

## Original Research Article

# Evaluation of serum amyloid A, soluble E-selectin and soluble E-cadherin as lung cancer biomarkers

Varinder Saini<sup>1\*</sup>, Chikkahonnaiah Prashanth<sup>1</sup>, Jasbinder Kaur<sup>2</sup>,  
Ashok Kumar Janmeja<sup>1</sup>, Seema Gupta<sup>2</sup>, Shivani Jaswal<sup>2</sup>

<sup>1</sup>Department of Pulmonary Medicine, Government Medical College and Hospital, Chandigarh, India

<sup>2</sup>Department of Biochemistry, Government Medical College and Hospital, Chandigarh, India

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### \*Correspondence:

Dr. Varinder Saini,

E-mail: [saini\\_vks@yahoo.com](mailto:saini_vks@yahoo.com)

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## ABSTRACT

**Background:** Lung cancer screening is a challenge. Sputum cytology, chest X-ray, low dose computed tomography and other screening methods have not proved to be very effective. Serum biomarkers are a new hope in screening of lung cancer. The present study was planned to evaluate sensitivity and specificity of serum levels of amyloid A (SAA), soluble E-selectin (sE-selectin) and soluble E-cadherin (sE-cadherin) as lung cancer biomarkers.

**Methods:** An observational and cross-sectional study comprised of three groups with 20 subjects each of proven lung cancer cases, patients with non-malignant respiratory diseases and healthy controls. Levels of SAA, sE-selectin and sE-cadherin were measured by solid phase sandwich ELISA. Individual and collective sensitivity and specificity of these biomarkers was analysed and cut off values calculated by receiver operating curves.

**Results:** A statistically significant difference was found in the median levels (ng/ml) of SAA in patients of lung cancer, other non-malignant respiratory diseases, and healthy controls, the levels (Mean±SD) being 24980.50±6564.14, 9961.10±2000.24 and 580.95±334.94 respectively in the three groups. A sensitivity of 80% and specificity of 55% was found when SAA levels of 1068 ng/ml were taken as cut off for screening of lung cancer. However, no significant difference was found in the serum levels of sE selectin and sE cadherin between the three groups. Moreover, significant association of biomarkers could also not be established with lung cancer when they were used in combination.

**Conclusions:** In this preliminary report from India, SAA has been found to be a promising biomarker in screening for lung cancer.

**Keywords:** Biomarkers, Lung cancer, Serum amyloid A, sE-selectin, sE-cadherin

## INTRODUCTION

Lung cancer has remained one of the commonest cancers and is the most important cause of cancer related deaths.<sup>1</sup> Symptoms in patients of lung cancer are non-specific and include persistent cough, haemoptysis, shortness of breath, chest pain and debility, which are also seen in many other non-malignant lung diseases. Commonly

used investigations for the diagnosis of lung cancer are CT FNAC and bronchoscopy both being interventional. Despite the development of new treatment and therapies designed to increase the 5 year survival rate, lung cancer still remains the deadliest cancer.<sup>2</sup> Screening with low dose computed tomography (LDCT) has been advocated but detection of false positive cases with it has been found to be upto 50%, as it also detects non-calcified

nodules.<sup>3</sup> Thus evidence to use LDCT scan as a screening tool is insufficient, although it has been found to reduce the mortality among high risk smokers.<sup>4</sup> There is, therefore, a felt need for establishing newer screening techniques as well as novel biomarkers for the early detection of lung cancer.

Proteomes change in response to cancer and there is hope that onco-proteomic research may help in early cancer diagnosis, prognosis and individualized treatment. Currently there is no promising diagnostic biomarker for lung cancer.<sup>5</sup> Various studies have been conducted to identify biomarkers of lung cancer and serum amyloid A (SAA), soluble E-selectin (sE-selectin) and soluble E-cadherin (sE-cadherin) have generated encouraging results regarding their use in the diagnosis of lung cancer.<sup>6-10</sup>

The level of SAA which is an acute inflammatory marker is seen to increase as the cells progress to dysplasia and neoplasia.<sup>11</sup> sE-selectin is induced in tumor cells by release of cytokines like interleukin-1 and tumor necrosis factor- $\alpha$ . The tumor cells through their receptors bind to E-selectin on endothelial cells and cause tissue transmigration.<sup>9</sup> E-cadherin being an adhesion molecule, its dysregulation due to either reduced production or altered function is associated with metastasis. In addition to adhesion, its loss is also associated with induction of several transcription factors. Thus, it plays a crucial role in metastasis.<sup>12</sup> Existing data on the role of soluble adhesion molecules in lung cancer is limited from this part of the world.

Studies so far have shown that SAA is a specific, but less sensitive and sE-selectin is a sensitive, but less specific biomarker of lung cancer.<sup>8,9</sup> Studies have also shown sE-cadherin to be a marker of distant metastasis and poor prognosis.<sup>6,9</sup> Also there is evidence to suggest usage of sE-cadherin for monitoring of disease progression.<sup>13</sup>

Thus, the combination of these biomarkers is expected to improve the possibility of screening the patients for lung cancer. If the association is found to be strong enough, these markers may be used as first line non-invasive tests in patients having high clinical suspicion of lung cancer. Hence, the present study was planned to measure the serum levels of SAA, sE-selectin and sE-cadherin in patients of lung cancer and to evaluate their sensitivity and specificity in the diagnosis of lung cancer.

## METHODS

The study was designed as an observational and cross-sectional study. Based on the age standardized incidence of lung cancer in 2008 in India which was 10.9 and 2.2 cases per lakh population for men and women respectively, it was estimated that 20 subjects shall be required as cases and controls to give a power of 90%.<sup>14</sup> Therefore, twenty patients with freshly diagnosed and proven lung cancer, twenty age and sex matched patients

with other non-malignant respiratory diseases and twenty age and gender matched apparently healthy individuals formed the subjects of the study. Evidence of cancer was proven by demonstration of malignant cells on histopathology and the patients were diagnosed to have small cell carcinoma (SCC, n=5) and non-small cell lung cancer (NSCLC, n=15). There were 9 cases of lung cancer with metastasis (all were in stage IV) and 11 such cases without metastasis (6 patients of stage III-B and 5 cases of stage III-A).

In the category of non-malignant respiratory diseases, there were patients of pneumonia (n=4), interstitial lung disease (n=2), COPD (n=7), bronchial asthma (n=3) and tuberculosis (n=4).

Subjects below 18 years of age, patients either on treatment or having completed treatment for lung cancer, patients with secondaries in the lung with primary tumor elsewhere in the body, pregnant females and critically ill individuals were excluded.

Ethical clearance was obtained from the Institutional Ethics Committee. Informed consent was taken on a standard proforma from all subjects of the study. After a detailed history and thorough clinical examination, five ml of blood was collected using all aseptic precautions. Serum was separated and stored at  $-70^{\circ}\text{C}$  for SAA and sE-selectin and at  $-20^{\circ}\text{C}$  for sE-cadherin. The serum levels of biomarkers were measured in all the three groups by solid phase sandwich ELISA (SAA by Hycult Biotech, Netherland, sE-selectin by Diaclone SAS, France and sE-cadherin by Boster, California). The analysis was performed according to manufacturer's instructions.

The statistical analysis was carried out using SPSS. Data obtained was analysed by chi-square test. Statistical significance was considered for  $p < 0.05$ . Cut off value of the serum bio-markers was calculated by receiver operating characteristic curves. Based on this cut off value (derived by Youden's index) and considering demonstration of malignant cells as gold standard, sensitivity and specificity of each serum marker was calculated individually and collectively.

## RESULTS

The study comprised of three, age and sex matched groups of twenty subjects each of patients of lung cancer, patients with other non-malignant respiratory diseases and healthy controls. Demographic profile and symptomatology in subjects of the three groups is given in Table 1.

Cough was found to be the most common symptom in patients with lung cancer, while both cough and shortness of breath were the commonest symptoms in patients with other non-malignant respiratory diseases (Table 1). It was also found that the patients approached the hospital early

seeking treatment for hoarseness of voice. SAA was found to be significantly raised in patients of lung cancer, as compared to patients with other non-malignant

respiratory diseases or healthy individuals as shown in Table 2.

**Table 1: Demographic profile and symptomatology in subjects of the three groups.**

	Lung cancer (n=20)	Non-malignant respiratory diseases (n=20)	Healthy controls (n=20)	p-value
Mean age (years)	60.50±10.54	62.80±11.84	57.40±7.91	0.254
Number of males	16	16	16	1.0
Number of females	4	4	4	1.0
Number of smokers	17	13	16	0.814
Smoking index (pack years)	46.65	46.15	45.13	
<b>Incidence (%) and duration of illness (months) of symptomatology</b>				
Cough	85%	100%	-	
	3.19	1.21		
Haemoptysis	20%	0%	-	
	2.95	-		
Shortness of breath	75%	80%	-	
	3.37	1.18		
Chest pain	45%	5%	-	
	2.26	6.00		
Hoarseness of voice	5%	5%	-	
	1.00	2.00		
Fever	15%	10%	-	
	1.50	0.78		

**Table 2: Mean± SEM (ng/mL) levels of SAA, sE-selectin and sE-cadherin in the subjects of the three groups.**

	Lung cancer (n=20)	Non-malignant respiratory diseases (n=20)	Healthy controls (n=20)	p-value
SAA	24980.50±6564.14	9961.10±2000.24	580±334.94	0.0007
				0.0348
sE-selectin	80.77±23.17	107.47±15.33	103.66±13.58	0.399
				0.342
sE-cadherin	1042.97±200.62	1319.02±207.12	1324.35±241.35	0.375
				0.344

**Table 3: Mean±SEM (ng/mL) levels of SAA, sE-selectin and sE-cadherin in SCC and NSCLC and metastasis and with no metastasis lung cancer patients.**

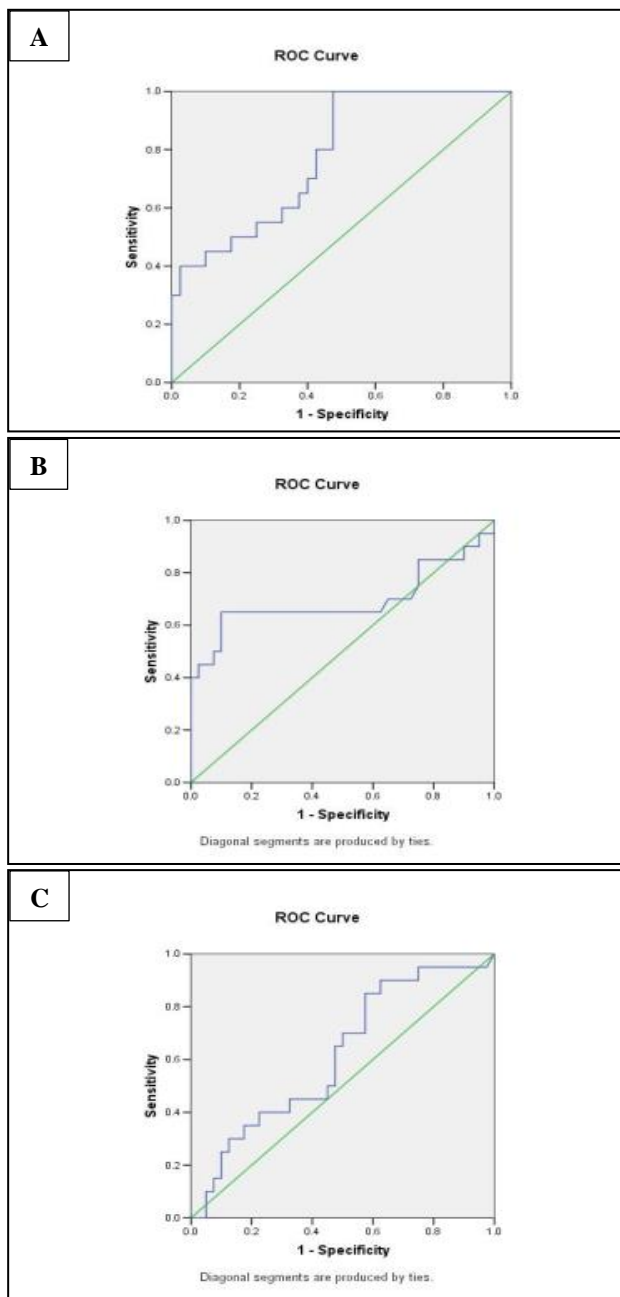
SAA	SCC (n=5)	NSCLC (n=15)	Metastasis (n=9)	No Metastasis (n=11)
	11888.00±7540.29	29344.67±8192.21 p=0.423	35612.22±12715.52	16281.82±5119.06 P=0.26
sE-selectin	75.04±34.24	82.67±29.30 P=0.884	55.13±20.25	101.74±38.58 P=0.869
sE-cadherin	911.42±342.69	1086.82±247.18 P=0.395	1046.80±402.63	1039.84±181.46 P=0.688

SAA showed a sensitivity of 80% and specificity of 53% in screening of lung cancer with a cut off value of 1068ng/ml (area under cover= 0.779) (Figure 1).

Patients with lung cancer had lower serum levels of sE-selectin and sE-cadherin as compared to patients with other non-malignant respiratory diseases and healthy

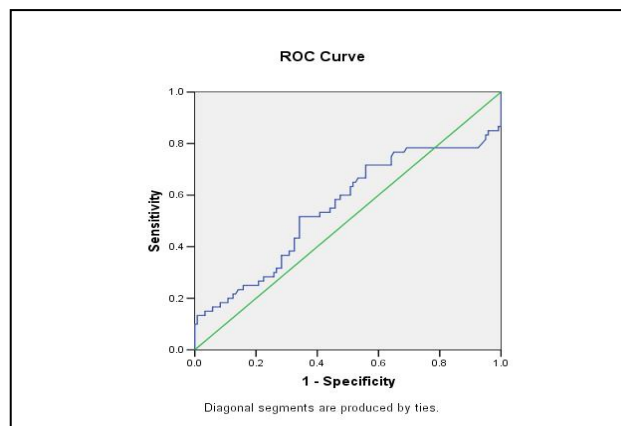
controls (Table 2). However, the difference was not found to be statistically significant. Moreover, sE-selectin and sE-cadherin were not found to be statistically significant in screening for lung cancer when used individually (area under curve=0.69 and 0.61 respectively) (Figure 1).

When SAA, sE-selectin and sE-cadherin were used in combination, the area under curve was 0.552 showing no significant association for screening of lung cancer (Figure 2).



**Figure 1: Sensitivity and specificity of SAA, sE-selectin and sE-cadherin for diagnosis of lung cancer. A) SAA (AUC: 0.77), (95% CI: 0.661-0.897). B) sE selectin (AUC= 0.69), (95% CI: 0.520-0.867). C) sE cadherin (AUC=0.61), (95% CI: 0.467-0.764).**

There was no statistical difference in serum levels of these biomarkers in metastatic or non-metastatic group of patients (Table 3). No significant difference was observed in the mean levels of different biomarkers when the mean levels were compared between SCC and NSCLC (Table 3).



(AUC=0.55, 95% CI: 0.456-0.647).

**Figure 2: Sensitivity and specificity of levels of SAA, sE-selectin and sE-cadherin in combination for the diagnosis of lung cancer.**

### DISCUSSION

Lung cancer has a high mortality rate as it is often detected in the advanced stage. The common symptomatology in lung cancer and other lung diseases often leads to misdiagnosis and hence, delay in treatment. Early detection of lung cancer by effective screening procedures is expected to improve the treatment outcomes.<sup>15,16</sup>

The present study was conducted to evaluate levels of SAA, sE-selectin and sE-cadherin as lung cancer biomarkers. The commonest symptom in lung cancer and diseased controls was found to be cough. The overlapping symptomatology fails to diagnose the condition precisely, leading to delay in diagnosis and treatment of patients of lung cancer, which may be an important cause of morbidity and mortality associated with this disease.

The mean duration of cough and shortness of breath in lung cancer patients was found to be 3.07 and 2.96 months respectively, while that of hoarseness of voice was 0.75 months. Thus, it was found that patients with hoarseness of voice approached the hospital early.

In the present study SAA levels were found to be significantly elevated in patients with lung cancer as compared to the patients with non-malignant lung diseases and healthy controls (Table 2). SAA levels were increased by about 40 times in patients with lung cancer as compared to healthy controls. The probable reason for this raise could be explained by the fact that several steps of tumorigenesis overlap with that of inflammation. These steps include cell adhesion and migration, induction of enzymes involved in the degradation of extracellular matrix and inhibition of cell adhesion to extracellular matrix proteins leading to an increase in SAA also in addition to the other acute phase proteins.<sup>10</sup> It has been suggested that SAA might be used to detect a

pattern of physiological events that reflect the growth of malignancy and host response. Increased levels of SAA have been found to distinguish patients of lung cancer from normal individuals.<sup>7</sup> Level of SAA has also been associated with poor prognosis in patients of lung cancer.<sup>10</sup>

The overall sensitivity and specificity of SAA for detecting lung cancer was found to be 80% and 53% respectively (AUC: 0.779; 95% CI: 0.661-0.897) (Figure 1). In a meta-analysis, reports from East Asia, Europe and America have shown SAA as a potential diagnostic biomarker for lung cancer having higher specificity for discerning lung cancer but not appropriate for screening because of lesser sensitivity.<sup>17</sup> Results of the present study reveal that SAA levels can be used as a non-invasive biomarker for screening of lung cancer. Our results are at variance from the findings of the meta-analysis that had suggested a better role in distinguishing lung cancer but not for screening in view of a high specificity but lower sensitivity.

There was no statistical difference in serum levels of these biomarkers in metastatic or non-metastatic group of patients (Table 3). While high levels of SAA have been demonstrated in NSCLC than SCC in meta-analysis, no significant difference was observed in the mean levels of SAA when the mean levels were compared between SCC and NSCLC patients of our study indicating that the biomarker could not be used for distinguishing between the different types and dissemination of the lung cancer (Table 3). These variations in our results could be due to the differences in the breakup of various types of lung cancers studied and a relatively smaller number of the same in our study. So study with a larger sample size involving different histological types of lung cancers in different stages needs to be undertaken to establish exact diagnostic and prognostic role of SAA.

sE-cadherin levels were found to be decreased in lung cancer patients as compared to patients with other non-malignant respiratory diseases or healthy controls, although this decrease was not found to be statistically significant. E-cadherin is the prime mediator of epithelial cell-cell adhesion acting in a homotypic fashion which participates in the development and maintenance of the epithelial tissues. Loss of function or/and the expression of E-cadherin/catenin complex impairs cell adhesiveness resulting in a loss of normal tissue architecture.<sup>6</sup> It has been found that loss of E-cadherin leads to reduced cell to cell adhesion and metastasis.<sup>18</sup> Reduced or absent expression of E-cadherin has been found in a variety of human cancers-medullary, gastric, head and neck, bladder, prostate, breast cancer.<sup>6,18</sup> Low levels of cadherins have also been associated with lung cancer.<sup>19</sup> Further studies including a larger number of patients are needed in order to establish the role of sE-cadherin as a useful biomarker in the diagnosis of lung cancer.

In the present study serum levels of sE-selectin were found to be decreased in patients of lung cancer as compared to patients of non-malignant lung diseases and healthy controls. However, this decrease was not found to be statistically significant.

E-selectin is not normally expressed by lung endothelium, but its expression can be induced after activation by inflammatory cytokines.<sup>9</sup> It mediates recruitment of neutrophils during inflammation, adhesion of cancer cells to endothelium and its expression in neoplastic tissue indicating vascular involvement.<sup>20</sup>

The role of sE selectin in lung cancer has been studied and increased levels have been found to be associated with lung cancer.<sup>9,21,22</sup> The results of this present study are however contrary to those available in the literature.

The present study is the preliminary report from India, to evaluate the role of two major adhesion molecules- sE-cadherin and sE-selectin along with an acute phase reactant protein known to be involved in tumour pathogenesis namely SAA, in patients of lung cancer. Results of the study highlight the importance of increased levels of SAA in distinguishing patients of lung cancer from patients with non-malignant lung diseases. The potential role of soluble adhesion molecules in detection of lung cancer was also evaluated. Low levels of sE-cadherin could be used as an adjunct to other diagnostic tests in detection of lung cancer. However, the role of sE-selectin could not be validated. Further studies with larger sample size are required to establish the use of these biomarkers in screening, diagnosis and prognosis of lung cancer.

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*Ethical approval: The study was approved by the institutional ethics committee*

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