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Original Article

Periostin as a predictor of prognosis in chronic bird-related hypersensitivity pneumonitis

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Abbreviations:

AE, acute exacerbation; AUC, area under the curve; BALF, bronchoalveolar lavage fluid;

DLCO, diffusing capacity for carbon monoxide; ELISA, enzyme-linked immunosorbent assay; FF, fibroblastic foci;

GGO, ground glass opacities;

HP, hypersensitivity pneumonitis;

HRCT, high-resolution computed tomography; HV, healthy volunteer;

IPF, idiopathic pulmonary fibrosis; KL-

6, Krebs von den Lungen-6;

NSIP, nonspecific interstitial pneumonia;

PFT, pulmonary function test; ROC, receiver operating characteristic; SP-D, surfactant protein D; TBE, traction bronchiectasis;

UIP, usual interstitial pneumonia; VC, vital capacity

ABSTRACT

Background: Periostin is an established biomarker of Th2 immune response and fibrogenesis. Recent research has indicated that periostin plays an important role in the pathogenesis of idiopathic interstitial pneumonias. To clarify the relationship between periostin and pathogenesis in chronic bird-related hypersensitivity pneumonitis (HP) and to reveal the usefulness of serum periostin levels in diagnosing and managing chronic bird-related HP.

Methods: We measured serum periostin in 63 patients with chronic bird-related HP, 13 patients with idiopathic pulmonary fibrosis, and 113 healthy volunteers. We investigated the relationship between serum periostin and clinical parameters, and evaluated if the baseline serum periostin could predict the prognosis.

Results: Serum periostin was significantly higher in patients with chronic bird-related HP compared to the healthy volunteers. In chronic bird-related HP, serum periostin had significant positive correlations with serum KL-6 levels, the CD4/CD8 ratio in bronchoalveolar lavage fluid, and fibrosis score on HRCT, and a significant negative correlation with the diffusing capacity of the lungs for carbon monoxide. Chronic bird-related HP patients with serum periostin levels exceeding ≥ 92.5 ng/mL and ≥ 89.5 ng/mL had a significantly worse prognosis and significantly higher frequency of acute exacerbation, respectively. Higher serum periostin (92.5 ng/mL or higher; binary response for serum periostin) was an independent prognostic factor in multivariate analysis.

Conclusions: Serum periostin may reflect the extent of lung fibrosis and play an important role in pathogenesis of chronic bird-related HP. Elevated serum periostin could be a predictor of prognosis in patients with chronic bird-related HP.

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Introduction

Hypersensitivity pneumonitis (HP) is an immune-mediated lung disease triggered by the inhalation of a wide variety of antigens.¹ The clinical presentation of HP is traditionally classified into

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acute and chronic types.² An epidemiologic survey of chronic HP in Japan demonstrated that bird-related HP was the most prevalent form, accounting for 60% of 222 chronic HP cases.³ Our previous study showed that Th2-predominant immune response might play an important role in the development of usual interstitial pneumonia (UIP) pattern.⁴ Among patients with chronic HP, those with UIP pattern have a poor prognosis and high incidence of acute exacerbation (AE).^{5,6} To date, however, none of the established serum biomarkers reflect the extent of fibrosis or overall prognosis in chronic bird-related HP.

Periostin is a 90-kDa extracellular matrix protein that belongs to the fasciclin family. Furthermore, periostin is characterized matrix-cellular protein which binds to several integrin molecules, such as $\alpha v \beta 1 / \beta 3 / \beta 5$, on cell surfaces providing signals for tissue development and remodeling. Takayama *et al.* reported that periostin was secreted by lung fibroblasts in response to Th2 cytokines, interleukin (IL)-4, or IL-13 and contributes to the formation of sub-epithelial fibrosis in patients with bronchial asthma.⁷ Other studies reveal that periostin contributes to the mechanism of pulmonary fibrosis.^{8–10} In a report from Okamoto *et al.*, serum periostin levels were higher in idiopathic pulmonary fibrosis (IPF) than in the other types of idiopathic interstitial pneumonias.⁸ Serum periostin levels may also be a marker of prognosis in patients with IPF.^{9,11}

From these lines of evidence, we hypothesized that periostin is associated with the pathogenesis of chronic bird-related HP and that serum periostin might be predictive of the prognosis of the disease. Our findings from this study demonstrated the potential of serum periostin as a good biomarker of fibrosis and predictor of prognosis in chronic bird-related HP.

Methods

Subjects

Eight hundred-eight patients were hospitalized at our center for the treatment of interstitial lung disease between January 2004 and December 2013. Chronic HP was diagnosed in this population based on clinical, radiological, and histological criteria, as previously described.¹² The inhalation provocation test with an avian antigen was performed to support and refine the bird-related HP diagnosis.¹³ Sixty-six patients were diagnosed with chronic bird-related HP based on positive results in the inhalation provocation test. Three out of the 66 patients were excluded: one with a medical history of connective tissue disease and two whose samples had not been stored. Finally, 63 chronic bird-related HP patients were recruited to this retrospective study. We also recruited IPF patients as disease controls during the same study period. The diagnosis of IPF was based on the criteria established by the American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association.¹⁴ We excluded patients who had a positive titer of antibodies against the avian antigen who had environmental factors known to cause HP, such as the use of feather products, breeding pigeons and so on, and patients whose samples were not stored. Finally, thirteen patients with IPF were evaluated in the study. None of the patients with chronic bird-related HP or IPF had medical histories of atopic dermatitis or bronchial asthma or were receiving treatments at the time of diagnosis. One hundred-thirteen staff members from our hospital and department were evaluated as healthy volunteers (HVs). After this cohort study, we also conducted an additional retrospective study to confirm the cutoff of serum periostin that was predictive of survival and AE derived from the first study. Eleven patients were diagnosed with chronic bird-related HP based on positive results in the inhalation provocation test between January 2014 and March 2017. The study conformed to the Declaration of Helsinki and was

approved by the Medical Research Ethics Committee of Tokyo Medical and Dental University (No. 2265). In adherence with the Ethical Guidelines for Medical and Health Research Involving Human Subjects, information on the study implementation was made public to ensure that the subjects had the opportunity to withdraw their consent at any time.

Study design

Medical records, pulmonary function tests (PFTs), high-resolution computed tomography (HRCT) findings, and analyzed blood sample data of patients with chronic bird-related HP and IPF were reviewed as baseline data at their first admission to our institution. Follow-up data on PFTs, AE, and survival status were also obtained. The criteria of Kondoh were used to define the AE of chronic bird-related HP.¹⁵ The follow-up period was the period from the first admission to our institution to the final observation (July 8, 2015). In the additional cohort, the follow-up period was the period from the first admission to the final observation (July 15, 2017). Death or AE were evaluated based on whether they occurred within the follow-up period. Survival time was defined as the first admission to the final visit or death. The AE-free interval was defined as the first admission to the final visit or the date of onset of AE.

High-resolution computed tomography

Three thin-slice HRCT images at the levels of the aortic arch, carina, and inferior pulmonary vein were extracted. The respective slices from the right and left lungs were reviewed independently by two observers (M. M., a pulmonary specialist, and M. K., a chest radiologist) who had no knowledge of the patients' clinical information. The fibrosis area and ground glass opacities (GGO) were respectively assigned a fibrosis score and GGO score by Kazerooni's method.¹⁶ Reticulation, centrilobular nodules, consolidation, and emphysema were separately quantified as proportions of lung parenchyma between 0% and 100%, and censored at 5%. Traction bronchiectasis (TBE) was scored as previously reported: grade 0 = none, 1 = mild, 2 = moderate, 3 = severe, based upon the most severely affected airways in that pattern.¹⁷ The global score was calculated as the mean of the six zones composed of each slice from the right and left lungs. Averages of all of the values assessed by the two observers were calculated for each subject.

Pulmonary function tests

The PFT data included vital capacity (VC) and diffusing capacity for carbon monoxide (DL_{CO}). Declines in the PFTs at 6 months from baseline were determined by calculating the differences in the PFT measurements ($\Delta\%VC$ and $\Delta\%DL_{CO}$).

Bronchoalveolar lavage

Bronchoalveolar lavage was performed using three 50-ml aliquots of sterile 0.9% saline. The cellular profile of bronchoalveolar lavage fluid (BALF) was determined by counting 200 cells in a cytospin smear with Wright's stain. The lymphocyte phenotypes were analyzed by flow cytometry using monoclonal antibodies for CD4 and CD8. BALF were stored at $-80^{\circ}C$ until use.

Measurement of periostin in serum and bronchoalveolar lavage fluid

Serum and BALF periostin levels were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using two rat anti-periostin monoclonal antibodies (clones nos. SS18A and SS17B), as

previously described (Supplementary Methods).^{8,18} Immunohistochemical analyses were performed by a previously reported method using antiperiostin monoclonal antibodies (clone nos. SS19C, 1 µg/mL) (Supplementary Methods).^{8,19} BALF periostin levels were also adjusted using total protein of BALF.

Statistical analysis

Data were described as the mean $\pm$ standard deviation. Two-tailed *P* values of less than 0.05 were considered significant. The two groups were compared using the Mann–Whitney *U* test or Fisher's exact test. Correlation coefficients including inter-observer variation for the extent of various abnormalities were obtained using Spearman's correlation coefficient test. The most appropriate cutoff values were defined by receiver operating characteristic (ROC) analysis using the Youden index, and the area under the curve (AUC) values were calculated. Univariate and multivariate Cox proportional hazard survival models were used to examine the serum periostin level and other clinical variables. Multivariate analysis was performed using variables with univariate *P* values of less than 0.05 and clinically relevant covariates (age, gender, smoking status, and %VC). Forward selection was used for model building in multivariate analysis. Cumulative survival curves and AE-free interval curves were constructed using the Kaplan–Meier method. Comparisons of the cumulative survival rate between two groups was based on the log-rank test. The cumulative rate of being free from AE was based on Gray's test because there was a competing risk between AE and death. The univariate and multivariate analyses were performed using SPSS Statistics version 22.0 (IBM Corp., Chicago, IL, USA). Gray's test was performed using EZR.²⁰ The other analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA).

Results

The patient characteristics are shown in Table 1. Patients with chronic bird-related HP and IPF were significantly older than the healthy volunteers (*P* < 0.001). The number of cigarette pack-years

Table 1
Characteristics of patients.

Characteristic	Chronic bird-related HP (n = 63)	IPF (n = 13)	HV (n = 113)
Gender			
Male	35	11	76
Female	28	2	37
Age, yr.	62.0 $\pm$ 11.4 [#]	67.7 $\pm$ 8.3 [#]	41.2 $\pm$ 10.1
Pack years	20.3 $\pm$ 29.8 [*]	42.5 $\pm$ 26.3	ND
Surgical lung biopsy	21	2	ND
Histological pattern	UIP 11/fNSIP 9/cNSIP1	UIP 2	ND
Serum KL-6, U/ml	1755.0 $\pm$ 1651.0	1212.0 $\pm$ 583.0	ND
Serum SP-D, ng/ml	310.9 $\pm$ 310.7	176.2 $\pm$ 77.6	ND
PFTs			
VC, L	2.3 $\pm$ 0.8	2.5 $\pm$ 0.7	ND
%VC	76.7 $\pm$ 15.7	78.7 $\pm$ 16.0	ND
DLco, ml/min/mmHg	9.9 $\pm$ 4.2	9.9 $\pm$ 5.8	ND
%DLco	55.2 $\pm$ 20.1	51.9 $\pm$ 24.5	ND
Acute exacerbation	19 (30%)	3 (23%)	ND
Treatment	46 (73%)	11 (85%)	ND

Data are given as number or mean $\pm$ SD.

[#]*P* < 0.05 vs. HV. ^{*}*P* < 0.05 vs. IPF.

ND, not determined; HP, hypersensitivity pneumonitis; IPF, idiopathic pulmonary fibrosis; HV, healthy volunteers; UIP, usual interstitial pneumonia; fNSIP, fibrotic nonspecific interstitial pneumonia; cNSIP, cellular nonspecific interstitial pneumonia; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D; PFTs, pulmonary function tests; VC, vital capacity; DLco, diffusing capacity of the lung for carbon monoxide.

was significantly higher for IPF patient than chronic bird-related HP patients (*P* < 0.001). There was no statistically significant difference in the following parameters between patients with chronic bird-related HP and IPF: Krebs von den Lungen-6 (KL-6), surfactant protein D (SP-D), PFTs, and the numbers of patients who experienced AE and received medication during the follow-up period. The CD4/8 ratio and percentage of lymphocytes in BALF were significantly higher for chronic bird-related HP than IPF, while the percentage of macrophages in BALF was significantly lower (Supplementary Table 1). Twenty-one chronic bird-related HP patients underwent surgical lung biopsy: 11 had a UIP pattern, 9 had a fibrotic nonspecific interstitial pneumonia (fNSIP) pattern, and 1 had a cellular NSIP (cNSIP) pattern. All chronic bird-related HP patients were instructed to avoid not only direct exposure (breeding birds) but also unrecognized exposure (feather products, wild birds, and breeding of birds by neighbors). Forty-six chronic bird-related HP patients received medication during the follow-up period. Twenty-six chronic bird-related HP patients were treated with a corticosteroid and immunosuppressants, and 20 patients were only treated with a corticosteroid. Eleven patients were treated with antifibrotic agents in addition to a corticosteroid or immunosuppressants.

The serum periostin level was significantly higher in patients with chronic bird-related HP and IPF compared to the healthy volunteers (chronic bird-related HP vs. HV, 101.5 $\pm$ 33.9 vs. 89.7 $\pm$ 21.2 ng/mL, *P* = 0.010; IPF vs. HV, 106.1 $\pm$ 40.1 vs. 89.7 $\pm$ 21.2 ng/mL, *P* = 0.030). There was no difference in serum periostin between the chronic bird-related HP and IPF patients (*P* = 0.639) (Fig. 1). When we examined the differences in serum periostin among the different histological patterns of chronic bird-related HP, the level tended to be higher in the UIP pattern compared to the fNSIP and cNSIP patterns, but not to a significant degree (UIP pattern vs. fNSIP pattern, 107.7 $\pm$ 34.9 vs. 75.6 $\pm$ 28.7 ng/mL, *P* = 0.087; cNSIP pattern, 56 ng/mL) (Supplementary Fig. 1).

In our analysis of the correlations between serum periostin and the clinical parameters for chronic bird-related HP, serum periostin was significantly but weakly correlated with the serum KL-6 level (*r* = 0.272, *P* = 0.031) and DLco (*r* = −0.312, *P* = 0.016) and was moderately correlated with the CD4/8 ratio in BALF (*r* = 0.565, *P* = 0.006) (Table 2). In our analysis of the correlations between

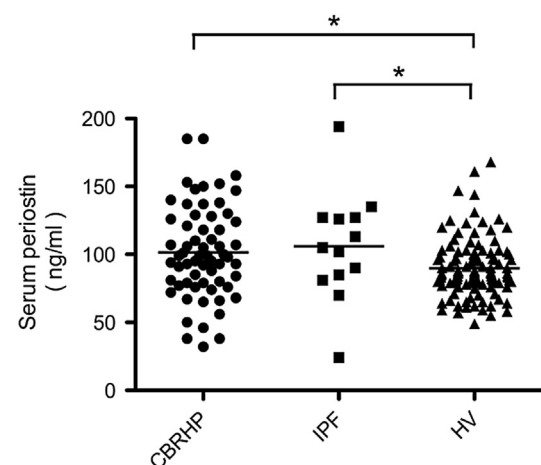


Fig. 1. Serum periostin level in patients with chronic bird-related hypersensitivity pneumonitis, idiopathic pulmonary fibrosis, and healthy volunteers. CBRHP, chronic bird-related hypersensitivity pneumonitis; n = 63; IPF, idiopathic pulmonary fibrosis; n = 13; HV, healthy volunteers; n = 113. The solid lines represent mean values. ^{*}*P* < 0.05.

Table 2
Correlation between serum periostin and the clinical parameters in chronic bird-related HP.

	Mean	r	P
KL-6, U/ml	1755.0 ± 1651.0	0.272	0.031*
SP-D, ng/ml	310.9 ± 310.7	0.139	0.277
PFTs			
VC, L	2.3 ± 0.8	−0.017	0.897
%VC	76.7 ± 15.7	−0.024	0.852
DL _{CO} , ml/min mmHg	9.9 ± 4.2	−0.312	0.016*
%DL _{CO}	55.2 ± 20.1	−0.211	0.110
Δ%VC	−0.3 ± 7.4	−0.178	0.222
Δ%DL _{CO}	−0.9 ± 8.2	−0.072	0.662
BALF			
Total cell counts, 10 ⁵ /ml	5.4 ± 8.7	−0.106	0.563
Macrophages, %	68.7 ± 24.4	0.000	0.992
Lymphocytes, %	20.4 ± 20.9	0.178	0.287
Neutrophils, %	9.2 ± 13.6	−0.231	0.203
Eosinophils, %	1.6 ± 3.3	−0.217	0.233
CD4/CD8 ratio	4.7 ± 4.2	0.565	0.006**

Data are given as mean ± SD.

P* < 0.05. *P* < 0.01.HP, hypersensitivity pneumonitis; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D; PFTs, pulmonary function tests; VC, vital capacity; DL_{CO}, diffusing capacity of the lung for carbon monoxide; BALF, bronchoalveolar lavage fluid.

serum periostin and HRCT findings, the inter-observer correlations in the extent of the various radiological abnormalities were statistically significant ($r = 0.511$ – 0.901 , all $P < 0.001$) (Supplementary Table 2). Serum periostin was also significantly correlated with the fibrosis score ($r = 0.298$, $P = 0.023$) (Table 3).

We measured the BALF periostin levels by ELISA in 32 out of 63 patients with chronic bird-related HP and in 8 out of 13 patients with IPF. No significant difference in the BALF periostin level was found between the chronic bird-related HP and IPF patients (2.6 ± 10.1 vs. 0.5 ± 0.4 ng/mL, $P = 0.390$) (Supplementary Table 1). There were no significant correlations between the serum periostin and BALF periostin levels in patients with chronic bird-related HP ($r = 0.318$, $P = 0.076$).

Figure 2 shows representative UIP and fNSIP patterns from our immunohistochemical analysis of lung tissues obtained from 8 UIP pattern and 6 fNSIP pattern patients with chronic bird-related HP. Periostin was positively stained within the area of the fibrotic lesion including the fibroblastic foci (FF) (Fig. 2A–C). Periostin was also stained in the thickened alveolar walls (Fig. 2D, E). By contrast, no periostin was detected in the alveolar epithelium.

Twenty-four patients with chronic HP died during the follow-up period. The causes of death were AE ($n = 14$), chronic respiratory failure ($n = 6$), lung cancer ($n = 1$), infection ($n = 1$) and unknown ($n = 2$). A ROC analysis was conducted to define the cutoff of serum periostin that was predictive of survival, which produced an optimal AUC and cutoff value of 0.603 and 92.5 ng/mL, respectively. Next, predictive factors associated with prognosis in chronic bird-

Table 3
Correlation between serum periostin and HRCT findings in chronic bird-related HP.

	Mean	r	P
GGO score	1.04 ± 0.76	−0.037	0.780
Fibrosis score	1.17 ± 0.55	0.298	0.023*
Reticulation, %	7.08 ± 7.70	0.013	0.924
Centrilobular nodules, %	1.74 ± 4.03	−0.045	0.738
Consolidation, %	1.57 ± 2.13	−0.106	0.429
Emphysema, %	3.21 ± 8.74	−0.044	0.743
TBE grade	0.62 ± 0.46	0.140	0.294

Data are given as mean ± SD.

**P* < 0.05.

HP, hypersensitivity pneumonitis; GGO, ground glass opacity; TBE, traction bronchiectasis.

related HP were examined using univariate and multivariate Cox proportional hazard models. In univariate analysis, the serum periostin level (92.5 ng/mL or higher; binary response for serum periostin), reticulation score, and TBE grade were all significantly associated with mortality (Table 4). Then, multivariate analysis was performed using variables with univariate *P* values of less than 0.05 (the serum periostin level [binary and continuous], reticulation score, and TBE grade) and clinically relevant covariates (age, gender, smoking status, and %VC). Forward selection was used for model building in multivariate analysis. The serum periostin levels and TBE conferred an elevated risk of mortality (Table 4). Survival in chronic bird-related HP patients divided into the higher-periostin group ($n = 39$, serum periostin ≥ 92.5 ng/mL) and lower-periostin group ($n = 24$, serum periostin < 92.5 ng/mL) were analyzed using Kaplan–Meier survival curves. The patient characteristics of the higher-periostin group (serum periostin ≥ 92.5 ng/mL) and lower-periostin group (serum periostin < 92.5 ng/mL) are shown in Supplementary Table 3. The log-rank test showed a significant association between the serum periostin level and survival time ($P < 0.001$, Fig. 3A). The higher-periostin group had a mean survival time of 34.8 months (95%CI: 25.2–44.5) and the lower-periostin group had a mean survival time of 65.0 months (95%CI: 46.0–84.1).

A ROC analysis conducted to define the cutoff serum periostin level predictive of AE produced an AUC of 0.554 and optimal cutoff value of 89.5 ng/mL. The patients were divided into the higher-periostin group ($n = 41$, serum periostin ≥ 89.5 ng/mL) and lower-periostin group ($n = 22$, serum periostin < 89.5 ng/mL) to analyze the incidence of AE. The patient characteristics of the higher-periostin group (serum periostin ≥ 89.5 ng/mL) and lower-periostin group (serum periostin < 89.5 ng/mL) are shown in Supplementary Table 4. Grey's test showed a significant association between serum periostin and the AE-free interval ($P = 0.037$, Fig. 3B). The mean AE-free interval was 35.2 months in the higher-periostin group (95%CI: 26.0–44.5) and 62.0 months in the lower-periostin group (95%CI: 42.4–81.5).

Furthermore, in an additional study, we validated the value of the cutoff serum periostin that was predictive of survival and AE derived from the first cohort study. Eleven patients with chronic bird-related HP were recruited to the additional study (Supplementary Table 5). Two of the 11 patients developed AE and died shortly afterward. These patients were in the higher-periostin group (with serum periostin values of 130 and 139 ng/mL). Survival in chronic bird-related HP patients was also analyzed using Kaplan–Meier survival curves ($n = 7$; serum periostin ≥ 92.5 ng/mL, $n = 4$; serum periostin < 92.5 ng/mL). Though the log-rank test did not show a significant association between the serum periostin level and survival time ($P = 0.225$, Supplementary Fig. 2A), the mean survival time tended to be shorter in the higher-periostin group compared to the lower-periostin group (higher-periostin group vs. lower-periostin group, 34.2 months [95%CI: 6.6–61.8] vs. 57.4 months [95%CI: −31.4 - 146.2]). The incidence of AE was also analyzed using Kaplan–Meier survival curves ($n = 7$; serum periostin ≥ 89.5 ng/mL, $n = 4$; serum periostin < 89.5 ng/mL). Though Grey's test did not show a significant association between serum periostin and the AE-free interval ($P = 0.227$, Supplementary Fig. 2B), the mean AE-free interval tended to be shorter in the higher-periostin group than in the lower-periostin group (higher-periostin group vs. lower-periostin group, 34.1 months [95%CI: 6.6–61.7] vs. 57.4 months [95%CI: −31.4 - 146.2]).

Discussion

Patients with chronic bird-related HP often develop pulmonary fibrosis and have a poor prognosis.⁵ The clinical course of chronic bird-related HP with fibrosis is similar to that of IPF. New prognostic

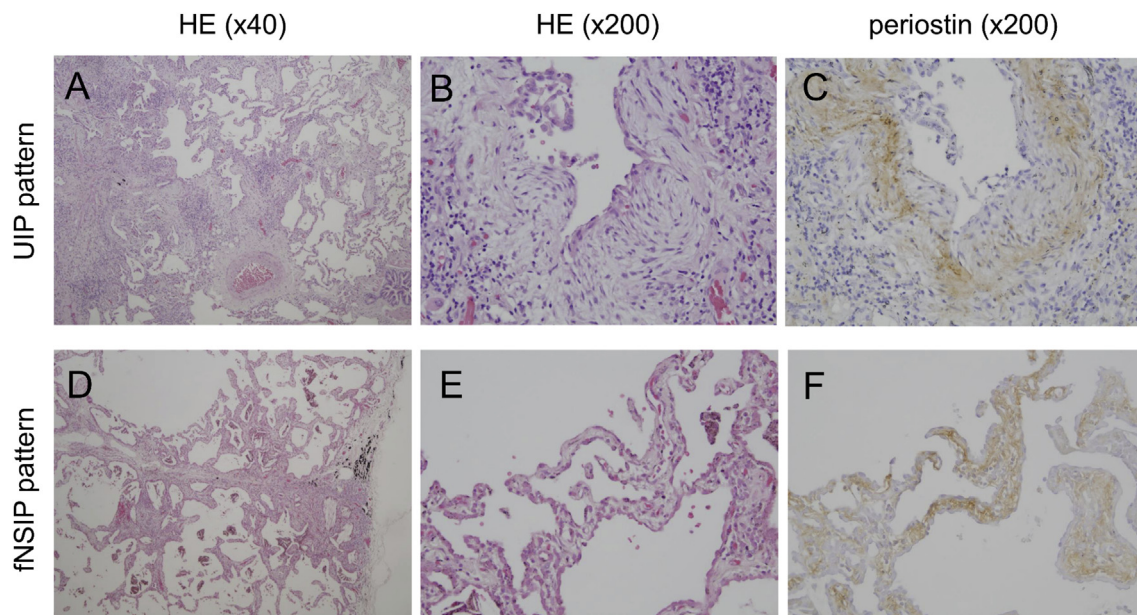


Fig. 2. Representative picture of immunohistochemistry of periostin in the surgical lung biopsy specimen. (A)–(C), usual interstitial pneumonia (UIP) pattern and (D)–(F), fibrotic nonspecific interstitial pneumonia (fNSIP) pattern. The tissues were stained with hematoxylin and eosin (HE) or periostin, as shown.

biomarkers are needed for the treatment and management of patients with chronic HP. In this study, which is the first to investigate serum periostin levels in chronic bird-related HP, serum periostin was significantly higher in patients with chronic bird-related HP compared to healthy volunteers. Serum periostin was also positively correlated with DLCO and the fibrosis score on HRCT and appeared to be potentially predictive of prognosis and AE in patients with chronic bird-related HP. On the other hand, the levels of serum periostin among patients with chronic bird-related HP, IPF, and healthy volunteers were partially overlapped. This finding suggests that serum periostin is less useful as a marker for the diagnosis of chronic bird-related HP but also confirms the existence

of a fibrosing pattern in histology. In this study, the number of patients with IPF was relatively small. As the radiological and histological features of chronic HP overlapped IPF, there was a possibility that many patients with chronic HP were diagnosed as IPF.²¹ As shown in the methods section, patients who may have had

Table 4
Survival analysis with Cox proportional hazards model in chronic bird-related HP.

	Hazard ratio	95% Confidence interval	P
Univariate analysis			
Serum biomarkers			
Periostin, binary	9.302	2.704–32.001	<0.001***
Periostin, continuous	1.022	1.008–1.036	0.002**
KL-6	1.000	1.000–1.000	0.224
HRCT findings			
GGO score	1.296	0.740–2.272	0.365
Fibrosis score	2.437	0.919–6.464	0.074
Reticulation	1.079	1.017–1.146	0.012*
Centrilobular nodules	0.305	0.823–1.063	0.305
Consolidation	1.167	0.957–1.424	0.127
Emphysema	0.963	0.901–1.030	0.272
TBE grade	4.621	1.642–13.005	0.004**
Multivariate analysis			
Serum biomarkers			
Periostin, binary	8.398	2.374–29.703	0.001**
HRCT findings			
TBE grade	4.095	1.530–10.959	0.005**

Covariates with $P < 0.05$ in univariate analysis and clinical relevant covariates such as age, gender, smoking status, and %VC were included in multivariate analysis (Cox regression).

Cutoff point of binary serum periostin: 92.5 ng/mL.

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

HP, hypersensitivity pneumonitis; KL-6, Krebs von den Lungen 6; HRCT, high-resolution computed tomography; GGO, ground glass opacity; TBE, traction bronchiectasis.

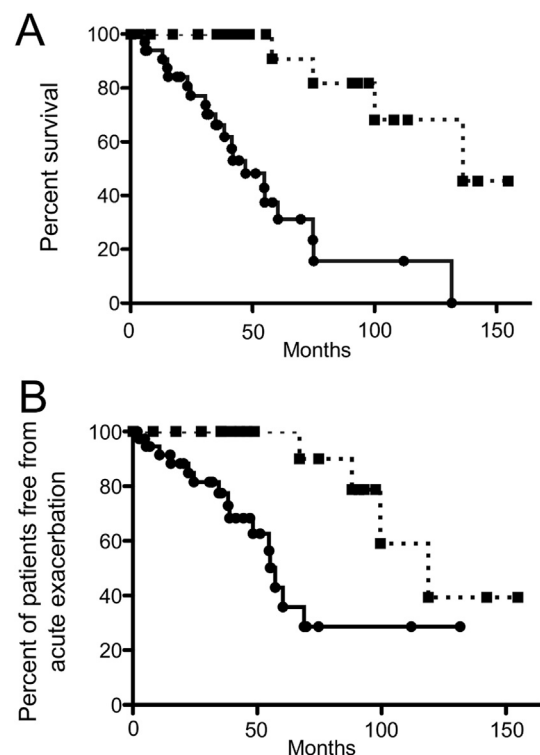


Fig. 3. Kaplan–Meier curves for survival (A) and acute exacerbation-free interval (B) in chronic bird-related hypersensitivity pneumonitis. (A) Solid line represents higher-periostin group (serum periostin levels ≥ 92.5 ng/mL) dotted line represents lower-periostin group (< 92.5 ng/mL) (log-rank test, $P < 0.001$). (B) Solid line represents higher-periostin group (serum periostin levels ≥ 89.5 ng/mL) dotted line represents lower-periostin group (< 89.5 ng/mL) (Grey's test, $P = 0.037$).

chronic HP were excluded from the population considered to have IPF. Okamoto *et al.* reported that the serum periostin level in 37 IPF patients was 107.1 ± 11.9 ng/mL.⁸ This result was similar to the outcomes of our study. In this study, the healthy volunteers were younger than the patients with chronic bird-related HP and IPF, and there was a deficit in the data on smoking status in healthy volunteers. Okamoto *et al.* also reported that the serum periostin level in 66 healthy volunteers (40 males and 26 females aged 60.7 ± 2.1 years) was 39.3 ± 3.0 ng/mL. They also reported that smoking status was not associated with the levels of serum periostin in patients with idiopathic interstitial pneumonias and healthy controls.⁸ Noting that earlier reports showed a minimum decrease of levels in serum periostin along with advancing age in adulthood, we considered that the results of our study were less influenced by the age difference between the patients and healthy volunteers.²²

Serum periostin was significantly correlated with DL_{CO}, the CD4/8 ratio in BALF, and the fibrosis score in the HRCT findings. In a previous report, the CD4/8 ratio in BALF was significantly higher in HP with lung fibrosis compared to HP without fibrosis.²³ Chronic HP patients with a greater extent of FF had more honeycombing on HRCT and a lower %DL_{CO} compared to patients with a lesser extent of FF.²⁴ Okamoto *et al.* reported that periostin was highly expressed in the FF of lung specimens from patients with IPF.⁸ In our immunohistochemical analysis of lung specimens from chronic bird-related HP patients, the area positive for periostin expression was similar to the previously reported positive area. The correlations of the serum periostin levels with DL_{CO} and the fibrosis score might be related to the existence of FF. In this study, although there was no statistically significant difference, the levels of serum periostin were higher in the chronic bird-related HP with the UIP pattern than with the fNSIP and cNSIP patterns. The finding of higher serum periostin in the UIP pattern was similar to the result of a previous study in idiopathic interstitial pneumonias.⁸ Our data suggest that the serum periostin level may reflect fibrogenesis in chronic bird-related HP as well as in IPF. It was sometimes difficult to differentiate between the two histological patterns for UIP and fNSIP. Our previous study of HP showed that patients with the UIP pattern had a significantly higher frequency of AE than those with the fNSIP pattern.⁶ On the other hand, the percentage of patients with FF were higher in the chronic bird-related HP with the UIP pattern compared to fNSIP, but not significantly.⁵ In our previous study of the Th1/Th2 balance in the pathogenesis of chronic bird-related HP, the ratio of thymus- and activation-regulated chemokine, TARC (Th2-type chemokines), to interferon-inducible protein, IP-10 (Th1-type chemokines), in the serum of patients with the UIP pattern was higher than in those with the fNSIP pattern, but that increase was also not significant.⁴ No significant difference in the serum periostin levels between the UIP pattern and fNSIP pattern were responsible for the partial similarity of pathogenesis as described above. Because the number of patients who underwent surgical lung biopsy was small in this study, the relationship between the serum periostin level and histological pattern did not go beyond speculation. Further histopathological analysis is needed to reveal the difference in the serum periostin levels in the histopathological pattern.

We also found a significant correlation between the serum periostin level and serum KL-6 level, which is a finding that is inconsistent with a previous report on IPF.⁸ KL-6 is produced by regenerating type II pneumocytes,²⁵ whereas periostin is secreted by lung fibroblasts in response to IL-4 and/or IL-13.⁷ Our group previously found that the serum KL-6 levels were higher in chronic HP patients than in IPF patients and were correlated with the percentage of lymphocytes in BALF from chronic HP patients.²⁶ These data on KL-6 suggested that the epithelial cell impairment

by lymphocytic alveolitis might increase the KL-6 levels in serum. Lymphocytic alveolitis has also been reported to be significantly more frequent in chronic HP patients with the UIP pattern than in patients with IPF.²⁷ This controversial correlation between the serum periostin and KL-6 levels may contribute to the difference in lymphocytic alveolitis observed between chronic HP and IPF.

We detected periostin in BALF from both chronic bird-related HP and IPF patients, albeit at far lower levels than periostin detected in serum. The levels of periostin in bronchial lavage fluid and BALF have been measured in earlier studies.^{8,18,28} The mechanism through which periostin secretes or leaks into BALF has yet to be determined. Further studies to clarify the meaning and mechanisms of periostin in BALF are needed.

The serum periostin level was related to an elevated risk of mortality in the present study. Periostin was recently reported to be a prognostic biomarker in IPF. A cohort study of North American IPF patients showed the potential utility of serum periostin as a biomarker of prognosis in IPF by demonstrating its power to predict disease progression within 48 weeks.⁹ Evidence from Tajiri *et al.* also demonstrated the potential of serum periostin to serve as a good predictive biomarker of prognosis.¹¹ Serum periostin was related to several clinical parameters in our own study, such as the fibrosis score and DL_{CO}, although the levels of correlation were weak. A CT finding of parenchymal fibrosis is associated with increased mortality and performs well as a prognostic indicator in HP.²⁹ Another recent study by our group found that patients with a greater extent of FF had more reticulation, honeycombing, and TBE on HRCT, as well as a higher risk of mortality.²⁴ DL_{CO} has also been shown to be valuable as a prognostic factor in IPF.³⁰ Taken together, these findings suggest that serum periostin might be a prognostic biomarker in chronic bird-related HP.

We also examined the relationship between serum periostin levels and AE. Our previous study showed that higher serum levels of the Th2 chemokine CCL17 might be predictive of AE in chronic HP.³¹ The relationship between higher serum periostin levels and the AE frequency in chronic bird-related HP may be associated with the predominance of a Th2 immune response.

This study had several limitations. First, this study was performed in a single center. Furthermore, there was a referral bias because our hospital is a clinical center for chronic HP. Second, pulmonary function may not have been remarkably deteriorated in the patients we recruited because we diagnosed chronic bird-related HP based on the result of the inhalation provocation test. Munoz and colleagues recommend that the inhalation provocation test not be performed on patients with a low forced vital capacity and DL_{CO} with respiratory insufficiency ($\text{PaO}_2 < 60$ mmHg).³² Third, the subjects of this study were limited to bird-related HP among some forms of HP, even though bird-related HP is the most prevalent form in Japan. Fourth, in an additional study, the higher-periostin group tended to have a worse prognosis and higher frequency of AE, but the difference was not significant. This result may be caused by the small sample size of the additional study. We will need to conduct a prospective study to certify the results.

In conclusion, this study demonstrated that periostin might reflect the extent of lung fibrosis and play an important role in chronic bird-related HP. Serum periostin levels could be a novel predictor of prognosis in patients with chronic bird-related HP.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2019.02.007>.

Conflict of interest

KI was supported by grants from Shino-Test, Astra Zeneca and received lecture fees from Astra Zeneca. JO is an employee of Shino-Test. The rest of the authors have no conflict of interest.

Authors' contributions

YM had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. YM and YN contributed to the conception and design of the study; YM, YN, MM, TO, HF, TT, MK, UT, and NI contributed to the analysis and interpretation of the data; JO, SO, and KI contributed to establishment of periostin monoclonal antibody and method for measuring periostin.

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