

# The value of plasma netrin-1 in non-small cell lung cancer patients as diagnostic and prognostic biomarker

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**Abstract** Netrin-1 is found to be elevated and purposive as a diagnostic biomarker in many human cancers. We evaluated serum netrin-1 concentrations in patients with advanced non-small cell lung cancer compared with those in a healthy group. Thirty patients with advanced non-small cell lung cancer and 30 healthy people were included in the study. Serum netrin-1 concentrations were measured by quantitative ELISA method in both groups. The mean serum netrin-1 concentrations were found to be significantly higher in patients with non-small cell lung cancer than in healthy controls. The mean serum netrin-1 concentrations were found to be significantly higher in patients with non-small cell lung cancer before the beginning of chemotherapy when compared after the completion of the third cycle. Our results represented that netrin-1 concentrations elevated in advanced non-small cell lung cancer compared to a healthy control group, and netrin-1 concentrations decreased with chemotherapy.

**Keywords** Netrin-1 · Gastric cancer · Treatment · Biomarker

## Introduction

Biomarkers are various tools used for diagnosis of cancer, monitorization of treatment, and determining prognosis. Use of biomarkers in clinical oncology may have great effects if they can be detected before the emergence of clinical symptoms or really sufficient for response to medications. Today, only a few biomarkers are available which have the abovementioned characteristics [1, 2]. Vast majority of biomarkers cannot be used in early diagnosis of cancers due to low sensitivity and specificity. Besides, most of them can elevate non-specifically in many other diseases [2]. Therefore, development of and introducing novel biomarkers to clinical use is of importance. Malignant transformation includes the alterations in protein expression and subsequent clonal proliferation. These altered proteins are secreted by tumor cells and released into circulation, and they can be monitored qualitatively and quantitatively.

Netrin-1 is a diffusible, laminin-related protein identified as neuronal guidance cues during development of the nervous system. Netrin-1 shows its biological effects through binding to two receptor families: deleted in colorectal cancer (DCC) and uncoordinated-5-homolog (UNC5H) [3–5]. As shown in previous studies, netrin-1 is also expressed out of the nervous system and contributes to cell adhesion, motility, proliferation, and differentiation during developmental process of epithelial tissues like breast gland, pancreas, and lungs [6–11]. Moreover, netrin-1 is also known to have a role in angiogenesis, inflammation, and apoptosis [7, 11–15]. Netrin-1 expression is induced after epithelial cell injury, and this may be used as biomarkers of organ injury or disease [16, 17].

Over-expression of netrin-1 is seen in many cancer tissues, and downregulation of netrin-1 expression or receptor inhibition leads to apoptosis in tumor cells resulting in tumor regression [18–20]. In a study, immunohistochemical analysis was

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done for netrin-1 expression in 92 non-small cell lung cancer (NSCLC) tumor tissues. Netrin-1 was observed to be over-expressed both in adenocancers and squamous cell cancers [21]. Ramesh et al. analyzed netrin-1 levels both in tumor tissues and plasma in various tumors and they did not find plasma netrin-1 levels high in lung cancer patients [22].

Therefore, we investigated serum netrin-1 level in non-small cell lung cancer patients, alterations with treatment, and its relationship with survival.

## Materials and methods

### Patient population

A total of 30 patients newly diagnosed with advanced stage (locally advanced or metastatic) non-small cell lung cancer were selected from the Department of Medical Oncology in Dr. Lütfi Kırdar Kartal Education and Research Hospital. The study inclusion criteria were having ECOG performance status  $\leq 2$ , normal serum bilirubin, AST, ALT levels, and renal function tests, WBC  $>4000/\text{mm}^3$ , absolute neutrophil count  $>1500/\text{mm}^3$ , and platelets  $>100,000/\text{mm}^3$ . Thirty age- and sex-matched healthy people without any known malignancy who were selected between healthy relatives of patients were constituted as the control group. Information about age at diagnosis, sex, performance status, histopathological subtype, and stage were obtained from the medical records of the patients.

### Collection of samples

Five milliliters of peripheral blood samples was taken into dry tubes from the 30 patients with locally advanced or metastatic non-small cell lung cancer. Blood samples were centrifuged at 1000g for 15 min to gain serum within 30 min after blood sampling. Serum samples were stored at  $-20\text{ }^\circ\text{C}$  until the biochemical analysis was performed. Netrin-1 levels were measured from the blood samples obtained before the chemotherapy and after the third cycle. All the samples were analyzed at the same time.

### Measurement of netrin-1 levels

Netrin-1 concentrations were measured by using a netrin-1 ELISA kit (CSB-E11899h, CUSABIO BIOTECH Co., Hubei, China). First, netrin-1 standard and plasma samples were added to antibody well plates and incubated for 2 h at room temperature, followed by the addition of biotin-antibody working solution and an additional 1 h. Then, plates were washed and incubated with avidin conjugated to horseradish peroxidase for 1 h. Each was aspirated and washed for five times. Color was developed using tetramethylbenzidine

substrate, and reaction was arrested by adding sulfuric acid. Solution, which was provided inside the kit, was added, and the optical density (OD) was determined by using a microplate reader set to 450 nm. The concentration of netrin-1 in the samples was determined by comparing the OD of the samples to the standard curve, with a minimal limit of detection of 7.8 pg/ml. Plasma netrin-1 concentration was expressed as picograms per milliliter.

### Statistical analysis

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software. Descriptives of the parameter analyses were presented using medians and standard deviations for normally distributed variables. The mean concentrations of the netrin-1 plasma values and its relationship with clinicopathologic factors were calculated by using the Mann-Whitney *U* and Wilcoxon tests. Survival estimates were analyzed according to the Kaplan-Meier method.

## Results

### Patient characteristics

Thirty patients with advanced stage non-small cell lung cancer and 30 healthy volunteers were analyzed. The median age of patients was 61 years. Twenty-six patients (86.7 %) were men and 4 (13.3 %) were women. The Eastern Cooperative Oncology Group performance status was 0–1 in 28 (93.3 %) of patients and 2–4 in 2 (6.6 %). Histopathological analysis revealed that 14 patients (46.7 %) had adenocarcinoma, 12 patients (40 %) had squamous cell carcinoma, and 4 patients (13.3 %) had NOS type. Twenty-two patients (73.3 %) had stage IV disease, and the 8 (26.7 %) had locally advanced stage. Patient characteristics are summarized in Table 1. In the control group, the median age was 57 years and 23 subjects were men.

### Netrin-1 concentrations

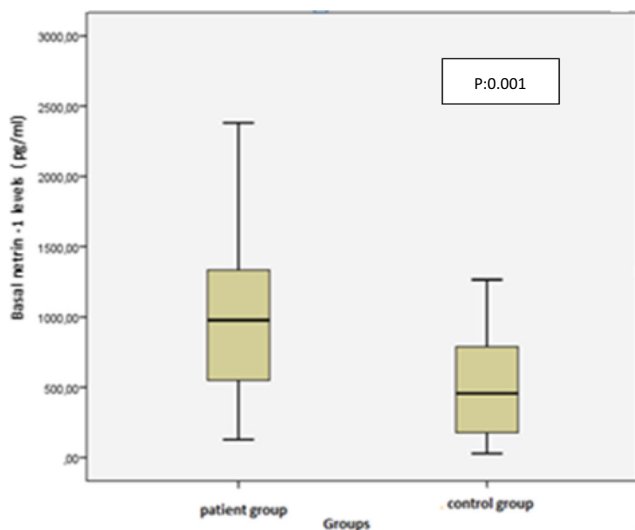
The mean netrin-1 concentrations were found to be significantly higher in patient group as compared to healthy group ( $856.98 \pm 615.33$  vs  $461.09 \pm 411.58$ ,  $p < 0.001$ ) (Fig. 1). The mean netrin-1 values were found to be higher in patients with non-small cell lung cancer before the beginning of chemotherapy than the values after the completion of the third cycle ( $856.98 \pm 615.33$  vs  $589.95 \pm 385.42$ ,  $p < 0.001$ ) (Fig. 2). There was no relationship between basal netrin-1 levels and gender, age, ECOG performance status, histopathological subtypes, and stage, and  $p > 0.05$ .

**Table 1** Patient characteristics

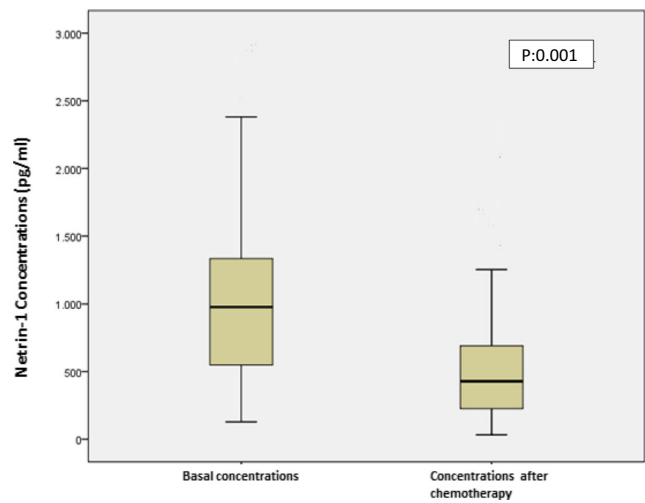
Characteristics	<i>n</i>	<i>p</i>
<b>Age</b>		
<60 years	6 (20%)	0.49
>60 years	24 (80%)	
<b>Gender</b>		
Men	26 (86.7 %)	0.75
Women	4 (13.3 %)	
<b>Performance score</b>		
PS 0–1	28 (93.3 %)	0.23
PS 2–4	2 (6.6 %)	
<b>Clinical stage</b>		
Locally advanced	8 (26.7 %)	0.75
Metastatic	22 (73.3 %)	
<b>Histopathology</b>		
Adenocarcinoma	14 (46.7 %)	0.20
Squamous cell carcinoma	12 (40%)	
NSCLC NOS	4 (13.3 %)	

**Survival analysis**

The median follow-up was 1 year (8–16 months). The median progression-free survival and overall survival of the patients were 5.86 months (95 % CI=5.02–8.39) and 13.8 months (95 % CI=10.94–14.28), respectively. There was no significant correlation between age, gender, histopathological subtypes, stage, and PFS and OS ( $p>0.05$ ) (Table 2). Similarly, we could not find any relationship between basal netrin-1 and decline in netrin-1 levels and PFS and OS ( $p>0.05$ ).



**Fig. 1** Mean levels of netrin-1 in patients compared with healthy group



**Fig. 2** Mean netrin-1 levels in patients before the beginning and after the third cycle of the chemotherapy regimen

**Discussion**

In the literature, various biomarkers have been evaluated to determine the response to the treatment and prognosis of lung cancer. Despite the advances in oncology in recent years, a significant improvement in the treatment of lung cancer still cannot be provided. Thus, possible new targets for treatment are intended to achieve with this study. Today, few biomarkers have properties for clinical use and most of them have low specificity and sensitivity. Therefore, more extensive researches are needed for further use of biomarkers.

**Table 2** The relationship between basal netrin-1 levels and clinicopathological characteristics of the patients group

	Netrin-1 (mean ± SD)	<i>p</i>
Patients group	856.98 ± 615.33	0.012
Healthy group	461.09 ± 411.58	
<b>Gender</b>		
Women	707.22 ± 527.17	0.758
Men	919.85 ± 635.36	
<b>Age</b>		
≤60	752.43 ± 603.48	0.49
>60	992.78 ± 638.90	
<b>Performance score</b>		
0–1	982.72 ± 617.56	0.23
2–4	478.86 ± 438.27	
<b>Histopathology</b>		
Adenocarcinoma	788.76 ± 554.63	0.20
Squamous cell carcinoma	1053.50 ± 691.83	
NSCLC NOS	689.84 ± 542.21	
<b>Stage</b>		
Locally advanced	919.88 ± 396.56	0.75
Metastatic	797.14 ± 695.91	

Netrin-1 is a protein that guides the migration of axons during embryogenesis, via the DCC, DSCA, UNC5H receptor families.

Studies showed that netrin-1 plays a role in the development of not only the nervous system but also epithelial tissues such as mammary gland, pancreas, and lungs by regulating cell adhesion, motility, proliferation, and apoptosis. Netrin-1 regulates inflammation, apoptosis, and angiogenesis at the same time and has been shown to increase the expression in many cancer tissues.

Ramesh et al. showed that netrin-1 is determined not only in the tumor tissue but also in circulation. Based on this study, we investigated serum netrin-1 levels in NCCLC before treatment and its prognostic value [22].

Patients' basal plasma netrin-1 levels were measured before treatment, and these results were compared with those in the control group. Mean netrin-1 values were statistically significantly higher in patients compared to healthy group ( $p=0.012$ ).

Regarding the relationship between the subtypes with netrin-1 level, mean netrin-1 plasma levels were found to be higher in squamous cell carcinoma than in adenocarcinoma but these values did not reach statistical significance. Contrary to this finding, Bernet et al. showed increased expression of netrin-1 on adenocarcinoma tumor tissue.

Bernet et al. show that adenocarcinoma tissue samples express higher levels of netrin-1 while Ramesh et al. did not find high plasma netrin-1 levels in lung adenocarcinoma [5, 22]. According to these, results have considered the question of whether all the netrin-1 has been released to plasma from the tumor tissue or not. This suggests that plasma netrin-1 level does not always represent the netrin-1 level on tumor tissue.

In our study, there was not a statistically significant difference between age, sex, performance status, stage, and plasma netrin-1 levels.

Plasma samples were collected before treatment to exclude false netrin-1 high levels according to tumor lysis after chemotherapy. The relationship between the response to treatment and plasma netrin-1 levels after treatment was analyzed.

Despite the fact that patients who respond to treatment (complete response, partial response, and stable disease) were found to have higher plasma netrin-1 levels than those without response to treatment (progressive disease) (respectively,  $1053.54 \pm 785.97$  and  $672.48 \pm 465.71$ ), this difference did not reach the statistical significance ( $p=0.09$ ).

Considering the pathogenesis of metastasis, antiapoptotic, and antigenic properties of netrin-1, we actually expect higher levels in metastatic disease.

In our study, netrin-1 plasma levels were significantly higher in patients than in healthy people regardless of age, gender, stage, and the histological subtypes of NSCLC. This may represent a strong relationship between plasma netrin-1 levels and the presence or absence of tumor and that is why it can be used as a biomarker.

Our study has some limitations. First, we had a limited number of patients ( $n=30$ ). Second, we do not know whether it is correlated with other plasma biomarkers. To answer all these questions, more comprehensive studies with higher number of patients are needed.

**Compliance with ethical standards** The study was approved by the Local Ethics Committee of our hospital. Informed written consents were obtained from each patients and healthy controls.

**Conflicts of interest** None.

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