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Serum surfactant protein A as a surrogate biomarker of a negative heart sign among patients with interstitial lung disease

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ABSTRACT

The mechanisms underlying interstitial lung disease (ILD) are characterized by variable inflammation or fibrosis of the pulmonary interstitium. A negative heart sign (NHS) on ^{67}Ga scintigrams of patients with ILD is due to considerably increased inflammatory activity in the lungs. We retrospectively analyzed relationships between NHS and established biomarkers of disease severity in patients with ILD. Among 81 consecutive non-smoking patients with ILD (mean age, 63 years) who had been hospitalized between April 2009 and October 2011, we selected 52 who had been assessed by ^{67}Ga scintigraphy. We then evaluated relationships between NHS and blood biomarkers, pulmonary function and high-resolution computed tomography (HRCT). Among these 52 patients, 10 showed idiopathic pulmonary fibrosis and 42 had other ILD. Multivariate analysis with stepwise variable selection, serum surfactant protein (SP)-A (OR (odds ratio), 1.026; 95%CI (confidence interval), 1.003–1.050; P = 0.024) and inflammation index calculated from HRCT findings (OR, 1.358; 95%CI, 1.079–1.709; P = 0.009) were significant predictors of an NHS. Serum SP-A offered 85% sensitivity and 75% specificity for predicting NHS at an optimal cut-off of 45.8 ng/mL. Serum SP-A concentrations correlated positively with inflammation index (r = 0.344, P = 0.015). In conclusion, serum SP-A might serve as a surrogate biomarker for predicting an NHS in patients with ILD.

Keywords: inflammation, idiopathic pulmonary fibrosis, surfactant protein, treatment, gallium uptake

Abbreviations:
%DLco: percentage predicted diffusion capacity of carbon monoxide
%FVC: percentage predicted forced vital capacity
%VC: percentage predicted vital capacity
AE: acute exacerbation
AUC: area under the receiver operating characteristics curve
CI: confidence interval
CTD-ILD: connective tissue disease-associated ILD
ELD: eosinophilic lung disease
HP: hypersensitivity pneumonitis
HRCT: high-resolution computed tomography

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IgG4-RD: immunoglobulin G4-related disease
ILD: interstitial lung disease
iNSIP: idiopathic nonspecific interstitial pneumonia
IPF: idiopathic pulmonary fibrosis
NHS: negative heart sign
OP: organizing pneumonia
OR: odds ratio
ROC: receiver operating characteristics
SD: standard deviation
SP: surfactant protein

INTRODUCTION

The pathological mechanisms underlying interstitial lung disease (ILD) are characterized by various inflammatory processes or fibrosis of the pulmonary interstitium.[1] Evaluation of the severity of lung inflammation in patients with ILD is crucial when predicting disease activity and deciding therapeutic strategies. Various parameters for detecting lung inflammation (alveolitis) have been reported, including blood biomarkers (surfactant protein (SP)-A and SP-D[2,3]), and high-resolution CT (HRCT) scores (inflammation index[4]). However, parameters allowing accurate evaluation of lung inflammation in clinical practice have yet to be established.

Increased uptake of ^{67}Ga on pulmonary scintigrams offers a reliable indicator of strong lung inflammation, with such uptake appearing brighter than cardiac (blood flow) images.[5-7] This is referred to as a negative heart sign (NHS) (Figure 1).[8] However, ^{67}Ga scintigraphy is unsuitable for rapid and simple assessments of lung inflammation in terms of labor and cost.

The present study retrospectively investigated data from patients who had been assessed using

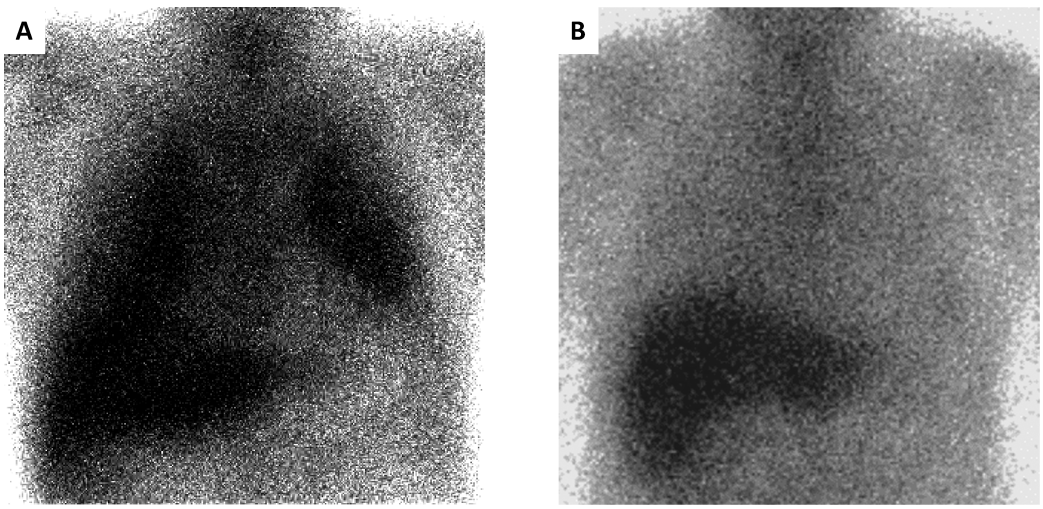

Fig. 1 Representative images for presence and absence of a negative heart sign (NHS)
Fig. 1A: Presence of an NHS.
Fig. 1B: Absence of an NHS.

^{67}Ga scintigraphy, with the aim of identifying parameters available in clinical practice that can help to predict an NHS.

MATERIALS AND METHODS

Study location and patients

This study proceeded at the National Defense Medical College Hospital in Japan. We selected 52 non-smoking patients with ILD who had been admitted to hospital and assessed by ^{67}Ga scintigraphy between April 2009 and October 2011. Because serum SP-A is reportedly increased due to the effects of smoking, current smokers were excluded from this research.[9] Patients were then assigned to groups depending on the presence or absence of an NHS on ^{67}Ga scintigraphy (Figure 2). Medical history, physical findings, blood biomarkers, pulmonary function and HRCT findings were compared between groups.

Diagnosis of ILD

Idiopathic pulmonary fibrosis (IPF) was diagnosed based on the IPF consensus classification.[10] Patients without IPF were subdivided according to whether ILD was stable at the time of evaluation or exacerbated non-IPF was present, defined as acute, progressive disease requiring steroid pulse therapy and accompanied by fever, dry cough, and/or dyspnea.[11] Connective tissue disease-associated ILD (CT-ILD) was diagnosed in patients without IPF based on physical, serological and HRCT findings that were consistent with ILD. Lung biopsy specimens were histologically

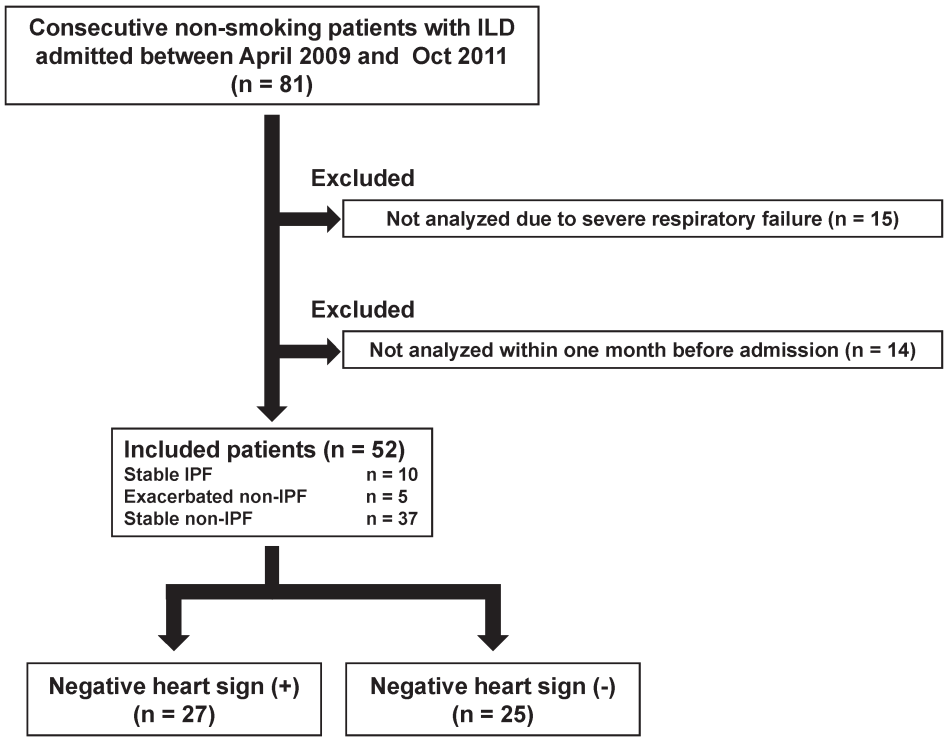

Fig. 2 Flowchart of patient recruitment and analysis

evaluated to exclude other specific diseases. Idiopathic nonspecific interstitial pneumonia (iNSIP), organizing pneumonia (OP), eosinophilic lung disease (ELD), hypersensitivity pneumonitis (HP), pulmonary sarcoidosis and immunoglobulin G4-related disease (IgG4-RD) were diagnosed based on established criteria.[10,12-16]

Pulmonary function tests and blood biomarkers

Blood samples were collected at the time of admission from all patients, and serum SP-A (normal < 43.8 ng/mL), SP-D (normal < 110 ng/mL) and KL-6 (normal < 500 U/mL) were measured. Lung function was tested within 1 month before admission and percentage predicted forced vital capacity (%FVC), percentage predicted vital capacity (%VC), and percentage predicted diffusion capacity of carbon monoxide (%DLco) were determined.

HRCT and ^{67}Ga scintigraphy

Patients were evaluated by HRCT and ^{67}Ga scintigraphy within 1 month before admission and images were independently assessed by two pulmonologists and one radiologist. Findings from HRCT were evaluated using the semi-quantitative scoring method described by Ooi et al.[4] Abnormalities on HRCT of the lungs were categorized as ground glass opacity, mixed ground glass and reticular disease, or reticular fibrosis and honeycomb lung, then scored based on ratios (%) of disease in each of the six lung lobes (Figure 3). Global scores were calculated by adding the scores for each anomaly in all lobes. An inflammation index was derived from the sum of scores for ground glass opacity, mixed ground glass and reticular disease. Fibrosis index was

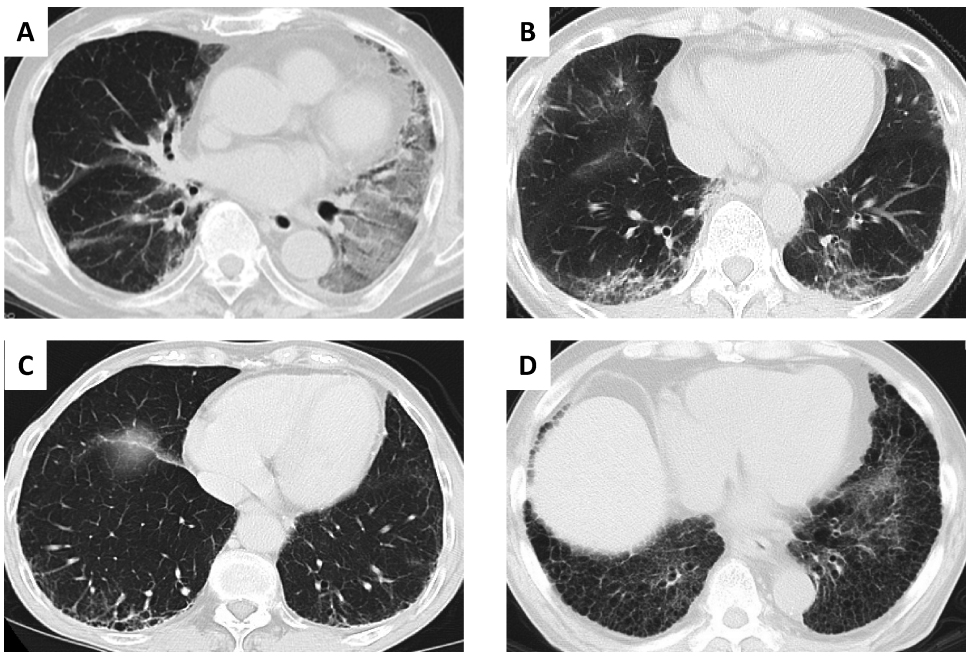

Fig. 3 Abnormalities on HRCT of the lungs
Fig. 3A: Ground glass opacity.
Fig. 3B: Mixed ground glass and reticular disease.
Fig. 3C: Reticular fibrosis.
Fig. 3D: Honeycomb lung.

calculated as the sum of reticular fibrosis and honeycomb scores.

Statistical analysis

Data were statistically analyzed using JMP version 11 software (SAS Institute, Cary, NC) and are expressed as mean ± standard deviation (SD). Groups were compared using Wilcoxon rank-sum tests. Optimal parameter cut-off values were determined from receiver operator characteristics (ROC) curves. Primary predictors for NHS were determined using multiple stepwise regression analysis. Nonparametric Spearman's rank correlation coefficients were calculated to assess correlations between SP-A and other clinical parameters. Values of $P < 0.05$ were considered significant.

Ethics approval

The institutional review board at the National Defense Medical College approved this study (approval number: kan-75; approval date: 19 October 2011). In all patients, consent for participation of this retrospective study was obtained by disclosing a clinical study including the description of opt-out (http://www.ndmc.ac.jp/wp-content/uploads/2016/03/test_state75.pdf).

RESULTS

Patient characteristics

Among 81 consecutive non-smoking patients with ILD, 52 were assessed by ^{67}Ga scintigraphy within 1 month before hospitalization. Among the remainder, 15 patients with severe respiratory failure who did not undergo scintigraphy and 14 patients who did not undergo scintigraphy within 1 month before hospital admission were excluded. Ten of the 52 patients were diagnosed with IPF. The remaining 42 patients had other types of ILD, comprising CT-ILD (n = 3; including polymyositis/dermatomyositis, rheumatoid arthritis and autoimmune hepatitis (n = 1 each)), iNSIP (n = 2), OP (unknown etiology n = 5; drug-induced, n = 1), ELD (eosinophilic granulomatosis with polyangiitis, n = 1; eosinophilic pneumonia n = 1), HP (n = 6), pulmonary sarcoidosis (n = 19) and IgG4-RD (n = 4). ILD was stable in 37 patients, with the remaining 5 patients showing exacerbated ILD (CT-ILD, n= 2; OP, n = 1; ELF, n = 2) at the time of evaluation.

Comparison of patients with and without NHS

Mean age of the 52 patients was 63 years, with 31 males (62%) and 48 patients (92%) showing pathologically confirmed ILD. Patients were categorized from ^{67}Ga scintigraphy as showing presence (n = 27) or absence (n = 25) of an NHS (Figure 2). Table 1 shows the characteristics of patients. Age, sex, smoking status, pathologically confirmed disease, diagnostic details and outcomes did not differ significantly between patients with and without an NHS. In contrast, serum SP-A, SP-D, KL-6, %VC, %FVC, %DLco, inflammation index and fibrosis index differed significantly between groups.

Predicting an NHS using ROC curves

Areas under ROC curves (AUCs) for serum SP-A, SP-D, KL-6, %VC, %FVC, %DLco, inflammation index and fibrosis index to predict an NHS were 0.83, 0.75, 0.78, 0.73, 0.71, 0.77, 0.75 and 0.67, respectively (Table 2). The AUC of all variables showed that serum SP-A could predict the presence of an NHS the most accurately among the measured parameters. At an optimal cut-off 45.8 ng/mL, serum SP-A offered 85% sensitivity and 75% specificity for predicting an NHS.

Table 1. Patient's characteristics

Characteristics	All patients	[a]NHS +	NHS –	P NHS+ vs. NHS–
Total (n)	52	27	25	
Age (y)	63±12	64.9±9.4	60.0±14.2	0.193
Male sex, n (%)	31 (62)	18 (67)	13 (52)	0.282
Smoking status (Former / never)	30 / 22	18 / 9	12 / 13	0.173
Pathological proven disease, n (%)	48 (92)	24 (89)	24 (96)	0.336
Parameters				
Serum surfactant protein-A (ng/mL)	66.4±43.5	78.2±43.9	44.0±30.5	< 0.001
Serum surfactant protein-D (ng/mL)	212.2±223.8	287±272	131±116	0.003
Serum KL-6 (U/mL)	1182.8±1080.2	1565±1120	753±868	< 0.001
Vital capacity (%predicted)	95.2±24.1	86.0±24.4	105.2±19.7	0.007
Forced vital capacity (%predicted)	93.8±25.3	84.6±26.4	103.8±20.0	0.013
Diffusion capacity of carbon monoxide (%predicted)	73.9±23.0	64.8±24.9	83.3±16.7	0.001
Inflammation index	4.4±4.2	6.1±3.9	2.5±3.8	0.001
Fibrosis index	0.9±3.1	2.3±4.3	0.2±1.0	0.004
Diagnosis n (%)				
Idiopathic pulmonary fibrosis	10 (19)	6 (22)	4 (16)	0.570
Other interstitial lung diseases	42 (81)	21 (78)	21 (84)	0.570
Outcome				
Follow-up, days	707±402	667±396	749±413	0.404
Death, n (%)	5 (10)	2 (7)	3 (12)	0.575

Footnote:
[a]NHS: negative heart sign

Table 2. Analysis of ROC curves to predict negative heart sign.

Variable	AUC	Best cut-offs	Sensitivity	Specificity	P
Serum surfactant protein-A (ng/mL)	0.83	45.8	85	75	0.002
Serum surfactant protein-D (ng/mL)	0.75	184	74	75	0.011
Serum KL-6 (U/mL)	0.78	603	81	67	0.015
Vital capacity (%predicted)	0.73	80.9	56	87	0.009
Forced vital capacity (%predicted)	0.71	90.4	68	78	0.012
Diffusion capacity of carbon monoxide (%predicted)	0.77	68.5	75	87	0.010
Inflammation index	0.75	7	59	88	0.003
Fibrosis index	0.67	1	37	96	0.086

Table 3. Multiple stepwise regression analysis of primary predictor of negative heart sign.

Variable	Odds ratio	95% Confidence interval	P
Surfactant protein-A (ng/mL)	1.026	1.003–1.050	0.024
Inflammation index	1.358	1.079–1.709	0.009
Fibrosis index	1.433	0.903–2.274	0.127

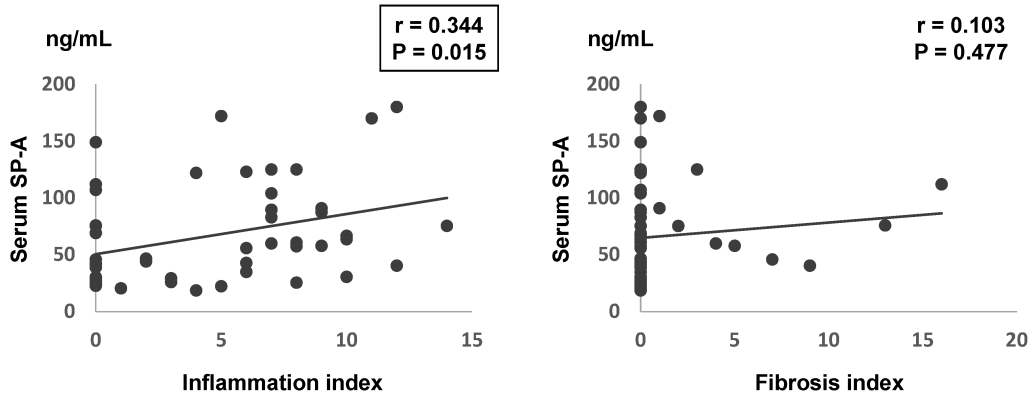

Fig. 4 Relationship between serum SP-A concentrations and HRCT scores
Serum SP-A concentrations correlate significantly with inflammation index (r = 0.344, P = 0.015), but not with fibrosis index (r = 0.103, P = 0.477).

Stepwise multivariate analysis

The variables of age, sex, smoking status, presence of pathologically confirmed disease, IPF or other ILD, serum SP-A, SP-D, KL-6, %VC, %FVC, %DLco, inflammation index and fibrosis index were assessed using stepwise multiple logistic regression. Serum SP-A (odds ratio (OR), 1.026; 95%CI, 1.003–1.050; P = 0.024) and the inflammation index calculated from HRCT findings (OR, 1.358; 95%CI, 1.079–1.709; P = 0.009) were significant predictors of an NHS (Table 3).

Relationship between serum SP-A and HRCT scores

Among patients with and without an NHS, inflammation indices were 6.1 ± 3.9% and 2.5 ± 3.8%, respectively, while fibrosis indices were 2.3 ± 4.3% and 0.2 ± 1.0%, respectively. Serum SP-A concentrations correlated with inflammation index (r = 0.344, P = 0.015), but not with fibrosis index (r = 0.103, P = 0.477) (Figure 4).

DISCUSSION

We aimed to determine an accurate, cost-effective and simpler means of detecting the severity and extent of lung inflammation in patients with ILD.

Lung inflammation due to the accumulation of lymphocytes, neutrophils, or eosinophils influences the pathogenesis of ILD. As a result, assessing lung inflammation in patients with ILD is thus important for evaluating disease activity.[17-20] Actually, 27 (52%) of 52 patients with an NHS

as determined from ^{67}Ga scintigraphy in the present study displayed strong lung inflammation. Values for blood biomarkers (serum SP-A, SP-D, and KL-6), pulmonary function tests (%VC, %FVC, %DLco), and HRCT findings (inflammation and fibrosis indices) were worse among patients with, than without an NHS. Also, 4 of 5 patients (80%) with exacerbated non-IPF lung diseases requiring steroid pulse therapy had an NHS. We therefore speculated that in ILD patients with NHS, attention should be paid to the presence of strong lung inflammation, not only as an AE of ILD, but also in NSIP, OP, and ELD as pathologies for which the standard treatment is steroid administration. However, ^{67}Ga scintigraphy is not an appropriate modality in terms of radiation exposure and labor for patients in the clinical setting who have such strong lung inflammation that requires immediate and intensive treatment. Persistent lung inflammation due to oxidative stress worsens the prognosis for patients with ILD and accumulating evidence is showing that supportive therapy with anti-inflammatory macrolides and steroids (in addition to antifibrotic agents) is useful.[21-24] However, the present study did not investigate relationships between anti-inflammatory therapy according to the presence or absence of an NHS and disease prognosis.

Serum SP-A as a surrogate biomarker of lung inflammation in patients with ILD might be able to replace ^{67}Ga scintigraphy, which can accurately detect lung inflammation, but is too laborious and expensive for the clinical setting.[5-7] SP-A is a member of the collectin family, and alveolar epithelial type II pneumocytes comprise the major source of surfactant apoproteins. Alveolar epithelial type II pneumocytes in lungs with alveolitis secrete SP-A, which is detectable in serum.[1,2] Furthermore, concentrations of SP-A correlate with the extent of alveolitis (confirmed as HRCT findings of ground glass opacity), but not with progression of fibrosis.[3] Consistent with this, we found that serum SP-A correlated with the inflammation index, which represents a robust indicator of alveolitis.[4] As a prospective study of lung biopsy-confirmed IPF has associated elevated serum SP-A with risk of mortality,[25] whether baseline serum SP-A correlates with long-term prognosis in patients with ILD warrants investigation.

The previous and present findings indicate that serum SP-A could provide a cost-effective, simple and rapid alternative to ^{67}Ga scintigraphy. However, this single-institution study of a small number of patients shows some clear limitations that need to be kept in mind when interpreting our results. The repeatability of our findings requires evaluation in a multi-center prospective study. Clinical diagnoses of the enrolled patients were heterogenous, and the clinical relevance of serum SP-A values should therefore be evaluated for specific histopathological diagnoses (for example, IPF alone), although ILD subtypes were not identified as significant predictors of an NHS in the present study. In addition, evaluating the relationship between the cellularity in bronchoalveolar lavage fluid and serum SP-A may identify subtypes of inflammatory cells (neutrophilic or lymphocytic) that more closely correlate with increases in serum SP-A concentrations.

CONCLUSION

Serum SP-A might serve as a surrogate biomarker for predicting an NHS in patients with ILD.

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DISCLOSURE

None of the authors have any real or perceived conflicts of interest to declare regarding the subject of this manuscript.

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