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Diagnostic Utility of Serum Krebs von den Lungen-6 (KL-6) and Surfactant Protein-D (SP-D) Levels in Hypersensitivity Pneumonitis

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Abstract

Background: *This study aimed to investigate the diagnostic and prognostic values of serum levels of Krebs von den Lungen-6 (KL-6) and surfactant protein-D (SP-D) in patients with hypersensitivity pneumonitis (HP).* **Methods:** *Serum samples were collected from patients diagnosed with HP and from a healthy control group. KL-6 and SP-D levels were measured using ELISA. HP cases were further compared across fibrotic and non-fibrotic subgroups and between those receiving treatment and those not. The relationships between respiratory function tests (DLCO, FEV_1, FVC) and biomarker levels were examined.* **Results:** *Both KL-6 (median 5.95 ng/ml vs 5.4 ng/ml, p<0.001) and SP-D (median 14.87 ng/ml vs 14.72 ng/ml, p<0.05) levels were significantly higher in HP patients than in the control group. In non-fibrotic HP patients, KL-6 levels were higher than in the fibrotic group (median 6.07 ng/ml vs 5.62 ng/ml, p<0.05), while no significant difference was observed for SP-D (p=0.71). KL-6 levels were significantly higher in untreated cases than in treated cases (median 6.30 ng/ml vs 5.65 ng/ml, p<0.01), whereas the difference in SP-D was not significant (p=0.26).* **Conclusions:** *KL-6 emerges as a sensitive biomarker for the diagnosis of HP, assessment of disease activity, and monitoring of treatment response. SP-D, although reflecting inflammatory processes, seems to have limitations in evaluating fibrotic progression.*

1. Introduction

Hypersensitivity pneumonitis (HP) is an inflammatory and/or fibrotic interstitial lung disease (ILD) that affects the lung parenchyma and small airways. It develops in susceptible individuals following exposure to inhaled antigens [1, 2]. HP is the third most common form of ILDs, accounting for up to 47.3% of all cases [3–8]. More than 50 different occupations and exposures associated with HP have been identified [1, 2]. The main causative agents in the disease's etiology are microorganisms, animal proteins, and organic and inorganic dust particles. HP is classified as fibrotic or non-fibrotic based on imaging and histopathological findings. The 7-year survival rate for fibrotic HP is 40.8%, indicating a poorer prognosis compared to many types of cancer [9–11].

Clinically, cough, dyspnea, fever, and fatigue are prominent symptoms [12–14]. While some patients present with acute symptoms, others may progress to the fibrotic stage [6]. History, exposure

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determination, and high-resolution computed tomography (HRCT) are critical for diagnosis [15]. Antigen removal is the basis of treatment for HP. Survival is poorer in cases where exposure cannot be identified and continues [13, 16]. Serum-specific IgG, bronchoalveolar lavage (BAL) lymphocytosis, and lung biopsy are supportive methods in the diagnosis of HP [4]. Due to the high mortality and rapid progression of fibrotic HP, there is a need for biomarkers to identify high-risk patients at an early stage [5, 8].

In this context, KL-6, which reflects type II alveolar epithelial damage, and SP-D, which is involved in the immune response, are prominent biomarkers. KL-6 is a high molecular weight glycoprotein found on the membrane of type II alveolar epithelial cells. It is released during cell damage and has been associated with activity and prognosis in interstitial lung diseases [5, 8, 17, 18]. SP-D is a collectin secreted from type II pneumocytes and involved in the innate immune response [8, 19]. Serum KL-6 and SP-D levels are used in the diagnosis and follow-up of various ILDs, including HP, particularly in Japan. They have been associated with epithelial damage and mortality [8, 13, 20, 21]. However, studies specific to HP are limited [1, 5]. Although KL-6 and SP-D biomarkers have been evaluated in hypersensitivity pneumonia in previous studies, many of these studies have not thoroughly analyzed biomarker levels in conjunction with clinical findings, radiological features, and treatment strategies. This study aimed to evaluate serum KL-6 and SP-D levels in patients diagnosed with HP and to determine the factors affecting these levels.

2. Methods

This cross-sectional study included 42 patients with hypersensitivity pneumonitis (HP) and 42 age- and gender-matched healthy volunteers. The Ethics Committee of Ankara Sanatorium Training and Research Hospital approved the study (2024-BCEK/247), and all participants provided informed consent. The HP diagnosis was established through a multidisciplinary evaluation by an experienced pulmonologist, radiologist, pathologist, and occupational disease specialist at the Interstitial Lung Diseases Council.

The control group comprised individuals in whom HP was ruled out. Their occupations and environmental exposures (e.g., dust, chemicals, animal allergens) were investigated, and they had no connective tissue, autoimmune, or malignancy-related interstitial lung diseases. Patient data included demographics, exposure history, occupation, smoking status, comorbidities, HRCT findings, pulmonary function tests (FEV_1, FVC, FEV_1/FVC, DLCO), 6-minute walk test (6MWT), and lab results. Patients were classified as fibrotic or non-fibrotic HP based on radiological and histopathological features.

Serum KL-6 and SP-D levels were measured in ng/mL using commercial ELISA kits (Shanghai Coon Koon Biotech) according to the manufacturer's instructions. Washing was performed with HUMAN COMBI WASH, and optical density was read with the NEXT LEVEL ALISEI device. All analyses followed the manufacturer's protocols; raw results were multiplied by the dilution factor to obtain final concentrations. Internal quality control results were within the reference ranges. Samples were collected via centrifugation at appropriate conditions, and measurements at 450 nm confirmed ELISA accuracy and reliability. No additional accuracy tests were conducted. The study evaluated the relationships between KL-6 and SP-D levels in fibrotic and non-fibrotic HP groups and controls.

Using GPower 3.1, the minimum sample size required to achieve 90% power with an effect size of 0.6 was calculated as 39 patients with hypersensitivity pneumonia and 39 healthy controls. To account for potential data loss, 42 patients and 42 healthy volunteers were included. Data were analyzed using IBM SPSS Statistics 22.0 software. Categorical variables were presented as counts (%), and numerical variables as mean ± standard deviation. Normality of distribution was assessed using the Kolmogorov-Smirnov test. The chi-square test was used for categorical variables, the Kruskal-Wallis and Mann-Whitney U tests for intergroup comparisons, and the Spearman correlation coefficient for correlation analyses. ROC analysis was performed for the diagnostic accuracy of biomarkers. A p-value < 0.05 was considered statistically significant.

3. Results

The study included 42 healthy individuals in the control group and 42 individuals with HP in the study group. Smoking history was detected more frequently in the HP group (64.3% vs. 50%), but the difference was not statistically significant (p=0.27). Symptoms were detected in 83.3% of the HP group, while all individuals in the control group were asymptomatic. The most common symptoms were cough (61.9%), shortness of breath (69.0%), sputum (33.3%), fever (19.0%), and weight loss (16.7%). The presence of comorbidities was similar in both groups (control 42.9%, HP 45.2%). When exposure types were examined in the HP group, animal protein exposure was the most common (54.7%). This was followed by unknown exposure (26.1%), metal exposure (7.1%), fungal-mold exposure (7.1%), and plant protein exposure (4.7%).

Laboratory tests revealed significantly elevated WBC and CRP levels in the HP group (p<0.001 for both). There was no difference in LDH values (p=0.40). In respiratory function tests, FEV1, FVC, and FEV1/FVC were similar, while DLCO/SB was significantly lower in the HP group (82.1±24.0 vs 98.1±9.9; p<0.01).

The 6-Minute Walk Test (6-MWT) distance was similar in both groups (p=0.21), but the HP group had lower start and end oxygen saturations (O_2 sat) (p<0.01 and p<0.001, respectively), a lower start heart rate, and a significantly higher end heart rate (p<0.01 for both).

High-resolution computed tomography (HRCT) findings revealed that ground-glass opacity was the most frequently observed finding in HP patients (41.7%), followed by centrilobular nodules, linear reticular pattern, mosaic attenuation, honeycomb pattern, traction bronchiectasis, and fibrosis. 66.7% of HP patients were recorded as non-fibrotic and 33.3% as fibrotic type. In terms of treatment, 42.8% of HP patients received no treatment, while 57.1% received treatment. Among the treated patients, 83.3% (n=20) received steroid treatment, and 16.6% (n=4) received a combination steroid and antifibrotic treatment (Table 1).

When HP patients were classified as fibrotic (n=14) and non-fibrotic (n=28), the age distribution was similar between groups (p=0.36). A significant difference was observed by gender: the proportion of women in the non-fibrotic group was 39.3%, whereas in the fibrotic group it was only 7.1% (p < 0.05). Smoking status and presence of comorbidities did not differ between groups (p=0.30 and p=0.74, respectively). There was no significant difference in the presence of symptoms between the two groups (non-fibrotic 85.7%, fibrotic 78.6%; p=0.66). The two groups were similar in terms of exposure types; animal protein exposure was the most common (non-fibrotic 57.1%, fibrotic 50.0%; p=0.74).

Laboratory findings showed that WBC values were slightly higher in the fibrotic group (Table 2), but the difference was borderline significant (p=0.05); there was no significant difference between the groups in CRP and LDH values.

In respiratory function tests, FEV1, FVC, and FEV1/FVC were similar; however, DLCO/SB was significantly lower in the fibrotic group (69.2±19.1 vs. 88.5±23.9; p<0.05). There were no differences between the groups in 6-Minute Walk Test distance and oxygen saturation values. In HRCT findings, centrilobular nodules were more common in the non-fibrotic group (92.9% vs 57.1%; p<0.05), while honeycombing was more common in the fibrotic group (85.7% vs 7.1%; p<0.001). Traction bronchiectasis (85.7% vs 17.9%; p<0.001) and fibrosis (100% vs 0; p<0.001) were predominant in the fibrotic group. Ground-glass opacity was similar in both groups.

When examining the diagnostic methods for cases diagnosed with hypersensitivity pneumonitis (HP), it was determined that 61.9% of patients (n=26) were diagnosed based on clinical and radiological findings, 30.9% (n=13) were confirmed histopathologically, and 7.1% (n=3) were diagnosed based on bronchoalveolar lavage (BAL) findings. Regarding treatment status, approximately half of both groups had received treatment (non-fibrotic 57.1%, fibrotic 57.1%). (Table 2)

In terms of KL-6 and SP-D biomarkers, both KL-6 (median 5.95 ng/ml vs 5.4 ng/ml, p<0.001) and SP-D (median 14.87 ng/ml vs 14.72 ng/ml, p<0.05) levels were significantly higher in HP patients than in the control group. In non-fibrotic HP patients, KL-6 levels were higher than in the fibrotic

Table 1. Sociodemographic Characteristics of Patients

			Without Hypersensitivity Pneumonitis (Control Group) n=42	With Hypersensitivity Pneumonitis (Study Group) n=42	
			n(%)	n(%)	*p* value
Age (decade)	30-39		6(14.3)	5(11.9)	0.97[a]
	40-49		10(23.8)	11(26.2)	
	50-59		12(28.6)	12(28.6)	
	≥60		14(33.3)	14(33.3)	
Gender	Female		11(26.2)	12(28.6)	>0.99[a]
	Male		31(73.8)	30(71.4)	
Smoking	No		21(50.0)	15(35.7)	0.27[a]
	Yes		21(50.0)	27(64.3)	
		Former	0	17(40.5)	
		Current	21(100)	10(23.8)	
Symptoms	No		42(100)	7(16.7)	**<0.001**
	Yes[1]		0	35(83.3)	
			Cough	26(61.9)	
			Sputum	14(33.3)	
			Dyspnea	29(69.0)	
			Fever	8(19.0)	
			Weight loss	7(16.7)	
Comorbidities	No		24(57.1)	23(54.8)	>0.99[a]
	Yes[2]		18(42.9)	19(45.2)	
		DM	12(28.6)	13(31.0)	
		HT	11(26.2)	11(26.2)	
		other	0	6(14.3)	
Exposure Type			Animal protein	23(54.7)	
			Unknown	11(26.1)	
			Metal	3(7.1)	
			Fungus-mold	3(7.1)	
			Plant protein	2(4.7)	
Laboratory[3]	WBC		6(5-8)	7(5-13)	**<0.001**[b]
	CRP		2(1-5)	4(1-81)	**<0.001**[b]
	LDH		174.5(139-210)	174.5(26-436)	0.40[b]
Pulmonary function test[4]	FEV1		83.0±6.7	85.2±21.8	0.44[b]
	FVC		84.5±8.2	88.4±22.7	0.21[b]
	FEV1/FVC		79.4±3.3	78.6±5.2	0.37[b]
	DLCO/SB		98.1±9.9	82.1±24.0	**0.001**[b]

		Without Hypersensitivity Pneumonitis (Control Group) n=42	With Hypersensitivity Pneumonitis (Study Group) n=42	
		n(%)	n(%)	*p* value
6 MWT	Not completed	0	2(4.8)	0.49[a]
	Completed	42(100)	40(95.2)	
	Distance (meters)	480(400-600)	480(400-720)	0.21[b]
	Start sat O_2	97(92-98)	95(82-99)	**0.002**[b]
	End sat O_2	96(90-102)	93(65-99)	**<0.001**[b]
	Start heart rate	96(83-99)	91.5(70-119)	**0.005**[b]
	End heart rate	97(83-108)	105(71-142)	**0.001**[b]
High-Resolution Computed Tomography findings (HRCT) [5]		Ground glass	35(41.7)	
		Centrilobular nodule	34(40.5)	
		Linear reticulation	29(34.5)	
		Mosaic attenuation	22(26.2)	
		Traction bronchiectasis	17(20.2)	
		Honeycombing	14(16.7)	
		Fibrosis	14(16.7)	
HP type	Non-fibrotic		28(66.6)	
	Fibrotic		14(33.3)	
Treatment status	No		18(42.8)	
	Yes		24(57.1)	

[1]A patient has more than one symptom; [2]A patient has more than one comorbidity; [3]median(min-max); [4] mean±standard deviation; [5]A patient has more than one radiology finding; [a] χ^2; [b] Mann-Whitney U test; DM: Diabetes Mellitus; HT: Hypertension; 6 MWT: 6-minute walk test

Table 2. Comparison of hypersensitivity pneumonitis subgroups

		Non-fibrotic n=28	Fibrotic n=14	
		n(%)	n(%)	*p* value
Age (decade)	30-39	4(14.3)	1(7.1)	0.36[a]
	40-49	6(21.4)	5(35.7)	
	50-59	10(35.7)	2(14.3)	
	≥60	8(28.6)	6(42.9)	
Gender	Female	11(39.3)	1(7.1)	**0.03**[a]
	Male	17(60.7)	13(92.9)	
Smoking	No	12(42.9)	3(21.4)	0.30[a]
	Yes	16(57.1)	11(78.6)	

Table 2 (*Continued*)

		Non-fibrotic n=28	Fibrotic n=14	
		n(%)	n(%)	*p* value
Symptoms	No	4(14.3)	3(21.4)	0.66[a]
	Yes [1]	24(85.7)	11(78.6)	
Comorbidities	No (n=23)	16(57.1)	7(50.0)	0.74[a]
	Yes [2] (n=19)	12(42.9)	7(50.0)	
Exposure types	Animal protein (n=23)	16(57.1)	7(50.0)	0.74[a]
	Unknown (n=11)	8(28.6)	3(21.4)	
	Metal (n=3)	1(3.6)	2(14.3)	
	Fungus-mold (n=3)	2(7.1)	1(7.1)	
	Plant protein (n=2)	1(3.6)	1(7.1)	
Laboratory[3]	WBC	7(5-13)	8(5-11)	0.05[b]
	CRP	3.5(1-28)	4.5(3-81)	0.07[b]
	LDH	171.5(99-436)	187.5(26-260)	0.28[b]
Pulmonary function test[4]	FEV1	87.5±22.1	80.5±21.1	0.29[b]
	FVC	92.3±23.5	80.5±19.6	0.13[b]
	FEV1/FVC	77.8±5.1	80.2±5.2	0.06[b]
	DLCO/SB	88.5±23.9	69.2±19.1	**0.01**[b]
6 MWT [3]	Not completed (n=2)	2(7.1)	0	0.54[a]
	Completed (n=40)	26(92.9)	14(100)	
	Distance (meters)	480(400-720)	480(400-640)	0.80[b]
	Start sat O_2	95(89-99)	94.5(82-98)	0.33[b]
	End sat O_2	94(80-98)	91(65-99)	0.16[b]
	Start heart rate	92(70-111)	90(76-119)	0.33[b]
	End heart rate	105(71-140)	105(85-142)	0.95[b]
High-Resolution Computed Tomography findings (HRCT) [5]	Ground-glass (n=35)	24(85.7)	11(78.6)	0.66[a]
	Centrilobular nodule (n=34)	26(92.9)	8(57.1)	**0.01**[a]
	Linear reticulation (n=29)	17(60.7)	12(85.7)	0.15[a]
	Mosaic attenuation (n=22)	17(60.7)	12(85.7)	0.23[a]
	Honeycomb (n=14)	2(7.1)	12(85.7)	**<0.001**[a]
	Traction bronchiectasis (n=17)	5(17.9)	12(85.7)	**<0.001**[a]
	Fibrosis (n=14)	0	14(100)	**<0.001**[a]
Treatment status	No (n=18)	12(42.9)	6(42.9)	>0.05[a]
	Yes (n=24)	16(57.1)	8(57.1)	

[1] A patient has more than one symptom.
[2] A patient has more than one comorbidity.
[3] median(min-max) [4] mean±standart deviation.
[5] A patient has more than one radiology finding.
[a] Chi sqare test.
[b] Mann-Whitney U test.

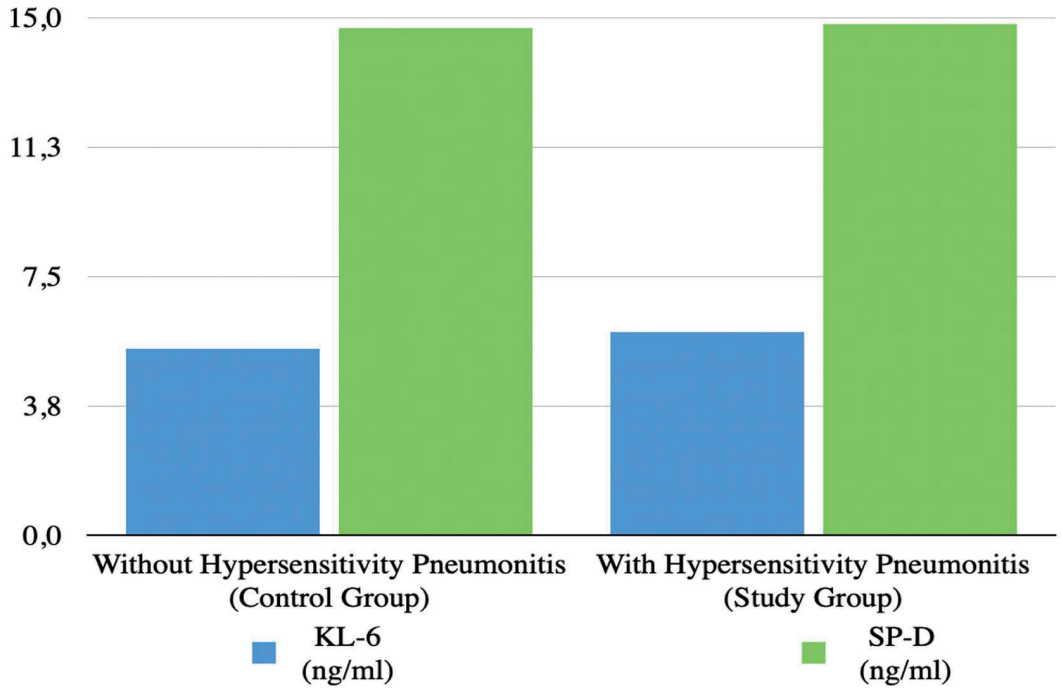

Figure 1. Median values of serum KL-6 and SP-D levels in the hypersensitivity pneumonitis (study) group and the non-hypersensitivity pneumonitis (control) group.

group (median 6.07 ng/ml vs 5.62 ng/ml, p<0.05), and SP-D values did not differ significantly between the groups (p=0.71).

Serum KL-6 and SP-D levels in the hypersensitivity pneumonitis group and the control group are shown in Figure 1.

KL-6 values were significantly higher in untreated patients than in treated patients (median 6.30 ng/ml vs 5.65 ng/ml, p<0.01), whereas there was no significant difference in SP-D levels (p=0.26). (Table 3)

The median KL-6 value was 5.70 (4.05–11.60) in the comorbidities group and 5.50 (3.05–37.30) in the non-comorbidities group. The median SP-D value was 14.95 (5.0–17.80) in the group with comorbidities and 14.65 (7.10–94.95) in the group without comorbidities. KL-6 and SP-D values did not differ significantly between the groups based on the presence of comorbidities (p = 0.17 and p = 0.33, respectively). When examined by exposure type, KL-6 and SP-D values also did not differ (p = 0.41 and p = 0.06, respectively). (Table 4)

When KL-6 and SP-D values were evaluated based on HRCT findings in HP patients, it was found that KL-6 values were significantly higher in individuals without linear reticulation compared to those with linear reticulation (median 6.85 ng/ml vs 5.65 ng/ml; p<0.01), while no difference was observed in SP-D values (p=0.24). Additionally, KL-6 values were significantly higher in patients without fibrosis than in those with fibrosis (6.07 ng/ml vs 5.62 ng/ml; p<0.05), whereas SP-D levels were not statistically different (p=0.07).

Honeycomb and traction bronchiectasis were found to be associated with significantly higher SP-D levels compared to those without (p<0.05 for both), while no significant association was observed for KL-6 levels. There was no significant relationship between the markers and ground-glass opacity, centrilobular nodules, or mosaic attenuation (p>0.05 for all). (Table 5)

Spearman correlation analysis revealed a weak positive correlation between KL-6 and SP-D values

Table 3. Comparison of KL-6 and SP-D values between patients with and without hypersensitivity pneumonitis.

	KL-6* (ng/ml)	p value**	SP-D* (ng/ml)	p value**
Without Hypersensitivity Pneumonitis (Control Group) n=42	5.4(3.05-6.20)	**<0.001**	14.72(5.0-16.80)	**0.03**
With Hypersensitivity Pneumonitis (Study Group) n=42	5.95(4.15-37.30)		14.87(11.8-94.5)	
Non-fibrotic (n=28)	6.07(4.15-37.3)	**0.04**	15.07(14.0-94.95)	0.71
Fibrotic (n=14)	5.62(4.75-6.35)		14.45(11.8-17.05)	
No treatment (n=18)	6.30(5.35-37.3)	**0.005**	14.95(13.35-94.95)	0.26
Treated (n=24)	5.65(4.15-7.30)		14.82(11.8-19.75)	

**median(min-max); **Mann Whitney-U test; KL-6: Krebs von den Lungen-6; SP-D : Surfactant Protein-D.*

Table 4. Association of KL-6 and SP-D markers with comorbidities and exposure.

		KL-6[1] (ng/ml)	p value	SP-D[1] (ng/ml)	p value
Comorbidities	No (n=47)	5.50(3.05-37.30)	0.17 [2]	14.65(7.10-94.95)	0.33 [2]
	Yes (n=37)	5.70(4.05-11.60)		14.95(5.0-17.80)	
Exposure type	Animal protein (n=23)	6.05(4.15-37.30)	0.41[3]	15.10(14-94.95)	0.06 [3]
	Unknown (n=11)	5.90(5.20-8.55)		14.75(14.15-19.75)	
	Metal* (n=3)	6.0(5.65-6.25)		14.15(13.36-14.25)	
	Fungus-mold (n=3)	5.5(4.75-5.50)		14.5(11.80-15.45)	
	Plant protein (n=2)	8.2(4.8-11.6)		16.95(16.70-17.20)	

*[1] median(min-max); [2] Mann Whitney-U test; [3] Kruskal Wallis test; * Occupations of the cases with metal exposure were metal processing (n=1) and hard metal cutting (n=2). Identified exposure agents were metalworking fluids (boron oil, n=1) and cobalt (n=2).*

Table 5. Comparison of HRCT findings with KL-6 and SP-D values.

HRCT Findings[1] (n=42)		KL-6[2] (ng/ml)	p value[3]	SP-D[2] (ng/ml)	p value[3]
Ground-glass	No (n=7)	5.65(5.35-6.35)	0.32	14.6(14.15-16.80)	0.91
	Yes (n=35)	6.05(4.15-37.3)		14.9(11.8-94.95)	
Centrilobular nodule	No (n=8)	5.82(4.80-8.55)	0.91	14.42(13.35-16.70)	0.14
	Yes (n=34)	5.95(4.15-37.3)		14.92(11.8-94.95)	
Linear reticulation	No (n=13)	6.85(4.15-37.3)	**0.009**	15.05(14.05-94.95)	0.24
	Yes (n=29)	5.65(4.75-7.95)		14.85(11.8-17.05)	
Mosaic attenuation	No (n=20)	5.65(4.15-37.30)	0.14	14.75(14.15-94.95)	0.27
	Yes (n=22)	6.20(4.75-9.45)		14.92(11.8-17.8)	
Honeycombing	No (n=28)	6.07(4.15-37.3)	0.07	15.07(14.0-94.95)	**0.02**
	Yes (n=14)	5.62(4.75-7.30)		14.45(11.8-16.7)	
Traction bronchiectasis	No (n=25)	6.15(4.15-37.3)	0.06	15.10(14.0-94.95)	**0.02**
	Yes (n=17)	5.65(4.75-7.30)		14.6(11.8-16.8)	
Fibrosis	No (n=28)	6.07(4.15-37.30)	**0.04**	15.07(14.0-94.95)	0.07
	Yes (n=14)	5.62(4.75-6.35)		14.45(11.8-17.05)	

[1] A patient may have more than one HRCT finding; [2] median (min-max);[3] Mann-Whitney-U test.

in HP patients, but this correlationwas not statistically significant (rho=0.212; p=0.178).

Correlation analysis was also conducted between KL-6 and SP-D levels and respiratory function parameters, showing no statistically significant relationships with DLCO-SB, FEV_1, or FVC.

3.1. ROC Analysis

The diagnostic performance of the KL-6 and SP-D biomarkers for hypersensitivity pneumonitis was evaluated using ROC analyses. KL-6 was shown to provide moderat diagnostic value for the diagnosis of HP (AUC=0.76; SE=0.05; 95% CI 0.66–0.86; p<0.001). The cut-off value was >5.42 ng/ml, with a sensitivity of 78% and specificity of 42%.

SP-D had more limited but significant diagnostic performance(AUC=0.63; SE=0.06; 95% CI 0.51–0.75; p=0.03). The cut-off value was >14.57 ng/ml, with a sensitivity of 64% and a specificity of 52% (Figures 2 and 3).

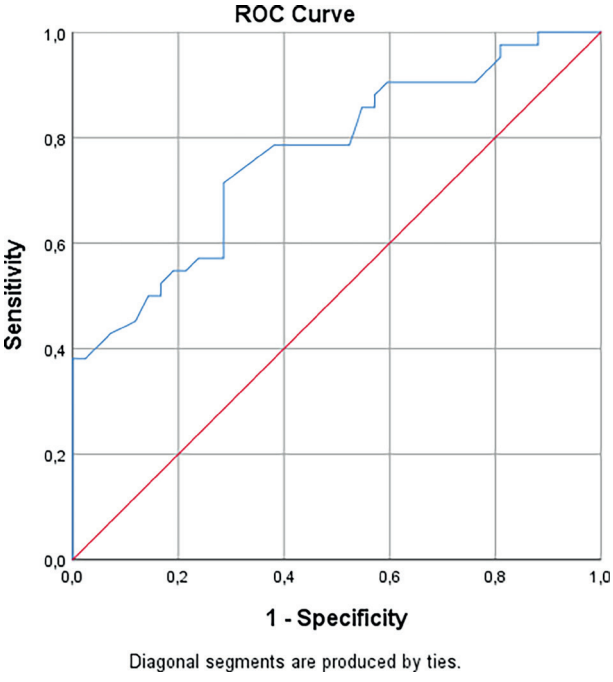

Figure 2. KL-6 marker ROC curve (AUC=0.76; SE=0.05; 95% CI 0.66–0.86; p<0.001).

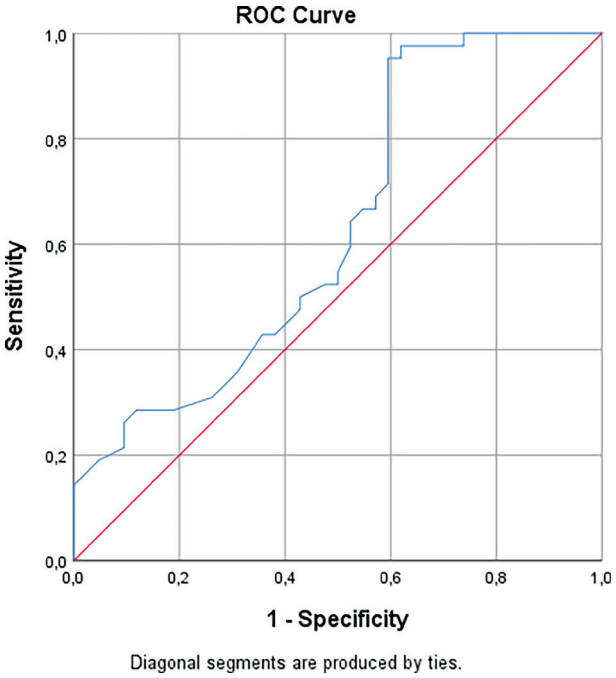

Figure 3. SP-D marker ROC curve (AUC=0.63; SE=0.06; 95% CI 0.51–0.75; p=0.03).

4. Discussion

Hypersensitivity pneumonitis (HP) is an interstitial lung disease that requires early diagnosis and regular follow-up. Its fibrotic form, in particular, can clinically mimic idiopathic pulmonary fibrosis (IPF) and carries a high mortality risk. In this context, KL-6 and SP-D are gaining importance as potential biomarkers for assessing disease activity and course. In our study, significant differences in serum KL-6 and SP-D levels were observed between patients with HP and healthy controls. Important findings related to subtypes and treatment status were also noted. The highest levels of both biomarkers were observed in the non-fibrotic HP group, whereas lower levels were observed in patients receiving systemic steroid treatment, suggesting that KL-6 and SP-D may be associated not only with disease presence but also with inflammatory activity and treatment response. Therefore, it was emphasized that these biomarkers have the potential to serve as helpful indicators of disease course. ROC analyses indicate that KL-6

may show supportive performance in distinguishing between the HP and control groups, whereas SP-D has more limited discriminative value. However, due to the study design, variability in biomarker levels, and sample size, these results should be interpreted with caution.

Although some significant correlations between KL-6 and SP-D and respiratory function parameters have been reported in previous studies [22], these relationships were limited to specific parameters or were weak [23, 24]. Other studies found no significant correlations [25, 26]. In our study, no statistically significant relationships were observed between serum KL-6 and SP-D levels and DLCO, FEV_1, or FVC, suggesting that measurements of biomarkers at a single time point may not reveal a clear linear relationship, which might be more evident when monitoring changes over time or assessing specific patient subgroups. Additionally, factors like population heterogeneity, small sample size, the timing of measurements, treatment status, and disease stage may also contribute to the inability to statistically demonstrate these relationships.

The literature reports that carbon monoxide diffusion capacity (DLCO) is significantly lower in patients with fibrotic HP than in those with non-fibrotic HP [27]. Consistent with these findings, our study also found DLCO values to be lower in the fibrotic HP group than in the non-fibrotic HP group. Furthermore, KL-6 levels were significantly higher in HP patients without a linear reticular pattern or fibrosis, and SP-D levels were significantly higher in those without honeycomb and traction bronchiectasis. Conversely, although increases in both biomarkers were observed in cases with ground-glass opacities, centrilobular nodules, and mosaic attenuation, these differences were not statistically significant. In cases where linear reticulation and fibrotic patterns dominate, low KL-6 levels indicate loss of type II alveolar epithelial cells and decreased cellular activity; whereas in advanced fibrotic stages accompanied by honeycomb and traction bronchiectasis, decreased SP-D levels can be explained by destruction of alveolar architecture and a reduction in surfactant-producing cells. The literature reports that BAL KL-6 levels in HP patients are associated with HRCT findings and lymphocytosis [3], and that elevated serum KL-6 levels correlate positively with reticular patterns and honeycombing [28]. These data suggest that KL-6 may serve as a potential biomarker for monitoring disease activity and prognosis in both HP and IPF. In fibrotic HP, structural HRCT findings have been linked to poor prognosis [13], while inflammatory findings are associated with a better prognosis [29]. Our current study also indicates that KL-6 and SP-D levels may be associated with distinct pathological patterns observed on HRCT and that these biomarkers may vary by disease phenotype. Overall, these findings suggest that both biomarkers are associated with pathophysiological processes reflecting different radiological stages of HP.

In our study, serum KL-6 levels were significantly higher in non-fibrotic HP patients than in fibrotic HP patients, whereas no significant difference was observed in SP-D levels. The interaction between inflammation and fibrosis plays a crucial role in the pathogenesis of HP. The inflammatory process, which is predominant in the early stages, leads to alveolar damage and activation of type II pneumocytes, resulting in increased release of biomarkers such as KL-6 and SP-D. Prolonged or recurrent inflammation triggers fibroblast activation and matrix accumulation through interactions with epithelial-structural cells, leading to fibrotic transformation. Therefore, prolonged disease duration or inadequate inflammation control may cause initially elevated KL-6 and SP-D levels to exhibit different patterns over time as fibrosis develops [30].

In the literature, KL-6 has been linked to the prognosis of fibrotic hypersensitivity pneumonitis (HP), and its serial measurement is valuable for early identification of patients at risk of progression [5]. KL-6 is a cell membrane protein secreted by type II pneumocytes and is elevated in interstitial lung diseases (ILDs) with significant inflammation. It can serve as a prognostic and diagnostic marker to distinguish between non-fibrotic and fibrotic HP. It has been reported to correlate with disease activity in both acute and chronic HP, and in non-fibrotic HP, early alveolitis and elevated serum levels may indicate mild alveolar damage [31, 32]. Similarly, high serum KL-6 levels have been observed in cases diagnosed in both domestic and occupational

contexts. It has been emphasized that alveolitis may be present in the early stages of non-fibrotic HP[3]. KL-6 differs from SP-D structurally; it's a cell membrane protein, and increased serum levels indicate membrane damage and enzyme activation. If SP-D increases without a rise in KL-6, it may suggest mild alveolar damage [33]. Our study supports KL-6 reflecting disease activity and being higher in non-fibrotic HP than in fibrotic cases, whereas SP-D is less sensitive for this distinction.

In our study, we found that serum KL-6 levels were significantly lower in HP patients receiving corticosteroid treatment compared to those not receiving treatment. Although a decrease in SP-D levels was also observed, this difference was not statistically significant. Increased KL-6 production in plasma is considered a sensitive indicator of alveolitis, and serum KL-6 levels in interstitial lung diseases (ILD) have prognostic and diagnostic value, reflecting both disease activity and severity [34]. KL-6, produced during the regeneration of type II alveolar epithelial cells, supports epithelial repair in response to alveolar damage, and high plasma levels are considered an indicator of increased airway epithelial permeability [35]. Therefore, targeting KL-6 therapeutically may help achieve optimal effect before fibrosis develops when corticosteroids are administered in the early stages of the disease. Consequently, the detection of elevated plasma KL-6 levels in early-stage HP patients with normal spirometry is clinically significant [36].

In the literature, higher KL-6 levels were observed in the non-fibrotic HP group compared to fibrotic cases, suggesting a link with pneumocyte renewal in response to alveolar damage [37]. Previous studies have shown that HP exacerbations are associated with increases in KL-6 and SP-D levels, while corticosteroid treatment and avoidance of the antigen decrease these biomarker levels [20]. Our study demonstrates that treatment lowers KL-6 and SP-D levels, with a more significant and stronger effect on KL-6. Fibrotic HP has been reported to have a 7-year survival rate estimated at 40.8% in recent studies and to exhibit worse outcomes than many cancers [10, 11]. Increased mortality has highlighted the need for accurate and timely diagnosis and exposure assessment to improve disease outcomes [8]. Research indicates that serial measurements of KL-6 can predict survival, which is associated with the poor prognosis and high mortality of fibrotic HP [38]. Additionally, acute exacerbations are considered negative prognostic indicators, and simply avoiding the antigen is not always enough [13, 39]. These findings support the clinical value of KL-6 and SP-D in HP diagnosis, disease phenotype differentiation, and monitoring disease activity. They also emphasize the importance of biomarker panels for early diagnosis and prognostic prediction.

One of the strengths of our study is that serum KL-6 and SP-D levels in patients with HP were evaluated in detail across fibrotic and non-fibrotic subgroups. Furthermore, the inclusion of a healthy control group contributed to a clearer understanding of the diagnostic value of these biomarkers. The study's scope was broadened and its clinical relevance increased by examining KL-6 and SP-D in Hypersensitivity Pneumonitis not only biomarker levels but also relationships with pulmonary function tests, HRCT findings, exposure types, and treatment response. The fact that our findings are largely consistent with the current literature also supports the value of KL-6 and SP-D as both diagnostic and prognostic biomarkers in clinical practice for HP.

Our study has limitations, including a small sample size reducing statistical power, especially in subgroup analyses. Unlike previous studies reporting KL-6 and SP-D in U/mL, ours used ng/mL, limiting direct comparison, though findings align with literature [40, 41]. Biomarkers measured at a single time point prevented assessment of disease activity changes. Variations in treatment, exposure, and measurement units hindered interpretation. Non-standardized corticosteroid doses and treatment durations may explain the lack of significant SP-D changes. Being cross-sectional without longitudinal follow-up, conclusions on KL-6 are based on literature. As a single-center study, generalizability may be limited.

5. Conclusion

In conclusion, serum levels of KL-6 and SP-D were higher in HP patients than healthy controls. KL-6 was elevated in non-fibrotic HP cases compared to fibrotic ones, indicating it reflects disease activity. SP-D, though sensitive for monitoring,

showed less difference than KL-6. The decline in serum KL-6 with steroid treatment suggests it helps monitor alveolar damage and treatment response. Our study supports the diagnostic and prognostic value of KL-6 and SP-D in HP, potentially linked to diagnosis and disease phenotype. Lower levels in treated patients indicate these markers vary with treatment and may reflect decreased inflammation.

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Institutional Review Board Statement: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration (as revised in 2013) and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Ankara Sanatorium Training and Research Hospital (2024-BCEK/247).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

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