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Prognostic effects of SuPAR and Neopterin Levels on Patients with Lung Cancer

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Abstract: Background: Two unique biomarkers, soluble form of the urokinase-type plasminogen activator receptor (suPAR) and neopterin, play a crucial role in inflammatory processes. This study aimed to reveal whether it is possible to utilize these biomarkers in predicting tumor prognosis in patients with lung cancers.

Methods: The present study was designed as a single center, prospective, and controlled research. The study was conducted with forty patients with lung cancer (case group) and 41 healthy individuals (control group) in Kırıkkale University, Faculty of Medicine between 2016-2020. The case group was also divided into two of the early and advanced stages. The blood samples were drawn to evaluate suPAR and neopterin levels, and these parameters were compared between the case and control groups. Also, the prognostic effects of age, stage of the tumor, and the levels of mentioned parameters were investigated with the survival analysis.

Results: The median duration of the follow-up was 32 (4-75) months. suPAR and neopterin levels were found to be higher in the case group than in the control group. Cox regression showed that the high levels of neopterin and suPAR increased mortality risk [p=0.002, HR: 1.25 (1.08-1.45 95%CI) and p=0.023, HR:1.07 (1.01-1.13), respectively]. Finally, age and stage of the tumor were found to have no relationship with survival.

Conclusion: suPAR and neopterin as members of the inflammatory pathway were found to be higher in cancer cases. Furthermore, both suPAR and neopterin levels were found to be predictive for the mortality of patients with lung cancers; therefore, they are thought to be used for the management of cancer.

Keywords: suPAR; neopterin; lung cancer.

Introduction

Lung cancer is the most frequent cancer type and a leading cause of mortality in all around the world. Although new diagnostic methods and therapeutic advances, such as immunotherapy, have been introduced to treat the disease, the overall 5-year survival rates are still low. Unfortunately, the majority of the cases were diagnosed on the advanced stage. Therefore, lung cancer is a serious burden on healthcare systems [1].

Neopterin (6-D-erythro-trihydroxypropyl-pterin-triphosphate) is synthesized from guanosined-erythro-192939-trihydroxypropyltriphosphate, and it is released into serum and excreted in urine after induction of monocytes/macrophages by interferon-γ [2]. Neopterin is an almost exclusively macrophage-originated biomarker thought to be related to acute and chronic immune activation. In cancer patients, a high level of neopterin is related to poor prognosis, as a reflection of the failure of the host response to the tumor [3]. In 1979, Wachter et al [4] demonstrated high neopterin levels in the urine of cancer patients. Over time, a raised interest in neopterin has led to reveal its robust association with infectious diseases, autoimmune disorders, acute coronary syndrome, and even atherosclerosis [5–9].

The soluble form of the urokinase-type plasminogen activator receptor (suPAR) is a glycosyl-phosphatidylinositol linked membrane protein and found in body fluids, such as blood, urine, etc. Also, the membrane-bound form of suPAR is present on different types of cells, including monocytes, lymphocytes, macrophages, fibroblasts, and megakaryocytes. suPAR has various actions on adhesion, proteolysis, and cell

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migration [10]. suPAR was first described for involving a biomarker for cancer progression and various infections in the 1990s. Subsequently, it has been investigated whether to be used as a diagnostic and prognostic tool [11].

Inflammation has a key role in tumorigenesis and cancer progression [12]. The presence of some laboratory tests, which predict prognosis, for inflammation at the time of diagnosis may be helpful for the management of the disease. The present study aimed at revealing whether serum neopterin and suPAR levels predict the prognosis at the time of diagnosis. It was also investigated whether these levels differ in cancer patients and healthy people.

Materials and Method

Study Design and Population

This single-center cohort study was conducted in Kırıkkale University between 2016-2017. Newly diagnosed 40 patients with lung cancer and 41 healthy individuals participated in the study. Stages IA, IB, IIA, IIB, and IIIA non-small cell lung cancer were defined as the early stage, while stages IIIB and IV were considered the advanced stage. To generate a common data pool, patients with small cell lung cancer with limited stages were included in the early stage group, and the others were assigned to the advanced stage group. Patients with any inflammatory disease, infection, and organ failure, such as kidney diseases, were excluded from the study.

Patients retrospectively scanned from "the National Notification and Registry System of the Death," and then survivors and non-survivors were compared. The tests were compared in healthy and control groups, and they were evaluated for predictivity of prognosis in survivor and non-survivor groups.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Sample collection and measurements

Blood samples were drawn from the case and control groups to detect neopterin, suPAR, and other biochemical parameters. The samples were centrifuged and stored at –20°C in a refrigerator, and all samples were processed at the same time. Plasma suPAR levels were determined with the suPARnostic AUTO Flex ELISA (ViroGates A/S, Birkerød, Denmark) on an automated Siemens BEP2000 platform at the Department of Clinical Biochemistry according to the manufacturer's instructions. Serum neopterin levels were determined by a commercially available ELISA (BRAHMS Diagnostics, Henningsdorf, Germany).

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 15.0 (Chicago, SPSS Inc.). Descriptive statistics for continuous variables were given as mean, standard deviation, minimum, maximum, and median. The Mann Whitney-U test was used for two-group comparisons of non-normally distributed data. Fisher's exact test was used for categorical variables. The Kaplan-Meier plot and Cox regression analysis with backward selection were used to determine the predictors of survival. The proportional hazards assumption and model fit were checked with residual analysis. ROC analysis was used for determining the cut-off values of tests. The significance level was accepted as $p<0.05$ in all statistical analyses.

Results

The mean ages of the case and control groups were found to be 61.0±7.48 and 43±5.48 years, respectively, and the difference was statistically significant. The control group consisted of 17 (41%) female and 24 (59%) male individuals, while the case group was composed of 3 (7.9%) female and 35 (92.1%) male patients. suPAR and neopterin levels showed a non-homogenous distribution. Clinical features of subjects included in the study and histological subtypes of lung cancer were given in Table 1. The median duration of the follow-up was 32 (4-75) months. Twenty-six patients (65%) were found to be deceased. By two-group comparisons of the survivor and non-survivor groups, age and sex distributions were similar, but the numbers of cases with advanced stage and neopterin and suPAR levels were found to be higher in the non-survivor group, and neopterin levels were significant in the 95% confidence interval (Table 2). The Cox regression model showed that high neopterin and suPAR levels had effects on mortality,

Table 1: Clinical and laboratory features of the subjects.

Variables		Case, n=40	Control, n=41	Significance
Age, *years*		61.5 (41-83)	41.5 (22-73)	p<0.01
Sex, male/female		37/3	24/17	p=0.001
Histological type	Squamous	16 (42%)	NA	
	Adenocarcinoma	7 (18.4%)		
	Undifferentiated	7 (18.4%)		
	Small Cell	8 (21.2%)		
Survivor		14 (35%)	NA	
suPAR, ng/ml		18.70 (1.64-35.75)	3,6 (0.95-11.47)	p<0.001
Neopterin, nmol/L		19.7 (4.27-59.73)	9.37 (2.66-51.77)	p=0.004

Table 2: The comparison of survivors and non-survivors.

Variables	Survivor n=14	Non-survivor n=26	Significance
Age, *years*	62 (47-73)	60.5 (41-83)	p=0.90
Female sex, n (%)	1 (6.3%)	2 (8.3%)	p=0.80
Advance stage, n (%)	5 (31.3%)	17 (70.8%)	p=0.016
suPAR, ng/ml	16.05 (1.64-27.32)	19.62 (11.20-35.75)	p=0.05
Neopterin, nmol/L	8.56 (2.66-13.54)	12.11 (3.36-51.70)	p=0.004

Table 3: Cox regression analysis.

	Univariate		Multivariate	
	HR (95% CI)	**Significance**	**HR (95% CI)**	**Significance**
Advanced stage	2.28 (0.94-5.51)	p=0.06	1.02 (0.34-3.04)	p=0.96
Neopterin levels	1.25 (1.07-1.44)	p=0.001	1.25 (1.08-1.45)	p=0.002
suPAR levels	1.07 (1.01-1.13)	p=0.013	1.07 (1.01-1.13)	p=0.023

while age and stage of the tumor had no significant relationship with survival (Table 3). The ROC analysis determined the cut-off values as 9.36 nmol/L (AUC=0.77) for neopterin and 18.84 ng/ml for suPAR (AUC=0.69). The survival plots of patients for high and low levels of tests were shown in Figure 1 and Figure 2.

Discussion

A non-invasive test capable of informing about prognosis is always desirable. Neopterin and SuPAR are two molecules that have unique roles in acute and chronic inflammation pathways. These two molecules were tested in lung cancers and found to be statistically higher in the case group than in the control group; therefore, they are thought to have a prognostic predictivity. Their high levels bear mortality risk.

Neopterin has been investigated in numerous studies as a prognostic tool in different cancer types. The setting and the role of neopterin in immune response have led clinicians to study this biomarker in various primary tumors, such as gynecological, gastrointestinal, urological, hematological malignancies, head-neck carcinomas, and breast cancers. High levels of neopterin have been found to be associated with poor prognosis [13–17].

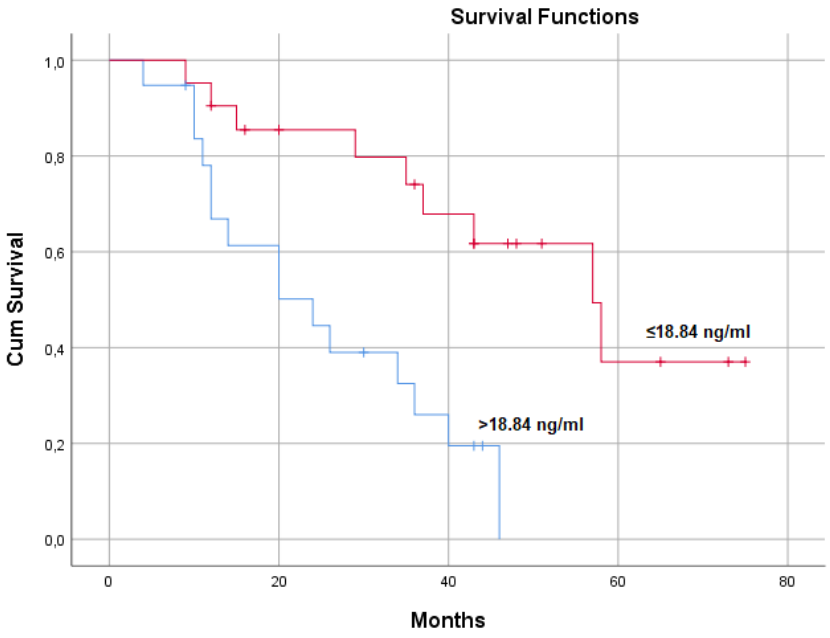

Figure 1: The survival rates with Kaplan-Meier plot for levels of suPAR.

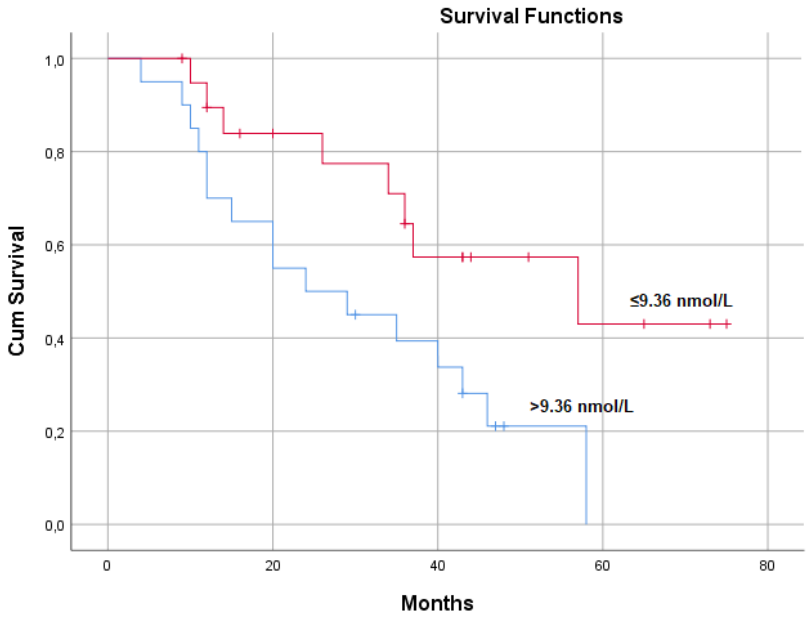

Figure 2: The survival rates with Kaplan-Meier plot for levels of neopterin.

There are few studies examining neopterin in lung cancer. El-Akawi et al. [18] found that neopterin levels and alpha-1 antitrypsin were higher in patients with lung cancer or prostate cancer than the control group, and these markers were elevated with advanced stages. Prommegger et al. [19] showed that high urinary neopterin levels were related to worse prognosis in operations for lung cancer.

Kronberger et al. [17] concluded that urinary neopterin was a significant predictor of prognosis at the time of diagnosis in addition to lymph node status, stage, and grading, according to a 10-year follow-up. However, in contrast to the study, the stage of the tumor was not a significant predictor of survival in the present study.

Mohamed et al. [20] investigated bronchoalveolar lavage (BAL) fluid and serum neopterin in tuberculosis and lung cancer. They found that BAL neopterin was mostly elevated in tuberculosis, but it was also higher in cancer patients than in the control group. They also revealed that neopterin levels differed by the types of lung cancer, especially in small cell cancer.

SuPAR has been found to be elevated in inflammatory diseases and various cancer types and to have an association with poor prognosis [21,22]. Moreover, a recent study indicated suPAR had a cancer-associated diagnostic value with CRP in individuals with severe non-specific symptoms [23]. Moreover, preoperatively high levels of SuPAR can independently predict patients' survival in colorectal cancer [24]. A recent systematic review and meta-analysis of 12 studies revealed that suPAR was a potentially promising biomarker in the prediction of prognosis in patients with colorectal, ovarian, breast, and prostate cancers [25]. Interestingly, in some studies, overall mortality has been found to have a strong association with cardiovascular diseases in cancer patients [26]. There is a lack of knowledge about suPAR on the diagnosis and prediction of prognosis. Cobos et al. [27] conducted a study with patients with advanced lung cancer and found that suPAR was higher in cancer patients than the control group, while there was no difference between the subtypes of lung cancer.

The present study has several limitations. The distributions of age and sex were different in the case and control groups, which generated a serious limitation in terms of the analyses. The sample size was small, and the tests were studied only once. Moreover, many other parameters (C-reactive protein, interleukin-6, etc.) could not be analyzed. Despite different staging criteria, the presence of various subtypes of lung cancer with different stages in a single pool made it difficult to make evaluations.

In conclusion, cancer prognosis is related to inflammatory processes since cancer patients have higher inflammation levels than healthy people. Neopterin and suPAR levels indisputably can contribute to the assessment of inflammatory diseases, as well as cancers. They are blood-drawn and easy-to-perform tests and may be helpful for predicting mortality of patients with lung cancer. Further investigations are needed to clarify the efficacy of these parameters and to explain the prognosis predictivity of inflammatory processes.

Conflict of interest: Authors state no conflict of interest

Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

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