Tumor Markers in Blood and in Bronchoalveolar Lavage Fluid in Patients With Lung Cancer

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Abstract

Objective: This prospective study aimed to compare the diagnostic value of several tumor markers in blood and bronchoalveolar lavage (BAL) fluid in patients with lung cancer.
Methodology: Fifty-one patients diagnosed as cancer and 44 patients with a benign lung disease were included in the study. Blood and BAL fluid samples were collected from all subjects. Levels of carcinoembryogenic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), carbohydrate antigen 15-3 (CA 15-3) and lactate dehydrogenase (LDH) activity were determined in both samples. The sensitivity and specificity of the markers were analyzed by means of receiver-operating curves.
Results: Serum CEA and CA 15-3 levels were significantly higher in the malignant group ($p<0.05$), whereas CA 19-9 and LDH levels were comparable. Concentrations of CA 19-9, CA 15-3 and LDH in BAL fluid were higher in the malignant group compared to the benign group ($p<0.05$). CEA levels in BAL fluid did not differ between the two groups. The sensitivity and specificity of CEA, CA 19-9, CA 15-3 and LDH were calculated as 21-50%, 8.6-68.6%, 34.9-84.3% and 61-56% in the blood samples, respectively. In BAL fluid samples the sensitivity and specificity of CEA, CA 19-9, CA 15-3 and LDH were 81.8-45.1%, 29-69%, 81.8-68% and 70-25% in the same order. CA 15-3 levels displayed a high specificity both in BAL fluid and blood samples.
Conclusions: Although these markers are not hundred percent specific in the diagnosis of lung cancer, we suggest that measurement of CEA in BAL fluid and CA 15-3 assessment in both body fluids will be useful as complementary laboratory tests.

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Introduction

Lung cancer is the most common cause of cancer mortality in both males and females. It constitutes 12.8% of cancer cases and causes 17.8% of cancer deaths worldwide (1). Overall, the global incidence of lung cancer is increasing at a rate of 0.5% per year (2). Rising incidence of lung cancer has been shown to be closely linked to increased cigarette smoking. Lung cancer is a rapidly increasing problem in developing countries also. In contrast to the decline of smoking in many developed countries, the prevalence of smoking in Turkey has increased significantly during the past three decades. A more recent study on cigarette consumption showed a prevalence of 63% for males and 24% for females (3). Lung cancer is the most common cancer encountered in Turkey and constitutes 42.3% of all cancers in males. The

annual age-standardized incidence rate is reported to be 61.6 per 100 000 in males, 5.1 per 100 000 in females (4).

Flexible bronchoscopy is the most widely used technique for diagnosis of lung cancer. The diagnostic yield ranges from 79% to 98% in central lesions but it decreases to 48-80% in peripheral lesions (5). Since differentiating malignant disease from benign disease in some lesions may be difficult, some additional methods are necessary to increase the diagnostic yield.

A tumor marker is a substance present in or produced by a tumor itself or produced by the host in response to a tumor, that can be used to differentiate a tumor from normal tissue or to determine the presence of a tumor based on its measurements in blood or secretions. Tumor markers can be determined easily in body fluids like blood and/or in bronchoalveolar lavage (BAL) fluid, therefore many physicians consider them as useful tools for differentiating malignant disease from benign disease. They may have different applications; screening, monitoring disease progress, detecting relapse and serving as prognostic indicators, and as diagnostic tools (6).

Tumor markers related to lung cancer are many. Among the best known are carcinoembrogenic antigen (CEA), carbohydrate antigen 15-3 (CA 15-3), carbohydrate antigen 19-9 (CA 19-9) and lactate dehydrogenase (LDH). A large series of studies have been undertaken to interpret the diagnostic value of these markers, although none of them proved to be hundred percent specific (7-12). Since in some studies only blood samples, in some studies only BAL fluid have been used and the number of studies including both are very few, the choice of sample type is still contentious (12).

This prospective study was designed to investigate the diagnostic value of CEA, CA 19-9, CA 15-3 and LDH in blood and BAL fluid of subjects with suspicion of lung cancer and to determine the correlations between blood and BAL fluid levels.

Methods

Subjects

Between January 2000 and December 2001, venous blood and BAL samples were collected from ninety-five patients who underwent diagnostic bronchoscopy. Forty-four patients (46.3%) were diagnosed as having benign pulmonary disease, and 51 (53.7%) were diagnosed as having lung cancer by bronchoscopy, fine-needle aspiration, video-assisted thoracoscopic surgery or thoracotomy results.

BAL samples

All the bronchoscopies were performed with the patient in supine position. The tip of the fiberoptic bronchoscope was wedged into the affected bronchus or into the bronchus closest to the lesion in patients in the malignant group and into the lingula or middle lobe in patients in the benign group with diffuse disease. In patients suspected of having focal benign disease, the bronchoscope was wedged into the affected lobe bronchus. Subsequently, 100 ml 0.9% sterile saline was instilled in five 20-ml aliquots. The fluid of each one was recovered by gentle suction. The total aspirated volume was transferred to the clinical biochemistry laboratory where the samples were centrifuged (500 g, 10 minutes at 4°C) to separate the cellular components from the supernatant. The supernatant fractions were used to determine the levels of different tumor markers.

Blood samples

After an overnight fast, venous blood samples were collected from all subjects just before bronchoscopy. CEA, CA 19-9 and CA 15-3 concentrations were determined on automated immunassay systems (Maggia, France), and LDH activity was determined on an automated analyzer by a commercially available kit (Dax-48, Toshiba, Japan).

Statistics and study design

SPSS 10 was used (software package for Windows version 10, IL, USA) for the statistical evaluation of the data. The accuracy of each tumor marker was defined as a function of two characteristics: sensitivity and specificity obtained from receiver operating characteristic (ROC) curves, which correlate the percentages between true and false positives. The accuracy of the system was validated by calculating the area under the ROC curve for each site. The confidence interval for accuracy refers to the range of values containing the true accuracy in the population considered, with a probability of 95%.

Results

The demographic data of the patients included in the study are summarized in Table 1. Malignant patients had a higher mean age (p=0.042). Male gender was dominant in the malignant group, whereas female gender was dominant in subjects with benign pulmonary disease.

In patients with malignant disease, there were 17 (33.3%) squamous cell carcinomas, 11 (21.6%) unspecified non-small cell carcinomas, 5 (9.8%) adenocarcinomas, 5 (9.8%) small cell carcinomas and 1 (2%) large cell carcinoma. Twelve (23.5%) patients belonged to a mixed group or had an indif-

Table 1. The characteristics of the patients

	Malignant disease	Benign disease	Total
Number of patients	51 (53.7%)	44 (46.3%)	95
Sex			
Female	6 (11.8%)	45 (88.2%)	27 (61.4%)
Male	17 (38.6%)	33 (34.7%)	62 (65.3%)
Age (years)	63.54±8.9*	59.15±11.73	

*p=0.042 as compared to benign subjects

Table 2. Mean values of the markers CEA, CA 19-9, CA 15-3, and LDH in samples of blood and BAL in the study groups

	Patients with malignant disease	
	Blood	BAL
CEA (ng/ml)	24.2±8.7*	6.91±7.57**
CA 19-9 (U/ml)	192.33±113.2**	231.39±49.61*
CA 15-3 (U/ml)	33.29±8.08*	15.14±8.49*
LDH (U/l)	614.84±302.46**	128.31±88.09*
	Patients with benign lesions	
	Blood	**BAL**
CEA (ng/ml)	4.78±6.19	6.11±13.98
CA 19-9 (U/ml)	68.08±12.9	127.72±16.07
CA 15-3 (U/ml)	13.19±1.49	5.07±1.38
LDH (U/l)	519.78±403.96	51.36±34.17

All results are expressed as means±standard deviation
*p<0.05 as compared to patients with benign lesions
**non-significant as compared to benign subjects

ferent tumor. A total of 4 (8%) tumors were classified as stage I, 2 (4%) as stage II, 16 (32%) as stage III and 28 (56%) as stage IV.

In patients with benign lesions, there were 15 (34%) cases of interstitial pulmonary disease, 10 (22.7%) pneumonia with late resolution, 6 (13.6%) sarcoidosis, 3 (6.8%) active pulmonary tuberculosis, 3 (6.8%) pulmonary abscesses, 2 (4.5%) granulomas, 2 (4.5%) with pulmonary involvement of rheumatologic disease and 3 miscellaneous cases (COPD, hydatic cyst, alveolar proteinosis).

Table 2 summarizes the levels of the investigated tumor markers in a comparative fashion. Blood levels of CEA and CA 15-3 were significantly higher in the malignant group, whereas CA 19-9 concentrations and LDH activities were not different in the two groups. BAL concentrations of CA 19-9, CA 15-3 and LDH activities were higher in the malignant group. CEA levels in BAL did not differ between the two groups.

Before performing the ROC analysis, cut-off values for the markers in blood samples were determined. These consisted of the upper values of the reference values claimed by the clinical biochemistry laboratory and were as follows; for CEA the cut-off value was 5 ng/ml, for CA 19-9 the cut-off value was <39 U/ml, for CA 15-3 the cut-off value was < 25 U/ml and finally, for LDH the cut-off value was 460 U/l. In blood, CA 15-3 displayed the highest specificity (84.3%), whereas LDH displayed the highest sensitivity (61%). In BAL samples, CEA levels displayed 82% sensitivity and 45% specificity for the cut-off value of 1.35 ng/ml, and CA 15-3 displayed 82% sensitivity and 68% specificity for the cut-off value of 2.2 U/ml. The diagnostic yields of the investigated tumor markers are summarized in Tables 3 and 4.

Discussion

In this study, the diagnostic yields of tumor markers, measured in blood and BAL fluid samples, in discriminating benign lung disease and malignant lung tumors were investigated. Blood levels of CEA and CA 15-3 and BAL levels of CA 19-9, CA 15-3 and LDH activities were significantly higher in the malignant group. The most important result we obtained was the high sensitivity and specificity of CA 15-3 in blood and its high sensitivity in BAL samples. CEA showed a higher sensitivity in BAL than in blood.

In recent years, different biochemical parameters and tumor markers have been studied in the differential diagnosis of lung cancer (6-8,11-13). However, there is no tumor marker that alone has sufficient diagnostic accuracy, especially in

Table 3. Diagnostic yields (sensitivity and specificity) of the tumor markers in BAL

	Cut-off value	Sensitivity	Specificity	Accuracy
CEA	1.35 ng/ml	82%	45%	67%
CA 19-9	64 U/ml	28.6%	68.8%	44%
CA 15-3	2.2 U/ml	81.8%	68%	75%
LDH	33 U/l	70%	25%	55%

Table 4. Diagnostic yields (sensitivity and specificity) of the tumor markers in blood

	Cut-off value	Sensitivity	Specificity	PPV	NPV
CEA	5 ng/ml	21%	50%	26%	42%
CA 19-9	<39 U/ml	28.6%	68.6%	43%	54%
CA 15-3	<25 U/ml	34.9%	84.3%	65%	61%
LDH	460 U/l	61%	56%	53%	63%

PPV: positive predictive value
NPV: negative predictive value

differentiating malignant disease from benign disease.

The most investigated tumor marker in lung cancer is CEA. Various study groups working on serum, bronchial fluid or BAL samples reported sensitivity range of 29-55% and specificity range of 78-97% for this tumor marker (10,13-15). As in our study, the cut-off value of 5 ng/ml was used in these studies. For CEA we determined a sensitivity of 21% and a specificity of 50% in the serum samples, values which are lower than those previously reported. However, in another study using 10 ng/ml as the cut-off value, a sensitivity of 52% was found (16). In studies involving BAL as sample type, different cut-off values were used in discriminating between benign and malignant lung disease. Lemaire et al accepted 10 ng/ml as the cut-off value in BAL in their study and concluded that 3% of patients with benign lesions and 61% of the malignant patients revealed higher values. The same study group found higher levels in BAL samples, and proposed that CEA levels determined in BAL were of value in the diagnosis of lung cancer (15). Another study group, Pina et al, compared serum and BAL fluid concentrations with biopsy material and concluded that CEA concentrations in BAL were the most useful marker with 90% specificity and 88% sensitivity (12). Obtaining BAL fluid is not an easy procedure and various techniques exist. Lemaire et al and Pina et al both used 150 ml of saline in obtaining BAL fluid, whereas we have used 100 ml. Different volumes may influence the dilution and lead to conflicting results.

Serum levels of CA 19-9 in the diagnosis of lung cancer have been investigated in a small number of studies, and studies involving BAL samples are even scarcer. Berthiot et al found a sensitivity of 41% (17). We found a low sensitivity, 29%, and a moderate specificity, 69%, in the blood samples. Niklinski et al reported a cut-off value of 62 U/mg protein in BAL samples, and found higher values in patients with malignant lung disease (18). In our study, from the ROC data we concluded a cut-off value of 64 U/ml which is similar to the above mentioned study. The specificity and the sensitivity of the marker were similar to those in blood samples, but the accuracy was lower (44%).

The diagnostic use of CA 15-3 has not yet been clarified in lung cancer, although this marker was shown to have a very high specificity in the diagnosis and follow-up of breast cancer. In 1990, Nutini et al found that the sensitivity of this marker was similar to that of neuron specific enolase in small cell lung cancer, and even had a better sensitivity than CEA in non-small cell lung cancer (19). After 10 years, Zimmerman et al investigated the diagnostic value of CA 15-3 in detecting adenocarcinoma in body cavity fluids and reported a high sensitivity in breast, ovary and lung cancers (20). Alataş et al investigated the discriminating power of this marker in malignant pleural effusions and reported 80% sensitivity and 93% specificity (21). On the basis of these data, CA 15-3 may also prove to be useful in the differentiation between malignant and benign lung diseases. As far as we know, there are no published studies on CA 15-3 levels in BAL samples. In our study, blood levels of this marker displayed high sensitivity, 84%, and BAL fluid levels showed high sensitivity and specificity (82% and 68%, respectively). Although our patient group is relatively small and the type of lung cancer is not homogenous, we suggest CA 15-3 may be more useful in determining malignant lung disease than the more traditional tumor markers.

LDH is an enzyme which shows a non-specific increase in different cancer types. Increased enzyme activity in the serum is usually due to cell death and the fast turnover rate observed in malignant cells (22). LDH is more often used to detect the prognosis rather than diagnosis. In one study, LDH was found to be more valuable than neuron specific enolase in predicting the prognosis in small cell lung cancer (23). In our study, we did not test LDH as a prognostic tool. We investigated the LDH levels at the time of diagnosis in different body fluids and assessed any correlations between. We found low sensitivity and specificity of LDH in blood. Although BAL levels were significantly higher in malignant cases, the accuracy was low.

The most important results of our study were the high sensitivity and specificity found for CA 15-3 levels in BAL samples, the high specificity of CA 15-3 in blood samples, and last of all, the higher sensitivity of CEA levels in BAL samples. In the malignant group, among the four tumor markers, only CA 15-3 levels showed a correlation between BAL fluid and serum values.

In conclusion, this study shows that CA 15-3 levels in blood and BAL fluid have a high sensitivity and specificity in the diagnosis of lung cancer. This tumor marker was already shown to have a high specificity in breast cancer. Its value in the diagnosis of lung cancer needs to be tested in larger series. We suggest that measurement of CEA in BAL fluid and of CA 15-3 in both blood and BAL fluid will be useful as complementary laboratory tests.

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