

# Plexin C1: A novel screening test for lung cancer

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## Ethics Committee Approval

Ethics committee approval was received from the  
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All procedures in this study involving human  
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## Conflict of Interest

No conflict of interest was declared by the  
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## Abstract

**Background/Aim:** Lung cancer, where early diagnosis is particularly important, is one of the leading causes of death worldwide. Unfortunately, patients with lung cancer present at advanced stages. Biomarkers are needed to detect cancer at an earlier stage. In the present study, we aimed to emphasize that Plexin C1 level can be used in the early diagnosis of patients with lung cancer.

**Methods:** This prospective case-control study included 50 patients with lung cancer who presented between May 2020-September 2020 (25 males and 25 females) in the patient group and 40 healthy individuals (23 males and 17 females) in the control group. All patients with lung cancer underwent routine preoperative tests. Additionally, the preoperative Plexin C1 levels of all patients were measured with the ELISA method and compared between the patient and control groups, and with respect to cancer staging.

**Results:** The median Plexin C1 levels in the patient and control groups were 9.5 ng/mL and 4.0 ng/mL, respectively ( $P<0.001$ ). The patients with Stage 4 tumors had significantly higher serum Plexin C1 levels than those with Stage 1, Stage 2, and Stage 3 tumors ( $P<0.001$ ). Also, Plexin C1 levels were higher in patients with greater depth of invasion ( $P<0.001$ ), more lymph node involvement ( $P<0.001$ ), and distant metastasis ( $P<0.001$ ).

**Conclusion:** Plexin C1 can be used as a predictive biomarker at the time of diagnosis for lung cancer, and for cancer stage discrimination to show early, advanced, or metastatic disease.

**Keywords:** Lung cancer, Plexin C1, Biomarker, Tumoral stage

## Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide [1]. In contrast to other cancers, the 5-year survival rate in lung cancer, as well as pancreatic and gastric cancers, only mildly increased in recent years [2]. A lower rate of diagnosis at earlier stages is a significant factor affecting the low survival rate. Although the challenges associated with the early diagnosis of lung cancer underline the significance of imaging technologies, the economic burden of such advanced radio-diagnostic methods, radiation exposure, and false-positivity rates cause significant obstacles in their use.

Biochemical markers, which are becoming a popular and promising alternative in cancer diagnostics, stand out since they help in understanding the body's condition better than genetic biomarkers such as DNA. The discovery of these biomarkers and the clinical need were extensively examined and are considered by many to be clinically useful [3].

In previous studies, several biomarkers were identified for lung cancer, including carcinoembryonic antigen (CEA), cytokeratin-19 fragments (CYFRA 21-1), cancer antigen-125 (CA-125), and neuron-specific enolase (NSE) [4-8]. However, the development of a new biomarker for cancer diagnosis, experimental processes, and clinical applications require a considerable amount of effort and time. The approval of a biomarker as an indicator of diagnosis or survival in an organ cancer depends on its confirmation by several studies. Although proposed biomarkers often fail to meet the target criteria, innovative clinical applications continue to aim for the early diagnosis of malignancies [9].

The studies to develop novel lung cancer biomarkers suffer from similar problems as in the studies for other cancers. Protein biomarkers found in serum tend to show cross-reactivity with other cancers. Large-scale studies are needed before the ultimate confirmation of a molecule as a biomarker in clinical settings [10]. Moreover, immunoassay-based diagnostic methods are heavily dependent on the availability of monoclonal antibodies to detect these biomarkers. Thus, a few of several molecules identified as potential biomarkers eventually advance to be of clinical use.

This study aimed to show that the serum levels of Plexin C1 can be used for lung cancer stage discrimination.

## Materials and methods

This prospective case-control study included 50 lung cancer patients enrolled between May 2020 and September 2020 (25 males and 25 females, 30 with adenocarcinoma and 20 with squamous cell carcinoma), and the control group included 40 healthy individuals (23 males and 17 females). After the surgery, the TNM classification for malignant tumors was used for pathological staging of cancer as described by the Union for International Cancer Control (eighth edition) [11].

The ELISA for Plexin C1 was performed with commercial kits (MyBiosource, Catalog No: MBS944227) according to the manufacturer protocol by using plates that were pre-coated with the primary antibody. Serum was transferred to wells, incubated with the antibodies, washed, and substrate solution was added, followed by the color-reagent and quenching

solution. Finally, absorption at 450 nm wavelength was measured. The mean absorption value for duplicates was used for the analysis of each sample. The assay has high sensitivity and excellent specificity for human Plexin C1, with a typical limit of detection of 0.078 ng/mL and a detection range of 0.312-20 ng/mL. The assay was found to have no significant cross-reactivity with or interference from Plexin C1 analogs.

### Statistical analysis

Normal distribution of the data was tested with the Shapiro-Wilk test, histograms, Q-Q plots, and box plot charts. The data were presented as median (25<sup>th</sup> percentile-75<sup>th</sup> percentile) and frequency (percentage). The Mann-Whitney U test was used for the comparisons of two groups. The Kruskal-Wallis one-way analysis of variance was used for the comparisons of three or more groups. The Dunn test was used for multiple comparisons. Nominal variables were evaluated with the Pearson chi-square test. The limit of significance was set as  $P < 0.05$  and bidirectional. The analyses were performed with NCSS 10 software (Kaysville, Utah, USA).

### Ethics

Ethics committee approval was received from the Non-invasive Clinical Research Ethics Committee of Van Yuzuncu Yil University, Van, Turkey (Decision No: 2020/03-17, Date: 22.05.2020). All procedures in this study involving human participants were performed following the 1964 Helsinki Declaration and its later amendments.

## Results

This study included 50 patients with lung cancer (25 males and 25 females) with a median age of 54.0 years (range: 50-60 years) as the patient group and 40 healthy individuals (23 males and 17 females) with a median age of 59.0 years (range: 49-69 years) as the control group. The most common T, N, and M stages were T<sub>4</sub> (64%), N<sub>2</sub> (64%), and M<sub>1</sub> (82%). According to TNM staging, 7 patients were Stage 1, 7 patients were Stage 2, 11 patients were Stage 3, and 25 patients were Stage 4. Table 1 summarizes the clinicopathological characteristics of all patients.

Table 1: The clinicopathological parameters of the patients and controls

Parameters	Control group (n=40)	Patient group (n=50)
Age (year)	59.0 (55.0-61.0)	54.0 (53.0-55.0)
Gender (M/F)	23 / 17	25 / 25
Data of patient group		
TNM stage (n, %)		
• Stage 1		7 (14.0%)
• Stage 2		7 (14.0%)
• Stage 3		11 (22.0%)
• Stage 4		25 (50.0%)
Depth of invasion (n, %)		
• T <sub>1</sub>		5 (10.0%)
• T <sub>2</sub>		5 (10.0%)
• T <sub>3</sub>		8 (16.0%)
• T <sub>4</sub>		32 (64.0%)
Lymph node metastasis (n, %)		
• N <sub>0</sub>		9 (18.0%)
• N <sub>1</sub>		9 (18.0%)
• N <sub>2</sub>		32 (64.0%)
Metastasis (n, %)		
• M <sub>0</sub>		9 (18.0%)
• M <sub>1</sub>		41 (82.0%)

The median Plexin C1 levels of the patient and control groups were 9.5 ng/mL, and 4.0 ng/mL, respectively ( $P < 0.001$ ). Serum Plexin C1 levels are presented in Table 2.

The patients with Stage 4 tumors had significantly higher serum Plexin C1 levels than those with Stage 1, Stage 2, and Stage 3 tumors ( $P < 0.001$ ). In addition, Plexin C1 levels

were higher in patients with greater depth of invasion ( $P<0.001$ ), more lymph node involvement ( $P<0.001$ ), and distant metastasis ( $P<0.001$ ). Serum Plexin C1 levels of the patients stratified by their clinicopathological variables are presented in Table 3.

Table 2: Preoperative serum Plexin C1 levels of the patients and controls (Median (IQR: 25<sup>th</sup> percentile-75<sup>th</sup> percentile))

Parameters	Control group (n=40)	Patient group (n=50)	P-value
Age (mean, year)	59.0 (55.0-61.0)	54.0 (53.0-55.0)	<0.001
Gender (M/F)	23 (57.5%)/17 (42.5%)	25 (50.0%)/25 (50.0%)	0.479
Plexin C1 (ng/mL)	4.0 (3.0-4.0)	9.5 (6.5-12.5)	<0.001

Table 3: Preoperative serum Plexin C1 levels stratified by the patients' clinical and pathological variables (Median (IQR: 25<sup>th</sup> percentile-75<sup>th</sup> percentile))

Parameters	Plexin C1 (ng/mL)	P-value
TNM stage		<0.001
• Stage 1	5.5 (5.5-6.0)	
• Stage 2	7 (6.5-7.0)	
• Stage 3	8.5 (7.0-9.5)	
• Stage 4	12.5 (11.0-14.5)	
Depth of invasion		<0.001
• T <sub>1</sub>	5.5 (5.3-5.8)	
• T <sub>2</sub>	6.5 (5.8-7.3)	
• T <sub>3</sub>	6.8 (6.5-7.0)	
• T <sub>4</sub>	11.5 (9.6-13.8)	
Lymph node metastasis		<0.001
• N <sub>0</sub>	5.5 (5.5-6.3)	
• N <sub>1</sub>	7.0 (6.5-7.3)	
• N <sub>2</sub>	11.5 (9.6-13.8)	
Distant metastasis		<0.001
• M <sub>0</sub>	5.5 (5.5-6.3)	
• M <sub>1</sub>	11.0 (7.8-12.8)	

## Discussion

Lung cancer is a global health problem. In the light of the Cancer Statistics report of Siegel et al. [1], 235,760 new lung cancer cases are expected to be seen in the USA. The same report also predicted that approximately 131,880 people will die due to gastric cancer in the USA. Since the exact mechanism of lung cancer is not known, most patients present with advanced disease. Therefore, early diagnosis is important in the diagnostic process, and biomarkers are needed to solve the diagnostic problem. Plexin C1 proteins were studied in human studies before in different cancer types such as hepatocellular carcinoma, gastric carcinoma, and melanoma. However, no human studies report that Plexin C1 protein can be used as a prognostic biomarker in lung cancer. We aimed to solve this problem in the literature.

Plexin C1 is a type-1 transmembrane receptor within the extracellular segment that has homology to the Met family tyrosine kinase receptors [12-14]. Plexin C1 is commonly involved in actin cytoskeleton rearrangements and focal adhesions. Focal adhesions are dynamic structures that bridge cell-to-extracellular matrix adhesions in an integrin-dependent manner [15]. Plexin C1 signaling influences focal adhesion assembly/disassembly and induces cytoskeletal remodeling. Thereby, it influences the cellular shape, extracellular matrix adherence, and cell motility and migration [16-18].

It can be speculated that understanding Plexin C1 levels of a patient facilitates an approach to establish the aggressiveness or metastatic potential of cancer where its increase represents aggressive cancer or cancer with high metastatic potential. In lung cancer, progression in tumor invasion and depth are significant indicators of the severity of the disease. Increased level of Plexin C1 in the sample, compared to that in a control, is associated with a higher depth of invasion.

The cell-matrix adhesion is coupled to the cytoskeletal dynamics during cell migration. The activation of Plexin C1 may

uncouple these processes, prevent the formation of adhesive complexes and lamellipodia, thereby hinder directional cell migration [19-20].

There are some studies about Plexin C1 level and cancer correlation in the English literature. According to the previous studies, Plexin C1 protein is upregulated in hepatocellular carcinoma cells [15, 21]. On the other hand, Ni et al. [22], showed that the Plexin C1 gene was highly upregulated in gastric cancer with a poor prognosis. The expression level of PLXNC1 could serve as an independent biomarker to predict a patient's overall survival according to the study of Chen et al. [23].

The molecular effect of Plexin C1 protein in cancer pathogenesis is still limited. In addition, there was no human study about plexin C1 serum levels among cancer patients. In this study, the Plexin C1 levels in patients with lung cancer were measured with the ELISA assay, and the levels in the patients with different stages of lung cancer were systematically analyzed. The study suggested that Plexin C1 expression was significantly higher in lung cancer compared to that in healthy controls, especially at advanced stages, which suggests its potential role in lung cancer pathophysiology.

## Limitations

One of the limitations of this study is that the role and mechanism of action of expression of the Plexin C1 protein in lung cancer have not been confirmed; therefore, further analysis of the clinical features and mechanism of action as well as larger patient populations are required to confirm the results of the current study.

## Conclusion

Delay in the diagnosis and treatment of lung cancer is an important problem. Plexin C1 may have an important role in lung cancer and elevated Plexin C1 expression may serve as a new biomarker.

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