

Research Article

Lymphocyte Stimulation Test and Serum KL-6 as Adjuvant Diagnostic Markers of Bird-Related Chronic Hypersensitivity Pneumonitis

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Abstract

Background: The differential diagnosis of Idiopathic Pulmonary Fibrosis (IPF) and chronic hypersensitivity pneumonitis (HP) is difficult. The lymphocyte stimulating test (LST) for chronic HP is occasionally used, but its sensitivity is not high enough. Krebs von den Lungen 6 (KL-6) is known to be a serum marker for interstitial pneumonia. But usefulness of combination of LST and serum KL-6 for the diagnosis of chronic HP is still remained uncertain.

Objective: The purpose of this study was to analyze the usefulness of LST as a diagnostic marker for chronic HP, and LST and serum KL-6 levels were compared in chronic HP cases.

Methods: Serum KL-6 and LST on peripheral mononuclear cells were measured in subjects with IPF (n = 18; 10 males and 8 females, 68.1 ± 1.3 years old), bird-related chronic HP (n = 9; 5 males and 4 females, 69.2 ± 4.1 years old), and controls (n = 10; 5 males and 5 females, 62.4 ± 3.4 years old).

Results: KL-6 and the LST stimulation index were higher in chronic HP cases than in IPF and control cases. When KL-6 was greater than 1,000 U/mL and the LST stimulation index was greater than 200.0%, the sensitivity and specificity for chronic HP were 85.7% and 90.0%, respectively.

Conclusions: These findings suggest that presence of a higher serum KL-6 with a positive LST may increase the possibility of chronic HP in the present study.

Keywords

Chronic Hypersensitivity Pneumonitis, KL-6, Lymphocyte Stimulation Test

Abbreviations

LR(+): Positive Likelihood

CRP: C-Reactive Protein

HP: Hypersensitivity Pneumonitis

HRCT: High Resolution Computed Scan

IP: Interstitial Pneumonia

IPF: Idiopathic Pulmonary Fibrosis

KL-6: Krebs Von Den Lungen 6

LDH: Lactate Dehydrogenase

LST: Lymphocyte Stimulating Test

SI: Stimulation Index

Sn: Sensitivity

Sp: Specificity

VC: Vital Capacity

Introduction

Chronic Interstitial Pneumonia (IP) is a heterogeneous disease entity that includes Idiopathic Pulmonary Fibrosis (IPF) and Chronic Hypersensitivity Pneumonitis (HP) [1,2,3]. Although many recent studies have been reported, its prognosis remains poor [1,2,3]. In particular, the clinical characteristics of chronic HP resemble those of IPF, and it is difficult to distinguish them [2,3,4]. HP is an immunologically mediated interstitial lung disease characterized by exposure to inhaled antigens or mold [2]. We previously reported that serum IL-17A and integrin $\alpha 4$ on peripheral lymphocytes were higher in chronic HP cases than in IPF cases [5]. Chronic HP develops insidiously or recurrently due to stimulation by environmental antigens. In chronic HP, diagnosis of the early stage and avoiding environmental antigens are important to prevent the development of pulmonary fibrosis. In Japan, more than half of chronic HP cases are bird-related chronic HP cases [6]. Bird-related chronic HP is induced by bird deposits and feathers, the major component of which is considered to be Avian-Derived Mucin [3-7]. While the antigen inhalation provocation test makes diagnosis easier, it might increase the risk of exacerbation of pulmonary fibrosis [8,9]. Measuring serum bird-related antibody titers, serum precipitant testing with bird-related antigen, or the antigen-induced peripheral Lymphocyte Stimulating Test (LST) is useful for the adjuvant diagnosis of chronic HP, but their sensitivity (Sn) and specificity (Sp) are not high enough in chronic compared to acute HP cases [10]. According to previous reports [5,6,7], High-Resolution Computed Tomography (HRCT) findings and serum Krebs von den Lungen 6 (KL-6) levels also contribute to the diagnosis of interstitial pneumonia, including HP. The serum KL-6 level was shown to be increased in patients with IP [11]. Therefore, we hypothesized that the combination of serum KL-6 levels and the LST Stimulation Index (SI) might affect the Sn and/or Sp for diagnosing chronic HP. To address the usefulness of the LST in chronic IP, both the LST and the serum KL-6 level were compared.

Materials and Methods

Study Design

A total of 27 cases of interstitial pneumonia were recruited from Shimane University Hospital (Shimane, Japan) and Akiota Hospital (Hiroshima, Japan) from April 1, 2008 to July 31, 2014. During the period, 64 interstitial pneumonia cases were enrolled as the candidate. As the exclusion criteria for the subjects was as following; (1) cases which did not meet the criteria for IPF or chronic HP; (2) cases which rejected for participating the study; (3) younger (less than 20 years old) or older cases (more than 85 years old). The subjects with interstitial pneumonia comprised the following: IPF (n = 18) (10 men and 8 women, 68.4 ± 1.3 years old) and chronic HP (n = 9) (5 men and 4 women, 69.2 ± 4.1 years old) (Table 1).

Although chronic HP includes two types, the insidious type and the recurrent type, all cases were the insidious type. Ten subjects (5 men and 5 women, 62.4 ± 3.4 years old) (sex- and age- matched) without respiratory disease were recruited from Akiota Hospital as the control group. At the time of diagnosis, HRCT, pulmonary function testing, and Serum C-Reactive Protein (CRP), Lactate Dehydrogenase (LDH), LST, and KL-6 levels were evaluated. All subjects gave their written, informed consent for participation in the study, which was approved by the institutional review boards of Akiota Hospital and Shimane University Faculty of Medicine. The diagnosis of IPF was based on the criteria of the American Thoracic Society [11], and the diagnosis of chronic HP was based on the previously reported criteria [3,4,6,12-15]. The criteria for chronic HP were: history of inhalation of antigen; positive for specific antibodies and/or LST; episode of respiratory symptoms related to chronic HP by an inhalation challenge test; histopathological evidence of pulmonary findings; compatible findings on HRCT; and chronic duration of symptoms more than 6 months. Pathological findings of open lung biopsy or transbronchial lung biopsy specimens were evaluated.

	IPF	Chronic HP	Control	p value
Number of subjects	18	9	10	
Age (years)	68.4 ± 1.3	69.2 ± 4.1	62.4 ± 3.4	0.671
Gender (Male: Female)	10:8	5:4	5:5	0.813
LSI (S.I. %)	158.4 ± 1.5	324.0 ± 2.6	140.0 ± 2.2	0.026
CRP (mg/dL) (< 0.5)	0.32 ± 0.26	0.43 ± 0.15	0.17 ± 0.07	0.473
LDH (IU/L) (106-211)	174.1 ± 6.0	201.5 ± 21.0	158.1 ± 34.8	0.128
KL-6 (U/mL) (< 499)	678.1 ± 47.3	$1,862.8 \pm 345.6$	287.8 ± 83.5	0.002
%VC (%)	76.18 ± 2.41	80.46 ± 0.79	91.7 ± 7.4	0.061

Table 1: Clinical characteristics of subjects.

All data is expressed as means \pm standard deviation of the means. P values showed statistical significance between IPF group and chronic HP group. IPF: Idiopathic Pulmonary Fibrosis; HP: Hypersensitivity Pneumonitis; LSI: Lymphocyte Stimulation Index; SI: Stimulation Index; CRP: C-Reactive Protein; LDH: Lactate Dehydrogenase; KL-6: Krebs von den Lungen 6; VC: Vital Capacity.

Lymphocyte Isolation and LST

Lymphocytes were purified from 10ml of peripheral venous blood and isolated by Ficoll-Hypaque density-gradient centrifugation [5]. Isolated lymphocytes (2.0×10^5 /well) were incubated in a 96-well-plate with or without 1.0% pigeon sera (purchased from Yamamoto Chemical Company, Hiroshima, Japan). The cells were then incubated with ^3H -thymidine for 24 hours, and radioactivity was counted using a scintillation counter. An S.I. (count stimulated cells/count unstimulated cells) greater than 200.0% was considered positive [9,10,12].

Statistical Analysis

Statistical analysis was performed using computer software (Excel Statistics 2012, SSRI Co., Ltd., Tokyo, Japan). The data are expressed as means \pm standard deviation (SD) of the mean. One-way analysis of variance followed by Fisher's least significant difference test was used to detect differences among groups. A probability value of less than 0.05 was considered significant.

Results

The subjects' clinical characteristics are shown in table 1. There were no significant differences in age and sex among the groups. The LST S.I. (%) was significantly higher in the chronic HP group

than in the IPF group ($p = 0.026$). The serum KL-6 concentration was also significantly higher in the chronic HP group than in the IPF group ($p = 0.002$). Serum CRP and serum LDH levels and pulmonary function testing (%VC) did not show significant differences between the IPF group and the chronic HP group.

The results for KL-6 and LST S.I. (%) were then compared. Serum KL-6 was divided into 3 categories: less than 500 U/mL; greater than or equal to 500 U/mL and less than 1,000 U/mL; or greater than or equal to 1,000 U/mL. LST SI (%) was divided into 2 categories: less than 200.0%, or greater than or equal to 200.0%. The number of cases in each category among the groups and the Sn/Sp for chronic HP are shown in (Table 2).

When KL-6 was greater than or equal to 1,000 U/mL; Sn and Sp were 77.8% and 92.9%; respectively, and when LST S.I. (%) was greater than or equal to 200.0%; Sn and Sp were 61.5% and 95.8%; respectively. Next, serum KL-6 was compared with LST S. I. (%) less than 200.0% or greater than or equal to 200.0%. Serum KL-6 was divided into 3 categories: less than 500 U/mL; greater than or equal to 500 U/mL and less than 1,000 U/mL; or greater than or equal to 1,000 U/mL. The results for LST S.I. (%) and Sn/Sp for chronic HP are shown in (Table 3).

When KL-6 was greater than or equal to 1,000 U/ml and LST S.I. (%) was less than 200.0%, Sn and Sp were 50.0% and 77.1%,

	IPF (n=18)	Chronic HP (n=9)	Control (n=10)	Sn (%)	Sp (%)	LR (+)
KL-6 < 500	2	0	10	0	42.9	0
500 ≤ KL-6 < 1,000	14	2	0	22.2	50	0.44
1,000 ≤ KL-6	2	7	0	77.8	92.9	11
LST < 200 (S.I. %)	14	1	9	4.2	38.5	0.07
200 (S.I. %) ≤ LST	4	8	1	61.5	95.8	14.6

Table 2: Sensitivity and specificity of chronic hypersensitivity pneumonitis.

IPF: Idiopathic Pulmonary Fibrosis; HP: Hypersensitivity Pneumonitis; LSI: lymphocyte Stimulation Index; SI: Stimulation Index; KL-6: Krebs von den Lungen 6; Sn: sensitivity; Sp: specificity; LR (+): Positive likelihood Ratio.

	IPF (n=18)	Chronic HP (n=9)	Control (n=10)	Sn (%)	Sp (%)	LR (+)
LST < 200 (S.I. %)						
KL-6 < 500 (U/mL)	2	0	9	0	60.7	0
500 ≤ KL-6 < 1,000 (U/mL)	11	0	0	0	60.7	0
1,000 (U/mL) ≤ KL-6	1	1	0	50	77.1	2.18
LST ≥ 200 (S.I. %)						
KL-6 < 500 (U/mL)	0	0	1	0	96.4	0
500 ≤ KL-6 < 1,000 (U/mL)	3	2	0	22.2	89.2	2.06
1,000 (U/mL) ≤ KL-6	1	6	0	85.7	90	8.57

Table 3: Comparison between lymphocyte stimulation index titer and diagnosis of chronic hypersensitivity pneumonitis.

IPF: Idiopathic Pulmonary Fibrosis; HP: Hypersensitivity Pneumonitis; LSI: Lymphocyte Stimulation Index; S.I.: Stimulation Index; KL-6: Krebs von den Lungen 6; Sn: sensitivity; Sp: specificity; LR (+): Positive likelihood Ratio.

respectively. When KL-6 was greater than or equal to 1,000 U/ml and LST S.I. (%) was greater than or equal to 200.0%, Sn and Sp were 85.7% and 90.0%, respectively. The positive likelihood ratio was 8.57.

Discussion

In this study, the Sn for chronic HP of each of KL-6 or LST was not high, but the Sn for chronic HP was increased when both KL-6 and LST S.I. were high. These results indicate that the combination of KL-6 and LST S. I. (%) might lead to suspecting the possibility of chronic HP. Furthermore, lung biopsy is necessary to confirm the diagnosis of chronic HP [2,3,4,14,15,16], but it has the risk of exacerbation of disease activity. However, when the possibility of chronic HP is highly suspected before lung biopsy, lung biopsy could be recommended, since administration of corticosteroid is necessary for such chronic HP cases to minimize the risk of lung fibrosis after diagnosis [7,8,9,15,16].

The limitation of the present study was that the chronic HP subjects all had only the insidious type, with none having the recurrent type. In most insidious type bird-related chronic HP cases, weak but continuous inhalation of bird-related antigens might lead to the development of pulmonary fibrosis, which is different from recurrent type chronic HP cases. Therefore, further examination will be necessary to confirm whether the recurrent type shows a similar tendency to the insidious type. According to a previous report, seasonal variation of the KL-6 concentration is related to the possibility of HP [17]. They showed that the KL-6 concentration in bird-related chronic HP increased in winter. In the present study, there were no data about the seasonal variation of the KL-6 concentration, but seasonal variation of KL-6 might also be a candidate to increase the Sn and Sp for bird-related chronic HP. And another limitation is that number of the sample was rather small. Further examination would be necessary to figure out the involvement of LST and KL-6 in the diagnosis of chronic HP in detail. Although we have limitation in this study, we speculate that measuring LST titer and serum KL-6 may have some clinical relevance in chronic HP patients. For example, systemic corticosteroid therapy might be appropriate candidates as therapeutic options in chronic HP cases not IPF cases after considering the results of LST titer and serum KL-6.

Conclusion

In summary, the present study suggests that the presence of a higher serum KL-6 with a positive lymphocyte stimulation test may increase the possibility of insidious type chronic HP.

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