necessary. Three patients in the control group were excluded as further investigation revealed that they had been treated recently for other tumours or were suspected of having other tumours on clinical grounds. Clq binding activity was considered to be abnormal if it was more than two standard deviations greater than the mean level of Clq binding activity in 20 healthy laboratory personnel —that is, greater than 11% Clq binding activity.

Table 1 *Results of Clq binding activity tests*

	Lung cancer	*Benign conditions*
Number tested	41	38
Mean age (yr)	61±9	63±14
Mean Clq binding activity±SD	11·5±11·8%	7·3±5·0%
Number of tests positive (>11·1%)	14	8
Number of tests negative (<11·1%)	28	30

Results

Thirty-four per cent of lung cancer patients and 24% of hospital control subjects had abnormally high levels of Clq binding activity (table 1). Although the highest levels were seen in the lung cancer patients and the mean value was greater than that of the controls, this did not reach statistical significance (Mann-Whitney U test). As the values of Clq binding activity in the two control groups were not normally distributed, the results from the lung cancer patients and the healthy and hospital control subjects combined were compared after log transformation.

There was no association between an increased Clq binding activity and histology or survival (table 2). However, there was a significant association between disease extent and an abnormally high test (table 3). All seven patients with limited disease had negative tests while 42% of patients with extensive disease had positive tests (p=0·03). The mean Clq binding activity in patients with extensive disease (12·7±13·0%) was significantly higher than that in patients with limited disease (6·0±1·0%, p<0·005, Student's *t* test). Eleven of the 14 patients with elevated Clq binding activity were dead within six months of the test. The remaining three patients all had stage 2 squamous cell carcinoma treated with radical surgery.

Table 3 *Association between extent of disease and test results*

Stage	*ClqBA>11·1%*	*ClqBA<11·1%*	
Limited	0	7	7
Extensive	14	19	33
	14	26	40*

*One patient extent not known. p=0·03 Student's *t* test.
Limited=stage 1, extensive=stages 2 and 3.

There was no correlation between increased Clq binding activity and either a raised ESR or raised white cell count, or between survival and these factors.

Discussion

Although Clq binding may be increased by factors other than circulating immune complexes, such as infection, or macromolecules such as DNA and heparin, these were eliminated as far as possible both in patients and controls. Nevertheless there is almost complete overlap between the carcinoma and control groups which cannot be explained entirely by minor infection in the latter as these include one healthy staff member who has a persistently raised Clq binding activity, a diabetic with hypoglycaemia and no evidence of infection, and a patient with a recent stroke and no evidence of infection. Although other patients in the control group with raised Clq binding activity had exacerbations of chronic obstructive airways disease, by no means all patients with clinical evi-

Table 2 *Relation between Clq binding activity and histology or survival*

Survival				*Histology*			
Time	*ClqBA>11·1%*	*ClqBA<11·1%*	*Total*	*Type*	*ClqBA>11·1%*	*ClqBA<11·1%*	*Total*
>4/12	7	17	24	Squamous	8	11	19
<4/12	7	8	15	Adeno	0	2	2
	14	25	39* NS	Oat cell	4	7	11
				Anaplastic	1	5	6
				Other/not known	1	2	3
					14	27	41

*Two patients' survival not known.
Mean ClqBA in those who survived <4/12=12·57±9·4
Mean ClqBA in those who survived >4/12=8·89±7·5
P<0·02, Students' *t* test.

dence of chest infection had a raised Clq binding activity. It seems unlikely, therefore, that measurement of Clq binding activity will be either a useful screening or diagnostic test for lung cancer. However, patients with extensive disease (stage 2 or 3) and a raised Clq binding activity seem to have a particularly poor prognosis, and if this is confirmed Clq binding activity may prove to be a useful marker for the experimental prediction of outcome and serial monitoring of disease.

The overall prevalence of elevated serum Clq binding levels found in lung cancer patients (34%) is much lower than that reported previously in other studies. For instance, Rossen *et al*[11] detected positive reactions in 18 of 20 (90%) patients with clinically apparent lung cancer and although Heier *et al*[3] reported a lower prevalence of elevated values, 16 of 24 (67%) patients had positive results. This rose to 18 of 24 (75%) in patients with disseminated disease. In both these studies, the control populations were healthy blood donors, and the upper normal limit was taken as 4·0 and 4·7% Clq binding respectively. This is considerably lower than the base line levels established in this study using the modified Clq assay with appropriately age-matched controls (mean 7·3±5·0%) and also with the normal value established in the laboratory with healthy donors (6·1±2·5%). In this respect, it is pertinent that there was no significant difference in the serum Clq binding levels of the young healthy control population and the age-matched controls with the lung cancer patients, this group comprising inpatients with benign conditions. From these considerations it is clear that the lower prevalence of elevated Clq binding sera in the lung cancer patients reported here is to some extent the result of the control baseline. This is further emphasised by the observed mean Clq binding value in our lung cancer patients (11·5±11·8%) when compared to that reported by Rosen *et al*[11] (13·66±9%) and Heir *et al*[3] (12·0±18·1%). In our other studies elevated serum Clq binding levels compared to the normal base line value of 6·1±2·5% have been observed in carcinoma of breast[12] and osteogenic sarcoma.[13] For example, 42 of 62 (68%) sera from patients with osteogenic sarcoma showed elevated Clq binding values (mean 30·3±11·4%). Accordingly, it is concluded that there was no overall increase in serum Clq binding levels in patients with lung cancer. The numbers of patients with different histological types were not sufficient for analysis. However, considering disease stage it is evident that only patients with extensive disease showed elevated serum Clq values.

The absence of a correlation of serum Clq levels with total white blood cell count or erythrocyte sedimentation rate suggests that a raised Clq binding activity in patients with lung cancer is caused by factors other than intercurrent infection. The association with extent of disease confirms that reported previously[3 11] and explains the correlation with survival, patients with extensive disease having a poorer overall prognosis.

From our data it seems unlikely that measurement of Clq binding activity will be of value in initial diagnosis and staging of lung cancer, but it may prove useful in association with other tests of tumour burden for prognosis and assessing response to treatment.

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