ORIGINAL ARTICLE

Evaluation of a Panel of Biomarkers in the Diagnosis of Lung Cancer: An Observational Study

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Received on: 21 April 2023; *Accepted on:* 31 August 2023; *Published on:* 30 October 2023

Abstract

Aims and background: Carcinoma of lung features is one of the most frequently encountered malignancies in the world, which accounts for most cancer deaths. To buckle down this deadly disease, novel tools for early diagnosis need to be explored. Biomarkers, being a simple and noninvasive method, are being researched all over the world for their role in screening, early detection, and confirmation of the diagnosis of specific types of cancer, as well as to predict the prognosis, monitor response to treatment, and detect recurrence.

Materials and methods: The current study evaluated the values of neuron-specific enolase (NSE), cytokeratin fragment 19 (CYFRA 21-1), carcinoembryonic antigen-related cell adhesion molecule (CEACAM), C-reactive protein (CRP), and lactate dehydrogenase (LDH) were evaluated in 75 participants, 25 each of lung cancer, benign disease, and healthy controls respectively, both individually as well as together as a panel in various combinations of set of two/three/four/five biomarkers for diagnosis, accuracy, sensitivity, and specificity of all the different combinations was calculated.

Results: Mean serum levels of all these biomarkers were elevated in patients with lung cancer vs normal healthy controls. It was inferred that the best panel was a combination of two biomarkers, CRP and NSE, together, which yielded a specificity of 94%, accuracy of 82.67%, and sensitivity of 60% (*p*-value of <0.001).

Conclusion: Biomarkers, both individually and as a combination as a panel may serve as useful tools for early detection of this deadly disease.

Keywords: Biomarkers, C-reactive protein, Lung cancer, Neuron-specific enolase.

Indian Journal of Respiratory Care (2023): 10.5005/jp-journals-11010-1061

Introduction

Lung cancer, once a rare disease, is now among the most common malignancies with poor survival rate.[1] Smoking is the strongest risk factor linked with the occurrence of lung cancer.[2] Symptoms of lung cancer (cough, chest pain, dyspnea, and hemoptysis) usually occur when the tumor has spread to distant sites. The detection of lung cancer entails a combination of imaging tests like computed tomography of the thorax and tissue diagnosis attained by fine needle aspiration cytology or endobronchial biopsy. However, these invasive tests have certain limitations and associated complications like pneumothorax, hemoptysis, and an increase in breathlessness.

Discovering different biomarkers for the finding of this malignancy has always garnered attention and has been considered for a long time. Being noninvasive and safe, there is a pressing need to find a sensitive and specific biomarker for the early diagnosis of this malignancy. Also, the diverse mechanisms of biomarker synthesis could give new prospects for research that could aid in the development of new drugs for this potentially fatal disease.

Many novel molecular biomarkers are continuously emerging and are being investigated for their vital part in the detection of this malignancy as well as for establishing prognosis.[3] Cytokeratin fragment 19 (CYFRA 21-1) is a soluble circulating tumor marker that increases in lung cancer subjects.[4] Similarly, carcinoembryonic antigen-related cell adhesion molecule (CEACAM), a biomarker implicated in the course of cell adhesion and modulation, has been shown to have raised levels in lung cancer.[5] Neuron-specific enolase (NSE) is an isoenzyme of enolase and is currently being delved into for the diagnosis of lung cancer.[6] Lactate dehydrogenase (LDH) is another sensitive marker of the cellular state as well as of tumor growth.[7] C-reactive protein (CRP) is an acute phase reactant that is linked with inflammation. Lung cancer patients have chronic inflammation, therefore explaining the raised levels of CRP.[8]

Previous literature has shown that these biomarkers have been evaluated independently for the analysis of lung malignancy.

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How to cite this article: Bhatia C, Jaswal S, Sodhi MK, *et al.* Evaluation of a Panel of Biomarkers in the Diagnosis of Lung Cancer: An Observational Study. Indian J Respir Care 2023;12(3):244–247.

Source of support: Nil

Conflict of interest: None

However, the maximum number of these studies have been conducted in the West. Moreover, very limited studies have been done evaluating the diagnostic role of these biomarkers together as a panel. Hence, the present study evaluated these five biomarkers, namely CYFRA 21, CEACAM, NSE, LDH, and CRP, individually and in various combinations for their sensitivity and specificity in the uncovering of this malignancy.

Neuron-specific enolase (NSE), when clubbed together with CRP, yielded the highest specificity (94%) and accuracy among all biomarker combinations used, thus inferring their use may help in the early detection and timely management of this disorder.

Materials and Methods

The study was conducted in the Department of Pulmonary Critical Care, and Sleep Medicine in collaboration with the Department of Biochemistry, Pathology, and Radiology at Government Medical College and Hospital, Chandigarh. It was an observational cross-sectional study conducted over a period of 1 year and 6 months (October 2018–June 2020). A total of 75 subjects formed the study group. The optimum sample size was established by seeing the percentage of lung cancer cases with increased levels of biomarkers available in the literature, assuming a 95% confidence level and 20% absolute precision.[5] The objective of the current study was to measure levels of LDH, CYFRA 21-1, CRP, NSE, and CEACAM and to explore their utility both individually and as a panel in the diagnosis of lung cancer. Informed consent was received from all subjects. The Institutional Ethics Committee of GMCH approved the study.

Out of 75 subjects, 25 patients had histologically/cytologically proven treatment for lung cancer (group I). A total of 20 patients of noninfectious other respiratory diseases like chronic obstructive pulmonary disease and asthma were enrolled from the outpatient department (group II). The remaining 25 were age and gender-matched, apparently healthy adults who wished to voluntarily participate in the study (group III). Subjects in groups I and II who had superadded infection or patients with diabetes, human immunodeficiency virus coinfection, those taking immunosuppressive drugs, and critically sick patients were excluded from the study.

Baseline demographic as well as clinical information of the patients was recorded. Thereafter, 5 mL of blood was collected under strict aseptic conditions from participants. Serum CYFRA 21-1 (kit—ElabSciences), NSE (kit—DRG), and CEACAM (kit—RavBiotech) were estimated by enzyme-linked immunosorbent assay, while LDH and CRP were measured by spectrophotometric methods on random access fully automated chemistry analyzer (Modular P-800 and Rx Imola).

Serum levels of the biomarkers were compared (both individually and in combinations) among the three groups of subjects for any statistically significant difference. Patients in groups II and III who had deranged levels for any of the biomarkers were given the option for further evaluation.

Statistical Analysis

Data were statistically described in terms of mean ± standard deviation, median with range or frequencies, wherever suitable. Comparison of continuous variables between two groups was done using student's *t*-test for independent variables/Mann–Whitney *U* test. The cut-off value of serum biomarkers was calculated by the receiver operating characteristic curve. Each biomarker was gauged for sensitivity, specificity, and accuracy to assess the utility of these in the diagnosis of lung cancer. $p < 0.05$ was taken as significant. The statistical analysis was performed using Statistical Package for the Social Sciences software.

Results

The mean age of patients in groups I, II, and III was 55.88 ± 5.71, 55.04 ± 8.97, and 48.96 ± 4.95 years, respectively ($p = 0.695$). No significant alteration was seen in the gender distribution in the study cohort (males = 43; females = 32; $p = 0.059$). Out of 49 (65.3%) ever smokers in the study subjects, 19 were in the lung cancer group, followed by 16 and 14 in the groups II and III, respectively ($p > 0.05$). In contrast to the number of smokers, the smoking index was statistically different in the three study groups ($p = 0.001$), implying a strong association of smoking with lung cancer.

Comparison of Individual Biomarkers in the Three Study Groups

As we can see in Table 1, out of the five markers, LDH levels were not statistically diverse in the three groups. Levels of both CEACAM and CRP were meaningfully raised in lung cancer patients when compared to those with benign lung diseases as well as healthy adults, separately ($p < 0.05$). Both NSE and CYFRA 21-1 levels were elevated in subjects with lung cancer as compared to healthy adults ($p = 0.019$ and 0.035, respectively). However, these were not statistically different when equated with patients with benign lung diseases (group II).

As we can interpret from Table 2, the best serum cut-off levels of NSE for diagnosis of lung cancer were estimated to be 11.35 ng/mL,

Table 1: Mean serum values of LDH, CRP, NSE, CEACAM, CYFRA 21-1 in three study groups

Biomarker	*Group I*	*Group II*	*Group III*	*p-value* *Groups I and II* *Group I and III*
LDH (IU/L)	480.68	439.56	354.32	0.19 0.08
CRP (mg/mL)	85.48	35.60	2.32	<0.001 <0.001
NSE (ng/mL)	23.35	10.80	8.31	0.061 0.019
CEACAM (ng/mL)	527.91	419.10	433.56	0.034 0.042
CYFRA 21-1 (ng/mL)	1.00	0.61	0.50	0.12 0.035

CEACAM, carcinoembryonic antigen-related cell adhesion molecule; CRP, C-reactive protein; CYFRA 21-1, cytokeratin fragment 19; LDH, lactate dehydrogenase; NSE, neuron-specific enolase

Table 2: Sensitivity and specificity of individual biomarkers

Biomarker	*Sensitivity*	*Specificity*	*NPV*	*PPV*	*Accuracy*
CRP	76%	90%	88.24%	79.17%	85.33%
NSE	76%	76%	86.36%	61.29%	76%
CYFRA 21-1	40%	88%	74.58%	62.50%	72%
CEACAM	60%	66%	76.74%	46.88%	64%
LDH	40%	84%	73.68%	55.56%	69.33%

CEACAM, carcinoembryonic antigen-related cell adhesion molecule; CRP, C-reactive protein; CYFRA 21-1, cytokeratin fragment 19; LDH, lactate dehydrogenase; NPV, negative predictive value; NSE, neuron-specific enolase; PPV, positive predictive value

with sensitivity, specificity, and accuracy of 76%. The levels had a high negative predictive value in the recognition of lung cancer (86.36%).

The levels of CRP were found to be augmented in both the patients of lung cancer and benign lung diseases. A statistically significant difference was extant in subjects with lung malignancy when equated to healthy controls ($p < 0.001$), which was highest among all biomarkers. The best cut-off value for CRP 35.4 mg/mL was taken with an area under the curve of 87.2%, which shows it had a very good performance. CRP showed a high specificity of 90%, a high sensitivity of 76%, and a high accuracy of 85.33%.

For CYFRA 21-1, the best serum cut-off levels for diagnosis of lung cancer were observed to be 0.98 ng/mL. The sensitivity of CYFRA 21-1 was 40%, specificity of 88%, and accuracy of 72%.

Levels of CEACAM were found to be higher in patients with lung cancer as compared to the patients with benign lung diseases and healthy controls. On comparing values among groups I and III, the change was statistically significant ($p < 0.042$). The best cut-off value for CEACAM was reserved as 333.34 ng/mL with sensitivity and specificity of 60 and 66%, respectively.

Table 3 shows the outcomes of different possible combinations of these biomarkers as a panel. We made a combination of sets of two/three/four/five biomarkers together. The best sensitivity (60%) and accuracy (82.67%) were found with CRP and NSE together. Specificity was 100% for the amalgamation of both CRP and CYFRA 21-1 and also for NSE and CYFRA 21-1 together. CRP had the best specificity of 90% and accuracy of 85%.

Discussion

Carcinoma lung is encountered as the chief cause of cancer-related mortality worldwide.[9] Presentation at a progressive stage leads to dismal survival. Molecular targeted therapy has greatly revolutionized the management of these tumors. However, the need of the hour is to develop noninvasive tests for early detection. Biomarkers are one such group of noninvasive diagnostic tests that seem a promising tool not only in screening but also in the diagnosis and prognosis of a lung cancer patient.[5,8] To date, many biomarkers have been considered in lung cancer; however, the search for an ideal biomarker continues as one that can accurately and reliably diagnose lung cancer at an initial step continues. In this context, many studies have explored the utility of a single vs a combination of biomarkers. Due to the heterogeneity of the respiratory system and varied presentation of different kinds of lung histologies in lung cancer, the utility of a combination of biomarkers seems more promising, and hence, the present study was conducted to discover the role of a panel of biomarkers, namely LDH, CYFRA 21-1, NSE, CEACAM, and CRP in the diagnosis of lung cancer in Indian patients. The utility of biomarkers was evaluated and compared among three groups of patients (groups I, II, and III), as detailed above. Their mean serum levels were found to be elevated in subjects with lung cancer when equated to subjects with benign lung diseases and healthy controls. A statistically significant difference was ascertained between patients with lung cancer vs those with benign lung diseases and healthy controls for four markers, namely CRP, NSE, CYFRA 21-1, and CEACAM ($p < 0.05$).

C-reactive protein (CRP) was found to be significantly higher in both benign lung disease as well as lung cancer groups. CRP, being a marker of inflammation, could be attributed to its increased levels in benign lung diseases like asthma, where the basic pathology is inflammation of airways. Thus, implying that it should only be utilized in amalgamation with other biomarkers for diagnosis of lung cancer to enhance the accuracy of results. Divergent to this, no statistically significant increase was seen in LDH levels among any group. However, various systematic reviews and research have put forward its role in prognosis and median survival rate of advanced lung cancer patients rather than diagnosis, especially in patients of nonsmall cell lung cancer (NSCLC).

A study was conducted in 2004 that studied CYFRA 21-1, CEA, and NSE individualistically as prognostic factors in advanced-stage

Table 3: Analysis of various combinations of biomarkers

	Different panels of biomarkers	*Sensitivity (%)*	*Specificity (%)*	*Negative predictive value (%)*	*Positive predictive value (%)*	*Accuracy (%)*
1	CRP and NSE	60	94	82.5	83.3	82.67
2	CRP and CYFRA 21-1	32	100	77.3	100	77
3	CRP and LDHE	4	98	67	50	66.7
4	NSE and LDH	8	98	68	66	68
5	NSE and CYFRA 21-1	36	100	75.8	100	78.6
6	CEACAM and LDH	0	97	71	0	70
7	CEACAM and NSE	8	90	66	28	62
8	CEACAM and CRP	4	98	67	50	66
9	CEACAM and CYFRA 21-1	0	98	66	0	65.3
10	CYFRA 21-1, CEACAM, and NSE	4	100	67	100	68
11	CYFRA 21-1, CEACAM and CRP	4	100	67	100	68
12	CYFRA 21-1, CEACAM, and LDH	0	98	66	0	65
13	CYFRA 21-1, NSE, and CRP	6	100	71	100	76
14	CRP, LDH, and NSE	8	94	67	40	65
15	CRP, CYFRA 21-1, NSE, and CEACAM	8	100	68	100	69
16	CRP, CYFRA 21-1, NSE, and LDH	8	98	68	100	68
17	CRP, CYFRA 21-1, CEACAM, LDH, and NSE	8	100	68.5	100	69.3

CEACAM, carcinoembryonic antigen-related cell adhesion molecule; CRP, C-reactive protein; CYFRA 21-1, cytokeratin fragment 19; LDH, lactate dehydrogenase; NSE, neuron-specific enolase; PPV, positive predictive value

NSCLC patients and also used a panel combining all of them. All three, that is, CYFRA 21-1, CEA, and NSE, were raised in these patients. The study concluded that compounding all three yielded better results in the diagnosis and screening of lung cancer.[10] We found similar results in our study in which the values of CYFRA 21-1, NSE, and CRP levels were upstretched in lung cancer patients, and when combined, accuracy, specificity, and negative predictive value of the test was higher than using individual markers thus being better in diagnosis of lung cancer. Further, in a study by Yu et al., different panels of biomarkers were used as follows (CEA, NSE, and CYFRA 21-1), (CEA, NSE, and LDH), and (CEA, NSE, and CRP) in carcinoma lung subjects. The best result was obtained when CEA, NSE, and CYFRA21-1 were combined.[11] Similarly, our study also found the combination of CRP and NSE as the most efficient panel for the diagnosis of lung cancer.

Zamay et al. reviewed the role of serum biomarkers in diagnosing lung cancer and found statistically significant alterations between subjects with lung cancer and healthy people using a panel of CEACAM, cancer antigen 125, CYFRA 21-1, and New York esophageal squamous cell carcinoma (cancer-testis antigen). Heightened accuracy of lung cancer diagnosis of around 94.8% was observed by using LDH, CRP, CEACAM, NSE, and CYFRA 21-1 collectively as a panel.[5] Also, the study concluded that CEA is a biomarker specific for all lung cancer types, NSE of NSCLC, and metastasis, while CYFRA21-1 is a biomarker for screening squamous lung cancer. In our study, we did not focus on other histological types than nonsmall cell carcinoma lung; however, the results were reinforcing as the levels of NSE were found to have significant differences in patients of lung cancer with a high accuracy of 86%, sensitivity and specificity of 76%. The highest accuracy of 82.6 with 94% specificity was found when NSE and CRP were used together.

The current study inferred that the biomarkers, namely CYFRA 21-1, NSE, CEACAM, LDH, and CRP, could prove to be efficacious in the detection of lung cancer. A panel comprising two biomarkers, namely CRP and NSE, together showed the best results. The study had its limitations. A small sample size may have altered the outcomes of the study. The absence of histopathological analysis specifically for different types of lung cancer was another limitation of the study. Further research, taking a high sample size and measuring levels of biomarkers in cytologically different types of lung cancer to find out the best available panel could further aid in the diagnosis as well as prognosis of this potentially fatal disease. We can also explore the role of biomarkers for comparing with CT scan findings to determine false positive cases.

Conclusion

Our study showed that biomarkers could prove to be a useful modality to help in the timely diagnosis of lung cancer. The difference in values of NSE, CRP, CYFRA 21-1, and CEACAM in lung cancer patients was inferred as statistically significant than other groups. The accuracy in diagnosing lung cancer was best when CRP and NSE were combined. This needs further research with high sample size and further categorically differentiating histology of lung cancer subtypes to find out their individual role in each type.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68(6):394–424. DOI: 10.3322/caac.21492
2. Peto R, Lopez AD, Boreham J, et al. Mortality from tobacco in developed countries: indirect estimation from national vital statistics. Lancet 1992;339(8804):1268–1278. DOI: 10.1016/0140-6736(92)91600-d
3. Du Q, Li E, Liu Y, et al. CTAPIII/CXCL7: a novel biomarker for early diagnosis of lung cancer. Cancer Med 2018;7(2):325–335. DOI: 10.1002/cam4.1292
4. Okamura K, Takayama K, Izumi M, et al. Diagnostic value of CEA and CYFRA 21-1 tumor markers in primary lung cancer. Lung Cancer 2013;80(1):45–49. DOI: 10.1016/j.lungcan.2013.01.002
5. Zamay TN, Zamay GS, Kolovskaya OS, et al. Current and prospective protein biomarkers of lung cancer. Cancers (Basel) 2017;9(11). DOI: 10.3390/cancers9110155
6. Isgrò MA, Bottoni P, Scatena R. Neuron-specific enolase as a biomarker: biochemical and clinical aspects. Adv Exp Med Biol 2015;867:125–143. DOI: 10.1007/978-94-017-7215-0_9
7. Jurisic V, Radenkovic S, Konjevic G. The actual role of LDH as tumor marker, biochemical and clinical aspects. Adv Exp Med Biol 2015;867:115–124. DOI: 10.1007/978-94-017-7215-0_8
8. Shrotriya S, Walsh D, Bennani-Baiti N, et al. C-Reactive Protein Is an Important Biomarker for Prognosis Tumor Recurrence and Treatment Response in Adult Solid Tumors: A Systematic Review. PLoS One 2015;10(12):e0143080. DOI: 10.1371/journal.pone.0143080
9. Mohan A, Garg A, Gupta A, et al. Clinical profile of lung cancer in North India: a 10-year analysis of 1862 patients from a tertiary care center. Lung India 2020;37(3):190–197. DOI: 10.4103/lungindia.lungindia_333_19
10. Barlési F, Gimenez C, Torre JP, et al. Prognostic value of combination of CYFRA 21-1, CEA and NSE in patients with advanced non-small cell lung cancer. Respir Med 2004;98(4):357–362. DOI: 10.1016/j.rmed.2003.11.003
11. Yu Z, Chen XZ, Cui LH, et al. Prediction of lung cancer based on serum biomarkers by gene expression programming methods. Asian Pac J Cancer Prev 2014;15(21):9367–9373. DOI: 10.7314/apjcp.2014.15.21.9367