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ORIGINAL ARTICLE

Diagnostic Role of Tumour Markers CEA, CA15-3, CA19-9 and CA125 in Lung Cancer

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Abstract The aim of this study was to assess the diagnostic yield of the tumour markers carcinoembryonic antigen, carbohydrate antigen 15-3, carbohydrate antigen 19-9 and carbohydrate antigen 125, in serum and bronchoalveolar lavage fluid in a group of patients with bronchogenic carcinoma. Serum and bronchoalveolar lavage fluid samples were collected in a group of 90 patients with benign or malignant pulmonary diseases. After appropriate processing, tumour markers were determined by enzyme immunoassay. The diagnostic yields (sensitivity, specificity and predictive values) in each environment (serum and bronchoalveolar lavage fluid) were obtained by using "Receivers operating characteristic" curve. Determined individually, carcinoembryonic antigen, carbohydrate antigen 19-9 and carbohydrate antigen 125, showed the greatest diagnostic accuracy in bronchoalveolar lavage fluid. Carbohydrate antigen 15-3 did so in serum. Carcinoembryonic antigen was the most relevant marker in bronchoalveolar lavage fluid. For the factors evaluated in this study, determination of carcinoembryonic antigen, carbohydrate antigen 19-9 and carbohydrate antigen 125 in bronchoalveolar lavage fluid were clinically more useful markers in comparison with serum, although the latter may also be helpful in certain situations. Although there is no specific tumour marker for lung cancer, the combination of several can be used to diagnose most patients with lung cancer and also to rule out false positive and negative cases.

Keywords Tumour markers · Lung carcinoma · Bronchoalveolar lavage fluid · Serum

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Introduction

The term lung cancer is used for tumours arising from the respiratory epithelium (bronchi, bronchioles and alveoli). According to the World Health Organization classification four major cell types make up 88 % of all primary lung neoplasm. Adenocarcinoma consists of 32 %, squamous cell carcinoma 29 %, small cell carcinoma 18 % and large cell carcinoma account for 9 % of all cases. Lung cancer accounts for 29 % of all cancer death (31 % in males and 26 % in female) in the world [1]. Emerging technologies for early detection of lung cancer includes low dose helical computed tomography scan [2], computer aided digital radiography [3–5], sputum immunostaining and sputum polymerase chain reaction based oncogene detection [5–8], auto fluorescence and virtual bronchoscopy. Many studies have targeted mass spectrometry aided secretome analysis in cancer cells for screening purpose [9–12].

Tumor markers are molecules occurring in blood or tissue that are produced by a tumour associated with a cancer or by the host in response to the cancer whose measurement or identification is useful for clinical diagnosis or patient management. Tumor markers can be used

for screening a high risk population for cancer, making a diagnosis and prognosis in a specific cancer and monitoring the course in a patient in remission or while receiving surgery, radiation, or chemotherapy. The ideal marker would be a "blood test" for cancer in which a positive result would occur only in patients with malignancy, one that would correlate with stage and response to treatment and that could be easily and reproducibly measured.

Carcinoembryonic antigen (CEA) the first oncofetal antigens to be described, consists of a large family of cell surface glycoprotein (molecular weight: 150–300 kDa) found in many types of cells but associated with tumors and the developing fetus. The normal range is <2.5 μg/l in an adult non-smoker and <5.0 μg/l in a smoker. It is associated with plasma membrane of tumor cells, from which it may be released into the blood. Although CEA was first identified in colon cancer, an elevated CEA level is also found in a variety of cancers like lung, pancreas, gastric, and breast. It is also detected in benign conditions including cirrhosis, inflammatory bowel disease, chronic lung disease, and pancreatitis.

Carbohydrate antigen 125 (CA125) is a carbohydrate-related high molecular mass glycoprotein (molecular weight: >200 kDa) present in 80 % of nonmucinous ovarian carcinomas. It is defined by a monoclonal antibody (OC125) that was generated by immunizing laboratory mice with a cell line established from human ovarian carcinoma. In healthy population the upper level of CA125 is 35 U/ml. The CA125 is elevated in other cancers including lung, endometrial, pancreas, breast, and colon and in menstruation, pregnancy, endometriosis, and other gynecologic and non gynecologic conditions.

Carbohydrate antigen 19-9 (CA19-9) is a monoclonal antibody generated against a colon carcinoma cell line to detect a monosialoganglioside found in patients with gastrointestinal adenocarcinoma (molecular weight: >1,000 kDa). The upper reference range is <37 U/ml in healthy subjects. It is found it to be elevated in cases of gastric cancer, lung cancer, colon cancer and pancreatic cancer and has been proposed to differentiate benign from malignant pancreatic disease.

Cancer antigen 15-3 (CA 15-3) is a murine monoclonal antibody produced by normal breast cells (molecular weight: 300–450 kDa). In many patients with cancerous breast tumors, there is an increased production and shedding of CA 15-3 by the tumor cells. As it enters the bloodstream, is determination in blood makes it useful as a tumor marker to follow the course of the cancer. In healthy subjects the upper limit of CA 15-3 concentration is 25 U/ml. CA15-3 may also be elevated in individuals with other cancers, conditions, or diseases, such as lung cancer colorectal cancer, cirrhosis, hepatitis, and benign breast disease [13].

Levels of tumour markers in biological fluids like pleural effusion have been used to establish diagnosis of pleural malignancy [14, 15]. This study aims to evaluate the possible role of tumour markers like CEA, CA125, CA19-9 and CA15-3 in serum as well as in bronchoalveolar lavage (BAL) fluid of suspected cases in diagnosis of lung cancer.

Materials and Methods

The case control study was conducted at Calcutta National Medical College and Hospital in Kolkata, West Bengal, India, during the period August 2010–August 2011. The study was approved by the institutional ethical committee and an informed consent was taken from each participant. The study included 92 subjects in the age group 30–70 years attending the Chest Medicine department of the hospital. The cases and controls consisted each of 46 age and sex matched subjects. Patients with clinico-radiological suspicion of lung malignancy and later confirmed by computed tomography guided fine needle aspiration cytology (FNAC) of peripheral lymph node or fibre optic bronchoscopy (FOB) guided biopsy were considered as cases. Controls were individuals admitted in chest department for various other respiratory ailments like pneumonia, diffuse parenchymal lung disease, pulmonary tuberculosis (sputum smear negative) etc. in which there was indication for diagnostic bronchoscopy. Patients less than 15 years of age with history of adenocarcinoma of colon, pancreas, breast ovary, hepatocellular carcinoma, gonadal germ cell tumours, pancreatitis, hepatitis, cirrhosis of liver, inflammatory bowel disease and pregnancy were excluded from the study. Serum was separated from 3 ml of venous blood samples collected aseptically from all the cases and controls. 20 ml of BAL fluid was collected from all the diagnosed cases and controls by performing FOB under local anesthesia. The supernatant fluid was separated from the precipitated cell debris. The serum and BAL fluid from all the subjects were analyzed for estimation of levels of various tumour markers like CEA, CA-125, CA 19-9 and CA 15-3 using third generation enzyme linked immunosorbant assay (ELISA) kits (Can Ag, Canada).

Data Analysis

The statistical software namely SPSS 16.0 was used for the analysis of the obtained data. Difference between mean values of different tumour markers was assessed by 'paired *t* test'.

Strength of association between different parameters was analyzed by 'Pearson's bivariate correlation analyses.

p value was considered to be significant at value <.05, at a confidence limit of 95 %.

Effect of smoking on the levels of tumour markers in cases and controls in BAL and serum was assayed by 'Mann–Whitney test'.

Results

After fulfilling the inclusion and exclusion criteria 46 subjects were selected as cases, while 42 healthy people were selected in the control group. Statistically the matching of age between these two groups was checked by independent t test ($t = 1.61$, $p = .109$). The cases comprised of 30 males and 16 females. The control group had 24 males and 18 females in it. The gender matching between these two groups was checked by Chi-square test ($\chi^2 = 1.001$, $p > .05$, Table not shown).

The study demonstrated that the levels of all the tumour markers were higher in the cases compared to the controls. Among the cases, the BAL fluid had significantly higher values for the individual markers, than that of serum. The BAL fluid and serum values for all the measured tumour markers were compared with each other and the correlation was found to be statistically significant (Table 1).

ROC Analysis

Figures 1 and 2 show the receiver operating curve (ROC) curves for BAL and serum respectively. Based on the ROC curve, cut-off values for BAL fluid and serum have been set for all the individual tumour markers (Table 7) and the respective sensitivity, specificity and predictive values have been calculated (Table 8).

Discussion

In this study we have compared the BAL and serum levels of certain tumour markers such as CEA, CA15-3, CA19-9 and CA125 in lung cancer cases. The results showed definite elevation in levels of all the parameters in both BAL fluid and serum of all the cases compared to the controls (Table 2).

Among the cases, BAL fluid CEA levels are more increased in smokers. It indicates that tobacco induces

Table 1 Age of cases and controls

	Cases	Controls	p value
No. of subjects	46	42	
Mean age (years)	56.54 ± 2.13	53.3 ± 5.4	.109

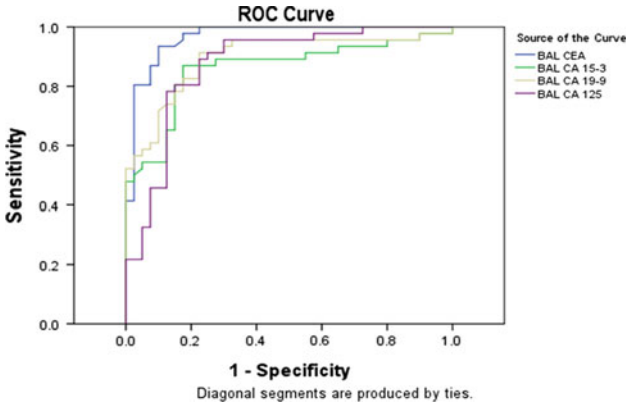

Fig. 1 Test result variable(s): ROC for CEA, CA15-3, CA19-9 and CA125 in BAL fluid

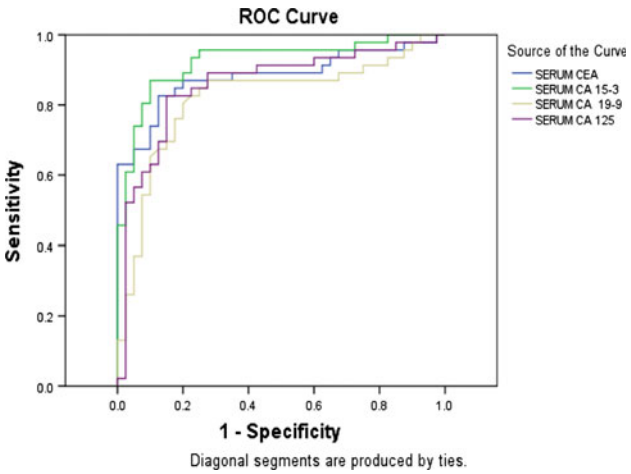

Fig. 2 Test result variable(s): ROC for CEA, CA15-3, CA19-9 and CA125 in serum

cellular alterations in the bronchial cells among the case group causing increased secretion of CEA. These results are in close agreement with other studies [16]. Moreover both age and smoking history are found to influence the evaluation of CEA levels [17, 18] (Table 3).

In our study Tables 4, 5, and 6, showed the effect of smoking on tumor marker levels in cases and control subjects. Among the cases, values for different tumour markers in BAL fluid were similar among smokers and non smokers except that of CA19-9 which was high only in smokers. Similarly, serum CA15-3 values were higher among smokers as compared to the non smoker cases. For the control subjects, however no such differences were observed for BAL or serum tumour marker values among the smokers and non smoker groups. When the entire case–control population were considered together, smoking was not found to alter the tumour marker values in BAL fluid and serum (p value <.001 for all).

ROC analysis is now a standard tool to asses, define and compare the diagnostic validity of tests. This statistical

Table 2 Mean levels of tumour markers in BAL and serum in cases and controls

Mean ± SD	CEA (μg/l)	CA15-3 (U/ml)	CA19-9 (U/ml)	CA125 (U/ml)
Cases				
BAL	31 ± 3.2	80.29 ± 10.6	141.83 ± 19.97	136.96 ± 19.03
Serum	20.55 ± 3.06	61.01 ± 4.53	62.39 ± 6.89	54.99 ± 4.74
Controls				
BAL	4.65 ± 3.77	21.66 ± 13.2	25.26 ± 15.1	29.6 ± 2.99
Serum	4.1 ± 2.1	21.94 ± 10.9	23.88 ± 18.14	25.9 ± 2.8

Table 3 Correlation data within the tumour markers in the cases

Sl. no	Parameters	*r* value	*p* value
1	BAL vs. serum CEA	.113	.001
2	BAL vs. serum CA15-3	.261	.025
3	BAL vs. serum CA19-9	.01355	<.001
4	BAL vs. serum CA125	.00658	<.001

parameter (Figs. 1, 2) had shown higher sensitivity and specificity levels for all the markers in BAL fluid compared to controls except for CA15-3 (Table 7). BAL fluid analysis in this study possessed higher predictive values compared to serum for all the markers except CA15-3. In the present study the cut off values for maximum sensitivity and lowest false positivity for the parameters CEA, CA15-3, CA19-9 and CA125 for BAL fluid were found to be <8 μg/l, <18.0 U/ml, <37.9 U/ml and <36.5 U/ml respectively. Similar cut off values for these parameters in serum were <4.5 μg/l, <28.8 U/ml, <16.0 U/ml and <24.5 U/ml respectively as evident by the ROC curves (Figs. 1, 2); (Tables 7, 8). Numerous studies have been performed to determine diagnostic or prognostic utility of tumor markers in patients with lung cancer. Charalabopoulos et al. and Niho [19, 20] in lung cancer cases had demonstrated higher BAL fluid and serum CEA levels

Table 4 Mann–Whitney test to assess the influence of smoking on tumour marker levels among cases

Case test statistics[b]								
	Serum CEA	Serum CA 15-3	Serum CA 19-9	Serum CA 125	BAL CEA	BAL CA 15-3	BAL CA 19-9	BAL CA 125
Mann–Whitney U	186.000	143.000	114.000	171.000	127.500	115.000	191.000	143.000
Wilcoxon W	252.000	209.000	180.000	801.000	757.500	181.000	257.000	773.000
Z	−.167	−1.275	−2.022	−.554	−1.674	−1.996	−.039	−1.275
Asymp. Sig. (2-tailed)	.867	.202	.043	.580	.094	.046	.969	.202
Exact Sig. [2*(1-tailed Sig.)]	.879[a]	.210[a]	.043[a]	.594[a]	.094[a]	.046[a]	.980[a]	.210[a]

[a] Not corrected for ties

[b] Grouping variable: smoking

Table 5 Mann–Whitney test to assess the influence of smoking on tumour marker levels among controls

Control test statistics[b]								
	Serum CEA	Serum CA 15-3	Serum CA 19-9	Serum CA125	BAL CEA	BAL CA 15-3	BAL CA19-9	BAL CA 125
Mann–Whitney U	127.500	139.000	140.000	105.000	145.000	119.000	120.000	110.500
Wilcoxon W	182.500	604.000	605.000	570.000	200.000	174.000	175.000	575.500
Z	−.703	−.344	−.312	−1.406	−.156	−.968	−.937	−1.234
Asymp. Sig. (2-tailed)	.482	.731	.755	.160	.876	.333	.349	.217
Exact Sig. [2*(1-tailed Sig.)]	.488[a]	.747[a]	.770[a]	.167[a]	.890[a]	.346[a]	.363[a]	.221[a]

[a] Not corrected for ties

[b] Grouping variable: smoking

Table 6 Mann–Whitney test to assess the influence of smoking on tumour marker levels among both cases and controls

All subject statistics[a]								
	Serum CEA	Serum CA 15-3	Serum CA 19-9	Serum CA125	BAL CEA	BAL CA 15-3	BAL CA19-9	BAL CA 125
Mann–Whitney U	61.500	259.500	194.500	231.000	217.000	138.000	335.000	262.000
Wilcoxon W	881.500	1079.500	1014.500	1051.000	1037.0	958.000	1155.000	1082.000
Z	−7.433	−5.719	−6.281	−5.965	−6.087	−6.771	−5.065	−5.697
Asymp. Sig.(2-tailed)	.000	.000	.000	.000	.000	.000	.000	.000

[a] Grouping variable: grouping

Table 7 Cut-off values for individual tumour markers in BAL fluid and serum

Cut-off values		
Tumour markers	BAL fluid	Serum
CEA	<8 μg/l	<4.5 μg/l
CA15-3	<18.0 U/ml	<28.8 U/ml
CA 19-9	<37.9 U/ml	<16.0 U/ml
CA 125	<36.5 U/ml	<24.5 U/ml

respectively, compared to benign lung lesion controls. Lazarev et al. [21], however have doubted the utility of estimation of biological tumour markers like CEA for its low sensitivity for diagnosis and treatment of non small cell lung cancer (NSCLC) cases. On the contrary retrospective review upon two hundred and seventy-seven diagnosed NCLC patients had shown significantly elevated serum CEA and CA125 levels at baseline [22]. Moreover CEA levels at baseline was found to correlate with overall survival time in both small and non small cell lung carcinoma cases [22, 23]. Similar studies conducted upon 51 lung cancer patients and 44 patients with a benign lung disease showed serum CEA, CA-125, CA 19-9 and CA 15-3 levels were significantly higher in the malignant group ($p < .05$). The results have validated the cut off values for CEA, CA15-3 and CA19-9 in BAL and serum at a level of 1.35 μg/l, 5 μg/l; 2.2 U/ml, <25 U/ml and 64 U/ml; <39 U/ml respectively [24]. In comparison to these values we found the cut off values at a higher degree of sensitivity and false positivity that might increase their diagnostic values. Studies conducted to evaluate the diagnostic usefulness of the tumor markers CA125, CEA in BAL fluid in 30 NSCLC have shown CEA and CA125 concentration in BAL fluid were significantly higher in NSCLC patients than in healthy volunteers and patients with sarcoidosis [25]. The sensitivity and specificity of CEA and CA 125 in BALF were 100 %, 84 % and 92 %, 80 %, respectively.

Similar examination conducted upon seven tumour markers CEA, AFP, CA19-9, squamous cell carcinoma antigen (SCC), neuron-specific enolase (NSE),CA125, cytokeratin 19 fragment (CYFRA) in 200 patients with adenocarcinoma have concluded that CEA had the highest positivity rate (46.5 % of patients) followed by CA125, the positivity rate increasing with advancing stage [26]. Other study results have shown that CEA and CA125 have different diagnostic yield for lung carcinoma in different environment like BAL, serum and cytosol biopsy [27].

This study has limitations that must be considered. Number of patients in the study groups was not large. Thus, care must be taken in extrapolating the present findings to other populations. Despite these limitations, we believe that our study will be helpful as assay of the above mentioned tumor markers is simple and will complement other tests in diagnosis of lung cancer.

In conclusion, the results suggest that among the chosen markers, combined measurement of CEA, CA19-9 and CA125 in BAL fluid and CA15-3 in serum is useful in diagnosis of lung cancer. These may be useful in patients in

Table 8 Sensitivity, specificity and predictive values for individual tumour markers based on ROC

Parameters	CEA		CA 15-3		CA 19-9		CA 125	
	BAL (%)	Serum (%)	BAL (%)	Serum (%)	BAL (%)	Serum (%)	BAL (%)	Serum (%)
Sensitivity	91.3	89.13	89.13	93.48	91.3	89.13	89.13	91.3
Specificity	90	65	45	80	77.5	32.5	75	62.5
Positive predictive value	91.3	74.55	65.08	84.31	82.35	60.29	80.39	73.68
Negative predictive value	90.3	83.87	78.26	91.43	88.57	72.22	85.71	86.21

whom tumour cannot be visualized by bronchofibroscopy or to rule out false positive cases. These results need further confirmation in larger groups of patients.

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